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RESEARCH ARTICLE

Investigating Psychological Well-being and Emotional Regulation in Indian Youth with Hearing Impairments

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ABSTRACT: Hearing-impaired individuals experience significant psychological challenges that often go unrecognized due to the lack of awareness surrounding their experiences. Individuals struggle with communication, education, and employment barriers, yet their difficulties are not adequately addressed. Limited literature exists on the psychological well-being and emotional regulation of hearing-impaired youth in India. Thus, this study aims to fill this gap and shed light on the psychological obstacles experienced. Using a mixed-methods approach, quantitative data were obtained using 2 scales, the BERQ (Behavior Emotion Regulation Questionnaire) and SBW (Subjective Well-being Scale), with the hearing-impaired youth. Qualitative data was obtained by conducting interviews with the school's faculty for the hearing-impaired. Analysis revealed that positive relationships with others were moderately correlated with self-acceptance and purpose in life, while seeking social support was also linked to a clearer sense of purpose. Additionally, distraction as a coping strategy showed a moderate positive relationship with both personal growth and social support-seeking behaviors. The qualitative findings highlighted psychological challenges faced by hearing-impaired students, including social stigma, feelings of isolation, low self-esteem, and difficulty expressing emotions. Findings indicate the need for increased family support, better communication training, and structured initiatives to improve emotional well-being.

KEYWORDS: Behavioural and Social Sciences, Clinical Psychology, Hearing Impaired, Emotional Regulation, Psychological Well-being, India.

Introduction

According to the WHO, individuals between the ages of 15 and 24 years are classified as "youth."[1] As of 2018, 20,00,000 Indians between 15-24 years of age are estimated to have reported being hearing impaired.[2] Given the significant number of individuals impacted by hearing impairment, studying their psychological well-being is of utmost importance.[3] Psychological well-being is defined as the presence of positive emotions (e.g., high self-esteem) and the absence of negative emotions (e.g., symptoms of depression).[4] Subjective well-being refers to an individual's overall cognitive and emotional assessment of their personal life; it encompasses cognitive evaluations of life satisfaction and both positive and negative emotional responses to specific life events.[5] The mental well-being of people with hearing impairments specifically is significantly threatened, which may lead to a decline in their quality of life as well. In a study investigating the impact of hearing loss on cognitive and emotional aspects, most participants indicated that hearing impairment carries a negative connotation and results in poor well-being.[7]

Apart from psychological well-being, to get a balanced perspective of the emotional health of those with hearing impairment, it is important to study their patterns of emotion regulation. According to APA, emotional regulation is defined as "the ability of an individual to modulate an emotion or set of emotions."[8]

In a study, participants with hearing impairments, aged 16-25, reported that the emotional regulation strategies employed by the majority of the sample included positive coping strategies such as refocusing on planning and positive reappraisal. Notable correlations between emotion regulation strategies and psychological issues were identified, suggesting that negative emotion regulation strategies are linked with psychological conditions.[9]

The study above used the Behavioral Emotion Regulation Questionnaire (BERQ), which investigates the behavioral aspects of emotional regulation. It has also been reported that adults with hearing loss are more likely to experience increased symptoms of depression, anxiety, psychological distress, and emotional sensitivity as compared to those with normal hearing.[10]

The most notable contribution to the theory of emotional regulation was made by James Gross, who claims that "much of what we feel is a consequence of our reactions to the world."[11] Emotion regulation constitutes any effort to modify any emotional experience (positive and negative emotions)[12] and categorizes five strategies for managing emotions: situation selection (e.g., approach/avoidance of situations), situation modification (e.g., altering emotion-eliciting aspects), attentional deployment (e.g., shifting focus within the situation), cognitive change (e.g., rethinking a situation), and response

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which occur before or during the emotion experience, and response-focused strategies, which occur after the emotion is fully developed.[14] The process model suggests that individuals can employ strategies to regulate their emotional intensity at various stages.[15]

Like Iwagami's paper listed above, similar results were found in a study carried out by Carolien Rieffe for hearing-impaired children.[16] This study compared the level of emotional understanding between hearing-impaired children and their hearing peers. A total of 26 hearing-impaired children (mean age: 11 years) and 26 hearing children of the same age and gender participated in tasks to evaluate their comprehension of emotions and their ability to manage them across the four fundamental emotions: happiness, anger, sadness, and fear. The findings revealed that hearing-impaired children are equally adept at recognizing their own emotions as well as identifying the triggers of these emotions. They can also understand the coexistence of opposite-valence emotions, such as feeling happy and sad simultaneously. However, the study found that these individuals struggled to distinguish between different negative emotions. Their emotional regulation strategies tended to focus on actively addressing the situation. However, the use of any avoidance tactics to lessen the negative impact of the situation was barely reported by the children. These strategies appeared less effective than those of their hearing peers.

To counter that, it is necessary to focus on the nature of interventions. Based on the works of Iwagami, Rieffe, Gross, and other researchers, a study conducted by Danadel and Ashori[21] noted that being able to emotionally self-regulate has a significant implication on the problem-solving abilities for those with hearing impairment. This has the potential to create directed interventions and opportunities to increase both emotion regulation and problem-solving skills to improve overall well-being as well. For individuals with hearing impairment, families play a significant role in regulating their emotional security. Here again, work by Iwagami as well as Brown and Cornes[22] highlights the varying degrees of struggles and mental health concerns for those with hearing impairment vs. their hearing peers. This emphasises the need to understand the role of families in emotional regulation and well-being for the hearing impaired and to curate remedies accordingly.

Significant gaps exist in the area of emotional health of the hearing-impaired. To begin, there is limited research that explores how Indian youth with hearing impairments define their own well-being as well as the nature of emotional regulation strategies that they use to cope with everyday struggles in their daily lives. Additionally, to provide the study with a more holistic perspective, it is essential to interview informants who can help give a deeper understanding of the stigma experienced by these youth. Due to India being a large geographical landscape, this study will be limited specifically to the city of Mumbai, as it hosts a multitude of different cultures and lifestyles. Mumbai is also reported to have one of the largest populations of people with hearing impairments, making it an ideal city for this research. The key takeaway and contribution of this work is to offer new literature in a niche research area of psychological health in youth with hearing impairment.

To summarize, the primary aim of this study is to evaluate the psychological well-being of individuals with hearing impairments by examining factors such as their psychological and subjective well-being and emotional regulation. A key focus of this study is to understand how the experience of social stigma associated with hearing impairment affects their mental and emotional state, including their self-esteem, relationships, and sense of belonging. Additionally, the study aims to explore the emotional regulation strategies they employ by investigating how they cope with emotions in situations that involve stigma or other stressors and how these strategies contribute to their overall psychological well-being.

The study examined the psychological well-being and emotional regulation of hearing-impaired youth in India by sending questionnaires to these youth for quantitative data and conducting interviews with teachers for qualitative data.

Methods

Participants:

An exploratory study was conducted to investigate the relationship between psychological well-being and emotional regulation. A convenience sample of youth between 18 and 23 years of age who all attended TeachEDU, a higher education institute for the hearing impaired, was selected. They were sent a Google form with the consent form, BERQ (Behavior Emotion Regulation Questionnaire), and SBW (Subjective Well-being Scale). The inclusion criteria for the participants were as follows: they must reside in Mumbai, be between 18 and 23 years of age, and have experienced hearing impairment for a minimum of 5 years. The exclusion criteria for this study were that participants should not have received any alternate treatments for either their hearing impairment or any sort of medication for their psychological health. The questionnaire was then filled out by 30 participants, with a mean age of 21.5 years, all of whom were from Mumbai, India.

Procedure:

Prior permission was first obtained from the management and faculty at TeachEDU. No incentives were provided to the school or the participants participating in the questionnaires. After students consented, an online Google form asking for demographic information and the 2 questionnaires, BERQ & SWB, was sent to 30 participants via the school counselors. Google Forms were selected to overcome communication barriers with the hearing-impaired participants and to garner personalized and realistic data.

Measures:

The SWB questionnaire, created in 1989, evaluated the participants' overall mental health by examining key aspects such as emotional stability, life satisfaction, coping strategies, and relationship management. For this questionnaire, the Cronbach's alpha ranged between 0.62 and 0.91 across the six subscales. It included questions such as "to what extent do you agree to the following questions", " When I look at the story of my life, I am pleased with how things have turned out so far", "In many ways I feel disappointed about my achievements in life" and, I

live life one day at a time and don't really think about the future The BERQ, created in 2019 by Kraaij and Garnefski had Cronbach's Alpha ranging in value from 0.86 to 0.93. BERQ assessed how individuals regulate their emotions through specific behavior in challenging situations. Sample questions from this questionnaire included: "I often think about how sad I feel about what happened.", "I worry that this will have terrible consequences for the future," and " I accept that this has happened and cannot be changed". After collecting the quantitative responses, interviews were scheduled with 5 faculty members working at TeachEDU. These interviews were scheduled over 2 weeks on the online platform, Google Meet. In these structured interviews, the faculty members were asked 10 questions, including the common psychological challenges faced by the hearing-impaired students, the intensity of their emotional issues, the impacts of social stigma, and how the school supports and promotes mental well-being.

Results

The study incorporated a mixed-methods design. This data was collected from the questionnaire responses and interviews with the school faculty. A correlational research design was also employed to investigate relationships between subjective well-being and emotional regulation. Furthermore, JASP (Jeffrey's Amazing Statistics Program) was a tool chosen for data analysis for this research paper due to its ability to handle various statistical tests and visualizations. Lastly, the author carried out content analysis, a research method used to identify and analyze the occurrence of specific words, themes, or concepts within qualitative data to derive themes from our interview transcripts.[17]

Quantitative:

— Descriptive Statistics:

Table 1: Descriptive statistics for each subscale of the well-being scale (SWB) and the emotional regulation scale (BERQ), providing insights into trends and variability within the data. 30 participants with a mean age of 21.5 years filled the SWB with skewness and kurtosis analyses indicating a normal distribution of data.

	WB-PIL	WB-EM	WB-PG	WB-SA	WB-PRO	WB-A	B-SD	B-W	B-SSS
Mean	10.567	14.4	14.600	16.867	12.467	13.267	10.5	11.533	11.862
SD	2.648	2.401	2.486	1.907	3.421	2.05	3.127	3.776	3.944
Skewness	0.994	-0.669	0.089	0.076	0.241	-0.463	0.783	0.554	0.163
Kurtosis	2.743	-0.086	0.713	-0.161	-0.046	-0.841	-0.133	-0.809	0.049

	WB-PIL	WB-EM	WB-PG	WB-SA	WB-PRO	WB-A	B-SD	B-W	B-SSS
Mean	10.567	14.400	14.600	16.867	12.467	13.267	10.500	11.533	11.862
Std, Deviation	2.648	2.401	2.486	1.907	3.421	2.050	3.127	3.776	3.944
Skewness	0.994	-0.669	0.089	0.076	0.241	-0.463	0.783	0.554	0.163
Std. Error of Skewness	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.434
Kurtosis	2.743	-0.086	0.713	-0.161	-0.046	-0.841	-0.133	-0.809	0.049
Std. Error of Kurtosis	0.833	0.833	0.833	0.833	0.833	0.833	0.833	0.833	0.845
Minimum	5.000	9.000	9.000	13.000	6.000	9.000	6.000	6.000	4.000
Maximum	19.000	18.000	20.000	21.000	21.000	16.000	17.000	19.000	20.000

The questionnaire was filled out by 30 participants, with a mean age of 21.5 years, all of whom were from Mumbai, India. Table 1 summarises the descriptive statistics across the subscales of subjective well-being and emotional regulation, providing insights into participants' psychological well-being and their emotion regulation strategies: WB-PIL (Well-being Purpose In Life), WB-EM (Environmental Mastery), WB-PG (Personal Growth), WB-SA (Self-Acceptance), WB-PRO, WB-A (Autonomy). WB-SA (Self-Acceptance) has the highest mean score, 16.867, and WB-PIL (Purpose In Life) has the lowest mean score, 10.567. Among the Behavioral Emotion Regulation Questionnaire (BERQ) subscales (B-SD, B-W, B-SSS), B-SSS (Seeking Social Support) has the highest mean, 11.862, and B-SD (Self-Distraction) has the lowest mean, 10.500. This indicates that the higher the scores, the higher the level of psychological well-being.

Based on the skewness and kurtosis scores, the population was found to be normally distributed (the skewness value lies between -2 and +2; the range of kurtosis is -7 and +7). Therefore, the Pearson correlation was used to find the relationship between well-being and emotional regulation.

Table 2: Pearson correlation between the subscales of SWB (subjective psychological well-being scale)[18] and BERQ (behavioral, emotional regulation questionnaire).[19]

Pearson's Correlations

Variable		WB-SA	WB-EM	WB-PIL	WB-PG	WB-PRO	WB-A	B-SSS	B-W	B-SD
WB-SA	Pearson's r	--								
	p-value	--								
WB-EM	Pearson's r	0.110	--							
	p-value	0.563	--							
WB-PIL	Pearson's r	-0.217	-0.151	--						
	p-value	0.250	0.426	--						
WB-PG	Pearson's r	-0.128	0.126	0.376*	--					
	p-value	0.500	0.507	0.041	--					
WB-PRO	Pearson's r	-0.603***	-0.124	0.438*	0.384*	--				
	p-value	<.001	0.513	0.016	0.036	--				
WB-A	Pearson's r	0.133	0.104	0.060	0.171	0.134	--			
	p-value	0.484	0.586	0.752	0.368	0.480	--			
B-SSS	Pearson's r	0.198	0.161	0.446*	0.284	-0.064	0.265	--		
	p-value	0.303	0.403	0.015	0.135	0.740	0.164	--		
B-W	Pearson's r	-0.042	0.116	-0.242	-0.072	0.060	0.262	-0.236	--	
	p-value	0.824	0.540	0.198	0.705	0.752	0.163	0.219	--	
B-SD	Pearson's r	-0.064	0.161	0.239	0.470***	0.158	0.113	0.410*	0.061	--
	p-value	0.738	0.396	0.202	0.009	0.404	0.552	0.027	0.747	--

*p<.05, **p<.01, ***p<.001

Table 2 summarises the correlation between the two standardised psychological assessments used in the study, specifically related to mental well-being and emotional regulation among youth with hearing impairment. The analysis indicates that PRO (Positive Relationship to Others) was significantly correlated with SA (Self-Acceptance), PIL (Purpose in Life) and PG (Personal Growth); PG was significantly correlated with PIL and SD (Seeking Distraction); SSS (Seeking Social Support) was significantly correlated with PIL and SD.PRO (Positive relationship with others) was positively moderately related to SA (Self-acceptance), $r = .0.60$, $p < 0.001$. PRO (Positive relationship with others) was also moderately related to PIL (Purpose in life), $r = 0.43$, $p < 0.05$, highlighting the moderate positive relationship between hearing-impaired individuals self-reporting having found their purpose in life and maintaining a good relationship with other people experiencing hearing impairments.

Lastly, PRO (Positive relationship with others) had a low correlation with PG (Personal growth), $r (28) = 0.38$, $p < 0.05$, establishing that there is no direct link between the personal growth of hearing-impaired individuals and the relationships they have with others.

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PG (Personal growth) had a low correlation with PIL (Purpose in life), r (28) = 0.37, p < 0.05. This indicates a low positive relationship between the hearing-impaired experiencing personal growth and finding their purpose in life.

SSS (Seeking social support) was moderately related to PIL (Purpose in life). This relationship indicates a moderate positive relationship between hearing-impaired individuals seeking external social support and having a clear idea of their purpose in life.

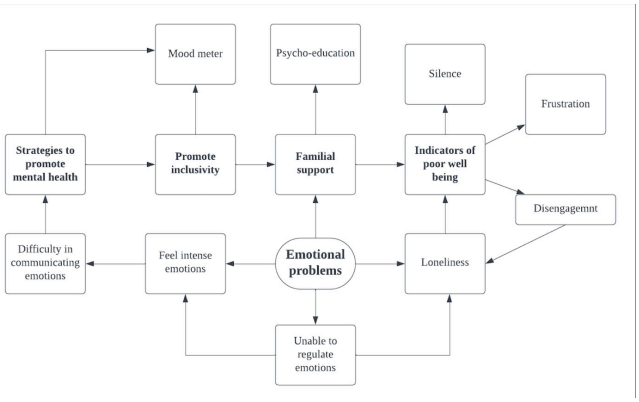
SD (Seeking distraction) was moderately related to PG (Personal growth), r(28)= 0.47, p < 0.01. This indicates that there is a moderate positive relationship between the hearing-impaired seeking distraction to occupy themselves and experiencing personal growth.SD (Seeking distraction) was also moderately related to SSS (Seeking social support), r(28)= 0.41, p < 0.05, highlighting the moderately positive relationship between the hearing-impaired community seeking distractions and seeking social support.

Qualitative:

The researcher used semi-structured interviews to explore pertinent areas of concern in this given population, which were analyzed using content analysis. The duration of the interviews was 45 minutes, and they were recorded with the consent of the participants. Sample questions presented to the participants included: "can you describe the common emotional and psychological challenges that hearing-impaired youth face in their daily lives", "In what ways have you observed/ seen them deal with their own emotional issues and are they usually of mild, moderate or severe intensity", "If you could implement one program or initiative to enhance the emotional wellbeing of hearing-impaired students, what would it be and why?" and "If a student is feeling restless or anxious, have you noticed any particular coping strategy that they employ to help calm themselves down." Table 3 gives a brief overview of the common concerns.

Discussion

Table 3: Themes and subthemes were generated from content analysis of interviews conducted with faculty at the TeachEDU school (school for the hearing impaired). Through qualitative analysis, the following key themes emerged: 1) strategies to promote mental health, 2) promote inclusivity, 3) familial support, and 4) indicators of poor well-being.



The study evaluated the psychological well-being and emotional regulation of hearing-impaired youth in India. The demographic for this study was hearing-impaired youth, ranging from ages 18 to 23. A mixed-method design was incorporated to obtain a holistic perspective that integrates diverse viewpoints and captures the full complexity of the research problem. Quantitative and qualitative data were obtained for this study through questionnaires and interviews.

Quantitative section:

Data from the 'BERQ' and 'SWB' scales provided insights into participants' psychological well-being and their emotion regulation strategies.

Among the Subjective Well-being subscales, WB-SA (Self-Acceptance) has the highest mean score (16.867), indicating that participants possibly scored this aspect of well-being the highest. For the Behavioral Emotion Regulation Questionnaire (BERQ) subscales, B-SSS (Seeking Social Support) has the highest mean (11.862), indicating its significance and importance in emotional regulation for hearing-impaired youth.

PRO subscales had no relationship with BERQ subscales, indicating that positive relationships with others had limited implications on emotional regulation. It was noted that within positive relationships with others, there is a moderate positive relationship between positive relationships with others (PRO) and self-acceptance (SA) and purpose in life, indicating that positive relationships help them direct their life. However, a low correlation with personal growth indicates that relationships with others are not necessary for mapping their inner growth; they are more individualistic journeys. PRO (Positive relationship with others) was also moderately related to PIL (Purpose in life). Positive relationships often provide emotional support and encouragement, inspiring individuals to pursue their goals and discover meaning in their lives. Similarly, having a clear purpose or direction can enhance the quality of relationships, as individuals with a strong sense of purpose are more likely to approach their social interactions with optimism.

PG (Personal growth) had a low but significant correlation with PIL (Purpose in life). This indicates that the connection between personal growth and purpose in life is not particularly strong. This could be because personal growth and a sense of purpose usually occur at different times; for instance, personal growth might arise from overcoming challenges or self-reflection, whereas purpose in life is more tied to long-term goals, relationships, or alignment with values.

SSS (Seeking social support) was moderately related to PIL (Purpose in life). This relationship suggests that individuals seeking support from others are more likely to experience a stronger sense of purpose. This connection may stem from the role of social interactions in shaping a person's goals and values. Seeking social support often involves building and maintaining meaningful relationships, which can provide encouragement and a sense of belonging, all contributing to a clearer sense of purpose. This relationship is moderate rather than strong, which is likely because PIL is also influenced by various other factors, such as personal achievements and experiences, beyond just social support.

SD (Seeking distraction) was moderately related to PG (Personal growth). This suggests that individuals who engage in distraction as a coping mechanism may experience growth opportunities. Seeking distraction often involves engaging in activities that shift focus away from stress or challenges, such as hobbies or creative pursuits. A possible explanation is that these activities can foster skill development, self-discovery, or a renewed perspective, all contributing to personal growth.

SD (Seeking distraction) was moderately related to SSS (Seeking social support). This relationship indicates that individuals who seek distraction to cope with challenges may also turn to social connections as part of their strategy. This could include wanting to spend time with friends or family, or participating in group activities, hence displaying the connection between seeking distraction and wanting to interact with others.

Qualitative section:

Through interviews with faculty members, several recurring themes emerged that shed light on the challenges associated with supporting hearing-impaired students' emotional and psychological needs. Similar results were found in a study (Qi Dong *et al.*, 2024),[20] which explored the emotional regulation of college students. The study showed that hearing-impaired students showed higher emotional intensity and weaker emotion regulation abilities than hearing students when using cognitive reappraisal and expression suppression strategies.

A prominent recurring theme identified among the students was a lack of familial initiative to engage meaningfully with their needs. This gap in understanding often results in significant barriers to effective communication, especially regarding the expression of complex or intense emotions that the students face. Hence, as a result, many students reported experiencing profound feelings of loneliness and isolation. As seen in Table 3, the perception of being viewed as "burdens" by their families emerged as a significant stressor, contributing to heightened vulnerability to suffer from emotional distress, including stress and symptoms of depression. Typically, when students struggle emotionally, they tend to withdraw from social interactions and become noticeably quieter and more reserved in class. They may avoid interacting with teachers and peers and choose to isolate themselves instead. Sometimes, they distract themselves by doing other activities or appearing "lost in thought" in the middle of the class. Additionally, as seen in Table 3, the psychological aspect of the hearing-impaired community is severely negatively impacted due to the social stigma faced by these individuals through both social discrimination and marginalization. Many individuals hesitate and display reluctance to engage with other people, which leads to feelings of isolation and, at times, a sense of being "unwanted." Such social exclusion is aggravated by offensive labels such as "deaf and dumb," which lowers their self-image and, in turn, their self-esteem. A pervasive societal bias against the hearing-impaired community creates substantial mental barriers, hence eroding the students' self-worth and resulting in heightened mental health concerns.

For hearing-impaired students, those from stable family backgrounds, i.e., with financial and familial security, generally experience fewer high-intensity challenges. However, when they do encounter difficulties, their emotional responses can be sudden and intense, often manifesting as anger. According to Table 3, hearing impairment also has a large impact on the student's sense of self-worth, which has a substantial effect on their self-image. Students with hearing loss often internalize a lack of status, feeling infe- rior or "less than" relative to other people without hearing impairments. This is further enhanced by the significant social stigma associated with hearing loss. This internalized stigma may result in increased feelings of rage and resentment. These students tend to show reluctance to seek psychological support, which can be attributed to a lack of familiarity or comfort with such services. They often conversely withdraw and become quiet and disengaged from school activities. This pattern underscores the demand for individualized practices that meet individual needs and promote a supportive and inclusive environment.

To address these challenges, it is extremely necessary to establish an environment in which students are both adequately supported and encouraged. Significant efforts will be required to establish trust and motivate students to contact faculty members with problems. Thus, by creating an inclusive and accepting environment for its students, pedagogy can be implemented to mitigate these negative emotions and promote a healthier sense of self-esteem among hearing-impaired students.

The faculty at TeachEDU, as reported in Table 3, noticed that if a student was feeling restless or anxious, they employed coping strategies to manage their emotions and calm themselves down. One notable pattern was their tendency to immediately divert their attention by participating in another activity or even something simple, such as using their mobile phones more often, as it serves as a "distraction from their distress." Since these students rely heavily on their friends for comfort, they prefer communicating with them via text messages rather than confronting their challenges directly. This indirect approach allows students to process their feelings in a "less overwhelming" way. Most often, their immediate reaction is to display sudden, intense emotions of anger, often stemming from weeks of pent-up frustration. Following this emotional release, they tend to reflect and rationalize their feelings, which helps them calm down. Many students even prefer keeping to themselves and isolating themselves from their peers to regain their composure. Through interviews with teachers, it became evident that many faculty members believed hearing-impaired students often used distraction as a primary method for emotional regulation. Teachers observed that these students frequently appeared to avoid confronting distressing emotions by engaging in other activities or shifting their focus. However, quantitative data from the questionnaire contradicted this perception. The BERQ scores indicated that self-distraction was the least utilized strategy among hearing-impaired students.

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Strengths of the study:

A significant strength of the study is that the topic of psychological well-being amongst hearing-impaired youth in India is an under-researched area, making the study unique due to the lack of prior research regarding this subject area. Despite the growing recognition of the importance of mental health, this specific subgroup has not been extensively explored within the Indian context. Thus, the study provides valuable insights that have yet to be made available to the public, hence contributing to a knowledge gap and offering fresh perspectives on the emotional and psychological challenges faced by this community.

Through this study, specifically tailored interventions can be implemented to directly address the needs of hearing-impaired youth. Understanding their unique psychological challenges facing such marginalisation would help in the creation of appropriate support systems and the provision of resources in mental health tailored toward their specific needs. Such interventions could empower the community to develop the tools to successfully navigate their psychological well-being and the social and emotional challenges they face.

The mixed-methods approach of this study also significantly strengthened its findings. By combining qualitative and quantitative data, a wide range of experiences and perspectives were captured, allowing for a more in-depth understanding of the psychological issues experienced by hearing-impaired youth.

Limitations of the study:

One notable limitation of this study is the relatively small sample size of hearing-impaired youth. While the insights gathered are valuable, the limited number of participants restricts the generalizability of the findings to a specific geographical landscape. A larger sample size would have allowed for more statistically significant conclusions and a better representation of the diverse experiences within this community.

The limited availability of existing studies on the emotional well-being of hearing-impaired youth in the Indian context presented a challenge. Without a body of prior research to draw upon, it was difficult to establish a theoretical framework that is specifically tailored to the unique cultural, social, and educational realities of hearing-impaired youth in India.

To overcome the limitations, the next step for this project would involve expanding it to a pan-India level, aiming to examine regional variations and broader trends across the country. By including participants from diverse states and socio-economic contexts, we could identify how factors such as language, accessibility to resources, and societal attitudes toward hearing impairment influence the emotional well-being of youth. This nationwide approach would allow us to explore whether specific regions or communities experience unique challenges or demonstrate distinct coping mechanisms compared to others. Including families during interviews might give more balanced insights regarding the struggles of those with hearing impairments.

Conclusion

The study used quantitative and qualitative methods to examine the relationship between subjective well-being and emotional regulation among hearing-impaired individuals. Findings showed that positive relationships with others were linked to higher self-acceptance and a stronger sense of purpose in life. Seeking social support was also associated with a clearer purpose, while using distraction as a coping mechanism was connected to both personal growth and seeking social support. The qualitative analysis revealed key emotional challenges, including social isolation, difficulty expressing emotions, and a lack of family support. Many students struggle with self-esteem, stress, and feelings of being perceived as inferior due to societal stigma. The faculty interviews emphasized the importance of parental involvement, structured support programs, and daily emotional check-ins. This study sheds light on the unique psychological and emotional challenges faced by the hearing-impaired, such as difficulties in social interaction, communication barriers, and coping with social stigma or isolation. It contributes to the limited scientific literature in the niche area of the emotional struggles of the hearing-impaired. The findings of this study can guide the development of tailored interventions and therapies that address the distinct needs of the hearing-impaired, such as specialized cognitive-behavioral approaches or community-based support systems that can be used by schools and organizations that include the hearing-impaired as well.

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REVIEW ARTICLE

CAR T-Cell Therapy: Structural Variability, Clinical Challenges, and Future Advances

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ABSTRACT: As of 2024, approximately 2.0 million people in the United States are projected to be diagnosed with cancer, with over 600,000 cancer-related deaths expected, primarily due to relapse or advanced metastasis. Since 2017, CAR T-cell therapy, which involves genetically engineering a patient's T cells to target and destroy cancer cells, has revolutionized cancer treatment. This treatment has shown remarkable success, particularly in patients with hematological malignancies, achieving complete remission rates of up to 90% in some studies. As the therapy continues to advance, it holds promise for becoming a standard treatment for all cancer types, with tailored modifications for specific cancers. This review focuses on the mechanisms behind the effectiveness of CAR T-cell therapy in targeting cancer cells, highlighting both the clinical successes for various types of blood cancers and challenges, particularly for solid tumors. This paper also covers how the advancements in CAR T-cell technology could significantly reduce relapse rates, improve patient outcomes, and overall increase survival. By creating an overview of the current clinical achievements and struggles, this review aims to highlight the potential of CAR T-cell therapy to enhance cancer treatment widely.

KEYWORDS: Translational Medical Sciences, Disease Treatment and Therapies, Personalized Medicine, Blood Cancers, CAR T-cell Therapy.

Introduction

Immunology is the branch of medical science that studies the immune system, including its components and functions in protecting the body from foreign pathogens and harmful microorganisms. It involves the study of both innate and adaptive responses and includes the different immune cells, molecules, and different bodily defenses that could identify and attack the pathogens. The innate response provides an immediate, non-specific defense against pathogens, while an adaptive response is much slower but highly specific and capable of forming immunological memory. Gaining a deep understanding of how the immune system interfaces with autoimmune diseases, allergies, infectious diseases, and cancer can allow scientists to develop specific treatments.

Cancer is the uncontrolled growth of abnormal cells in the body. These cells form tumors and invade distant organs of the body through a process known as metastasis. Once metastasized, the cancer becomes much more life-threatening. These metastasizing tumor cells can surface on almost any organ or tissue, even if the tumor cells originated in a completely different region by migrating through the circulatory and lymphatic systems. However, in the bloodstream, various types of immune cells are circulating actively, trying to seek out and destroy foreign bacterial pathogens or native human cells.[1] Tumor cells have various mechanisms to evade these lymphocytes to achieve their metastatic potential, which refers to their ability to spread from the primary tumor area and form secondary lesions in other organs. [1,2]

Common defense techniques used by tumor cells include down-regulating the expression of tumor antigens on the cell's surface and developing resistance to apoptosis, even when targeted by cytotoxic immune cells. These evasion methods relate to the tumor's intra-tumoral niche, the microenvironments within a tumor that allow the growth and spread of cancer cells. These areas typically have low oxygen levels, unusual pH levels, and signals that weaken immune responses, all of which help tumor growth and make it easier for cancer cells to spread to other parts of the body.[3] The most commonly used methods of treatment are radiation therapy, chemotherapy, and surgery.[4] All of these techniques have been effective in reducing the symptoms or eradicating tumors across most cancer types, but disease mortality still poses a major challenge.

A recently discovered therapy, known as Chimeric Antigen Receptor (CAR) Therapy, has emerged as a groundbreaking advancement in cancer research. This form of therapy has only been FDA-approved since 2017. During its short lifespan, it has made radical changes in how cancer treatment helps patients.[5] CAR T-cell therapy is a form of immunotherapy engineered to treat particular types of cancer, specifically blood cancers like lymphoma and leukemia. The treatment uses the patient's own T cells. Unlike traditional forms of cancer therapy, CAR T-cell therapy is designed to primarily target cancer cells by recognizing the antigens on the surface of the cancer cells, enabling a more precise attack on the tumors while not impacting the healthy cells.[6] This allows the CAR T cells to overcome evasion strategies found in cancer, leading to impressive remission rates in many types of cancer, particularly in hematological cancers.

While many cancers exist today, this review primarily focuses on hematological cancers such as Diffuse Large B-cell Lymphoma (DLBCL), Multiple Myeloma, and B-cell Acute Lymphoblastic Leukemia (B-ALL). These cancers are well-

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suited for CAR T-cell therapy because they originate from immune cells like B cells, which effectively express well-defined surface markers like CD19 or B cell maturation antigen (BCMA). These markers make the cancers an ideal target for CAR T-cells to recognize and destroy.[7] However, solid tumors often have challenges like a lack of specific markers, a denser tumor microenvironment, and immune evasion methods, which overall limit the efficacy of CAR T-cell therapy.[8]

Even though the testing and clinical trials of this treatment are still in their early stages, CAR-T cell therapy shows promise as a new method of cancer treatment. This paper will discuss the current state of CAR T-cell therapy, new advances on the horizon, the complications, the structure and shape of the CAR T-cell, and the clinical successes regarding this form of therapy. By reviewing the advancements, challenges, and successes of CAR T-cell therapy, we can better understand the significant potential it has in revolutionizing cancer treatment and paving the way for more targeted and effective therapies in the future.

Discussion

Background of CAR T-cell therapy:

CAR T-cell therapy became a groundbreaking cancer treatment that has revolutionized immunotherapy treatment. The person responsible for this breakthrough is Dr. Carl June. He is regarded as the "Father of CAR T-cell therapy" and serves in leadership positions at both the Center for Cellular Immunotherapies and the Parker Institute for Cancer Immunotherapy at the University of Pennsylvania. His work on genetically engineered T cells for treating acute lymphoblastic leukemia led to the FDA approval of tisagenlecleucel for ALL in 2017, specifically for younger patients.[5] He is a lead researcher on lymphoblastic leukemia activation mechanisms related to immune tolerance and cancer immunotherapy. As mentioned above, Dr. Carl June contributed to developing the first FDA-approved therapy for children and young adults with acute lymphoblastic leukemia (ALL). Even though his first successful therapy was in a teenager in 2012, the pioneers of CAR T cell did not receive FDA approval until 2017.[12]

The CTL019 trial, tested by Dr. Carl June, overcame the risk of Cytokine Release Syndrome and became the first chimeric antigen that was FDA-approved in 2017. This treatment used tisagenlecleucel as the medication for patients up to 25 years old who had B-cell Acute lymphoblastic leukemia. These patients didn't respond to the standard treatment, like chemotherapy and radiation, and, if improved, relapsed at least twice during their cancer journey. In the results of this clinical trial, 83% of the young adults and children being treated had a complete remission.[24]

Dr. Carl June hypothesized that tocilizumab, a monoclonal antibody-based drug commonly used to treat rheumatoid arthritis, could mitigate the life-threatening side effects of cytokine release syndrome (CRS) that endangered many patients, a hypothesis later confirmed. Tocilizumab reduced CRS within a short time frame for 69% of the patients involved.[25] Dr. Carl June significantly advanced the path to FDA approval, developing a solution that not only secured acceptance for CAR T-cell therapy but also paved the way for its application in treating other related diseases.

Difference between T-Cells and CAR T-Cells:

The difference between T cells and CAR T-cells lies in how they target foreign and abnormal material in the body, despite both having this capability. Normal T cells target a wider variety of threats because they are integral to the body's natural immune system. In contrast, CAR T-cells are engineered specifically to target antigens on the surface of tumor cells. T cells can only recognize certain antigens based on the Major Histocompatibility Complex (MHC) molecules, which ensure the cells only respond to specific antigens and avoid an immune response against the body's own healthy cells, also known as self-cells, by recognizing markers that identify them as part of the body. CAR T-cells completely avoid the need for MHC molecules because they are only programmed to target the molecules on a cancer cell's surface. However, one of the differences in T cells is that they can adapt to new threats over time and can change their function based on the invasion of the body. In contrast, CAR T-cells only have a fixed target solely dependent on the CAR's design. T cells develop and mature in the thymus, where they generate a diverse repertoire of T cell receptors (TCRs), enabling them to recognize a wide range of antigens and adapt to various targets. CAR T-cells have a fixed receptor engineered to recognize specific antigens, such as CD19, which is expressed on the surface of B-cell malignancies.[4] CAR T-cell therapy has emerged as a ground-breaking form of cancer therapy that enhances the immune system's ability to recognize and destroy cancer cells, particularly in blood-related cancers.

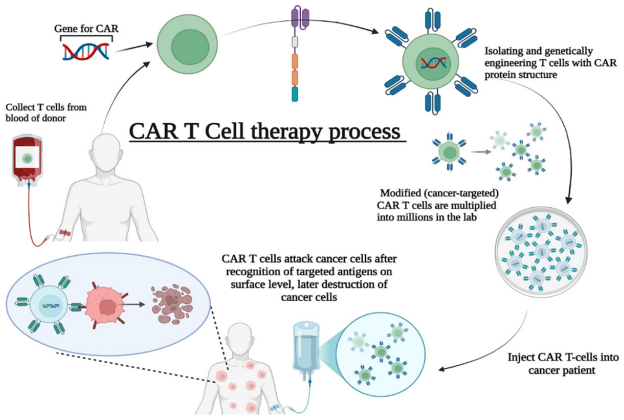

Figure 1: A diagram illustrating the steps involved in CAR T-cell therapy. The diagram details the main steps: Blood is drawn from the patient, extracting the T cells; T cells are isolated and genetically engineered to express CARs; Engineered T cells are multiplied in the lab; and Modified T cells are infused back into the patient to destroy cancer cells. This figure was created through BioRender.

CAR T-Cell Therapy and Structures Involved:

The process of CAR T-cell therapy involves modifying and genetically engineering a patient's T-cells to target and attack the cancer cells found in the body. Before the T-cells can be reprogrammed, the patient's white blood cells are collected

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through leukapheresis. (Figure 1)4 This is a process where a machine separates the T-cells from a person's blood while returning the red blood cells, platelets, and plasma back to the body.

After the T-cells are isolated from the white blood cells, they are sent to a lab to be re-engineered with a gene that provides instructions for making proteins that can bind to the surface of cancer cells, known as chimeric antigen receptors (CAR). (Figure 1)[4] To introduce the proteins, scientists use a method called gene transfer where a vector, commonly a lentivirus (a type of modified virus), is used to safely deliver the genetic material into the T-cells. Lentiviral vectors are often used in CAR T-cell therapy because they can stably integrate the transgene into the "host genome", allowing them to transduce both dividing and non-dividing cells, and offer long-term expression of the CAR receptor. Once successfully integrated, the modified T cells now express a CAR receptor. However, before patients are infused with these modified CAR T-cells, the efficacy of the receptor needs to be tested.

The most effective method for validating the therapy involves *in vitro* testing. In these experiments, modified T-cells are evaluated to determine if they can recognize and bind to cancer cells in a controlled environment. Researchers also confirm that the CAR T-cells retain their ability to express the CAR protein and effectively target and destroy cancer cells.[9] To identify what CAR proteins need to be configured for the T-cell, researchers first identify the surface proteins that are expressed more frequently on a cancer cell's surface compared to normal, healthy cells, like CD19 and HER2. For example, CD19 is commonly found on B cells, including malignant ones in leukemia and lymphoma, while HER2 is overexpressed in certain breast and gastric cancers, but also is present at lower levels in normal tissues. Once the specific CAR protein required for targeting cancer cells is identified, they can stimulate and culture the modified T cells with cytokines to promote proliferation, increasing the number of CAR T cells used for the therapy.[10] After this protein is configured to the cell and efficacy is tested, these T-cells can be infused back into the patient and actively destroy cancer cells once returned to the bloodstream. (Figure 1)

Prior to the introduction of CAR T-cells into the bloodstream, the patient must undergo chemotherapy rounds to reduce the number of T cells and create a more favorable environment for the infused cells. Now, when an antigen is bound, the CAR T-cell is activated and can destroy the cancer cells that have this marker.[4] However, this process requires the involvement of four key structures within the CAR T-cell: an antigen-binding domain (scFv), a hinge region for flexibility, a transmembrane domain, and intracellular signaling domains like CD3ζ and co-stimulatory molecules.

Antigen-Binding Domain Recognizes Antigen:

To begin with, the Antigen Binding Domain is the part of the CAR that determines what the cell targets. It's made from the parts of antibodies called the VH (variable heavy) and VL (variable light) chains. These chains are essential because they allow the CAR to bind specifically to a target antigen (Figure 2) They connect to form a single-chain fragment called scFv, which maintains the specificity and flexibility required for antigen recognition.[11,12] The chain can target the proteins present on the surface of the cancer cells and effectively attack these cells.[13] However, how the VH and VL chains interact with one another impacts the CAR's ability to bind to its intended target. For instance, the strength of interaction between the VH and VL chains affects the binding affinity between the CAR and the antigens on the cancer cell.[11] A high-affinity interaction can improve the CAR T-cell's ability to bind to low levels of antigens on the tumor cells, making the T-cell much more effective in destroying cancer cells. (Figure 2) To affect how CAR binds to its target, scientists also need to consider factors like how much of its surface is present and where CAR binds to the target.[11]

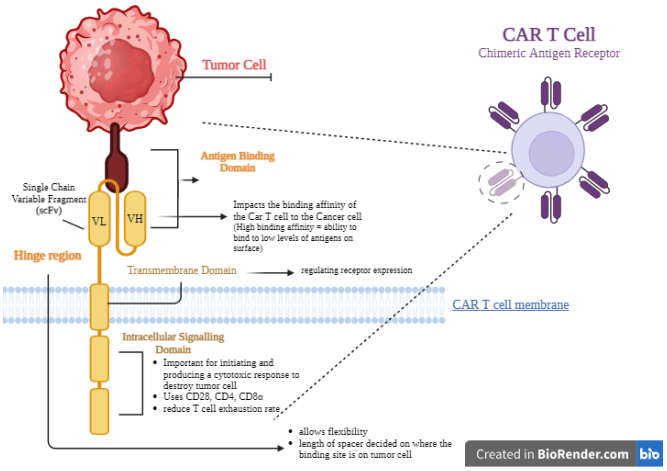

Figure 2: In this figure, the antigen receptors on the CAR T cells are focused on to give a deeper understanding of how each part of the receptor works together to bind to the CAR T cell and induce Apoptosis in the tumor cell. Without each of these structures, CAR T cells cannot play their part in targeting and attacking the tumor cells. This figure was created through BioRender.

Hinge Region & Transmembrane Domain:

For the antigen-binding domain to reach the targeted epitope on the antigen molecule and connect the binding unit to the transmembrane domain, the CAR T-cell needs to use the hinge region. The shape and length of these spacers influence the CAR's functionality, including its flexibility to navigate physical barriers, its expression efficiency, and its ability to accurately identify the region where the antibody binds to the antigen. (Figure 2) [4,11]

The length of the hinge region depends on where the target is located on the cancer cell, long spacers are more suited to targets situated closer to the cell membrane (membrane-proximal; more useful for antigens with sugar molecules attached), while short spacers are better used for targets farther away from it (membrane-distal).[11,13] While scientists should test different hinge lengths to choose the best one for each CAR structure, donor sequences from proteins like CD8, CD28, IgG1, and IgG4 are commonly included in viral builds to create synthetic hinge regions. Some spacers, like ones from IgG, can create problems when interacting with immune system components, so while making the chimeric antigen receptors, scientists

must be careful to avoid creating even more problems for the patient's immune system.[11]

The next structure needed is the transmembrane domain, which is used to anchor the CAR to the cell membrane and influence the function of the CAR T-cells. These domains are commonly found in CD3ζ, CD4, CD8α, or CD28 T-cells.[13] CD4 and CD28 are integrated into the receptor as activation switches, enhancing the T-cell's capacity to recognize and attack cancer cells. This leads to stronger and more sustained anti-tumor immune responses compared to using only the primary signaling domain. CD4 can be used to target specific tumor antigens, while CD28 can enhance T-cell proliferation and cytokine production when bound to the ligand.[14] The transmembrane domain is one of the least studied components, but without the transmembrane domain, CAR T-cells wouldn't be able to link the scFv to the intracellular signaling domain.[11,13]

Mechanism of Action:

The intracellular signaling domain initiates activation inside the T cell after contact with the antigen. This signaling is crucial for T cell activation and producing a cytotoxic response that causes the destruction of the tumor cell using, as seen in CD19-targeted CAR T-cell approaches. (Figure 2, Figure 3) This cytotoxic response releases granzymes and perforins that induce the tumor cell to undergo apoptosis.[15]

Initially, perforins form a pore at the junction between the cancer cell and the immune cell membrane, allowing cytotoxic proteins like granzymes to enter. These granzymes can then damage the tumor cell's DNA, causing irreversible harm.[15] Some of the major cytokines released due to this cytotoxic response are IFN-y and Il-2.[16] As for IFN-y, this cytokine can induce an increase in MHC I molecules so that the immune system can better identify this invader and help the CAR T cells as a whole recognize and destroy cancer cells. (Figure 3)[16] IL-2 can stimulate T cells to attack the cancer cells so that the memory T cells can remember what the specific tumor cell type looks like, and if there is a cancer relapse, IL-2 would enable a faster and effective immune response. (Figure 3)

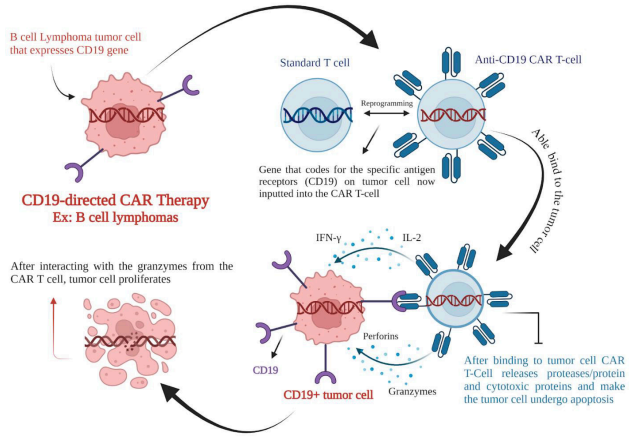

Figure 3: This figure depicts how CAR T-cell therapy works for B-cell lymphomas. First, the T cell needs to be embedded with the CD19 antigen receptor so the T cell can become an Anti-CD19 CAR T-cell. After, the T cell can bind to the cancer cell and release a cytotoxic reaction to cause the cell to proliferate. This figure was created through BioRender.

However, like all other T cells, CAR-T cells have to undergo T-cell exhaustion, a progressive loss of function that occurs after prolonged exposure to antigens, such as those on cancer cells. In normal immune responses, T-cell exhaustion helps prevent overactivation and protects healthy tissue from immune damage. However, in CAR T-cell therapy, this exhaustion limits the cell's ability to continue attacking tumors, reducing long-term efficacy. Exhausted CAR T-cells have reduced cytotoxic capabilities.[17] This is one of the main challenges regarding CAR T-cell therapy, and a sustained reduction in tumor growth and stability. Only by understanding the complex process and structure of the CAR T cells can one advance their design, enhance their efficacy, and avoid issues like T-cell exhaustion.

Structural Milestones: Future Generations of CAR T-Cells:

The concept of infusing cells into patients with hematologic cancers started coming into focus in the 1950s. It was not until the early 2000s that doctors were exploring the idea of genetically advancing T-cells by embedding genes to affect certain factors. The development of CAR T cell therapy since the 1990s has been divided into different generations to show how it has advanced over time. (Figure 4)

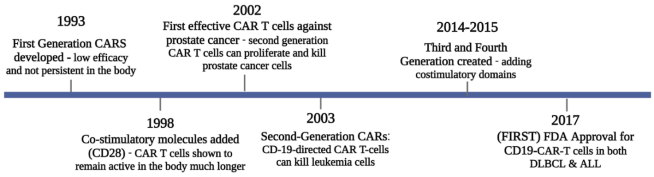

Figure 4: Timeline of the progression of CAR T cell therapy and its generations from 1993 to 2017 and further on as generations improve further to better accommodate different illnesses beyond cancer. This figure was created through BioRender.

The first generation (1993) included the two key structures in a CAR T cell, the antigen-binding domain region and the intracellular domain. The former contains a single-chain variable fragment that helps the cell recognize and bind to target cancer cells, while the intracellular domain contains a CD3ζ signaling domain to help coordinate signals through the CAR T cell. The early design of these CAR cells allowed them to bind to the cancer cell and release cytokines like IL-2 to activate other immune cells at the tumor site and induce apoptosis.[18] But without co-signaling domains like in the present design, the first-generation CAR T cells had reduced proliferation, meaning there would be a smaller number of active CAR T cells, limited cytokine release that created a weakened immune response, and reduced persistence in the body. (Figure 4)[19] Due to these limitations, the first generation of CAR T cells was far less effective at destroying large numbers of tumor cells than was considered the primitive design.

By identifying these limitations, the new design of second-generation CAR T cells included both the intracellular domain and co-stimulatory molecules like CD28 or 4-1BB. As shown in clinical studies, 4-1BB-based CARs had a longer persistence and strong response but a weaker activation compared to CD-28-based CARs, which had a quicker response, greater T cell expansion and survival, and quicker signal

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ing.[20] This created the conclusion that CD-28 CARs would be more efficient in targeting cancer cells, especially with CD-19 antigens. This additional signaling domain enhanced T cell activation, increased proliferation, and cytokine production.[21]

The CAR T-cells can now target CD19 on B-cell malignancies and are being implemented in clinical practices.[18] All current FDA-approved CAR T-cell therapies use this generation's designs as of 2023.[22] However, there were still issues regarding the persistence of the CAR T-cells and cancer relapsing in single co-stimulatory signaling domains, which is what scientists decided to improve in the third generation. (Figure 4)[23]

CAR T-Cell Therapy for Blood Cancers and Solid Tumors:

Individual cancers have varying responses to CAR T-cells due to different tumor structures, antigen expressions for other types, and the tumor microenvironment. Hematologic malignancies like certain leukemias and lymphomas often respond well to CAR T cell therapy, which can target specific antigens that reside on the surface.[7] However, solid tumors present additional challenges due to the expression of multiple antigens, immune suppression created by the tumors, and the barriers within the tumor microenvironment. These solid tumors form a hostile microenvironment with physical barriers like dense extracellular matrix and angiogenesis, which can prevent the CAR T-cells from binding to tumor cells.[8]

We performed a survey of clinical trials on clinicaltrials.gov using search terms "CAR T" or specifically "multiple myeloma" to obtain these studies. This data is not an exhaustive list of all ongoing or completed trials, of which there were 166 as of January 2021.[26] While CAR T-cell therapy has shown achievements in treating blood cancers such as Diffuse Large B-cell lymphoma, Multiple Myeloma, and B-cell Acute Lymphoblastic Leukemia, ongoing research is necessary to overcome the limitations and improve clinical trial results even with solid cancer types.

Table 1: A brief survey of published clinical trial data was conducted on clinicaltrials.gov. (Table 1) Here, we have outlined the posted results. This table was created through BioRender.

Cancer Type	Patients Enrolled	CR/CRi%	Phase	Generation of CAR T	Name and ID of Trial
Diffuse Large B-Cell lymphoma	93	40 / 12 %	Phase II	Second	JULIET; NCT02445248
Multiple Myeloma	113	67 / 30 %	Phase II / I b	Second	CARTITUDE-1; NCT03548207
B-Cell Acute Lymphoblastic Leukemia	36	81% achieved CR, only 26% stayed in CR	Phase II / I b & Phase I	Third	Pooled Analysis from ALLCAR19 and FELIX Phase Ib; NCT02935257 & NCT04404660
Solid Tumors	≈ 12	Still Recruiting	Phase 1	Fourth	MAGNETO; NCT05990751

Diffuse Large B-cell Lymphoma:

CAR T-cell therapy is particularly effective for hematologic cancers, including Diffuse Large B-cell lymphoma (DLBCL). Especially for patients whose DLBCL hasn't responded to other treatments, CAR T-cell therapy is a promising option since the therapy is incredibly personalized. The treatment directly takes the patient's T cells and modifies them to target antigens on cancer cells. This is much more effective than treatments like chemotherapy, where healthy cells may also be negatively impacted. CAR T-cells can target CD19, an antigen commonly expressed on malignant B cells in DLBCL but not on most normal cells. This targeted approach directs the CAR T-cells specifically to the cancer cells.[27] (Figure 3). This provides a precise and potent immune attack on the tumor antigens and avoids all other cells.

This therapy has shown successful results, especially in the JULIET trial using the CAR T-cell therapy, tisagenlecleucel (also known as CT019). This study involved 93 patients who received CAR T cell therapy and were evaluated after 14 months. In this trial, 52% of patients responded to the treatment, experiencing a reduction in the disease. Among them, 40% achieved complete remission, with no cancer detected upon re-assessment, while 12% showed only partial responses after receiving tisagenlecleucel.[28]

At 12 months post-treatment, 65% of the patients who first responded to the treatment were relapse-free, and the ones who reached complete remission had a relapse-free rate of 79%. The results suggest that a substantial portion of the patients maintained their response throughout the trial. However, this does not mean there were no adverse side effects during the trial; over 20% of patients experienced severe CRS, and 12% faced significant neurological issues, primarily immune effector cell-associated neurotoxicity syndrome (ICANS), which can include symptoms like confusion, seizures, and speech complications.[28] The trial was very effective, with overall response rates around 50% or higher in the trial. CAR T-cell therapy shows promising responses after relapses, with notable remission rates, even when using second-generation CAR T-cells.

Multiple Myeloma:

Another excellent candidate for CAR T cell therapy is Multiple Myeloma (MM). This type of blood cancer affects plasma and, consequently, can affect the production of antibodies created to protect against harmful invaders of the body. In Multiple Myeloma, CAR T-cells target B-cell maturation antigens (BCMA); just like in Diffuse Large B-cell Lymphoma, the protein is found on the surface of harmful B cells (plasma) and makes an easy target for the CAR.[29,27] To distinguish between normal cells and multiple myeloma cells, it's important to note that, compared to other cancers, MM expresses very high levels of BCMA, while normal tissues show little to no expression of this antigen. Engineered CAR T cells can be designed to recognize the B-cell maturation antigens on myeloma cells, enabling them to activate and kill the malignant cells.[29]

One of the key trials showing positive results for CAR T-cell therapy in treating MM is CARTITUDE-1, which uses JNJ-4528 to target BCMA antigens. The trial consisted of 113 patients, of whom only 97 were given CAR T cell therapy. A year post-treatment, results showed that 97% of patients responded to the treatment, and 67% of patients had a complete response where cancer could not be detected after receiving treatment.[30]

Positive results started even in the first month after treatment, with heavy responses increasing over time, and the survival rate was fortunately stable at 89% at the end of the

experiment. While the positive responses produced by this clinical trial were high and made up the majority of the patients, there was also a significant risk of adverse effects like DLBCL. 95% of the patients experienced CRS after receiving treatment. While the cases were resolved only 24 days after seeing symptoms, this seemed to be the most common adverse event. Only 4% experienced severe grades of CRS.[30]

B-cell Acute Lymphoblastic Leukemia:

A third promising application of CAR T cell therapy is in B-cell Acute Lymphoblastic Leukemia (B-ALL), also due to its high expression of CD19 antigen. B-ALL is an acute leukemia, so the disease progresses fast, and diagnosis and treatment need to follow closely with each other to reduce the mortality rate of the patients.[7] CAR T-cell therapy can be a much more reliable form of treatment since the consequences affecting healthy self-cells will not be as much of an issue.[18]

In the trials ALLCAR19 and FELIX Ib, the use of the CAR T cell therapy (obe-cell) had a significant positive impact on the remission rates of the patients. The trial states that there were 36 patients in total from the two trials, and 81% of these patients achieved complete remission with CR or CR with an incomplete hematologic recovery after receiving the obe-cell transfusions. After a follow-up of 43 months, 26% of patients remained in remission, with continued observation in 13 patients to check for signs of remission. Notably, 91% of the patients who continued to respond well to the treatment still had CAR T-cells in their bodies and did not show signs of remaining cancer, and the overall survival rates were 39% after 4 years.[31]

While the statistics from the trials were overall negative, and the number of people who remained in remission was quite low, B-ALL itself is an aggressive cancer that progresses quickly and has a high relapse rate.[7,31] Common treatments like chemotherapy and radiation often do not lead to significant remission, whereas CAR T cell therapy shows more promise. As research and technology continue to advance, CAR T cell therapy is expected to undergo significant improvements that could enhance its effectiveness in treating B-ALL.[7] Future developments include improvement in the design of CAR constructs to target additional antigens such as CD22 and BCMA, which is useful in overcoming antigen escape and resistance methods in leukemia and other cancers. Some strategies also involve dual targeting techniques, where CAR T-cells are engineered to recognize both CD19 and CD22 to increase treatment efficacy and reduce relapse rates.[32]

Solid Tumors:

While CAR T-cell therapy has shown significant progress in treating blood cancers, its application to solid tumors remains challenging, and most research in this area is still in the development phase.[8] Researchers have started to experiment with using CAR T cell therapy for neuroblastoma. Neuroblastoma overexpresses antigens like the ganglioside GD2, and CAR T cells can be designed to target these specific antigens while disregarding normal cells. Unlike other solid tumors, neuroblastoma has a more favorable immune microenvironment that can allow for better T-cell infiltration and activity. This solid tumor can produce pro-inflammatory cytokines that can attract immune cells and eventually induce an immune response, unlike some tumors that constantly suppress immune activity.[8] Because of this, multiple clinical trials regarding Neuroblastoma have been taking place with CAR T cell therapy. However, all of these trials still don't have results available to the public, indicating that even though steps have been made to start including solid tumors as a category for CAR T cell use, there is still a long way before this becomes a viable treatment.

Navigating the Obstacles of CAR T-Cell Therapy:

One of the biggest limitations that has hindered the progression of CAR T-cell therapy is the inability to penetrate solid tumors.8 One of the reasons for this obstacle is the difficulty in finding antigens on the surface of solid tumors. Solid tumors have a dense and complex microenvironment containing connective tissue, immune cells, and blood vessels, which creates physical restrictions for CAR T-cells penetrating to tumor tissue from the surface. Solid tumors also express heterogeneity in their cell surface antigens, meaning not all cancer cells express the same antigens. This makes it difficult for CAR T-cells to target all the cancer cells on a solid tumor's surface.[35,36]

Engineered T cells are designed to recognize a specific antigen, and if that antigen is not expressed throughout the tumor, some cancer cells will be able to evade destruction. Another immune evasion mechanism from solid tumors could be down-regulating the expression of harmful antigens or increasing the activity of inhibitory molecules that weaken T-cell function. This limits the effectiveness of CAR T-cells and makes it harder to identify harmful antigens in cancer cells.

The tumor's microenvironment can also be immunosuppressive, as cytokines can inhibit T cell activation and may not be able to maintain their activity after reaching the tumor.[36] Some immunosuppressive molecules in tumor cells worth mentioning are program death 1 (PD-1) and program death ligand 1 (PD-L1). Solid tumors express PD-L1, which binds to PD-1 on CAR T-cells; this interaction can reduce the efficacy of CAR T-cells and prompt T cell exhaustion within a tumor's microenvironment. Some trials are currently being developed to inhibit the binding of PD-L1 to PD-1 interactions, like combining CAR T-cells with checkpoint inhibitors to increase anti-tumor activity.[35]

Complications regarding Treatment:

One of the complications regarding CAR T-cell therapy is CRS. CRS is one of the major side effects that occurs when activated immune cells release large amounts of cytokines into the blood, leading to an extreme inflammatory response. Some causes of CRS are after T cell activation and high tumor load.[10] When CAR T-cells bind to their tumor antigens, they activate and signal the CAR T-cells to proliferate for an immune response against the cancer cells. After being activated, CAR T-cells can release cytokines like IL-2, TNF-alpha, and IL-6, creating even more immune activation in the form of inflammatory responses.[37]

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Patients with many tumor cells can have a more unstable immune response because more tumor cells can lead to greater CAR T cell activation, increasing cytokine release. Some of the symptoms of this side effect include mild or high-grade fever, nausea, severe migraines, respiratory distress, etc. This side effect can range from grade 1 severity (not requiring treatment) to grade 4 (with life-threatening symptoms needing emergency medical care). Still, regardless of the severity, CRS is a very common side effect after CAR T cell therapy.[4] 77-93% of patients with leukemia and 37-93% of patients with lymphoma who received CAR T cell therapy had any grade of CRS.[38]

Mild CRS can be managed with IV fluids and antipyretics, while more severe cases of CRS might need corticosteroids to hinder the immune response and reduce inflammation; however, there is no definitive cure for CRS.[4]

In addition to CRS, immune effector cell-associated neurotoxicity syndrome (iCANS) is also a significant toxicity that can be seen during the CAR T-cell treatment. After the infusion of CAR T-cells, the engineered immune cells become activated after recognizing and attacking tumor cells, leading to the release of inflammatory cytokines. Some symptoms of iCANS are confusion, seizures, and other cognitive defects, showing the impact of an activated immune system on the central nervous system. The occurrence and severity of iCANS are correlated to the severity of CRS that may occur at the same time, and just like CRS, corticosteroids with the most efficient way to reduce the inflammatory response affecting the body.[10] This occurrence of toxicity highlights the balance between the efficacy of CAR T cell therapy in targeting cancer cells and the potential for neurological side effects.

One of the causes of these forms of toxicity is on-target, off-tumor toxicity, where CAR T-cells may attack healthy self-cells that express low levels of the same target antigen present on the surface of the tumor cells.[39] Since CAR T cells are programmed to recognize these specific antigens, the cells might inadvertently bind to healthy tissues and destroy them in the process of targeting cancer cells.

For example, in some therapies targeting CD19 antigens found in blood cancers, CAR T-cells sometimes attack self-B cells, which typically also express CD19. This leads to a decrease in healthy B cells, known as B cell aplasia, and results in an increased risk of infections since B cells are necessary for antibody production. Specifically in cases of acute lymphoblastic leukemia/Lymphoma (expressing the CD19 antigen), when patients are treated with CAR T-cell therapy, nearly 100% of patients have some moderate toxicity symptoms in terms of CRS and iCANS.[7]

Some methods to combat this off-tumor toxicity are using dual-targeting CARs and safety switches. Dual-targeting CARs require CAR T-cells to recognize two antigens at once, reducing the chance of attacking normal cells that express only one of the two target antigens.[39] However, if toxicity occurs, scientists could engineer CAR T cells with "suicide genes" that allow them to deactivate the T cells before the toxicity spreads further to the organ systems.

Future Innovations: Car T-cell Therapy for Infectious Diseases:

On top of cancer-type malignancies, scientists have been trying to implement CAR T cell therapy into infectious diseases, specifically HIV. However, the progress isn't substantial, as CAR T-cells still have many flaws in incorporating CAR T-cells in HIV, such as viral escape, CAR T cell infectivity, and access to hidden viral reservoirs. Doctors first believed that, compared to vaccines and immune checkpoint inhibitors, chimeric antigen receptors might have more success in enhancing immune responses against HIV-infected cells.[33] The first clinical application, in 2022, tried to treat HIV by changing the cytolytic properties of CD8+ T cells to now express chimeric antigen receptors with a CD4 extracellular domain that can recognize and target specific antigens on HIV-infected cells.[34] This creates a specific immune response that is separate from the MHC, so even if the HIV cells reduce their expression of MHC I to evade detection from immune cells, the CAR T cells can target and destroy them.

Regardless of the laboratory studies showing that the therapy was safe and that using CAR T cells had a good survival rate in patients, it couldn't prevent the relapse of HIV infection. On top of this, compared to blood malignancies, there were much lower levels of the targeted antigen since not all the infected cells didn't express the HIV envelope glycoprotein (Env).[34] This prevented the full effectiveness of the CAR T-cell therapy. The issues above carried over throughout most of the clinical trials conducted with HIV patients, and HIV-infected cells might continue to evade the immune system's detection through viral escape, but CAR T Cell seems to be a feasible treatment for the patients compared to antiretroviral therapy or entry inhibitors that don't account for HIV reservoirs.[33] Overall, despite these challenges, some clinical trials in the future seem to show a promising future down the line for CAR T cells in HIV therapy.

Conclusion

Chimeric Antigen Receptor therapy has emerged as a groundbreaking advancement in the field of immunotherapy, progressing from oncology studies and providing significant survival opportunities for patients with blood cancer. This review paper has highlighted the current stage of CAR T-cell therapy, including the structure of the technology, clinical success, side effects, and future advancements in the field. Despite its current success in the oncology field, specifically in hematological malignancies, CAR T-cell therapy faces challenges such as the ineffectiveness against solid tumors and chronic side effects like CRS and iCANS that persist after treatment. Currently, ongoing research into multitargeted CAR T cells and combined therapies seems to be the most efficient ways to address these limitations. Although the treatment is still in its early stages, CAR T-cell therapy is paving the path for personalized cancer treatments, specific to the target antigens on cancer cells, and as our understanding of the anatomy of cancer and immune responses increases, its potential in cancer care will continue to expand and become a catalyst for future advancements in the battle against cancer.

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■ RESEARCH ARTICLE

Analysis of Human Focus Patterns Using Uniform Manifold Approximation and Projection

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ABSTRACT: Defining "focus" scientifically has been challenging due to the reliance on subjective self-reports with no objective measurement methods. The advent of AI for data analysis presents new opportunities to discover correlations in human behavior using techniques such as unsupervised clustering and dimensionality reduction. Our study analyzed an online dataset from 51 participants who reported their focus levels while completing tasks under four audio conditions: silence, Endel, Apple Music, and Spotify. The Uniform Manifold Approximation and Projection (UMAP) technique, utilizing gradient descent for dimensionality reduction, revealed two distinct clusters in the data. Brainwave indicated focus levels demonstrate a strong correlation with the revealed clusters, but other variables in the dataset, including participant gender, age, audio type, and their self-reported focus levels, do not correlate well with the clusters. The findings suggest that self-reports may not accurately reflect focus, highlighting the need to reconsider how focus is measured in research.

KEYWORDS: Behavioral and Social Sciences, Neuroscience, Human Focus, Unsupervised Clustering, Visualization.

■ Introduction

As a qualitative trait, focus has been hard to scientifically define, as there is no proven method established to identify it without the use of EEG devices. Because "focus"– and similarly, "hyperfocus"– levels are usually self-reported in studies, they are undefined as an observation with no scientific proof.[1] Historically, the measurement of focus has relied heavily on self-reported data due to the lack of established, objective methods. Surveys and subjective ratings have been the primary tools, with participants providing their perceived focus levels during various tasks.[2,3] While practical and straightforward, this approach is inherently limited by biases and individual differences in perception, making results unreliable and inconsistent.[4] In more recent studies, physiological data, such as eye tracking [5], heart rate variability,[6] and EEG recordings,[7] have been explored as potential objective measures to complement or replace self-reports.

The advent of artificial intelligence in data analysis offers a new way to explore correlations of multiple variables with human focus levels and find patterns in multidimensional data. Unsupervised clustering and dimension reduction are key techniques in data analysis and machine learning, particularly useful for understanding complex datasets without labeled outcomes. Unsupervised clustering is a technique that groups data points based on their inherent similarities, with the goal of identifying distinct clusters within the data that share common characteristics.[8] Dimension reduction is the process of reducing the number of variables under consideration while preserving as much information as possible.[9] The Uniform Manifold Approximation and Projection (UMAP) offers several significant benefits for dimension reduction and data visualization. First, it effectively preserves both local and global structures in the data, making it particularly useful for exploring complex datasets. UMAP is also scalable, allowing it to handle large datasets efficiently, which is essential for big data applications. Additionally, UMAP generally operates faster than other dimensionality reduction techniques, such as t-SNE, enabling quicker analysis. Furthermore, UMAP produces results in visually distinct clusters, enhancing the interpretability of complex data.[10]

This study aims to use UMAP to analyze the patterns and ambiguity of "focus" through a publicly available research dataset,[11] including their demographic information, self-reported focus level, and EEG data, when performing tasks under four different audio stimuli. We hypothesize that brainwave data demonstrates a stronger correlation with distinct clusters that emerged from UMAP than the self-reported focus data and participant demographic information.

While previous studies have explored measures of human focus, they largely relied on traditional methods that oversimplify complex focus dynamics. Our research fills this gap by introducing state-of-the-art machine learning models capable of capturing multidimensional associations with more insights.

■ Methods

Data Collection:

We utilized an online JSON public research dataset,[11] which recruited 51 participants from an opt-in screening panel–they were all ensured that their native language was English, they were all distributed evenly along the five major regions of the continental United States, and they all had normal hearing, normal vision or corrected normal vision. The participants included 34 males and 17 females. The age range of participants was between 18 and 53 years, with an average age of 36

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years and a standard deviation of 8.04 years. Brainwave data was recorded using Neuros, a headband that collects electroencephalogram (EEG) sensor data non-invasively, and then decoded using Neuros SDK decoding algorithms. Focus was measured using two methods in this dataset: brainwave data from EEG headbands (ranging from 0.0 to 1.0, with a neutral or baseline focus level at 0.5) and a cursor-based app (also ranging from 0.0 to 1.0). Deviations from 0.5, either above or below, indicated an increase or decrease in focus, respectively. Both focus levels were recorded for each of the four different audio stimuli in the dataset for every participant: silence, Endel, Apple Music, and Spotify. Among the various UMAP hyperparameter combinations tested, the 2D visualization using 15 neighbors and a minimum distance of 0.1 resulted in the most distinct and interpretable clustering, with clear correlation to brainwave-based focus levels. This configuration also provided the best visual separation for interpretability.

Data Analysis:

Brainwave data was trimmed to the minimum length present in the dataset (9297 timesteps). All timesteps past this length were removed for each participant. Figure 1 illustrates the brainwaves of one example participant in the dataset.

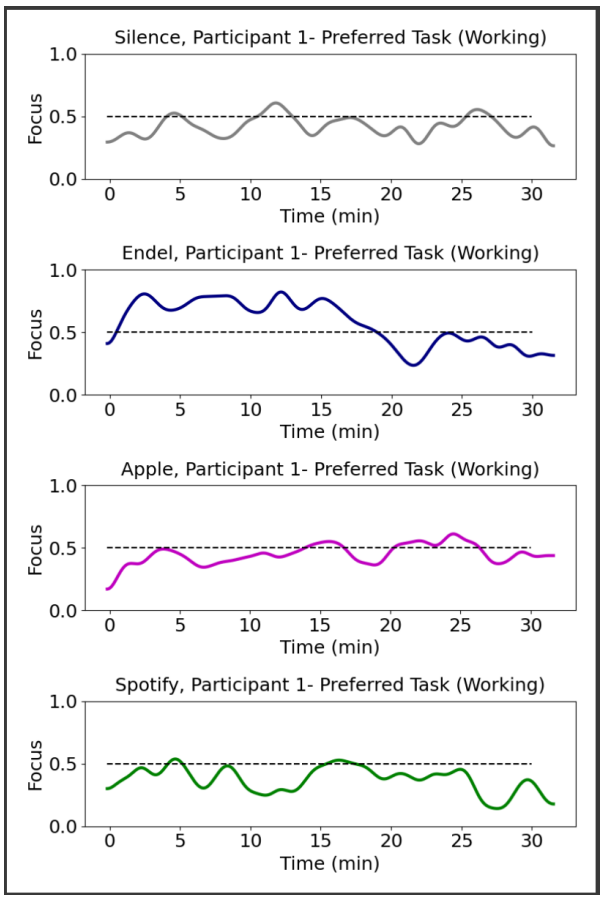

Figure 1: Brainwaves of one example participant. This was graphed utilizing the focus level data from EEG devices, which was then normalized to be centered around 0.5.

To categorize the brainwave data, the average of the 9297 timesteps was taken and binned into focus improved (>0.5) or focus did not improve (<=0.5). For self-reported focus levels, we took the difference between the self-reported focus (collected via a slider in the Neuros app, ranging between 0 and 1) after the study and the self-reported focus at the start of the study. If there was an increase, we labeled it "improved," and the latter "did not improve."

Various UMAP hyperparameters and settings were tested, including the number of neighbors (5, 10, 15, 20, 30, 40, and 50) and the number of reduced dimensions (3D, 4D, 5D, and 6D). We then ran a gradient descent, and the process was stopped after 200 iterations to check for convergence. The following variables were explored to determine which most correlated with the UMAP clusters: i) participant gender (M/F), ii) binned participants age (categorized into four groups: under 25, 25-30, 31-35, and over 35), iii) audio stimulus type: Apple (pure focus playlist), Spotify (focus flow playlist), Endel (personalized soundscape including a mixture of noise and musical properties engineered by the Endel app), and silence (no audible sound), iv) self-report focus improvement (improved/did not improve), and v) brainwave focus improvement (improved/did not improve).

Result and Discussion

The quick conversion of cross-entropy (CE) of 2D-UMAP, as shown in Figure 2, suggests the analysis results in reliable visual clusters and clear patterns. The 2D-UMAP visualization, shown in Figure 3, illustrates two distinct clusters that emerged from the analysis. Figures 4 through 8 show Figure 2 colored with respect to the different variables in the dataset. Figure 8 shows a clear correlation between the cluster and coloring.

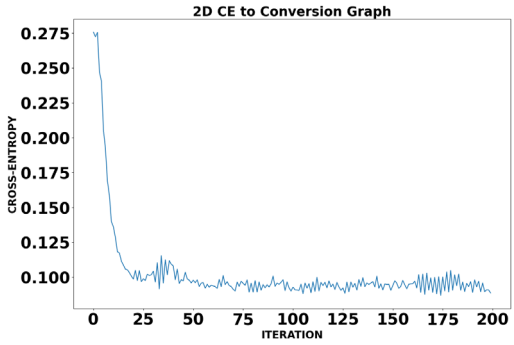

Figure 2: Conversion of the cross-entropy of 2D UMAP. Figure 2 shows the loss function of the algorithm converging successfully.

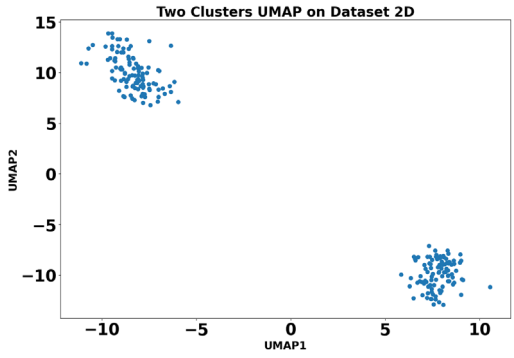

Figure 3: Visualization of 2D clustering using UMAP. Figure 3 shows that the data has been grouped into two distinct groups.

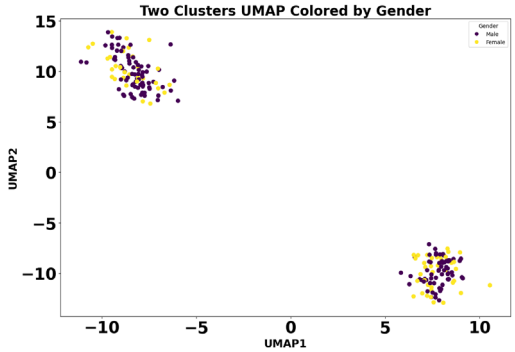

Figure 4: 2D UMAP visualization colored by gender. Figure 4 shows the 2D UMAP visualization, colored by gender, where purple and yellow dots represent male and female participants, respectively.

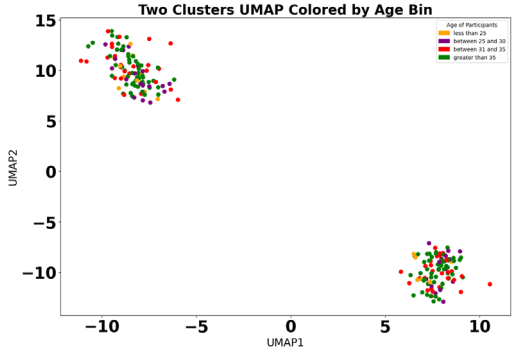

Figure 5: 2D UMAP visualization colored by age groups. Figure 5 shows the 2D UMAP visualization, colored by age groups, where orange, purple, red, and green dots correspond to the age groups of less than 25, between 25 and 30, between 31 and 35, and greater than 35, respectively.

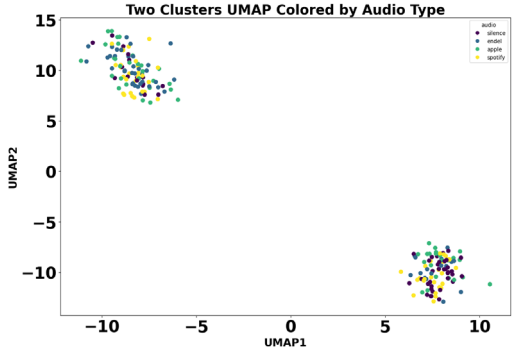

Figure 6: 2D UMAP visualization colored by audio type. Figure 6 shows the 2D UMAP visualization, colored by audio type, where purple, blue, green, and yellow dots correspond to the four audio types used in the dataset: silence, Endel, Apple, and Spotify, respectively.

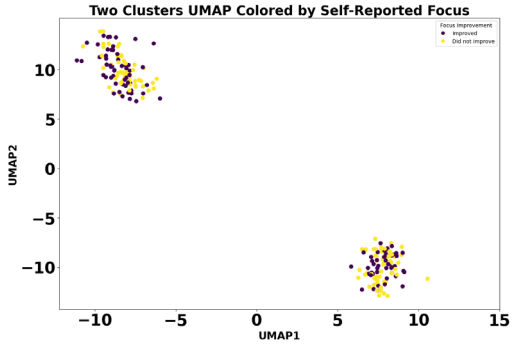

Figure 7: 2D UMAP visualization colored by self-reported focus. Figure 7 shows the 2D UMAP visualization, colored by self-reported focus levels, where purple and yellow dots correspond to participants' self-reported focus levels, improved and not improved, respectively.

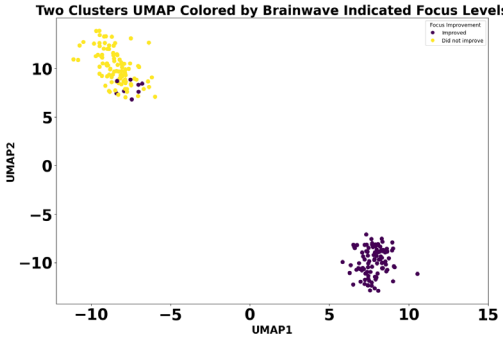

Figure 8: 2D UMAP visualization colored by brainwave indicated focus. Figure 8 shows the 2D UMAP visualization, colored by brainwave-indicated focus levels, where purple and yellow dots correspond to participants' self-reported focus levels, improved and not improved, respectively.

Comparing the distributions of data points in the 2D-UMAP figures, Figures 4-8, we can see that the brainwave data show the strongest correlation with the emerged clusters, which supports our hypothesis. Self-reported focus levels, however, do not correlate well with the clusters discovered through UMAP, suggesting that self-reported data may not accurately reflect patterns in the underlying data. These findings reveal the limitations of self-reported focus and suggest that objective data, such as brainwave analysis, can provide more reliable insights.

Conclusion

Focus is a critical trait in human performance, yet it lacks a clear scientific definition or objective measurement method, relying heavily on subjective self-reports. Using unsupervised clustering and dimensionality reduction, we analyzed data from 51 participants performing tasks under four audio stimuli (silence, Endel, Apple Music, Spotify). In particular, UMAP was used for dimensionality reduction and clustering patterns, with gradient descent applied to optimize cross-entropy convergence. Significant patterns emerged in 2D UMAP clusters, aligning with participants' decoded brainwave data. However, no associations were found between clusters and other variables, including age, gender, audio type, or self-reported focus levels. These findings suggest that self-reported focus may not accurately reflect objective patterns, raising questions about its validity in scientific research. This study highlights the need for more reliable, data-driven approaches to measuring focus in human studies. One limitation of this study is the relatively small and homogeneous participant pool (n = 51), which may affect the generalizability of the findings. Additionally, although EEG data provides an objective measurement, it may still contain noise or be influenced by environmental factors during data collection. Another limitation is the potential variability in individuals' reactions to audio stimuli, which was not controlled beyond categorizing stimulus types.

For future work, different audio genres and physical monitoring[5] as control variables should be used to explore the genres' unique effects on focus. Furthermore, future research should examine how the magnitude of focus levels varies with participants' individual preferences, such as the inclination to listen to streamlined music during work.[12] Lastly, self-reported

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variables can be explored alongside positive illusion studies,[13] leading to more reliable measures.

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RESEARCH ARTICLE

The Epidemiology and Social Determinants of Vaping in US Adolescents: Two Decades After the E-Cigarette Invention

Ayaan Seshadri

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ABSTRACT: Marketed as a safer cigarette and an aid to smoking cessation, nicotine vapes, or e-cigarettes, became popular in the early 2000s. The branding as a safer tobacco product, however, brought in a new generation of users, namely school-aged students and adolescents. This review article tracks a decade of United States (US) data on school-age student vaping and its trends, collating and critiquing publications of social triggers (determinants) of teenage vaping. Noting the impact of non-medical factors like race, socioeconomic status, mental health, and built environment on adolescent vaping rates, this article paves the way for these social determinants of e-cigarette use to be topics for deeper epidemiological analyses. Furthermore, the article calls on pediatricians across the US to be cognizant of the underlying influences that promote e-cigarette use in minors. This way, when vigilant pediatricians come across such youth in their practice for any medical reason, they can identify these "at-risk" teens and effect change.

KEYWORDS: Behavioral Science, Social Psychology, Nicotine, Vaping, E-cigarette, Adolescents, Epidemiology, Social Determinants.

Introduction

Few topics in pediatric public health are as debated as whether vaping is unequivocally injurious to health, or if it is partially beneficial, as it blocks users from a worse form of addiction - conventional (combustible) cigarettes.

Today, vaping is synonymous with Generation Z (those born between the years 1997 and 2012).[1] This population already understands that smoking conventional cigarettes, which contain nicotine within the tobacco leaf, is deleterious to their health,[2] yet paradoxically believes that inhaling vapors of this same nicotine is safe.

In 2003, four centuries after tobacco began to be sold commercially,[3] a Chinese pharmacist, Hon Lik, invented the electronic cigarette (e-cigarette), which was initially intended to be an alternative to the conventional cigarette, for smokers to use as a tool for smoking cessation. Vaping devices are cylindrical structures with a mouthpiece at one end consisting of a battery, an atomizer, and a cartridge containing a liquid solution (referred to as juice) composed of purified nicotine (**Figure 1**).[4,5]

In e-cigarettes, the battery generates the power to heat the liquid nicotine in the cartridge, and the atomizer vaporizes this liquid, emitting it as mist (aerosol) that the user inhales.[6] E-cigarettes, unlike their conventional counterparts, do not rely on the combustion of the tobacco leaf to release nicotine; therefore, they do not need to have certain chemicals called nitrosamines to cure the tobacco leaf and extend its shelf life.[7] Since tobacco-specific nitrosamines are known carcinogens and central to the link between smoking and cancer,[7] e-cigarettes were invented to reduce carcinogens, make smoking safer, and perhaps even aid in smoking cessation.

Paradoxically, the opposite occurred. The notion that "purified" nicotine must be harmless, and the absence of carcinogens must make it "safe," drew a new generation of first-time e-cigarette users: adolescents aged 13-18 years[8] and young adults. Additionally, the use of fruity flavors to mask the bitter stench of concentrated nicotine further resulted in nicotine vapes appealing to youth.[8] A 2018 report that an alarming 85% of young adults (18-29 years of age; **Figure 2A**) had used vape devices at least once stunned public health experts.[9] Aggravating matters, e-cigarette use had even trickled down to teenagers; a 3-year study published in the New England Journal of Medicine demonstrated that its usage doubled between 8th and 12th grade (**Figure 2B**).[10]

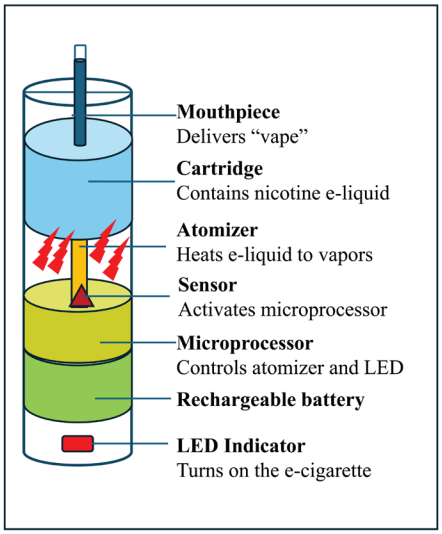

Figure 1: Schematic diagram of an e-cigarette. This figure shows how the vape "pen" works: the battery generates the power to heat the liquid nicotine in the cartridge, and the atomizer vaporizes this liquid, emitting it as mist (aerosol) that the user inhales.

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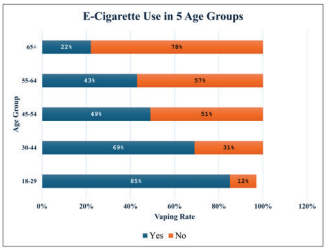
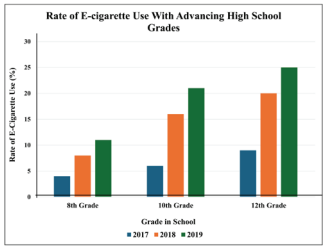


(A)



(B)

Figure 2A: Vaping and e-cigarette use by age. Adapted from "Percentage of adults in the U.S. who had used e-cigarettes as of 2018, by age" on Statista.[9] This figure spotlights data from a 2018 survey that 85% of young people (18-29 years old) had used vape devices at least once.

Figure 2B: Uptick in Adolescent Vaping Rates Between 2017 and 2019. Adapted from Miech *et al.*, New England Journal of Medicine.[10] The graphs show a doubling of adolescent e-cigarette users through the course of the high school years between 8th and 12th grade.

Written shortly after the 20th anniversary of the invention of the vape device, this article is one of the few that provide a comprehensive evaluation of the multiple, often interrelated, triggers that lead adolescents towards e-cigarette use. In doing so, the author seeks to examine the "why" of teenage vaping through an epidemiological lens. While causality analyses of each trigger of adolescent vaping are complicated, having reviewed over 100 relevant publications, it became apparent that looking at these factors in isolation is not enough, because "at-risk" teenagers often present with multiple complicating factors. Only by understanding the interplay of the multiple social determinants that impact e-cigarette dependency can health professionals, like pediatricians, flag vulnerable teens and effect sustainable change.

Discussion

Epidemiology and Social Determinants of Adolescent Vaping:

The advent of the e-cigarette saw the rise of a new demographic of users: first-time vapers, often teenagers or young adults.[8]

According to the 2024 annual National Youth Tobacco Survey (NYTS) from the Centers for Disease Control and Prevention (CDC), nicotine e-cigarettes were the most used tobacco product among middle and high schoolers, with 410,000 middle schoolers (3.5% of all US middle schoolers) and 1.21 million high schoolers (7.8% of all US high schoolers) using them nationwide.[11] Of them, 26.3% reported daily vaping, and 38.4% used an electronic cigarette at least 20 of the last 30 days.[11] In contrast, smoking of conventional combustible cigarettes was at an all-time low of 1.4% among middle- and high-school students.[12]

This literature review studies multiple key epidemiological factors and social determinants of health (SDOH) that result in these 1.63 million US middle and high schoolers using e-cigarettes each year.[11] The CDC defines SDOH as all the nonmedical factors that influence health outcomes. These determinants include ethnicity or race, gender, mental health, neighborhood, housing status, food security, family education level, income, and more. By analyzing multiple SDOHs both individually and interconnectedly, our work fills a gap in the literature, as these triggers are often interrelated.

Racial and Cultural Factors:

Racial identity is a noteworthy determinant of e-cigarette usage in adolescents.[13,14] From 2011, when e-cigarette-related questions were introduced into the NYTS, the prevalence of e-cigarette use has always been greater in white adolescents than in teenagers from the Black community. Since vaping costs ranged between $50 and $100 per month, e-cigarettes and nicotine vaping were considered a "vice of the wealthy". The CDC's annual Behavior Risk Factor Surveillance System (BRFSS) demonstrates that race may inform other SDOHs like socioeconomic status, education, income, food (in)security, and social connectedness; hence, its influence on substance dependency in general, and e-cigarette use in this case, cannot be underestimated.[15]

The racial distribution of adolescent vapers in the United States has changed in recent years. The NYTS 2024 data demonstrated that 6.6% of current (past 30-days) e-cigarette-using adolescents in the US identified themselves as multiracial, 5.9% as white, 6.1% as Hispanic or Latino, 7.0% as Black and 11.5 % as American Indian or Alaska Native [(AI/AN); **Figure 3A**].[16]

The NYTS 2024 is unique in the observation that for the first time, vaping rates in African American, Hispanic, and white communities are comparable to each other, whereas in prior years, e-cigarette use was most prevalent among white youth. This outcome could have several potential causes. On the positive side, it may have stemmed from the success of culturally inclusive youth awareness campaigns.

One such US teenager-centric educational campaign, "The Real Cost," is credited with reducing e-cigarette initiation in an estimated 444,252 US youth across all races in the 2023-2024 school year.[17] On the negative side, top-selling vape brands may be providing steep discounts to expand their clientele across teenagers from a wider spectrum of racial and socioeconomic backgrounds.[18]

While nicotine vaping rates have been down across all major racial groups in 2024 compared to 2023, their high prevalence (11.5%) in AI/AN youth remains a cause for concern. This is not a sporadic occurrence, as prior years of NYTS have corroborated this finding. The American Indian/Alaska Native (AI/AN) community faces unique stressors compared to other racial groups. The CDC BRFSS reports 31.9% of AI/AN people reporting "lack of support" and social unconnectedness.[15] This state of poor mental health in the community must make its youth more susceptible to e-cigarette dependency (see *Mental Health* section). What is paradoxical, however, is that an expensive habit like vaping is so prevalent in one of America's most impoverished communities, as measured by 21.3% of AI/AN people reporting needing food stamps.[15]

Mental Health:

In an era where great emphasis is placed on feigning perfection (on social media), the mental well-being of youth is undeniably impacted.[19] Nicotine is a highly addictive substance

that binds to nicotinic receptors in the brain to stimulate the release of euphorigenic neurotransmitters like dopamine and norepinephrine in a matter of seconds after inhalation.[20] Thus, teenagers use nicotine vapes hoping that this euphoric sensation will instantly alleviate their stressors of everyday life.[21] In one of the only publications that we think comprehensively addresses multiple SDOHs, Andrea Gentzke and colleagues use primary data from NYTS 2021 to show that school-aged teenagers who were getting D's and F's in classes had an alarming lifetime tobacco use rate of 36.7% and 41.7%, respectively, and 15.6% and 17.3% rate of current usage (**Figure 3B**). Their analysis also revealed that middle and high schoolers who were in moderate and severe psychological distress had 29.3% and 37.8% lifetime tobacco use rates and 11.2% and 14.2% current usage rates, respectively. Similar observations of elevated use of tobacco products were made among teenagers who identified as gay, lesbian, bisexual, or transgender in this survey. 81% of tobacco use in this study was in the form of e-cigarettes. In totality, these data depicted in Figure 3B underscore how academic, social-emotional, orientation-based, and general stressors may bring teenagers to tobacco products, specifically e-cigarettes.[22]

It is likely that these teenagers are misinformed about the adverse repercussions of using vaping devices and believe erroneously that e-cigarettes are a win-win solution: a way to improve mental well-being without deteriorating physical health.[23] However, given the short-lived psychoactive effect of the nicotine as the level of this agent decreases in the brain with time, nicotine cravings or withdrawal symptoms can set in, creating an urge to inhale nicotine again.[24,25] This marks the beginning of dependency; nicotine addiction is rooted in the teenagers need to enhance its positive effects (heightened vigilance, improved mood) on one side and the desire to reduce the negative impact of its absence (withdrawal symptoms such as anxiety, irritability, impaired concentration) on the other. This is the beginning of substance abuse, which can not only result in addiction from a mental health perspective (discussed above) but also acute conditions such as heart disease, stroke, and COPD, from a physical health perspective.[26]

Yet another factor affecting teenagers' mental well-being, and in turn their extent of vaping, is loneliness. In an online survey-based study, John E. Grant and his colleagues concluded that illicit drug or alcohol use, being single, and being an undergraduate student, all of which can cause or exacerbate mental health problems, were statistically significant determinants of e-cigarette use.[27] Once again, they demonstrated nicotine e-cigarette usage to be more prevalent in young adults (18-19 years of age) than older adults in college. Grant's team also reported that students who were current or past year e-cigarette users were more prone to untoward risk-taking behaviors, including their greater susceptibility to non-nicotine substance abuse and higher impulsivity scores. By delineating early undergraduate years and being single (both markers of loneliness) as determinants of e-cigarette usage, Grant's study corroborates Andrea Gentzke[22] analysis that academic and social stressors are relevant mental health precursors to e-cigarette addiction. Looking at how SDOHs can be interconnected, John Grant's work now contextualizes why AI/AN youth, whose community had the highest loneliness rates per the BRFSS,[15] may turn to e-cigarette use.

Socioeconomic Status, Housing, and Neighborhood:

Family income and socioeconomic status play a powerful role in adolescents' vaping in the US, but there is conflicting evidence on whether it is poverty or affluence that promotes e-cigarette use in minors.

Treating housing status as a proxy for socioeconomic stratum (SES), a paper by Patricia Simon and coworkers from Yale University surveyed 3473 urban and suburban Connecticut high schoolers to demonstrate a positive correlation between SES and prevalence of vape use: the higher the SES, the greater the rate of teenage vapers. Although the direct correlation between SES and e-cigarette use narrowly missed statistical significance ($p=0.07$), what was significant was a correlation between SES and exposure to advertising ($p<0.001$), which in turn had a significant ($p=0.05$) correlation with e-cigarette use (**Figure 3C**).[28] Another study, a secondary analysis of social determinants from NYTS 2021 by Andrea Gentzke, claimed otherwise showing no correlation of family affluence with tobacco and e-cigarette use (**Figure 3D**).[22]

In complete contrast, an analysis of the California Healthy Kids Survey by Jennifer Felner and colleagues concluded that the likelihood of vaping among youth living in transitional homes or shelters was 1.53-1.88 times greater than those residing in permanent homes, and these results were statistically significant.[29] Felner's work also showed that teens from a lower social class have a greater susceptibility to e-cigarette use. Since vaping nicotine is an expensive habit, costing $50 to $100 each month, Felner and colleagues' observation that the lower the SES, the greater the chance of vaping, is paradoxical.[29]

These contradictions underscore the central goal of this paper, which is to look at the matrix of SDOHs both individually and collectively. Perhaps that paradox can be explained by delving deeper into the neighborhood in which a teenager's home is located. Patricia Simon's[28] work has already demonstrated the direct impact of advertisement on adolescent e-cigarette use. Extrapolating it forward, the area in which one's permanent or even transitional home is located may perhaps have a triggering effect on e-cigarette use through the continuous vape shop advertising that a teenager sees around them in the community.

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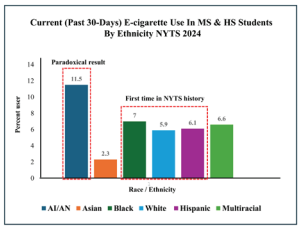

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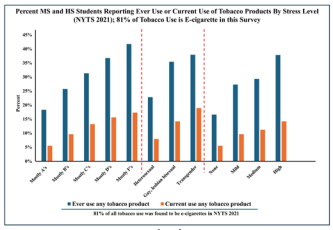

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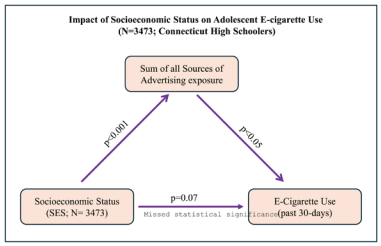

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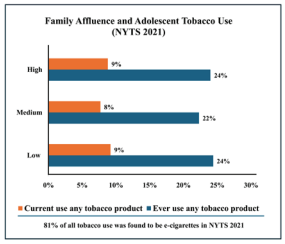

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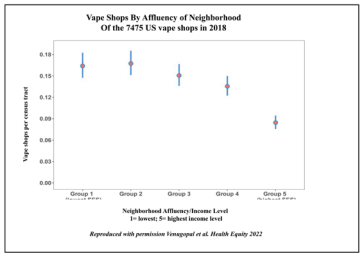


(E)

Figure 3A: Current (30 days) e-cigarette use in middle school (MS) and high school (HS) by student ethnicity. Adapted from Jamal *et al.*, NYTS 2024.[16] The figure depicts the racial representation of current adolescent e-cigarette users from NYTS 2024 and analyzes it as a percentage of all US middle and high schoolers of the same ethnicity.

Figure 3B: Percent of middle (MS) and high school (HS) students reporting ever use or current use of tobacco products analyzed by stressors of life. Adapted from Gentzke *et al.*, NYTS 2021.[22] (Note: 81% of all tobacco use was as e-cigarettes). This graph shows the impact of academic grades, sexual or gender orientation, and general mental health status of a teenager on their tobacco use.

Figure 3C: Association analysis of family socioeconomic status (SES) with e-cigarette consumption either directly or through advertising exposure. Adapted from Simon *et al.*[28] Using a Yale University survey conducted in Connecticut high schools, this schematic representation shows both a direct correlation between SES and current e-cigarette use and a more robust indirect correlation through the extent of exposure to advertising.

Figure 3D: Adolescent tobacco use (current or ever) by family affluence. Adapted from Gentzke *et al.*, NYTS 2021.[22] This graph demonstrates a lack of correlation between family affluence level (self-reported) and use of tobacco products (ever or current) from the NYTS 2021 dataset. (Note: 81% of all tobacco use was as e-cigarettes)

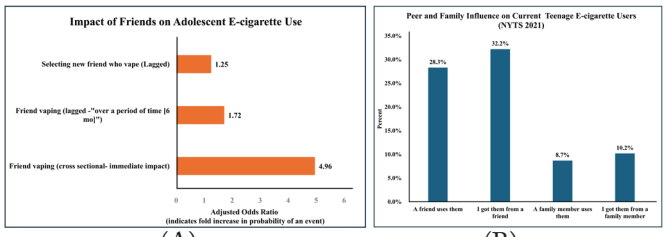
Figure 3E: US geospatial mapping of specialty vape shops analyzed as a function of SES of the neighborhood. Group 1 = lowest SES, group 5 = highest SES. Reproduced with permission from Dr. D Venugopal.[31] This diagram shows that the lower the SES level of an area, the higher the number of vape shops located there.

A spatial analysis of Tobacco Retail Outlets (TROs) and Vape Shop Outlets (VSOs), entitled "Neighborhood Disadvantage and Tobacco Retail Outlet and Vape Shop Outlet Rates," was published in April 2020 by David C. Wheeler and colleagues. This study concluded that the socioeconomic level of a neighborhood had a clear negative correlation with the number of TROs and VSOs in that area; on average, a low-income neighborhood had 63% more TROs and 64% more VSOs than an affluent one. This study also reported strong positive associations between the dependent variable of TRO/VSOs and independent variables such as percentage of renter-occupied housing versus owner-occupied homes, and percent vacant housing units.[30] This correlation supports the thesis that living in neighborhoods with several itinerant households, devoid of long-term ties within one's community, predisposes teenagers in those families to nicotine vaping. The authors suggest that, conversely, neighborhoods with entrenched multi-year, multi-generational families may likely create robust local laws that deter TROs and VSOs from springing up in the first place. Perhaps it is now possible to contextualize why Felner and colleagues saw the increased odds of vaping in teens living in transitional homes, as they would gravitate to the high number of VSOs in their area and get initiated into vaping.

Bringing our analysis a full circle, at the height of the adolescent vaping crisis in 2018-2019, a team from the Food and Drug Administration (FDA), Venugopal and colleagues performed geospatial mapping of US VSO's and analyzed their abundance in an area as a function of the SES of that neighborhood determined from census data. This data corroborated Wheeler's findings and showed an inverse proportionality between SES and number of vape shops (**Figure 3E**).[31] Together, the cited works establish the complexity of evaluating SES alone, and the value of studying it in the context of other SDOHs like housing, neighborhood and built environment to quantify its true impact on adolescent e-cigarette use.

Interpersonal Influences: Peer Pressure and Family Dynamics:

Interpersonal influences, specifically peer pressure, are a powerful predictor of the likelihood of nicotine vaping in American adolescents. In 2023, a paper entitled "Social Network Influences on Adolescent E-cigarette Use," by Valente and colleagues, analyzed the role this SDOH plays in adolescent e-cigarette use by interviewing 1,208 students in a Midwestern school district and asking them about exposure to vaping among their circle of friends. Valente's team concluded that both seeing e-cigarette use among a pre-existing (friendship duration ≥6 months) circle of friends (**Figure 4A**; "lagged" data) and selecting new friends (Figure 4A; "immediate" data) who vape were both positively correlated with one's own vaping initiation. In fact, new friends who vape dramatically increased the likelihood (4.96-fold) of a teenager initiating e-cigarette use (Figure 4A). This marked the first prospectively defined epidemiological study to quantify the impact of peer pressure and social dynamics on teenage e-cigarette initiation.



(A) (B)

Figure 4A: The likelihood (odds) of an adolescent vaping by immediate (cross-sectional) and 6-month (lagging) impact of their friends using e-cigarettes. Adapted from Valente *et al.*[32] Using data from a Midwest school district, this graph shows how having existing friends who vape impacts the likelihood of a teenager initiating e-cigarette use themselves. This impact is far greater (4.96-fold) when a teenager makes new friends who vape.

Figure 4B: The role of friends and family members who vape on adolescent e-cigarette use. Adapted from Gentzke *et al.*, NYTS 2021.[22] This data graphically represents student responses from the NYTS 2021, demonstrating the influence of friends and family members who vape on teenage e-cigarette users, including their role in sourcing the first vape for the adolescent.

Valente's data also corroborates the elegant analysis of the NYTS 2021 by Andrea Gentzke demonstrating that an 28.3% of current vapers indicated that they had a "friend who was using them" and 32.3% of the current vapers indicated that it was a friend who had "got it for them" (**Figure 4B**).[22] While overall nicotine vaping rates among school-aged youth have reduced since the time of this analysis (2021), the triggering influence of friends remains undeniable.

Not all pressure is peer pressure; a child's elders using e-cigarettes would likely increase their own affinity for vaping.[33] In fact, data mining of individual responses from the teenagers polled for the NYTS 2021 revealed 18.7% lifetime e-cigarette users, and 8.7% of current users admitted to family members using them at home. Far more concerning was that 10.2% of all current vapers indicated that they actually developed the habit because a family member actually "got it for them" (**Figure 4B**).[22] Similarly, in a 2018 meta-analysis, Jian-Wei Wang and her research team compiled 21 studies analyzing the effect of observing family members vape on a teenagers own e-cigarette usage and reported a positive correlation between teen vaping and e-cigarette usage in both family members and in friends. Adolescents are 1.47 times more likely to vape if their family members do so.[34]

Social Media and Tactical Marketing:

Valente's work already shows the dangerous (4.96-fold) impact of new friends on teenage e-cigarette initiation.[32] But not just physical friends, cyber peers also play a role in enticing adolescents towards e-cigarettes. A 2023 publication by Hopkinson and colleagues concluded that those spending 1-3 hours per day on social media are 92% more likely to vape, and those spending greater than 7 hours per day on social media increase their likelihood of smoking or vaping by nearly five-fold.[35] Once again, just like VSOs are an example of physical advertisements, Hopkinson's work ties back to Simon *et al.*,[28] that increased exposure to advertising (see Figure 3C), in this case e-advertisements through social media, correlates with a higher incidence of nicotine vaping.

The impact of tactical social media marketing by "big tobacco" companies is central to the US adolescent e-cigarette epidemic. The success of the two oldest corporations in the US vaping landscape, JUUL Labs (makers of the popular pod-based vape product, JUUL) and RJ Reynolds (the makers of Vuse, the rechargeable vape), monopolizing the nicotine e-cigarette market (through 2023), stemmed from their ability to convincingly market nicotine vaping products to adolescents through social media. Showcasing the fruit and candy flavorings in the vape through social media convinced adolescents that they are only inhaling fun flavors, when in reality, they are breathing in nicotine.[36] In their 2018 annual earnings release, JUUL reported expenses of $73 million on targeted teenage branding and marketing campaigns highlighting the breadth of flavors in which their vapes were available.[37] Not surprisingly hence that in the following year, the 2019 NYTS data demonstrated that 69.3% of American teenagers reported exposure to e-cigarette marketing directed at them.[38] Furthermore, in 2019 and every year since, > 8 out of 10 youth initiate their tobacco addiction with a flavored product, and 72% of high school students who vape regularly have expressed a preference for flavorings in their electronic cigarettes.[12]

Products like the Elf Bar have recently risen to prominence in 2023-2024 by savvy marketing of their fruit flavors, colorful packaging, easy disposability (single use), and their unique social media strategy targeting teenagers on TikTok.[18] In fact, 36.1% of all adolescent nicotine e-cigarette users surveyed in the NYTS 2024 used this product (**Figure 5**).[11] Since TikTok involves individuals disseminating their own videos, it is reported that Shenzhen iMiracle Technologies from China, the makers of Elf bars, pay teenage influencers to put out the Elf Bar content while remaining incognito themselves. [39] In this way, the company has been able to circumvent US regulations regarding marketing to minors. Similarly, the colorful packaging and cartoon characters on Elf Bars serve as both a ploy to import these products into the US as battery-operated toys, obfuscating US Customs enforcement, and as their fun-filled youth marketing strategy.

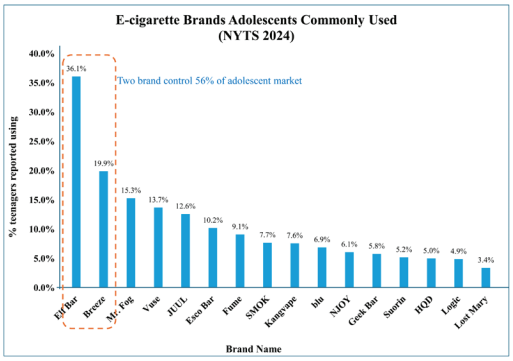


Figure 5: Best-selling nicotine vape brands among adolescent users in 2024. Adapted from NYTS 2024.[11] The bar graph compiles student responses from NYTS 2024 about what are the brand(s) of vape products they use routinely. Percentages cumulate to > 100% as the same student may use multiple products.

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Legislation and Enforcement:

One of the most powerful SDOHs of teenage vaping is, in fact, extrinsic to the teenagers themselves and in the hands of policymakers. Past 30-day e-cigarette usage rates among high school students have declined from 27.5% in 2019 to 10% in 2023 to 7.8% in 2024 (**Figure 6**).[40]

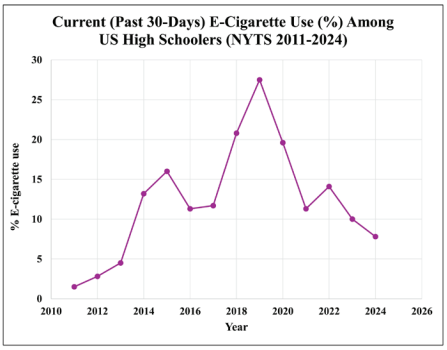

Figure 6: Past 30-day e-cigarette use from 2011-2024. Adapted from NYTS 2011-2023.[40] This graph shows the evolution of current e-cigarette use among teenagers over 14 years (2011-2024). E-cigarette use peaked in 2019 and has been trending downwards since. Yet, with 1.6 million adolescents using e-cigarettes in 2024, vaping remains a very serious pediatric public health crisis.

One of the most foundational reasons for this steady decline in adolescent e-cigarette use in the 2020-2024 period is the enactment and enforcement of diverse sets of legislation that restrict the actions of the major corporations in the vaping industry. **Table 1** lists five of the most consequential pieces of legislation related to e-cigarette use passed in the last 15 years, and details their ramifications.

Table 1: Existing e-cigarette-related federal legislation passed since 2009. The table shows the robust body of legislation passed in the last 15 years to regulate e-cigarette manufacturers selling products in the US markets, aimed specifically at blocking their sales to teenagers.

Name of Law/Month and Year of passage	Consequences of the legislation
Family Smoking Prevention and Tobacco Control Act June 2009	Allow the executive branch of the government to restrict and regulate sale and distribution of tobacco products to promote public health Restricts promotion of tobacco products by preventing tobacco brands from sponsoring social events (such as sports, entertainment, etc.)[41]
Deeming Rule May 2016	Extends the definition of "tobacco products" in Family Smoking Prevention and Tobacco Control Act to include electronic nicotine delivery systems and e-cigarettes.[42]
Tobacco 21 Dec. 2019	Raises the minimum age for purchasing tobacco and nicotine products from 18 to 21.[43]
Preventing Online Sales of E-Cigarettes to Children Act July 2020	This legislation subject's e-cigarette sales to taxation Requires US Postal Service to prevent mailing of nicotine e-cigarette products. Requires National Institutes of Health (NIH) to conduct a study on health impacts of nicotine vape usage.[44]
Premarket Tobacco Product Application (PTMA) Guidelines Amended 2021	Requires companies selling tobacco to submit clinical studies to the FDA that demonstrate that their products are not more injurious to public health than conventional cigarettes. In these reports, corporations must also prove that they are marketing tobacco products in an accurate and veracious manner.[45]

Since 2009, the US government, and specifically organizations like the FDA, have passed substantial legislation to combat the vaping epidemic in American adolescents. The implementation of these laws has also improved meaningfully in the last 5 years. For example, under the aegis of the Family Smoking Prevention and Tobacco Control Act of 2009 and the Deeming Rule of 2016, the FDA was able to launch an investigation on Juul in 2019, resulting in the first leg of the removal of some of its fruity flavored vapes from the market that same year.[46] The 2021 amendment of the PTMA Guidelines resulted in both Juul and Vuse receiving marketing denial orders (MDOs) for their PTMAs from the FDA that year.[47] Although both Juul and Vuse have been reinstated in US markets in 2024, the scope of their product offerings has been curtailed, and no longer contains any candy or fruit flavored vapes whatsoever.[48] Knowing that >80% of adolescents across all NYTSs report favoring fruity vapes, this legal enforcement was likely a primary contributor to the reduction in adolescent vaping since 2020.

With strong and proven legislation already in place, now it is behooved of the government agencies to galvanize their implementation even further by working with local level city law enforcement (police) to combat the sales of the remaining fruit flavored vape products like Elf Bar and Breeze, that together make up 56% of all e-cigarettes sold to US teenagers. One reason that these two brands have been able to avoid the national ban on fruit flavored vapes that Juul and Vuse were forced to comply with is that both are imported products brought in from China, hence able to circumvent the PTMA law relevant only for US-domiciled manufacturers.

■ Conclusion

Public health research is often confounded by the multifactorial nature of human decision-making. The epidemic of e-cigarette use among US adolescents is one such public health emergency where the unequivocal cause and effect as to what drives a teenager to their first vape is hard to establish. A matrix of life events may create conditions that entice teens to vape. Therefore, studying these triggering factors individually may leave unanswered questions. In this paper, race and ethnicity, socioeconomics, housing, neighborhood, peer-pressure, family dynamics, mental health, governmental legislation, and its implementation are all studied as determinants of teenage e-cigarette use. Throughout the paper, we have highlighted how understanding one SDOH may be impacted by clarifying the role of another. An example of the power of triangulation of information from various SDOHs is the case of minors in shelter and transitional homes, affording vapes when they have no money for food; a paradox when one looks at it as just an SES question. Looking through the multi-SDOH lens, we see their dilapidated neighborhood, potentially with high numbers of VSOs reinforcing vaping, in turn impacting their newfound peer group, perhaps family members with substance abuse, or their falling grades from moving to a transitional home, together taking a final toll on their mental health. Another unique aspect of our work was to show how seemingly unconnected things like VSOs and social media marketing can both serve as positive reinforcers to e-cigarette dependency; one physical, another digital.

One SDOH for future analysis is the impact of urban, suburban, or rural school settings on teenage vaping in the US. All the papers found were pre-pandemic, predating the social

media surge, or devoid of a dependable trendline. What we gleaned cursorily was that nicotine vaping can be high in both extremely rural and ultra-urban environments. As a future step, the authors intend to approach the CDC (under the Freedom of Information Act) for the zip codes of the roughly 300 schools that are chosen each year for NYTS, and the rate of current e-cigarette use by that school's zip code. A five-year lookback of such zip code level information, when cross-matched with census assignment of that area (rural, suburban, or urban), will shed light on the SDOH of the school setting.

Through this work, the authors also want to reach out to adults who interact with teenagers, including teachers, guidance counselors, coaches, and above all, pediatricians in community practices, to carefully evaluate adolescents through the multi-SDOH lens studied in this paper. By being cognizant of the interplay of underlying triggering causes that promote e-cigarette use in adolescents, vigilant pediatricians and educators can identify these "at-risk" teens quickly and effect change.

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REVIEW ARTICLE

Contributing Factors to Eating Disorders in Sexual and Gender Minorities

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ABSTRACT: This review explores the disproportionately high prevalence of eating disorders (ED) in sexual and gender minorities (SGM) compared to their cisgender heterosexual (CH) peers. It first explains the main contributing factors leading to disordered eating behaviors in SGM, including minority stress, gender/ sexual dysphoria and body dissatisfaction, desire to pass, and barriers to affirming and inclusive care. It then separates the discussion into sexual minorities and gender minorities to explore the specific contributing factors of these communities. The review highlights the need to develop more inclusive treatment models to support SGM individuals, as well as future research that expands to include underrepresented groups within SGM.

KEYWORDS: Behavioral and Social Sciences, Clinical and Developmental Psychology, Eating Disorders, Sexual and Gender Minorities, Minority Stress.

Introduction

Sexual and gender minorities (SGM) face certain unique and heightened challenges compared to cisgender heterosexual (CH) individuals, contributing to a higher prevalence of eating disorders (ED) among SGM populations. Recent studies support the greater prevalence of ED among SGM populations. For example, in a nationally representative sample of 35,995 U.S. adults who participated in a diagnostic review from 2012 to 2013, ED prevalence rates were significantly higher among SGM than among heterosexual cisgender individuals (anorexia nervosa—1.71% versus 0.77%; bulimia nervosa—1.25% versus 0.24%; binge eating disorder—2.17% versus 0.81%).[1,2] Another literature review published in 2021 found that SGM adults have between 2–4 times greater odds of being diagnosed with anorexia nervosa, bulimia nervosa, or binge eating disorder compared to cisgender heterosexual adults.[3] However, the majority of studies concerning ED do not include the unique experiences of SGM individuals, leading to a limited understanding of risk factors specific to them. Furthermore, current ED treatment models designed for CH individuals do not attend to the specific needs and unique experiences of SGM, resulting in a lack of access to affirming and effective treatment.[4] Understanding why SGM communities have a higher chance of ED is crucial for healthcare providers to develop specialized interventions that provide effective and inclusive care.

According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), eating disorders are "behavioral conditions characterized by severe and persistent disturbance in eating behaviors and associated distressing thoughts and emotions."[5] They can lead to serious physical, psychological, and social consequences. The most commonly recognized types of EDs include Anorexia Nervosa (an intense restrictive food intake or starvation driven by the fear of gaining weight), Bulimia Nervosa (cycles of binge eating, usually with a loss of control, followed by episodes of intense compensatory behaviors, such as vomiting, excessive exercising, or fasting, to lose weight), Binge Eating Disorder (recurrent episodes of binge eating, consuming large amounts of food, without the following compensatory behaviors), Avoidant Restrictive Food Intake Disorder (avoidance of food or restricted food intake that results in nutritional deficiency), and Other Specified Feeding and Eating Disorder (eating disorders that do not fit in any of the above categories but still pose physical, psychological, or social consequences). The studies included in this review conform to this definition of eating disorders. Several studies use ED screening tools that reflect the criteria in the DSM-5-TR, which contains the most conventional understanding of mental disorders. This review includes studies of individuals who are diagnosed with ED as well as those with disordered eating behaviors. The latter indicates that these individuals may not meet the full criteria for an ED diagnosis, but still exhibit irregular eating behaviors that match the definition above.

This review focuses on sexual and gender minorities (SGM), which are relatively underrepresented in ED studies. SGM is an umbrella term for LGBTQIA+ (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, and Asexual) populations whose sexual orientation or gender identity doesn't conform to traditional social norms.[6] Specifically, this review discusses transgender, gender nonbinary, gay, lesbian, and bisexual individuals, with studies mostly based on demographics in the United States and the United Kingdom. This review argues that SGM communities have a greater prevalence of ED compared to CH peers due to factors including minority stress, gender dysphoria, desire for passing, and barriers to accessing affirming and inclusive care. By analyzing, comparing, and contrasting studies from different demographics and perspectives, this review aims to raise awareness of ED among SGM and the unique stressors and contributors, thus offering insights that may result in more effective and inclusive treatment programs for this population.

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Discussion

Minority Stress Model:

The minority stress model is based on the theory that marginalized populations face greater mental health challenges due to increased social stress that results from their stigmatized status.[7] Minority stress differs from general stress because the former is rooted in stigma and prejudice experienced by minority communities. Minority stressors can be separated into 2 categories: distal and proximal.[8] Distal stressors are external events that SGMs experience due to discrimination and prejudice, typically transphobia and homophobia. These can be discriminatory policies or everyday microaggressions, including acts of harassment and violence. For gender minorities, one study from Project AFFIRM compared transgender and gender nonbinary participants who met the criteria for current ED-related symptoms with transgender and gender nonbinary participants who didn't, and found that the former had higher minority stress scores along with higher BMI and lower transgender congruence.[9] In this study, minority stress scores were calculated based on 3 tests: a 10-item version of the Everyday Discrimination Scale measuring enacted stigma, evaluating aspects of discriminative experiences like frequency and prevalence; a 10-item version of the Stigma Consciousness Questionnaire for felt stigma, in which participants had to indicate their level of agreement with statements like "Most non-transgender individuals have a problem viewing transgender individuals as equals"; and the Transgender Identity Survey for internalized transphobia.

For sexual minorities, one study from Tennessee, USA, using the Eating Disorders Screen for Primary Care and other screening instruments, found that lesbian individuals have a 66.7% ED proneness, whereas gay individuals had a 47.6% ED proneness.[10] The study found that perceived stigma has a direct relationship with ED proneness in gay individuals and a significant indirect relationship with ED proneness in lesbian individuals. Similarly, a literature review conducted in 2021 showed that experiences like minority stress, heterosexism, and sexual objectification can lead to eating disorder behaviors and body dissatisfaction among lesbian and bisexual women.[3] It also found that sexual minority adults who have experienced discrimination based on their weight, a common experience of minority stress, had a greater risk of eating disorder behaviors compared to those who did not experience weight discrimination.

On the other hand, proximal stressors are internal responses to these external pressures. These include internalized stigma, an expectation of stigmatization, and hiding sexual orientation or gender identity. According to the minority stress model, SGM individuals face increased stress as they do not fit into the traditional social norms of heterosexuality and cisnormativity. In turn, these individuals might develop disordered eating behaviors as a maladaptive coping mechanism for their emotional distress. Furthermore, internalized stigmas such as transphobia and homophobia exacerbate body dissatisfaction, increasing the drive to engage in ED behaviors to conform to social norms, and thus a greater prevalence of ED.[9,11] For gender minorities, the Project AFFIRM study found that individuals with internalized transphobia have 41% greater odds of being associated with ED, and individuals with anxiety have 3% greater odds of ED association.[9] It also found that the gender nonbinary individuals had greater levels of minority stress and psychological distress compared to the binary transgender individuals, suggesting that minority stress potentially leads to heightened ED symptoms for gender nonbinary individuals.

For sexual minorities, the study from Tennessee showed that depression was significantly associated with ED proneness in gay and lesbian individuals.[10] The logistic regression analyses from the study further indicated that depression and self-compassion were the predicting factors of ED proneness in gay individuals, and depression was the predicting factor of ED proneness in lesbian individuals. Similarly, the 2021 literature review found that depression was a significant predictor of a positive ED screen among lesbian individuals among a sample of 267 SGM adults in the United States.[3] It also found that depression and perceived stigma are predictors of ED behaviors among gay individuals among a sample of 317 gender minority adults.

Gender/Sexual Dysphoria and Body Dissatisfaction:

Gender dysphoria is the psychological distress felt by gender minorities, resulting from the incongruence between sex (assigned at birth) and gender identity.[12] For gender minorities, this distress can manifest as body dissatisfaction, as one's sex characteristics don't align with one's gender identity, yet one has already internalized the beauty ideals that contradict one's body. Therefore, this population may employ disordered eating to change the shape of their body or specific body parts in an attempt to suppress or accentuate gender.[11] Beauty standards of conventional masculinity and femininity are significantly amplified for transgender individuals, especially transgender youth, who are highly influenced by social media's reinforcement of these narrow beauty standards.[11] For example, the Project AFFIRM study found that 50% of transgender and gender nonbinary participants over-reported their weight, and more than 50% attempted to restrict food intake to change their body shape or weight.[9] The shape and weight concerns reported among the transgender participants were also greater than is typical in cisgender individuals, leading to further gender dysphoria within this community. One possible explanation for this finding is that in some cultures, masculinity may be linked to muscularity while femininity may be linked to thinness. According to this theory, transgender men could display ED symptoms like fasting, vomiting, or excessive exercise in an attempt to achieve a masculine physique, and transgender women could adopt restrictive diets to appear thinner, to match the conventional beauty standards for women, as shown in the 2021 literature review.[3]

For sexual minorities, all participants from a study published in 2022 said in the interview that gender dysphoria was a critical factor in the development of their eating disorder, but it was often overlooked.[13] The study showed how sexual minorities endorse higher body dissatisfaction because their idealized body image is different from heterosexual peers, with sexual minorities enduring heightened appearance pressure.

The study reported high levels of body dissatisfaction for gender-diverse individuals, suggesting that their bodies may often be a primary source of overall distress. ED behaviors are reinforced when these individuals feel decreased gender dysphoria and body dissatisfaction as their bodies change. For example, the 2021 literature review showed that gay and bisexual boys have higher chances of fasting, skipping meals, or using diet pills than straight boys.[3] Several studies on gay and bisexual men have found that men in sexual minorities report a greater prevalence of eating disorder behaviors related to weight loss or muscle-building, and one study found that gay and bisexual adolescents have greater rates of purging and restricting behavior. Similarly, lesbian and bisexual girls and women also have higher rates of disordered eating behavior, such as fasting, skipping meals, or using diet pills.

Desire for Passing:

Passing is when someone is perceived as their affirmed gender identity and not suspected as transgender. The desire to pass has a strong association with disordered eating behaviors, which can be used to change body shape to accentuate and suppress gender-related traits. This desire is strong for many people because those who pass are at a lower risk of discrimination, harassment, and violence.[14] Since gender nonconformity is not desired in a primarily cisnormative society, many individuals turn to ED behaviors in an attempt to gain social validation. A paper published in the Columbia Social Work Review showed that transgender individuals have the pressure to "pass" as cisgender to avoid being visibly identifiable as transgender, which would put them at a greater risk of transphobic discrimination, including harassment, social othering, microaggressions, and violence.[4] Therefore, transgender individuals are often hyper-aware of their appearance as a survival instinct in society, further increasing body dysmorphia and the possibility of engaging in ED behaviors.

However, passing is difficult because beauty standards for masculinity and femininity are amplified for transgender individuals, as shown in the Columbia Social Work Review.[4] The paper documented a study showing that participants with higher transgender congruence (more congruence between gender and appearance, i.e., a greater chance to pass) have lower rates of reporting ED symptoms. Gender nonbinary individuals report lower gender congruence than binary transgender individuals, which suggests heightened difficulties in aligning their identified gender with physical appearance, potentially leading to body dissatisfaction or decreased self-confidence, both of which are factors of ED behaviors. Moreover, when gender minority individuals fail to pass despite taking actions (including extreme measures such as disordered eating), their mental health might be further harmed, and in turn, they might resort to ED as a coping mechanism. In a book published in 2018, it's shown that the resulting sense of all control being lost can lead to intensified ED symptoms to reclaim a sense of control amid overwhelming distress due to the inability to resolve the conflict between sex and gender.[15]

Barriers to Affirming and Inclusive Care:

The ability to receive affirming and inclusive care is crucial for the well-being and health of SGM individuals. However, current treatment models present these communities with several obstacles that prevent them from getting the support they need. Firstly, few ED care providers are knowledgeable about the unique experiences and challenges faced by SGM individuals and thus aren't able to provide affirming and inclusive care.[16] The 2021 literature review found that a significant clinical challenge transgender youth face is the standard use of growth curves based on sex, preventing providers from establishing appropriate goal weights.[3] This can cause distress when these curves don't reflect the individual's identified gender, further exacerbating body dissatisfaction. Furthermore, the 2022 study showed that 70% of gender-diverse participants report treatment experiences impacted by gender dysphoria.[13] For them, ED is more about gender issues and trauma, which doesn't fit the conventional treatment model. For example, one participant said, "There is one thing they had me do, which was to stare into a mirror for half an hour… It seems okay if you're just dealing with body dysmorphia… but with gender dysphoria as well, that's also very triggering." Therefore, transgender youth might also be discouraged from seeking medical care in the future, resulting in a cycle of untreated ED.

Secondly, many of these individuals face discrimination in general healthcare settings, which can include misgendering, misnaming, or a direct refusal to provide healthcare services.[17] For example, the Columbia Social Work Review paper showed that transgender individuals often face discrimination, such as misgendering, misnaming, or direct refusal of service when seeking mental and medical healthcare.[4] These negative experiences discourage SGM individuals from seeking medical support in the future, contributing to the persistent cycle of ED behaviors. Thirdly, many of these individuals face geographic or financial barriers. For example, the Columbia Social Review Paper found that these individuals have a high possibility of living in a region where this specific type of healthcare is not provided or might not be able to afford such care.[4] The paper also showed that these individuals might also face social barriers such as a lack of support from family members, further increasing the difficulty in accessing affirming care. When the needs of these individuals are unmet, they might turn to disordered eating again in an attempt to gain control over their bodies since they can't exert control through acquiring treatment.

Conclusion

This review has cited several studies that support the disproportionately high prevalence of ED in SGM individuals compared to CH individuals. This prevalence can be explained by unique factors they face, including minority stress, gender or sexual dysphoria, body dissatisfaction, desire for passing, and barriers to affirming and inclusive care.

The current ED care system should be improved to provide more effective support to these individuals. Current treatment models are mostly tailored for CH individuals, especially white women, failing to consider the unique stressors faced by the

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SGM community. Healthcare providers should be trained to be aware of these factors and ensure that treatment environments are inclusive, such as adjusting sex-based growth charts for transgender patients. To take it one step further, there could be interventions targeted to address SGM patients, such as using patient-centered language and assessment methods to avoid assumptions based on gender or sexual orientation. From an institutional level, policy adjustments can assist in implementing inclusive practices across healthcare systems. For example, policies regarding growth charts can expand to include sex-based growth charts to suit more kinds of patients. It's important to note that diversity exists within the SGM community, so these interventions should be designed to meet the needs of different SGM groups.

Further research is also crucial for advancing our knowledge of the intersection between ED and SGM. First, future studies should expand the scope of SGM groups. As seen in this review, a lot of current research is focused on transgender individuals, while other SGM individuals are underrepresented. This means that important factors, like barriers to affirming care faced by sexual minorities, may not be well-documented due to insufficient research in this area. Second, future studies can explore the intersectionality of SGM, race, ethnicity, and socioeconomic status, and how the interrelation may heighten one's chance of developing ED. Addressing these knowledge gaps helps raise awareness of the challenges these individuals face and enhances healthcare systems, helping them become more inclusive, affirming, and effective.

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RESEARCH ARTICLE

Application of Pulse Width Modulation to Boron-Doped Diamond Wastewater and Water Treatment

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ABSTRACT: Wastewater and water treatment using boron-doped diamond (BDD) electrodes has garnered significant attention due to their distinguishing properties. In this study, we describe the application of a pulse width module (PWM) technique to a wastewater and water treatment system utilizing BDD electrodes prepared via hot-filament chemical vapor deposition (HFCVD). To investigate the efficiency of PWM, PWM square waves with a 50% duty cycle and a frequency of between 1 Hz and 500 Hz were applied to the BDD electrodes in 600 ml of tap water, and the resulting ozone concentrations were measured. Specifically, at frequencies above 10 Hz, the PWM technique generated higher ozone concentrations than the continuous DC voltage method while using only 50% of the electrical energy. The resulting treatment efficiency was 14.3 ppm/mW in DC mode and 34.9 ppm/mW in PWM mode, highlighting that PWM application significantly enhances ozone generation efficiency. These results demonstrate that the PWM technique not only reduces energy consumption but also enhances the treatment efficiency of BDD-based wastewater and water treatment systems.

KEYWORDS: Chemistry, Environmental Chemistry, Wastewater Treatment Using Boron-doped Diamond, Pulse width Module.

Introduction

Wastewater and water treatment using boron-doped diamond (BDD) electrodes, grown using the hot-filament chemical vapor deposition (HFCVD) method, is well-known for its efficiency and has been extensively studied.[1,2] BDD electrodes possess exceptional properties, including excellent corrosion resistance, chemical stability, high wear resistance, and outstanding electrical conductivity. Additionally, they exhibit unique electrochemical characteristics, such as low surface reactivity toward generated reactive chemical species.[1]

Numerous studies have focused on enhancing the efficiency of the treatment using BDD electrodes. Key factors influencing this efficiency include the thickness of the BDD thin films, the electrical conductivity of the electrodes, the properties of the electrolyte solution, and its temperature. However, there are still limitations to further improving efficiency, showing the need for new technological breakthroughs.

In this study, the application of the pulse width modulation (PWM) technique to a BDD wastewater and water treatment system is investigated. This is a new approach to enhancing the energy efficiency and performance of BDD treatment systems. PWM is a switching technique that controls analog devices with digital circuits.[3] A PWM switching controller was implemented in the BDD treatment system to evaluate the approach. Its effectiveness was evaluated by measuring the dissolved ozone concentrations in the treated water. Experimental results demonstrate that PWM can reduce energy consumption and enhance the treatment performance of the BDD treatment system.

Methods

BDD electrodes were prepared by depositing BDD films onto the surface of a silicon (Si) electrode body using an HFCVD at Pusan National University. HFCVD is widely known as an effective technique for depositing diamond films on substrate surfaces. Figure 1 presents a schematic illustration of the HFCVD system used in this study. A gas mixture of CH_4/H_2 was used for diamond film deposition on the Si substrate. B_2H_6 was introduced into the vacuum chamber during the deposition process, resulting in the doping of boron into the diamond films.[4] Hot filaments were heated to 2000 °C using an electrical power supply, and the temperature of the Si electrode body was raised to 750 °C by the heated filaments. The chamber pressure was maintained at 5.3 kPa (refer to Ref. 5 for more detailed conditions). The deposition time for the BDD films was 15 hours.

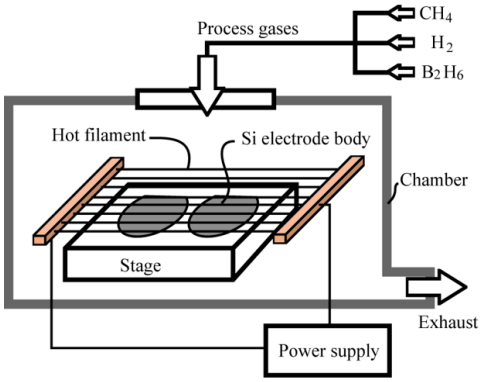

Figure 1: A schematic illustration of a hot-filament chemical vapor deposition (HFCVD) system used for the preparation of boron-doped diamond (BDD) electrodes. A gas mixture of CH_4 and H_2 was introduced for diamond film growth on a Si electrode body. B_2H_6 was added to achieve boron doping into the growing diamond film. The hot filaments were electrically heated to 2000 °C, raising the substrate temperature to 750 °C. The growth of diamond films was carried out at a chamber pressure of 5.3 kPa.

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Figure 2 (a) and (b) show a picture and a schematic illustration of the water treatment system with a PMW switching controller, respectively. The water treatment system has a 1000 ml glass beaker as the reactor container, a set of BDD electrodes consisting of cathode and anode electrodes, an ion exchange membrane (Nafion™ #117 membrane film) placed between them, a DC power supply, and components for PWM switching. The distance between BDD electrodes is fixed at 3 mm. The PWM switching controller is composed of two devices: a PWM signal generator and a MOSFET (Metal oxide semiconductor field effect transistor) module. The PWM signal generator provides a square wave pulse of 50% duty cycle with a frequency range between 1 Hz and 20 kHz, with a DC of less than 30 mA. The generated square wave pulse is input to the MOSFET module. The MOSFET module (Operating voltage: DC 5 V-36 V, Max current: 15 A) acts as a switch by continuously turning on and off according to the input square wave pulses. The DC voltages supplied by the DC power supply to the MOSFET module are converted into DC square waves based on the input PWM signal and are then supplied to the BDD electrodes. 600 ml of tap water in the glass beaker was used for experiments without any added electrolyte, except when treating water contaminated with water-based ink. BDD electrodes have been reported to facilitate ozone generation without the use of any electrolytes.[6] After the treatment, the ozone concentration in the treated water was measured using an ozone colorimeter (EUTECH instruments, C105) within 1 minute. To estimate the efficiency of applying the PWM technique to the BDD water treatment system, tap water in the glass beaker was treated with a DC voltage of 20 V and DC square waves with a 50% duty cycle and a frequency range of 1 to 500 Hz.

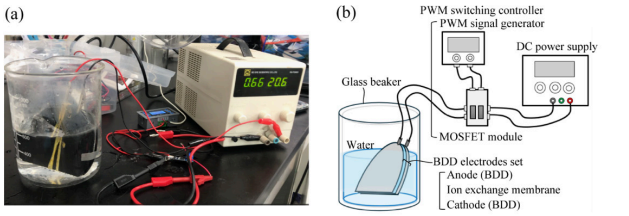

Figure 2: (a) Photograph and (b) schematic illustration of boron-doped diamond (BDD) water treatment system equipped with a pulse width modulation (PWM) switching controller. The system includes a gas beaker filled with tap water, BDD electrodes set (anode and cathode) with an ion exchange membrane in between, a DC power supply, and a PWM controller consisting of a signal generator and a MOSFET module. The PWM signal generator outputs a square wave signal, which is used to control the MOSFET module, converting the supplied DC voltage into a square wave. This modulated voltage is applied to the BDD electrode.

Results and Discussion

Figure 3 (a), (b), and (c) show a picture of a BDD electrode prepared by an HFCVD, a scanning electron microscope (SEM) image of a BDD surface, and a cross-sectional SEM image of BDD, respectively. The SEM images show that polycrystalline diamond films with a grain size of around 7 μm and a thickness of 14 μm were deposited on the Si electrode body. It is well known that CVD-grown diamonds without any doping have a high electrical resistance. Boron doping in diamond enhances the electrical conductivity by making it a conductor.[7] The sheet resistance of the prepared BDD electrodes in this work, measured using a 4-point probe method, is around 4.3 Ω/sq.[5]

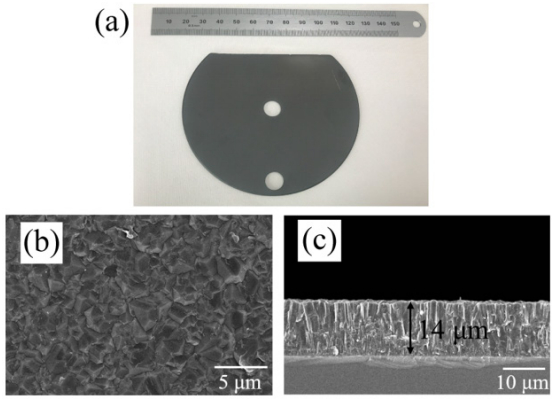

Figure 3: Characterization of a boron-doped diamond (BDD) electrode prepared by hot-filament chemical vapor deposition (HFCVD). (a) Photograph of the BDD electrode. (b) SEM image of a BDD surface, displaying polycrystalline diamond films. (c) Cross-sectional SEM image of a BDD electrode. The BDD electrode exhibits enhanced conductivity due to boron doping.

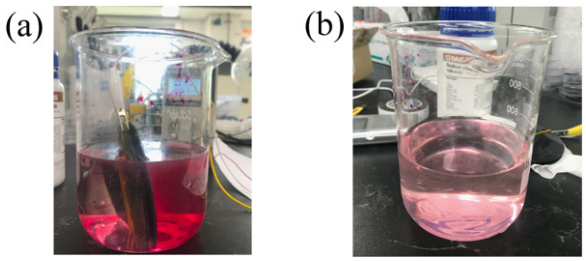

Figure 4: Photographs of ink wastewater (a) before and (b) 5 minutes after the electrochemical treatment using boron-doped diamond (BDD) under DC voltage. The discoloration results from the oxidative degradation via direct anodic oxidation and indirect oxidation by reactive oxygen species such as OH and O_3. Gas bubble formation on the electrode surface indicates oxygen and hydrogen evolution.

The BDD treatment system prepared in this work has been successfully operated, as confirmed by the treatment of water-based ink wastewater. When DC voltage was applied to BDD electrodes, bubbles formed on the surfaces of the electrodes, and the wastewater became gradually colorless and transparent, as shown in Figure 4. Figure 4 (a) and (b) show pictures of ink wastewater before treatment and 5 minutes after treatment began, respectively. Electrochemical wastewater and water treatment using BDD electrodes is governed by a combination of direct and indirect oxidation mechanisms.[8] Direct anodic oxidation involves an outer-sphere electron transfer at the surface of the electrode, does not involve adsorbed intermediates, and typically proceeds in a quasi-reversible manner.[8,9] Hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), and ozone (O_3) can also be formed at the BDD anode surface.[9] The primary mechanism for organic degradation shifts from a direct to an indirect oxidation under appropriate electrochemical conditions.[8] The colored components in ink wastewater are broken down by hydroxyl radicals and ozone through oxidative reactions. During the treatment, bubbles are observed on the

electrode surfaces, most likely due to the evolution of oxygen at the anode and hydrogen at the cathode.[8]

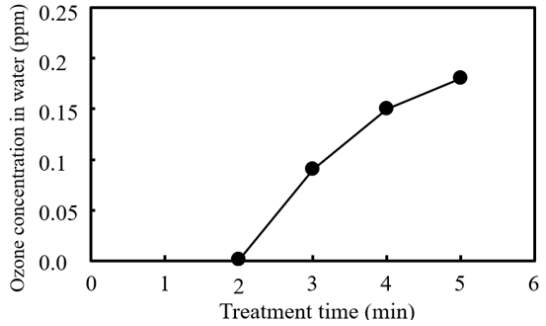

Figure 5: Ozone concentration in water as a function of treatment time using boron-doped diamond (BDD) electrodes under a constant 20 V DC. The ozone level increased steadily with treatment time, reaching 0.18 ppm after 5 minutes in 600 mL of water.

Figure 5 shows the ozone concentrations in water treated using the BDD treatment system, where a DC power supply was directly connected to the BDD electrodes. A 20-V DC voltage was applied between the electrodes. Ozone concentrations in 600 ml of water treated for 2 to 5 minutes were measured. As shown in Figure 5, the ozone concentration in water treated for 2 minutes was 0.001 ppm. The ozone concentration was increased with the treatment time. It was 0.18 ppm in water treated for 5 minutes.

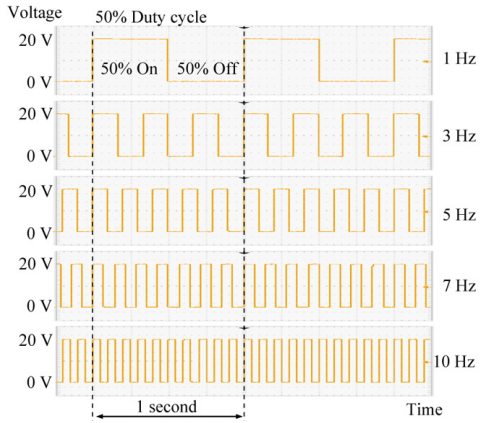

Figure 6: Oscilloscope measurements of pulse width modulation (PWM) with square wave voltages generated at 1, 3, 5, 7, and 10 Hz with 50% duty cycle. Each signal alternates between 0 V (off) and 20 V (on) for equal durations. As frequency increases, the number of on-off cycles per second increases. With a 50% duty cycle, the average voltage is 10 V, reducing the average power input to the electrodes by half compared to continuous DC voltage.

Next, the voltage shapes generated by the PWM switching controller shown in Figure 2 were measured using an oscilloscope (Tektronix, TBS1064). Figure 6 shows voltage shapes of PWM square waves with a 50% duty cycle with a frequency of 1 Hz, 3 Hz, 5 Hz, 7 Hz, and 10 Hz. As shown in Figure 6, a PWM square wave is on for half of the time and off for the other half. The number of times per second of a PWM signal is increased as the frequency of the PWM signal increases, where the frequency of a PWM signal is the number of times per second that the on-and-off cycle is repeated. When the 20 V DC voltage is supplied on the BDD electrodes with a duty cycle of 50%, then the average voltage over time would be 10 V, resulting in the reduction of average power wasted in a resistive load by half.

Finally, experiments were carried out to investigate the efficiency of the application of a PWM system to a BDD water treatment. A 600 ml of water in a glass beaker was treated by applying the PWM signals, shown in Figure 6, to the BDD electrodes for 5 minutes, and the ozone concentrations in the treated water were measured. The dependence of ozone concentration on the frequency of PWM signals is shown in Figure 7 (a). Ozone concentration was 0.05 ppm at 1 Hz. Ozone concentration gradually increased with the increase in the frequency of the PWM signal. It was 0.08 ppm, 0.09 ppm, and 0.11 ppm at 3 Hz, 5 Hz, and 7 Hz, respectively. As the frequency increased further to 10 Hz, ozone concentration was doubled, 0.22 ppm, which is higher than the value in water treated with a continuous DC voltage shown in Figure 5. Next, the change of ozone concentration in treated water was investigated when the frequency was further increased from 10 Hz to 500 Hz as shown in Figure 7 (b). As the frequency increases further than 10 Hz, ozone concentration tends to saturate at around 0.22 ppm.

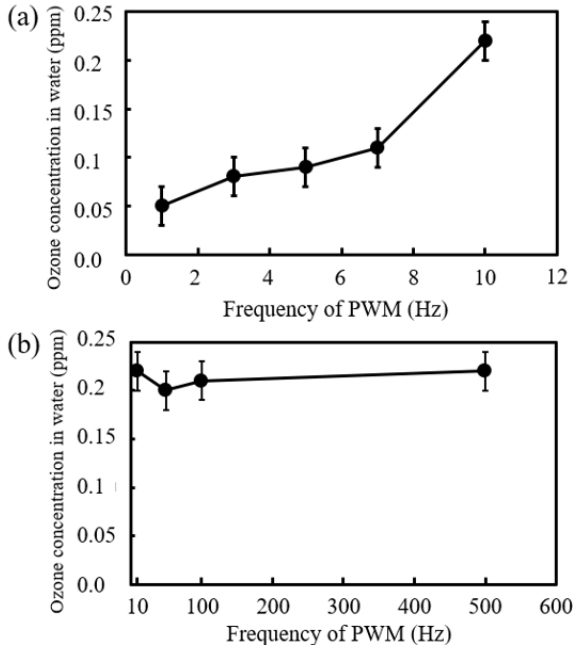

Figure 7: Ozone concentration in water as a function of pulse width modulation (PWM) signal frequency applied to boron-doped diamond (BDD) electrodes during 5-minute treatments. (a) At low frequencies (1-10 Hz), ozone concentration increases with frequency, reaching 0.22 ppm at 10 Hz. (b) At higher frequencies (100-500 Hz), ozone concentration plateaus near 0.22 ppm. In both DC and PWM modes, the current was approximately 0.63 A at a voltage of 20 V; however, due to the 50% duty cycle in PWM mode, the power consumption was halved compared to DC mode. The resulting treatment efficiency was 14.3 ppm/mW in DC mode and 34.9 ppm/mW in PWM mode, highlighting that PWM application significantly enhances ozone generation efficiency while reducing energy consumption.

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In the experiments, the current flowing through both electrodes was approximately 0.63 A in both DC mode and PWM mode. The electrical power, calculated using the formula P=V×I, is 12.6 W in DC mode. On the other hand, when applying PWM with a 50% duty cycle, the power is 6.3 W, as calculated using the formula P= $V \times I_{peak} \times$Duty cycle. Treatment efficiency is 14.3 ppm/mW in DC mode and 34.9 ppm/mW in PWM mode at 50% duty cycle and 10 Hz, respectively. This indicates that the use of PWM can reduce energy consumption and significantly improve treatment efficiency in electrochemical water treatment technology.

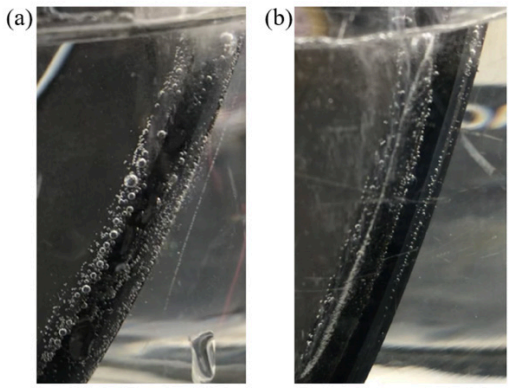

Figure 8: Pictures of BDD electrode surfaces during water treatment: (a) under conventional DC mode, and (b) under PWM mode. Compared to DC mode, PWM results in the formation of smaller bubbles that detach more rapidly from the electrode surface, which can contribute to reduced activation, ohmic, and concentration overpotentials, thereby enhancing overall energy efficiency.

In addition, in the experiments, it is observed with the naked eye that bubbles produced on the surface of BDD electrodes are smaller and quickly fall away from the electrode surfaces when a PWM switching controller is used (Figure 8). Bubbles can affect the energy efficiency of electrode processes, detailing the bubble's impact on activation, ohmic, and concentration overpotentials.[2] Persistent bubbles on the electrode surface can hinder electrochemical reactions, disrupt ionic conduction paths in the electrolyte, and obscure mass transport phenomena. This shows that the application of the PWM technique can influence the formation of bubbles and allow the reaction efficiency on the electrode surfaces. Further research into that is needed to better understand its mechanism as future work. Additionally, investigating a wider range of duty cycles could offer deeper insights into PWM behavior. Unfortunately, further experiments were not feasible due to limited access to the laboratory at the time. Future work may involve exploring additional duty cycles to provide a more comprehensive understanding of how PWM parameters influence performance.

Conclusions

This study investigated the application of the PWM technique in a BDD water treatment system. PWM square waves with a 50% duty cycle, generated by a PWM switching controller, were applied to the BDD electrodes, achieving ozone concentrations higher than those produced with continuous DC voltage. The results demonstrate that this new approach is an effective technology for reducing energy consumption and improving treatment efficiency in BDD-based wastewater and water treatment systems.

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RESEARCH ARTICLE

ATHENA Science Enabled by Cryocooled Space Detectors

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ABSTRACT: This study examines how cryogenic systems power the ATHENA mission's X-ray observations. The X-ray Integral Field Unit (X-IFU) requires cooling to 50 mK for high-resolution spectroscopy. This paper reviews key cryocoolers—Stirling, Joule-Thomson, and Adiabatic Demagnetization Refrigerators—and their roles in space applications. A detailed review of the multi-stage cryochain explains how it achieves the ultra-low temperatures needed for X-IFU. Transition Edge Sensors (TES), vital for X-ray detection, operate at the core of this system. These technologies enable ATHENA to study black hole physics, cosmic feedback, and the growth of large-scale cosmic structures. By combining advanced cooling methods with precise instrumentation, this study shows how ATHENA will deepen our understanding of the hot and energetic universe.

KEYWORDS: Physics and Astronomy, Astronomy and Cosmology, ATHENA, X-ray Astronomy, X-IFU, Space Instrumentation.

Introduction

The universe is a complex and nebulous subject to explore, filled with mysterious things that challenge our understanding. To unravel these cosmic enigmas, the Advanced Telescope for High-ENergy Astrophysics (ATHENA) mission, selected by ESA in June 2014, will explore a plethora of astronomical subjects. This includes the examination of stellar formation and evolution, supernova explosions, and gamma-ray bursts.[1] Furthermore, it will study the growth of supermassive black holes and their influence on galaxy formation and evolution. It will also explore how the universe is taking shape through the study of how baryonic matter formed large-scale structures such as supermassive black holes.[2] These black holes largely influence their surroundings through a process known as Cosmic Feedback. Therefore, X-rays are crucial to observing this hot and energetic universe as they can penetrate through obscured environments, revealing various ionization states of abundant elements.[3]

To achieve its scientific objectives, ATHENA will carry the X-ray Integral Field Unit (X-IFU) instrument, which can provide spatially resolved high-resolution spectroscopy, granting the ability to observe bright X-ray sources. X-IFU aims to tackle key scientific concepts related to the Hot and Energetic Universe and enable critical observation of the universe's chemical evolution and the physical processes behind the growth of large-scale structures. Overall, X-IFU's high-resolution X-ray spectroscopy will provide insights across a wide range of astronomical objects, from planets and stars to supernovae, compact objects, and the interstellar medium.[4]

The X-IFU consists of a large array of X-ray absorbers on top of the Transition Edge Sensors (TES). Each X-ray photon creates a distinct current pulse, with its amplitude reflecting the photon's energy. These pulses are measured by the TES microcalorimeter to sense the pulses generated by X-ray photons and determine the measurement of photon energy and arrival time. The heat capacity of the TES, the thermal conductance to the cold bath, and the electrical properties of the TES circuit all impact the shape and timing of these pulses.[3] Therefore, to accurately measure this change in electrical resistance, TES needs to be cooled to below 100 mK, with the thermal bath at 50 mK.[4] This cooling is achieved through a multi-stage cryogenic chain consisting of five 15 K pulse tube coolers, two 4 K Joule-Thomson coolers, two 2 K Joule-Thomson coolers, and a final stage Adiabatic Demagnetization Refrigeration (ADR) 100 mK, with the thermal bath at 50 mK.[5]

Many criteria must be met before cryocoolers can be approved for use in space, such as cooling power at the required cold end temperature with a reduced input power, reliable and maintenance-free operation, compactness, minimum vibration and noise, and long shelf life.[6] In addition, cryocoolers for space applications are generally qualified for a ten-year lifetime.[7] Lastly, the cost is undoubtedly an important factor, especially when maintenance opportunities are difficult, therefore, it is often justified to invest more in the initial capital cost to reduce the risk of failure.[8] Understanding the advantages and limitations of each type of cryocooler is essential for selecting the most appropriate technology for space applications, which ensures optimal performance and reliability. Therefore, this paper will focus on the various types of cryocoolers and their role in the ATHENA mission.

Background and Theory

Stirling Cryocoolers:

There are many ways to classify cryocoolers, and the primary distinguishing features are based on the types of thermodynamic cycles that are being run to achieve refrigeration: recuperative or regenerative.[9] In the recuperative cycles, the working fluid moves around a loop in one direction at fixed high and low pressures, with JT (Joule-Thomson) and Brayton cycles as prime examples of these types.[10] Due to the steady flow and pressure, temperature oscillations and vibrations

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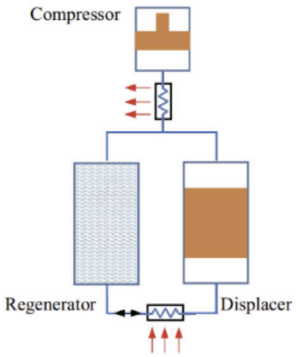
are low. In contrast, the regenerative cycle uses an oscillating flow of gas, and its pressure cools the cold end of the system; Stirling, Gifford-McMahon, and pulse tube cycles are prime examples of such types.[11] Temperatures of 3 K up to 300 K can be achieved with regenerative cryocoolers, although temperatures below 150 K are the most common.[8]

Each cryocooler has its distinct advantages and disadvantages, and these are considered for each space mission. One of the most unique properties of pulse tube cryocoolers is the lack of a moving part; they utilize Stirling or GM-type compressors, which makes them more reliable and minimizes vibration, at the cost of reducing efficiency.[12] Therefore, pulse tube cryocoolers for space applications typically operate at high frequencies for efficiency, which makes it challenging to achieve very low temperatures due to regenerator performance degradation.[13] For single-stage coolers, temperatures have been known to reach 40-70 K,[9] but multi-stage coolers can cool to much lower temperatures.[14]

Stirling coolers were the first coolers to be successfully utilized in space and proven to be reliable and highly efficient. Recent developments of two-stage devices allow lower temperature ranges from 60-80 K down to as low as 15-30 K.[11,15] However, to reach 4 K temperatures, it is more common for Stirling cycles to be integrated with other coolers. Some of the most common pairs are Stirling and JT coolers, previously mentioned, and hybrid Stirling-type pulse tube coolers.



(a) Stirling/Pulse Tube

Figure 1: A schematic showing the Stirling cycle operation for a Stirling cryocooler. (Reference numbers have been modified from the original source.)[16] Figure 1 shows the four thermodynamic processes of the Stirling cycle: isothermal compression, isochoric cooling, isothermal expansion, and isochoric heating. It emphasizes how these steps enable efficient closed-cycle cryogenic cooling.

In the Stirling cycle, the flow of the working fluid, commonly helium, is controlled with the use of pistons and displacers.[16] The cycle consists of four heat transfer procedures: two isothermal and two isochoric processes. First, during isothermal compression, the fluid is compressed and the heat is transferred from the fluid to a warm heat exchanger. Next, in the constant-volume regenerative cooling phase, the heat is transferred from the fluid into the regenerative matrix through the motion of displacers, lowering the temperature. Then, during the isothermal expansion, the compressor piston and displacer move backward, expanding the gas and lowering the pressure.[17] The working fluid also absorbs heat from the required external source. Finally, in the constant volume regenerative heating phase, the gas is moved to the warm end of the cooler, and the fluid absorbs heat from the regenerative matrix, completing the cycle.[18] Figure 1 presents the schematic for the Stirling cycle as run in a Stirling cryocooler.[16]

Net cooling power is the amount of cooling power that is available for cooling the item that is attached to the cold tip.[17] This can be derived as:[19]

$$\dot{Q} = \dot{W} + \dot{H}_e - \dot{H}_i + \frac{d < U >}{dt} \quad (1)$$

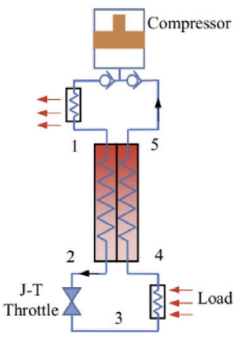
Carnot efficiency is used as a standard for comparing heat engine cycles, as it represents the highest efficiency in a given temperature range.[9] For the cryocooler that operates the Stirling thermodynamic cycle, the equations can be expressed as this:[19]

$$\eta_r = (\frac{Q_c}{\dot{W}_{in}})(\frac{T_h}{T_c} - 1) \quad (2)$$

Qc represents cooling power, and the term W signifies displacer input power; the ratio is also known as the coefficient of performance, which measures how much cooling can be achieved per unit of work input. The latter part of the equation represents the temperature ratio between the hot and cold sides, as Tc is the cold end temperature while Th is the heat rejection temperature.[20]

The JT cycle:

JT cryocoolers are valued for their stability, compact structure, simplicity, reliability, and low electrical and mechanical noise levels.[15,21] Furthermore, the JT cycle, operating with no moving parts at the cold end, can easily be made compact. However, similar to the pulse tube coolers, high-efficiency heat exchangers are essential to reach cryogenic temperatures due to the temperature changes in the cycle being relatively small compared to the temperature difference between ambient and cryogenic temperatures.[13] Therefore, hybrid JT cryocoolers working at liquid helium temperature are widely used in space detectors working at 4 K due to their flexibility and relatively high efficiency.[21] Therefore, the ATHENA project is currently developing a two-stage Stirling refrigerator, which provides 4.5 K temperatures. This 20 K two-stage Stirling cryocooler, consisting of a cold head with two cold stages, is first employed as a pre-cooler, which is then connected to the JT heat exchangers so that the helium gas can be cooled below 20 K.[21]



(c) Joule-Thomason

Figure 2: A schematic showing the JT cycle operation for a JT cryocooler. (Reference numbers have been modified from the original source.)[16] Figure 2 illustrates the Joule-Thomson cycle, highlighting isenthalpic expansion through a throttling valve as the core cooling mechanism. It demonstrates how high-pressure gas undergoes a temperature drop as it expands without external work.

The JT cycle achieves cooling without external heat input or work production, and therefore, expansion happens at constant enthalpy.[22] First, the high-pressure gas first passes through a primary heat exchanger, where it is precooled by transferring heat to an external cooling system.[23] The cooled, high-pressure gas undergoes isenthalpic expansion upon entering the JT orifice or a porous plug.[17] This expansion causes a pressure drop, and the temperature decreases when the gas is in the cooling region. Then, this cooled, low-pressure gas passes through a secondary heat exchanger, where it is further cooled to cryogenic temperatures.[24] Figure 2 presents the schematic for the JT cycle as run in a JT cryocooler.[16]

For recuperative cryocoolers, such as JT coolers, net cooling power is the difference between the gross cooling power produced by the expander and the losses from the heat exchanger. This is expressed as:

$$\dot{Q}_L\max = \dot{m}\left(h(T_a, p_1) - h(T_a, p_h)\right) \quad (3)$$

This equation calculates the maximum cooling power expressed through the mass flow rate (m) of the working fluid and on the enthalpy differences of the high and low-pressure fluids at constant temperature (Ta).[25]

Based on the Colburn-Chilton analogy, an analytical formula can be derived for Carnot efficiency upon considering the loss during the compression process:

$$\eta_{\text{comp,isoth}} = \frac{(T_{\text{H}} - T_{\text{L}})}{T_{\text{H}}} \quad (4)$$

The formula shows that the efficiency of Carnot depends on the temperature difference between the hot and cold reservoirs, relative to the temperature of the hot reservoir.[23]

Note: *While sorption coolers have been used in previous space missions as passive or pre-cooling stages, ATHENA does not currently incorporate a sorption cooler in its baseline cryochain. Instead, it relies on a two-stage Stirling system for pre-cooling, followed by a Joule-Thomson cooler and multi-stage ADR.*

The two-stage Stirling cooler pre-cools helium gas to ~20 K, which is then transferred via heat exchangers to the JT cooler. The JT system further cools the gas to ~4 K before it enters the ADR stage. The ADR is thermally connected to the JT cold tip and activated through magnetic cycling. Thermal switches are used to isolate and engage stages during the cooldown and recycling processes.

Adiabatic Demagnetization Refrigerators (ADR):

ADRs are a type of magnetic cooler that is capable of providing milli-Kelvin cooling for space applications. To achieve cooling, the magnetization process is carried out isothermally. The heat of the magnetization is promptly removed to maintain the substance at a constant temperature. When the system is in an ordered state, it is thermally isolated, which allows an isentropic demagnetization process, where the magnetic field is gradually reduced without heat exchange, further cooling the substance. One of its unique properties is the lack of moving parts with no vibration or motion forces, making it highly reliable. There are various ADR configurations, such as cycle, two-stage, double, and continuous ADR, each suited for specific purposes.[26] For the ATHENA mission, a five-stage ADR is used, replacing earlier two-stage concepts, to provide increased cooling capacity and thermal stability.[27]

Table 1: Comparison of Cryocooler Technologies Used in ATHENA.[16,26,28,29]

Cryocooler Type	Cooling Principle	Typical Temperature Range	Moving Parts at Cold End	Vibration Level	Key Advantages	Common Use Case in ATHENA
Stirling Cooler	Regenerative cycle with pistons	~60–15 K	Yes	Moderate	High efficiency; compact	Pre-cooling for JT stage
JT Cryocooler	Isenthalpic expansion via throttling	~4 K	No	Low	No moving parts; compact; stable	Intermediate cooling before ADR
ADR	Magnetic entropy reduction via demagnetization	~50 mK	No	None	Reaches mK range; no vibrations	Final cooling for TES detectors

ATHENA Instrumentation

Cryochain at 4 K and 50 mK:

The Focal Plane Assembly (FPA) includes all the cryogenic components of the XIFU detector, positioned on the coldest part of the instrument to capture incident X-rays. These X-rays are absorbed by the detector's materials, in which their energy is measured by the Transition Edge Sensors (TES). Therefore, to optimize performance and maintain the superconducting state of the TESs, the FPA is kept at a temperature of 50 mK.[30]

To reach the desired temperature, X-IFU's cooling system relies on a multi-stage cryochain consisting of several mechanical cryocoolers, divided into upper and last-stage coolers.[31] The upper cooling chain provides cooling power at 2 K, consisting of a 15 K pulse tube, 4 K-JT, and the 2 K-JT coolers. The last stage cooler cycles at regular intervals and it cools to 50 mK, through the usage of the 300 mK sorption cooler and the ADR.[32,33]

In the cryochain, the 2 K-JT and 4 K-JT coolers are connected to a hybrid cooler using thermal straps to function as pre-coolers. However, integrating continuous cooling JT coolers into a hybrid cooler that operates in alternating phases can lead to parasitic heat flow. This unwanted heat transfer, especially during the recycling phase, can increase the thermal load on the 2 K cooler, potentially affecting its performance. Therefore, controlling parasitic heat flow can help manage the amount of heat in the JT cooler and avoid overloading. This cool-down process involves the gradual activation of the pulse tube and JT pre-coolers. As the temperature drops, the Stirling cooler provides more cooling power, ensuring a controlled and efficient method to reach its intended operating conditions.[28] The 2 K JT cooler is pre-cooled by the 15 K pulse tube coolers, while the 4 K JT is pre-cooled by the two-stage Stirling coolers. In addition, a commercial 4 K pulse tube cooler is responsible for cooling the radiative shields and the 4 K JT interface through the usage of a gas gap heat switch to control heat transfer.[29] Finally, the desired temperature of 50 mK is

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achieved through the ADR, which is connected to the 2 K stage via a heat switch. When the ADR cools to 2 K, a magnetic field is applied, which aligns the ion spins in the salt pill. This, in turn, produces heat, which is released through the heat switch until the system returns to 2 K. Once the ADR stage is insulated by opening the heat switch, the magnetic field is reduced, randomizing the paramagnetic ions once again, further lowering its temperature. The ADR's temperature is regulated by adjusting the magnetic field, which must be decreased over time to compensate for the heat loss of the system.[34]

TES Detectors:

The X-IFU system is based on an array of X-ray absorbers linked to Transition Edge Sensors (TES), which function as micro-calorimeters. The TES detects the heat pulses generated by absorbed X-ray photons, measuring the temperature variation (a 300-400 microsecond pulse) through changes in its electrical resistance. Therefore, TES needs to be cooled to temperatures below 100 mK, with the system's thermal bath set at 50 mK, maintained by an ADR and a pulse tube cryocooler. It is also adjusted to transition between superconducting and normal states for optimal sensitivity.[35] Furthermore, the main TES array is operated using frequency domain multiplexed (FDM) SQUID readout electronics, which are used to achieve low-noise, low-dissipation readout of the low-impedance TES pixels.[33]

As TES detectors are highly sensitive to mechanical vibrations, a "50 mK snout" is suspended from the refrigerators' mixing chamber using a two-stage Kevlar vibration isolation system. This minimizes lower-frequency noise that could cause power dissipation from sources such as the pulse tube cryocooler.[36]

Furthermore, a small coil is placed over the TES chip to generate a small magnetic field when a current is passed through the wire. This helps reduce any residual external magnetic field. The field coil is mounted on a mechanical support that also serves as an X-ray collimator, which ensures that only the active pixels are exposed to X-rays, reducing interference between them. This improves the accuracy and sensitivity of TES and helps detect X-rays more effectively. The shape of the resistive TES transition depends on the current, temperature, and the magnetic field. TESs are highly sensitive to both static and dynamic magnetic fields, with greater sensitivity in the direction perpendicular to the TES.[37] Therefore, the FPA features magnetic and stray light shielding, electromagnetic compatibility (EMC) shielding, and filtering. This protects TES from environmental disturbances during ground tests and in-flight operations and reaches its required performance.[33]

Science Goals

Aims 1 (Accretion Physics around Compact Objects:

The ATHENA mission is structured around three main scientific missions. The first is the physics of accretion around black holes, neutron stars, and other compact objects. The second is cosmic feedback, the process by which accretion and star formation are interconnected to the formation and evolution of galaxies. Lastly, the third mission investigates the physical nature and evolution of the large-scale structure, particularly through the study of hot baryonic matter. Furthermore, ATHENA will also provide a major contribution to our understanding of the nature and influences of hot cosmic plasmas in all astrophysical objects, through spatially resolved high-resolution X-ray spectroscopy and sensitive wide-field X-ray imaging.[38]

Some of the most extreme physical conditions in the Universe are found in and around compact objects. For instance, black holes create the strongest gravitational fields, while neutron stars are the densest observable form of matter. Matter trapped in these extreme gravitational conditions is spun and heated, causing it to radiate X-rays. X-ray observations can measure the physical conditions in the innermost regions around black holes and neutron stars, revealing how the laws of general relativity apply under extreme circumstances and how matter behaves under such intense pressure and density. Furthermore, X-ray observations can also explore the process of accretion, where they will investigate how matter accretes into compact objects by mapping the innermost flows around black holes and measuring their spins.[38]

According to the "no hair" theorem, astrophysical black holes are characterized by two main parameters: mass and spin. However, its effects are only evident in the very innermost regions, specifically through X-ray emissions. Black hole spins are an important energy source in astrophysics as they could serve as an energy source, affecting various phenomena such as the behavior of Active Galactic Nuclei (AGNs), the powering of jets, and the production of gravitational waves when black holes are being merged. Unlike measurements of black hole mass, spin measurements can also reveal information on stellar evolution and the histories of galaxy mergers. Therefore, ATHENA will measure black hole spins of many bright Galactic Black Holes (GBHs) and AGNs, allowing the studies of fainter AGNs, which are linked to the evolution of supermassive black holes (SMBHs) and their host galaxies.[38]

Aims 2 (AGN Feedback and Galaxy Evolution):

Energy injections from growing SMBHs, known as AGN feedback, are key to understanding the formation and evolution of galaxies. This feedback can heat or expel cold gas from galaxies, driving a "downsizing" effect, in which massive galaxies form first, followed by smaller ones.[39] AGNs play a crucial role in the evolution of galaxies, where AGNs can contribute significantly due to their ability to penetrate denser gas with X-rays. Despite its significant impact on galaxy evolution models, the underlying physical processes behind its feedback are still unclear. Therefore, the ATHENA mission aims to tackle these gaps through high spectral and spatial resolution instruments to study AGN activity in galaxies and their relationship between key galaxy properties such as stellar mass, gas mass, and star-formation rates, revolutionizing our understanding of early SMBHs and their impact on galaxy evolution.[39]

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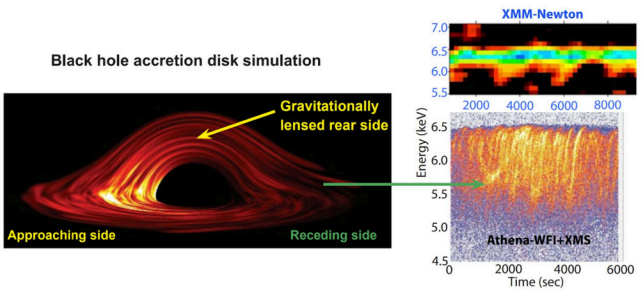

Figure 3: Left: Snapshot from a time-dependent Magneto-Hydrodynamic (MHD) simulation of an accretion disk around a BH (Armitage & Reynolds 2003). Rings and hotspots of emission are seen due to turbulence, the emission from which should be modulated on the orbital timescale. Right: Because the features are variable in both flux and energy, the disk can be mapped out in the time-energy plane. The first hints of this behavior have been seen in XMM-Newton data (Iwasawa *et al.* 2004; upper right), but the factor ~ 4 improvement in throughput and ~ 40 in energy resolution offered by Athena are needed to sample weaker and (possibly) narrower features on suborbital timescales, allowing us to map out the inner accretion flow. Only unusually strong and slow flares can be detected by XMM-Newton. (Reference numbers have been modified from the original source.)[38] Figure 3 presents a snapshot from a magneto-hydrodynamic (MHD) simulation, showing turbulent gas flows and variability in density and velocity. It reflects the dynamic environments Athena aims to observe with high time-energy resolution.

Reverberation mapping is a technique used to study the inner accretion flows by measuring the light travel time it takes for X-ray light to affect the emission lines produced in the accretion disk. The ATHENA will observe matter within a few gravitational radii of the event horizon of black holes. Under these extreme environments, large gravitational redshifts, extreme light bending, and time delay effects will cause spectral and timing signatures in X-ray emission, which can reveal the black hole's spin and the effects of gravity near the black hole.[38] X-rays provide a unique opportunity to study the innermost regions around SMBHs in AGNs. Therefore, the X-IFU of the ATHENA mission can provide precise measurements of black hole spins and properties of AGN outflows through its unprecedented sensitivity.[40] As illustrated in Figure 4, reverberation mapping provides insight into the structure near black holes by measuring time lags in X-ray emission.

Aims 3 (Large-Scale Structure and Baryonic Matter):

Large-scale structures are the largest arrangement of matter in the universe, which are shaped by gravity over billions of years. These structures grow over cosmic time through the accretion of gas from the intergalactic medium, which constitutes the majority of the baryonic matter in the local universe. Tracing the physical evolution of galaxy clusters and groups from their formation epoch to the present day can reveal insights into their formation and evolution.[41] However, the majority of the hot baryons are not in clusters, and their location and physical states are currently unknown. More than 30 percent of the baryons in today's universe are expected to reside in the Warm/Hot Intergalactic Medium (WHIM), a region in the universe filled with warm and hot gas. Baryons align with the filamentary structure of dark matter, thus, their location is influenced by the distribution of dark matter. Therefore, determining baryonic matter requires detecting resonance absorption lines, which occur when x-rays from a bright background source pass through hot diffuse plasma,[42] revealing its composition, properties, and origin.[41]

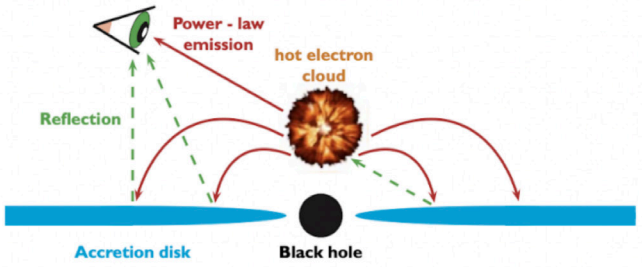

Figure 4: Cartoon of a black hole and accretion disk (blue) above which the power-law continuum is generated by Comptonisation of thermal disk photons in a hot electron cloud (orange). Power-law emission (red) irradiates the disk, producing the reflection spectrum (green). Intrinsic variations in the power-law source create delayed, blurred changes (reverberation) in reflection. Thanks to Athena, it will be possible to translate these signals into a physical picture of the inner region around the black hole. The reflection features and thermal disk emission both provide an independent estimate of the inner accretion disk radius via the maximum gravitational redshift of emission features and the disk temperature. (Reference numbers have been modified from the original source.)[38] Figure 4 visualizes how X-ray echoes from the accretion disk are used to map structures close to a black hole. The time lag between direct and reflected X-rays provides information about the disk's geometry and inner radius.

The universe contains large amounts of hot gas, which exists at extremely high temperatures. On the largest scales, clusters of galaxies form in regions where dark matter created the deepest potential wells, which are regions with strong gravitational pull. These wells trap the hot gas, which causes it to accumulate within the clusters, which are in a state of hydrostatic equilibrium. Hydrostatic equilibrium is when the pressure of the hot gas balances the gravitational pull of the cluster, preventing it from collapsing further. Therefore, how baryons are pulled into these clusters and how they evolve gives insight into the cosmological evolution of the universe.[42] X-ray observations can reveal information on the velocities, thermodynamics, and chemical composition of the gas, which can provide insight into how baryons evolve inside the gravitational potential wells of galaxy clusters.[43]

Supersonic events release huge amounts of energy and elements, impacting the interstellar medium through heating, turbulence, heavy element enrichment, particle acceleration, and magnetic field amplification. X-ray emissions from hot plasma offer key insights into its compositions and processes, such as its ionization states.[44] Heavy elements produced in stellar processes, such as oxygen, neon, magnesium, silicon, sulfur, argon, calcium, and iron, are crucial to understanding the chemical evolution of the universe. The X-IFU would be able to observe these elements and create maps of metal distribution in clusters. These maps will cover unprecedented distances, showing how metals are distributed in the entire cluster volume.[41]

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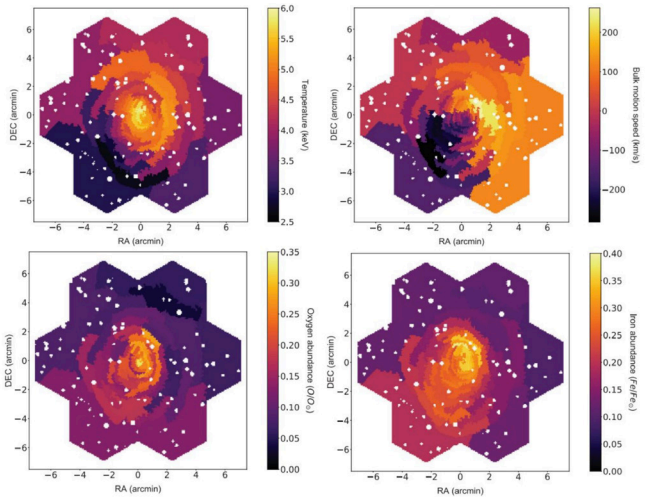

Figure 5: Physical parameter maps reconstructed from seven 100-ks X-IFU observations of a galaxy cluster over regions of S/N 300 (90,000 counts), simulated using the instrument end-to-end simulator SIXTE (Dauser *et* al. 2019). The cluster is located at z = 0.1, with R500 = 1.1 Mpc, and T500 = 4.2 keV. Input clusters are taken from large-scale hydrodynamic simulations performed using the GADGET-3 smoothed-particle hydrodynamics code· (From top left to bottom right) Spectral-like temperature (keV), bulk velocity deduced from line-shift with respect to the cluster's average redshift (km/s), and emission-measure-weighted oxygen and iron abundance (with respect to solar). The image is taken from (Cucchetti *et al.* 2018), and references therein. White holes in the maps are associated with subtracted point sources. (Reference numbers have been modified from the original source.)[44] Reconstructed parameter maps reveal temperature, metallicity, and velocity variations within a simulated galaxy cluster. Figure 5 demonstrates Athena's capability to spatially resolve complex intracluster medium dynamics.

The ATHENA mission would provide the necessary angular resolution, spectral resolution, throughput, detection sensitivity, and survey grasp to revolutionize our understanding of these issues. With X-IFU's spatially resolved high-resolution X-ray spectroscopy and the WIFI's wide field X-ray imaging, ATHENA would be able to map the outskirts of nearby galaxy clusters in detail. These observations will measure the emission, temperature, and metallicity of the ICM. This will reveal the physical state of the accreted material, leading to a comprehensive understanding of how galaxy structures assemble and grow over time.[41] Specifically, ATHENA will use the X-IFU to reveal the location, chemical composition, physical state, and dynamics of baryons in the local universe, focusing specifically on the Warm-Hot Intergalactic Medium (WHIM). This can be achieved through nominal resolution, where gamma-ray bursts afterglows are used as background sources shining through the cosmic web.[44] Thus, the ATHENA mission would be able to study how baryonic gas accretes and evolves in the dark matter potential wells by measuring motions and turbulence in the ICM.

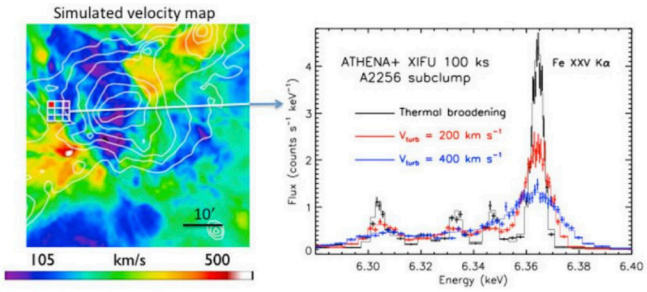

Figure 6: Athena+ X-IFU spectrum of a subclump in the galaxy cluster A2256, demonstrating the high precision measurements possible for the ICM velocity field. Left: Velocity map of a cosmological hydrodynamical simulation of a perturbed galaxy cluster of about M200 ~ 1015M⊙ with X-ray surface brightness contours overlaid. Right: Simulated spectrum for a 100 ks observation with Athena+ XIFU for a 1.5 arcmin region (one of the 9 small regions shown on the image), showing the turbulent broadening of the Fe XXV Kα line. Simulated data with vturb = 200 km s−1 are shown in red· Black and blue represent the model with vturb = 0 and vturb = 400 km s−1, respectively. For an input turbulent velocity of 0, 200, 400 km s−1, the 1σ statistical uncertainty is ±20, ±5, and ±10 km s−1, respectively. (Reference numbers have been modified from the original source.)[41]

Figure 6 shows simulated spectral data distinguishing chemical elements and physical conditions across a supernova remnant. It highlights Athena's ability to dissect remnants at high spatial and spectral resolution.

Discussion

The multi-stage cryochain for ATHENA demonstrates how combining Stirling coolers, Joule-Thomson systems, and Adiabatic Demagnetization Refrigerators successfully achieves the 50 mK temperatures required for high-resolution X-ray spectroscopy. This cooling system represents an engineering achievement that directly enables ATHENA's scientific capabilities. For instance, cryogenically cooled TES detectors onboard Hitomi enabled the most precise measurement to date of turbulent motion in the Perseus galaxy cluster's hot gas45 — a discovery that reshaped models of how energy spreads in large-scale structures. The selection of appropriate cryocoolers involves balancing cooling capacity with reliability, compactness, vibration control, and longevity—all critical factors for space applications where maintenance is impossible. Each technology contributes distinct advantages: Stirling coolers provide reliable medium-temperature cooling, JT coolers offer stability without moving parts at the cold end, and ADRs achieve the final cooling step with minimal vibration.

ATHENA's cryogenic technologies represent a substantial improvement over previous X-ray missions, with approximately 40 times better energy resolution than XMM-Newton. This advancement will revolutionize our understanding of cosmic phenomena from black hole physics to the formation of large-scale structures. The relationship between cryogenic engineering and astronomical discovery highlights how technological innovation directly enables scientific breakthroughs in space exploration.

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Conclusions

The ATHENA mission represents a significant advancement in X-ray astronomy, made possible through innovative cryogenic cooling technologies. This study has demonstrated how the multi-stage cryochain—combining Stirling coolers, Joule-Thomson systems, and Adiabatic Demagnetization Refrigerators—enables the X-IFU instrument to achieve the ultra-low temperatures of 50 mK required for high-resolution spectroscopy.

The selection of appropriate cryocoolers for space applications involves careful consideration of cooling power, reliability, compactness, vibration control, and longevity. Each cryocooler type offers distinct advantages: Stirling coolers provide reliability and efficiency for medium-low temperatures; Joule-Thomson coolers offer stability and compact design for reaching liquid helium temperatures; and ADRs enable the achievement of milli-Kelvin temperatures with minimal vibration.

The Transition Edge Sensors at the core of the X-IFU detector system demonstrate how these cooling technologies directly enable scientific discovery. By operating at 50 mK, these sensors achieve the exceptional energy resolution needed to study the physics of black holes, cosmic feedback mechanisms, and the formation of large-scale cosmic structures.

This research highlights the critical relationship between advanced cooling technologies and breakthrough astronomical observations. By pushing the boundaries of cryogenic engineering, ATHENA will provide unprecedented insights into the hot and energetic universe, revolutionizing our understanding of cosmic phenomena from black hole physics to galaxy evolution and large-scale structure formation.

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■ RESEARCH ARTICLE

A Study on the Relationship Between Student Management Systems and Psychological Safety in High School Students

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ABSTRACT: This study aimed to compare the administrative systems of public high schools in the United States and South Korea to analyze differences in psychological safety levels in American and Korean high school students. In the United States, teachers are in charge of a student's academic learning, while counselors manage the other aspects of student management, including college applications, grades, and student well-being. In contrast, in South Korea, homeroom teachers are assigned to classes of 30-40 students and are in charge of both academics and student well-being, while counselors have comparatively specialized duties. For this study, we conducted interviews and surveys with 20 high school students attending school in the United States and South Korea to understand how administrative differences can affect students' psychological well-being. The results showed that American students receive more academic education from their teachers and counselors, but experience relatively low psychological safety due to weak teacher-student bonds and limited access to their counselors. Meanwhile, Korean students reported high psychological safety regarding their relationship with their homeroom teacher and class; however, psychological safety levels also varied depending on the students' perceived competence of their teacher. The results of this study suggest ways in which American public high schools can improve students' psychological well-being while maintaining the current education quality by developing teachers' competency, assigning specific roles, and introducing mentor networks.

KEYWORDS: Behavioral and Social Sciences, Psychology, Psychological Safety, Student Management Systems.

■ Introduction

The Reality of American High Schools and Mental Health Issues:

American high school students have struggled heavily with mental health issues in recent years. Youth suicide rates have increased significantly over the past decade, with 37% of students showing mental health warning signs during the COVID-19 pandemic due to isolation by quarantine, remote learning, and other detrimental factors.[1] The COVID-19 pandemic not only impacted students during quarantine but continues to negatively affect the mental health of children and adolescents, with feelings of isolation caused by the pandemic appearing to exacerbate depression and anxiety, particularly in adolescents, for whom feelings of belonging are vital.[2] Mental health refers to an individual's ability to cope with the environment and circumstances in which they find themselves, with the appropriate control of negative emotions, including psychological stress, depression, anxiety, and tension being crucial to maintaining a state of psychological well-being. According to a recent mental health survey by the National Youth Health Behavior Survey,[3] 34.2% of high school students reported perceived stress, 25.2% reported depression, 10.9% reported suicidal ideation, and 2.0% reported suicide attempts. This indicates that more than one-third of youth are exposed to stress. These emotional and psychological issues experienced by high school students become increasingly problematic as they begin to manifest as disruptive behaviors in high school students, negatively impacting their mental health.[4] Moreover, the mental health of students affects not only their high school experience but also their lives after adolescence.

As such, it is crucial to give students the resources they need to lead happy and fulfilling lives as productive members of society.[5] In addition, the United States is currently facing a serious mental health crisis in its adult population. In the aftermath of the COVID-19 pandemic, over 40% of American adults have reported symptoms of anxiety and depression. As a result, these illnesses are expected to become the leading chronic health conditions among adults in the United States.[6] Furthermore, mental health issues are extremely prevalent in the United States, with one in five adults living with a mental health disorder. This makes the prevention and awareness of mental illness in high school all the more important.[7] Not only does mental illness have a negative impact on physical health, but it also puts people at a higher risk for a myriad of diseases, including heart disease, diabetes, and Alzheimer's disease. The healthcare costs of people with both a chronic and mental illness are also two to three times that of people with just a chronic disease. Lastly, poor mental health among high school students is considered a significant issue because of the strong correlation between mental health and academic performance. One survey done by a specialized student survey organization found that nearly half of the students reported that their mental health was a barrier to their ability to study.[8]

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The Relationship Between Mental Health and Psychological Safety:

Past research on factors that influence adolescent mental health suggests that teenagers' self-esteem, social support at school, and relationships with parents are crucial to maintaining their mental stability.[9] This is especially true for high school students, who spend the majority of their lives at school, making it all the more important to address mental health in the classroom setting and ensure their psychological safety. Psychological safety has been defined by multiple studies as a feeling of self-esteem and social support and includes the ability to function productively with the goal of growth and development,[10] adapting to daily life. It has also been defined as a sense of well-being based on subjective experiences perceived by an individual as a result of their response to the environment around them or the interactions they have with the environment's stimuli.[11] In addition, it has been defined as an individual's belief or shared belief within a team that his or her ideas or opinions will not be shut down or negatively perceived within their social relationships, making it a key factor that enhances an individual's speaking and learning behaviors, improving their performance within a group.[12] It has additionally been defined as the state of being able to freely express opinions due to the belief that expressing one's thoughts will not result in negative consequences.[13] Increased psychological safety is also associated with improved mental health and a greater likelihood of proactive behavior.[14] Previous research on psychological safety has shown that psychological safety enhances knowledge sharing and learning, creative engagement and performance, task performance, and academic performance.[15] Though psychological safety was initially noted as a concept to explain team effectiveness and learning behavior, recent research on psychological safety has evolved beyond studying psychological safety's link to freedom of expression, treating it as a complex concept that operates through structural trust, interdependence, and relational context. These studies reflect research trends of aiming to understand psychological safety as a product of interactions and group dynamics within an organization rather than through the limitations of individual perception.[16] Furthermore, the concept of psychological safety is currently actively discussed in the context of interactions between networking and relational job crafting within organizations.[17] Rather than viewing psychological safety as an isolated variable, this approach uses recent research trends to understand interactions and changing dynamics within organizations.

The Necessity of Research:

Psychological well-being is crucial not only to mental health but also to a productive high school experience, fostering creativity, mutual trust, and knowledge sharing. However, much of the research on psychological well-being has been conducted in the workplace, and there have been no studies on the effects of high school student management systems on psychological safety. In this study, student management systems refer to the support system that high school students rely on throughout their schooling experience, including classes, academic information, career counseling, friendships, and grievance counseling. The current American school teacher and counseling system has been a cornerstone of American high school public education since the 1990s, allowing teachers to focus solely on teaching. The current counseling system was established in 1997 alongside the American School Counselor Association model, which was created to provide professional services in three categories: academic, career, and personal/social.[18] However, although the counselor system was designed with students' overall school life in mind, it is highly ineffective in meeting the personal needs of students.

According to this study's surveys and interviews, the counseling system effectively deals with issues pertaining to academics and careers, but offers little personal/social support. Teachers have limited time to connect with students and are not individually assigned, with their primary responsibility being to teach students academically. As a result, high school students in the United States generally have no adult figures to go to with personal problems. They have no one to talk to about conflicts with friends, parents, health problems, and other critical issues. This is especially true for transfer students or foreign students, making them all the more anxious. Meanwhile, South Korea operates using a system of specialized teachers (teachers who teach a specific subject), homeroom teachers, and counselors. Homeroom teachers are responsible for the overall life of students within the boundaries of a class of typically 30 students. They work as both a class teacher and supportive figures, managing their class with the responsibility of both teaching their students and supporting them personally. Figure 1 models the American student management system in comparison to the Korean student management system, presenting the different pillars of support each system has for its students. This study aims to analyze how the differences in education systems between the United States and South Korea affect psychological safety and, by extension, psychological well-being, something that is critical to the mental health of high school students and carries heavy implications for public education in the United States.

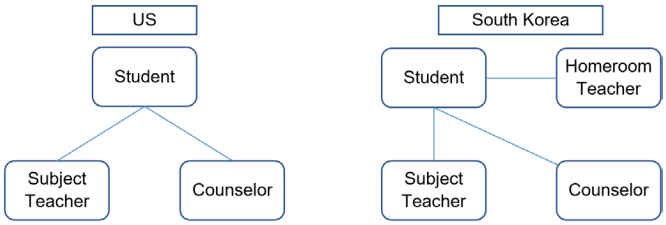

Figure 1: The typical South Korean student management system has three main components: a homeroom teacher, a specialized teacher (instructor of a specific subject), and a counselor. In comparison, the typical American student management system has only two: a specialized teacher and a counselor. The American student management system emphasizes academic and career support, but the Korean student management system places more emphasis on personal support, leading to greater levels of observed psychological safety in Korean high school students.

Methods

The object of study:

The study was conducted at six public high schools in the US and Korea. To obtain objective data, the research was con-

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ducted across multiple schools, with surveys and interviews being done at two schools in the U.S. and four in South Korea. Table 1 presents 20 respondents, including their age, gender, and grade. Surveys and interviews were conducted with the tenth to twelfth graders. Among them, 11 were male and 9 were female, offering a diverse range of perspectives.

Table 1: Interviewees and survey participants were selected from varying grade levels across high schools in the United States and South Korea. The majority of Korean students rated survey questions on a higher scale than American students.

Interviewee	Grade	Age	Gender	Country
1	10	16	Female	USA
2	10	16	Male	
3	10	16	Male	
4	10	16	Male	
5	10	16	Female	
6	11	17	Male	
7	11	17	Male	
8	11	17	Female	
9	11	17	Female	
10	12	18	Female	
11	10	16	Male	Korea
12	10	16	Male	
13	10	16	Male	
14	10	16	Female	
15	11	17	Female	
16	11	17	Male	
17	11	17	Male	
18	11	17	Male	
19	12	18	Female	
20	12	18	Female	

Research tools:

This study utilized a combination of qualitative and quantitative research to compensate for the lack of a survey population. The qualitative research method consisted of in-depth interviews to understand the realistic experiences of American and Korean high school students, as well as to refine and improve the questions asked as interviews were carried out. Interviews were conducted in person as much as possible, with phone and email interviews conducted with Korean students who were unable to meet face-to-face. Questions were asked via email and supplemented with additional interviews via video conference and email. Table 2 presents the interview questions posed to each participant. For American students, questions were sent via email a day prior to the interviews, which were interactive and in-person. The emails explained the purpose and objectives of the study in depth, the definition of psychological safety, and the student management systems in South Korea and the United States, and only proceeded with the study with the participants' consent

Table 2: Interview questions about perceived psychological safety were presented to each participant. Questions focused on perceived psychological safety levels, factors that impacted psychological safety, and suggestions for improving psychological safety at schools in both the United States and South Korea.

No.	Interview Questions
1	Do you feel a sense of psychological safety at your high school?
2	What factors affect your sense of psychological safety at school?
3	In particular, how do relationships affect your sense of psychological safety at school?
4	In elementary school, did having a homeroom teacher add to your psychological safety?
5	Would having a mentor whom you can talk to about your school life add to your sense of psychological safety?
6	What could be improved at your school to increase your psychological safety?

※ Survey questions are attached as a separate file

Data Analysis:

The interview results were recorded completely during the interview process, and the study results were synthesized and derived from the participants' responses. To ensure objectivity in analyzing interview responses, students from multiple schools were interviewed, and the results of the interviews were verified by a professional with a Doctor of Business Administration degree and extensive research experience. The survey results were used to complement the interview responses by utilizing the average value of the respondents' answers on the 5-point scale questionnaire prior to the interview. Four of the survey items on the questionnaire were revised versions of questions appearing on a previously validated survey questionnaire[19] to measure psychological safety.

Research Ethics:

In conducting this study, we followed the following criteria to ensure compliance with established research ethics. First, before conducting the interviews, we explained the purpose of the study, the purpose of use, and the sharing of future results in detail to the participants via email and phone calls to confirm their informed voluntary participation. Second, we explained in advance that if the participant felt uncomfortable during the interview or did not wish to have their responses shared in the study, the interview would be stopped, and their responses would not be used. Third, to ensure the privacy of the participants, we did not inform other participants of others' participation, and all names were anonymized. Fourth, we informed participants that the data collected during the interviews and questionnaires would be used solely for the study and its research. Fifth, the results of the study were reviewed by an expert to ensure the objectivity of the collected data due to a limited survey population.

Results

This study is directly quoted from the interviewees' thoughts and opinions about their school experience. The results are organized by interview question order and responses to the survey.

Psychological safety in high school:

Table 3 presents the average rating of psychological safety at the participants' schools. Both American and Korean high school students reported above-average levels of psychological safety, but there were differences between the countries. Korean high school students scored an average of 4.3 out of 5, while American students scored slightly lower at 4.0. Most of the students interviewed entered high school after the COVID-19 pandemic, so the impact of the pandemic was minimal. During the interviews, many students mentioned their experience beginning high school, particularly the unfamiliarity and anxiety they felt, and its impact on their sense of psychological safety. Due to the structure of Korean student management systems, Korean students' sense of psychological safety was well-established prior to their high school experience, allowing them to feel safer and more supported in the classroom.

"My anxiety was highest right after entering 9th grade. I expected someone to explain and guide me through high school, but there was no one. I had to change classes mid-semester because I didn't get enough explanation, and it's harder to make friends when you move from out of town. You can get the information you need

through emails and posts, but it took me at least a couple of months to figure everything out on my own. Everything was too unfamiliar to feel psychologically safe." (10th, United States)

"The student management system is similar in elementary school, middle school, and high school. You can start with new friends in your class and get the answers you need by asking your homeroom teacher. You can participate and voice your opinion if you want to, but it's culturally not expected. There is a high level of psychological stability because the familiar environment continues." (10th, South Korea)

Table 3: Level of psychological safety in high school (N=20, U.S and Korea). Korean students, on average, felt more psychological safety compared to American students.

Survey Question	Average (US / KOR)
Do you feel a sense of psychological safety at your high school?	4.2 (4.0 / 4.3)

※ Five-point rating scale: 1point (Strongly Disagree) ~ 5point (Strongly Agree)

The connection between high school relationships and psychological safety:

While classroom atmosphere and structure, commuting distance, method of transportation, and other factors contribute to psychological safety, the most important factor is relationships. Relationships at school include those with friends, teachers, and counselors. Compared to American students, Korean students' relationship with their homeroom teachers was more impactful on their psychological safety.

Table 4 presents the participants' rating of the importance of different relationships on psychological safety. First, relationships with friends were the most important factor in both the United States and South Korea, with an average score of 4.7 on the survey, showing the heavy influence it has on psychological safety.

"Overall, I get the most psychological safety from my friends. I feel comfortable talking to them when I'm in trouble, and I talk to them the most about choosing classes and going to college. However, when it comes to official information such as college admissions information, they are not as helpful." (10th, United States)

"My friends have the biggest impact on my psychological safety. They are more trustworthy than teachers and parents, and I can talk to them whenever I want. The information exchange between friends is especially important because I can get the information I need at the right time and in the most relevant way. " (10th, South Korea)

However, there is a big difference between South Korea and the United States when it comes to teacher influence. Korean students scored a 4.1, while the US scored a 3.5, which is relatively low in comparison. Students were asked about specialized teachers and homeroom teachers separately to check for differences in the education systems between the two countries, and American students who do not have homeroom teachers were asked if they think they would benefit from having a homeroom teacher in high school, just as they did in elementary school. While both groups scored similarly for specialized teachers, when homeroom teachers were included, both groups scored significantly higher (4.3).

"I can always talk to my teachers and get help when I need it. It was hard for me to talk to them at first, but after I got comfortable, I got enough help. However, since she is not responsible for helping me, it is not easy to talk to her about small problems. In particular, since the face-to-face time is not long, I have to meet before or after class or during lunch." (10th, United States)

"Specialized teachers don't really interact with you unless it's subject-related. However, when it comes to the subject matter, I feel comfortable expressing my opinions and getting the information I need. I get a sense of psychological safety from my homeroom teacher. You can ask them anything about school life, and they are always available via phone or social media. Also, because she has a lot of experience, she helps and guides me a lot when it comes to college admissions." (11th, South Korea)

Finally, the influence of counselors was low in both countries, with an average score of 3. Students did not see counselors as someone they could easily meet with, but rather as administrators who helped them choose classes and get into college.

"I can only see them by appointment, and they don't contact me outside of class for career advice. They have specialized knowledge and experience in their field, which makes me feel safe. I don't think the counselors are there to talk about problems. There is a mental health counselor, but I don't think I've ever contacted them to discuss my problems." (10th, United States)

"There is an academic counselor and a mental health counselor, but I have never met with them. I don't think it's necessary because my homeroom teacher covers the counselor's role well enough. I feel like the counselor is more of an administrative support rather than a professional." (11th, South Korea)

Table 4: Effect of relationships on psychological safety in high school (N=20, US and South Korea). Korean students, on average, rated the importance of relationships on psychological safety higher than American students. Friends had the greatest impact on psychological safety, while counselors had the least impact.

Survey Question	Average (US / KOR)
Does your relationship with friends affect your sense of psychological safety?	4.7 (4.6 / 4.7)
Does your relationship with teachers affect your sense of psychological safety?	3.9 (3.5 / 4.1)
Does your relationship with the counselor affect your sense of psychological safety?	3.0 (2.9 / 3.1)

※ Five-point rating scale: 1point (Strongly Disagree) ~ 5point (Strongly Agree)

How to increase psychological safety:

To feel more psychologically safe, people need to feel more comfortable, have a direct line of communication, and have a clear sense that someone cares about them. Participants claimed that having one-on-one relationships gives them a sense of belonging, security, and connection. However, because teachers have a strong influence on grades and college admissions, students are more likely to feel that a mentor with a looser, more detached relationship with academics can help them feel more secure, especially if the mentor is an alumnus who graduated from the same high school as the student, is pursuing higher education, and is active in society. Table 5 presents the importance of a mentor figure to psychological safety.

"Mentoring with an alumnus from the university I want to attend would help me feel more secure. I think I would be able to relate to someone who has been through the same experiences as me, and I would be able to approach them more easily than a teacher." (10th, United States)

"It would be nice to have a college alumnus mentor that I could easily communicate with, but it's pointless to just connect with them.

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I think the school should pay the mentors, and they should at least have counseling skills and expertise. Mentors who are not qualified and capable can be frustrating for students." (10th, South Korea)

Table 5: Effect of a mentor on psychological safety in high school (N=20, United States and South Korea). Korean students, on average, rated the need for a mentor higher than American students, but both demographics rated the need for a mentor highly.

Survey Question	Average (US / KOR)
Do you need a mentor (alumni, senior) with whom you can discuss your school life?	4.4 (4.3 / 4.5)

※ Five-point rating scale: 1 point (Strongly Disagree) ~ 5 point (Strongly Agree)

Possible improvements for American high schools:

The results of the study showed that among the factors that add to psychological safety, friendships have the most impact, but the impact of teachers and counselors is much less significant. Based on the responses of Korean students, it can be concluded that the role of a homeroom teacher significantly impacts psychological safety. However, adding a homeroom teacher in addition to specialized teachers can be expensive. Currently, the American public education system has great difficulty securing funding and compensating teachers. Meanwhile, in South Korea, homeroom teachers oversee their own classes and are also specialized teachers. Though it is a more demanding role, homeroom teachers are relatively well compensated for their work. In addition, homeroom teachers can be trained in counseling and can choose to be solely specialized teachers if they desire. Specialized teachers in the United States can be given a lighter role with fewer responsibilities than a homeroom teacher, but simply as mentors to designated students. In exchange, they could be provided with additional compensation and growth opportunities, creating a low-cost way to make students feel more psychologically secure.

"Currently, homeroom teachers in Korea have too many roles and responsibilities. There's even a phenomenon of young teachers avoiding homeroom duty. I don't think a homeroom system like Korea's could be applied to American high schools. It would be impossible to control the students. Instead, a loose homeroom system that only assigns a 1:1 communication role is acceptable. Just having a designated teacher that you can communicate with can give students a sense of psychological security. However, they need more compensation and support from the school than traditional specialized teachers." (11th Grade Homeroom Teacher, South Korea)

Another Way to Improve High School Students' Psychological Safety:

Many studies show that one-on-one mentoring has a positive impact on psychological safety.[9] During our interviews, we found that students preferred to have mentors who are alumni close to their age over teachers or parents. Alumni who attended the same school faced the same problems and went through the same hardships in high school, college, and adulthood can provide valuable advice for current students. However, basic mentorship qualities and knowledge are required. Mentors need to be trained in how to talk to and counsel high school students, who are often very impressionable. Mentors should also be provided with financial support to encourage them to take responsibility for advising students.

"There's a lot you can do when you have the opportunity to mentor a high school student. If you're an alumnus, you can be more compassionate, too. You can take them on a college tour to motivate them to go to college, or you can share your own high school struggles and tips on how to get into college. If mentees tell you about their problems at school or home, you can listen to them and help them with their problems." (Sophomore at the University of North Carolina at Chapel Hill, United States)

■ Discussion

This study offers meaningful results on the relationship between the school system and psychological safety in the United States and Korea, but there are some limitations that affect the study's analytic value. The small sample size makes it difficult for the survey results to be generalized and applicable to the overall school systems of the United States and Korea. As such, it is difficult to determine whether the observed patterns are reflective of broader trends in both countries and if they were shaped by respondent-specific biases. Nevertheless, given the nature of exploratory research, this study remains valuable in setting the foundation for future research. In future studies, quantitative analysis based on a larger sample will be beneficial to further strengthen study results. It is expected that the external validity of the results will be improved by conducting surveys and interviews that include larger and less homogeneous groups to consider various demographic factors such as grade, race, and gender. Applying statistical techniques such as regression analysis based on expanded data will lead to more in-depth and quantitative insights into the relationship between the variables introduced in this study. Furthermore, comparing the psychological safety experiences of students from various backgrounds will establish the basis for a more elaborate analysis of how cultural and institutional factors interact to affect psychological safety.

In addition, students' psychological safety is formed through complex socio-cultural contexts that are critical to consider when analyzing psychological safety in the classroom. For example, Korean society is characterized by a strong focus on academic success and social comparison; the resulting stress from societal pressures may hinder students' psychological safety. Moreover, unlike the United States, where factors such as race and familial background may heavily impact interpersonal relationships within an educational setting, Korea has a relatively homogenous population, limiting the influence of these factors. These cultural and demographic characteristics can cause significant differences in how students perceive and experience psychological safety.20 Therefore, rather than identifying these differences as limitations of research, this study intends to propose a research project that will be characterized by a more in-depth comparative cultural approach in the future. Future research will promote a more nuanced understanding of the formation of psychological safety and its influencing factors by comprehensively reflecting the social norms, education systems, and demographic compositions of various countries and cultures

Conclusion

Psychological safety at school plays a critical role in allowing students to thrive and enjoy their schooling experience. The influence of a homeroom teacher and classroom boundaries can help students feel more psychologically safe, but given the current situation of American higher education, especially limited education budgets and teacher shortages, it is rather unrealistic to expect teachers to take on more responsibilities and completely the general public education system. Rather, this study suggests ways to motivate teachers and develop their capabilities, including counseling and mentoring, and the impact alumni can have as mentors to high school students. Finally, as it has been established that friendships have the greatest influence on psychological safety, it is also necessary to foster clubs and other school activities (ex., school-wide events and social activities) and provide time and space for more conversations and social interactions between students at school.

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■ RESEARCH ARTICLE

When is Sex Selection Morally Permissible? Considering Family Balancing & Disease

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ABSTRACT: Sex selection is a budding set of technologies intended to allow prospective parents the ability to choose the desired sex of their fetus. This technology may seem harmless in the short term, but long-term consequences are staggering, as the literature shows. While some authors try to justify using sex selection, research shows that sex selection leads to a high number of negative consequences, both personal and social. Because of this, sex selection is only morally permissible for medical reasons, such as preventing sex-linked diseases. It is not ethically permissible for non-medical reasons, such as family balancing. In the following paper, I outline the negative consequences of sex selection. Moreover, I show that only medical needs justify allowing the use of sex selection technologies.

KEYWORDS: Cellular Biology, Genetics, Genetic Engineering, Sex Selection, Bioethics.

■ Introduction

Sex selection technologies have recently been created and made available, allowing potential parents to choose the sex of their child before its birth. These technologies include but are not limited to Preimplantation Genetic Diagnosis (PGD) and sperm selection through the process of in vitro fertilization (IVF).[1] These sex selection technologies help parents choose a child of their preferred sex for a variety of reasons, ranging from family balancing to preventing life-threatening sex-linked diseases such as muscular dystrophy and Barth syndrome.[2] Some of the most popularly used sex selection techniques include sperm selection and PGD.[3] Sperm selection is a process where sperm is collected and analyzed in the lab. The lab identifies sperm that will create either a male or female embryo, according to the client's desires. Then, the sperm is used to fertilize the ovum through IVF.[4] This means that in the process of choosing the desired sperm, the parents can choose the desired sex of the baby. PGD is a process in which scientists examine the fertilized embryo and determine its sex.[6] In this method of sex selection, parents can choose whether they want to implant the embryo into the uterus or not, depending on whether they want to have a child of that sex.[5] PGD is one of the most popularly used forms of sex selection as it ensures nearly 100% accuracy of sex selection.[6]

With this powerful technology arises an important question: For what purposes is it morally permissible to select embryos for implantation based on their sex? Many bioethicists have proposed a variety of arguments around the ethical circumstances of sex selection technology. Scholars like Birging and Shahvisi provide strong arguments against permitting sex selection and illustrate the long-term consequences of sex selection technology on society. On the opposing side, Sureau and Etzioni both argue in favor of allowing sex selection and examine how risks in these situations must be taken for the sake of progress in technology and science. Authors like Kalfoglou produce arguments for both sides with a more deontological perspective. The argument that I wish to propose here is that sex selection is morally permissible only when it is used for medical reasons, such as preventing sex-linked diseases. However, sex selection is not morally permissible for non-medical reasons. This is because when it comes to cases of sex selection for non-medical reasons, the negative consequences greatly outweigh any positive outcomes.

In the next section, I discuss the negative consequences of sex selection. In later sections, I argue that given these negative consequences, sex selection is not morally permissible for family balancing reasons and is only morally permissible for medical reasons. This is because a parent's moral obligation is to protect their children's health. In contrast, parents have no inherent moral obligation to balance the sex ratios within their families. Hence, only in cases where sex selection serves a medical purpose do the positive consequences outweigh any adverse consequences of sex selection.

■ Introduction

A) The Negative Consequences of Sex Selection:

Many negative consequences occur with sex selection. One issue is sexism towards women. Sexism is the practice of discriminating, stereotyping, or using prejudice against someone because of their sex.[7] When families wish for a child of a particular sex, they do not wish for this because of the anatomical differences, but because they believe that the child will act according to gender stereotypes.[6] For example, in a family with three boys, if the parents want their next child to be a girl, it is not because of the bodily differences that separate a male from a female; in reality, it's because of the stereotypical roles they assign to a girl in comparison to a boy.[8] Some families may expect a girl to be more docile than their boys, and others may expect boys to be more energetic and sportier than girls. This just reinforces all the heteronormative gender stereotypes that society has been trying to escape for ages. Sex selection for a

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balanced family is sex selection for heteronormative reasons, which, in turn, is just another form of sexism. The long-term consequences of such ideals could result in the reinforcement of oppressive and sexist social norms that we pride ourselves on escaping.

Another negative consequence of sex selection is medicalization.[9] Medicalization is a concept in which medicine is used to treat issues that are not real medical problems at all; instead, medicine is misused to treat the aspects of human life that don't need to be 'fixed,' as there is nothing wrong with the body in the first place. Given that most sex selection occurs for family balancing reasons and that a sex imbalance in a family is not a physiological problem, sex selection contributes to medicalization. A good amount of social resources has been devoted to sex-selective technologies, but the demand for them outstrips the supply. There are lengthy waitlists as long as 18 months for people waiting for the opportunity to use PGD for the sex selection of embryos.[10] This is yet another reason why sex selection for non-medical reasons is not ethical. Thousands of people from many countries, primarily countries in Asia, travel around the world to use sex selection technologies purely for sexist reasons (the desire to have a male child as opposed to a female) or other personal preferences.[11] This limits the availability of sex selection technologies that are essential to making life-saving decisions for babies who are potentially susceptible to chronic disease.[12] Using sex-selective resources for medical issues should be prioritized to the greatest extent possible, as sex-selection usage in such cases could determine the physical well-being and survival of the child. However, there is nothing of such a dangerous magnitude that can be lost when using sex selection for non-medical reasons. We have seen medicalization happen in the past, with medicines provided to 'correct' the LGBTQ+ population.[13] It is well known how problematic and unethical it was to use medicines to 'fix' the LGBTQ+ population; sex selection for family balancing is quite similar.[14] In this case, we still try to 'correct' the child by ensuring it is not a particular sex. In reality, a child's gender does not reflect a problem with their physiology. Hence, it is wasteful to squander resources to solve a manufactured medical problem.[15] Therefore, sex selection is not morally permissible for family balancing because there isn't a medical problem to fix, and it takes resources from conditions that are genuinely life-threatening and can be solved with medical care.

Yet another damaging consequence of sex selection is the high possibility of an unbalanced sex ratio in a population. In countries with strong sexist stereotypes, some wish for their child to lead the best life as the "better" sex, which is generally taken to be the male sex.[16] When a pregnancy occurs with a fetus of the undesired sex, they take drastic measures such as preventing the birth of the child.[17] With sex selection technologies made available, this only reinforces such beliefs and increases their consequences.[18] This can result in potential social problems due to the increased objectification of women and the growing presence of them in trafficking schemes.[19] Asian countries, such as India and China are well known for having deep-rooted sexist ideals, but having sex selection available would make matters worse. Women would become more 'valuable,' but not in an ethical way—they would become more valuable to families who are desperate to get their sons married off to achieve elite status, contributing to sexist objectification

Sex-selective infanticide is the leading cause of an unbalanced sex ratio in Asian countries.[20] With sex-selective technologies becoming increasingly available to many populations, this allows for more room to choose the preferred sex for sexist reasons, furthering the already dangerously imbalanced sex ratio in such countries. The consequences of an imbalanced sex ratio can lead to the death and maltreatment of the targeted sex group. Let's take a look at the current circumstances in certain countries in Asia, with a growing unbalanced sex ratio, such as China, which has 112 males for every 100 females born.[21] Because there are so many sons than daughters, there's not an equal ratio, meaning there are too many unmarried men. As a response to this, some groups responded by trafficking young women. These programs were called 'bride trafficking' and were influenced by an imbalanced sex ratio. Simultaneously, we also saw a massive increase in abusive child marriage from an unreasonably young age in girls. Although this is just an example in Asia, a similar phenomenon could occur in other countries, partly driven by imbalanced sex ratios.[s] Presently, there is a massive sex trafficking crisis in the United States; if we allow limitless sex selection, we will be contributing to social conditions that lead to more trafficking scenarios, which hurt the well-being of many social groups, in particular women.

There are also a multitude of ways that sex selection can harm a child, both emotionally and physically. On the rare occasion that sex-selective technologies fail to select the embryo of the preferred sex, parents tend to resort to abortion. The parents are essentially deciding that this embryo isn't worth existing due to its sex. Most abortions tend to happen due to reasons that endanger the life of either the parent or fetus, but in this case, it's because the fetus isn't the proper sex, even though the sex of the fetus is not a medical problem. The entire idea of aborting the fetus undermines the concept of unconditional love for a child. This can also mean an increased potential for post-natal child maltreatment. If a child isn't what the parents had hoped for, this could lead to feelings of resentment due to the sheer amount of money, effort, and hope spent on the program. Consequently, this could result in abusive or neglectful treatment of the child and lead to malnourishment, for instance.

Family balancing can not only lead to sexist outcomes but also transphobic ones. Parents pay thousands of dollars to have a child of a particular sex; however, if the child chooses a different sexual identity later, then the parents are not likely to support the child's decision due to the amount of work they put into determining the sex of their child beforehand. [6]Sex selection can, therefore, lead to a transphobic society that makes it harder for children to be loved and show their identities.[6]

B) Discussion: Sex Selection is Not Morally Justified for Family Balancing:

Family balancing is a goal many families aim for. It is based on the idea that each family should have a 'balanced' number of children, gender-wise. Every family interprets this norm

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differently. Some families with all girls might wish for just one boy to keep everything 'balanced,' whereas other families with one girl might favor having just one boy and one girl. However, most families who desire a balanced family have a common goal: to achieve a balanced family with the least number of children possible.[6] That is why many families that want a balanced family look into sex selection technologies, like sperm sorting or PGD. Because of the high accuracy of such programs, families can get the desired result with the fewest children possible.

Given the multitude of negative consequences due to the usage of sex selection technologies, sex selection is not morally permissible for nonmedical reasons. Sex selection for non-medical reasons is unnecessary. It is just in the parents' interest because it regards what kind of child they want, even though the child does not need it to lead a healthy life. Sex selection for non-medical reasons can lead to a multitude of issues, such as enabling sexism, increasing trafficking schemes, heteronormativity, and many other negative consequences.

Furthermore, when asked why families want to be balanced, the parents usually respond simply, saying that siblings of the opposite sex often have a better social life when they grow up. However, there is virtually no scientific literature proving this true. Studies only talk about the benefits of having a sibling, disregarding gender.[6] This study indicates that parents have no problems with having their child's friend group be full of children of the same sex, but they only seem to have that problem with the family.[6] Siblings of the opposite sex do not appear to give the family any additional benefits, meaning that the preference for a child of a particular sex may simply be internalized sexism. This is because families do not want a child of a particular sex because of the physical differences, but because of their stereotypical beliefs of what they believe a girl will be like versus a boy. In actuality, this means that internalized sexism is the leading reason for family balancing, as they follow their pre-existing stereotypes of how they believe girls and boys are different emotionally. This is highly unethical reasoning, making the whole process unethical under non-medical circumstances.

Not only is the thought process behind family balancing detrimental to society, but the outcomes can also be detrimental to the children produced. Like any other medical procedure, family balancing doesn't have a 100% success rate.[22] There is always a possibility that the child can still be of the unwanted gender, and this doesn't always end well for the child. In drastic situations, this can lead to malnourishment of the child, post-natal murder, etc.[20] There have been multiple instances of 'extermination' procedures like these happening in countries in Asia, proven through the mass female infanticide in China for the past 2,000 years.[6] This destroys the idea that parental love should be unconditional rather than dependent on the child's characteristics. Parents have a moral obligation to ensure the best life for their children, regardless of the child's gender.

C) Sex Selection is Morally Justified for Medical Reasons:

Sex selection is morally permissible for medical reasons, however, such as preventing sex-linked diseases, because the moral imperative of preventing serious diseases outweighs the negative consequences of the use of sex selection technologies.[11] Medical issues could potentially threaten the life of a child, and we have moral reasons for preventing them. Indeed, parents have a moral obligation to ensure their child's health to the best of their ability. Sex-linked diseases are diseases that only occur in a particular sex or primarily affect a specific sex. Examples of diseases that are sex-linked include hemophilia, which is an X-linked recessive disorder. Because males have only one X chromosome, they are more likely to get it.[23] At the same time, females are only carriers, meaning they carry the disease and can pass it on to their children, but they usually do not experience any symptoms.[11] This means males, or individuals with XY chromosomes, are more likely to have hemophilia than females, who have XX chromosomes, are. Hemophilia, at times, can be life-threatening, as can many other sex-linked diseases.[11] They are taking all of this into consideration: if two prospective parents have a gene that carries or has hemophilia, their child, if male, is very likely to get the life-threatening disease. Because parents are morally obligated to ensure the healthiest life for their children, it is morally permissible for them to use sex selection to have a female child so they can prevent the risk of their child having a life-threatening disease. Furthermore, even if the sex-linked disease is not life-threatening, it still threatens the quality of life of the individual, so it is ethical for the parents to use sex selection to reduce the likelihood of such diseases.

■ Discussion

A) Counterarguments:

The idea that family balancing is beneficial persists. This is one of the most popular reasons parents cite when asked why they want a balanced family. They state that having children of opposite sexes interact with each other from a young age will make it easier for the kids to grow up and be more social.[6] However, studies have shown little to no empirical evidence to support this idea. Studies do show that having a sibling, no matter the sex, can help the child to grow up more socially adept, but there is no compelling scientific literature to support what these parents are stating. Since there is neither a material nor medical reason behind why parents want a child of a particular sex, the parents are likely driven by sexist stereotypes to justify their reasoning behind family balance.

Another common counterargument is that sex selection must be used to provide the best life for the child.[24] According to this logic, this technology should be allowed to select a male child because men tend to have better lives in some regions of the world.[25] This, however, reflects a selfish and immoral androcentric worldview. By allowing sex to promote a "better life" for some individuals, we will only be reinforcing the problem of unequal treatment of the sexes. Furthermore, this mindset is immoral because families that do not have the opportunities and money to use the technology will face greater impacts of existing problems that are caused by an imbalanced sex ratio. There is already a bride-trafficking epidemic in Asia, and if sex-selective technologies are found to be morally acceptable, they are likely to be legalized across the globe. In that case, poor families are at higher risk of being targeted by

sex trafficking schemes.[21] By accepting sex selection in sexist countries, we knowingly risk subjecting the children of the poor to abusive practices. Therefore, sex selection is not the solution to the problem of gender inequality and could make the situation worse. And most importantly, it makes women's lives worse. Because there are fewer women in society, they are forced to deal with even more domestic labor, and the pressure of their normative, stereotypical roles in society increases. This leads to even more sexual exploitation.

One unique argument supporting sex selection is that having more men than women in society can be beneficial for society,[14] in particular, for women, as men can begin taking on what has usually been seen as women's roles and thus contribute to undermining sexist gender norms. However, this has no likelihood of occurring, considering past events. Referencing the imbalanced sex-ration argument, it is seen that in countries such as China, which has one of the largest imbalanced sex ratios in the world, the imbalanced sex ratio did not lead to better lives for the oppressed gender, women.[21] Men never took on the societal, domestic roles that were forced upon women. Women just had more of a burden to deal with as they slowly became a minority. Worst of all, women became victims of dangerous trafficking schemes and suffered greatly. This had significantly adverse effects on the lives of women, both physically and mentally; in many instances, the women died from their unfortunate circumstances.[21] The argument that having fewer women in a population can lead men to help with their roles lacks evidential support, rendering the argument weak.

Conclusion

Sex selection is morally permissible only for medical reasons because the consequences of not using sex selection in such situations could potentially result in death or disease. Death is the heaviest negative consequence, and no other consequence outweighs it. Potential parents' failure to try to prevent disease to give their child the best quality of life possible reflects moral neglect. They are not using sex selection for non-medical reasons, such as family planning, which, on the other hand, results in little to no adverse consequences. In the context of non-medical reasons, the negative consequences of using sex selection technologies easily outweigh any positive ones.

If we decide to allow sex selection with no limitations, the consequences may be similar to those of past eugenics policies. Eugenics is a 'race science' that was quite predominant in the late 19th century and the early 20th century. The idea behind this program was to create the best future generations by creating the best children. This was a program where minorities were labeled 'unfit' to reproduce. These groups included LGBTQ, disabled individuals, low-income people, etc.[6] Dominant groups ensured that minorities couldn't contribute to society by mandating sterilization and going to extreme measures to bring their utopian but highly oppressive worldview to life. Eugenics in the past was a very destructive pseudoscientific approach that resulted in all sorts of racial and sexist consequences. Our 'modern' practices of controlling reproduction, which include sex selection technologies, could result in outcomes similar to those of past eugenics programs. Eugenics programs created unthinkable consequences, for instance, the non-consensual sterilization of minority women, which incomparably destroyed lives. Later, several movements were launched in the United States to eradicate such biased and oppressive practices.[6] However, if we allow sex selection, we would likely see a great increase in sexism and potentially other biases. Suppose we wanted reproductive practices not to result in similar consequences to past eugenics programs. In that case, we must place limitations on the use of sex selection technologies and employ them only for morally justified reasons.

With the quickening development of modern sex selection technology, even more questions are left unanswered. If we don't carefully place limitations, it is impossible to predict what other consequences could result from the adoption of sex selection technologies. Furthermore, we must also consider which countries it is safest to consider for sex selection. Some countries have poor medical standards that are inadequate for controlling this kind of technology; however, despite all of these concerns, few safeguards are placed on this new technology.

Unfortunately, even with significant differences in the levels of necessity, there is no clear mechanism to prioritize access to medical resources. Resources may not exist for those who need sex selection to save their children from diseases linked to sex. Therefore, the minority of parents who genuinely need sex selection for valid medical purposes are often neglected. Eventually, this could lead to a depletion of medical resources for medically necessary treatments. Another concern is that since there is such a large number of people who want to take advantage of sex selection, this can result in a lack of resources for the families that are in desperate need to prevent life-threatening diseases from adversely affecting their kids' lives.[22]

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RESEARCH ARTICLE

Literature Review on *Helicobacter pylori* as a Major Risk Factor for Stomach Cancer

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ABSTRACT: *Helicobacter pylori (H. pylori)* is a prevalent bacterium that infects approximately 50% of the world's population. *H. pylori* colonization of the gastric mucosa with the help of its motility organ, the flagella, triggers immune responses, and important toxins are released, which play a role in the disease. These virulence factors and molecular mechanisms of *H. pylori* could lead to the development of gastric cancer. Important virulence factors of *H. pylori* include the cytotoxin-associated gene A (CagA), vacuolating cytotoxin (VacA), and outer inflammatory protein A (OipA). H. pylori is a major pathogenic contributor to gastric diseases. Therefore, further research into these virulence factors can help develop effective prevention of gastric cancer. Eradication methods of *H. pylori* infection related to the flagella reveal promising treatment methods worldwide despite antibiotic resistance. This review paper discusses the importance of *H. pylori* flagella and virulence factors as possible anticancer targets.

KEYWORDS: Behavioral and Social Sciences, Psychology, Psychological Safety, Student Management Systems.

Introduction

Gastric cancer is the fifth most common cancer worldwide, accounting for 5.6% of all cancer cases. It is more prevalent in East Asia, Central Asia, and South and Central America, with around 75% of all new stomach cancer cases and deaths reported from Asia. It also accounts for 7.7% of all cancer deaths. In the US, the disease occurs more frequently among Black, Hispanic, Asian/Pacific Islander, and American Indian/Alaska Native individuals, with increasing rates in younger females.[1] One major risk factor is *Helicobacter Pylori*, which is responsible for 70% of all gastric cancer cases. In addition, stomach cancer remains a major health problem due to its aggressive nature and diverse characteristics, such as its role in pathogenicity.[1]

H. pylori is a spiral-shaped, gram-negative bacterium that lives in the mucus layer of the human stomach. Its survival in the hostile, acidic environment of the stomach is aided by adaptation mechanisms such as neutralizing the stomach acid and attaching itself to the mucus layer.[2] Although the exact route of transmission of *H. pylori* and how it gets into the stomach is still under investigation, studies suggest infections occur due to either human-to-human transmission or environmental contamination. They indicated that human-to-human transmission occurs within families due to close contact and shared genetics. Infections are often acquired in childhood, typically through gastric fluids or saliva transmission, where *H. pylori* can colonize the mouth. Oral-to-oral transmission may be the primary route, but *H. pylori* can sometimes be passed from mother to child through contaminated oral secretions. Poor hygiene increases the risk, as contaminated water can carry *H. pylori* and spread infections.[3] The majority of infected individuals remain asymptomatic, and usually remain chronic unless treated with antibiotics. Over time, about 10%-15% of infected individuals develop duodenal ulcers, and around 1% develop gastric cancer. In fact, *H. pylori* infection is a significant risk factor for the development of gastric cancer.[4] The current prevalence of chronic *H. pylori* infections in the U.S. population is estimated to be around 36%.[5]

Chronic *H. pylori* infections develop slowly and persist over time, while acute *H. pylori* infections are very rare and clearance of such infections is extremely rare. Many acute *H. pylori* infections tend to turn into chronic infections because they are asymptomatic, and although they do activate the immune system, its response is not effective in clearing the infection.[6] Although the reason behind *H. pylori's* asymptomatic behavior is unclear, it is believed that some people may be born with resistance against *H. pylori*.[7] Persisting *H. pylori* infections cause chronic inflammation by manipulating the host immune system, which leads to chronic gastritis.[8]

The infection's outcome of chronic gastritis can increase the risk of stomach cancer by 0.1%. In addition, specific host genetic mutations can affect the intensity of the inflammation of the gastric tissue, which also influences the risk of subsequent development of stomach cancer.[6]

A causal relationship between *H. pylori* infection and gastric cancer was first proposed in the 1980s through the work of Australian scientists Barry Marshall and Robin Warren. Their research included isolating *H. pylori* and showing how stomach ulcers are caused. This led to further studies on the association between chronic *H. pylori* infections and the development of gastric cancer. In 2005, Marshall and Warren were awarded the Nobel Prize in Physiology for discovering *H. pylori* and its role in gastric diseases.[9]

H. pylori pathogenesis is related to several mechanisms. First, *H. pylori* produces urease to neutralize the stomach acid to survive and colonize. *H. pylori* uses its flagella for motility, allowing it to reach the gastric cells, where it attaches to host

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receptors through bacterial adhesins. This leads to successful colonization and infection. Lastly, *H. pylori* can produce the cytotoxins, CagA and VacA, both of which are associated with a higher chance of developing stomach cancer.[2]

H. pylori flagella are a motility organ composed of protein subunits, primarily the flagellin. The pathogenesis of *H. pylori* depends on colonization, with the flagella playing a major role in allowing *H. pylori* to navigate the gastric mucosal layer. Flagellar motility is essential for colonization.[10]

This review discusses the role of *H. pylori* flagella in colonization and inflammation, and the mechanisms associated with increased risk of gastric cancer following *H. pylori* infection.

Discussion

Roles of H. pylori Flagella in Colonization:

H. pylori has a helical motility organ of four to eight unipolar and distinct flagella that are composed of multiple protein subunits, including FlaA, FlaB, and FliD as shown in Figure 1.[10]

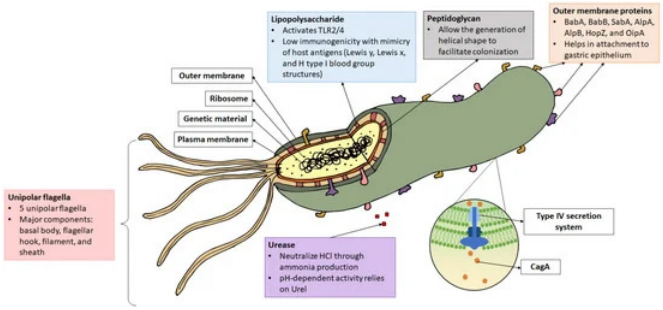

Figure 1: *H. pylori* morphology. Important outer membrane proteins, urease production, CagA, and structural composition of *H. pylori* are indicated. These important features allow for successful bacterial colonization.

Its helical shape helps its invasion into the mucous layer of gastric epithelial cells.[6] Once *H. pylori*, with the help of the flagella, reaches the gastric epithelium cells, it attaches firmly and establishes permanent colonization. In addition, the helical shape helps the bacteria to swim through the mucus gel present in the stomach. Its helical shape helps the bacteria to create a screw-like rotation while the flagella produce the force needed to move forward.[11]

The flagella are crucial for successfully colonizing the gastrointestinal mucosa.[10] The flagellar filament's structural proteins play an important role in bacterial motility to drive the bacterium through the mucus layer. The FaaA protein is found in the flagellar sheath, which protects the flagella from the effects of acid. Optimal flagellar function enables efficient colonization.[10]

Another role of the flagella is to protect *H. pylori* from the stomach's acidic environment. The pH within the stomach can reach pH 1-2, which is essential for digesting food. Without specialized mechanisms, this acidity harms most organisms by disrupting cellular homeostasis and damaging membranes and proteins. However, the mucus layer that lines the stomach has a pH 6-7 gradient, allowing the bacteria to survive near the stomach lining.[12] *H. pylori* can thrive in the stomach by protecting itself from the harmful stomach acid with the help of specialized systems. One such system is the presence of a flagellar sheath, which protects the flagellar filaments from damage. The structure of the flagella, with its outer sheath to protect against the acid, ultimately results in enhanced motility of *H. pylori*.[12]

Motility allows *H. pylori* to survive and thrive in the hostile environment of the stomach. *H. pylori* possesses 4-8 flagella. Motility plays a crucial role in the bacteria's ability to survive in harsh gastric conditions, thus enabling stable colonization of the gut.[14]

H. pylori senses acid, and flagellar motility is essential in enabling the bacteria to swim away from acid and reach the gastric mucus layer, where the pH is close to neutral. Thus, bacterial motility, particularly its flagella, is critical in enabling the bacteria to persist under these extreme conditions. In addition, bacterial urease production is also essential for acid resistance.

Once *H. pylori* colonizes the stomach, it triggers an inflammatory response.[6] Flagella in *H. pylori* are involved in both *H. pylori*-induced inflammation and immune evasion. Specific flagellin proteins that compose the flagella, such as FlaA and FlaB, can promote humoral immunity and trigger the immune system to produce antibodies that target *H. pylori* infection.[15]

H. pylori inflammation can evolve into chronic gastritis, a lifelong illness that causes inflammation and potentially progresses to atrophic gastritis. Chronic inflammation results from the inability to effectively clear the bacteria from the stomach. This leads to prolonged infection and continuous immune cell recruitment.[16]

Though the exact transmission route of *H. pylori* to the stomach is still being studied, research indicates that infections are the result of human-to-human transmission within families due to close contact and shared genetics.[3] Since *H. pylori* infections are often asymptomatic, infected patients are unaware of the ongoing infection until severe tissue damage occurs. Therefore, infections are usually diagnosed when chronic.[16] Some potential therapeutic vaccines have been used to target urease subunits; however, few have demonstrated protection against the infection. Thus, it is important to study the early stages of *H. pylori* infections by targeting certain pathways, such as NF-kB, which are one of the pathways *H. pylori* activates during the onset of gastric cancer.[16]

H. Pylori Motility and Survival in the Stomach and Links to Stomach Cancer:

Outer membrane proteins (OMPs) act as a barrier to help *H. pylori* bacteria withstand their harsh environment.[16] Three *H. pylori* outer membrane proteins are linked to gastric cancer: OipA, HomB, and HopQ. The host cell JAK/STAT signaling pathway functions as a signaling mechanism from the cell membrane to the nucleus and also triggers various mediators of cancer and inflammation.[24] When *H. pylori* adheres to gastric epithelial cells, OipA stimulates the JAK/STAT signaling pathway by triggering JAKs (a non-receptor tyrosine kinase) to phosphorylate STAT (Signal Transducers and Activators of Transcription). This causes STAT to translocate to the nucleus to bind to specific DNA sequences called GAS (Interferon γ-Activated Sequence), leading to inflammatory gene expression.[25] Dysregulation of these pathways by OipA can disrupt

normal cell functions, which may ultimately contribute to the development of gastric cancer.[6]

HomB is another outer membrane protein of *H. pylori* that binds to gastric epithelial cells and initiates inflammation in the gastric mucosa. HopQ facilitates the transfer of CagA protein into gastric epithelial cells through a cell surface receptor called CEACAM (carcinoembryonic antigen-related cell adhesion molecule). However, the exact mechanism by which HopQ's interaction with CEACAm facilitates CagA transfer is unclear. Once inside gastric epithelial cells, CagA alters normal cell signaling and promotes inflammation. This also causes DNA damage, which increases the risk of gastric cancer.[25]

H. pylori produces intracellular urease, around 10% of the total protein produced, indicating that it's crucial to the bacteria's survival. Urease is also found extracellularly and contributes to the external neutralization of stomach acid. Urease-catalyzed urea hydrolysis into ammonia (NH3) and carbon dioxide (CO_2). Ammonia neutralizes the stomach acidity around the bacteria, while carbon dioxide helps maintain a balanced pH around the bacteria. NH_3 and CO_2 production are necessary for creating an environment for *H. pylori*'s survival. Ammonia production can disrupt cell junctions and damage the gastric epithelium.[14] Urease was recently found to contribute to tumor growth by promoting angiogenesis.[17] *H. pylori* urease can also activate the PI3K-AKT-mTOR, a major signaling pathway in regulating cell growth and cell cycle in gastric cells. Increased mTOR activity increases the level of a protein called HIF-1α, and urease was found to trigger the differentiation of endothelial cells by generating reactive oxygen species and activating lipoxygenase, an inflammatory pathway.[14] These mechanisms aid *H. pylori*'s survival in the stomach's acidic environment while simultaneously increasing the risk of gastric cancer.

H. pylori is a spiral organism, but coccoid forms can be found, depending on environmental conditions, including pH. *H. pylori* exists in its spiral form, known as spiral viable culturable form (SVCF), only under favorable conditions; it is capable of causing infections. However, under harsh conditions, *H. pylori* can transform into its coccoid form known as coccoid viable non-culturable form (CVNCF), which can persist for a prolonged time, enabling it to survive under unfavorable conditions. Due to this unique mechanism, the spiral/helical shape promotes motility through the corkscrew mechanism. This mechanism and flagellar motility allow *H. pylori* to migrate quickly to the gastric epithelial surface.[28]

The flagellum of *H. pylori* allows the bacterium to move efficiently through the basal layer of the gastric epithelium. In this context, the flagellum is a virulence factor because its increased motility enables initial colonization. In addition, once *H. pylori* moves through the basal layer of the stomach, it can obtain nutrients and metabolic substrates from the host's cells and release toxins to damage the host cells, contributing to gastric cancer.[19]

H. Pylori Virulence and Links to Stomach Cancer:

Virulence factors are molecules or structures produced by pathogens, such as bacteria, viruses, or fungi, enabling them to infect and cause disease in a host. *H. pylori*'s virulence factors trigger several mechanisms that allow it to invade, colonize, and stimulate inflammation within the gastric mucosa. The severity of *H. pylori* infections is associated with the type of virulence factors expressed since they control and regulate inflammatory responses, promoting immune evasion. The sequence of events that occur in *H. pylori* colonization and infection is shown in Figure 2.[15]

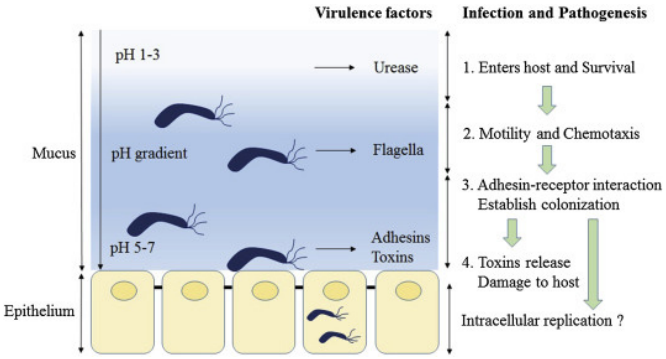

Figure 2: *H. pylori* infection and pathogenesis. Flagella-mediated motility, adhesions, toxins, and urease production contribute to successful bacterial colonization, which leads to the survival of *H. pylori* in the stomach.

Major toxins that *H. pylori* releases are cytotoxin-associated gene product A (CagA), vacuolating cytotoxin A (VacA), and high-temperature requirement A (HtrA). Important adhesions that mediate binding of *H. pylori* to gastric cells are blood group antigen-binding adhesion (BabA), outer inflammatory protein (OipA), outer membrane protein (OMP), outer membrane vesicles (OMV), neutrophil-activating protein A (NapA), and sialic acid-binding adhesins (SabA), as shown in Figure 3.[18]

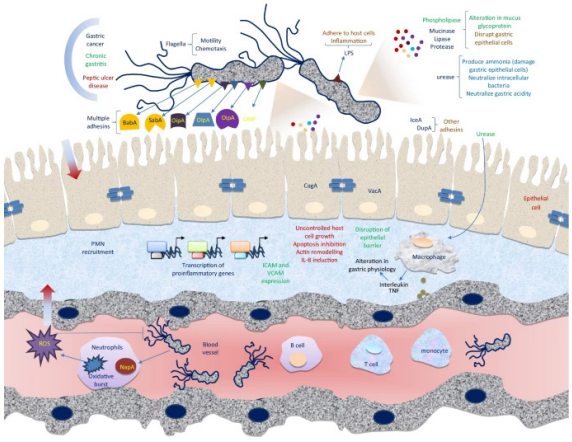

Figure 3: Pathogenic mechanisms of *H. pylori*. Four key stages are involved in H· pylori colonization: adapting to the stomach's acidic environment, moving to the epithelial cells, penetrating and attaching to the epithelial cell barrier, and causing tissue damage.

Once *H. pylori* successfully colonizes the gastric epithelium, the toxins produced by *H. pylori* damage host tissues. The damage triggers the immune system to release signaling molecules called chemokines. Chemokines recruit neutrophils to the infection area, which causes inflammation.[18] They are also involved in promoting angiogenesis and influencing tumor growth.[26]

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Bacterial colonization triggers humoral and cellular responses by recruiting immunoinflammatory cells such as lymphocytes, neutrophils, etc. This damages the stomach's epithelial cells. In addition, *H. pylori* causes NF-kB activation, which leads to long-term chronic infection. NF-kB is a proinflammatory pathway activated to release various proinflammatory cytokines and chemokines, playing a role in evading apoptosis.[27]

CagA (cytotoxin-associated gene A) protein is highly immunogenic and is present in around 50 to 70% of *H. pylori* strains. The CagA protein is part of a larger genomic element called the cag pathogenicity island (PAI). The PAI encodes components of the Cag T4SS, which is required for the translocation of CagA from the bacteria into host cells. It was found that patients with CagA+ strains have an increased inflammatory response, leading to a higher risk of developing gastric cancer.[21] The CagType 4 secretion system (-T4SS) is encoded for genes on the cag PAI, and it functions to inject the CagA protein directly into the gastric epithelial cells.[25]

CagA is then phosphorylated at a specific motif (EPIYA), enabling it to bind to the SHP-2 protein. This activates oncogenic signaling processes, which cause abnormal changes to cell polarity, cell proliferation, proinflammatory responses, etc., which is shown in Figure 4.[16]

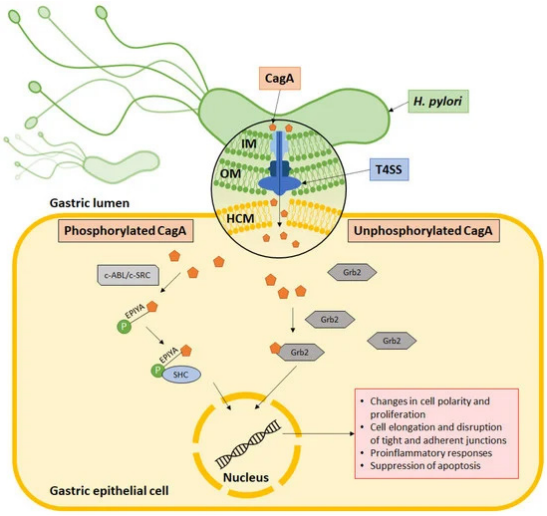

Figure 4: Bacterial virulence factor, CagA. Cag PAI influences *H. pylori*'s pathogenicity by facilitating the transfer of virulence factors, including CagA, into the host cells.

The Cag PAI influences *H. pylori*'s pathogenicity by facilitating the transfer of virulence factors into cells. Non-phosphorylated CagA from *H. pylori* can manipulate cell signaling by disrupting E-cadherin (a cell adhesion protein) and β-catenin interactions. Non-phosphorylated CagA binds to E-cadherin, which results in the dissociation of the E-cadherin-β-catenin complex.[16] The E-cadherin-β-catenin complex is important for maintaining epithelial integrity.[23] B-catenin accumulates in the cytoplasm and moves to the nucleus to form a complex with Tcf (T cell factor). This activates the transcription of genes, including Cyclin D1 and c-Myc, which promote cell growth and division. Non-phosphorylated CagA also interacts with SOS (guanine nucleotide exchange factor) to activate the Ras/MEK/ERK pathway. The Ras/MEK/ERK pathway is a signaling cascade that regulates cellular functions, but dysregulation can lead to cancer.[25] Overall, this leads to loss of cell polarity, mitogenic responses, and proinflammatory signaling involved with gastric cancer development. CagA protein was also found to promote DNA double-stranded breaks and activate Hippo signaling (a pathway that regulates cell proliferation and apoptosis), contributing to genome instability, and is involved in cancer development. CagA was further observed to induce epithelial-mesenchymal transition (EMT), a cellular process that increases tumor invasiveness and metastatic activity.[22] Along with the activation of YAP (Yes-associated protein) signaling, this accelerates carcinogenesis and the spread of cancer.[16]

Once CagA is injected inside the cell, it can be phosphorylated and bind to a phosphatase known as SHP-2. This binding can influence cell adhesion, spread, and migration. In addition, CagA can affect host cell behavior by inducing cytoskeleton rearrangements and cell proliferation. Nonphosphorylated CagA can activate the phosphatidylinositol 3-kinase/Akt pathway, which promotes gastric cell proliferation and leads to the development of chronic gastritis and eventually, gastric cancer.[19]

VacA causes vacuolization (formation of vacuoles that disrupts cell function), necrosis (induces cell death), and apoptosis. VacA can integrate into the host cell membrane and act as an anionic selection channel to release bicarbonate and organic anions into the cytoplasm. As a result, it can help *H. pylori* colonization by providing metabolic substrates for growth. VacA can also target various organelles within the host cells; for example, it can enter the endosome to release cytochrome C and trigger apoptosis. In addition, it was observed that VacA can cause stress in the endoplasmic reticulum (ER). This further promotes apoptosis in gastric epithelial cells, which can influence gastric atrophy and cancer development.[19] Furthermore, another virulence protein called OipA is known to adhere to gastric epithelial cells, causing mucosal damage and host cell apoptosis. It was also found that OipA-positive strains improved CagA's translocation, contributing to cell proliferation and damage.[14]

H. pylori is a spiral organism, but coccoid forms can be found, depending on environmental conditions, including pH. *H. pylori* exists in its spiral form, known as spiral viable culturable form (SVCF), only under favorable conditions; it is capable of causing infections. However, under harsh conditions, *H. pylori* can transform into its coccoid form known as coccoid viable non-culturable form (CVNCF), which can persist for a prolonged time, enabling it to survive under unfavorable conditions. Due to this unique mechanism, the spiral/helical shape promotes motility through the corkscrew mechanism. This mechanism and flagellar motility allow *H. pylori* to migrate quickly to the gastric epithelial surface.[28]

The flagellum of *H. pylori* allows the bacterium to move efficiently through the basal layer of the gastric epithelium. In this context, the flagellum is a virulence factor because its increased motility enables initial colonization. In addition, once *H. pylori* moves through the basal layer of the stomach, it can obtain nutrients and metabolic substrates from the host's cells and

release toxins to damage the host cells, contributing to gastric cancer.[19]

***H. pylori*-Induced Prolonged Inflammation and Its Link to Stomach Cancer:**

Prolonged inflammation of the gastric tissue due to *H. pylori* generates large amounts of nitric oxide, which can damage the gastric epithelial cells.[6] In addition, *H. pylori* secretes a bacterial protein called *H. pylori* neutrophil-activating protein (HP-NAP), which can activate neutrophils (a type of white blood cell) and influence immune responses that contribute to tissue damage and cancer development. Once HP-NAP activates neutrophils, it generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) to adhere to endothelial cells. ROS and RNS induced by *H. pylori* can cause DNA damage and mutations, which can lead to gastric cancer.

H. pylori infections can trigger DNA damage in host cells, negatively affecting how DNA repair processes work, resulting in genetic instability and chromosomal abnormalities.[19] The *H. pylori* protein HP-NAP activates neutrophils, which produce ROS and RNS.[20] Overproduction of ROS and RNS induces several types of DNA damage, including point mutations, DNA adducts, 8-OHdG, and DSB (single or double-strand DNA breaks). APE1 is a crucial protein that helps cells respond to oxidative stress by repairing damaged DNA. However, *H. pylori* can alter normal APE1 functions, including DNA repair and gene regulation, in various ways. Although increased oxidative stress from *H. pylori* infection can increase APE1 levels to repair DNA damage, a chronic *H. pylori* infection can eventually reduce APE1 expression, which results in genetic instability. In addition, genetic and epigenetic changes can impact the repair of DNA. Altogether, these changes cause inaccurate DNA repair, genomic instability, and chromosomal aberrations, which promote gastric cancer.

Conclusion

This review depicts the importance of *H. pylori* motility, virulence factors, and inflammatory response and their links to stomach cancer. About 50% of the world's population and approximately 30-40% of the population in the US are infected with *H. pylori*. Recent improvements in *H. pylori* infection rates are largely due to improved hygiene and reduced transmission. However, novel treatment mechanisms are necessary to combat resistance, and much remains unknown about *H. pylori*'s pathogenicity and its link to stomach cancer. In this respect, the flagella's structure may be a crucial target for successful bacterial eradication. Associated mechanisms such as urease production and inflammatory responses or toxins (CagA, VacA, etc.), differences in shapes, and DNA damage influence the severity of *H. pylori* infections and thus, cancer formation. Therefore, further research on *H. pylori*'s pathogenic mechanisms, including the role of flagella, can allow for more effective personalized treatment methods and prevention.

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REVIEW ARTICLE

Proton Beam Therapy on Pediatric Cancer: A Review

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ABSTRACT: Proton beam therapy (PBT) is an advanced radiation modality that offers significant benefits in pediatric oncology, particularly in reducing radiation-induced toxicities and secondary malignancies. Unlike conventional photon-based therapies such as intensity-modulated X-ray therapy (IMXT) and volumetric-modulated arc therapy (VMAT), PBT utilizes the Bragg peak to deliver radiation precisely to tumors while sparing surrounding healthy tissues. This advantage is crucial in children, where long-term survivorship is a major concern. Studies show that PBT lowers the lifetime attributable risk (LAR) of secondary cancers by up to 50% in whole central nervous system (CNS) treatments and significantly reduces organ-at-risk (OAR) exposure, particularly in the brain, spinal cord, and optic structures. Despite challenges such as high costs, limited accessibility, and technical complexities, growing clinical and dosimetric evidence demonstrates that PBT is superior to conventional radiotherapy, providing safer and more effective treatment for pediatric cancer patients.

KEYWORDS: Biomedical and Health Sciences, Physical Medicine, Pediatric Cancer Oncology, Proton Beam Therapy, Secondary Cancer Prevention.

Introduction

Cancer is one of the leading causes of death among children worldwide, with approximately 400,000 new cases diagnosed annually.[1] Unlike adult cancers, which often result from long-term environmental exposures and accumulations of genetic mutations, pediatric cancers arise due to developmental disruptions, making them biologically distinct and often highly aggressive.[2] Common types include leukemia, brain tumors, lymphomas, and sarcomas, which typically require surgery, chemotherapy, and radiation.[3] Advances in treatment have raised survival rates to over 80% in many high-income countries, but they also bring long-term risks.[4] Children's developing tissues are highly sensitive to radiation, increasing the likelihood of chronic health issues, cognitive impairment, and secondary cancers.[5] These concerns highlight the need for safer treatments — an area where PBT is gaining attention due to its ability to reduce radiation-induced damage.[6]

Radiation therapy has been a cornerstone in cancer treatment for over a century, offering a targeted method of destroying malignant cells through DNA damage. By inducing breaks in cellular DNA, radiation disrupts the ability of cancer cells to proliferate, leading to tumor shrinkage and eradication.[7] However, while radiation therapy is highly effective, normal tissues in the treatment field are also exposed to radiation. This is particularly concerning in pediatric patients, whose rapidly dividing cells are more susceptible to radiation-induced damage, increasing the risk of both acute and long-term toxicities.[3] Children who receive radiation therapy are at a higher risk of developing secondary cancers later in life because of radiation exposure to healthy tissues.[5] This has led to significant efforts to refine radiation delivery techniques to improve targeting accuracy while reducing exposure to surrounding normal structures.[3]

Radiation therapy can be broadly categorized into two main types: external beam radiation therapy (EBRT) and internal radiation therapy, or brachytherapy.[2] EBRT is the most commonly used form, delivering high-energy radiation from an external source to the tumor site, while brachytherapy involves placing radioactive material directly within or near the tumor to provide localized radiation exposure.[6] Within EBRT, there are two primary subtypes: photon therapy and proton therapy. Photon therapy, which includes X-rays and gamma rays, has been the traditional standard for radiation treatment. It works by delivering energy that ionizes atoms in the target tissue, leading to DNA damage and cell death.[7] However, a major limitation of photon therapy is that it deposits energy continuously along its entire path, making both healthy tissues before and beyond the tumor receive radiation exposure.[4] This increases the likelihood of side effects and long-term complications, particularly for pediatric patients.[8] Advanced photon-based techniques, such as intensity-modulated radiation therapy (IMRT), including Intensity-modulated X-ray Therapy (IMXT), and volumetric-modulated arc therapy (VMAT), have improved dose conformity to some extent, but they still exhibit the fundamental limitation of exit dose beyond the tumor.[9]

Then, Three-Dimensional Conformal Radiation Therapy (3D-CRT) provides improved dose targeting over conventional methods but lacks the precision of IMXT or PBT by shaping photon beams to match the tumor's contour. While it provides better dose distribution than conventional radiation, it lacks the precise modulation of IMXT and VMAT, leading to higher radiation exposure in nearby tissues. Helical Tomotherapy, a variation of IMXT, delivers radiation in a spiral pattern for complex tumors but still irradiates more normal tissue than PBT.[10] Lastly, Electron Beam Therapy (EBT) uses electrons

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and is effective for superficial cancers but lacks penetration for deeper tumors.[11]

In contrast, PBT's main distinction lies in its highly precise radiation delivery and superior dose distribution.[3] Unlike photons, protons are charged particles that gradually deposit energy until reaching a sharp peak, known as the Bragg peak, where they release most of their energy and stop.[2] This enables targeted radiation confined to the tumor, sparing surrounding tissues and reducing unnecessary exposure.[3] Such precision is particularly valuable in pediatric oncology, where minimizing toxicity is essential. Tumors near critical organs—like brain tumors close to the brainstem and spinal cord, or sarcomas near the heart and lungs—are especially suited for PBT, as photon-based therapies would expose these areas to potentially harmful doses.[5]

The increasing recognition of PBT's potential in pediatric oncology has led to growing interest in evaluating its clinical benefits. In this review, an analysis of PBT in pediatric oncology will be conducted, evaluating its physical and biological mechanisms, clinical applications, and potential advantages over conventional photon therapy. Also, current challenges in implementing PBT—including financial barriers, accessibility issues, and the need for further comparative studies—will be explored to provide a balanced perspective on its viability.

Discussion

Physics of PBT:

To draw conclusions about PBT, it is essential to understand the underlying physical principles of the therapy.

Proton therapy uses high-energy protons to destroy cancer cells by interacting with atoms in tissue and gradually losing energy. The main mechanism is Coulomb collisions, interactions between positively charged protons and negatively charged electrons, which lead to ionization and molecular damage. This energy loss is quantified as stopping power, which increases as protons slow down, causing them to release most of their energy at a specific depth known as the Bragg Peak.[12]

Another way protons lose energy, although it happens less frequently in PBT, is through bremsstrahlung radiation losses. This occurs when a moving charged particle, such as a proton, is suddenly deflected by the strong electric field of an atomic nucleus. When this happens, the particle loses some of its energy in the form of electromagnetic radiation, which is typically in the X-ray range. However, because protons are much heavier than electrons, they do not lose as much energy through bremsstrahlung radiation as lighter particles do.[7]

The loss of energy by protons does not happen in a perfectly predictable way. Instead, it is a stochastic process, meaning that it involves an element of randomness. Although the overall energy loss follows a general pattern, individual protons may lose slightly different amounts of energy due to variations in their interactions with electrons. This randomness leads to energy straggling, which means that even if all protons start with the same energy, they will not all stop at the same depth. Some protons will stop slightly earlier, while others will travel a bit farther. This makes it necessary to carefully account for variations when planning PBT treatments as those variations broaden and reduce the sharpness of the Bragg Peak.[12]

Another key effect in proton therapy is multiple Coulomb scattering. In addition to electrons, protons interact with positively charged atomic nuclei, causing repulsion and slight deflections. Repeated scattering events lead to beam broadening, reducing dose precision and altering energy delivery at various depths, especially in heterogeneous tissues like bone adjacent to soft tissue. This spread can be modeled using Gaussian approximations to predict deflection angles statistically.[12]

Besides Coulomb interactions, protons can also trigger nuclear reactions when they collide directly with atomic nuclei, rather than just being deflected by them. These nonelastic nuclear reactions are significant because they alter the energy and composition of the proton beam. About 21% of the energy lost by a 250-MeV proton beam in water is due to nuclear reactions, while for a lower-energy 70-MeV beam, this fraction decreases to about 4%. When a high-energy proton, such as a 250-MeV proton, interacts with an oxygen-16 nucleus, about 66% of the proton's energy is transferred to newly created secondary protons, 21% goes to secondary neutrons, and smaller fractions contribute to other nuclear fragments such as alpha particles and recoil nuclei.[12]

Near the end of their range, protons lose energy differently through nuclear reactions. At around 10 MeV, about 17% of this energy goes to secondary protons, with minimal transfer to neutrons. Alpha particles and recoil nuclei still contribute slightly. These heavy fragments have high Linear Energy Transfer (LET), meaning they deposit energy densely and are highly damaging to cells. In contrast, secondary neutrons spread energy over larger areas with less local damage due to infrequent interactions.[7]

As protons travel deeper into tissue, their stopping power increases, peaking at the Bragg Peak where energy deposition reaches up to 80 keV/μm. This concentrated dose enables effective tumor targeting while sparing surrounding tissues. At the distal edge of the Bragg Peak, the dose-averaged LET exceeds 10 keV/μm, enhancing precision. In contrast, Cobalt-60 gamma rays have a much lower LET (<2 keV/μm), dispersing energy over a wider area and increasing damage to healthy tissue.[12]

So, since LET plays a role in how effective radiation is at killing cancer cells, PBT has a higher relative biological effectiveness (RBE) compared to photon radiation. RBE is a measure of how effectively a particular type of radiation causes biological damage compared to standard X-rays or gamma rays. Studies at the Harvard Cyclotron Laboratory and other research centers have found that the RBE of proton beams increases near the Bragg Peak, leading to the adoption of a universal RBE factor of 1.1. What this says is that the dose from a proton beam is 10% more effective than an equivalent dose from standard photon radiation. However, at very high LET values (above 100 keV per micrometer), the RBE decreases because too much energy is being deposited into individual cells, leading to overkill effects, where excess radiation is wasted.[7]

Protons' ability to stop at a specific depth based on their initial energy allows clinicians to use different proton beam energies to control exactly where the Bragg Peak occurs, ensuring that the highest dose is deposited precisely in the tumor.[12] However, a single-energy proton beam would only treat a thin slice of the tumor. Hence, the solution is to create a Spread-Out Bragg Peak (SOBP), to cover a larger tumor volume, multiple proton energies are combined, effectively superimposing multiple Bragg Peaks to provide uniform radiation across the tumor while still preserving the sharp dose falloff beyond it.[10]

In contrast, X-ray therapy delivers radiation in an exponential decay pattern, meaning healthy tissues both before and after the tumor receive a significant dose. This is because photons interact probabilistically, which causes energy to spread unpredictably throughout the body.[9]

Quantitative differences between PBT and other forms of radiotherapy:

PBT significantly lowers the lifetime attributable risk (LAR) of radiation-induced secondary cancers compared to intensity-modulated X-ray therapy (IMXT), especially in pediatric patients. Reported reductions range from 1.02% to 50%, with an average of 22.73%, depending on the treatment area (Table 1).[9] For example, LAR differences are modest in brain and head-and-neck cancers but much greater in whole CNS treatments, where IMXT irradiates broader regions. This advantage stems from PBT's Bragg peak, which delivers radiation precisely to the tumor while sparing nearby tissues, unlike IMXT's wider, less selective dose distribution that raises long-term complication risks.[13]

Table 1: LAR (Lifetime Attributable Risk) differences between PBT and IMXT, and the number needed to treat (NNT) for each organ at risk. Category A: brain, head, and neck; B: chest; C: abdomen; D: whole central nervous system.[9]

Organ at Risk	A. Brain, H&N (LAR Diff. % ± SD)	NNT	p-value	B. Chest (LAR Diff. % ± SD)	NNT	p-value	C. Abdomen (LAR Diff. % ± SD)	NNT	p-value	D. Whole CNS (LAR Diff. % ± SD)	NNT	p-value
Brain	0.77 ± 0.44	131	0.0036**	0.00 ± 0.01	-20000	0.230	-	-	-	-	-	-
Female Breast	-	-	-	7.46 ± 13.34	13.4	0.158	0.59 ± 1.19	171	0.207	15.9 ± 14.2	6.3	0.0960
Lung	-	-	-	3.23 ± 1.41	31.0	0.0003**	3.76 ± 2.59	26.6	0.0642	-	-	-
Colon	-	-	-	9.22 ± 14.96	10.8	0.125	12.5 ± 19.7	8.0	0.115	22.19 ± 6.94	4.5	0.0156*
Stomach	-	-	-	2.02 ± 1.95	49.6	0.0220*	1.89 ± 1.58	53.0	0.0118*	3.45 ± 2.44	29.0	0.0671
Small Intestine	-	-	-	0.63 ± 0.78	160	0.0589	0.72 ± 0.80	139	0.0384*	2.30 ± 2.79	43.4	0.145
Liver	-	-	-	0.60 ± 0.42	166	0.0046**	0.49 ± 0.25	204	0.0009**	1.10 ± 0.14	90.6	0.0026**
Bladder	-	-	-	0.17 ± 0.32	584	0.171	0.14 ± 0.25	698	0.211	-	-	-
Thyroid	0.01 ± 0.01	18900	0.356	-0.03 ± 0.13	-3590	0.565	1.09 ± 0.18	91.8	0.0046**	-	-	-
Bone	0.08 ± 0.05	1210	0.0043**	0.03 ± 0.04	2950	0.0455*	0.08 ± 0.07	1310	0.0183*	0.03 ± 0.02	3230	0.0608
Soft Tissue	0.17 ± 0.16	596	0.0335*	0.10 ± 0.09	1030	0.0231*	0.20 ± 0.17	506	0.0121*	-	-	-
Cumulative LAR	1.02 ± 0.52	98.0	0.0021**	23.3 ± 17.2	4.3	0.0065**	16.6 ± 19.9	6.0	0.0497*	50.0 ± 21.1	2.0	0.0274*

Beyond pediatric oncology, PBT also shows clear benefits over volumetric modulated arc therapy (VMAT) and electron beam therapy (EBT), especially when it comes to reducing the dose to organs at risk (OARs), as shown in Figure 1. Dosimetric studies have found that PBT can lower both the average and peak radiation doses to critical structures, in some cases by as much as 100%, particularly when those organs are centrally located and contralateral OARs. The precision of PBT enables superior dose conformity, making it highly effective for treating superficial skin cancers like angiosarcoma without requiring a bolus.[9] Clinical outcomes also support these findings; for instance, a case study on a patient treated with PBT for recurrent scalp angiosarcoma showed no signs of recurrence six months post-treatment and experienced only mild, manageable side effects such as eyelid dryness.[11] VMAT and EBT, while offering comparable target volume coverage, result in higher unintended radiation exposure to adjacent healthy tissues, increasing the likelihood of toxicities.

PBT also demonstrates clear dosimetric benefits in breast cancer treatment, which is globally one of the most recurrent cancer types, particularly in reducing radiation exposure to the heart and lungs. Compared to 3D-conformal radiation therapy (3D-CRT), intensity-modulated radiotherapy (IMRT), and tomotherapy, PBT reduces radiation exposure to non-target breast tissue by 40.9%, 33.3%, and 22.8%, respectively.[10]

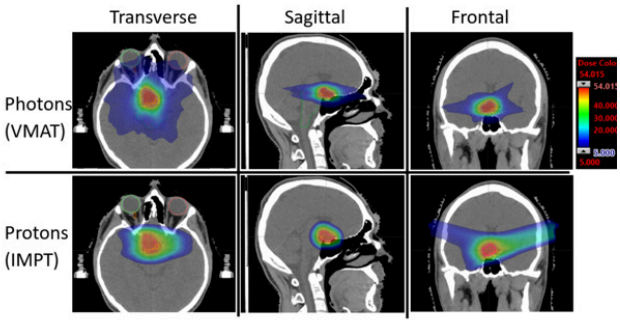

Figure 1: Comparison of dosimetry between VMAT and IMPT on three orthogonal planes. Overlaid structures include targets as well as organs-at-risk (OARs).[14]

Additionally, PBT minimizes the mean heart dose by up to 50% relative to photon-based therapies, which is crucial for mitigating long-term cardiovascular complications associated with breast irradiation. By sparing the non-planning target volume (PTV) breast tissue from receiving 50% of the prescribed dose, PBT enhances treatment safety without compromising tumor control. The dosimetric superiority of PBT over photon-based approaches is a result of its ability to deliver highly conformal radiation while limiting dose spillage to adjacent critical structures.[10]

A significant concern in left-sided breast cancer treatment when using radiotherapy is reducing radiation-induced cardiotoxicity. When conventional radiotherapy often exposes the heart to ionizing radiation, PBT enhances treatment safety, making it the preferred option for breast cancer patients with preexisting cardiovascular conditions or increased susceptibility to radiation-induced heart disease.[15]

In terms of survival outcomes, PBT has demonstrated superior efficacy in specific cancer types. For glioblastoma multiforme, a meta-analysis identified PBT as the modality most likely to improve overall survival (OS) and progression-free survival (PFS), with a 72.6% probability of yielding the best

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effective across multiple tumor types, has a 66.5% probability of improving OS for glioblastoma patients. EBT, primarily used for superficial malignancies like basal and squamous cell carcinomas, achieves high cure rates (90–98%) in these cases but lacks efficacy for deeper or more complex tumors.[13]

Finally, a dosimetric study comparing PBT to IMRT and VMAT found that proton therapy reduced mean and maximum doses to critical organs in pediatric brain tumors by over 50%, particularly in regions like the hippocampus and optic chiasm, which are highly sensitive to radiation.[3] Similarly, in breast cancer irradiation, PBT reduced heart and lung exposure by nearly 90% compared to IMRT, an essential factor in minimizing long-term cardiac toxicity.[10]

Challenges and Limitations:

PBT faces multiple challenges and limitations that can hinder its widespread adoption and clinical effectiveness. One of those challenges is its prohibitively high cost. Establishing a single-room proton therapy center costs approximately $20 million, while multi-room facilities exceed $225 million.[13] Additionally, pre-treatment costs for PBT are estimated to be 1.5 to 3 times higher than those of photon-based therapies such as Intensity-Modulated Radiation Therapy (IMRT) and Volumetric Modulated Arc Therapy (VMAT).[5] This cost puts it out of reach for many patients, especially in low- and middle-income countries where even standard radiation therapy often lacks enough funding.

Also, the uncertainty surrounding its radiobiological effectiveness (RBE) is an issue. Unlike photon therapy, which has a well-established relative biological effectiveness (RBE) of 1.0, the RBE of protons varies across different tissues and tumor types, typically estimated at 1.1 but potentially higher at the Bragg Peak.[4] This makes it difficult to precisely predict biological effects in different patients, complicating dose calculations.

Additionally, there is still a lack of long-term clinical outcome data for PBT, especially in pediatric patients. Most large-scale randomized trials comparing PBT with IMRT or VMAT are still ongoing or in the early stages.[4]

Finally, the physical properties of protons, while advantageous in dose localization, introduce complexities in treatment planning and beam delivery. One major issue is range uncertainty, where small errors in tissue heterogeneity, CT calibration, or proton beam energy can cause significant deviations in where the Bragg Peak occurs.[5]

Radiosensitivity of developing tissues in children and long-term side effects:

Children are particularly susceptible to the long-term adverse effects of radiation therapy since their developing tissues exhibit heightened radiosensitivity. Proton therapy, with its superior dose distribution and reduced radiation exposure to surrounding normal tissues, has been proposed as a more favorable alternative to conventional photon therapy for pediatric patients. The significance of this approach is particularly evident in pediatric brain tumors, where radiation exposure can lead to cognitive decline, growth disturbances, and endocrine dysfunction. Studies have demonstrated that radiation-induced cognitive impairments are more pronounced in younger children, and PBT has been modeled to reduce this risk by limiting radiation exposure to non-targeted brain regions.[3] The impact of radiation on the developing brain has been observed in various pediatric tumors, including medulloblastoma, where cognitive function, IQ scores, and reading abilities tend to decline post-radiation. Proton therapy, by delivering radiation more precisely, has shown promise in reducing these neurocognitive deficits, particularly in young patients who are at the highest risk for developmental delays.[5]

The risk of secondary malignancies is one of the most critical concerns in pediatric radiation oncology. Given that children have a longer post-treatment life expectancy compared to adults, minimizing radiation exposure to normal tissues is essential in reducing the likelihood of radiation-induced cancers. Dosimetric studies estimate that PBT decreases the likelihood of secondary tumors by a factor of 8 compared to intensity-modulated radiation therapy (IMRT) and by a factor of 15 compared to conventional photon therapy. Consequently, pediatric oncology places strong emphasis on investigating late treatment effects, as demonstrated by long-term studies on survivorship, which indicate that many children treated with traditional radiation modalities develop secondary malignancies decades after treatment. This reinforces the necessity of PBT or some other therapy that is safer.[3]

Additionally, growth abnormalities remain a major concern in pediatric radiation therapy, particularly for patients requiring craniospinal irradiation. The spine and nearby muscles and bones are very sensitive to radiation, and exposure during treatment can sometimes cause lasting problems with spinal growth. How serious these effects are can depend on the patient's age, sex, and which part of the spine is treated. Some studies suggest that avoiding the spine's central canal during irradiation may mitigate these risks, although long-term impacts remain incompletely understood.[4] PBT reduces the risk of exposure to adjacent structures such as growth plates, which are crucial for normal skeletal development, by sparing these structures and is expected to result in fewer skeletal deformities and an overall improvement in quality of life for pediatric cancer survivors.[3]

Neurocognitive and developmental effects are major long-term concerns in pediatric oncology, especially for children with central nervous system (CNS) tumors. Radiation to the developing brain is linked to deficits in memory, attention, and processing speed. Studies in medulloblastoma patients show that lowering radiation to non-targeted brain regions improves outcomes, with PBT outperforming conventional photon therapy.[4] This is particularly important in younger children, where brain plasticity is still forming. Modeling studies also suggest PBT reduces memory loss and IQ decline.[5] By minimizing exposure to healthy brain tissue, PBT helps preserve key cognitive functions, supporting better long-term quality of life.[4]

Also, particularly in pediatric patients with central nervous system (CNS) malignancies, acute toxicities are generally mild to moderate, with fatigue, alopecia, nausea, and dermatitis being the most common. The severity of these toxicities depends on factors such as tumor location and concurrent treatments,

with infratentorial tumors more frequently leading to headaches and nausea. Quantitatively, fatigue was reported in 67% of patients, alopecia in 73%, and nausea in 46%, but these effects were manageable with supportive care.[16] Although PBT reduces long-term toxicities compared to conventional radiotherapy, the evidence shows that continuous monitoring is essential.

In nonsmall cell lung cancer (NSCLC) patients, PBT enables the delivery of higher radiation doses with lower rates of severe esophagitis and pneumonitis, showing a good improvement over 3D-CRT and IMRT, which exhibit much higher toxicity rates.[17]

Thus, overall toxicity across multiple organ systems is lower with PBT than with photon-based therapies, though some unique complications have been reported in case studies, such as radiation necrosis, Moyamoya syndrome, and increased sensitivity to range uncertainties. Despite a lower frequency of pulmonary, cardiac, and gastrointestinal toxicities, long-term effects still require further study.[15]

In pediatric patients, acute toxicities associated with PBT are well-tolerated and manageable. The precise targeting of tumors significantly reduces radiation exposure to normal brain tissue, lowering the risk of cognitive and endocrine dysfunctions compared to conventional radiotherapy.[18] This is particularly relevant for younger patients, as minimizing radiation to the developing tissue is of great importance.

Conclusion

Summarising, PBT represents a major advancement in radiation oncology, offering great precision, superior dose distribution, and significantly reduced toxicity compared to conventional photon-based therapies such as IMXT, VMAT, and EBT.[4] The Bragg peak phenomenon allows PBT to deliver radiation precisely to the tumor while sparing surrounding healthy tissues, making it particularly beneficial in pediatric cancer treatment, where reducing secondary malignancies and long-term toxicities is crucial.[9]

Quantitative data confirm the advantages of PBT, as studies show that it reduces the lifetime attributable risk (LAR) of secondary cancers by up to 50% in whole central nervous system (CNS) treatments, while in breast cancer cases, it minimizes mean heart dose by 50% compared to photon-based therapies.[10] To add, PBT decreases radiation exposure to organs at risk by up to 100%, particularly in tumors near the brainstem, spinal cord, and optic nerves.[11] Its physical properties set it apart from conventional photon-based treatments.[6]

Despite its benefits, PBT faces several challenges. Its high cost remains the biggest barrier, as multi-room proton therapy centers exceed $225 million, and per-treatment costs are up to three times higher than IMXT or VMAT.[13] Accessibility is another issue, with fewer than 100 centers worldwide, leading to long wait times for patients in need.[4] Technical difficulties, such as range uncertainties and motion sensitivity, complicate treatment planning, requiring advanced imaging and real-time tracking to ensure accuracy.[5] Additionally, while dosimetric models predict fewer toxicities, large-scale randomized clinical trials comparing PBT and photon therapy are still ongoing, making some oncologists hesitant to fully replace conventional radiotherapy.[4]

Despite its current limitations, PBT is positioned to become the gold standard in precision radiation therapy. Its consistent superiority in CNS treatments highlights its value in pediatric oncology, where safety margins are narrow. Advances in proton delivery, cost-reduction strategies, and expanding clinical evidence continue to strengthen its role.[5] Innovations like proton-immunotherapy and AI-based planning may further improve its efficacy and accessibility.[13] The long-term benefits of PBT in minimizing complications and enhancing outcomes make it a transformative tool in cancer care. While challenges related to accessibility and cost remain, continued investment and innovation in PBT hold great promise for expanding its reach, ultimately providing more patients with a safer and more precise alternative to conventional radiotherapy. While challenges related to accessibility and cost remain, continued investment and innovation in PBT hold great promise for expanding its reach, ultimately providing more patients with a safer and more precise alternative to conventional radiotherapy.[17]

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REVIEW ARTICLE

Impact of the COVID-19 Pandemic on Women's Entrepreneurship in the European Union: Challenges and Opportunities - A Literature Review

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ABSTRACT: Women's entrepreneurship drives economic growth by launching new businesses, which boosts economic activity and spurs innovation. Female entrepreneurs frequently tackle social challenges, resulting in wider societal advantages. Additionally, they offer unique insights, enhancing diversity and strengthening resilience. This article aims to analyze the impact of the COVID-19 pandemic on women's entrepreneurship in the European Union (EU) by reviewing recent and relevant literature, studies conducted in member states, and official statistical data. The COVID-19 pandemic intensified existing challenges and brought about new difficulties, yet it also created fresh opportunities. The lasting effects are still emerging and are expected to influence the future of women's entrepreneurship in Europe for years to come. A range of policy responses and support measures issued by the governments of member states aimed to mitigate COVID-19's severe impact on businesses, including those owned by women. Further research is needed to provide invaluable insights into how future global crises can have minimal impact on women entrepreneurs, guiding policymakers and stakeholders in designing and building an inclusive, equitable, resilient, and sustainable entrepreneurial ecosystem for women in the EU.

KEYWORDS: Business, Entrepreneurship, Women, European Union, Covid-19.

Introduction

The COVID-19 pandemic has fundamentally reshaped economic landscapes across the globe, with particularly profound impacts on entrepreneurship.[1] When, on March 11th, 2020, the World Health Organization declared the pandemic, government-mandated containment policies, such as social distancing, widespread lockdowns, school closures, and restrictions on travel, movement, and people gatherings, resulted in economic activities being severely curtailed, trade being disrupted and an estimated 114 million jobs being lost in 2020 worldwide.[2] Scholars argue that the pandemic-related crisis can be at least comparable with the global financial crisis (GFC) of 2007-2009.[3–6]

In the European Union (EU), entrepreneurs faced unique challenges, such as vast uncertainty, existential risks, liquidity problems, threats of bankruptcy, and massive declines in income and sales as they navigated this unprecedented socio-economic crisis.[7–10] Thus, the COVID-19 pandemic imposed an important exogenous multiple-period shock on entrepreneurial activities, particularly in the service industry and in small and medium-sized (SME) enterprises.[11-14] Female enterprises within the European Common Market mostly operate in the services sector, retail, and tourism, which were the most exposed to the economic and social effects of the pandemic.[15,16] Therefore, scholars agree that during the pandemic, women entrepreneurs, regardless of their socio-economic status and vulnerability, had to face a disproportionately larger impact, compared to men.[17-20] Nevertheless, scholars conducting qualitative and quantitative research with SME business owners and managers from different sectors of the economy found that some enterprises even benefited from the pandemic by exploiting new opportunities and establishing practices of crisis management, showcasing a speed of adaptation towards the new technology and digital solutions, and, ultimately, resilience and sustainability.[21-25]

This literature review aims to deliver a thorough and systematic analysis of the COVID-19 pandemic's impact on women's entrepreneurship within the EU, emphasizing the diverse challenges they have faced and the new opportunities that have emerged. It also seeks to introduce a fresh perspective to entrepreneurship studies by acknowledging that gender issues are often overlooked during economic crises. Identifying gender-specific barriers and opportunities during the pandemic and understanding the impact of female-led businesses is crucial for overall economic recovery efforts in the EU. Reviewing the recent scholarship, compiling, and analyzing secondary data on women entrepreneurs is crucial for understanding their needs and informing policymakers about the specific requirements of female entrepreneurs to develop effective and equitable support policies. This is essential for minimizing the negative impact of future crises.

Methodology

A systematic literature review was conducted using Google Scholar, searching for relevant papers published in English between 2019 and 2024. The following keywords were used: "COVID-19," "Pandemic," "Women and Entrepreneurship,"

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and "European Union." Additionally, data from Eurostat databases, the Organization for Economic Co-operation and Development (OECD) databases, and the Global Entrepreneurship Monitor were processed. Studies that met the eligibility criteria were assessed based on their titles, abstracts, methodologies, discussions, and conclusions. Ultimately, 55 papers were considered eligible and included in this literature review.

To analyze the identified papers on the impact of the COVID-19 pandemic on women entrepreneurship in the EU, a structured and systematic approach was applied. Relevant data were extracted from each paper, focusing on research objectives, methodologies, key findings, and conclusions. The extracted data were then organized and analyzed into four key categories: (1) the state of women's entrepreneurship in the EU before the pandemic, (2) the impact of the COVID-19 pandemic on women's entrepreneurship in the EU, (3) opportunities for women entrepreneurs during the pandemic, and (4) policy responses and support measures. A thematic analysis revealed several key themes, including the disproportionate burden of caregiving on women entrepreneurs, the acceleration of digital transformation as a survival mechanism, and the importance of targeted policy support to foster resilience and recovery. These themes were compared and contrasted within each category to provide broader insights into the challenges and opportunities faced by women entrepreneurs in the EU during the pandemic.

To further assess the impact of the COVID-19 pandemic on female entrepreneurship in the EU, eight specific research papers involving surveys and statistical analysis of responses collected during the pandemic were analyzed. These papers were selected because they specifically focused on women entrepreneurs in the EU and were large-scale studies, providing comprehensive and statistically significant insights into the impact of the COVID-19 pandemic.

The European Union was chosen for this study due to its diverse economic and social landscape, offering a valuable context for examining the impact of COVID-19 on women's entrepreneurship. With 27 member states, the EU allows for a comparative analysis of how different national policies and economic conditions have influenced entrepreneurial outcomes during the pandemic. Additionally, the EU's significant role in global trade and policymaking makes the findings from this region particularly relevant and applicable on a broader scale.

Literature Review

Many researchers view women's entrepreneurship as a significant phenomenon of the 21st century, particularly in developing countries.[26, 27] The dynamics of entrepreneurship play a vital role in driving economic growth and social development, contributing to economic stability during challenging times with high unemployment. By fostering new startups, women's entrepreneurship enhances an economy's creativity, competitiveness, and overall productivity.[28] Women entrepreneurs tend to search for change and its exploitation as an opportunity, using innovation as a specific entrepreneurial tool. An increase in the rate of female entrepreneurs generates positive effects on the economic conditions and social well-being of both developed and underdeveloped countries.[25]

The OECD and European Commission report that men are more likely than women to start and oversee new businesses.[29] From 2016 to 2020, 8% of the men's labor force in the European Union (EU) was involved in total entrepreneurial activity (TEA), compared to 5% of women. Similarly, in OECD countries, 13% of men and only 9% of women were involved in starting and managing young firms.[29] According to The Startup Heatmap Europe, only 15.5% of Europe's founders are female, and only 7% of Venture Capital (VC) funding in Europe goes to startups with a female founding team member. In particular, the share of women working in the Information and Communication Technology sector in Europe was 17% in 2019.[30] In 2019, more than 10 million women were entrepreneurs in the EU Member States.[19]

Immediately after Severe Acute Respiratory Syndrome Coronavirus-2 was declared a pandemic, every European government imposed a lockdown that led to the total closure of schools and all educational institutions, public places, and most companies. During this period, all EU Member States closed their borders. However, the severity of these measures varied by country.[19] Following this initial measure, there was a phase of gradual reopening, during which people had to live with the virus by adopting specific security measures. This phase also included the reopening of businesses.

Most companies across the EU were negatively impacted.[31] For instance, 43.2% of Italian businesses reported severe financial difficulties, and 97.2% were significantly affected by the pandemic's negative economic consequences.[32] Even with the introduction of government support measures, 51% to 58% of EU companies reported financial difficulties after three months of lockdown.[11] Small and medium-sized enterprises (SMEs) suffered greater revenue declines relative to their total assets compared to larger companies. Sectors reliant on human interaction, such as the cultural and tourism industries, were particularly hard-hit by the crisis.[33] For instance, Greece's tourism sector experienced a dramatic decline, with a 63.3% drop in activities during the first half of 2020, contrasting with a slight 0.7% increase in the same period the previous year.[34]

Recent research has extensively examined how small enterprises respond to crises and build resilience. The ability to prepare and implement effective crisis management strategies is crucial for the survival of these businesses. While preparedness is key to resilience, the unprecedented nature of the COVID-19 pandemic made it nearly impossible for most enterprises to anticipate. Nonetheless, a robust crisis management approach could have facilitated quicker recovery or even growth. Past crisis experiences indicate that small enterprises benefit from their flexibility to adapt to changing circumstances. Equipped with self-executed methods, they mobilize available resources and continually adjust plans to seize opportunities. Unlike larger companies, small enterprises often do not follow rigid management schemes.

Female entrepreneurs responded to the pandemic in various ways. In their large-scale study, Koltai *et al*. report that half of the respondents sought adaptation strategies, one-third intro-

duced new products or services, bolstered their online sales, or explored new markets.[19] Over one-third focused on long-term developments to enhance prospects, such as maintenance and development activities during lockdowns, employee training, procuring new production means, or implementing home delivery. Additionally, over half of the respondents utilized some form of support measure. The correlation between the crisis measures adopted and the ability to overcome the pandemic's challenges varied significantly depending on the extent to which enterprises were affected. For instance, enterprises less impacted by the crisis often did not take action yet managed to maintain or improve their economic situation, while those facing negative impacts were more likely to adopt adaptive or reduction measures to mitigate losses.[19]

Since the onset of the COVID-19 pandemic in 2019, governments worldwide have faced the dual challenge of addressing immediate health concerns and managing the economic and welfare implications of pandemic measures, restrictions, and closures. The European countries were affected differently by the course of the pandemic; therefore, the protective measures were very different in every country. The declaration of a state of emergency was one of the first decisions in most countries, as well as the restriction of public events and the introduction of quarantine requirements for those coming from abroad. In many countries, strict police controls were also introduced along with the restrictions. All countries encouraged remote working (home office), where possible. Also, childcare and educational institutions were closed everywhere, and distance education was introduced.

Policy support measures for SMEs in the EU have not been differentiated based on the owner's gender. The primary goal of the support measures was to ensure the survival of businesses. In the first two to three months following the pandemic's outbreak, 50% of the surviving businesses reported income losses. According to the OECD 2020 report, countries implemented various support schemes for businesses and the self-employed, including reductions, deferrals, or cancellations of social contributions and taxes. Other forms of support included emergency capital, specific unemployment aid, tax moratoriums for SMEs and self-employed individuals, and temporary mortgage payment suspensions. Structural policies were not widely employed and, when used, primarily focused on remote working and digitalization.

Results

State of Women Entrepreneurship in the EU Before the Pandemic:

In the European Common Market, female-led enterprises predominantly function as small and medium-sized businesses, with many women entrepreneurs being self-employed. Experts contend that these small and medium-sized enterprises are more vulnerable to social distancing measures due to their inherent fragility and lower resilience. These enterprises possess fewer amounts of capital and reserves to mobilize, or they are less flexible in adapting to changes in supply chains.[35,36] Despite this, their significance is highlighted by the fact that small and medium-sized enterprises constitute 99.2% of all companies in the EU, employing 66.4 million people and contributing significantly to economic value (Eurostat).

According to GEM, women in developing economies are much more likely to start their own businesses than those in high-income countries, with approximately 25% of women in low-income countries and 13% in lower-middle-income countries compared to approximately 10% globally.[23,37] The reason for this is the fact that these women evaluate entrepreneurship as a way to find better incomes for their families, underlining the major distinction between opportunity and necessity entrepreneurship, a push and pull factor, respectively.[24] Opportunity entrepreneurship aims to capitalize on perceived market possibilities and takes advantage of new business opportunities. It is more commonly seen in developed member states in the EU, with higher levels of Gross Domestic Product (GDP), mainly located in Western and Northern Europe.[38] Necessity entrepreneurship occurs when there are few or no alternative possibilities for successful labor market involvement, and starting a new business is the best option available.

Although the negative viewpoint of most European societies about women's attainment of independence and wealth is being left behind, female entrepreneurs continue to face multiple obstacles in their journey.[39] Discrimination against women's participation in the workplace persists and is greatly influenced by social stereotypes and cultural norms.[40] Gender biases affect women's perception of their entrepreneurial abilities and access to opportunities. Women are generally less confident in their capabilities and more likely to be deterred by failure, suggesting that cultural factors may be at work influencing this gender gap and some social network differences in ecosystems as well.[41] Brieger *et al.* argue that higher socioeconomic, cultural, and institutional gender egalitarianism supports women's empowerment.[8] They underline a substantial gap in this area between Eastern and Western Europe, with the latter ranked highest for gender equality in the 2020 Global Gender Gap Index.[8] Child and elderly care norms are highly gendered within all European societies; women often shoulder a larger share of domestic and care work than men. Among parents, mothers are traditionally the main providers of childcare and accumulate a disproportionate amount of housework, which poses additional obstacles to female entrepreneurship.[42] Balancing entrepreneurial pursuits with family responsibilities is a significant challenge for many women.

There are significant differences between the entrepreneurial income of women and men. This difference might be explained by the lower wages of women and the higher proportion of women in part-time jobs. Differences among males and females in enterprising also appear in terms of obtaining the initial capital to fund their businesses. Scholars argue that insufficient access to funding during the startup phase is the most significant issue for entrepreneurs.[43] Surveys and questionnaires completed by women entrepreneurs across several European member states highlight a persistent gender gap in access to credit.[19, 23, 44] Women frequently report facing significant challenges in securing funding for their businesses, underscoring ongoing disparities in financial support. Female borrowers often receive fewer loans and face higher

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interest rates. Gender bias is evident when finance providers evaluate startup proposals, with women more frequently questioned about their commitment, legitimacy, and credibility. Additionally, women are required to provide more detailed information about their businesses compared to men. According to Martínez-Rodríguez *et al.*, institutional discrimination is a primary factor behind the financial challenges women face.[24]

Evidence from the world of equity investment suggests that women business owners and leaders face discrimination in the equity investment pipeline. According to the Women's Entrepreneurship Report 2018-2019, released by GEM, women in Europe cited a lack of financing about 10% more often than men, at 11.3% compared to 10%.[37] The situation is the same when accessing training and resources, which are extremely important for starting a business. OECD data from 2019 underlines one of the main challenges that women face before launching their business: in each European country covered, the rate of women having access to training is lower than their male counterparts.[35]

Nevertheless, the average global business discontinuance rate is approximately 10% lower for women (2.9%) compared to men (3.2%). Business discontinuance rates decrease as country-level income increases, with the lowest rates for women being found in Europe at 1.4%.[41] These findings also highlight that women entrepreneurs are exceptionally resilient and passionate about sustainability, working diligently to integrate sustainable practices into their businesses.

Impact of the COVID-19 Pandemic on Women Entrepreneurs in the EU :

As shown in Table 1, the analysis of qualitative and quantitative studies, involving surveys and statistical analysis of responses, reveals the impact of COVID-19 on female entrepreneurship in the EU.

Table 1: Analysis of qualitative and quantitative studies on the impact of COVID-19 on female entrepreneurship in the E.U.

		Country	Sample	Results
1	De Simone et al., 2021	Italy	137 women entrepreneurs	• Women experience greater conflict between work and family roles than men • When family negatively interferes with work, perceived firm performance success decreases. • Personal fulfillment must be sacrificed to enhance perceptions of financial success. • Establishing good relationships is a key factor in influencing the success of women entrepreneurs.
2	Stephan et al., 2020	UK	361 entrepreneurs 272 men / 88 women	• 60,9% of Business' existence was threatened • Trading: 9,2% suspended / 61% decreased / 10,3% increased • 7% of males / 16% of females expanded into online trading/delivery • Male: 16,7/40 Female: 18,5/40 perceived stress
3	Birsan et al., 2022	Romania	320 entrepreneurs 112 men / 208 women	• Women entrepreneurs are opportunity-driven • 63,3% small business model • Impact: 39,3% Moderate decline 32% Steep decline 14,7% Moderate growth 12,7% Stagnation / no effect
4	Gergely et al., 2021	Romania (Transylvania)	84 women entrepreneurs	• Changes in the operations of a firm and changes in its activity. • Temporarily close or suspend businesses. • 90% are unable to continue activities the way they used to do before. • Often, employees had to be fired or required to take compulsory leave. • 21% adapted to online sales and promotion • 1/3 were positive about the future
5	Brieger et al., 2023	27 European countries (Eurofound' s Living, Working and COVID-19 e-Survey)	5,754 entrepreneurs 2,244 men / 3,510 women	• Women entrepreneurs report more work-family conflict than men entrepreneurs. • Work-family conflict mediates the relationship between being a female entrepreneur and psychological well-being. • Larger gender gaps in work-family conflict in macro-level contexts with low socio-economic, institutional, and cultural gender egalitarianism. • The negative indirect effect of gender on psychological well-being is weaker at higher versus lower levels of socio-economic, institutional, and cultural gender egalitarianism.
6	Goldstein et al., 2022	50 countries globally	150,000 business owners	Female-led firms are: • 4% more likely to close their businesses than male-led • 1,2% more likely to report revenue reduction • 3,9% more likely to cut their workforce • 12,9% lower sales than 2019
7	Koltai et al., 2020	7 European countries (Andalusia in Spain, Austria, Bulgaria, Czech Republic, Hungary, Serbia, Transylvania)	1,681 women entrepreneurs	• 79% of the women-led businesses were negatively affected • 8% believed that the pandemic improved their businesses' opportunities • 35% significantly worsened the situation of the enterprise • 31% slightly worsened the situation of the enterprise • 13% not affected • 13% improved the situation of the enterprise • 10% decrease in the total number of employees
8	Graeber et al.,2021	Germany	155 Female / 156 Male self-employed	Self-employed are: • 42% more likely to report losses of gross income • 30% more likely to report reduction in working hours than employees. • Self-employed women are 33% more likely to experience income losses than men

Female entrepreneurs in the EU faced greater work-family conflict, higher business closures, and financial instability during COVID-19, with most experiencing moderate to steep declines in business performance.

According to De Simone *et al.*, women entrepreneurs in Italy encountered more significant challenges than men, including liquidity issues, supply chain disruptions, job losses, access to credit, and technological difficulties.[32] Their research also suggests that women entrepreneurs understand the need to sacrifice family time and prioritize work to achieve entrepreneurial success and enhance firm performance, especially during the pandemic, which increased women's workload both in their occupations and in their housework. They conclude that difficulties related to risk management and uncertainty in managing one's own company, especially during the pandemic, can negatively impact perceived entrepreneurial success in terms of personal financial rewards and fulfillment. This highlights that interpersonal resources are a decisive factor for women entrepreneurs during this crisis.

A study conducted in London revealed that 93% of respondents were negatively impacted by the COVID-19 pandemic, while 4% reported no impact. Among those affected, 44% had temporarily suspended operations, and 3% had permanently ceased them.[20]

According to a King's Business School study on 361 entrepreneurs, 88 of whom were women, "on balance the Covid-19 pandemic impacted women entrepreneurs more adversely than men entrepreneurs".[20] Women-led businesses suffered a greater reduction in trading and women entrepreneurs scored higher in perceived stress than their male counterparts, slightly more often raising concerns about the survival of their businesses. However, their social response to the crisis was stronger. They more often than men gave money and volunteered their personal time and business offerings to support charities or noble causes.

In their empirical research, undertaken in March 2021, Birsan *et al.* state that their hypothesis, which suggests that the female entrepreneurial business model has helped keep Romania's unemployment rate below the European average, has proven to be true.[45] Their study also confirms that women entrepreneurs often choose to rely on their own strengths rather than government support, which can create vulnerabilities during times of crisis, such as the COVID-19 pandemic. However, it also highlights the resilience of female entrepreneurship.[45]

In another Romanian survey, all respondents reported being significantly impacted by the COVID-19 situation. Specifically, 20% reduced their activities, 18% partially interrupted them, and 19% ceased their activities entirely.[46] Gergely *et al.*, in their study on the responses of 84 Romanian female

entrepreneurs, highlight that administrative and bureaucratic obligations doubled due to the pandemic.[47] They assert that reduced sales and the associated loss of income were major concerns for female entrepreneurs, who were not prepared for an income loss of this magnitude. It is reported that 21% of female entrepreneurs implemented adaptation strategies due to the COVID-19 situation. These strategies primarily involved shifting to online platforms and rapid digitalization, as well as using online networks, which became their primary means of communication with clients.

Evaluating the gender differences in entrepreneurs' work-family conflict and well-being during COVID-19, Brieger et al. highlight that women entrepreneurs with employees faced greater challenges juggling family and employee demands during the pandemic.[8] Women entrepreneurs with children were particularly stressed, while those without employees benefited more from socio-economic, institutional, and cultural gender egalitarian contexts, which helped reduce their work-family conflict during the pandemic. In their study, they found that psychological well-being during the COVID-19 pandemic was positively correlated with gender egalitarianism, but negatively correlated with being a woman and work-family conflict. Specifically, work-family conflict showed a positive correlation with being a woman and socio-economic and institutional gender egalitarianism, but a negative correlation with cultural gender egalitarianism.

Goldstein *et al*. conducted a study using data collected from over 150,000 business owners via Facebook across 50 countries during 2020-2021.[42] Their findings highlight that globally, female-led firms were, on average, 4% more likely to close their businesses than male-led firms in 2020. In addition, women entrepreneurs were 1.2% more likely to report a reduction in revenues compared to their male counterparts. Women-led businesses also experienced a larger drop in sales, averaging 12.9% lower sales relative to 2019, compared to male-led businesses. Furthermore, women-led businesses were 3.9% more likely to completely cut their workforce than male-led businesses. These findings underscore the disproportionate impact of economic challenges on women entrepreneurs, further contributing to gender inequalities in the entrepreneurial landscape. The authors emphasize that policy decisions made due to COVID-19's impact will have significant long-term implications for women's economic empowerment and gender equality. They advocate for governments to actively engage in promoting social dialogue that challenges traditional gender roles where men are seen as breadwinners and women as caretakers, and supports workplace flexibility.[42]

Koltai *et al*. examined 1681 responses from women entrepreneurs across seven European countries.[19] 79% of the women-led businesses were negatively affected, while 68% of the responders reported a 28% decrease in their annual income in 2020. However, women entrepreneurs working in the field of tourism and hospitality claimed a 50% loss in their annual income. The number of employees in the responding enterprises run by women decreased by 10%. Nevertheless, some respondents reported that their businesses will benefit in the long run. Specifically, 23% managed to introduce new products, and 14% saw growth in online sales. Half of the respondents sought adaptation strategies, and over a third began initiatives that could enhance their business prospects in the long run. Based on several indicators, it appears to be significant that enterprises more active in the online space before the pandemic were more resistant to the effects of the pandemic.[19]

Lastly, Graeber *et al*. indicate that self-employed individuals face a higher risk of income losses due to the COVID-19 pandemic compared to employees, with women being about one-third more likely than men to experience such losses.48 This gender gap was not observed among employees. Their findings indicate that the disparity among the self-employed is largely due to women being more concentrated in industries most severely impacted by the pandemic. Furthermore, their analysis reveals that women are disproportionately affected by government-imposed restrictions, such as reduced operating hours.[48]

Reflecting a global trend, women in Europe are much more likely to engage in businesses in wholesale and retail trade or government, health education, and social services. These industries tend to have lower entry barriers, lower margins, and higher failure rates, specifically in times of crisis, which can be constraints on sustainability and performance.[7] A significant portion of female-led enterprises operate in sectors severely impacted by pandemic restrictions, such as tourism, hospitality, and personal services. Many of these small businesses lack the liquid capital necessary to weather crises and do not possess the tools to digitize their services. Startups and small enterprises are among the most vulnerable players in the economy.[49] As a result, since most female-led businesses are small and many women entrepreneurs are self-employed, their ventures are especially vulnerable to the economic downturn brought on by the pandemic. Research shows that self-employed women are about one-third more likely to experience income losses due to COVID-19 compared to men.[50,51] The most significant gender disparities among the self-employed stem from the fact that self-employed women are more frequently employed in industries hit harder by the COVID-19 pandemic than their male counterparts. Women-owned businesses face substantial revenue declines, cash flow problems, and challenges in fulfilling financial commitments. While several government support measures were implemented across Europe, they were not always effectively targeted towards women-owned businesses, leading to disparities in access to aid and securing loans and investments.

Moreover, evidence indicates that gendered household responsibilities also play a role in the income loss disparity. Notably, 19% of households saw a change in the primary earner during the pandemic.[19] In most cases, this shift involved men from couples with roughly equal salaries becoming the main earners. However, in some instances, women relinquished their role as the primary earners. Studies indicate that women entrepreneurs, on average, dedicate nearly 2 hours less to their business activities on a typical weekday compared to their male counterparts. Instead, they allocate these 2 hours to fulfilling caregiving responsibilities and household duties. This time disparity highlights the additional domestic burdens women face,

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which can impact their business productivity and growth. The extra caregiving responsibilities not only reduce the time women can invest in their entrepreneurial ventures but also underscore the broader societal expectations and pressures placed on women to manage both professional and domestic roles. Specifically, female entrepreneurs in their 40s appear to have faced the most challenging situation during the pandemic, as they reported the highest proportion of childcare responsibilities at 72%. Additionally, they also reported a significant share of regular elderly care duties, at 39%. This pandemic-induced blurring of work and personal and family life may have long-lasting effects on women's ability to balance entrepreneurial pursuits with family responsibilities.

Opportunities for Women Entrepreneurs during the Pandemic:

An impact analysis conducted by the Canadian Women Entrepreneurship Knowledge Hub in 2020 found that female entrepreneurs are less likely to use online tools than their male counterparts.[52] However, the majority of women entrepreneurs in the EU had already been using more online tools before the pandemic. Over half of them regularly used three to five online tools. Nonetheless, 27% of female enterprises had not used any online tools before the pandemic.[19] During the pandemic, women entrepreneurs frequently used online tools such as offering services via online sales, working from home, setting up home offices, facilitating internal online communication, and leveraging digital marketing tools like social media and Google. Social media was the most popular online tool, with 82% of European women entrepreneurs using it to some extent. Besides specialized online software, online communication tools within the company, and online sales, remote work was the least utilized, with most entrepreneurs reporting they had never used it in their business operations.[19] However, 15% of them worked exclusively remotely, which helped them adapt more flexibly to the restrictions.

In several instances, respondents reported impacts that could potentially strengthen their enterprises. Specifically, 23% of the respondents were able to introduce new products during the pandemic, and 14% saw an increase in online sales. Additionally, 23% of respondents launched new product services, while 10% identified new market opportunities.[19] These developments highlight how the pandemic has driven innovation and market expansion for some women-led businesses, despite the broader challenges faced.

There was a significant correlation between the effects of the pandemic and the use of online tools. Female entrepreneurs whose businesses were not negatively affected or were positively impacted by the pandemic had already been using online tools more frequently before the pandemic began. This finding may be partly explained by the fact that sectors less affected by the pandemic were those where the nature of the activities allowed for the use of online tools. In contrast, the sectors most severely impacted by pandemic restrictions were those requiring personal presence. The correlation between online presence and the effects of the pandemic is also evidenced by the fact that enterprises that used more online tools anticipated smaller income losses compared to those that used fewer or no online tools before the pandemic. Various indicators suggest that businesses more active in the online space were more resilient to the pandemic's impacts.

Therefore, it is accurate to say that the pandemic solidified the shift to e-commerce.[55] Many women entrepreneurs viewed the pandemic as an opportunity to accelerate the existing trend towards more digital offerings and trading, from B2B marketplaces to increased demand for digital advertising, search engine optimization, and remote learning. Entrepreneurs saw increased digitalization as a chance to access new, geographically distant markets that were previously costly to reach. The pandemic accelerated the shift to online shopping, allowing women entrepreneurs to set up online stores and broaden their customer base.

Women entrepreneurs are innovating in how they run, produce, and transform their businesses, with a strong focus on leveraging technology effectively. They are developing websites, exploring online marketing strategies, and seeking online solutions. In response to the pandemic, various sectors have adapted their approaches: professional services, training, and education have embraced online communication channels, while retail and food manufacturing sectors have focused on reaching customers through delivery services. Additionally, the pandemic has opened new market opportunities for products and services related to health, hygiene, remote work, and online education. As customer preferences shifted towards local and sustainable products, women entrepreneurs in these areas have benefited significantly.

The extent to which women entrepreneurs were able to capitalize on new opportunities during the pandemic varied significantly across EU member states. Countries with robust digital infrastructure and supportive policy environments saw greater success, while women entrepreneurs in less-equipped regions often struggled due to a lack of resources and support. Despite the crisis creating new avenues for growth, these disparities highlighted existing inequalities that need to be addressed to foster women-led economic advancement. Nevertheless, the pandemic may have also sparked the emergence of a new generation of resilient and adaptable women entrepreneurs, equipped to overcome adversity and seize opportunities in the evolving business landscape.

Policy Responses and Support Measures – Implications:

It is important to note that all European countries implemented a mix of measures addressing labor regulations, tax and loan deferrals, and financial instruments. However, most of these measures were aimed at businesses with employees, whereas many female entrepreneurs and self-employed individuals, as previously noted, do not have employees.

The support measures utilized by female entrepreneurs in the EU, listed in order of popularity, were wage subsidies, tax discounts, deferred tax payments, and various credit schemes, including deferred payment and supported credit options.[19] Across Europe, various sector-specific support measures were introduced, including discretionary monthly lump-sum payments for SMEs. These fixed-amount payments were potentially more accessible for small enterprises and the self-employed, requiring less administrative effort compared

to wage subsidies, which were more commonly used in other countries.

This review has significant policy implications not only for future pandemics but also for other disruptions, such as natural disasters and civil political unrest, which could disproportionately impact women at a scale similar to or approaching that of a pandemic. Entrepreneurs, particularly women, have been disproportionately affected by the systemic shock of the COVID-19 pandemic, partly due to the policy measures implemented to control the virus's spread. This issue is particularly relevant given the gradual increase in women choosing entrepreneurship. If women feel less supported by policies during such crises compared to female employees, there is a risk they might turn away from self-employment, potentially widening the gender gap in this sector. This could have negative repercussions for economic growth, especially in areas that heavily rely on female entrepreneurship.

Therefore, policy measures designed to alleviate economic hardships during future crises should consider these disparities. Since government-imposed restrictions contribute to this unequal impact, targeted policies to restore gender equity are especially important. Additionally, since entrepreneurs have been particularly affected by the COVID-19 pandemic, policymakers might consider specific measures to support them. In a broader sense, any support scheme for entrepreneurs could have both negative and positive externalities that need to be balanced. Self-employment and entrepreneurship positively influence growth, so support schemes that help individuals remain self-employed during crises could aid in economic recovery post-pandemic.

Discussion

The World Economic Forum estimated that COVID-19 would push back the timeline for closing the global gender gap across key dimensions—economic participation and opportunity, educational attainment, health and survival, and political empowerment —from 99.5 years to 135.6 years.[50] To provide a thorough review of the pandemic's impact on women-led businesses and the challenges female entrepreneurs face in balancing their business and family roles, eight primary studies were examined and analyzed.

In all the European countries studied, the vast majority of women entrepreneurs are leaders of micro or small enterprises. Many of these entrepreneurs are self-employed, with some being necessity-driven or forced into entrepreneurship. Due to their size, these women-led businesses are more vulnerable. In addition, a substantial portion of female entrepreneurs operate in sectors severely impacted by coronavirus restrictions, such as tourism and personal services. In sectors where personal presence plays a bigger role, pandemic measures had a major negative impact. SMEs in the service sector, i.e., retailing, tourism, hospitality, and transport, suffered the most from the restrictive measures, which significantly limited the service retail and were compounded by the closure of educational institutions.51 Those working in tourism and hospitality were in the most demanding situation, with some claiming losses of up to half of their annual income in 2020. Women's enterprises in education, trade, and personal services anticipated losing one-third of their annual revenues in 2020. Additionally, female entrepreneurs in industry, handicrafts, healthcare, and social services also expected a 25% decrease in their revenues. For many of these women, the reduction in business income threatened their families' livelihoods.

Women faced multiple challenges during the months of the pandemic. Female entrepreneurs dealt with difficulties caused by the virus on both economic and family fronts during the closure period. Balancing entrepreneurial activities with private and family responsibilities is a complex management task even in normal times. This situation was exacerbated by restriction measures, the introduction of distance learning, and increased caregiving responsibilities.[53] Female entrepreneurs with children under 7 years old were seven times more likely to reduce their business-related activities to prioritize caring for their children and other family members.[19] Globally, women faced increased domestic burdens following the closure of educational and healthcare institutions.[42] The reconciliation of work and family life, just like before the pandemic, was a significant challenge for female entrepreneurs. Goldstein *et al*. provide sufficient global evidence of a motherhood penalty and childcare constraints to help explain gender inequalities in an entrepreneurship context.[42] Despite this, women who run businesses have received significantly less attention from scientists and researchers, even though they have been challenged in numerous ways during the pandemic. As entrepreneurs and employers, they had to navigate the constraints of epidemiological measures and their economic impacts. Simultaneously, as women and mothers, they often bore the brunt of household and home care tasks, including helping children with their studies, preparing family meals, and caring for elderly relatives.

While progress had been made in advancing women's entrepreneurship in the EU before the crisis, the pandemic exacerbated existing challenges and exposed vulnerabilities in the ecosystem.[54,55] Key findings from this literature review indicate that women entrepreneurs experienced disproportionate economic losses, increased work-life balance challenges, and reduced access to finance compared to their male counterparts. The pandemic particularly affected women-dominated sectors, leading to business closures and job losses. Despite these challenges, the crisis also presented opportunities for digital transformation and the exploration of new market niches. However, the ability of women entrepreneurs to capitalize on these opportunities was often hindered by factors such as limited access to digital skills, financial resources, and support networks.

In the past decade, female entrepreneurship has developed as a key part of economic growth. Evidence shows that female entrepreneurship models can greatly foster resilience during crises.[45] According to Birsan *et al.*, female entrepreneurship models are characterized by adaptability, innovation, and sustainability, which are particularly vital in navigating pandemic-related challenges. These models often emphasize leveraging local resources, fostering community-oriented initiatives, and prioritizing flexible business structures. Such approaches not only address immediate crises but also build long-term resilience and sustainability in business operations.

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The policy responses and support measures implemented at both the EU and national levels were critical in easing the pandemic's effects. While some initiatives proved successful, more targeted and continuous support is necessary to address the particular challenges faced by women entrepreneurs. The long-term impact of the pandemic on women's entrepreneurship in the EU remains complex and unclear. There is a risk of expanding gender gaps, rising debt burdens, and loss of human capital. Nonetheless, the crisis could also give rise to a new generation of resilient and adaptable women entrepreneurs. To fully understand the long-term implications, further research is needed, particularly in the areas of intersectionality, policy evaluation, and the impact on specific sectors. By addressing research gaps and investing in targeted support measures, policymakers and stakeholders can contribute to building a more inclusive and equitable entrepreneurial ecosystem for women in the EU.

The COVID-19 pandemic has provided invaluable insights into the challenges and resilience of women entrepreneurs in the EU. Key lessons learned highlight the vulnerability of women-owned businesses, which often operate in more vulnerable sectors with smaller business sizes and less financial cushion. Women entrepreneurs are disproportionately affected by economic downturns and crises, underscoring the need for targeted support. One significant lesson is the importance of digital transformation. The pandemic emphasized that digital skills are crucial for business survival and growth. Investing in digital infrastructure and training for women entrepreneurs is essential to ensure their future success in an increasingly digital economy.

Another critical area is the need for flexible and inclusive policies. Policies should be crafted with a focus on women entrepreneurs, addressing their unique needs and challenges. Support measures need to be accessible, timely, and tailored to various business stages and sectors to be effective. Additionally, work-life balance is crucial for the success of women entrepreneurs, making it essential to support them in managing both work and caregiving responsibilities. Investments in childcare and flexible work arrangements can significantly impact women's economic participation and entrepreneurial success.

At the policy level, women entrepreneurs might be more sensitive to pandemic-related crises. Without proper attention or effective recovery strategies, existing gender-based well-being differences are likely to intensify. Policymakers should consider implementing supportive infrastructures, such as egalitarian entrepreneurial ecosystems, to help both women and men entrepreneurs balance work and family responsibilities during times of crisis.[8]

From a practical perspective, this review highlights the need for flexible work arrangements, societal support, and government benefits, such as childcare subsidies, especially when remote work becomes the standard. Additionally, promoting gender equality benefits not only women entrepreneurs but all women, especially mothers, who are particularly vulnerable in times of crisis. Enhancing the business climate, career opportunities, parental leave, and anti-harassment policies can significantly improve women's overall well-being.

Support networks have proven to be powerful assets for women entrepreneurs. Mentorship, networking, and peer support are vital for their growth and resilience. Building strong support networks can help women overcome challenges and access necessary resources. A long-term perspective is essential when considering the impact of crises on women entrepreneurs. The effects are often enduring, and sustainable support and recovery strategies are necessary to rebuild and strengthen women-owned businesses.

Finally, data-driven policymaking is essential for understanding the needs of women entrepreneurs and crafting effective policies. Gathering and analyzing data on women entrepreneurs, as well as regularly monitoring and evaluating policies, are critical for assessing their impact and making necessary adjustments. These insights emphasize the importance of targeted support and comprehensive strategies to foster a more inclusive and equitable entrepreneurial ecosystem for women in the EU.

Conclusion

The landscape of women entrepreneurship in Europe before the COVID-19 pandemic showcased significant progress in certain areas, yet persistent challenges remained. The situation varied significantly among EU member states, with Nordic countries generally having higher rates of women entrepreneurship and more robust support systems compared to Southern European countries. Countries with stronger social safety nets and higher gender egalitarianism tended to experience less severe impacts on women entrepreneurs during the pandemic. When women are empowered socioeconomically (through education and financial means), institutionally (via governmental support and key rights), and culturally (through equality-focused attitudes and norms), they are better positioned to access the resources and support needed to overcome resource drainage.[8] This empowerment also enables women entrepreneurs to more effectively balance work and family responsibilities, mitigating the negative impacts of crises such as the pandemic.

The economic consequences of the COVID-19 pandemic were particularly devastating for women entrepreneurs, emphasizing the fragility of women-owned businesses and the urgent need for targeted support measures. The ability of women entrepreneurs to seize opportunities during the pandemic was uneven across the EU, with better outcomes observed in countries possessing superior digital infrastructure and supportive policy environments. Despite the potential for new opportunities, such as digital transformation and emerging markets, many women entrepreneurs lack the necessary resources and support to fully take advantage of them. Key findings from before and during the pandemic reveal that women entrepreneurs experience greater economic losses, more significant work-life balance challenges, and limited access to financing compared to men. Women-dominated sectors were particularly hard hit, resulting in widespread business closures and job losses.

Despite the challenges, both EU and national-level policy responses and support measures were vital in reducing the pandemic's impact. Although some initiatives were successful, there is a clear need for more extensive and targeted support to

address the specific difficulties faced by women entrepreneurs. The long-term consequences of the pandemic on female entrepreneurship remain complex and uncertain, with potential risks including widening gender gaps, increased debt burdens, and loss of human capital. However, the crisis also has the potential to foster a new generation of resilient and adaptable women entrepreneurs.

To fully understand and address these long-term effects, further research is needed, particularly focusing on intersectionality, policy evaluation, and sector-specific impacts. By closing these research gaps and investing in targeted support, policymakers and stakeholders can foster a more inclusive and equitable entrepreneurial ecosystem for women in the EU. Addressing these challenges is essential for fostering women-led economic growth and building a more resilient, inclusive, and equitable economy.

Limitations

This literature review on the impact of the COVID-19 pandemic on women entrepreneurs in the EU has limitations, as it only considered open-access articles written in English.

Several research gaps persist, including the scarcity of long-term studies tracking the effects on women entrepreneurs. Additional research is needed to understand the specific challenges faced by women entrepreneurs from diverse socio-economic, ethnic, and racial backgrounds. Cross-country comparisons through comparative studies that examine the experiences of women entrepreneurs across different EU member states could offer valuable insights into best practices and policy implications.

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REVIEW ARTICLE

Analysis of CD16$^{+/-}$ Monocytes and CD4^{+} T Cells to Identify Novel Gene Signatures and Develop a Diagnostic Tool for SLE

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ABSTRACT: Systemic lupus erythematosus (SLE) is an incurable chronic autoimmune disease that causes widespread inflammation and organ damage. Due to the lack of a single test to diagnose SLE, doctors use multiple general methods to diagnose the disease. This study evaluated gene expression in CD16^{+} monocytes, CD16^{-} monocytes, and CD4^{+} T lymphocyte cells to identify signatures unique to SLE, to improve diagnostic processes. Gene expression profiles from individuals diagnosed with SLE and females aged 24-29 controls were obtained from the Gene Expression Omnibus, and 54,675 gene probes were compared between healthy and SLE patients. The top five gene probes with increased differential expression between healthy and SLE patients were associated with the *ATP6V0C, UBA1, TGFB1, STAT1*, and *NFYC* genes. Quantile-quantile plots confirmed statistical appropriateness for genetic analysis. Further evaluation determined that the *ATP6V0C, UBA1, STAT1, NFYC*, and *TGFB1* genes associated with the CD16^{-} monocyte cell type represent a novel gene expression signature for SLE identification. Gene expression ranges were established for these probes, serving as a diagnostic tool for SLE. This tool can detect SLE in a single blood sample, which may improve diagnostic outcomes and reduce healthcare costs.

KEYWORDS: Biomedical and Health Sciences; Genetics and Molecular Biology of Disease; Systemic Lupus Erythematosus; Gene Expression Signatures; Transcriptomic Biomarkers.

Introduction

Systemic lupus erythematosus (SLE) is an incurable autoimmune disease in which the immune system generates antibodies that attack the body's own tissues, causing widespread inflammation and tissue damage.[1] SLE can affect the joints, skin, brain, lungs, kidneys, and blood vessels. Poor access to health care, late diagnosis, poor effectiveness of treatments, and imperfect adherence to therapeutic regimens may increase the damaging effects of SLE, resulting in complications and an increased risk of death. Between 2010 and 2016, the average number of deaths per year of US residents where SLE was identified as the underlying cause of death was 1,176. During the same 7-year period, SLE was recognized as a contributing cause of death in an average of 2,061 deaths per year.[1]

On average, it takes almost six years for people with SLE to be diagnosed with the disease from the time they first notice their symptoms.[2] SLE is known as "the great imitator" because its symptoms mimic many other illnesses. SLE symptoms can also be unclear, come and go, and change over time. In addition, SLE is diagnosed far more frequently in females than in males, a pattern that reflects a well-documented sex bias in both clinical presentation and existing datasets.

Furthermore, based on current resources and findings, it is important to note that the diagnosis of SLE currently cannot solely rely on a single test. Instead, doctors apply various methods to discover the presence of the disease. A thorough examination of the patient's medical history focuses on any genetic occurrences of SLE or other autoimmune disorders. Additionally, a comprehensive physical examination is performed to identify potential indicators such as skin rashes or other abnormal signs associated with SLE.[3]

Blood and urine tests, specifically the antinuclear antibody (ANA) test, are commonly used to assess the likelihood of the patient's immune system producing autoantibodies associated with SLE. While a positive ANA test is common among SLE patients, it does not confirm a SLE diagnosis conclusively. In the event of a positive ANA test result, the doctor typically orders further tests for antibodies specific to SLE.[3]

A skin or kidney biopsy, involving the removal of a tissue sample for microscopic examination, is sometimes recommended to detect potential signs of an autoimmune disease. However, knowing that none of these methods definitively determines SLE is crucial. Instead, their primary utility lies in excluding other conditions that may be mistaken for SLE. Recognizing that these diagnostic approaches do not offer a conclusive verdict on whether an individual has SLE is essential. Instead, they assist healthcare professionals in eliminating potential misdiagnoses and narrowing down the possibilities. Therefore, the great complexity of SLE diagnosis emphasizes the need for ongoing medical evaluation and collaboration between patients, healthcare providers, and medical researchers. SLE and other autoimmune disorders tend to run in families, but the inheritance pattern is unclear. People may inherit a gene variation that increases or decreases the risk of SLE, but in most cases, do not inherit the condition.[2] If a robust gene expression signature is identified for patients with SLE or at risk of SLE, this can be used as a diagnostic tool to improve diagnostic patient outcomes. A diagnostic tool that can conclusively identify SLE in patients would be novel and signi-

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ficantly impact patients and the health care system. Patients would have earlier access to treatments to help control symptoms and prevent further health decline. Healthcare costs to identify SLE would be substantially lower since many of the current testing regimes would not be necessary.

This investigation distinguishes itself from prior studies by focusing on the gene expression profiles of $CD16^-$ monocytes in systemic lupus erythematosus (SLE), a cell type subset that has been less explored in the context of SLE diagnostics. While previous research has highlighted the proinflammatory role of $CD16^+$ monocytes in SLE pathogenesis, particularly their involvement in T-cell activation and B-cell differentiation,[9] this study uniquely identifies a gene expression signature in $CD16^-$ monocytes.

$CD4^+$ T cells play a central role in coordinating the adaptive immune response. They act as "helper" cells that activate and direct other immune cells, including B cells, cytotoxic T cells, and macrophages, by secreting cytokines. In the context of systemic lupus erythematosus (SLE), $CD4^+$ T cells are critically involved in the loss of immune tolerance, which leads to the production of autoantibodies. Dysregulated $CD4^+$ T cells in SLE patients often exhibit abnormal activation, impaired regulatory function, and excessive help to B cells, leading to the formation of pathogenic autoantibody-producing plasma cells. Studies have also shown that $CD4^+$ T cells in SLE patients exhibit altered gene expression patterns linked to interferon signaling and pro-inflammatory cytokine production, further contributing to tissue damage and systemic inflammation.[17]

$CD16^+$ monocytes, also known as non-classical monocytes, are a subset of circulating monocytes that exhibit pro-inflammatory properties and are involved in patrolling the endothelium. In SLE, $CD16^+$ monocytes are found in elevated numbers and have been implicated in tissue infiltration and inflammation. These cells express higher levels of inflammatory cytokines such as TNF-α and IL-1β and contribute to the dysregulation of immune responses seen in SLE.[18]

In contrast, $CD16^-$ monocytes, or classical monocytes, are primarily involved in phagocytosis, the process by which a phagocyte (a type of white blood cell) surrounds and destroys foreign substances (such as bacteria) and removes dead cells.[10] These classical monocytes respond to infection and injury. While often considered less inflammatory, $CD16^-$ monocytes are important for understanding early immune activation and homeostasis. Interestingly, recent studies suggest that transcriptional reprogramming in these classical monocytes may occur early in SLE pathogenesis, even before overt clinical symptoms, making them valuable targets for early diagnosis and biomarker discovery.[19]

The purpose of this study was to evaluate gene expression in $CD16^+$ monocytes, $CD16^-$ monocytes, and $CD4^+$ T lymphocyte cells to identify gene expression signatures unique to systemic lupus erythematosus (SLE), which may offer an approach to improve diagnostic processes and outcomes for patients with SLE or at risk of acquiring SLE. Here it was investigated whether gene expression differed in $CD4^+$ T cells, $CD16^{+/-}$ monocytes between individuals with and without SLE.

Methods

Determine Gene Probe Values:

Initial research for the project included evaluating available gene expression data from various open-source repositories. The raw gene probe data chosen for this project were obtained from the Gene Expression Omnibus (GEO) hosted by the US National Center for Biotechnology Information (NCBI). GEO is a public genomics repository meant as an open source for scientific research. The datasets obtained from the GEO repository and used in this project were records GDS4888 ($CD4^+$ T lymphocytes), GDS4889 ($CD16^-$ monocytes), and GDS4890 ($CD16^+$ monocytes).

Gene expression differences between people with and without SLE were determined using microarray analysis. A total of 50 mL of peripheral blood was collected from each person. For $CD4^+$ T lymphocyte cells, this included six people with SLE (average age: 29.0±7.6) and four healthy people (average age: 24.8±0.5). CD16– monocyte cells included four people with SLE (average age: 26.5±1.7) and four healthy people (average age: 24.8±0.5). For $CD16^+$ monocyte cells, this included four people with SLE (average age: 26.5±1.7) and three healthy people (average age: 24.7±0.6). All people in the study were female. Erythrocytes were lysed in EL buffer, and then granulocytes were depleted using CD15-conjugated microbeads. The CD15-depleted fraction was stained with a CD14-fluorescein isothiocyanate antibody. Using a FACSAria cell sorter, the $CD4^+$ T cells, CD16- monocytes, and $CD16^+$ monocytes were isolated. After sorting, the cells were lysed with RLT buffer and frozen at -70°C. Total RNA was then isolated using an RNeasy mini kit. The generation of cRNA was accomplished by sample hybridization using HG-U133 Plus 2.0 arrays and scanning. The clinical characteristics of SLE and healthy persons are summarized in Table 1 below.

Table 1: Clinical characteristics of the female study participants, including CD^{4+} T lymphocyte cells from six SLE patients and four healthy individuals, CD^{16^-} monocyte cells from four SLE patients and four healthy individuals, and CD^{16+} monocyte cells from four SLE patients and three healthy individuals.

SLE / ND[a]	ID#	Collected cell type	Age	Sex	Disease activity: SLEDAI	ANA[b]	Anti-dsDNA[c]	Therapy
SLE	2 / M1	CD4+ T / CD16- Mo[d], CD16+ Mo	27	f	8	1:10240	48	MMF[e] 2000 mg/day
SLE	4 / M4	CD4+ T / CD16- Mo, CD16+ Mo	24	f	22	1:640	39	Pred.[f] 7 mg/day, HQ[g] 200mg/day, MMF 2000 mg/day
SLE	7	CD4+ T	42	f	6	1:5120	89	Pred. 10 mg/day, HQ 300 mg/day
SLE	8	CD4+ T	22	f	8	1:10240	1542	None
SLE	9	CD4+ T	34	f	8	1:2560	39	None
SLE	12	CD4+ T	25	f	10	1:5120	23	Pred. 10 mg/day, HQ 300 mg/day, CYC[h] 800 mg/month
SLE	M2	CD16- Mo, CD16+ Mo	28	f	2	1:2560	73	None
SLE	M3	CD16- Mo, CD16+ Mo	27	f	16	1:2560	130	None
ND	54	CD4+ T, CD16- Mo	25	f				
ND	55	CD4+ T, CD16- Mo, CD16+ Mo	24	f				
ND	56	CD4+ T, CD16- Mo, CD16+ Mo	25	f				
ND	57	CD4+ T, CD16- Mo, CD16+ Mo	25	f				

[a]ND: healthy donor.
[b]ANA: anti-nuclear antibody with cutoff for ANA titer <1:160.
[c]dsDNA-AK (U/ml) with cutoff 20 U/mL.
[d]Mo: monocytes.
[e]MMF: mycophenolate mofetil.
[f]Pred.: predonisolone.
[g]HQ: Hydroxychloroquine.
[h]CYC: cyclophosphamide.

The microarray data were analyzed through a multi-step process. First, data normalization and the generation of cell files were conducted using Affymetrix GCOS software. These cell files were then analyzed using the BioRetis database to perform group-wise comparisons and to filter for differentially expressed probe sets. To identify interferon (IFN)-regulated transcripts, the differentially expressed probe sets were compared with published reference lists. Finally, hierarchical cluster analysis was carried out using Genesis version 1.7.5. This comprehensive analysis produced gene probe expression values

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that were subsequently used for further investigation in the study.[16]

Identify Statistically Significant Gene Probes:

Gene probe expression differences were evaluated between SLE and healthy samples using a t-test. P-values of <0.0001 were identified as statistically significant, indicating a meaningful difference in gene expression between the two groups. Statistical comparison of the differentially expressed genes for the healthy versus the SLE cohort was performed using a one-tailed t-test to determine the resultant p-value. The value considered to be a statistically significant difference is a p-value less than 0.05. Variation in the data, which can affect the p-value calculation, was dealt with by using either a heteroscedastic or homoscedastic t-test. Before choosing the appropriate t-test, the variance of the data was calculated. The standard threshold for choosing a heteroscedastic t-test is 1.5 or greater.[4] This meant that a one-sided t-test was more appropriate. All the p-values were recalculated using a one-sided t-test. The process of performing the one-sided t-test and calculating the p-values for differentially expressed genes involved several key steps, as outlined below.

The datasets labeled “GDS4888”, “GDS4889”, and “GDS4890” were accessed through the website https://www.ncbi.nlm.nih.gov/, each representing a different cell type. In the "Downloads" box on the right side of the screen, the link to download the entire SOFT file was clicked for each of the three datasets. Each SOFT file was then opened in Excel after being downloaded. A new column titled “Variance Lupus” was created in the Excel spreadsheet. The following equation was inserted in the cell below: =VAR.S(C4:H4). This calculates the variance of the SLE data for that gene probe. A column labeled "Variance Healthy" was added beside "Variance Lupus," with the equation =VAR.S(G4:I4) inserted for variance calculation. After "Variance Healthy," a column labeled "Ratio" was created with the following equation: =IF(N4>O4, N4/O4, O4/N4). This equation ensures that if the Variance Lupus is greater than the Variance Healthy, the value for Variance Lupus is divided by the Variance Healthy; if the opposite is true, Variance Healthy is divided by Variance Lupus. The output represents the ratio between the variances of the Healthy and Lupus data. Next, a column titled "Homoscedastic T-test" was added to conduct the t-test if the variance was less than 1.5. The equation =TTEST(C4:H4, I4:L4,1,2) was used to compare the data from C4 to H4 with that from I4 to L4, with "1" indicating a one-sided t-test and "2" specifying a homoscedastic t-test. A column labeled "Heteroscedastic T-test" was created for use if the variance exceeded 1.5. This t-test equation was similar to that for the homoscedastic t-test, with the last number changed to "3" to denote a heteroscedastic t-test. Another column, titled "P Value Actual," was created with the equation =IF(T4>1.5, W4, V4). If the ratio was greater than 1.5, the heteroscedastic p-value was selected; otherwise, the homoscedastic p-value was chosen. Steps #1 to #11 were repeated for each gene probe in each dataset, with each dataset preferably placed on separate spreadsheets. Each spreadsheet contained over 54,000 rows.

Following this, the gene probes with the lowest p-values were identified by extracting all p-values, ID_REFs, and IDENTIFIERs for each gene probe across the datasets into a new Excel spreadsheet for comparison. Using Excel’s Sort function, p-values were sorted from smallest to largest to identify the smallest values. A new tab was created to filter for gene probes with p-values less than 0.001, and duplicates were removed using a formula. A combined list of unique gene probes was created, and VLOOKUP was used to match p-values for each gene probe across the datasets. A "Code" column was added to identify probes with p-values below 0.001. Data from this step were transferred to a new tab where redundant values were removed and gene probes were ranked based on their p-value significance. The final dataset included the top 5 gene probes with the lowest p-values, which were ranked and organized based on their average p-values across the datasets. Line graphs were then created for each of these top gene probes, comparing probe values between the healthy and SLE groups across the datasets.

Determination of Novel Gene Expression Signatures:

The top five gene probes were evaluated to identify those associated with vital functions, based on findings from current genetic research. These probes were selected for their critical roles in maintaining essential cellular and physiological processes. The next step involved defining a novel gene expression signature by identifying the cell type(s) with the lowest p-values for each selected gene probe. A gene expression signature refers to a specific gene, or a set of genes, that shows a strong statistical association with Systemic Lupus Erythematosus (SLE) and is linked to vital cellular functions within specific immune cell types. This signature is considered novel if it has not been previously described in scientific literature and demonstrates unique or previously unreported associations with SLE. To confirm novelty, the expression patterns of the identified genes were compared to existing publications, ensuring that the signature represents a new contribution to the understanding of SLE pathogenesis.

The three statistical tools used for analysis were QQ plots, histograms, and graphical cohort comparison. QQ plots assessed the distribution of p-values against a theoretical distribution, histograms visualized p-value distribution across cell types, and graphical analysis compared the differences between healthy and SLE cohorts.

The procedure used to create Quantile-Quantile (QQ) plots began with downloading and installing Python 3.12.2 (64-bit) from the official Python website (https://www.python.org/). For reference, the website https://support.minitab.com/en-us/minitab/21/integration/python-integration-guide/example-qq-plot/ was opened and left on-screen, as it provided guidance on how Minitab interfaces with Python to generate QQ plots. However, an alternative method proved more effective for this study. Visual Studio Code was downloaded from https://visualstudio.microsoft.com/ and installed following the latest instructions on the site. Next, Command Prompt was opened, and the command pip install mtbpy numpy matplotlib was entered to install the necessary Python module packages. Minitab Statistical Software was then downloaded and insta-

lled via a free trial from https://www.minitab.com/en-us/products/minitab/free-trial/, with care taken to use the latest version and follow the installation instructions. Minitab was pinned to the laptop, and the desktop version was launched. A zip file from the earlier support site was downloaded and unzipped in a designated folder. The file qq_plot.py from this archive was opened in Visual Studio Code and served as the bridge for Python-Minitab integration. In Minitab, the "Open" option was used to load the "Hospital test runs" data file, although its content was immediately deleted from the worksheet to prepare for new input. Relevant data was copied and pasted below the worksheet, and the Command Line was used to run the script with the customizable command PYSC "qq_plot.py" "Hospital A" "Hospital B". After clicking the "Run" button, QQ plots were generated and displayed. These plots could be copied by right-clicking and selecting "Copy Image." Additional functions were available through this interface. The Minitab-Python Interface code is given as a supplementary file to this journal. In addition to QQ plots, histograms were used to determine the distribution of p-values across the three immune cell types. Graphical comparisons between healthy and SLE cohorts were also conducted to assess whether the data differences trended positively or negatively.

Develop Gene Probe Expression Ranges for Diagnostic Tool:

This section describes the methods to identify the diagnostic tool's most significant gene probes and associated cell types. It also describes the methods used to develop gene probe expression ranges for the most important gene probes that would result in a p-value less than $1x10^{-4}$. These gene probe expression ranges have the potential to serve as a novel diagnostic tool for assessing individuals for the presence of SLE, or potentially identifying a genetic predisposition to developing SLE in the future. However, it is important to note that this application remains hypothetical and would require extensive clinical validation and large-scale studies before it could be implemented in practice.

The raw and p-value data for the 4889 (CD16-) datasets were opened in an Excel spreadsheet, and the gene probes used in the diagnostic tool were identified. Data for these probes were copied into a new spreadsheet, where a "Value" column was added. The p-value equation was modified to incorporate the "Value" column in the SLE portion of the p-value calculation. This process involved incrementally increasing the value in the "Value" column and generating a new p-value for each SLE value. The gene probe data, Value quantity, and p-value were then transferred to a Data Chart spreadsheet, where a "Criteria for P-Value" column was added with a value of 0.001, and a "Code" column was created with an equation to identify probes with p-values below 0.001. A line chart was created with gene probe values on the x-axis and a maximum p-value of 0.003. The range of gene probe values resulting in a p-value of 0.001 was considered statistically significant, and these steps were repeated for all evaluated gene probes.

In conclusion, regarding all methodology, the data obtained from the Gene Expression Omnibus (GEO) represented a full gene expression profile (54,675 probes) for healthy persons and persons with systemic lupus erythematosus (SLE). The data obtained measured differences in expression in 3 different cell types, which were $CD16^+$ monocyte, $CD16^-$ monocyte, and $CD4^+$ T lymphocyte cells for each cohort. T-test p-values (p-value) were calculated using Microsoft Excel for all gene probes in all three cell types. Variation in the data was accounted for by choosing either a heteroscedastic or homoscedastic t-test based on a variance test result threshold of 1.5. The variance calculation was performed using Microsoft Excel.

If interested in an in-depth methodology, a supplemental file is added to this journal.

Results and Discussion

Determine Gene Probe Values:

Due to the large amount of data generated during this study, including the raw and p-value data tables in this report was impossible. The raw data tables, including associated p-values, were labeled Table 2A, Table 2B, and Table 2C, one for each cell type. Due to the large data tables, it was impossible to include them in this publication.

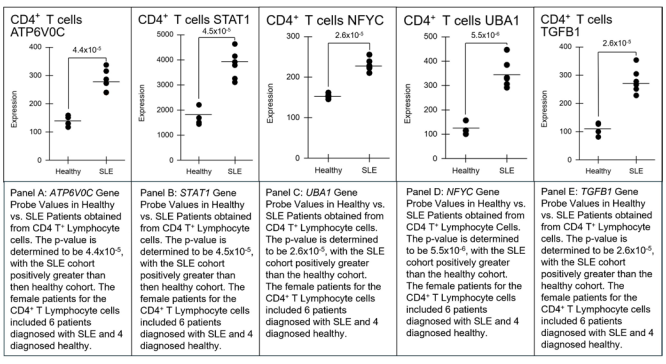
Identify Statistically Significant Gene Probes

The most significant differences between the SLE and healthy cohort gene expression across monocyte and CD4+ T cell subtypes were identified for the genes A*TP6V0C, TGFB1, STAT1, NFYC*, and *UBA1*. The lowest p-values for these genes ranged from $1.9x10^{-4}$ to $7.3x10^{-7}$. These p-values are very low and demonstrate that the difference in gene expression between the SLE and healthy cohorts is statistically significant.

Table 2: Lowest p values by cell type across different cell types, with each gene linked to its respective gene probe identifier (ID_REF). The table highlights the comparison between healthy individuals and those with SLE, revealing five gene probes with p-values ranging from 1.9×10^{-4} to 7.3×10^{-7}, indicating statistically significant differences in gene expression.

ID_REF	IDENTIFIER	p-Value		
(Gene Probe)	(Gene)	4888 (CD4+T)	4889 (CD16-)	4890 (CD16+)
200954_at	ATP6V0C	4.4E-05	1.4E-06	1.9E-04
200964_at	UBA1	2.6E-05	4.6E-06	3.8E-04
203085_s_at	TGFB1	2.6E-05	7.3E-07	4.3E-04
202215_s_at	NFYC	5.5E-06	2.0E-05	2.7E-04
200887_s_at	STAT1	4.5E-05	3.7E-04	3.9E-05

Scatterplots showing probe values for the diagnosed healthy and SLE cohorts, including associated p-values, for each of the top five gene probes and the three cell types were prepared. See Figures 1 to 3 below.



Panel A: *ATP6V0C* Gene Probe Values in Healthy vs. SLE Patients obtained from CD4 T+ Lymphocyte cells. The p-value is determined to be $4.4x10^{-5}$, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD4+ T Lymphocyte cells included 6 patients diagnosed with SLE and 4 diagnosed healthy.

Panel B: *STAT1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD4 T+ Lymphocyte cells. The p-value is determined to be $4.5x10^{-5}$, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD4+ T Lymphocyte cells included 6 patients diagnosed with SLE and 4 diagnosed healthy.

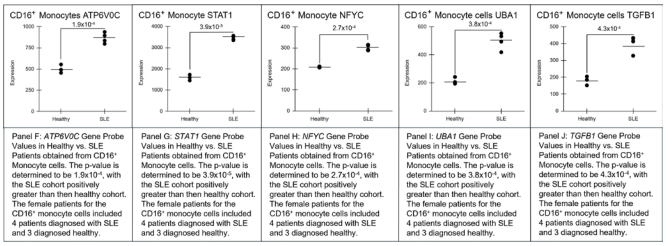
Panel C: *UBA1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD4 T+ Lymphocyte cells. The p-value is determined to be $2.6x10^{-5}$, with the SLE cohort positively greater than the healthy cohort. The female patients for the CD4+ T Lymphocyte cells included 6 patients diagnosed with SLE and 4 diagnosed healthy.

Panel D: *NFYC* Gene Probe Values in Healthy vs. SLE Patients obtained from CD4 T+ Lymphocyte Cells. The p-value is determined to be $5.5x10^{-6}$, with the SLE cohort positively greater than the healthy cohort. The female patients for the CD4+ T Lymphocyte cells included 6 patients diagnosed with SLE and 4 diagnosed healthy.

Panel E: *TGFB1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD4 T+ Lymphocyte cells. The p-value is determined to be $2.6x10^{-5}$, with the SLE cohort positively greater than the healthy cohort. The female patients for the CD4+ T Lymphocyte cells included 6 patients diagnosed with SLE and 4 diagnosed healthy.

Figure 1: The top five gene probes regarding the CD^4+ T Lymphocyte cell type, healthy vs. SLE patients gene expression.

DOI: 10.36838/v7i9.80



Panel F: *ATP6V0C* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 1.9x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

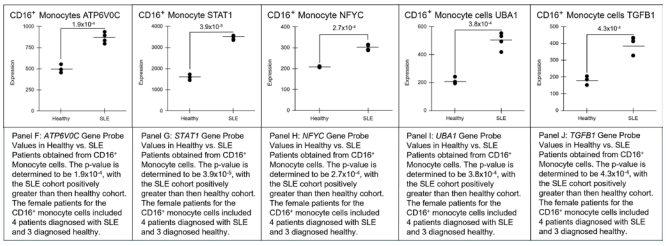
Panel G: *STAT1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 3.9x10-3, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel H: *NFYC* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 2.7x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel I: *UBA1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 3.8x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel J: *TGFB1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 4.3x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Figure 2: The top five gene probes regarding the CD16+ Monocyte cell type healthy vs. SLE patients gene expression.



Panel F: *ATP6V0C* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 1.9x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel G: *STAT1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 3.9x10-3, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel H: *NFYC* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 2.7x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel I: *UBA1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 3.8x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Panel J: *TGFB1* Gene Probe Values in Healthy vs. SLE Patients obtained from CD16+ Monocyte cells. The p-value is determined to be 4.3x10-4, with the SLE cohort positively greater than then healthy cohort. The female patients for the CD16+ monocyte cells included 4 patients diagnosed with SLE and 3 diagnosed healthy.

Figure 3: The top five gene probes regarding the CD16- Monocyte cell type healthy vs. SLE patients gene expression.

Determination of Novel Gene Expression Signature

1) Identify Gene Probes Related to Vital Functions:

The top five gene probes with differential expression, *ATP6V0C, UBA1, TGFB1, NFYC*, and *STAT1*, were further evaluated to determine how they affect the human body and known interactions with other diseases.

ATP6V0C encodes a subunit of the vacuolar H+-ATPase (V-ATPase), a proton pump responsible for acidifying intracellular compartments such as lysosomes and endosomes. This gene plays a crucial role in maintaining cellular pH balance, which is essential for processes like endocytosis, protein degradation, and the proper functioning of organelles. The proper functioning of V-ATPase, and by extension *ATP6V0C*, is vital for cellular homeostasis and vesicular trafficking.[5]

UBA1 encodes the ubiquitin-activating enzyme 1, which is imperative for the ubiquitination process. *UBA1* activates ubiquitin molecules by attaching them to proteins, marking them for various fates, including degradation, alteration in activity, or changes in cellular location. This process is central to regulating cellular processes such as the cell cycle, stress responses, and protein turnover, ensuring that proteins are appropriately controlled within the cell.[7]

*TGFB*1 encodes Transforming Growth Factor Beta 1 (TGF-β1), a multifunctional cytokine that plays a pivotal role in regulating cell growth, differentiation, and immune function. TGF-β1 is involved in tissue repair and fibrosis by promoting extracellular matrix production and influencing immune responses. It also plays a significant role in immune suppression, helping regulate inflammation and maintain immune homeostasis, which is important for tissue homeostasis and wound healing.[12]

NFYC (Nuclear Transcription Factor Y Subunit C) is a transcriptional regulator that binds to the CAAT box sequence in the promoter regions of various eukaryotic genes. While it does not produce RNA directly, it assists in regulating transcription, the process of copying DNA into RNA, by RNA polymerase activity. This regulation can increase or decrease RNA transcript levels, thereby affecting the expression of target genes.[13]

STAT1 is another gene that encodes a transcription factor involved in the immune response, particularly in activating genes triggered by interferon, signaling proteins that help the body fight infections and cancer. Interferon is a natural substance produced in the body by white blood cells that help the body's immune system fight infection and other diseases, such as cancer.[6] The protein encoded by *STAT1* immune system is involved in transmitting signals within cells, particularly in response to interferons, which are signaling proteins that play a key role in the immune response to viral infections. When interferons bind to their receptors on the surface of a cell, they activate *STAT1* and cause it to move into the cell's nucleus.[14]

All five genes are on the list of priority gene probes for further evaluation.

2) Define Novel Gene Expression Signature:

The p-value data for the top three gene probes were evaluated to determine whether one or more cell types would be used as a diagnostic tool.

In this analysis, the CD16- monocyte cell type was selected not solely based on having the lowest p-values, but rather because the p-values reflected consistent and statistically robust differences across all three gene probes. While p-values indicate the likelihood that observed differences are not due to random variation, they do not capture the magnitude of expression change. To address this, complementary analyses assessing effect sizes (e.g., fold-change) could be performed to evaluate the biological relevance of the differences. However, to establish a statistically sound candidate cell type in this initial assessment, statistical significance was prioritized to ensure that any observed differences were reliable across all gene probes.

Further research identified a list of the current known genes associated with SLE. These genes are shown in Table 3. The three genes identified in this study, *ATP6V0C, UBA1, TGFB1, UBA1*, and *STAT1*, are not included in Table 3. Therefore, the *ATP6V0C, UBA1, TGFB1, UBA1*, and *STAT1* genes associated with the CD16- monocyte cell type represent a unique and novel gene expression signature for identifying SLE in patients.

Table 3: Genes Associated with SLE[8]. However, the three genes identified in this study, *ATP6V0C, UBA1, NFYC, STAT1*, and *TGFB1*, are not included, highlighting their unique and novel role in gene expression signatures for identifying SLE in patients with CD16- monocyte cell types.

Table 1: The top five gene probes regarding the CD16+ Monocyte cell type healthy vs. SLE patients gene expression.

Gene	Location	Odds ratio	Best *P* value	Population
PTPN22	1p13.2	1.4	3.4 × 10−12	EU, HA
FCGR2A, FCGR3B	1q23	0.74	6.8 × 10−7*a*	EU, AA, AS
NCF2	1q25	1.19	4.62 × 10−20	EU, AS
CRP	1q21	0.49	9.2 × 10−14	EU, AA
TNFSF4	1q25	1.46	2.5 × 10−32	EU, AS, HA
IL10	1q31–q32	1.19	4.0 × 10−8	EU, AA
Complement genes	1p36		Convincing	EU
RASGRP3	2p24.1	0.7	1.3 × 10−15	AS
IFIH1	2q24	1.11	1.6 × 10−8	EU

STAT4	2q32.2	1.55	5.17 × 10−42	EU, AA, AS, HA
PXK	3p14.3	1.25	7.1 × 10−9	EU
TREX1	3p21.31	44.65	8.5 × 10−11	EU
BANK1	4q24	1.31	2.62 × 10−13	EU, AA, AS, HA
IL2/IL21	4q26	1.16	2.2 × 10−8	EU, AA, AS
TNIP1	5q32	1.27	1.67 × 10−9	EU, AA, AS
Mir146a	6q5	1.29	2.74 × 20−8	AA, AS
HLA and other genes	6p21.3	2.35	1.27 × 10−51	EU, AS, AA, HA
ATG5	6q21	1.25	5.2 × 10−12	EU, AS
TNFAIP3	6q23	1.72	1.3 × 10−17	EU, AA, AS
IKZF1	7p13	0.72	2.8 × 10−23	AS, EU
JAZF1	7p15.2	1.19	1.5 × 10−9	EU
IRF5	7q32	1.54	3.611 × 10−19	EU, AA, AS, HA
XKR6	8p23.1	1.23	2.5 × 10−11	EU
BLK	8p23	0.69	2.1 × 10−24	EU, AA, AS, HA
LYN	8q13	0.77	5.4 × 10−9	EU, AA, AS
LRRC18, WDFY4	10q11.23	1.24	7.2 × 10−12	AS
CD44	11p13	0.71	4.0 × 10−12	EU, AS, AA
PHRF1/IRF7/KIAA1542	11p15.5	0.78	3 × 10−10	EU, AA
ETS1	11q24.3	1.37	1.8 × 10−25	AS
SLC15A4	12q24.32	1.26	1.77 × 10−11	AS
ELF1	13q13	1.26	1.5 × 10−8	AS
ITGAM	16p11.2	1.62	1.61 × 10−23	EU, AS, HA
PRKCB	16p11.2	0.81	1.4 × 10−9	AS
IRF8	16q24.1	1.16	2.3 × 10−9	EU
TYK2	19p13.2	1.2	3.88 × 10−8	EU
CD40	20q12	0.63	2.0 × 10−8	EU
UBE2L3	22q11.21	0.78	1.48 × 10−16	EU, AS
TLR7	Xp22.3	1.67	6.5 × 10−10	AS
IRAK1/MECP2	Xq37	1.39	6.65 × 10−11	EU, AS, HA

Table 3 above from the Journal of Leukocyte Biology (2012) presents a comprehensive overview of over 50 genes statistically associated with SLE. Each entry includes the gene's name, chromosomal location, odds ratio indicating the strength of association with SLE, p-value demonstrating statistical significance, and the specific populations in which the gene variant was studied: European (EU), African American (AA), Asian (AS), and Hispanic American (HA). An odds ratio greater than 1 reflects an increased risk of developing SLE, while values below 1 suggest a potential protective effect. The chart was selected for analysis to benchmark newly identified genes, *ATP6V0C, UBA1, STAT1, NFYC*, and *TGFB1*, against existing SLE-associated genes. The absence of these genes in Table 3 supports their novelty and strengthens the claim that they represent a unique gene expression signature. This comparison validates the identification of previously unreported genetic markers in CD16- monocyte cells as well.

3) Final Statistical Analysis:

A) QQ Plots:

When reporting probe values for genes, there are only examples of genes where the value is higher in SLE patients compared to healthy patients. Generating a Q-Q plot is a common way to showcase that the test has a proper significance distribution. The results of the QQ plots, as shown in Figures 4 to 11, determine that the data is statistically appropriate for analysis.

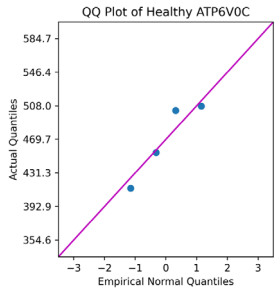


Figure 4: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the ATP6V0C gene, for the Healthy cohort, with respect to the CD16- monocyte cell type. cell type, healthy vs. SLE patients gene expression.

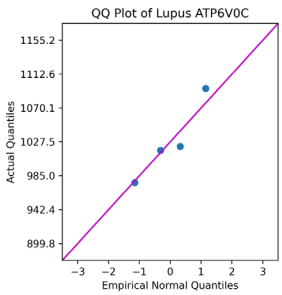


Figure 5: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the ATP6V0C gene, for the Healthy cohort, with respect to the CD16- monocyte cell type. cell type, healthy vs. SLE patients gene expression.

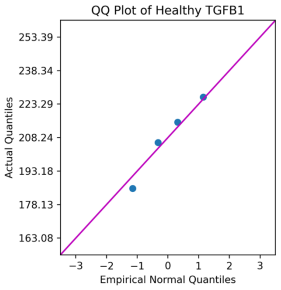


Figure 6: Quantile-Quantile plot graph for gene probe values regarding the probe associated with the *TGFB1* gene, for the Healthy cohort, with respect to the CD16- monocyte.

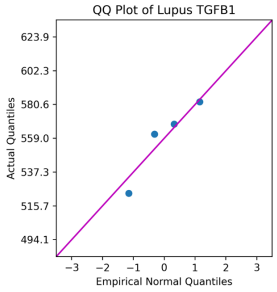


Figure 7: Quantile-Quantile plot graph for gene probe values regarding the probe associated with the *TGFB1* gene, for the SLE cohort, with respect to the CD16- monocyte.

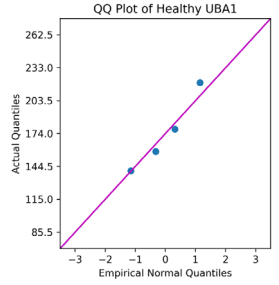


Figure 8: Quantile-Quantile plot graph for gene probe values regarding the probe associated with the UBA1 gene, for the Healthy cohort, with respect to the CD16- monocyte.

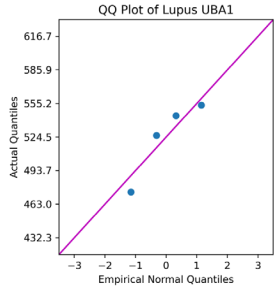


Figure 9: Quantile-Quantile plot graph for gene probe values regarding the probe associated with the UBA1 gene, for the SLE cohort, with respect to the CD16- monocyte.

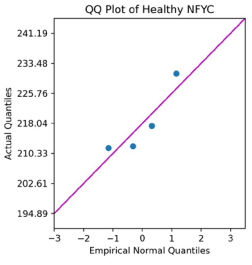


Figure 10: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the NFYC gene, for the Healthy cohort, with respect to the CD16- monocyte cell type.

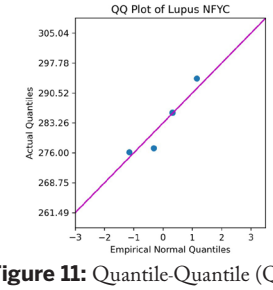


Figure 11: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the NFYC gene, for the SLE cohort, with respect to the CD16- monocyte cell type.

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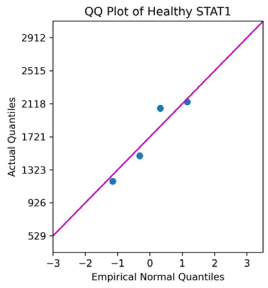

Figure 12: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the STAT1 gene, for the Healthy cohort, with respect to the CD16⁻ monocyte cell type.

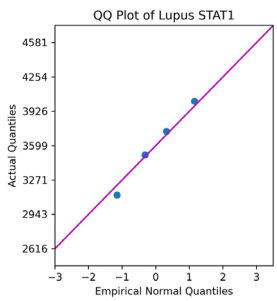

Figure 13: Quantile-Quantile (QQ) plot graph for gene probe values regarding the probe associated with the STAT1 gene, for the SLE cohort, with respect to the CD16⁻ monocyte cell type.

A standard test to determine if genetic data is statistically appropriate and follows a normal distribution is a test called a Quantile-Quantile (QQ) plot. A QQ plot plots a distribution's observed quantiles versus the ideal distribution. Where quantiles are regular, equally spaced intervals of a random variable divide the random variable into units of equal distribution.[11] Above are the QQ plots for the healthy and SLE cohorts associated with the top three gene probes evaluated.

B) Histograms:

Histograms were created to visualize the distribution of p-values derived from differential gene expression analyses between healthy controls and SLE patients. While p-values alone do not reflect the magnitude or biological relevance of gene regulation, they remain a useful tool for identifying statistically significant patterns within large-scale expression datasets. The raw gene probe data in Table 1 are shown in Figures 12, 13, and 14, as frequency histograms for each cell type, CD16- monocyte, CD16+ monocyte, and CD4+ T lymphocyte. These histograms show the number of times the p-values for a specific range of values occur in the dataset. These histograms show relatively high p-values in the very low p-value ranges. This is normal for gene probe data and indicates that there are outliers that have statistically significant p-values.

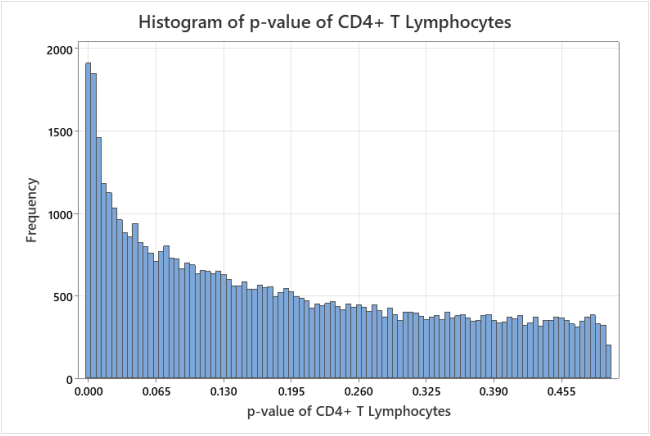

Figure 14: Histogram of p-values for CD4⁺ lymphocyte dataset. The dataset represented full gene probe analysis results (54,675 probes x 1 cell type CD⁴+ T Lymphocyte) for healthy persons and persons with systemic lupus erythematosus (SLE).

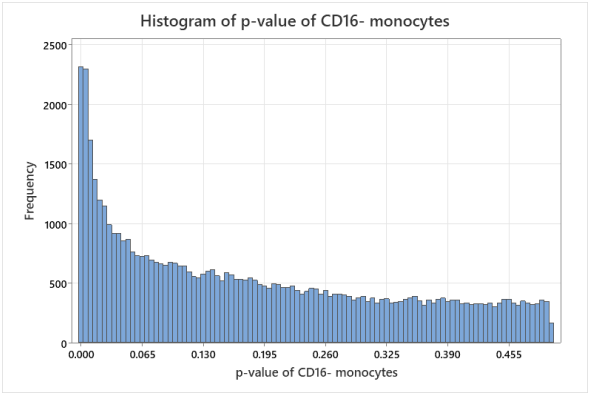

Figure 15: Histogram of p-values for CD16⁻ monocyte dataset. The dataset represented full gene probe analysis results (54,675 probes x 1 cell type CD16⁻ Monocyte) for healthy persons and persons with systemic lupus erythematosus (SLE).

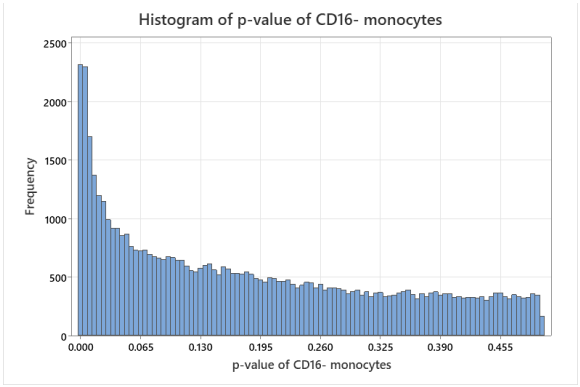

Figure 16: Histogram of p-values for CD16⁺ monocyte dataset. The dataset represented full gene probe analysis results (54,675 probes x 1 cell type CD16⁺ Monocyte) for healthy persons and persons with systemic lupus erythematosus (SLE).

C) Healthy versus SLE Charts:

It was noted previously that all the line graphs in Figures 1 to 3 showed gene probe data for the SLE cohort, which was higher in value than the healthy cohort. Two gene probe scatterplots, Figure 17, show that there are gene probes for which the healthy cohort has higher values than the SLE cohort. Many more gene probes with this trend demonstrate a variety in the data. Each bar is the expression value in an individual T cell.

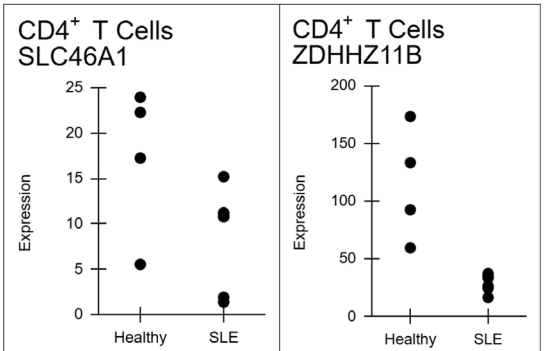

Figure 17: Two Gene Prove Values in Healthy vs. SLE Patients to Demonstrate Different Cohort Positioning.

4. Develop Gene Probe Expression Ranges for Diagnostic Tool:

Two gene probe values were identified, resulting in a p-value of $1x10^{-4}$ for each of the top three gene probes from the $CD16^-$ monocyte cell dataset. These two gene probe values represent the highest and lowest gene probe expressions, resulting in a statistically significant difference between the healthy and SLE cohorts, based on minimum p-value criteria of $1x10^{-4}$. Charts were prepared for each of the top three gene probes to show this data. See Figures 18 to 22.

To note, expression ranges are method-dependent, with different gene expression platforms (e.g., microarray, RNA-seq, qPCR) potentially producing varying absolute values. In this analysis, ranges were derived specifically based on the methodology employed to ensure internal consistency. The analysis was performed to provide a structured framework for interpreting expression differences and selecting the most suitable candidate cell type.

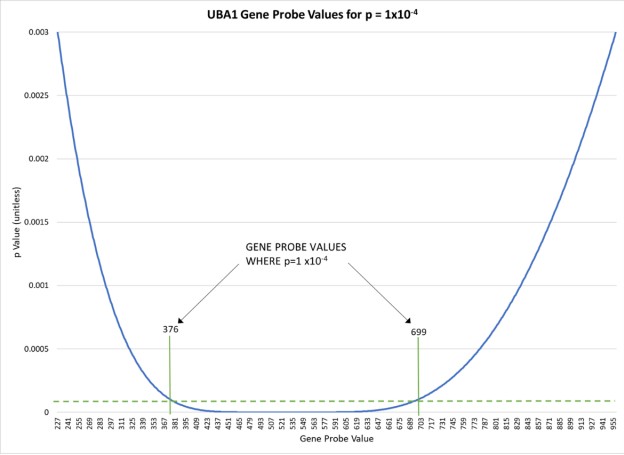

Figure 18: *UBA1* Gene Probe Values for p = $1x10^{-4}$. Displays the statistically significant UBA1 gene probe range (p<0.0001) for detecting the presence of SLE using $CD16^-$ monocyte cells. Specifically, the *UBA1* gene probe range is between 376 and 699.

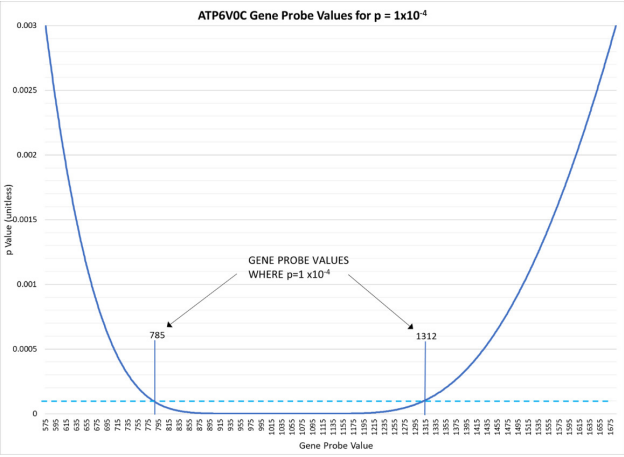

Figure 19: *ATP6V0C* Gene Probe Values for p = $1x10^{-4}$. Displays the statistically significant *ATP6V0C* gene probe range (p<0.0001) for detecting the presence of SLE using $CD16^-$ monocyte cells. Specifically, the *ATP6V0C* gene probe range is between 785 and 1312.

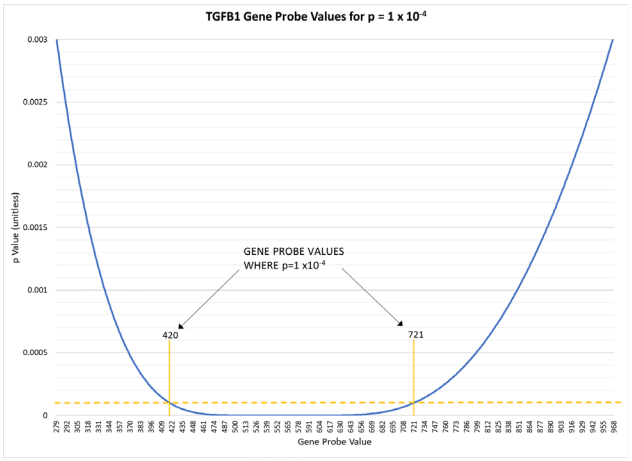

Figure 20: *TGFB1* Gene Probe Values for p = $1x10^{-4}$. This figure provides a comprehensive overview of the statistically significant TGFB1 gene probe range (p<0.0001) for detecting SLE through the analysis of $CD16^-$ monocyte cells, with gene probe values spanning from 420 to 721.

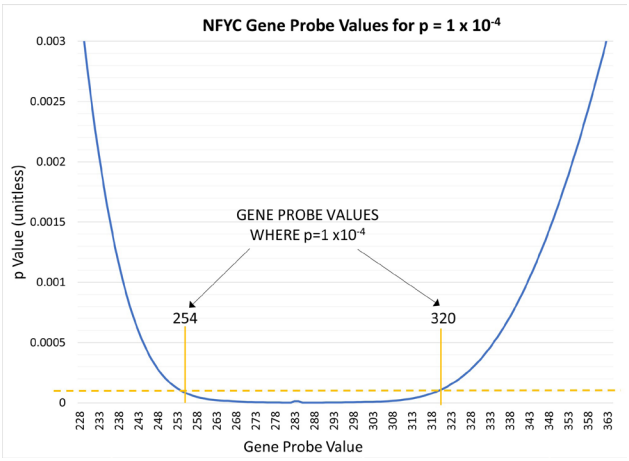

Figure 21: *NFYC* Gene Probe Values for p = $1x10^{-4}$. Displays the statistically significant NFYC gene probe range (p<0.0001) for detecting the presence of SLE using $CD16^-$ monocyte cells. Specifically, the *NFYC* gene probe range is between 254 and 320.

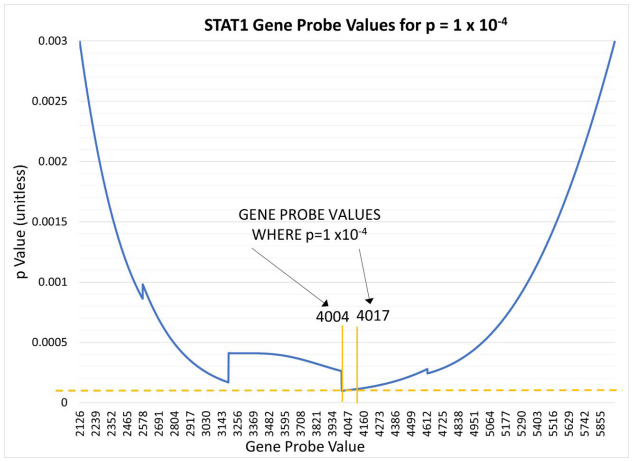

Figure 22: *STAT1* Gene Probe Values for p = $1x10^{-4}$. Displays the statistically significant STAT1 gene probe range (p<0.0001) for detecting the presence of SLE using $CD16^-$ monocyte cells. Specifically, the STAT1 gene probe range is between 4004 and 4017.

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The graph above shows "abnormality" in comparison to the other graphs due to the variance set to 1.5. Below is a graph for *STAT1* with Variance disregarded, and therefore, the homoscedastic t-test is only regarded as the final p-value to analyze. This will remove the "jagged edges" visually displayed.

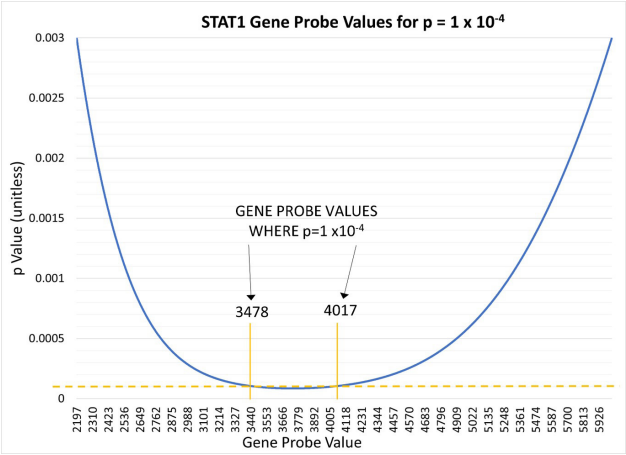

Figure 23: *STAT1* Gene Probe Values for p = 1x10^{-4}. P-values are produced through only the homoscedastic t-test. Displays the statistically significant STAT1 gene probe range (p<0.0001) for detecting the presence of SLE using CD16^{-} monocyte cells. Specifically, the STAT1 gene probe range is between 3478 and 4017.

Conclusion

The following are the significant conclusions from this study.

The study determined a statistical difference in the gene probe values for genes *ATP6V0C, UBA1, NFYC, STAT1*, and *TGFB1* between healthy people and people with SLE. The t-test p-values (p-value) from this analysis for these genes were less than 1 x 10-4 for all cell types in the study, CD16^{-} monocyte, CD16^{+} monocyte, and CD4^{+} T lymphocyte.

Furthermore, it was determined that the CD16- monocyte cell type is the best indicator of statistical difference in gene probe expression in this study for SLE in the *ATP6V0C, UBA1, NFYC, STAT1*, and *TGFB1* genes. The p-values from this analysis ranged between 1.4 x 10^{-6} to 7.3 x 10^{-7}.

The study results were compared to a list of known genes associated with SLE. It was determined that the gene probes in this study, related to the *ATP6V0C, UBA1, NFYC, STAT1*, and *TGFB1* genes and the CD16^{-} monocyte cell type, represent a novel gene expression signature for the identification of SLE.

The criteria for a novel diagnostic test method were developed to detect the presence of SLE in patients. The criteria are based on the high and low gene probe expression values identified in this study as statistically significant in SLE patients. Using these high and low gene probe values that define the gene probe expression ranges that are statistically significant in SLE patients a diagnostic test method could be developed to test patients for the presence of SLE. First, a blood sample will be obtained from a patient. The CD16^{-} monocyte cells would be isolated from the blood sample. Then, the CD16^{-} monocyte cells would be analyzed for the three gene probes defined in this study and associated with the *ATP6V0C, UBA1, NFYC, STAT1*, and *TGFB1* genes. This diagnostic tool would be a quick and relatively simple way to determine if a person has SLE. Introducing this innovative diagnostic method could transform the lives of SLE patients globally. It quickly and accurately detects SLE, so patients can receive timely treatment, vastly improving their quality of life and potentially saving lives. This test could ease the financial burden on healthcare systems by simplifying diagnosis, allowing resources to be redirected toward patient support and research into new SLE treatments and a possible cure.

The sample size for this study was relatively small. Further analysis with additional persons in the SLE and healthy cohorts would improve the study's statistical power and increase confidence in rejecting the null hypothesis. Further long-term research, including persons who are asymptomatic for SLE, is required to determine if this test can also determine whether a healthy person is genetically predisposed to SLE in the future. This is a very interesting study area since I am unaware of any quantitative methods for determining a person's predisposition to SLE.

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All glory be to God.

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REVIEW ARTICLE

Understanding the Etiology of Astigmatism: A Literature Review

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ABSTRACT: Astigmatism is caused by the irregular curvature of the eye's cornea, affecting how light is refracted. Though astigmatism has been researched, and its effects are understood well, etiology is not fully characterized, and there are few effective treatment options. This review will characterize the current literature on the etiology and treatments of astigmatism and identify gaps for future research. Both genetic and environmental components contribute to the development of astigmatism. In our review, we found several genes with evidence of association with the development of astigmatism, including PDGFRA, FRAP1, SHH, and VAX-2. Additionally, we identified a few genes with mixed evidence of their connection to the development of astigmatism. We also identified evidence indicating that environmental factors, such as birth during periods of increased sunlight exposure and specific age-related variables, significantly influence the outcomes. In our review, we identified specific gaps in astigmatism etiology research. These gaps include the lack of clinical trials and limited additional research on these factors. Adding more GWAS studies that are wide in range can allow us to pinpoint a robust and evidence-backed cause of astigmatism. This review summarizes the current literature on astigmatism's genetic, environmental, and age-related factors and identifies gaps for future research.

KEYWORDS: Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Astigmatism, Corneal Curvature.

Introduction

Astigmatism is a common refractive abnormality that has affected millions of individuals worldwide. In the United States alone, it affects 1 in 3 people.[1] Astigmatism is caused by the irregular curvature of the eye's cornea, affecting how light is refracted and ultimately resulting in distorted vision.[2] While astigmatism can arise from irregularities in either the cornea or the lens, this review will focus specifically on corneal astigmatism. If left uncorrected, astigmatism can lead to blurred vision, eye strain, and, in severe cases, contribute to amblyopia, especially in children. Though astigmatism has been researched and its effects are understood well, the etiology is not fully characterized, and there are few effective treatment options. This review will characterize the current literature on the etiology of astigmatism and identify gaps for future research.

Discussion

Genetic Components of Astigmatism:

The genes PDGFRA, FRAP1, SHH, and VAX-2 have a relevant link to the development of astigmatism. Other instances of relevance are recorded but have little to no link to the development of astigmatism.[3]

Though little research has been conducted into its direct effect on astigmatism, the PDGFRA protein-coding gene plays a known role in corneal curvature. Genome-wide association studies (GWAS) are commonplace in genetic research that use large quantities of genomic data to identify associations between genotypes and phenotypes. One major GWAS meta-analysis involving five Asian cohorts (N = 8513) demonstrated that a cluster of single-nucleotide polymorphisms (SNPs) called rs7677751 located on PDGFRA was linked to the relevance of abnormal corneal curvature.[4] It is known that irregular corneal curvature causes astigmatism due to the distortion of the eye's focus and uneven light refraction, leading to blurred vision.[5] Another large GWAS study for corneal (n = 86,335) and refractive astigmatism (n = 88,005) was conducted on European populations that reported a significance of a cluster of SNPs on rs4864857, located on the 4q12 promoter region of PDGFRA.[6] Thus, future research is needed to investigate the impact of the PDGFRA-influenced abnormal corneal curvature on the development of astigmatism.

Several smaller studies investigated the link between PDGFRA and corneal curvature.[7–10] Most of these studies found a moderate link between the two,[11] though some reported no significance.[7,9] The scope of studies on the relationship between PDGFRA and corneal astigmatism demonstrates how further research is needed to establish a firm cause and effect between the two, due to the trend of some studies reporting significance and some reporting no significance.

In addition to PDGFRA, another gene has been implicated in the development of astigmatism, known as the FRAP1 gene, or mTOR. A GWAS study (n = 4289) conducted on Asian populations in Singapore found FRAP1 to be associated with corneal curvature development, and variations in FRAP1 can alter corneal curvature development.[12] The authors found this unsurprising as FRAP1 is known to influence metabolic pathways that affect epithelial growth, which is a main component of the cornea. At this time, little research has been performed to assess possible links between FRAP1-induced corneal curvature abnormality and astigmatism, and thus, more research is needed to establish a potential relationship.

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The sonic hedgehog (SHH) gene is generally associated with eye patterning in embryonic development. The evidence of a direct relationship between SHH and astigmatism is weak but largely unexplored. One analysis determined that a mutation of SHH caused an autosomal dominant syndrome in children, resulting in cataracts, vitreoretinopathy, primary open-angle glaucoma, and asymmetric myopia with astigmatism.[13] SHH and other genes in the hedgehog (HH) family are known to influence the regeneration of the corneal epithelium.[14] As astigmatism can have onset during any age, it is possible that variants in SHH that affect the regeneration of the corneal epithelium may contribute to astigmatism and its age of onset.[14] Future research should explore this potential connection. Additionally, there is evidence that the PAX6 gene interacts with SHH and influences the regeneration of corneal epithelium.[14–16] The current literature does not describe a relationship between SHH and astigmatism; thus, future research should aim to better characterize the link between the two.

Another gene that is potentially implicated in astigmatism onset is the VAX2 gene. The VAX2 gene is a protein-coding gene that forms the retina and the dorsoventral axis during eye development. According to a meta-analysis involving seven studies and (n = 22100) individuals, a susceptibility locus with SNP rs3771395 on Chromosome 2p13.3 was identified in the VAX2 gene. A susceptibility locus occurs when an allele is detected to increase the risk, but does not fully indicate disease expression.[17] This study suggests a connection between VAX2 and astigmatism, but the details of this connection are yet to be understood.3 Another study explored VAX2 overexpression and its role as a transcription factor, suggesting a particular link to VAX2 and neural retina development. When overexpressed, the VAX2 gene tends to alter other parts of the eye.[18] This could unveil a potential link between VAX2 overexpression and its effect on the cornea and the development of astigmatism, but more research is needed to confirm this.

In a large GWAS study with 14 European (n = 22250) cohorts and 9 Asian (n = 9120) cohorts, three new genes, CLDN7, ACP2, and TNFAIP8L3, were identified to have a possible contribution towards astigmatism. These genes have not been identified in any other study and represent areas that should be further investigated in future research.[19]

Environmental Components of Astigmatism:

Genetics alone may not explain astigmatism onset. Environmental factors could also play a role, as exposure to different conditions during development may affect corneal curvature at/before birth. A large study conducted in Israel (n = 67899) suggests multiple environmental factors are associated with astigmatism development.[20] An interesting finding shows that people born in longer perinatal photoperiods, which refer to people born in the summer months, are more likely to be diagnosed with the rule (WTR) astigmatism,[20] which is a subset of astigmatism where the vertical meridian of the eye is steeper.[21]

Additionally, some studies did not find evidence for a link between astigmatism and genetic factors. Namely, the Tehran Eye Study did not link the two, suggesting that astigmatism is not inherited and may result from environmental factors.[22] The development of astigmatism is complex and does not seem to be tied to one factor alone. However, evidence establishes a possible link for and against the environmental component of astigmatism development. There is insufficient research for definitive conclusions, emphasizing the need for future research.

Another potential factor that may lead to the development of astigmatism is age-related determinants. Many studies suggest that with age, the prevalence of astigmatism increases due to changes in the shape of the corneal curvature. This is because of a shift in the axis of the eye, which simultaneously encourages a shift from WTR to against-the-rule (ATR) astigmatism, the other subset of astigmatism where the horizontal meridian of the eye is steeper than the vertical meridian.[21,23,24] Increased astigmatism with age was also found in a population of myopic patients, suggesting this trend may occur independently of refractive error.

Limitations of Review

This review acknowledges several limitations inherent in both the extant literature and the present analysis. A considerable proportion of studies addressing the etiology of astigmatism focus on specific populations or age cohorts, thereby limiting the external validity of their findings. Moreover, genetic association studies often exhibit variability in outcomes. Environmental and behavioral factors remain underrepresented in literature, and their contributions to astigmatism development are not yet fully elucidated. Recognizing these limitations is essential to contextualize the findings and to inform future research directions aimed at elucidating the multifactorial etiology of astigmatism.

Conclusion

This review summarizes the current literature on astigmatism's genetic, environmental, and age-related factors and identifies gaps for future research. These gaps include the lack of clinical trials and limited research on these factors. Adding more GWAS studies that are wide in range can allow us to pinpoint a robust and evidence-backed cause of astigmatism. Current research that includes but is not limited to genes such as PDGFRA, SHH, FRAP1, VAX2, environmental factors such as perinatal photoperiod, and age-related causes opens pathways for these gaps in astigmatism research to be closed and identified. Future research should focus on conducting larger, diverse population-based GWAS, more clinical trials to validate findings, and investigations into how environmental and age-related factors interact with genetic predispositions.

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REVIEW ARTICLE

Gender Disparity and Representation of Women and the LGBTQIA+ in the Political Sphere

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ABSTRACT: This study is an analysis conducted to understand the ethos of gender disparity, particularly in political institutions and societal spheres. An emphasis on the misrepresentation of women and other genders included in the LGBTQIA+ Community is taken into consideration to understand and highlight inconsistencies in political bodies, and how it has affected their decision-making. The methodology of the paper includes both quantitative and qualitative analysis. The scope of the study ranges from the 1950s to the present, as well as some suggestions for the future, concluding that governing bodies including a diverse population of multiple genders and personalities can elevate political institutions. The analysis from the primary survey summarizes an overall structural mindset of our society, with aspects like awareness, activism, and passion for change given some thought. The study is supported by multiple graphs and pie charts to strengthen the construction presented in the paper and enhance the understanding of the results. Analysis from case studies of contrasting countries draws the inference that proportionate composition in their decision-making bodies can lead to more empathetic, relatable, and accurate policies, resulting in better governance and growth.

KEYWORDS: Social Sciences, Sociology, Gender Disparity, LGBTQIA+ and Women, Political Gender Representation.

Introduction

Gender inequality refers to a phenomenon where a specific gender is targeted and receives unusual treatment. It has been deep-rooted in our society for as long as anyone can recollect, and has taken shape from details to huge differences that mold society into the way it is today. It does not take a sharp eye to notice, still, analysis of illustrations drawn in the latest high school textbooks shows men being portrayed as engineers, doctors, scientists, and other professionals. In contrast, women's roles are limited to those of housekeepers and nurturers, mothers, and nurses.[1] This research paper delves into exactly these specifics of the structure, dissecting the gender disparity that is found in the status quo as the product of past conditions and workings.

Political inequality occurs when political power and authority are distributed unevenly among a particular group. Commencing from the ethos of gender inequality in governance, the paper explores the history of governmental patriarchy and the origin of misrepresentation, followed by the spike of change in political representation in the last few decades, highlighting the key milestones, gender rights, and political trends, including the rise of feminism and also the increasing popularity of the LGBTQIA+ (lesbian, gay, bisexual, transgender, queer, intersex, asexual) community.[2] The study is descriptive, historical, and qualitative in nature. Both primary and secondary data have been used. Secondary sources include books, government documents, journals, articles, and other similar materials. The primary data is collected using survey questionnaires and a semi-structured interview with an LGBTQIA+ expert.

Case studies of key political personalities who have created a huge impact in empowering women are conducted to explore the intricacies of historical disparity and their fight against it. The interview with LGBTQIA+ expert and activist Dr. Rimashree Borah is discussed to highlight the origin, progress, and many more details involving the community by exploring the breakthroughs and issues, and surveying grassroots-level analysis.

A primary survey with 50+ participants aims to infer about inequality at the local levels. Through the analysis presented, the paper aims to understand the underlying loopholes of the socio-political system regarding equal gender representation. The key focus of the survey revolves around the commonplaces of differentiation in tasks based on gender, public policy on women and the queer community as well as subjectivity on the question of 'how to improve the observable misogyny in our surroundings.' The intriguing patterns of answers found in varied genders and ages emphasize the importance of equal voice and the opportunity to present opinions.

The study also draws inferences on other fields that show traces of gender inequality, except in the political sphere. Further, the paper highlights a contrasting case study of two different countries, namely, New Zealand and the Maldives, to understand gender disparity in political institutions through different perspectives and circumstances, as well as acknowledge how egalitarian representation in political bodies can lead to better policy making and empathy in governments.[3] The reforms introduced till now in the field have been summarized in the next section, with the hope of resolution and a progressive attitude, alongside some recommendations that can be implemented.

Survey of Literature:

1. Barbara Smuts conducted a study titled The Evolutionary Origins of Patriarchy. The study aimed to argue that feminist analyses of patriarchy should be expanded to address the evo

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lutionary basis of male motivation to control female sexuality. Six hypotheses were proposed to conclude how this unusual gender inequality came about.[4]

2. Karen Celis conducted a study titled Substantive Representation of Women (and improving it): What it is and Should be About? Argues that more women MPs and the structural presence of attention for women's interests not only contribute to just and democratic politics but also enhance the quality of democratic decision-making and policymaking on a substantive level. It consists of quantitative improvements (for example, more support for women's interests by representatives) and qualitative improvements (for example, support for a broader range of women and women's interests). The paper concludes that substantive representation implies recognizing diversity and ideological conflict regarding women's interests and gendered perspectives.[5]

3. Melanie M. Hughes and Pamela Paxton conducted a study called The Political Representation of Women Over Time. It mapped trends in women's political representation from 1945 to 2015, stating that while some countries have made considerable progress, others still progress slowly. Some countries openly resent the idea. Hughes and Paxton conclude the paper by identifying and describing four basic paths to women's increased representation over time: (1) No Change, (2) Incremental Gains, (3) Fast-Track Growth, and (4) Plateau.[6]

4. Tyler J. Hatchel conducted a study on the Digital Development of LGBTQ Youth: Identity, Sexuality, and the ubiquity of digital contexts. It analyzes the limitations and disadvantages of using devices and reforms for how their use can be better utilized to achieve developmental tasks.[7]

5. Sherry Ortner conducted a study titled Patriarchy, stating that it is more than just "sexism." It is a social formation of male-gendered power with a particular structure that can be found with striking regularity in many different areas of social life. The paper concludes with statements about Right-wing politics and how it cannot be completely separated from normative heterosexuality. It closes with the question of how to formulate an activist's politics that addresses both immediate threats and long-term structural patterns.[8]

Objectives

The main objectives of the paper are to, firstly, understand and analyze whether the proper representation of various genders in a decision-making political body increases their relatability and leads to more empathetic policymaking. This has been done through case studies of pioneers and a comparative analysis between two contrasting countries.

The paper also observes how much awareness about the accurate definitions and gender equality, feminism, and the LGBTQIA+ Community has increased considerably. Additionally, it introduces some reforms as to how we can incorporate these concepts as more colloquial terms in our decision-making and domestic areas.

Methods

The surveys and case studies in this paper have been conducted in a modern setting. It involves results from a live interview as well as questionnaires taken through a telephonic conversation or written survey. The history of various pioneers has been taken from live anecdotes and experiences and used after verification.

History of Governmental Patriarchy:

Exploring the origins of patriarchy, in this section, the grassroots level and the start are discussed, delving into the causes of this inequitable domination and its prominence in earlier time periods. Going back to historical times, according to Engels' assumption, there was a natural division of labor in which women were 'producers of life' while men were 'producers of goods' and thus the primary producers in hunting, agricultural, and herding societies.[9]

Women's sexual subordination has been found to be institutionalized in many legal codes. Their cooperation in the system was ensured by various means: force, economic dependence on the males of the family, class privileges bestowed upon conforming, and the artificially created division into so-called respectable and non-respectable women. The book 'Creation of Patriarchy' by Gerda Lerner, widely known as the 'Godmother of Women's History' noted that women were dominant among the earliest slaves and that not only were their sexual services important in motivating their slavery but that rape in general was a major factor in keeping them enslaved and oppressed.[2]

The horrible manipulation and coercion involved in this process led to many generations of trauma and terror. Deep-rooted practices similar to this, if not to such a degree, continue to exist to this day in our social sphere.

As opposed to previous beliefs and myths that have not only undermined the strength and abilities of women but also reduced them and their roles to menial tasks, the feminist wave of ideologies has risen. This community, believing in not a matriarchy nor female domination, but in equal standing and equity, has taken hold over many. It is not only females who have been victims of insubordination, girls who have seen their mothers drain their lives to never be too good for society, and every other dreamer who never had the opportunity to live up to their potential; but all those willing to stand up for the fight who have envisaged the changes that are shaping the present societies.

Feminism has never been about the alternative recreation of the patriarchy, it has been about breaking it from its roots and eliminating all kinds of androcentric biases.[10] It is less known that patriarchy doesn't put men on the highest pedestal but creates an unbelievable image for them that builds up to high pressure and tries to classify any sort of behavior as 'normal' or excusable.

Many theories about the performing abilities of women have been debunked. For instance, the five-ounce difference in weight between male and female brains was widely thought to be the apparent cause of female cognitive inferiority, but this theory was later contradicted when it was brought to attention that absolute brain weight was not the right measure of intelligence, nor was cranial or body height.[11] Many such other theories have been made and erased simply based on assumptions and bias, yet they have morphed into prejudice and stereotypes with no scientific base, but are prominently observable in some groups of society more than others.

In today's political sphere, there is no doubt that a lack of representation of women prevails. However, should our politics only revolve around 2 genders as we progress into a world where gender identities are no longer tied down by conventionality, as our society grows diverse and inclusivity becomes a vital aspect to keep in mind in the upcoming generations?

In such a scenario, it has become increasingly important not to exclude the LGBTQIA+ community; our society must question whether all the development in the past decades is worth it when it cannot be fully utilized by a mass chunk of the population who is unable to gain equitable access to it.

So, several more questions continue to persist: Why are political institutions defined and controlled by male elites still the focus of narratives about political change? What justifies the continued lack of engagement with feminist deconstructionism? What would the global history of the recent past look like if one took these critiques to heart and attempted to build a different kind of epistemology, a human feminist one?[2]

The Rise of Women in Politics 1950s Onwards:

History has almost always been written from the perspective of men-philosophers that students have read about have also been gender-centric: from John Locke to Rousseau, the books used by the education system portray the contribution of men in textbooks when several female revolutionaries who shaped their future and the present we live in remain shadowed. Some of these are Empress Catherine of Great Russia, Rani Ahilyabai, and many other pioneers, some of whom have been explored below.

Regardless, women have spearheaded truly unprecedented and groundbreaking shifts; from new rights and cultural norms to greater agency as a category consisting of half the world population, women have slowly transformed into a rising community of politically enfranchised powers.

Their influence in the educational, healthcare, and legal fields has shifted the face of inequality and improved the standing position when it comes to medical autonomy, use of birth control, reproductive healthcare, financial independence, and manifold other facets. Women's increasing participation in the global market as not only consumers but also producers has made products more relevant, relatable, and efficient for other females due to their empathy, understanding, and accurate knowledge.[2]

Since the 1900s, there has been a gradual shift leading to the acceptance of women's sexuality, creating room for discussion and policy-making. One of the major grounds for remonstration was a denial of the most basic political right: the right to vote. The political movement for suffrage commenced with great exuberance among women, while most sections of men received the right to vote. The radicalness and intensity of the protests varied worldwide; while some countries witnessed petitions, others opted for outright civil disobedience.

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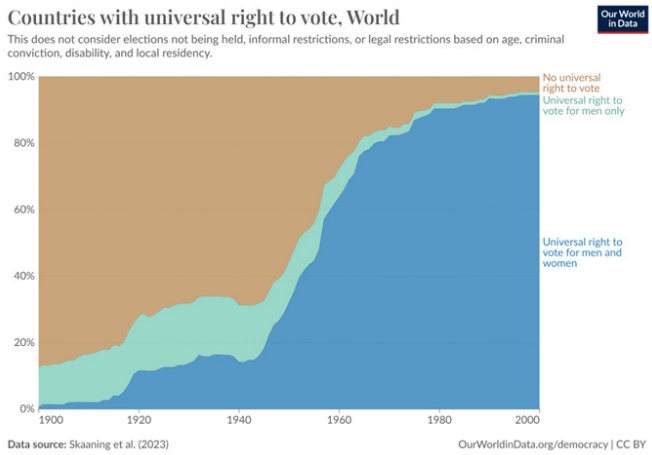

Figure 1: Visual representation of the gendered perspective towards the right to vote from the 1900s to the year 2000 in countries, depicting the rise of universal voting rights for both men and women.
Source: Our World in Data.[12]

With the right to vote, mostly after World War II, their voices and spirit amplified. Specifically in the 1920s and 30s, more and more women became interested in political participation, and there was more talk about gender inclusivity in the responsibilities of the state.[2]

It was found that the participation of women greatly depended on their race and the abilities and ideology of the breadwinner, i.e., the male head of the family. However, this was not the case with all women: pioneers like Mary McLeod Bethune (1884-1962) left no stone unturned to flip the trajectory of racial relations and improve the public and civil service sector.[13]

During the 1960s, many college-going women reported that they were influenced by the social movements of that era, even though they were not direct participants, but rather just engaged observers.[14] This decade opened with women's participation hovering at its fringes, but ended with a feminist movement that paced considerably with the second wave of feminism.

The 1970s included some major milestones, including the declaration of sex discrimination a violation of the 14th Amendment in the United States of America, and a surge in feminist art, literature, and research.[15]

In the 1980s, the Human Rights Campaign (HRC) was founded to mobilize the LGBTQIA+ community and their rights. In 1985, it merged with the Gay Rights National Lobby. Currently, with approximately 1.5 million members, it is the largest organization for lesbian and gay rights in the United States.[16] In the 1990s, violence against women emerged as one of the global challenges. The United Nations Declaration bridged the gap between women's rights, world peace, and the elimination of violence against women.[16]

Feminists Bennet and Cooke spoke out against the disparity in governmental policies and pressured the male-dominated institutions to introduce progressive politics and abate the stereotypes spewing that people of certain genders or races were inferior and not deserving of equal respect.[2]

Through the gradual shift in the century, transformation not only in the political field but in several others led to women

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in the paid workforce, economic independence that welcomed both partners sharing familial responsibilities, and several other key milestones. From every woman who had no choice but to succumb to the force of the patriarchal structure to those who sacrificed their own lives and souls to the movement, the trail of change held the footsteps of every person who contributed towards equality.

Case Study 1: Changemakers of our History:

The following are some more leaders who made a mark through their work in campaigning, governance, or erasing disparity. Their pioneering spirit inspired many to take a step forward and demand an egalitarian government. **Indira Gandhi** was the first female Prime Minister of India, in 1966, who entered a male-dominated political scene. While her term was not without controversy, she is known for her decisive leadership, and she has been instrumental in initiating transformations such as the Green Revolution, which strengthened India's agricultural self-sufficiency and played a crucial role in the Bangladesh Liberation War of 1971. Her declaration of Emergency from 1975 to 1977, which curtailed civil liberties, remains a polarizing chapter in Indian history. Her ascension to power marked a great moment for women in politics, inspiring future generations while also portraying the complexities of leadership.[17]

In Sri Lanka, **Sirimavo Bandaranaike** was the first female world prime minister elected in 1960, albeit due to her husband's assassination. Her reign has been full of notable social and economic reforms; one of the significant reforms during her tenure included nationalization and the encouragement of non-alignment with the Western and Eastern blocs during the Cold War. While her policies often invited criticism for economic challenges and growing ethnic tensions, her leadership was a milestone for women globally, showcasing their ability to lead nations during tumultuous times and breaking gender barriers in the political arena.[18]

Golda Meir of Israel is known as the "Iron Lady" of Israeli politics, having served as Israel's first woman prime minister in 1969. She took a lot of responsibility and showed unbelievable toughness as she commanded the defense of Israel in 1973 during the Yom Kippur War, making complex diplomatic moves worldwide. Criticisms of how prepared the country was before the war marred Meir's premiership, but she proved to be a tough woman. At such a point in the country, she ensured her leadership stood out among the most iconic women leaders of the 20th century.[19]

Margaret Thatcher led the United Kingdom as its first female Prime Minister from 1979 to 1990. With her policies strongly branded as 'Thatcherism,' emphasizing economic liberalization, deregulation, and privatization, they left an indelible mark on international politics. Thatcher's robust leadership was also displayed in the Falklands War in 1982 when she showed that Britain was a leading power worldwide. Although her policies were polarizing and caused significant domestic unrest, Thatcher's tenure proved that women could lead with conviction and decisiveness in both domestic and international affairs.[20]

Excerpts from Interview with Dr. Rimashree Borah, LGBTQIA+ Expert:

Homosexuality has been found in our history for longer than our textbooks want us to know, and traces are visible in many of our documents, lifestyles, and ideas at the grassroots level, where impact is vital to improve the current situation politically as well as socially. Understanding where the base influence of such a widespread community starts is crucial to building the stepping stones toward inclusivity. Thus, focusing more on exploring the frontier of representation and growth of the LGBTQIA+ community, a semi-structured interview with a renowned LGBTQIA+ expert, Dr.Rimashree Borah, was conducted.

Q. Do you believe that there is a lack of representation of the queer community in government bodies?

Dr. Reemashree: Yes, there is a lack of awareness leading to misrepresentation of the queer community in government bodies. There is a social stigma attached to non-heteronormative identities in our society, often resulting in a distorted view of homosexuality and discrimination against LGBTQIA+ persons in their workplace.

Q. Where and how, according to you, does this homophobia or disparity stem from historically and politically?

Dr. Reemashree: Indian culture has always exhibited the essence of pan-genderism and queerness: the autochthonous nature of our society was steamrolled with the advent of the British colonial government; the Victorian Puritans strove to regulate homoerotic desires by controlling bodies and repressing sexuality. In the post-colonial era, Hindu culture was reconstructed, based on social mores and heteronormativity. The Indian Nationalists, along with the Hindu extremist forces, tried to eliminate homosexuality from our society, declaring it as a "Western burrowing."

Q. How important is it to make an impact at the grassroots level to improve the current situation politically as well as socially? More importantly, where does the base influence of such a widespread community originate?

Dr. Reemashree: It is crucial to make an impact at the grassroots level for the advancement of LGBTQIA+ rights in our society. The current situation pertaining to LGBTQIA+ rights would improve with greater engagements in discussions around the pressing topics related to queer rights, mental health, sexual health, environmental challenges, and livelihood opportunities. If we can address the critical issues faced by the queer community at the local level, the existing condition would revamp at the political and societal level.

The base influence of the queer community starts with the social-cultural and socio-legal practices, nature of regimes, and lack of upright policies for the furtherance of inclusive laws towards the development of the LGBTQIA+ community. By working towards them, we can hope to advance in this field to a considerable length.

Q. What more can be done so that policies can be made in the right direction, and can the community be properly represented?

Dr. Rimashree: There should be just awareness concerning non-heteronormative identities and practices, and that we need to address the intersectionality of race, caste, class, re

gion, language, color, gender, and sexuality. The legal, social, and health-related challenges faced by the queer and transgender people are diverse, thus, to build effective policies for the LGBTQIA+ community we need to understand the diverse history and the gender consciousness within the community and the society at large. The legislative actions should support the autonomy and safety of the queer individuals. The government can create laws that protect LGBTQIA+ people from violence, discrimination, and hate crimes. This can include laws that protect transgender people and ensure that the LGBTQIA+ community has access to healthcare that meets their needs. It is important to educate people about LGBTQIA+ rights. Schools and universities should train staff to provide the necessary knowledge and skills to deal with abuse. Both the public and private sectors should create anti-discrimination policies and take steps to eliminate homophobic stereotypes. Organizations should implement policies to ensure that no one is discriminated against based on their sexual orientation, gender identity, or gender expressions. When creating policies, it's important to consider local conditions. It is important to get guidance from local LGBTQIA+ persons to identify the right approach and tone. In this regard, open and accessible forums for discussions among the queer community members including the allies could be a sensible approach.

Q. Do you think reservation is a viable solution to reduce this unequal gap of representation in government bodies?

Dr. Rimashree: Reservation would aid in reducing the unequal gap of representation of specifically transgender persons in government bodies since they face unique challenges, and to ensure they are represented inclusively, horizontal reservation could be a pertinent solution.

Q. Have the political trends been affected in any way as the community has become increasingly popular in the past years?

Dr. Rimashree: Yes, to some extent. Discussions and debate surrounding the rights and inclusive laws as well as policies that have been in the agenda of every queer organization and LGBTQIA+ persons. Referring to contemporary jurisprudence, although we observe a passive and facilitative role of the Indian judiciary in the current times, it seems to have a gradual impact on the legislative framework of the government concerning LGBTQIA+ laws. We can observe that the government's approach is characterized by several concerning actions that highlight a move towards hegemonic control, significantly affecting various sections of society. The legislative actions specifically targeting the queer community are particularly alarming, referring to The Transgender Persons (Protection of Rights) Act of 2019 and the Draft Trafficking in Persons (Prevention, Care and Rehabilitation) Bill of 2021.

Primary Survey Result :

The status of both these groups can be further understood through a primary survey conducted on approximately 60 participants of multiple genders spanning from ages 13 to 60+.

It was inferred that, of the total responders, almost 95% were aware of the terms 'misogyny' and 'gender disparity', while 1% were not aware, and the rest of the 4% were not sure of it.

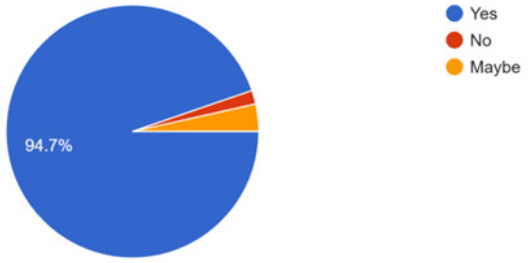

Figure 2: Composition of awareness of the terms 'misogyny' and 'gender disparity' among responders, with the majority responding in the affirmative while only a few take the opposite stand.

This, held in contrast to the unpopularity of these words during the 1990s due to taboos and misconceptions, depicts how their use has increased with a rise in awareness about gender disparity and woke culture.

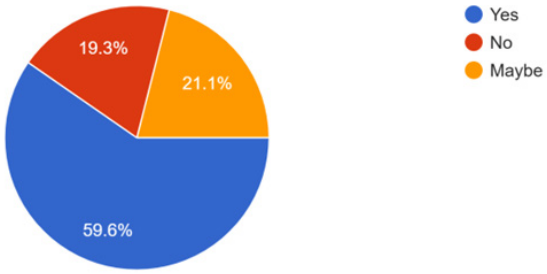

Figure 3: Responders who support the statement that there has been increased awareness of these terms and concepts. While almost half of them are unsure or reply in the negative, the rest acknowledge the surge in their usage.

Even more weight can be added to this argument by the finding that 100% of the responders, though they are not aware of the term misogyny or gender disparities, know that they are associated with a societal bias. It can be concluded that while the terms may be new, gender bias and disparities are age-old issues in our society.

An important discovery made through this survey showed that while 96% of females were aware of these terms, only 88% of males showed signs of knowing them, proving that more knowledge and campaigning are required to eradicate this gap and to assist more people in grasping the concept.

Among those aware of these terms, 81% believed that misogyny was prevalent in politics and that it is deep-rooted in Indian society through culture, traditions, and opinions being passed on to the next generations, starting from birth to the extent of education, marriage, and career paths.

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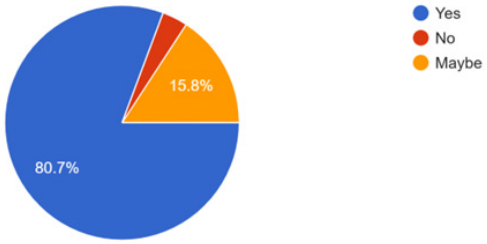

Figure 4: Composition of responders who believe misogyny is prevalent in politics, with only a few denying that its role is of any importance in the scenario, while most reply in the affirmative.

In terms of political leadership, 60% think that there have been significant changes in terms of gender representation at a political level all the years, claiming that given free access to education, women could pave a path for themselves.

Coming to how this rate of representation could be better, the percentage of responders supporting reservation for women in political bodies was 100%, portraying a paradigm shift in Indian history.

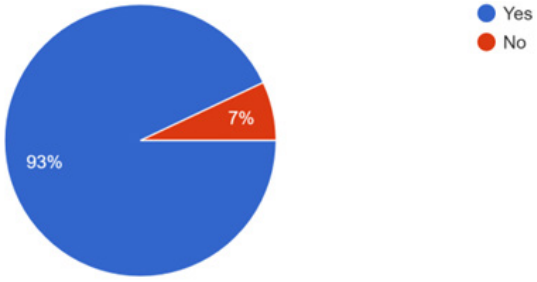

Figure 5: Composition of participants who do/do not believe that there should be reservations for women in poorly represented political institutions. A whopping majority affirm that there is such a need in the status quo, according to the survey.

Out of these, 33% supported the 1/3rd reservation mandate, while the other 67% believed that proper representation involved an equal proportion of genders in the committee. For instance, instead of just 33% of women in a political body, the ratio would be better suited as approximately 50% to depict the equal sharing of opinion from proportional gender communities.

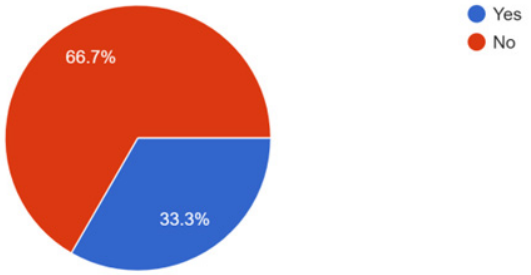

Figure 6: Percentage of those who believe it is right to reserve only 33% of the seats for women in governing bodies when they comprise approximately 50.0% of the total population. Most responders believe it is not right and that this aspect of the reservation system needs to change.

The agreements and disagreements regarding women's representation are found to be based on the historical context and the changes that were ushered in due to India's constitutional provisions for its citizens.

Considering the LGBTQIIA+ community, 96% of the respondents opined that their policies should be incorporated into the policies of the governing body. This displayed an inclusive society where everybody is accepted for who they are and gender-affirming practices are encouraged and followed.

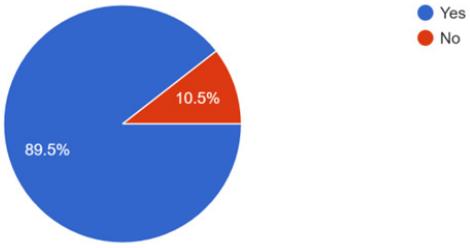

Figure 7: The Majority of the respondents believe the LGBTQIA+ community and their policies should be represented in the governing bodies (This does not necessarily mean having reservations).

When asked about the best countries that the participants would associate with having the most ideal representation in governing bodies, Canada was chosen as a classic example for LGBTQIA+, while many responded with New Zealand for women's representation.

Most of the responders said that the 3 places where they have witnessed gender inequality are: Home, Education (schools, textbooks), and the workplace.

Since these 3 situations are critical for the upbringing of a child to shape their thoughts and inculcate values of respect, equality, and inclusivity to mold them into compassionate and welcoming humans, there is a need to continuously inculcate a culture of diversity and embrace each other regardless of their identity in our homes and society.

■ Results of Case Studies of LBTQIA+ and Women's Representation in Government Bodies: Maldives and New Zeland

To understand more about the growth scenario of both women and the LGBTQIA+ communities in specific countries, the following case study involves the analysis of 2 countries. According to the primary survey conducted, New Zealand and Canada have proved their worth in creating inclusive policies for women and the LGBTQIA+ Community, while some other countries, such as the Maldives, still have a long way to go. By analyzing their governance, laws, and current situation, a clear picture of their status in representation and accuracy of policies can be gathered.

Maldives:

From legislation in the interests of women and the LGBTQIA+ to the number of seats occupied by them in the parliament, every political aspect of the Maldives has a lot of room for improvement. Out of the 77 seats in the Maldivian National Parliament, only 5 are held by women and no laws against domestic violence have been passed yet, even though according to the Maldives Study on Women's Health and Life Expectancy 2007, 1 in 3 women in the country have experienced physical or sexual violence in their lifetime.[22]

In fact, the situation had only worsened in 1968, when the freshly adopted Constitution of the Maldives barred women from running for the office of the President, emphasizing the

dire situation of women in the modern world. However, later in January 2008, the Constituent Assembly removed the gender ban.[23]

The reasons for poor representation of women in Maldivian politics involve factors such as a lack of information and financial resources, the deeply rooted patriarchal culture challenging those who dare to step outside the borders of traditional norms and values, as well as rigid mindsets refusing to welcome non-conforming ideas.

Article 17 of the constitution prohibits discrimination based on gender and legitimizes temporary special measures (TSM) to review any inequalities, yet no attempts to introduce TSM have worked despite the legal framework protection provided to do so. This situation speaks volumes about the lack of requisite political will to bring about structural changes to increase women's representation.[24]

A major reason for this poor portrayal of equality is the lack of financial backing and support from their families. Since women in the Maldives also have a lower employment rate than men, it is tussling for them to fund their campaigns personally as well.

This is a matter of heavy concern as according to a study, developing countries that have a higher share of women parliamentarians are more likely to pass comprehensive laws on sexual harassment, rape, divorce, and domestic violence.[25] With only 4.6% women's representation in the Maldivian Parliament, all those who have survived from the trenches of a lack of legal protection are constantly endangered.[25]

Key instances that highlight the burgeoning political status of women are issues with inheritance, where women receive less than their male relatives. This is derived from the Islamic values of male guardianship and responsibility for their female siblings and relatives, reinforcing the domestic role of women and the need for maintenance and protection.[26]

Another stringent measure is the Penal Code of 2014, criminalizing same-sex marriage as the provision carries a maximum penalty of eight years imprisonment and 100 lashes if found guilty.[27]

New Zealand:

On the other hand, the example of New Zealand depicts a significantly better representative state, as it was the first nation to grant female suffrage.[28] We can observe a 40:60 ratio of women and men in the parliament in 2019. New Zealand also made history in October 2022 when the 53rd New Zealand Parliament marked the milestone of having a majority of women Members for the first time, with 60 women and 59 men. As of May 2024, there were 57 women (46%) Members of Parliament elected to the current 54th Parliament of New Zealand.[25]

Women also hold several significant positions in the New Zealand Parliament, including former Governor-General Dame Patsy Reddy, former Prime Minister Jacinda Ardern, Deputy Speaker Anne Tolley, and former member of the parliament Poto Williams.[29]

The second youngest MP of the New Zealand parliament, representing Te Pāti Māori, Hana-Rawhiti Maipi-Clarke, has been widely recognized for her dedication towards popularizing her culture and proudly acting upon a recent bill that would define the principles of the treaty between Māori and The Crown.[30]

Some key figures that show improvement in the representation of women in public sector leadership according to the Stocktake of Gender, Māori, Pacific and Ethnic Diversity on Public Sector Boards and Committees (2023):

In June 2018, the Government set a target of 50% women's participation on public sector boards and committees. This target was met in 2021 and remained so for three consecutive years. As of December 2022, women held 53.1% of state sector board and committee roles–the highest ever proportion achieved. As of 30 June 2023, women made up 53.7% (22 of 41) of public sector Chief Executive roles, compared with 24.1% in 2012. The percentage of women in the top three tiers of public service roles is about 56% (up from 41.5% in 2013).[31]

As for the LGBTQIA+ community, Budget 2022 allocated NZD 4 million to establish engagement mechanisms for communities to work with the government in developing approaches to tackle family violence and sexual violence. This fund was specifically for communities that are disproportionately affected by family violence and sexual violence, such as the LGBTQIA+ community. Support for these groups has been found in New Zealand in abundance.[31]

Better comprehensive laws make the country open and friendlier to international students as well, especially those from the respective community.

Results and Discussion

As inferred from the detailed study of both countries, it is visible that the efficiency of creating empathizing and nurturing laws to cater to diverse communities is more apparent in countries that have more women representation as well as attention towards other groups as women in all levels of public office seem to move in a more feminist direction. Female legislators are more likely to understand and be aware of women's problems for two reasons: they have experienced them firsthand or through their association with other women. They may be experts in solving the types of problems women have confronted and can look at policymaking through their own experiences and expert perspectives.[32]

The gendered perspective between men and women prevails greatly as their inferences from different situations vary a lot. This divergence in outlook prevents the accuracy and number of insights each can have when it comes to making policies or impacts for the opposite gender, making it vital to introduce all genders in the policymaking body.

Many times, political bodies do not act on a problem simply because they are not aware of the intensity of it or that it exists at all. This can only be tackled by including as many people of diverse opinions and backgrounds as possible.

Progress in the Status Quo:

Moving from country-specific research, at a global level, to create a comprehensive framework of all the progress made in the status quo concerning representation and growth of women in political bodies, a compilation of some key facts to support the developments made has been presented.

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According to the World Government Summit (2023), the Beijing Declaration and Platform for Action (1995) was adopted by the UN at the end of the Fourth World Conference and set out an internationally agreed-upon target to achieve balanced political participation and power-sharing between women and men in decision-making.[3]

Moreover, figures state that women have been increasingly involved in political decisions, as they now make up 21% of government ministers, with 14 countries having achieved 50% or more women in cabinets. In 2019, the proportion of women in senior management roles, both public and private, grew to 29%, the highest number ever recorded and the number of women as heads of state, the highest level of public office, has been increasing over the past 50 years, even though it has not risen equally or evenly across regions.[3]

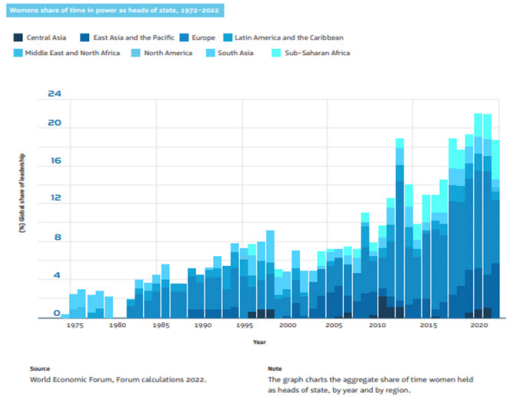

Figure 8: Times women have held the position of Heads of State in different regions over the years, portraying a spike in recent years. Source: World Economic Forum, Forum Calculations, 2022[33]

While it is true that a female leader can make a huge difference in a workplace, studies conclude that without at least 30% female representation, there will be no significant benefits of having more senior female leaders, highlighting how vital it is to introduce more of them in the task force for effectiveness and support.[34]

Figure 9: The Rise of Women as Chief Executives or Heads of State from 1789 to 2022, depicting increasing prominence and global leadership. Source: Our World in Data[12]

Data over the years states that women in government often prioritize social policies that improve health, education, and welfare. For example, a study in Germany found that adding just one woman to a local council increased the rate of childcare expansion by 40%, highlighting their role in addressing family-centric policies. This statement highlights how immensely a new perspective can influence policy-making and foster a positive environment for development.[3]

After considerable provisions and stipulations, the stagnancy in representation has abated significantly all these years, and by exploring some recommendations to target further growth, we can identify more key areas that require utmost focus to reach our goals for inclusivity.

Recommendations

Addressing the entrenched patriarchy and homophobia to foster gender equality in politics requires a comprehensive, multi-dimensional approach. The following recommendations outline actionable steps to mitigate patriarchal barriers and enhance women and queer participation and representation in political spheres:

1. Legal Reforms:

A. Gender quotas implementation – Incorporating a percentage of vulnerable social groups should be guaranteed in legislatures. For example, the African National Congress (ANC) in South Africa has instituted a minimum of 50% representation of women in all elected positions. This also keeps in mind proportional representation, something that has been emphasized earlier in the paper.[35]

B. Implement anti-discrimination policies: It is important to sensitize and reinforce laws that address discrimination and harassment based on gender orientation and sexual identities in social and political institutions. Such measures encourage a more inclusive and equitable political landscape.

C. Protection of Maternity and Parental Rights: The introduction of programs that encourage female politicians to have greater involvement in politics by allowing them to take maternity leave and adjust their working hours will help realize the goal. Making similar adjustments for queer people can also lead to more participation and inclusion in everyday roles.

2. Educational and Awareness Campaigns:

A. Raise the political consciousness of the public: Incorporating campaigns and workshops for women and LGBTQIA+ to enhance their understanding of their political rights and encourage active involvement could boost their participation in politics. For instance, UN Women and other institutions for social development are recommended to enhance the strength of these groups in politics.[36]

B. Challenge gender stereotypes: It is crucial to use media, training programs, and social conversations to eradicate patriarchal and homophobic stereotypes regarding leadership positions. Teaching journalists how to report can be an intervention that aims to lessen discrimination in the media.[37]

3. Institutional Support:

A. Establish representational equality committees: Setting up oversight bodies to monitor and address gender disparities in political institutions can ensure accountability and progress toward proportionate representation.

B. Provide mentorship programs: Matching aspiring diverse politicians with experienced mentors and activists in the field will guide their careers and help them navigate political landscapes effectively.

4. Economic Empowerment

A. Funding diverse candidates: Financial aid to women and queer candidates can eliminate many economic impediments afflicting their pursuit in the political arena. Programs that promote their political participation understand that economic empowerment is a means toward gender equality.[38]

B. Encourage them in leadership roles: Increasing the resources and training dedicated to preparing aspiring leaders for high-level political positions helps instill confidence and competency in leading properly.

5. Media Representation

A. Promote positive representation: Encourage media houses to depict women and queer politicians as effective leaders rather than dwelling on gendered and homophobic issues such as looks or personal lives. This can shift public opinion and eliminate stereotypes.

B. Counter misinformation and bias: Monitoring and countering the spread of sexist narratives and disinformation campaigns with no scientific base in politics is essential to creating a fair and unbiased media environment.

6. Community-Level Interventions

A. Engage grassroots movements: Mobilizing local communities to support equal political participation creates a bottom-up approach to equality, thus achieving widespread societal change.

B. Foster male allies: Encouraging men in politics and communities to support gender parity and actively challenge patriarchal norms can result in more inclusive political environments.

7. Global and Regional Collaboration

A. Learn from best practices: Sharing ideas and strategies with countries that have increased political participation can be valuable in terms of insights and models for implementation.

B. Encourage international support: The use of international organizations and frameworks to hold governments accountable for the promotion of equality in politics ensures that commitment and progress are sustained.

8. Technological Tools and Platforms

A. Leverage digital campaigning: Empowering politicians from different backgrounds with the ability to utilize social media and digital platforms for political outreach and advocacy will increase their visibility and engagement with constituents.

B. Protect against online harassment: Creating legal frameworks and technological solutions to counter cyberbullying and online threats against anyone in politics is important for their safety and participation.

9. Cultural and Social Transformation

A. Encourage gender-responsive education: Incorporating school curricula with discussions about gender equality and leadership can shape progressive attitudes from a young age toward a long-term change in society.

B. Celebrate women and queer leaders: Recognition of their work in politics can inspire and motivate future generations to pursue the leadership role, making the political landscape more gender inclusive.

By implementing these recommendations, political systems can move towards dismantling patriarchal and homophobic structures that ensure an environment is inclusive and equitable for the contribution of women and the LGBTQIA+ Community to governance and leadership. This is not just a necessity in terms of gender equality, but is also important to foster diverse and representative political decision-making processes. While they have created some extent of impact in all these years, consistency and adapting to the current situation are integral in ensuring the solution remains relevant. This can be done through avoiding rigid and inflexible solutions and understanding what tangibly benefits society. While digital campaigns and community support are spearheading change on a large scale, media representation and institutional support still require considerable attention, specifically encouraging news channels that provide accurate content without any institutional bias or political pressure.

Conclusion

The survey findings present a paradigm shift in varied age groups, ranging from teenagers to senior citizens over the years. It analyzes the current perspective of misogyny and gender disparity, specifically in the political sphere, centering on how the reservation policy present in India can be improved and whether it should be there at all, as well as some countries that have the most and least ideal policymaking for women and the LGBTQIA+ Communities. The interview with a renowned LGBTQIA+ activist and expert, Ms. Rimashree Borah, has been presented. A detailed discussion on their inclusion at the grassroots level as well as policymaking stresses the urgent need to make further progress, as well as explore more solutions, except for reservations in political institutions to promote accurate governance. Contemporary jurisprudence has occupied the foreground in the latter part of the interview as we question how political trends have changed with the increasing popularity of the community. Analyzing two countries; Maldives and New Zealand on their gender political representation, the key difference noted is the wide gap in policies related to diversity and inclusivity in comparison to the degree of women and queer representation in their political institutions and their position in society.

The overall conclusion of the study is that it is vital to include more perspectives in political institutions for the betterment of a state. Involving more genders in decision-making not only leads to diverse opinions but also encourages empathetic, multi-faceted laws for the well-being of all. It is important to act on this situation now so that we can create accurate policies for future generations to properly benefit from them and take advantage of their potential through equitable opportunities for the progress of the nation.

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REVIEW ARTICLE

Targeting the Undruggable: Small Molecule Modulation of Ras Proteins

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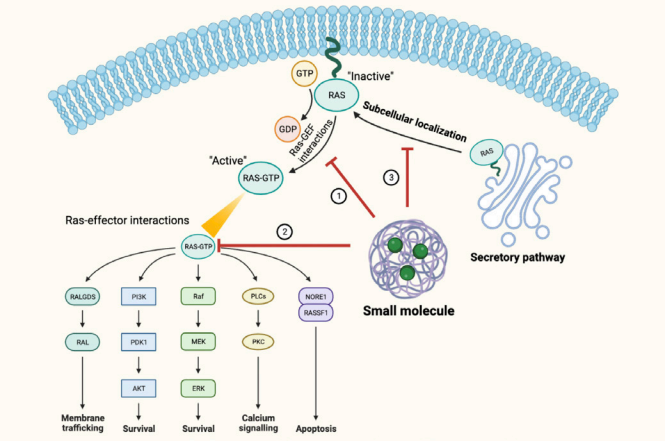
ABSTRACT: Ras proteins control critical signaling pathways that regulate cell growth and proliferation. Mutations in Ras are responsible for 30% of all human cancers, making them important targets for therapeutics. Despite enormous efforts in developing therapeutic agents against Ras, minimal success has been observed in clinical settings. Moreover, mutated Ras isoforms have been shown to be unresponsive to established therapies, leading to the notion that Ras may be "undruggable." This review examines several recent studies that utilized methods in chemical biology to target Ras signaling.

KEYWORDS: Chemistry, Other, Chemical Biology, Cancer, Ras Proteins.

Introduction

In our bodies, cell proliferation only happens under specific circumstances, which is possible due to the tight regulation by a network of complex signaling pathways. However, when there is a mutation in these signaling pathways, cells can grow in an uncontrolled manner, leading to cancer. The mutation usually involves the malfunctioning of proteins that possess the ability to regulate cell growth, widely known as proto-oncogenes. Among proto-oncogenes, Ras proteins have attracted much attention because they play essential roles in modulating the activity of many major signaling pathways required for normal cellular proliferation.[1] During signal transduction, Ras proteins switch between "off" and "on" states upon their binding to GTP, which is regulated by guanine nucleotide exchange factors (GEFs).[2] The binding of GTP induces conformational changes and activates Ras proteins, enabling them to interact with downstream effectors, such as PI3K and Raf (Scheme 1).[1] Ras signaling is heavily dependent on the correct cellular localization of Ras, in which the protein must be positioned in the plasma membrane.[3] The intracellular trafficking of Ras is determined by post-translational modifications, such as lipidation and interaction with solubilizing protein factors.[2] Specific mutations in the Ras gene induce the constitutive activation of Ras, with K-Ras being the most frequently mutated isoform.[2] Ras mutants are "stuck" in their active state, causing cells to grow uncontrollably and become unresponsive to apoptosis signals. Mutated Ras is perhaps one of the most significant drivers of cancer, as they are associated with 30% of all human cancers.



Scheme 1: Recent approaches in using small molecules to modulate Ras-signaling. The three main branches are: preventing the formation of the Ras-GTP complex by interfering with Ras-GEF interaction (shown as 1), developing inhibitors of Ras-effector interactions (shown as 2), and suppressing the subcellular localization of Ras proteins (shown as 3). These approaches ultimately aim to inhibit the activity of mutated Ras, preventing the uncontrolled growth of tumor cells.

Over the past 30 years, researchers have made great strides toward understanding the Ras signaling pathway at the molecular level. Accompanied by this progress, many scientists have attempted to target mutated Ras proteins for cancer therapy. However, no study has succeeded in clinical trials, causing the scientific community to consider Ras "undruggable."[3] Two factors that make Ras extremely difficult to target. First, these mutants have a picomolar affinity for GTP and thus are often frozen in their "on" state.[1] Second, Ras proteins lack suitable surfaces for small molecules to bind. Besides the nucleotide-binding pocket, these proteins do not possess any additional binding domains.[3] Even with in-depth structural analysis and iterative sounds of synthetic screening, scientists were not able to identify a feasible binding site on Ras proteins.[2] In addition, multiple studies have reported that tumors can quickly adapt to Ras inhibitors by inducing the lipidation

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of H-Ras and K-Ras isoforms, further hampering the success of Ras-targeted therapies.[5]

The status quo on Ras-targeted therapies has called scientists to develop new approaches. Recent advances in science, especially in the field of chemical biology, have provided scientists with powerful tools to combat this challenging problem. Recent attempts can be widely characterized into three groups: 1) inhibiting the formation of Ras-GTP complex, 2) blocking Ras-effector interactions, and 3) suppressing the cellular localization of Ras (Scheme 1). This paper will cover some of the illustrative examples of each approach and evaluate whether these endeavors offer hope in targeting the "undruggable."

Discussion

Approach I: Preventing the Formation of Ras-GTP:

Inspired by ATP-competitive kinase inhibitors, early studies attempted to develop Ras inhibitors by directly competing against binding with GTP molecules.[6,7] However, these inhibitors exhibited micromolar affinity toward Ras and could not out-compete the picomolar affinity between Ras-GTP.[8] Thus, instead of competing directly against the nucleotide, scientists turned to investigate strategies that prevent the initial formation of the Ras-GTP complex. Peri and Patgiri aimed to accomplish this task by inhibiting the GEF-catalyzed nucleotide exchange reaction.[9,10] Due to the high affinity of Ras to GDP, the conversion of GDP to GTP needs to be catalyzed by nearby GEFs, such as RasGRF1 and SOS.[3] During the RasGRF1-catalyzed reaction, the catalytic domain of RasGRF1 interacts with the switch I and switch II domains of Ras to open up the nucleotide-binding site,[11] allowing for the release of GDP (Figure 1a). Using virtual ligand docking methods,[12] Peri identified a series of bicyclic scaffolds derived from the natural sugar D-arabinose that bind to the Ras switch II region (Figure 1b). Molecular modeling results showed that the aromatic residues of these sugar-derived molecules form π-stacking interactions with the phenylhydroxylamine groups near the switch II region, forming a stable product.[9] The binding of small molecules to the Ras switch II domain disrupts the Ras-RasGRF1 interaction, thus preventing the conversion of GDP to GTP. When these Ras inhibitors were characterized in vitro, the nucleotide-dissociation assay demonstrated that the sugar derivatives inhibited the release of GDP in a concentration-dependent manner.[9] Notably, one of the lead compounds showed a similar nucleotide dissociation rate compared to that of intrinsic GTPase activity.[9]

Similarly, Patgiri developed an orthosteric inhibitor of Ras, but instead aimed to suppress the SOS-catalyzed nucleotide exchange reaction.[10] During the SOS-catalyzed reaction, the SOS helical hairpin domain is inserted into the switch regions of Ras, disrupting water-mediated intermolecular interactions between Ras and guanine nucleotide, ultimately destabilizing the GDP-bound state of Ras.[11] Based on structural and biochemical analyses of Ras-SOS interactions, researchers identified that F929 and N944 contribute most strongly to the binding of the hairpin domain to Ras.[11] Then, Patgiri utilized the hydrogen bond surrogate (HBS) approach[13] to design synthetic α-helix mimics that stabilize the GDP-bound state of Ras, preventing the release of GDP (Figure 1c). In *in vitro* assays, these α-helical peptides significantly suppressed nucleotide exchange as compared to the negative control, illustrating that they can act as an orthosteric inhibitor of Ras-SOS interactions.[10]

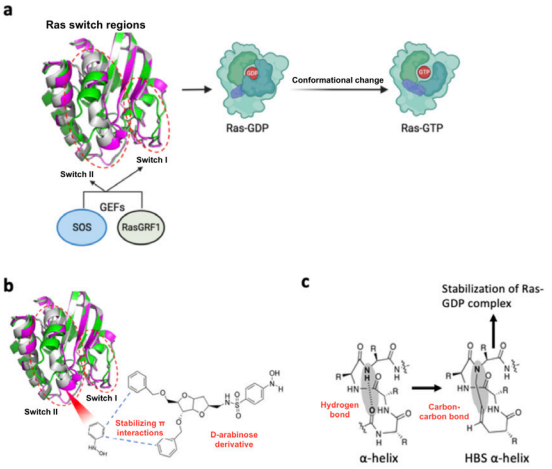

Figure 1: a) Conformation of Ras proteins showing the switch I and switch II regions (indicated with red dotted lines). Guanine nucleotide exchange factors (GEFs) catalyze the conversion of GDP to GTP by interacting with specific domains of the switch I and II regions, ultimately opening the nucleotide-binding site of Ras. (b) Inhibition of RasGRF1-catalyzed nucleotide exchange reaction by D-arabinose-derived bicyclic molecules. The two aromatic residues form stabilizing interactions with the phenylhydroxylamine group of the switch II region, disrupting Ras-RasGRF1 interaction. (c) Structural differences between a regular α-helix and a hydrogen bond surrogate (HBS) α-helix. The HBS strategy affords preorganized α-helices in which the N-terminal main chain hydrogen bond between the C=O of the "ith" amino acid residue and the NH of the "i+4th" amino acid residue is replaced with a carbon-carbon bond (shown by grey circles). HBS α-helix stabilizes the Ras-GDP complex, disrupting Ras-SOS interaction.

Both studies successfully identified small-molecule inhibitors that inhibit Ras-dependent cell proliferation at high micromolar concentrations. Orthosteric inhibitors of Ras-RasGRF1 and Ras-SOS interactions effectively downregulated cell growth in p21 human Ras and HeLa cells, respectively.[9,10] However, sugar derivatives are known to be unstable in organic solvents and water at room temperature.[14] Furthermore, Ras-RasGRF1 inhibitors showed poor results in vivo and even displayed toxic effects.[9] In the case of α-helix mimics, they had a tenfold lower affinity for Ras-GDP than the parent SOS itself, which would lead to limited therapeutic effects. Moreover, the exact mode of action remains elusive, hindering further optimization.[10] Most importantly, a fundamental limitation of these Ras-GEF inhibitors is that they do not discriminate between mutated and wild-type Ras proteins. Without targeting cancer-targeting moieties, the inhibitors will prevent the formation of the Ras-GTP complex in healthy cells, which can lead to undesirable outcomes, including cell death.[3]

Approach II: Inhibiting Ras-effector Interactions:

An alternative approach in Ras inhibition is to suppress the interactions between Ras and its downstream effectors, which regulate various signaling pathways (Scheme 1). When Ras proteins interact with their effectors, they readily transition between two distinct states, in which state 1 represents

a conformation with a reduced affinity for effector binding (Figure 2a). The affinity of state 1 for effectors is reported to be 20 times weaker.[15] Notably, multiple studies have discovered that the weak binding state possesses potential binding sites for small molecules on its surface.[15,16] Based on this finding, Rosnizeck developed allosteric inhibitors of Ras based on organometallic motifs.[17] Their goal was to stabilize the weak binding state to discourage its interaction with effector molecules. Through 31P NMR, the researchers identified that the zinc (II) complex of 1,4,7,10-tetraazacyclododecane complex ($Zn2^+$ cyclen) selectively binds to the surface of the weak-affinity state.[17,18] Importantly, the organometallic molecule significantly stabilized the weak affinity state, thus shifting the equilibrium toward state 1 and discouraging the formation of the strong affinity state (Figure 2b). However, $Zn2^+$ cyclen only showed millimolar affinity toward Ras, and the downregulation of Ras-dependent pathways was not observed in animal studies.[17] Moreover, organometallic compounds suffer from unfavorable pharmacological properties, including but not limited to formulation barriers, off-target issues, systemic toxicity, and engagement in redox reactions.[19] These limitations raise a critical concern for the use of organometallic inhibitors *in vivo*.

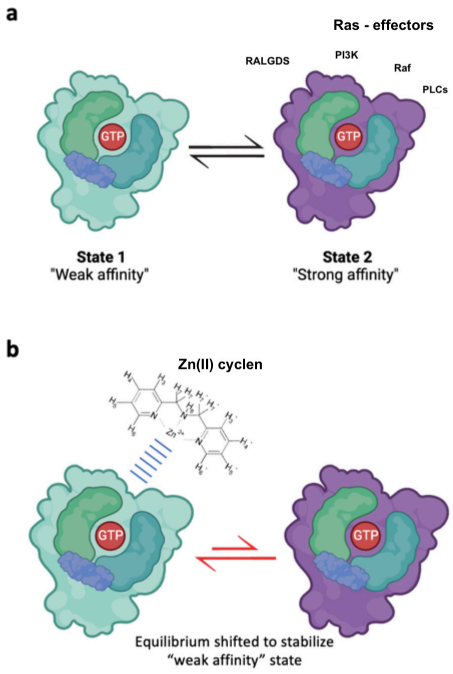

Figure 2: (a) Dynamic equilibrium between the weak-affinity (state 1) and strong affinity conformations (state 2). Ras-GTP complexes constantly switch between the two conformations. Effector molecules of Ras bind to the Ras-GTP complex when the complex is in state 2. (b) Allosteric inhibition of Ras-effector interactions using organometallic compounds. Zn^{2+}cyclen selectively binds and stabilizes the weak-affinity state. As a result, the equilibrium is shifted toward state 1, making the transition to state 2 thermodynamically unfavorable.

To overcome the unfavorable properties of organometallic inhibitors, Tanaka and Rabbitts explored different methods to inhibit Ras-effector interactions. In particular, they were interested in finding antibodies that bind to the Ras-GTP complex. The researchers screened a library of antibodies in yeast with an H-Ras mutant and isolated a single immunoglobulin heavy chain variable domain fragment (named iDab#6) that selectively binds to the weak affinity state of Ras-GTP (Figure 3a).20 When the binding of iDab#6 to Ras was characterized using luciferase assays, the antibody fragment was specifically bound to positions 12 and 61 of the switch I region of the Ras-GTP complex.[21] This was an important finding because major Ras-effectors, such as PI3K and Raf, bind to the switch I region of Ras,[22] meaning that iDab#6 can act as a competitive inhibitor of Ras-effector interactions. Furthermore, Tanaka and Rabbitts observed that iDab#6 interacts with mutant H-Ras with at least 10 times higher binding than with wild-type H-Ras, allowing the specific targeting of mutant Ras.[20] To test whether the antibody fragment can downregulate Ras-dependent signaling pathways, iDab#6 was introduced to mouse tumor models bearing lung cancer cells. Based on the results, the introduction of iDab#6 led to an increased survival rate and reduced tumor size, demonstrating the antibody's ability to suppress Ras-effector interactions.[21]

Other scientists aimed to discover peptide-based orthosteric inhibitors of Ras-effector interactions. In a recent study, Wu and Upadhyaya utilized a combinatorial screening method to identify a cyclic peptide that selectively binds to the weak-affinity state of Ras-GTP.[23] A library of 6×10^6 cyclic peptides was synthesized, in which each bead contained a random peptide sequence of four to six amino acid residues on its surface (Figure 3b). Each position was randomized with a 25-amino acid set to maximize structural diversity and protease resistance.[23] To further increase diversity, the researchers varied the ring size at each position by cyclizing an aliquot of the library peptides. Next, the library was screened against the G12V K-Ras, one of the most frequently observed K-Ras mutants.[3] Using this method, researchers were able to produce more than six million unique compounds and screened each of them, which speaks to the power of combinatorial science. Screening of the library produced around 20 lead compounds that selectively bind to state 1 of the Ras-GTP complex.[23] Interestingly, the lead compounds were dominated by larger rings and rich in aromatic residues. The exact reason behind this finding is unclear; however, large rings may form favorable interactions with aromatic residues of the switch I domain, establishing a steric block between Ras and its effectors. Homogenous time-resolved fluorescence (HTRS)[24] indicated that the cyclic peptides inhibit the Ras-Raf interaction with micromolar affinity, leading to the downregulation of the MAPK pathway (Scheme 1). In cellular assays, the small molecule inhibitors also led to a decreased phosphorylation of Mek and Erk, two downstream effectors of Ras, that promote tumor survival when phosphorylated (Scheme 1).

Even though orthosteric inhibitors developed by Wu and Tanaka displayed promising in vivo and in vitro results, there are several limitations. First, the size of these small molecules poses a problem, especially in the case of cyclic peptide inhibitors. Findings revealed that incorporating large aromatic residues favors binding with Ras,[23] meaning that the size of the resulting inhibitor will be relatively large. Size is a critical

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factor in therapeutic efficacy, and the large size of the peptide will likely pose problems when crossing the plasma membrane. Second, these studies targeted specific isoforms of Ras mutants. However, there are multiple Ras isoforms, and a single isoform is not responsible for driving tumor progression.1 There is no guarantee that an inhibitor that downregulates K-Ras will work for H-Ras or N-Ras. The genetic makeup of Ras isoforms will vary depending on the type of cancer and individual patients, which complicates the use of these inhibitors in clinical studies. Most importantly, no molecular-level evidence exists of the interaction between the small molecule inhibitors and the Ras-GTP complex. This is a crucial limitation that prevents further development of these Ras inhibitors.

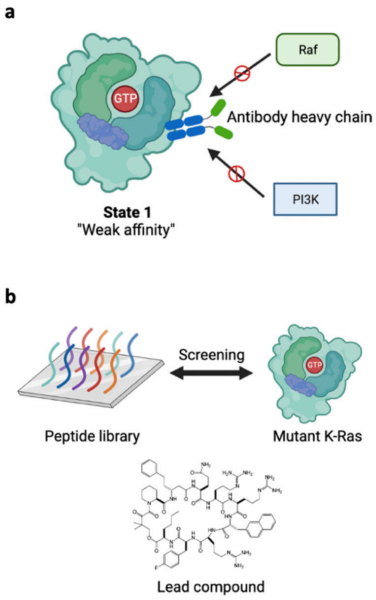

Figure 3: (a) Orthosteric inhibition of Ras-effector interactions by iDab#6. The antibody fragment selectively binds to the switch I domain of mutant H-Ras, a binding site of major Ras-effector molecules. The administration of IDab#6 into mouse xenograft models significantly reduced tumor growth and proliferation. (b) Synthesis of cyclic peptide inhibitors using combinatorial science. The library included about six million peptides, screened against mutant K-Ras molecules. The lead compound containing large aromatic residues led to notable downregulation of Ras-Raf interactions and inhibited tumor proliferation.

Approach III: Impairing Ras Subcellular Localization:

The final approach in small molecule modulation of Ras signaling is through impairing its intracellular localization. Ras-dependent signaling pathways that promote tumor cell survival and metastasis depend on the correct localization of Ras at the plasma membrane, 4 which is enabled by a series of post-translational processing reactions (Figure 4a). These modifications include cysteine S-farnesylation, cysteine S-palmitoylation, proteolysis, and C-terminal carboxymethylation.[25] Among them, the addition of farnesyl groups to the CAAX cysteine thiol by farnesyltransferase (FTase) has been reported as a major driver of Ras cellular localization, as highlighted by the inability of Ras mutants lacking the C-terminal cysteine to localize to the plasma membrane.[4,25,26] This observation prompted the development of farnesyltransferase inhibitors (FTIs) as potential anticancer drugs. Kohl and Mosser were among the first groups of scientists to design FTIs by designing CAAX motif derivatives.27 When a panel of tetrapeptide analogs of the CAAX motif was screened, N-2(S)-[2(R)-amino-3-mercaptopropylamino]3(S)-methylpentyl isoleucyl-homoserine lactone showed selective binding to FTase (Figure 4b). The N-terminal peptide bonds were reduced for resistance against hydrolysis by mammalian aminopeptidases, while the C-terminal serine was cyclized to facilitate membrane penetration by masking the anionic carboxylate.[28] In cellular assays, the tetrapeptide selectively inhibited FTase by out-competing the substrate farnesyl diphosphate, leading to an increased concentration of inactive, cytosolic Ras proteins. Its potency was also demonstrated in mouse models, in which the injection of the CAAX mimetic led to a significant decrease in tumor growth and proliferation.[27] Since Kohl and Mosser's work, there have been many efforts in optimizing the CAAX motif-based inhibition and elucidating its molecular mechanism.[4, 26,29]

Instead of competing directly with the substrate farnesyl diphosphate, some studies sought methods to compete against Ras proteins for binding with farnesyl-binding proteins within the plasma membrane. Campbell and Boufaied utilized DECIPHER technology,[30] a genomics and bioinformatics platform that predicts the structures of secondary metabolites based on bacterial genomic sequences, to discover a farnesyl cysteine mimetic.[31] Their lead compound, TLN-4601, is a farnesylated dibenzodiazepinone that selectively binds to the peripheral benzodiazepine receptor, which shares many structural similarities to farnesyl-binding proteins (Figure 4c).[32] Campbell and Boufaied hypothesized that TLN-4601 would interfere with Ras localization by binding to farnesyl-binding proteins. To evaluate whether the farnesyl mimetic interferes with Ras-dependent signaling, the researchers treated human pancreatic epithelial cells with 10 μM of TLN-4601.[31] As expected, TLN-4601 treatment resulted in decreased phosphorylation of Raf-1, MEK, and ERK1/2, all of which are downstream effectors of Ras that regulate tumor cell survival (Scheme 1).[31] Furthermore, promising anti-tumor effects were observed in mouse xenograft models, suggesting that TLN-4601 is a good candidate for future clinical studies.

Despite promising preclinical efficacy, FTIs and farnesyl mimetics were ineffective in clinical trials, and data suggest that only a small subset of patients respond to these Ras inhibitors.[33] The major reason for the discrepancy between laboratory findings and clinical data is the high mutation rate of K-Ras and N-Ras isoforms. Mutant Ras proteins can be alternatively prenylated by gamma-glutamyltransferase 1, obviating their dependence on farnesylation for their correct subcellular localization.[26] Recent findings suggest that there may be more enzymes that lapidate mutant Ras.[34] Moreover, these mutant Ras are fully functional and can bind to a series of lipid-binding proteins, significantly hampering the efficacy of farnesyl mimetics.[29] From this point, a major effort must be put into identifying tumors that depend on farnesylation for proliferation and characterizing the human prenylome to increase therapeutic efficacy in clinical trials.

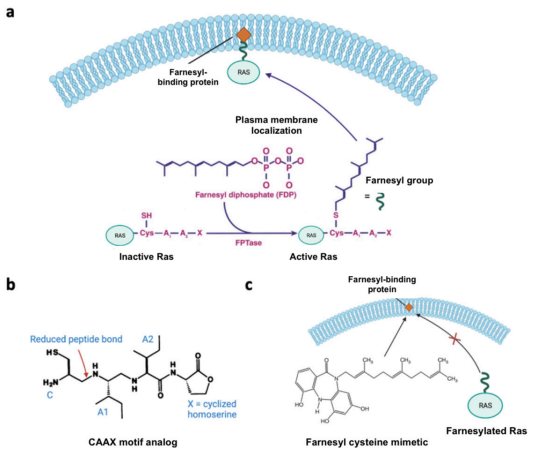

Figure 4: (a) Subcellular localization of Ras proteins· Ras is synthesized as a cytosolic precursor that ultimately localizes to the cytoplasmic face of the plasma membrane. Farnesyl transferase covalently adds farnesyl diphosphate to the C-terminal cysteine of inactive Ras. Farnesylated Ras is then localized to the plasma membrane by binding to farnesyl-binding proteins. (b) Structure of the CAAX motif analog, N-2(S)-[2(R)-amino-3-mercaptopropylamino]3(S)-methylpentyl isoleucyl-homoserine lactone. (c) TLN-4601 competes with farnesylated Ras in binding to farnesyl-binding proteins.

Conclusion

To sum up, the recent developments in the field of chemical biology have created segways to use small molecules to prevent Ras-signaling. Aberrant Ras signaling is a crucial problem to be solved, as it is one of the biggest drivers of cancer in humans, and most importantly, there is no method to control Ras signaling now. Small molecules can be used in three aspects: 1) preventing the formation of Ras-GTP, downregulating interactions between Ras and its effector molecules, and 3) stopping the subcellular localization of Ras onto plasma membranes. However, despite these promising approaches, the complete inhibition of Ras-signaling remains a challenge. Limited success in clinical trials raises the question of whether a single approach or a specific type of small molecule can downregulate Ras-signaling. In addition, multiple studies have reported how Ras-dependent pathways are upregulated in response to drug treatments.[34] Such a response ultimately leads to treatment evasion and increases the dose, causing pharmacokinetic complications.[35] Therefore, future research should aim to decipher the adaptive resistance mechanisms of cancer cells in response to small-molecule treatments. Moreover, structural investigation on Ras isoforms and interactions with their effector molecules is required, which can reveal novel sites for small molecule interventions and ways to develop personalized treatment strategies.[34] To translate these molecular findings into effective treatments, it will be crucial to bridge chemical biology with systems biology, AI-driven screening, computational biology, and clinical oncology.[36]

On the positive side, advances in combinatorial science and bioinformatics have allowed researchers to make further leaps in discovering novel inhibitors of Ras. For example, scientists have recently discovered small-molecule binding pockets in the switch regions of Ras.[37,38] Researchers have also shown that integrating nanotechnology with existing therapies can significantly enhance pharmacokinetics and drug delivery.[39,40] Noting that Ras is one of the major drivers of human cancer pathogenesis, continuous efforts should be made to develop multidisciplinary approaches to inhibit aberrant Ras-signaling. Although Ras was widely considered "undruggable" over the past decades, recent findings discussed above have revived the hope that Ras is "yet to be drugged." As chemical biology and related fields continue to advance, the likelihood of effective therapeutic intervention of Ras-signaling steadily increases.

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REVIEW ARTICLE

Pathophysiology, Diagnosis, and Therapeutic Strategies of Cytokine Storms in Autoimmune Diseases

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ABSTRACT: Autoimmune diseases are caused by the immune system mistakenly attacking its healthy cells and tissues. Sometimes, the body's production of the protein cytokine becomes dysregulated, generating an imbalance in proinflammatory and anti-inflammatory cytokines. Excessive release of proinflammatory cytokines causes a cytokine storm, characterized by unregulated inflammation that leads to impaired organ functions, damaged tissues, and increased mortality rate. Cytokine storms are associated with various conditions, including sepsis, COVID-19, and influenza. Strategies for the treatment of cytokine storms are still developing, with a particular focus on the inhibition of cytokine secretion. Despite the advancements, challenges in early detection and diagnosis remain, and further research to understand cytokine storms is taking place to discover personalized medicine and novel therapeutic methods for the future. This review discusses the mechanisms and consequences of cytokine storms and their interplay with autoimmune diseases and provides potential therapeutic strategies and future research directions to restore balance in the immune system.

KEYWORDS: Biomedical and Health Sciences, Pathophysiology, Cytokine Storm, Autoimmune Diseases, Hyperinflammation.

Introduction

Autoimmunity is when adaptive immune molecules are directed against the body components,[1] often due to the interaction between one's genetic predisposition and environmental factors. Autoimmune diseases are an array of conditions caused by autoimmunity, particularly the response of aberrant B cells and T cells towards host constituents. These diseases are widespread and affect all individuals, although they tend to be the most prevalent in women. It is frequently triggered by infection and microbiota-induced pathogenesis.[2]

A pathological reaction that autoimmune diseases can lead to is cytokine storm (CS), a set of medical symptoms and pathological responses resulting from an overstimulated immune response.[3] In 1993, the term was first used to describe the effects of an uncontrolled inflammatory response for graft-versus-host disease (GvHD).[4] However, it is now commonly seen as a condition in which the excessive production of cytokines prompts rampant, systemic inflammation, bringing about multi-organ failure and, in severe cases, death.[5] Cytokines are essentially small soluble proteins facilitating communication between cells and their surrounding environment and modulating immune responses vital for regulating immune homeostasis. Interleukins, interferons, and tumor necrosis factor (TNF) are all types of cytokines, and their dysregulation is prone to detrimental consequences.[6] Table 1 summarizes the sources and functions of common biomarkers affected in a CS.

Table 1: The sources and functions of common biomarkers affected during the cytokine storm. IL, interleukin; IFN, interferon; TGF, transforming growth factor; CCL, chemokine ligand; CXCL, CXC motif chemokine ligand; CRP, C-reactive protein.

Biomarker (abbreviation)	Source	Function
Cytokines		
IL-1	macrophages, pyroptotic cells, epithelial cells	Proinflammatory; pyrogenic function; activation of macrophage and Th17 cells
IL-4	Th2 cells, basophils, eosinophils, mast cells, NK cells	Anti-inflammatory; Th2 differentiation; adhesion; chemotaxis
IL-6	T cells, macrophages, endothelial cells	Proinflammatory; pleiotropic; pyrogenic function; acute phase response; lymphoid differentiation; increased antibody production
IL-10	regulatory T cells, Th9 cells	Anti-inflammatory; inhibition of macrophage activation; inhibition of Th1 cells and cytokine release
IL-13	Th2 cells	Anti-inflammatory; differentiation of B cells; mediator of humoral immunity
IL-17	Th17 cells, NK cells, group 3 innate lymphoid cells	Protection from bacterial and fungal infections; promotion of neutrophilic inflammation
IFN-γ	Th1 cells, cytotoxic T cells, group 1 innate lymphoid cells, NK cells	Proinflammatory; activation of monocytes and macrophages
TGF-β	Treg cells, monocytes, macrophages, fibroblasts, epithelial cells, cancer cells	Immunosuppressive; regulation of proliferation, differentiation, apoptosis, and adhesion; inhibition of hematopoiesis
TNF	T cells, NK cells, mast cells, macrophages	Pyrogenic; increasing vascular permeability
Chemokines		
CCL2	macrophages, dendritic cells, cardiac myocytes	Pyrogenic; recruitment of TH1 cells, NK cells, macrophages, eosinophils, and dendritic cells
CCL3	monocytes, neutrophils, dendritic cells, NK cells, mast cells	Recruitment of Th1 cells, NK cells, macrophages, and dendritic cells

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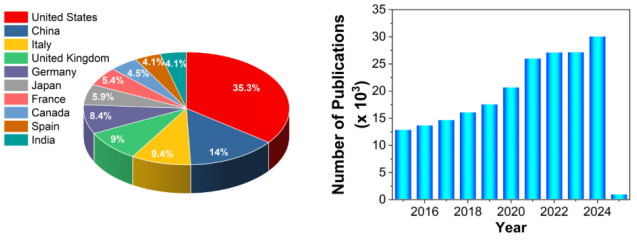
CXCL10	monocytes, endothelial cells, keratinocytes	Interferon-inducible chemokine; recruitment of TH1 cells, NK cells, and macrophages
CXCL13	B cells, follicular dendritic cells	Recruitment of TH1 cells, monocytes, dendritic cells, and basophils
Plasma proteins		
CRP	hepatocytes	Interleukin-6 increases CRP expression, interleukin-8 and MCP-1 secretion

The concept of CS has recently gained significant attention due to the COVID-19 pandemic, as its severe cases have medical features similar to a cytokine storm.[7] Early detection of CS is crucial in choosing appropriate treatment methods, as well as in the prediction of its progression and outcome. Sepsis pathology is complicated, with the dynamic nature of cytokines and the presence of anti-inflammatory intermediaries (molecules that aid in regulating and reducing inflammation) such as interleukin-4 (IL-4), IL-10, IL-13, and anti-IL-1ra at the starting point of inflammation. The initial proinflammatory phase may be absent in patients with pre-existing immunological impairments from chronic diseases or iatrogenesis (adverse health effects resulting from medical treatment). Nonetheless, the concentrations of pro- and anti-inflammatory cytokines are mutually dependent. When the pro-inflammatory response is heavily expressed, the production of anti-inflammatory cytokines increases to counteract the effect. The excess production of anti-inflammatory mediators can lead to immunosuppression,[8] a state of weakened immune system that increases risks of conditions such as cardiovascular diseases and cancer, in addition to heightened susceptibility to other infections.[9]

$CD4^+$ T cells that produce IL-17 (Th17 cells) generate tissue inflammation and autoimmunity because they recognize self-antigens as targets in dysfunction and are crucial in inducing autoimmune diseases.[10] When Th1 cells are exposed to innate inflammatory cytokines, they transform into pathogenic effector cells and cannot produce IL-10. They instead release IL-22 and IFN-γ, which contribute to hyperinflammation.[11] Similarly, large amounts of proinflammatory cytokines (TNF-α, IL-6, and IL-1β) are released during sepsis, triggered by the excited Toll-like receptor 4 (TLR4) by the microbial component lipopolysaccharides (LPS). Together, the cytokines cause vascular dysfunction, increasing their permeability extensively and lowering blood pressure, tissue necrosis, dysregulation of metabolic activities, and ultimately, systemic organ failure.[7]



(a) (b)

Figure 1: (a) Statistics illustrating the countries actively researching autoimmune diseases. (b) The graph depicts the surge in publications related to autoimmune disease research from 2015 to 2025 (Data courtesy: Scopus, February 2025).

Table 2: List of abbreviations used in the article..

Abbreviation	Full Form
CS	Cytokine Storm
IL	Interleukin
TNF	Tumor necrosis factor
TLR	Toll-like receptor
IIR	Inflammatory immune response
iNOS	Inducible nitric oxide synthase
TGF	Transforming growth factor
CAR	Chimeric antigen receptor
IFN	Interferon
TCZ	Tocilizumab
CRS	Cytokine release syndrome
HLH	Hemophagocytic lymphohistiocytosis
ELISA	Enzyme-linked immunosorbent assay
POC	Point-of-care
FET	Field-effect transistor
LoC	Lab-on-a-chip

Discussion

1. Pathophysiology of Cytokine Storms in Autoimmune Diseases:

Inflammation, immune protection, and immune suppression are the three functional categories of immune responses. In particular, inflammatory immune responses (IIRs), the ability of immune cells to adapt to changes in the external and internal environment, play a role in cytokine release. While moderate, IIR defends the body against diseases, overstimulation can cause harm.[12] During a viral infection or a disease, homeostasis in the immune system due to the balance between pro- and anti-inflammatory cytokines is thrown off, leading to the hyperactivation of diverse immune cells, such as macrophages, T and B lymphocytes, dendritic cells, and natural killer cells. This results in the production of an abundance of proinflammatory cytokines and chemokines that would generate a positive feedback loop to promote the immune response in the body, which can damage the body through a CS, for example.[13]

Interleukin-1β (IL-1β) plays an essential role in cytokine storms associated with COVID-19. It synthesizes enzymes like cyclooxygenase and inducible nitric oxide synthase (iNOS), with the nitric oxide from iNOS contributing to tissue impairment during airway inflammation. Moreover, it increases the expression of chemokines and adhesion molecules and stimulates the formation of interleukin-6 (IL-6).[13] IL-6 is a versatile cytokine with pro- and anti-inflammatory activity based on the circumstances. It assists B cells to mature into memory B cells and plasma cells. When IL-6 binds to its receptors, the JAK/STAT pathway is activated, which causes further production of inflammatory cytokines. Because IL-6, along with transforming growth factor β (TGF-β), prompts the differentiation of naïve T cells to Th17 cells, dysregulated IL-6 production is crucial to autoimmune diseases. IL-6 is the

primary cytokine responsible for the cytokine storm observed in some patients who underwent chimeric antigen receptor (CAR) T-cell therapy.[14] Interferon-gamma (IFN-γ) is another key mediator of excessive immune activation in inducing a CS. It links innate and adaptive immune responses and is vital in the body's defense against intracellular pathogens and tumor control. Combining IFN-γ and tumor necrosis factor (TNF-α) can induce pyroptosis, simply an inflammatory cell death. Together, they activate the JAK/STAT1/JRF1 pathway and produce nitric oxide, which triggers caspase-8/FADD-interceded inflammatory cell death, a process termed PANoptosis.[13]

Endothelial cells (ECs) are activated and produce tissue factors that stir up the extrinsic coagulation pathway, resulting in a hypercoagulable state. In the case of a CS, the ECs are likely to become impaired and dysfunctional. The dysfunction of the ECs can cause unregulated platelet aggregation and thrombosis from the abnormal production of ADP. It also activates the proinflammatory peptide C5a, triggering a coagulation cascade and the production of thromboxane A2, which contributes to platelet aggregation. Systemic multiorgan ischemia can be aroused by inappropriate pro-inflammatory mediators that provoke microvascular congestion and the coagulation cascade.[15]

Chemokines, such as CCL2, CCL3, CXCL10, and CXCL13, also significantly initiate a CS. Chemokines are chemoattractant cytokines or small proteins that bind to cell surface receptors and guide cells' movement around the body. They dictate the positioning of cells, steering stem cells and ECs through their route, and controlling leukocytes in extra- or intravasation.[16] Along with cytokines, they modulate the tumor microenvironment (TME) by activating signaling pathways, including nuclear factor kappa-B (NF-κB), which is crucial in enhancing activating B cells and driving inflammatory responses, and vascular endothelial growth factor (VEGF) that instigates epithelial-mesenchymal transition (EMT), and hence contributing to the resistance of therapeutic and promoting aggressive metastatic colorectal cancer (CRC) development.[17] Elevated levels of proinflammatory cytokines and chemokines are usually found in cases of SARS-CoV-2 infections. Bronchoalveolar lavage fluid (BALF) from critically ill patients shows high levels of inflammatory macrophages and chemokines in the lungs.[18] Viral infection, such as influenza, triggers excessive immune system activation to produce increased cytokine levels and chemokine levels. The amplification of the CS can cause severe inflammation and tissue damage in organs like the lungs **(Figure 2)**.

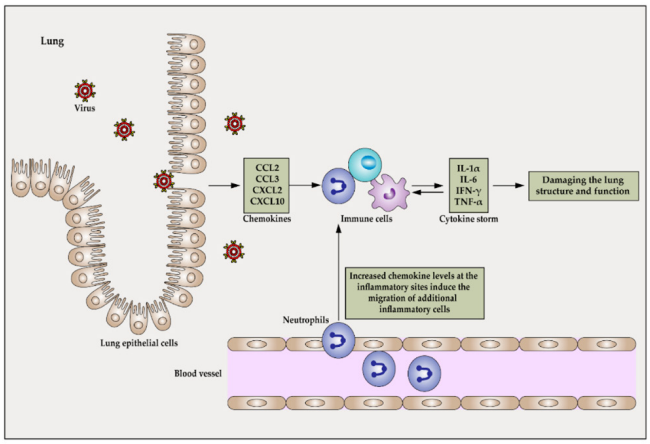


Figure 2: Influenza-induced cytokine storm in the lungs leads to amplified inflammation and lung tissue damage. Due to the viral infection, the immune response is triggered, and excess cytokines are released (Reproduced with permission,[19] Copyright 2021, MDPI).

2. Therapeutic Strategies:

Various therapeutic strategies are involved in treating CS in autoimmune diseases, such as suppressing the release of cytokines or reducing the inflammation already present in the body. Systemic corticosteroids are one method that is widely used for mechanically ventilated patients, ICU patients, and especially COVID-19 patients in grave conditions. It is an immunomodulatory agent and was used in a retrospective study involving 107 patients suffering from SARS. The study showed that 89% fully recovered, with 95 patients treated with high doses of methylprednisolone and hydrocortisone exhibiting signs of clinical improvement in their condition.[20] However, corticosteroids have a broad spectrum of adverse effects associated with them as well, divided into twelve categories: cardiovascular system, dermatological complications, endocrine glands, fluids and electrolytes balance, gastrointestinal tract, renal system, metabolism, musculoskeletal system, nervous system, ophthalmic complications, reproductive system, and allergic reactions.[21] Although its short course treatments (for example, up to 10 days) are clearly shown to provide benefits for those with severe ARDS, there is no evidence for positive outcomes in the case of extended corticosteroid therapy, and it may even have a higher mortality rate than those who are not treated with corticosteroids. It is essential to consider the risk-benefit ratio for each patient before its use and to avoid long-term treatments.[22]

Targeting proinflammatory cytokines like IL-1β, IL-6, and TNF-α is another therapy frequently adopted by patients suffering from CS. This type of therapeutics aims to inhibit the production of cytokines in an attempt to lessen hyperinflammatory conditions in the body.[23] Antagonists for IL-1β include anakinra and canakinumab. Anakinra, which blocks the receptor of IL-1β, was investigated in over 20 clinical trials of cytokine-induced multiorgan failures with a particular focus on the lungs. In an earlier study of anakinra by Cavalli et al., when combined with 4-aminoquinoline, the antagonist showed impressive results by decreasing the mortality rate while improving respiratory functions. However, since the results of extended studies have not been confirmed, regulatory

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use for anakinra in treatment has yet to be decided, unlike canakinumab, which is approved for treating autoimmune diseases.[24] Canakinumab is a monoclonal antibody that targets to neutralize IL-1β, reducing inflammation and hence treating various inflammatory diseases, namely adult-onset Still's disease (AOSD).[25] Tocilizumab (TCZ) is a recombinant humanized monoclonal antibody that interferes with immune responses by targeting IL-6 receptors. A case study evinced a notable decline in CRP levels (from 225 to 3 mg/L) in a COVID-19 patient after treatment with TCZ for four days. Other studies also found quick recovery and rapid TCZ reduction as well, but multiple administrations of medication may be required for some patients who are acutely ill.26 Siltuximab works similarly to TCZ in the way it binds to IL-6 directly to inhibit its effect. A 77-year-old patient with relapsed/refractory multiple myeloma (RRMM) developed cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). After 1 hour of siltuximab administration, CRS was cured, and within 7 hours of treatment, the patient recovered from ICANS as well, demonstrating the effectiveness of siltuximab in relieving CRS.[27]

High levels of TNF-α facilitate the pathway that allows virus penetration into host cells, or the TNF-α-converting enzyme (TACE)-dependent alteration of ACE-2, which can be stopped by TNF-α blockers.[28] TNF-α inhibitors tend to be monoclonal antibodies, such as adalimumab, certolizumab pegol (CDP87), and golimumab. New anti-TNF-α agents that address the limitations of the current inhibitors are also in the development process. For instance, ozoralizumab has nanobody characteristics, and ZINC09609430 has the potential to be used as a novel inhibitor for TNF-α in evaluating *in vitro* and *in vivo*.[29]

3. Challenges and Future Directions:

Symptoms of CS are nonspecific and resemble septic shock and other systemic diseases, making its diagnosis difficult. Diagnostic tools evolved from HLH-1991, based on five easily determined markers to identify CS, but lacked universality, to HLH-2004 by the International Histiocyte Society. These are diagnostic guidelines for hemophagocytic lymphohistiocytosis (HLH), a severe cytokine-driven inflammatory disorder. HLH-2004 was further refined by research into immunopathophysiology and advanced diagnostic techniques and is now the most widely accepted diagnostic criterion.[30] Studying biomarkers in laboratory settings is one method for their comprehensive evaluation. For example, procalcitonin and neutrophil-to-lymphocyte ratio, along with T cells and cytokines, and endothelial dysfunction markers, predict the severity of the inflammation and, hence, the disease. Depending on the different causes of CS, cytokine levels differ, with CS associated with CAR T cell therapy having an increased amount of IFN-γ compared to others, and IL-1β and markers of endothelial damage being reported in high levels for systemic infection-associated CS. Accurate measurement of cytokines is challenging due to biological factors, including fluctuating secretion patterns, low concentrations in body fluid, and barriers to accessibility, as the equipment required is costly and has limitations in implementing continuous monitoring of cytokine levels.[31]

Developed in 1971, enzyme-linked immunosorbent assay (ELISA) is a relatively versatile test using specific antibodies to locate a single analyte. It is sensitive to not only high but also low concentrations of body fluids, as well, and has a high accuracy rate with quick turnabout time for results, but it faces hardships in making it more efficient for multiplexing or the detection of multiple analytes at the same time.[32] Point-of-care (POC) testing allows on-site diagnosis of patients, making it a quick and affordable detection method and an attractive choice during the COVID-19 pandemic. It holds an advantage over ELISA because no specialized equipment is needed. POC can be paper-based, more lightweight, cost-effective, or transistor-based. In field-effect transistors (FET), an underlying semiconducting material covered by non-metalized gate dielectrics in contact with an electrolyte solution converts biological binding activities into quantifiable signals. This allows FET-based POCs to be highly sensitive to cytokines and track them in time.[33] Lab-on-a-chip (LoC) devices and biosensors are now utilized to overcome diagnostic limitations. The microfluidic structure of LoC devices permits biochemical reactions to occur faster by their small channels' high surface area to volume ratio, careful control from minimized reagent use, and multiplexed analysis. Moreover, by integrating nanotechnology and biosensors, biomarkers of lower quantity in the human body can be detected. Multiple methods exist to observe cytokines, such as fluorescence signals, electrochemical transduction, and color changes.[34]

Negative regulators of TLR signaling play a key role in recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) to activate pathways that induce a pro-inflammatory immune response. The effectiveness of their inflammatory response has been tested in animal studies and clinical trials. While it showed potency in animal models, organ damage and mortality rates did not reduce in sepsis patients. Seeking to solve this problem, researchers proposed targeting specific pathways, like the Wnt/β-catenin signaling pathway, which may be able to act as negative regulators. Therapeutic approaches considering the genetic variants in individuals, considering the patient's age and genetic background, is also a potential approach in developing personalized treatments for sepsis and CS.[35] Continuous research is taking place to analyze the cellular and molecular contributors of CS, and other anti-inflammatory strategies, including TLR4 antagonists, cyclooxygenase (COX) inhibitors, sphingosine-1-phosphate modulators, and protease-activated receptor (PAR) 2 agonists, are being tested in animal models, making advancements toward the progression of effective therapy for CS in various autoimmune diseases.[36]

■ Conclusion

In conclusion, cytokine storms are a critical factor in the pathogenesis of autoimmune diseases, driving excessive inflammation and contributing to disease severity. By identifying the key cytokines and signaling pathways involved, researchers have made significant strides in understanding how dysregulated immune responses lead to tissue damage and systemic

complications. Establishing a deeper connection between cytokine storm mechanisms, their harmful effects on the body, and their role in disease progression is essential for developing targeted treatments. Advancing this knowledge can pave the way for novel therapeutic strategies that mitigate the impact of cytokine storms and improve overall disease management, ultimately enhancing patient outcomes.

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REVIEW ARTICLE

Biofuel Manufacturing Potential Using Diverse Biomass Resources: Indian Perspective

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ABSTRACT: Increasing energy demands and escalating environmental concerns have brought biofuels into focus as a vital alternative to fossil fuels. In response to these challenges, India has prioritized developing and integrating biofuels to reduce dependence on non-renewable energy resources. Biofuels are a potential solution to the energy crisis in India, and biomass resources are available in plenty in the country and will continue to progress. The paper aims to estimate the market for liquid biofuels and evaluate the appropriateness of different materials. Owing to their high cellulose content, aquatic plants like water hyacinth have been identified as promising candidates for bioethanol production. Straw and husk used in bioethanol, biodiesel, and biogas analysis are checked for efficiency using gasification and fermentation techniques. The document also examines the applicability of the circular economy framework in biofuels, where waste and residuals are reclaimed and reintroduced into the production system, leading to improved resource utilization efficiency and a significant reduction in environmental impact. This paper highlights the economic and ecological benefits of potential export opportunities in India's biofuel industry. The Biofuel Report further underscores the critical role of biofuels in advancing a sustainable energy future for the country and fostering the development of a circular economy.

KEYWORDS: Energy: Chemical, Alternative Fuels, Biofuels, Circular Economy, Sustainable Energy, Economic Implications, Policy Support.

Introduction

Amid rising global concern for renewable energy alternatives, biofuels have emerged as a viable and sustainable energy resource. India, in particular, holds significant potential in this domain, owing to its abundant biomass availability, especially within the agricultural sector.[1] This report aims to assess the feasibility of biofuel production in India by exploring the potential use of raw materials such as water hyacinth and agrarian waste.

India has a wide range of biomass resources that offer both potentialities and difficulties in biofuel usage. Agricultural residues like straw, husks, water hyacinth, a common aquatic weed, and other materials can be converted to biofuels. Such materials are a renewable source of energy that supports the management of waste and the environment.[2]

This study incorporates several key concepts, particularly the circular economy, which emphasizes the reuse and recycling of materials to minimize waste.[3] In India, the biofuel sector aligns closely with circular economy principles by transforming biomass waste into valuable, renewable energy sources as an alternative to fossil fuels. Additionally, India's strategic geographic location and abundant biomass resources position it as a potential exporter of biofuels to energy-deficient regions. This research provides a systematic assessment of India's biofuel production potential, emphasizing the diverse range of raw materials available within the framework of a circular economy.[4]

One of the main policies driving this shift is the National Biofuel Policy (2018), which aims to include 20% ethanol in gasoline by 2030. As illustrated in Figure 1, this approach encourages the production of ethanol from a range of feedstocks, such as residual grains, sugarcane molasses, and biomass waste. It focuses on lowering crude oil imports, creating jobs through bio-refineries, and encouraging investment in rural areas. It also encourages environmental preservation and converts municipal and agricultural trash into electricity, thus converting "waste to wealth."[5] The biofuel sector in India has the potential to stimulate technological advancement, drawing in capital and creating fresh approaches to biomass conversion. The effective incorporation of biofuels into India's energy mix has the potential to greatly lower carbon emissions, increase energy independence, and provide rural communities with long-term economic prospects. Biofuels might be a key component in India's shift to a low-carbon economy with more research and development, supporting a resilient energy future and being in line with international environmental targets.[4]

This report will look at the practical biofuel market in India, given the increasing energy consumption and the implementation of renewable energy sources in the country. It will also examine the potential of biofuel exports, which aim to fulfil the energy demands of the world using India's biomass resources. The report seeks a coherent picture of biofuel manufacturing in India. It will also explore the potential and prospects in the general framework of a circular economy and the market. The study aims to provide a comprehensive picture of biofuel production in India and will investigate its prospects going forward in the context of sustainability, the circular economy, and government-led market assistance.

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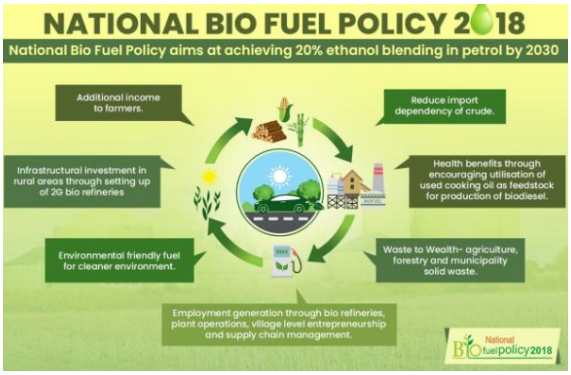

Figure 1: The Government of India introduced the National Biofuel Policy (2018) to encourage the use of biofuels as a renewable source of energy.[6] It seeks to attain a 20 percent blending of ethanol in petrol by 2030, hoping to cut down on fossil fuels and carbon emissions. This policy aims to promote ethanol production from different feedstocks, such as surplus grains and sugarcane molasses, and encourages research in advanced biofuels.

Discussion

1. Global Trends in Biofuels:

Biofuels generated from organic matter are renewable energy sources, thus playing an important role in energy security. There are first, second, and third-generation biofuels, the first generation of which is made from food crops, the second from non-food biomass, and the third from algae. Each category has its benefits and drawbacks depending on the feedstock supply, production mechanisms, and environmental effects. Research and development have focused on improving conversion technologies and supply chain efficiency to address the challenges associated with second-generation biofuels, which rely on non-food feedstocks such as agricultural residues, forestry cuttings, and energy crops.[7] Such biofuels have garnered attention because they reduce greenhouse gas emissions without contributing to food-versus-fuel conflict. Biomass is converted to biofuels using gasification, pyrolysis, and fermentation technologies, with common outputs including bioethanol and biodiesel. Advanced-generation biofuels are being developed to further enhance efficiency and sustainability beyond what first- and second-generation fuels offer. Despite these advancements, many developed nations, including Brazil and the United States, continue to invest heavily in second-generation biofuels due to their balance of scalability and environmental benefits.

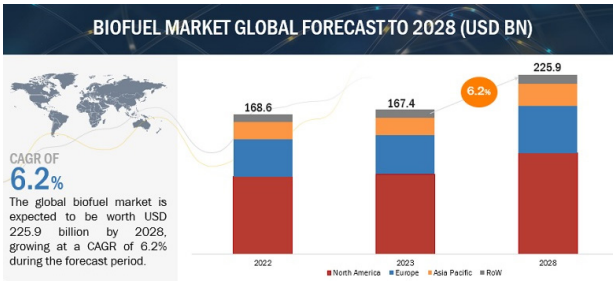

Figure 2: The graph illustrates the global biofuel market forecast to 2028 (USD BN), with an expected compound annual growth rate (CAGR) of 6.2% from 2023 to 2028. Biofuel production is predominantly shown in North America, next to Europe and the Asia-Pacific region. This market's expansion is driven by the growing demand for sustainable energy and government policies in favor of biofuels. It shows the potential and various areas of economic biofuel adoption.[8]

The third-generation biofuels utilize algae as the feedstock, and are a new advancement in the production of biofuels. Algae-based feedstock offers the possibility of producing substantial quantities of biofuel per unit area of land. Biofuels can be in the form of biodiesel, bioethanol, or biogas, which are derived from algae and are, hence, more efficient and renewable energy sources. In the experimental stages, third-generation biofuels have some indication of lower land and water use than other crops. The US, China, and a few European nations are researching how to bring the real algae biofuel business into existence, which can be seen as a projected market indicator in Figure 2. The fuel ethanol market has been defined by regulations supporting renewable energy sources, the most prominent examples of which have been established by the EU and the US, such as RED and the RFS. Such policies have established blending mandates for biofuel in transportation fuels, fostering demand and production. Asia and South America are turning to biofuels as a means of sourcing fuel locally and helping out the rural people. As seen in Table 1, Biofuels hold a promising position to fill the gap for cleaner energy sources in the future decades on a global scale.

Table 1: Presents biofuel production statistics across different regions. The rise of North America to 62 billion liters leads the pack with 38.5% contribution to the global blend, followed by Europe at 24.8% and Asia Pacific at 19.9%. In other words, Latin America and Africa & the Middle East account for 13.7% and 3.1%, respectively. The figure attests to the importance of developed regions in biofuel production and its need to expand in emerging economies.

Region	Biofuel Production (Billion Liters)	Percentage of Global Production (%)
North America	62	38.5
Europe	40	24.8
Asia-Pacific	32	19.9
Latin America	22	13.7
Africa & Middle East	5	3.1

2. Biofuels in India:

The biofuel market in India is in concordance with the emerging international trends, but is differentiated by the availability of biomass and energy requirements. Biofuels are one of the most promising renewable energy sources, and various policies of the Indian government support them. The National Biofuel Policy of 2018, as seen in Figure 1, contains a guide toward biofuel production focusing on non-food and waste feedstocks. The following policy focuses on funding research as well as development, encourages biofuel technology investment, and seeks to incorporate biofuels into the nation's energy capacity.[7] In addition, this policy aims to boost biofuel production in India by setting targets of 20% ethanol and 5% biodiesel blending by 2030, as seen in Figure 3. It promotes the use of diverse raw materials such as sugarcane juice, damaged grains, used cooking oil, and biomass. The policy supports ad-

vanced biofuels (2G and 3G), encourages R&D for efficiency, and offers financial incentives.

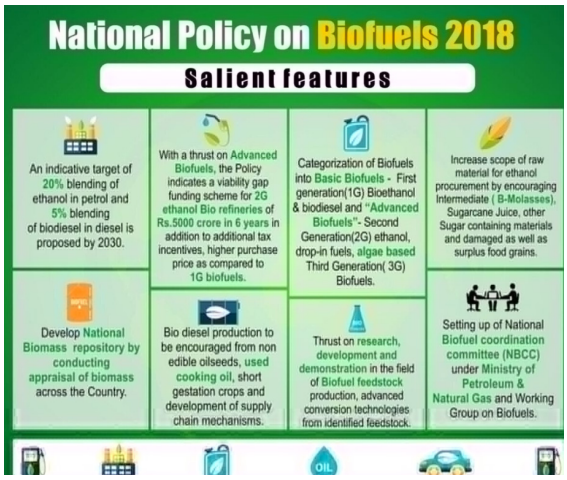

Figure 3: Salient features of India's national biofuel policy 2018, enlisting key initiatives that form a part of the policy – for ethanol blending and advanced biofuels, and sustainable production – are outlined in this figure. It is an emphasis on the development of biodiesel, biomass utilization, and research for efficiency improvement. In addition, there also remains the issue of institutional coordination that would enable smooth policy implementation and promotion of investment in biofuel technology.[9]

India has an extensive region of agroforestry and biomass resources that can be used for biofuel purposes. Some major agricultural states, including Punjab, Haryana, and Uttar Pradesh, have confirmed that burning crop residue and releasing large quantities of smoke cause major air pollution. Using these residues for bioethanol or biodiesel production reduces the pollution level, and farmers also have an extra source of income. Another resource is water hyacinth, which is a problematic weed in rivers and water bodies; its use for biofuel production also has a double advantage since it eradicates the weed while producing energy. Biomass biofuels, for instance, are also viewed as empowering the agricultural-based economies in India by offering employment opportunities in the collection of biomass and operating biofuel processing plants. With assistance from the government's subsidies and incentives, and also improvements in technology, the production of biofuel has been made cheaper and more sustainable. Biofuels have the potential to achieve energy security in the country, develop the rural economy, and promote sustainable development in the future. Government support and technological advances are key factors that may lead the country to the status of the leading producer of biofuels, including ethanol, as seen in Table 2.

Table 2: The Indian ethanol capacity is divided by the feedstock used for production. It is essentially aided by 4,500 million liters of sugarcane molasses, with 1,200 million liters contributed by the surplus rice and maize. Indicating India's dependence on traditional agricultural sources for ethanol production, India has a total ethanol capacity to produce, which stands at 5,700 million liters, and the ability to diversify into alternative feedstocks.

Feedstock	Ethanol Production Capacity (Million Liters)
Sugarcane Molasses	4,500
Maize and Surplus Rice	1,200
Total Ethanol Capacity	5,700

3. Raw Materials for Biofuels Production

3.1. Water Hyacinth:

Water Hyacinth, an invasive aquatic plant prevalent in many regions of India, presents significant potential as a biofuel feedstock. Owing to its high cellulose and hemicellulose content, it is well-suited for bioethanol production. It has been effectively processed through anaerobic digestion and fermentation methods to yield bioethanol. This not only aids in the control of excessive proliferation of water hyacinth in the water source but also creates a renewable energy source. The utilization of water hyacinth for biofuel production has been established to lower greenhouse gas emissions and is, hence, sustainable.

It has been noted that the utilization of water hyacinth in the production of biofuel has impacts that are beneficial to the environment.[10] Removing water hyacinth from water bodies will mean that the ecosystems can be corrected, and the health of aquatic life will be improved. The utilization of bioethanol production also helps to alleviate the dependency on other traditional sources of biomass, such as food crops, that cause competition between animal food and fuel. Bioethanol derived from water hyacinth alleviates emissions of greenhouse gases and the direct utilization of fossil fuels, and meets India's broad objectives of harnessing renewable energy and combating climate change. It will be important to note that there are considerable economic gains.[11] Manufacturing bioethanol from water hyacinth offers new business ventures, especially in agrarian regions where the plant thrives. This is because it would create employment chances for other personnel, hence employment in biomass collection, processing, and supply. The bioethanol from water hyacinth also finds its market in the Indian biofuel market, which is likely to expand due to the government's new policies on increasing bioethanol blending rates. Though the water hyacinth is considered an invasive species that threatens the aquatic environment, it is considered an excellent biofuel feedstock in India, amounting to 0.25 liters of bioethanol per kg of water hyacinth, as seen in Table 3.

Table 3: The bioethanol yield per kilogram of dry weight of different feedstocks is presented in this table. The corn stover has a yield of 0.30 liters per kg, the rice straw 0.28 liters per kg, and the water hyacinth 0.25 liters per kg. It shows the efficiency of the different agricultural residues in ethanol production and the ability to use non-food biomass for improving the sustainability of biofuels.

Feedstock	Bioethanol Yield (Liters/Kg of Dry Weight)
Water Hyacinth	0.25
Rice Straw	0.28
Corn Stover	0.30

3.2. Agricultural Waste:

There are a lot of residues from agricultural activities, such as straw, husks, and stalks, that can act as raw materials for biofuel in India. Several researchers have shown that agricultural residues have the potential for bioethanol, biodiesel production, and biogas. Some of the processes used to convert these residues to biofuels are gasification and fermentation. Studies have shown that incorporating agricultural waste makes sense in the circular approach, involves recycling wastes into energy sources, reducing pollution, and embracing sustainable agricultural systems. The copious nature of agricultural waste

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makes it a cheap feedstock for the biofuel industry of the country. Under-utilized crop wastes like straw, spoiled fruits, and vegetables can serve as effective raw materials for biofuel production, as can be seen in Figure 4.

Figure 4: The figure demonstrates some examples of underutilized agricultural lands that can be exploited for biofuel production. This encourages the conversion of rice straw, wheat straw, and sugarcane bagasse residues into bioethanol, reducing agricultural waste and environmental pollution. Such resources offer to improve energy security and help rural economies.[12]

From an economic standpoint, using surplus non-consumable agricultural residue for biofuel production offers new commercial opportunities and is relatively cost-effective compared to conventional energy resources. As shown in Table 4, from an environmental perspective, this approach reduces waste disposal issues, lowers greenhouse gas emissions, and promotes sustainable agricultural practices through the recycling of organic waste.[13] Using agricultural waste in biofuel production is advantageous in turning waste into valuable energy, leaving a minimal carbon footprint. Based on agricultural residues, India can boost the growth of the biofuels industry, which in turn will contribute to the energy security of the country, as well as provide more local employment and enhanced income for the farmers. The utilization of agricultural waste can thus be seen as consistent with the strategies supported by the government toward increased usage of renewable energy as opposed to conventional fuels. The use of agricultural waste in the genesis of biofuel is feasible and environmentally friendly in handling the problems of energy and the environment in India.

Table 4: The table indicates the annual availability of different agricultural wastes and their bioethanol potential. Similarly, bioethanol can be produced from rice straw available in the world at a level of 120 million tons per year, producing 30 billion liters. There is the potential of 18 billion liters for wheat straw and 12 billion liters for sugarcane bagasse. According to the data, agricultural residues in India have great untapped potential to contribute to the biofuel industry.

Agricultural Waste	Annual Availability (Million Tons)	Bioethanol Potential (Billion Liters)
Rice Straw	120	30
Wheat Straw	70	18
Sugarcane Bagasse	90	12

3.3. Other Biomass Resources:

Other biomass resources, such as wood chips, municipal solid waste, and dedicated energy crops, are also used in biofuel production. Forest and municipal residues, such as wood chips and municipal solid wastes, have large biomass potential for biofuel conversion, while switchgrass and miscanthus are chosen for high energy densities and efficient conversion. Studies have shown that these biomass resources can be transformed into bio-energy through conversion techniques such as pyrolysis and gasification. Some factors that make each type of biomass unique are energy content, processability, and environmental issues related to its use.

4. Economic and Environmental Implications:

The consequences of biofuels are mixed; economic and environmental factors are involved. From an economic point of view, biofuels have a lot to give. They generate new business opportunities and foster employment in areas like agriculture, ICT-enhanced technologies, and waste disposal. As biofuels are produced from locally available biomass feedstocks, they decrease reliance on imported fossil fuels, which can only be beneficial to energy security and may decrease the cost of energy in the long run. The advancement of biofuel technologies can create demand for innovation and spur investment, boosting the economy.[3] Agricultural residues and other biomass for biofuels can also bring additional income sources to the growers, thus reducing the risks of crop production ventures. On the environmental side, biofuels have been shown to lower the emission of greenhouse gases compared to traditional fossil fuels. Through the comprehensive process of generation, usage, and burning of biofuels, these vehicles are known to emit less carbon, hence reducing the effects of climate change. The process that converts biomass wastes into biofuels helps in waste disposal because most organic wastes, if not tapped for biofuel production, are dumped into waste disposal sites, leading to methane production from the rotting materials. The composting of the byproducts of biofuel back into agricultural soil has made it possible to improve soil quality through sustainable farming.[14] There are also some problems, including the problems of biomass preprocessing and high costs of the required technologies, and the issues of biomass provisioning, including the changes in land use and water consumption when biomass is grown in large amounts. This shows that managing these factors is important to reap the advantages of using biofuels while avoiding the negative influence of the same. It is necessary to promote biofuels as a new front for economic development, but only when it is initiated and managed.

5. Circular Economy and Policy Support:

Biofuels greatly improve energy sustainability when incorporated with a circular economy system. The principles of a circular economy include striving to optimize resources, minimize waste, and enhance recycling. Within the context of biofuels, it refers to the process whereby waste materials are transformed into useful energy resources, including agricultural residues and water hyacinth, reducing environmental effects, and utilizing all available resources.

5.1. Circular Economy in Biofuels Production:

The production of biofuel is a perfect example of a circular economy where biomass waste is turned into renewable energy. Miscellaneous agricultural residues, including straw and husks, and water hyacinth, among others, are predominantly a nuisance to waste management systems, but are utilized in

producing bioethanol, biodiesel, and biogas.[15] This process is applied to waste disposal, which aids in decreasing greenhouse gas emissions as a result of utilizing conservation energy rather than fossil energy. The circular economy approach helps environmental conservation as nutrients are recycled back into the soil with minimal artificial fertilizer use.

5.2. Economic and Environmental Benefits:

Applying the circular economy in producing biofuels has provided ample economic and ecological returns. In this respect, it creates new jobs and stimulates business activities toward biomass gathering and biofuel production at the local level. It also reduces reliance on fossil fuel imports, improving energy security. Environmental legislation and policies enhance the curbing of greenhouse gases and waste management. According to the circular economy, waste products are utilized in the production of energy to counter the impacts of environmental degradation and the drain of resources.[16]

5.3. Policy Support for Biofuels:

Government policies play an important role in developing the biofuels sector and the overall shift towards a circular economy system. India's National Biofuel Policy of 2018 sets an overall direction for biofuel policy, focusing on non-food feedstock and waste resources. This policy seeks to increase funding for research and development in the biofuels sector, encourage investors in the biofuel industry, and incorporate biofuels into the energy mix in the country.[17] At some state levels, it supports subsidies, tax credits, and infrastructure development for biofuel production.

5.4. Future Prospects:

Integrating biofuels with the circular economy and favorable policies fosters a positive prospect for sustainable energy solutions in the Indian context. Progress in technology, whilst supported by secure policy structures, assists the development of biofuels, optimizes the use of resources, and increases energy system resilience. Through awareness creation of a circular economy and enhancing policy support, India can fully maximize its biomass resources for energy security and sustainability.

6. Analysis of Various Raw Materials for Biofuels Production

6.1. Water Hyacinth:

Eichhornia crassipes, commonly known as water hyacinth, is one of the most terrible invasive water plant species and affects many water bodies in India. Even though the water hyacinth forms a nuisance resulting from its fast growth rate, it offers a good chance of providing biofuel. Due to its high cellulose, hemicellulose, and lignin content, it is suitable for converting into different biofuels such as bioethanol, biogas, and bio-oil. The general procedures for converting water hyacinth into biofuels involve its collection and preprocessing. The plant material is treated to reduce it into a pulp and thus eliminate the fibrous structure it possesses. Methods employed include anaerobic digestion, whereby the organic matter is treated with microorganisms that break it down into biogas, mainly methane and carbon dioxide. This biogas can be used directly as energy or converted to biofuel in second-generation strategies.[18] Water hyacinth can be subjected to pyrolysis, a thermal process that produces bio-oil, a liquid biofuel that can be further processed to produce different energy products. Apart from its high energy content, water hyacinth provides environmental benefits. Biofuel production offers a positive way of controlling its spread since it uses plants whose negative impact on aquatic systems is well known. Overgrowth of water hyacinth reduces water movement, reducing dissolved oxygen and affecting aquatic organisms. Using it for biofuel production not only affords a renewable energy source but also helps conserve the environment. The incorporation of water hyacinth in biofuels is therefore in line with India's overall vision about energy security, reducing reliance on fossil-based products while increasing the usage of renewable energy sources. It helps the country switch to using cleaner sources of energy while at the same time tackling the local environmental problems. Establishing technologies and structures for water hyacinth bioenergy may lead to new opportunities for economic development and wetland environmental management in affected areas.

6.2. Agricultural Waste:

Residues like straw, husks, stalks, and leaves of crops, crop stover, and agricultural waste are largely untapped resources for the generation of biofuels in India. This biomass is abundant due to the country's high levels of agricultural activities, such as rice, wheat, and other crops. The conversion of agricultural waste into biofuels is possible through different techniques, including gasification, pyrolysis, and fermentation.[19] Syngas generation entails a stream of agricultural remnants under controlled conditions to generate a gaseous mixture of hydrogen with carbon monoxide, which is popularly referred to as syngas. This syngas may then be utilized to produce biofuels such as bioethanol or biodiesel. Pyrolysis, in its part, involves the thermal decomposition of biomass at high temperatures in an inert atmosphere to yield bio-oil, which can be upgraded to various forms of biofuel. Another method is fermentation, which is based on the action of agricultural waste to convert the bioethanol from high cellulose residues.

The adoption of agricultural waste in the production of biofuel has several advantages. It provides a solution for handling large quantities of biomass, which, if dumped, burned, or allowed to rot, can result in pollution and emission of greenhouse gases. Through the conversion of these residues into biofuels, India will be able to reduce the usage of fossil fuel imports, thus improving energy security for the country and the environment. Biofuel production incorporating agricultural waste is consistent with the circular economy because it reuses what other people would consider waste to produce valuable energy sources. This approach is beneficial to environmental conservation, but at the same time, it has economic returns that embrace new employment opportunities within rural areas and strengthen local economies. It complies with government policies on renewable energy and enhanced waste management systems. As seen in Figure 5, the various agricultural waste sources suitable for biofuel production in India are categorized as crop residues (e.g., rice and wheat straw), livestock waste (e.g., manure, animal fat), agro-industrial waste (e.g., bagasse, seed cakes, fruit peels), and aqua-cultural waste

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(e.g., fish waste, algae). These diverse feedstocks enhance the scope for sustainable and efficient biofuel manufacturing.

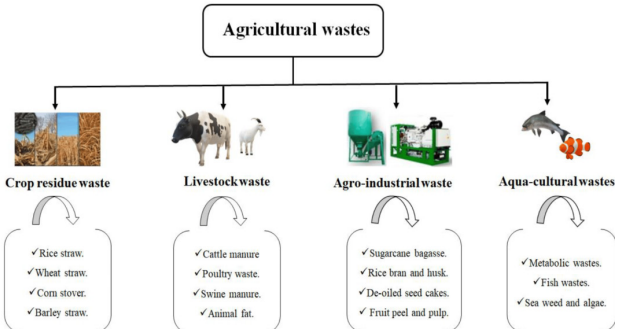

Figure 5: The Illustration shows how various agricultural waste sources can be used as biofuel resources and therefore constitute alternative energy sources. This shows the distribution of biomass feedstocks visually, which highlights the fact that biofuel raw material should be diversified. The second solidifies the use of agricultural waste in addressing energy demands and decreasing energy deprivation with fossil fuels.[20]

6.3. Other Biomass Resources:

Other biomass feedstocks besides water hyacinth and agricultural waste that are used to produce biofuels include woodchips, municipal solid waste, and dedicated energy crops. Woody biomass from forestry and sawmilling operations can be converted to bioethanol or bio-oil using pyrolysis or hydrolysis processes. Another form of biomass is municipal waste, which is from households and industries, including organic waste.[21] Technologies like anaerobic digestion and gasification are used to turn municipal waste into biogas or bioethanol. Energy crops are established for biofuel production because of the high production capacity of crops like switchgrass and miscanthus for the conversion process. These crops undergo various processes that result in bioethanol or biodiesel. This flexibility of these biomass resources shows that they have the potential to expand the array of India's biofuels. The advantages of different types of biomass in terms of availability, energy content, and processing technology are presented below. Energy crops offer most of the biomass with known characteristics on the energy content, whereas municipal solid waste is available in plenty but with fluctuating characteristics. These different types of biomass resources can be incorporated into the production process of biofuels to improve the effectiveness of the sector.[22] The wide variety of raw materials in India for biofuel production indicates the opportunities available for the development of a sound biofuels sector. Each of the sources, like water hyacinth, agricultural waste, and other biomass, has its advantages and disadvantages, but in combination, they provide an excellent scope for the growth of biofuels in India. Through these resources, India's renewable energy target, waste management, and biofuel economy can be achieved.

7. Possibilities of Circular Economy for Biofuels in India

7.1. Waste Management and Resource Efficiency:

Integration of the circular economy in the Indian Biofuel industry presents a transformative approach to managing agricultural and organic waste. At its core, the circular economy emphasizes the continuous reuse of resources to generate value, rather than allowing them to go to waste. Practical examples include the production of biofuels from agricultural residues such as straw, husks, and stalks. Utilizing these biomass resources to generate energy addresses the problem of waste disposal and adds value by converting waste into renewable energy.[23] Agricultural residues like ash often accumulate on farmland, degrading soil quality or being burned, contributing to greenhouse gas emissions and air pollution. Instead of relying on conventional waste disposal methods that harm the environment, India can adopt circular practices by transforming such residues into biofuels. A notable example is the use of water hyacinth, which clogs water bodies and disrupts local ecosystems. Instead of treating it as a nuisance, this biomass can be used to produce bioethanol and biogas, offering a dual benefit of efficient waste management and sustainable energy generation. This circular economy approach enhances environmental conservation by enabling the recycling of waste materials and promoting optimal resource utilization.

7.2. Economic Benefits:

The shift toward a circular economy within biofuel production substantially impacts India. Innovation of new biofuels from agricultural residues and wastes, such as Water Hyacinth, establishes new business ventures in rural areas where biomass feedstocks are easily accessible. Local people, especially farmers and other people in the communities where these raw materials are found, can get an extra source of income, which is very important to improve the employment rate in rural areas. The production of biofuels helps reduce the importation of fossil fuels, increasing India's energy security and economic stability.[16] The occurrence of volatile oil prices in the global market can be stabilized depending on the utilization of domestic biomass resources. Such a system can help promote the Indian energy regime to a more self-reliant and sustainable energy economy. The transition to biofuels is also a long-term cost-saving aspect because the cost of dealing with the ravages of fossil fuels to the environment and the global health of the population cannot be underestimated. Due to the implementation of the global circular economy, innovations in the production of biofuel technologies are enhanced due to efficiency gains, and hence, the introduction of lower costs. This can make Indian biofuels more attractive in the domestic and international markets, as shown in Figure 2, making exports to countries in dire need of renewable energy. Biofuels may significantly contribute to addressing environmental challenges in the country and stimulate the preparation for transforming different sectors in India toward a green economy.

7.3. Technological and Policy Support:

The biofuels sector's potential in India largely depends on the existing technologies and the use of policy developments. Advanced technology is therefore paramount in enhancing the transformation of multiple resources into biofuels and making the process the most cost-effective and scalable. Suggested technologies like gasification, pyrolysis, and bio-digestion are being enhanced to get better and higher biofuel yields from biomass resources, including biomass like agricultural waste, water hyacinth, etc. These technologies improve biofuel yields and reduce production costs in general, making biofuels more competitive with regular gasoline.[24] Investing in research and development (R&D) is important in improving these techno-

logies. Several research universities, institutions, and private organizations are devoting efforts and time to the research and development of new technologies to increase biomass conversion efficiency to biofuels. There is a possibility of enhancing the microbial fermentation techniques used in the synthesis of bioethanol from lignocellulosic feedstocks. The second-generation biofuels that can be produced from non-food biomass are another important research focus because feed competition has emerged as a major issue among critics of biofuel production.[25]

Government policies play an equally crucial role in advances made in biofuels as well as in the step towards a circular economy. India's biofuel policy, the National Policy on Biofuels, was launched in 2018 and lays down specific goals for biofuel production and their use. The policy requires a combination of petrol with 20% ethanol by 2025 and has given a much-needed push to the biofuels market. The government provides attractive stimuli, including subsidies, tax credits, and grants, for creating biofuels and developing bio-refineries. These initiatives create the right environment for investment and innovation in biofuel industries, extending the development area.[26] Some of the measures initiated at the state level to increase the production of biofuels from the available biomass resources are as follows. Some states provide subsidies to farmers for the delivery of agricultural residues, and others focus on developing the biofuels distribution chain. To overcome the obstacles of scaling up the biofuel technologies and supply chain management initiatives required for a sustainable economy, multiple stakeholders, such as government departments, private entities, and research organizations, need to cooperate.

Conclusion

The prospect of manufacturing biofuels in India is colossal as the country presents agricultural acreage and a rich biomass wealth. Some potential feedstocks include, but are not limited to, water hyacinth, agricultural waste, and other biomass that have great prospects in the energy sector and handling of environmental concerns. Possibly the most attractive angle on biofuels in India is that these fuels contribute greatly towards the practice of a circular economy. Agricultural residues, weeds, and organic waste biofuel can be utilized in India to change waste material into beneficial energy sources, giving a closed cycle that lessens the environmental footprint and offers an economic advantage. The conversion of waste to bioenergy results in effective resource optimization and is one of the country's greatest challenges for waste management. The production of agricultural waste helps conserve fossil fuels. It also assists in the reduction of the environmental risks from the open burning of crop residues. This has been a leading cause of air pollution, mainly in Northern India. The water hyacinth plant, which is used for water predominance and has been classified by most people as a weed, can also be used for the production of biofuels. By collecting this plant and then using it to produce bioenergy, India will have dealt with an environmental nuisance and come up with a clean energy source in the long run. This double advantage suggests that biofuels can play a significant role in the country's energy mix and contribute to the improvement of the environment at the same time.

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RESEARCH ARTICLE

Assessment of Infrared Spectral Data Availability for Carbon Chemistry Detection in Exoplanet Atmospheres

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ABSTRACT: As of February 2025, over five thousand exoplanets have been confirmed, many with carbon-containing atmospheres that remain largely unexplored. Infrared spectroscopy plays a crucial role in identifying carbon signatures. Carbon absorption signatures, key indicators of organic compounds, are prevalent largely in the near to mid-infrared range. This study aims to evaluate the factors limiting the detection of chemical signatures in exoplanetary atmospheric spectra by studying available datasets from the NASA Exoplanet Archive. A quantitative approach is utilized to assess the suitability of infrared spectra to detect and elucidate carbon chemistry in exoplanetary systems. We find that most data have limited spectral resolution, are sampled with fewer than 500 data points per micron, and are insufficient in differentiating specific carbon absorption features. Another key limitation found is the gap in data beyond five microns, largely attributed to engineering difficulties related to budget and technology maintenance. These findings highlight an exigent need for improved detectors and instruments to support the study of the chemistry of exoplanet atmospheres and life beyond Earth.

KEYWORDS: Physics and Astronomy, Atomic, Molecular, and Optical Physics, Spectral Data Processing, Exoplanet Atmospheres, Infrared Spectroscopy, Carbon Chemistry in Space.

Introduction

The field of exoplanetary discovery has witnessed a surge in recent years. As of December 2024, the total number of confirmed exoplanets has reached a remarkable 5,811.[1] The atmospheres of these extraterrestrial planets present an intriguing area of study; the composition of these astronomical bodies could uncover many mysteries. Investigations and studies can provide critical insights into planetary composition, formation, and potential habitability. Spectroscopic observations of exoplanets have revealed a diverse array of chemical compositions and processes not previously observed.[2] Organic molecules, made of carbon, are often referred to as the "molecules of life" as they are recognized as the backbone of life on Earth and are hypothesized to have potentially played a role in the emergence of life. Interestingly, organic molecules are also prevalent in exoplanetary atmospheres,[3] so their analysis can uncover vital clues about the processes that drive planetary habitability, offering valuable perspectives on the potential for life beyond Earth.

Carbon is an element that is omnipresent throughout Earth and the universe. Making up approximately 18.5% of the human body,[4] carbon is one of the few elements known since antiquity.[5] In space, these molecules tend to persist in the different forms of interstellar carbonaceous grains, such as graphite, nanodiamonds, and polycyclic aromatic hydrocarbons.[6] Having the maximum number of outer shell electrons capable of covalent bonds, carbon is known to be extremely versatile and can exist in various forms. Carbon-carbon single bonds are extremely diverse in nature, having been observed in hydrocarbons with lengths up to 1.71 angstroms.[7] On the other hand, carbon-carbon double bonds are shorter and more rigid, influencing the molecular shape and reactivity, while triple bonds are even shorter and stronger. Carbon-hydrogen bonds create a chemical species collectively known as hydrocarbons, which are prevalent in space, particularly in the polycyclic aromatic form. These molecules then go through various chemical reactions influenced by environmental conditions, creating a diverse spectrum of organic molecules.[8]

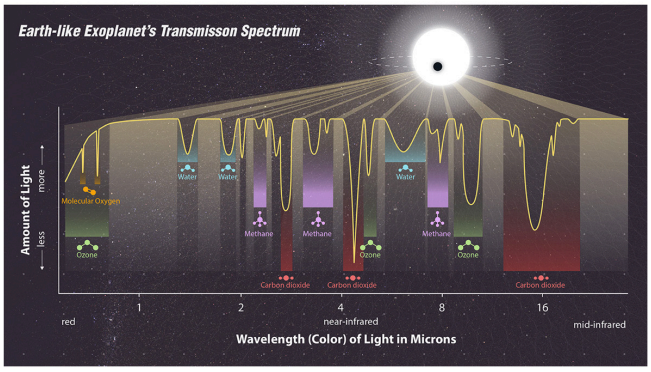

Figure 1: A simulated mid-infrared transmission spectrum of an Earth-like exoplanet as it transits its host star. The dips in the yellow curve indicate the wavelengths where molecules like oxygen, ozone, water, methane, and carbon dioxide absorb starlight. These features demonstrate how different molecules absorb starlight at specific wavelengths, allowing astronomers to identify different chemical signatures in exoplanet atmospheres. Figure credited to J. Olmsted.[9]

The measurement of such chemistries in space is not trivial. Chemistry beyond the solar system can be measured in various ways. Many modern Earth-based and satellite-based telescopes are equipped with spectrometers designed for astronomical observations. Spectrometers are instruments that can analyze the

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light emitted, absorbed, or scattered by astronomical objects, which can reveal crucial information about their composition, temperature, velocity, and more. These devices collect discrete samples of light across different wavelengths or frequencies, similar to how a prism splits white light into discrete visible base colors.[10-13] Transmission spectroscopy utilizes spectrometers to analyze the composition of an exoplanet's atmosphere by studying the starlight that passes through the exoplanet's atmosphere during a transit, which is when the planet crosses in front of its star. This process is depicted in Figure 1.

Spectral units are used to quantify and describe the properties of light: wavelength is commonly utilized as the independent variable, and the percentage of transmitted light is measured in response, which is derived from dividing the spectrum in transit by the spectrum out of transit, removing the stellar spectrum and leaving the transmission from just the exoplanet atmosphere. Such atmospheric spectral data can reveal a variety of information about the exoplanet, particularly its chemical composition, which is central to the analysis in this study. This is because spectral bands and lines emerge from the vibrational spectra and atomic structure of a molecule.[14] Figure 2 shows an example spectrum of a molecule, polysaccharide, displaying that different atomic bonds (e.g., the hydrocarbon C-H bond) can vibrate at distinct energies, which are absorbed by the spectral light, resulting in a spectral band related to each molecular fingerprint. Laboratory spectroscopy in the 1-15 micrometer range can help measure atomic spectral lines for organic molecules in astrophysical sources,[15] allowing for accurate observation of the chemical processes within an extraterrestrial atmosphere. In recent times, high-resolution spectroscopy has enabled the better detection of carbon molecules in exoplanetary atmospheres, especially using near-infrared spectroscopy.[16] Therefore, specific carbon bonds can be identified due to their difference in vibration energies, resulting in different spectral bands, which have helped scientists identify many carbon-bearing species in space.[17]

There exist numerous databases on the internet containing diverse spectral data of exoplanets; some examples include the Exoplanet Atmospheric Spectral Library (CREATES),[18] Stellar Planet Transmission Spectra Archive,[19] and the Virtual Planetary Laboratory's Spectral Database and Tools.[20] These datasets may vary widely in their characteristics, including the wavelengths covered, the number of data points, resolution, and even the type of spectrum recorded. Such variability arises because spectral data can be collected using different instruments, energy ranges, and observational techniques. NASA's Exoplanet Archive is chosen as the focus of this study due to its comprehensiveness, reliability, and integration with other space mission data, widely established as the most extensive repository of exoplanet spectra. However, there exists only a small subset of the data that is relevant to this study. Not all spectrometers are suited for the study of carbon chemistry in space, as carbon bond vibrations are primarily detectable in the infrared.

This study aims to quantitatively analyze and determine how suitable existing astronomical datasets from various infrared spectrometers are for the study of carbon chemistry in exoplanet atmospheres. Near to mid-infrared spectral data were obtained from NASA's Exoplanet Archive, loaded and processed using Python, and then classified based on suitability for the study of carbon-related vibrational bonds. The hypothesis is that spectral datasets from exoplanets are limited and not entirely targeted to the study of organic processes in exoplanet atmospheres. This quantitative study aims to identify hypothesized shortcomings of current datasets and guide future instruments of design parameters that are better suited for studies of carbon chemical processes in exoplanet atmospheres.

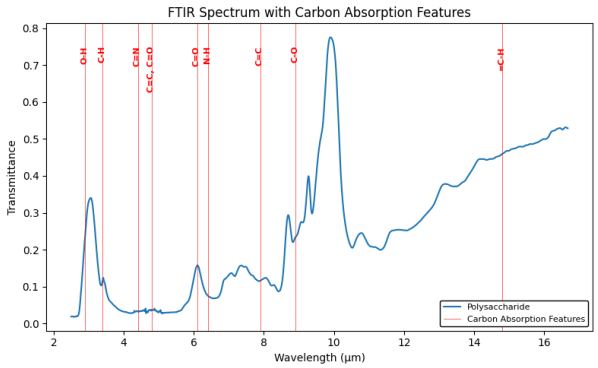

Figure 2: The infrared spectrum of polysaccharides, the most common organic structures in organic chemistry, shows light transmittance as a function of wavelength (μm). The spectrum illustrates the presence of various functional groups associated with the chemical structure of the organic species, providing insights into their chemistry and bonding characteristics. Additionally, the red vertical lines highlight the key carbon bond absorption features. Spectrum credited to Github.[21]

Methods

Data Collection:

Spectral data for the analysis were obtained from NASA's Exoplanet Archive, focusing only on exoplanets with publicly available transmission spectra within the infrared (IR) range. Only datasets containing greater than ten significant data points were considered to ensure validity. The near to mid-infrared range (1-15 μm) was selected due to its relevance in detecting chemical bonds, critical to this analysis. The absorption of IR light allows for the identification of the vibrational modes of carbon-based molecules, which are crucial in distinguishing carbon molecules present in exoplanetary atmospheres. A total of 75 spectra were collected for this study, primarily originating from instruments aboard the James Webb Space Telescope (JWST). We note that we did not consider eclipse or direct imaging datasets, but these could be considered in the future.

To confirm the completeness of the datasets, previously mentioned spectral databases like CREATES, the Stellar Planet Transmission Spectra Archive, and the Virtual Planetary Laboratory's Spectral Database were reviewed. No additional infrared spectra relevant to this study were found beyond those available in NASA's Exoplanetary Archive.

Data Loading and Processing:

The data was imported and analyzed using Python, utilizing the Astropy library, supplementing additional astronomy-specific Python packages necessary for data manipulation and analysis. The following preprocessing steps were conducted.

Data Pack Installation: Using Astropy (v6.1.6),[22-24] NumPy (v1.26.4),[25] and SciPy (v1.13.1),[26] the Specutils package (v1.19.0)27 is retrieved and installed. The NumPy library is imported for numerical computations, and the supplemental units module from the Astropy library is imported. Within the Specutils package, the Spectrum1D class is imported specifically to represent 1D spectral data.

Data Standardization: All spectra were carefully calibrated and normalized to consistent units of wavelength and flux density. Wavelengths are set in micrometers (μm) using Astropy's unit handling tools. Flux values, originally provided as transit depth in percentage, were, in this case, converted to a dimensionless form by dividing the values by 100. Transit depth represents the fractional decrease in stellar flux during the transit of a planet. This standardization allows for consistent comparison across datasets from different instruments.

Data statistical analysis:

A reference list of molecular vibrational modes relevant to carbon chemistry in the infrared range was compiled and referenced in Figure 2. These modes were identified based on each molecule's specific characteristic absorption features within the 1-15 [space]μm range. These bands are chosen based on their simplicity and possible correlations with biosignatures. Some vibrational modes include carbon-hydrogen stretching vibrations, carbon-carbon bond stretches, as well as overtones and combinations specific to organic molecules. This list of molecules we find of note includes:

1. Oxygen-Hydrogen (O-H) Stretch - 2.9 μm
2. Carbon-Hydrogen (C-H) Stretch - 3.4 μm
3. Carbon-Nitrogen (C≡N) Triple Bond Stretch - 4.4 μm
4. Carbon-Carbon (C≡C) and Carbon-Oxygen (C≡O) Triple Bond Stretch - 4.8 μm
5. Carbon-Oxygen (C=O) Double Bond Stretch - 6.1 μm
6. Nitrogen-Hydrogen (N-H) Stretch - 6.4 μm
7. Carbon-Carbon (C=C) Double Bond Stretch - 7.9 μm
8. Carbon-Oxygen (C-O) Stretch - 8.9 μm
9. Carbon-Hydrogen (=C-H) Bending Mode - 14.8 μm

This reference was derived from established molecular databases and spectroscopic literature, which are used to identify characteristic absorption features within the spectra. Accordingly, each spectrum was analyzed to identify the presence and intensity of molecular vibrational features corresponding to carbon-based molecules. We determined the number of carbon-related bands of the nine listed above that are covered by the wavelength range of each spectrum in question. Any spectrum whose wavelength range overlaps more than three distinct carbon-related vibrational bands from the reference template, contains greater than ten data points, and exceeds the range threshold of 2.9 microns is noted.

Results and Discussion

The spectral data were obtained from NASA's Exoplanet Archive and filtered as described in the previous section. Of the remaining spectra after filtering for those of interest, the only represented instruments are those aboard JWST: Near Infrared Spectrograph (NIRSpec), Near Infrared Imager and Slitless Spectrograph (NIRISS), and Near Infrared Camera (NIRCam). Filtration resulted in only 75 spectra that are viable for this study. This is only 8.23% of the 911 total available spectra within NASA's Exoplanet Archive as of December 2024.

Figure 3a involves a set of 75 spectra, only 39 exist with more than 100 data points, leaving only 52% of the subset applicable to the analysis of carbon features. The greater the resolving power of a spectrum, the more accurate, the more clearly carbon absorption features can be identified, as higher resolution allows sharper and more distinguishable spectral features. Resolving power is defined as:

$$R = \frac{\lambda}{\Delta\lambda}$$

where λ is the central wavelength and Δλ is the smallest difference in wavelength the instrument can distinguish at that wavelength. This relative measure of resolution is commonly used in spectroscopy to express how precisely an instrument can resolve fine spectral details. In this study, resolving power was estimated by computing the inverse of the median spacing between adjacent wavelength values in each spectrum. Figure 3b demonstrates a highly accurate positive linear correlation, with R^2=0.884%, between the resolving power and the number of data points present in a spectrum. Low resolving power results in the natively narrow spectral features being spread across a wider wavelength range, which results in a shallower absorption depth and more difficult line identification that may cause the signal to dip below background noise levels, or may blend the lines together and make their identification less certain. These carbon vibration bands have many sharp and localized lines at specific wavelengths, making high resolving power optimal for reliable identification. Ultimately, while NASA's exoplanet database includes promising datasets for the study of carbon chemistry, only 39 filtered datasets with high resolution, which lies only a small percentage of the overarching dataset with 911 accessible spectra. This shows only 4.28% of the total datasets are of relevance to the detection of

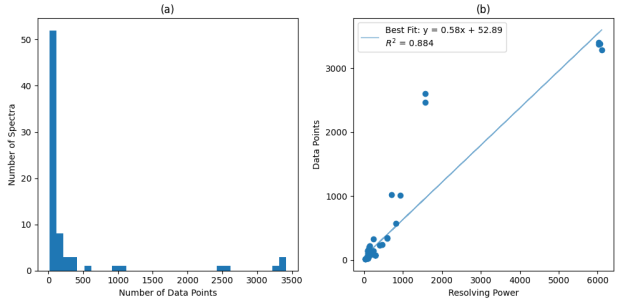

Figure 3: (a) Histogram depicting the distribution of the data points across the dataset, showing that the majority of spectra contain fewer than 500 data points. (b) Scatter plot demonstrating the positive correlation between the resolving power of each spectrum (wavelength/change in wavelength) and the number of data points, with a linear regression fit (R^2=0.884) indicating a relatively strong relationship. The high correlation emphasizes the

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dependence of spectral resolving power on the quantity of data points, which is crucial for accurate carbon feature identification.

carbon chemistry in exoplanet atmospheres, exemplifying the constraints in current instrumentation.

The NASA Exoplanet Archive comprises data over various energy ranges, and this study focuses on the near to mid-infrared range due to the vibration of carbon bonds. Figure 4 presents a power distribution of the percentage of datasets containing bands in the 2-15 μm wavelength range. The higher the wavelength, the lower the power; therefore, the fewer the spectra containing such power range. The green dotted line represents a cumulative histogram, highlighting a few significant drops in data. By the wavelength of 2.7 microns, approximately 13.33% of the data have been excluded. The most significant drop in the number of datasets is at 5 microns, with only 8.00% remaining. Most carbon bands are outside the range of detection, which emphasizes the exclusiveness of the database, especially for studies based on carbon-bond detection. There are ten useful carbon bands within the 2-15 μm range of detection; these includes the O-H band at 2.9 μm, the C-H band at 3.4 μm, C≡N at 4.4 μm, C≡C and C≡O at 4.8 μm, C=O at 6.1 μm, N-H at 6.4 μm, C=C at 7.9 μm, C-O at 8.9 μm, and =C-H at 14.8 μm. The carbon absorption features represented in red demonstrate the specific wavelength ranges in the electromagnetic spectrum that correspond to a specific vibrational mode of a molecular bond. This is crucial because these bonds are key components of complex organic molecules found in interstellar environments. Additionally, the band with the lowest absorption feature is the O-H band, occurring at approximately 2.9 microns. The cumulative histogram reveals that 21.33% of the spectra do not exceed the wavelength of 2.9

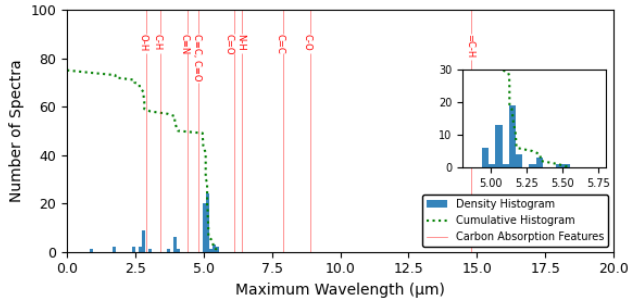

Figure 4: Density histogram of the maximum wavelength of each spectrum, overlaid with a cumulative histogram to illustrate the distribution of spectral coverage. Results indicate that the majority of the spectra are exclusive of wavelengths greater than 5.75 μm. An inset zooms in on the region containing the majority of the data for greater clarity. Red vertical lines indicate key carbon bond absorption features, highlighting the spectral regions relevant to carbon chemistry analysis.[20]

microns, and are thus useless for carbon band detection.

Based on absorption feature coverage, resolution, and wavelength range, the top datasets from NASA's Exoplanet Archive of Atmospheric Spectra include K2-18b,[28] LHS 475 b,[29] and WASP-96 b.[30] These data all originate from NASA's 6.5-meter JWST, in 2023, though the instrument utilized differs. Therefore, it can be inferred that datasets most suitable for the measurement of carbon chemistry in space originate from more recent studies, especially those from the JWST facility, due to its highly advanced and specific instruments designed for high-sensitivity infrared observations, enabling the detection of these features in unprecedented detail. Studies in more recent times are becoming more promising for carbon band detection in exoplanet atmospheres due to new space missions, improved telescopes, and new spectroscopy techniques. The JWST has had propitious breakthroughs in its infrared sensitivity with unprecedented resolutions through its Mid-Infrared Instrument (MIRI) and NIRSpec.[31] The detection of these bands will allow for the analysis and modeling of organic molecules in space. Finally, the limited range of carbon bonds detectable within the energy range offered by the NASA Exoplanet Archive demonstrates its finite relevance to detecting carbon chemistry. We propose that the ideal wavelength range for carbon detection is between 2.9 microns extending past 8.9 microns, which would encompass the spectral features from the bonds considered above. The data presented is sufficient to confirm detections of carbon in exoplanetary environments, but inadequate for the detection of specific carbon species. This is due to the limited resolution of a spectrum. While most of the spectra contain enough data points to reveal the presence of carbon species through the identification of peaks, they often lack the resolving power necessary to distinguish between closely spaced or overlapping spectral features. For example, the detection of two peaks with nearby peak wavelengths is detectable with a relatively lower resolving power. The N-H and C=O features, located at 6.4 and 6.1, respectively, are separated by 0.3 microns. Using the resolving power formula introduced earlier, this yields a required resolving power of approximately R=21 to distinguish them. On the other hand, the detection of carbon features that are virtually overlapping would require a resolving power of greater than R=1,000 to be distinguished accurately, like that of the C=C and C=O bonds. However, not all instruments considered in this study meet this threshold. The NIRISS/WFSS and NIRSpec/PRISM instruments, for example, have relatively low resolving powers of approximately 150 and 700, respectively. In contrast, instruments such as NIRSpec/M, NIRSpec/H, and NIRCam/WFSS offers significantly higher resolving powers of around 1000, 2700, and 1120-1680, respectively.[32] Datasets best suited for the measurement of carbon chemistry would have high resolving power to distinguish closely spaced emission lines, broad spectral range coverage to capture an adequate number of carbon bands, and a high signal-to-noise (SNR) ratio to ensure reliable detection of weak signals.

Ultimately, this research is limited in scope due to its sole coverage of NASA's Exoplanet Archive. Filtering the dataset according to the criteria listed in the Methods section XX results in a small number of spectra out of the full sample, showing the most datasets do not cover the ideal wavelength range we proposed for analyzing carbon chemistry. The required spectral data properties for detecting carbon species include broad wavelength ranges, high SNR, and high resolution. Achieving these data properties remains a significant challenge due to a gap in current instrumentation. The gap between ideal and current detection conditions highlights the

strategies to enrich our current understanding of extrasolar organic molecules. It is crucial to recognize these limitations to underscore the importance of interdisciplinary efforts to refine observational techniques, expand coverage, and improve modeling approaches to ameliorate future research in extrasolar carbon chemistry.

A hypothesis for the limitation of data greater than five microns is mission and telescope constraints. Many telescopes do not have instruments with mid-IR capabilities. Missions may choose to focus on the near-infrared range, as these shorter wavelengths can significantly improve the accuracy and precision of estimations and measurements of data.[33] For example, the Spitzer Space Telescope originally had mid-IR capabilities before its coolant ran out, causing it to operate only in the near-IR range.[34] Observing with MIR instruments results in large data volumes due to the nature of the detector readout, which also requires intense computing capabilities and sensitivity to thermal background noise, which then requires intensive cooling. All of these add complexity to operating in the mid-IR. Moreover, another reason for this gap is instrumental limitations. High spectral resolution has not yet been achieved; sending high-resolution spectrometers (R>10,000) into space is not yet possible due to payload weight limitations, and thus these instruments are currently limited to ground-based telescopes. Earth's atmosphere absorbs infrared radiation beyond 5 microns due to the presence of water vapor, carbon dioxide, and other gases, a phenomenon known as telluric absorption.[35] This significantly hinders the ability of ground-based telescopes to observe in the MIR, where even locations at high altitudes or dry sites leave deep absorption features that obscure the spectral signals of interest. Another possible reason for this limitation is the relationship between the SNR and the detection wavelength.[36] As the detection wavelengths increase, the SNR decreases due to thermal radiation from telescopes, instruments, and even interstellar dust. It is difficult to build and maintain a high-resolution detector due to engineering challenges, heat and thermal noise during data collection, large data volumes and processing power, as well as cost and material constraints. Ultimately, understanding these limitations is crucial for improving observational strategies and instrument design. Potential solutions to bridging this gap include further advancements in space-based infrared telescopes, enhanced cooling mechanisms, and higher-sensitivity detectors. Future missions like the Habitable Planets Observatory,[37] the Large Ultraviolet Optical Infrared Surveyor,[38] the Origins Space Telescope,[39] and next-generation technologies will play a crucial role in expanding our ability to study carbon chemistry in extrasolar environments, allowing us to enhance our understanding of molecular processes and the identification of complex organic compounds in exoplanetary atmospheres.[40]

Conclusion

We reviewed the detectability of carbon species in exoplanet transmission spectra by comparing current instrument capabilities to the wavelength coverage and sampling needed. Although SNR is a critical factor in spectral analysis, its impact was not directly investigated in this study. Ultimately, the majority of spectra available on the NASA exoplanet archive are not of relevance to carbon band detection due to two main constraints: limited wavelength range and low resolving power. We discuss ideal conditions for carbon detection, which would encompass broad and optimal wavelength ranges, high SNR, resolution, and detector sensitivity. Such conditions are arduous due to engineering challenges and budget constraints. Nonetheless, bridging this gap in our detection of carbon-chemistry and its analysis in exoplanets are acute as more and more exoplanets are being detected and analyzed. This study provides valuable insights for the advancement of future spectral detectors, as well as aiding future exploration of extraterrestrial carbon chemical processes. Studying the carbon chemistry in these interstellar environments will allow us to enhance our understanding of Earth-like worlds beyond as well as our own.

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RESEARCH ARTICLE

Antimicrobial-Resistant Bacteria and Strategies to Overcome Antimicrobial Resistance

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ABSTRACT: Antibiotic resistance is an escalating global health crisis that threatens to undo the remarkable advancements made in modern medicine. The overuse and misuse of antibiotics have led to the evolution of resistant bacterial strains, which are increasingly difficult to treat. This paper explores the mechanisms behind antibiotic resistance, categorizing it into intrinsic and acquired resistance and discussing key resistant bacteria such as Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococci, and Carbapenem-resistant *Enterobacteriaceae*. This paper also inspects methods to combat antibiotic resistance, including antibiotic modification, combination therapy, the use of adjuvants, and the innovative use of bacteriophages. Finally, the potential for emerging technologies to address the crisis, such as CRISPR-Cas systems and antimicrobial peptides, is highlighted. Continued research and the development of new strategies are vital to overcoming the challenges posed by antibiotic-resistant bacterial infections and are required to safeguard the health of the public.

KEYWORDS: Microbiology, Antimicrobials and Antibiotics, Antibiotic Resistance, Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococci, Carbapenem-resistant *Enterobacteriaceae*.

Introduction

Bacterial infections remain a major global health concern, contributing significantly to morbidity and mortality worldwide. Each year, millions of deaths are attributed to bacterial infections, with lower respiratory infections and bloodstream infections contributing to the majority. The causative agents of these infections include a wide variety of bacteria, such as *Streptococcus pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*, each of which poses unique treatment challenges due to varying resistance patterns.[1]

The discovery of antibiotics in the early 20th century by Sir Alexander Fleming revolutionized medicine; his discovery of penicillin marked the beginning of the antibiotic era, transforming the treatment of bacterial infections that were once fatal. This breakthrough drastically reduced the mortality rates of infections, leading to significant improvements in public health and an increase in life expectancy.[2] Antibiotics, which target specific processes in bacterial cells,[3] have been vital in treating a wide range of bacterial infections. They have additionally allowed for many modern medical procedures such as cancer treatment, organ transplants, and open-heart surgery.[4]

However, the widespread and sometimes inappropriate use of antibiotics has led to a critical problem: antibiotic resistance.[4] Over time, bacteria evolve mechanisms to bypass the effects of antibiotics, rendering them ineffective. This phenomenon is driven by both intrinsic genetic factors and acquired resistance.[5]

Infections caused by multidrug-resistant organisms, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), and Carbapenem-resistant *Enterobacteriaceae* (CRE), have become more common, complicating treatment options and increasing mortality rates. These "superbugs" are resistant to multiple classes of antibiotics, making them particularly difficult to treat and posing a serious threat to public health.[6]

To address this growing crisis, there is an urgent need for innovative strategies to reduce the impact of antibiotic resistance and protect the efficacy of current and future antibiotics.[7] The current article focuses on antibiotic resistance, antibiotic-resistant bacteria, and different strategies to overcome antibiotic resistance.

Antibiotics:

Antibiotics are powerful medications used to treat bacterial infections by inhibiting the growth of or killing bacteria. Discovered in the early 20th century, they revolutionized medicine and consequently significantly extended the average human lifespan by over 23 years. The discovery of penicillin by Sir Alexander Fleming marked the beginning of the golden age of antibiotic discovery, which peaked in the mid-1950s. This age was followed by a decline in the discovery and development of new antibiotics, and, by 1962, all major classes of antibiotics had been discovered.[2] This led to the rise of antibiotic resistance in numerous bacterial pathogens, resulting in the current pressing crisis of antibiotic resistance.[4]

Bacterial cells have unique structures that aid in their ability to survive and reproduce. The components of a bacterial cell (present in most but not all) include a cell wall made of peptidoglycan, a compact and flexible cell membrane, a cytoplasm containing ribosomes and plasmids, a nucleoid usually consisting of a single chromosome, polysaccharide or polypeptide capsules that help the bacteria adhere to surfaces and escape the immune system of a body, pili, and a flagellum.[8] Bacterial cells act in multiple ways: they can multiply rapidly, overwhelming host tissues and disrupting normal function;

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kill cells and tissues; or secrete toxins that can paralyze and destroy cells' metabolic machinery, among many other methods of action.[9] Antibiotics take advantage of the bacterial cell's components and features, limiting or inhibiting its functions to effectively kill or hinder the cell.[3]

Antibiotics are classified into largely 5 types based on their mechanism of action.[10] Figure 1 illustrates 5 classes of antibiotics.

Cell Wall Synthesis Inhibitors:

The β-lactam antibiotics are a family of bactericidal drugs containing the β-lactam ring in their chemical structure. Classified in penicillins, cephalosporins, carbapenems, penems (also known as thiopenems), and monobactams, they are amongst the most commonly prescribed drugs.[11]

β-lactam antibiotics work by inhibiting penicillin-binding proteins (PBPs), enzymes that are vital to forming cross-links in the peptidoglycan layer of bacterial cell walls and ensuring cell wall stability. Their similarity to the peptidoglycan precursor (D-Ala-D-Ala) allows them to bind to PBPs and disrupt cell wall synthesis. This binding forms a stable enzyme–antibiotic complex that inactivates the enzyme and prevents the formation of the cell wall, leading to bacterial death.[12]

Glycopeptide antibiotics, like vancomycin, work by targeting lipid II, a molecule used to synthesize the cell wall in Gram-positive bacteria. Vancomycin binds to the D-Ala-D-Ala sequence of lipid II, furthermore blocking the action of PBPs. This prevents the proper formation of the cell wall, leading to bacterial cell death due to osmotic shock.[13]

Cell Membrane Integrity Disruptors:

Cyclic lipopeptide antibiotics such as daptomycin work by binding to the bacterial membrane in the presence of calcium ions. The Ca^{2+}–daptomycin complex forms micelles that penetrate the inner membrane, bind to negatively charged phosphatidylglycerol groups, and neutralize them. The Ca^{2+}–daptomycin complex is then inserted into the membrane and undergoes phosphatidylglycerol-dependent oligomerization. This causes the leakage of ions, mainly potassium and sodium, disrupting the cell's membrane function and ultimately leading to bacterial cell death.[10]

Polymyxins, such as colistin, primarily target the outer membrane of Gram-negative bacteria. They interact with lipid A, a component of lipopolysaccharide, displacing stabilizing ions and increasing membrane permeability. This allows the antibiotic to enter the cell and also causes damage to the inner membrane.[14] Polymyxins can cause the fusion of the inner and outer membranes. This promotes the exchange of phospholipids between the membranes, furthermore causing osmotic imbalance and cell lysis.[10]

Nucleic Acid Synthesis Inhibitors:

Quinolones work by targeting DNA gyrase and topoisomerase IV enzymes in bacteria. These enzymes regulate the topological state of DNA during replication and transcription by removing the accumulated positive supercoils. DNA gyrase maintains negative supercoils, while topoisomerase IV helps unlink newly synthesized DNA. Quinolones bind to the topoisomerase-DNA cleavage complex, stabilizing the DNA break and preventing the replication fork from moving.[15] This blockage halts DNA synthesis, preventing bacterial cell division and leading to cell death.[10]

Rifamycins inhibit bacterial RNA synthesis. The presence of a macrocyclic ring in its structure targets the β-subunit of prokaryotic DNA-dependent RNA polymerase near its catalytic center, blocking its ability to initiate transcription.[10] This inhibition prevents the production of RNA and, subsequently, protein synthesis, which results in bacterial cell death.[10]

Protein Synthesis Inhibitors Protein Synthesis Inhibitors:

Tetracyclines and aminoglycosides, along with other antibiotic classes such as macrolides, lincosamides, streptogramins B, and oxazolidinones, work by targeting bacterial protein synthesis.[10] These antibiotics specifically target bacterial, prokaryotic ribosomes, which are structurally different from eukaryotic ribosomes in terms of size, structure, and the number of RNA molecules they contain. This ensures no harm to human cells.[10]

The structure of Tetracyclines consists of four flat aromatic hydrocarbon rings, and they work by binding to the 30S subunit of the bacterial ribosome. This binding prevents the transfer of amino acids during protein synthesis by blocking the attachment of aminoacyl-tRNA to the A-site of the ribosome. As a result, the bacterial cell is unable to build the proteins it needs to survive.[16]

Aminoglycosides, such as gentamicin and amikacin, also bind to the 30S subunit of bacterial ribosomes. This binding leads to the misreading of mRNA, causing the wrong amino acids to be incorporated into the protein chain. This results in faulty proteins which can disrupt the function of bacterial membranes, allowing more aminoglycosides to enter the cell and kill the bacteria. Some aminoglycosides also inhibit the formation of the initiation complex or block the movement of tRNA during protein synthesis.[10]

Metabolic Pathway Disruptors:

Sulfonamides, like sulfamethoxazole, are bacteriostatic antibiotics that inhibit bacterial growth by interfering with folic acid synthesis, a substance necessary for the synthesis of nucleic acids and proteins. They act by competing with para-aminobenzoic acid (PABA) for the active site of the enzyme dihydropteroate synthase (DHPS). DHPS is involved in converting PABA to dihydropteric acid, a precursor to folic acid.[17]

Trimethoprim also disrupts folic acid synthesis by inhibiting Dihydrofolate reductase (DHFR). DHFR converts dihydrofolate to tetrahydrofolate, an essential cofactor for nucleotide synthesis. By blocking this conversion, trimethoprim disrupts DNA and protein synthesis in bacteria.[18]

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Antibiotic Resistance:

Antibiotic resistance is a phenomenon that occurs when microorganisms evolve to resist the effects of medications designed to kill or inhibit them. This phenomenon arises from the overuse and misuse of antibiotics, both in human medicine and agriculture, leading to the emergence of "superbugs"–microorganisms, particularly bacteria, that have developed resistance to multiple antibiotics–that are harder to treat.[19] A growing health concern, most organizations consider this a serious problem.[20] However, despite the billions of dollars that have been put into research and solutions, the development of antibiotic resistance has proven to be relentless.[21] Antibiotic resistance falls largely into two categories: intrinsic resistance and acquired resistance.[22]

Intrinsic Resistance:

Intrinsic resistance is a natural ability found in certain bacteria that allows them to withstand the effects of antibiotics. This resistance is due to the bacteria's genetic makeup and exists independently of any exposure to antibiotics. Gram-negative bacteria exhibit a multidrug-resistant phenotype,[23] attributed to the presence of an additional outer membrane rich in lipopolysaccharides in their cell wall[13] that acts as a barrier and prevents many antibiotics from entering the cell while simultaneously aiding their ability to acquire resistance mechanisms from their surroundings.[23] Additionally, gram-negative bacteria have efflux pumps, which can actively push antibiotics out from inside their cells, making these drugs less effective.[24] Gram-positive bacteria, as well, often possess certain features that contribute to intrinsic resistance. Enterococci, for example, can exhibit resistance to penicillin through the overproduction of a low-affinity penicillin-binding protein (PBP5), which is capable of maintaining cell wall synthesis despite antibiotic pressure. In Enterococcus faecium, specific amino acid substitutions in PBP5 further reduce penicillin affinity, enhancing resistance to β-lactam antibiotics such as ampicillin.[25]

There is significant concern regarding intrinsic resistance. As infections caused by these resistant bacteria rise, treatment options become limited, leading to challenges in managing what were once treatable infections. This situation is especially significant due to the increasing frequency of infections caused by Gram-negative pathogens, which can be particularly hard to treat.[24] Environmental bacteria, such as those found in soil, are intrinsically resistant to many classes of antibiotics, with resistance that predates the clinical use of antibiotics. While these bacteria may not be direct threats, they carry along with them the added risk of transferring their resistant traits to pathogens, which further complicates the fight against antibiotic resistance.[24]

Acquired Resistance:

Acquired resistance refers to the ability of bacteria to develop resistance to antibiotics that were previously effective against them, typically through genetic mutations or the acquisition of resistance genes from other microorganisms. This can happen through selection pressure that causes mutations that give certain bacteria an edge in survival or through horizontal gene transfer (HGT). Additionally, the overuse and misuse of antibiotics in healthcare and agriculture create pressure that favors the growth of resistant strains. As a result, acquired resistance is a growing concern, especially for treating infections caused by multidrug-resistant Gram-negative bacteria, making effective treatment increasingly challenging.[26]

HGT is one method of acquired resistance in which bacteria can share genetic material with one another and, therefore, adopt each other's traits and abilities. This was first demonstrated by Frederick Griffith in 1928 when he discovered that harmless pneumococcus bacteria could become dangerous by taking up DNA from virulent strains. There are a few key ways this happens: transformation, where bacteria absorb free DNA from their surroundings; conjugation, which involves a one-way transfer of DNA through a structure called a sexual pilus; and transduction, where viruses that infect bacteria move DNA between cells. Once inside, this new DNA can either be degraded, remain as independent pieces, or integrate into the host's genome, furthermore possibly changing the host's characteristics.[27]

Along with HGT, there are a multitude of methods by which bacteria can acquire resistance. Bacteria can produce enzymes that degrade or chemically modify antibiotics, rendering them ineffective.[28] They can alter the target sites of antibiotics, preventing effective binding, which can allow the bacteria to develop certain resistance mutations.[28] The presence of antibiotics additionally creates a selective pressure, encouraging mutation as well as favoring the survival of resistant strains.[28]

Antibiotic-Resistant Bacteria:

Antibiotic resistance has become a challenging problem in the past few decades.[29] There are hundreds of different antibiotic-resistant bacteria today.[21] The current review article aims to focus on the most common antibiotic-resistant bacteria, namely Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE), and Carbapenem-Resistant *Enterobacteriaceae* (CRE). There are certain genes, either acquired or intrinsic, that allow for antibiotic resistance. The resistance can escalate to a serious threat, especially as a single gene is often able to render a multitude of different types of antibiotics useless against certain bacteria.[6] Prolonged hospital stays, higher treatment costs, and greater mortality rates are common consequences of infections involving antibiotic-resistant bacteria, highlighting the urgent need for improved infection control practices, careful use of antibiotics, and the development of new treatments to manage these dangerous infections.[20]

Methicillin-resistant Staphylococcus aureus (MRSA):

The Gram-positive Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat in healthcare settings due to its ability to resist β-lactam antibiotics. This resistance is mainly due to the mecA gene, which encodes the PBP2a protein that allows cell wall synthesis to continue even in the presence of β-lactams.[30] The *mecA* gene is located on the staphylococcal cassette chromosome (SCCmec),[31] facilitating horizontal gene

transfer between different bacterial strains. MRSA is naturally found, asymptomatically, on the skin, skin glands, and mucous membranes, including the nose and gut. However, when it breaches these barriers, it can cause a range of infections, from mild skin conditions to life-threatening conditions like pneumonia and bloodstream infections. Its ability to evolve further complicates treatment, with some MRSA strains acquiring additional resistance to other antibiotics. As a result, MRSA infections require alternative therapies, often involving the use of non-β-lactam antibiotics or combination treatments.[30]

Vancomycin-resistant Enterococci (VRE):

Out of the fifty-eight *Enterococcus* species that have been described to date, *Enterococcus faecalis* and *Enterococcus faecium* are responsible for the majority of human infections. Frequently found as normal members of the gastrointestinal microbiota, they sometimes become opportunistic pathogens, especially in critically ill and immunocompromised patients. They are known to cause a variety of infections, including skin and soft tissue infections, urinary tract infections, and bloodstream infections.[32]

The Gram-positive Vancomycin-resistant *Enterococci* (VRE) develop resistance through genetic changes, specifically by obtaining *vanA* and *vanB* genes. These genes alter the bacterial cell wall's peptidoglycan layer, which vancomycin typically targets to prevent bacterial growth. The *vanA* gene results in high-level resistance by replacing D-alanine-D-alanine,[30] a dipeptide located at the terminus of the pentapeptide stem in peptidoglycan precursors,[33] with D-alanine-D-lactate, a change that prevents vancomycin from binding. The *vanB* gene also alters the peptidoglycan, though its effects are more variable. Due to these genetic modifications, VRE can resist vancomycin's ability to disrupt their cell walls, furthermore making them much harder to treat. VRE are often found in the gastrointestinal tract and can spread to cause severe infections, especially in hospital settings.[32]

Carbapenem-resistant Enterobacteriaceae (CRE):

The Gram-negative Enterobacteriaceae, which includes common bacteria like *E. coli* and *K. pneumoniae*, have developed resistance to carbapenem antibiotics through several mechanisms, furthermore making them difficult to treat. The primary method is the production of carbapenemase enzymes, such as KPC, MBLs, and OXA-48-like enzymes, which degrade carbapenems and prevent them from working.[34] These enzymes are often plasmid-mediated, so they can transfer between bacteria and therefore spread resistance.[35] In addition to carbapenemase production, these bacteria use efflux pumps that actively remove antibiotics from the bacterial cell, and they may alter porin proteins, which act as entry points for the drugs. When these changes occur together, bacteria become resistant to multiple drugs, complicating treatment options. Efflux pump systems such as AcrAB-TolC, which is particularly common in *E. coli*, and CusABC both contribute to multidrug resistance.[36] Changes in porin synthesis or expression further block the ability of carbapenems to reach their targets inside the bacterial cell. This mechanism is particularly evident in *K. pneumoniae*, where carbapenem resistance is linked to both the production of carbapenemases and altered porins. While these resistance mechanisms do not typically spread easily in the community, hospital conditions are ideal for these bacteria to reproduce rapidly. The growing prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) is a major concern for public health.[36]

Strategies to Overcome Antibiotic Resistance:

Antibiotic resistance is directly linked to antibiotic consumption, which has been steadily increasing since the discovery of antibiotics. In terms of DDD (defined daily doses), global antibiotic consumption increased by 65% in a span of 15 years between 2000 and 2015 (21.1–34.8 billion DDDs), while the rate of consumption increased by 39% (11.3–15.7 DDDs per 1,000 inhabitants per day).[37] Reducing consumption is, therefore, the easiest, cheapest, and most effective method to prevent the outbreak of new resistant strains. The COVID-19 pandemic has additionally contributed to the increase in consumption of antibiotics. Despite COVID-19 being viral, many patients were treated for bacterial infections, causing overuse and misuse of antibiotics.[38] Secondary infections were a common consequence of the virus, and due to healthcare systems being overwhelmed by the pandemic, the infection control measures were relatively weak.[38] Extended ICU stays and delays in accurate diagnostics furthered the spread of resistant bacteria.[38]

New drug development is extremely expensive,[39] and not always possible, so the following strategies are commonly used or under research and have the potential to be commonly used in the future instead.

Researchers have been exploring and have found many promising approaches to combating the antibiotic resistance crisis. 19 promising approaches have been identified, 10 of which show good clinical potential.[40] These include targeting quorum sensing, a bacterial communication system, antimicrobial peptides, which disrupt bacterial membranes, among others.[41] CRISPR-Cas systems have also been experimented with, and they hold substantial promise in the fight against antimicrobial resistance.[42] It operates by guiding Cas nucleases to specific resistance genes, either on plasmids or chromosomal DNA, thereby inactivating or eliminating them. Studies have demonstrated the successful use of CRISPR-Cas9 to remove resistance genes such as *mecA*, *blaNDM*, and *mcr-1*, resensitizing pathogens like *E. coli*, *K. pneumoniae*, and *S. aureus* to last-line antibiotics.[43] The most common strategies employed are described as follows:

Structural Modifications:

One way to combat antibiotic resistance is to structurally modify currently available antibiotics to make them more effective against resistant strains. Research has been conducted to modify the peripheral structure of the antibiotic Vancomycin, which includes the resistant strain Vancomycin-Resistant Enterococcus (VRE).[41] These modifications have been conducted on the binding pocket and have additionally induced bacterial cell membrane permeability. Due to these changes, Vancomycin is now 6000 times more potent against VRE.[41]

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Combination Therapy:

Two or more different antibiotics can also be used in combination to increase their efficacy. Combination therapy, albeit dangerous due to the possibility of interaction between the drugs, is a common and necessary practice. The therapy is key to many cancer and HIV treatments and is used almost exclusively for the treatment of Mycobacterium tuberculosis infections, with combinations of up to four typical drugs. Combination therapy can be chosen to (1) inhibit targets in different pathways, like the combination of isoniazid, rifampicin, ethambutol, and pyrazinamide that is used to treat M. tuberculosis infections, (2) inhibit different targets in the same pathway, like the combination of sulfamethoxazole and trimethoprim that inhibits successive steps in the folic acid biosynthetic pathway (3) inhibit the same target with multiple drugs with, for example, streptogramins. Another example of inhibiting different targets in the same pathway is tunicamycin, which inhibits teichoic acid synthesis and works synergistically with β-lactam antibiotics to significantly lower the concentration needed for bacterial inhibition. Similarly, ticlopidine enhances the effectiveness of cefuroxime,[44] an antibiotic frequently utilized for empirical therapy in community-acquired infections,[45] by disrupting specific bacterial processes. Other inhibitors of early cell wall synthesis, such as fosfomycin and vancomycin, also exhibit synergy with β-lactams. These combinations reduce resistance and improve efficacy, showing great potential in overcoming resistant bacterial strains.[44]

Adjuvants:

Adjuvants are substances that, when administered in conjunction with vaccine antigens, enhance the immune response to the antigen by increasing its immunogenicity.[46] Adjuvants can also be used to enhance the effectiveness of antibiotics without directly killing bacteria. A well-known example is Augmentin®, which combines amoxicillin with clavulanic acid to inhibit β-lactamase enzymes that degrade the antibiotic. Additionally, avibactam, when combined with ceftazidime, significantly boosts its effectiveness against *P. aeruginosa* by inhibiting AmpC β-lactamase.[41] Researchers are also repurposing non-antibiotic drugs, such as antihistamines and anti-inflammatory agents, which have shown potential to enhance antibiotic activity against resistant bacteria like MRSA.[41]

Bacteriophages:

Bacteriophages, also known as phages, are viruses that target bacteria. They are also being developed, with treatments like Phico Therapeutics' SASPject™ showing rapid bacterial destruction without harming normal flora.[41] Phages have even been seen used in combination with antibiotics. The concept of "phage-antibiotic synergy" (PAS), coined by Comeau *et al.* in 2007, refers to the enhanced effectiveness of phages when combined with sublethal concentrations of antibiotics.[47] Studies have shown that antibiotics, such as β-lactams and quinolones, increase phage plaque size and replication efficiency by reducing the latent period and enhancing phage adsorption.[47] PAS has been observed in various bacteria, including Pseudomonas aeruginosa and *Escherichia coli*. This synergy can be particularly useful in treating drug- and phage-resistant bacteria. PAS has proven effective in reducing bacterial density and virulence factor production, even in resistant strains.[47] For example, a combination of ciprofloxacin and phage *ECA2* reduced *E. coli* colony counts significantly,[48] while phage-antibiotic combinations have been shown to prevent phage resistance in *P. aeruginosa*.[49] However, interactions between phages and antibiotics can vary, with some combinations showing antagonistic effects, such as when rifampicin inhibits phage replication.[47]

■ Conclusion

Antibiotic resistance represents a significant and escalating global challenge, posing serious risks to public health, healthcare systems, and economies. Strains such as MRSA, VRE, and CRE have developed mechanisms to resist conventional antibiotic treatments, complicating the management of infections. These bacteria evolve through genetic mutations that enable them to survive despite the presence of antibiotics. While traditional treatments are increasingly ineffective, innovative solutions such as drug combinations, modifications to existing antibiotics, and emerging therapies like bacteriophages and CRISPR-Cas offer promising alternatives. CRISPR-Cas especially stands out for its remarkable precision and transformative potential. Though it remains under development, it holds significant promise for future applications. These strategies have the potential to restore the effectiveness of antibiotics.

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