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Exploring Nasal Secretion Levels of Amyloid Beta 42/40 and Interleukin-6 as Diagnostic Biomarkers for Alzheimer's Disease

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ABSTRACT: Alzheimer's disease (AD) presents a significant global health challenge, affecting over 6 million individuals. This underscores the urgent need for early detection to improve treatment effectiveness and disease management. AD is characterized by the buildup of amyloid beta (Aβ) plaques and neurofibrillary tangles, resulting in progressive and irreversible neuronal degeneration. However, current diagnostic methods, like imaging scans, are often inaccessible and costly, limiting their usefulness for early detection. This study investigates the potential of nasal secretion analysis as a non-invasive diagnostic method for AD. Using Bicinchoninic acid (BCA) and Enzyme-Linked Immunosorbent Assay (ELISA) assays, we examined protein levels, focusing mainly on amyloid beta 42/40 (Aβ 42/40) and Interleukin-6 (IL-6) in nasal secretion samples. Blood and brain samples were also collected for comparison, providing insights into systemic biomarkers reflecting overall pathological processes and direct examination of AD-related neuropathology, respectively. Our findings highlight the promise of nasal secretion analysis as a diagnostic tool for AD, emphasizing the importance of Aβ 42/40 and IL-6. ELISA results revealed increased levels of Aβ 42/40 in nasal secretions from AD mice, exhibiting an average OD450 signal of 0.496, in contrast to healthy cohorts where the signal was 0.4895 for young mice and 0.46 for aged mice. Additionally, elevated levels of IL-6 were identified in nasal secretions of AD mice, averaging 0.1395 pg/mL, compared to healthy cohorts, where young mice averaged 0.0998 pg/mL and aged mice had 0.11 pg/mL. This underscores IL-6's potential as a biomarker for AD pathology and contributes to understanding its dynamics in AD. Moreover, the study's BCA assay results indicated a concentration of proteins in the fluid, expanding the potential for future research to discover more protein biomarkers. This study aims to facilitate further research to validate the diagnostic accuracy of nasal secretion analysis and its comparative effectiveness with existing methods. These advancements hold promise for early AD detection and improved patient outcomes.

KEYWORDS: Neuroscience; Alzheimer's Disease; Nasal Secretion; Interleukin-6; Amyloid Beta 42/40.

Introduction

AD currently impacts over 6 million people in the United States, with projections estimating a rise to 14 million by 2060. This type of dementia is a progressive neurological condition marked by the buildup of beta-amyloid plaques and neurofibrillary tangles in the brain. Beta-amyloid plaques consist of amyloid-beta peptides, which tend to aggregate outside neurons, while neurofibrillary tangles contain hyperphosphorylated tau protein, accumulating within neurons. These changes in the brain's structure lead to the loss of synapses and neurons, ultimately causing cognitive decline and dementia. Moreover, AD involves inflammation, oxidative stress, and further neurodegeneration, contributing to its intricate and multifaceted development. Current diagnostic modalities for this neurodegenerative disorder encompass a suite of advanced imaging techniques, notably Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET) scans. Additionally, refinements in PET imaging, particularly amyloid/tau PET scans, have significantly enhanced diagnostic precision. However, notwithstanding their diagnostic utility, these imaging methodologies present inherent challenges, predominantly in terms of cost and accessibility, particularly in resource-constrained environments. Furthermore, their efficacy in detecting the prodromal stages of Alzheimer's disease is limited. For instance, while MRI and CT scans may reveal structural alterations indicative of Alzheimer's pathology, their sensitivity in early-stage detection is compromised, often failing to capture the disease until considerable neuronal degeneration has transpired.[1]

The necessity for novel, earlier diagnostic methods has led to the emergence of identifying and utilizing biomarkers. These biomarkers provide vital insights into the underlying pathophysiological mechanisms of various neurological conditions, including Alzheimer's disease (AD). These biomarkers, such as Aβ42 and IL-6, serve as pivotal indicators for the early diagnosis of AD, often preceding the onset of overt clinical symptoms.[2] In the context of this study, particular attention is directed towards these biomarkers. The Aβ42/40 ratio is acknowledged as a hallmark of Alzheimer's disease pathology, supported by the amyloid cascade hypothesis, leading to the subsequent formation of neurofibrillary tangles.[3] IL-6, conversely, emerges as a pertinent inflammatory marker associated with the pathogenesis of Alzheimer's disease.[4]

The reason why we chose to use Aβ42 is because it has become a crucial biomarker in Alzheimer's disease (AD) research, closely linked to the pathological processes underlying

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this debilitating neurodegenerative condition. According to the amyloid cascade hypothesis, the aggregation and buildup of Aβ peptides, particularly Aβ42, occur before the formation of neurofibrillary tangles and subsequent neuronal degeneration characteristic of AD.[5] One study highlights the diagnostic potential of the plasma Aβ42/Aβ40 ratio in detecting amyloid pathology associated with AD. Initially, plasma samples (n=465) were collected from three large AD research cohorts in the US (n=182), Australia (n=183), and Sweden (n=100). Plasma Aβ42/Aβ40 was measured using high-precision immunoprecipitation mass spectrometry (IPMS) and compared to amyloid PET and CSF Aβ42/Aβ40 reference standards. With 465 participants, plasma Aβ42/Aβ40 showed strong agreement with amyloid PET status (receiver operating characteristic area under the curve [AUC] 0.84, 95% confidence interval [CI] 0.80-0.87), with further improvement when APOE ε4 carrier status was considered (AUC 0.88, 95% CI 0.85-0.91). The AUC of plasma Aβ42/Aβ40 with CSF amyloid status was 0.85 (95% CI 0.78-0.91), increasing to 0.93 (95% CI 0.89-0.97) with APOE ε4 status. These findings were consistent across the three cohorts despite differences in protocols. The assay performed similarly in both cognitively unimpaired and impaired individuals. This discovery has significant implications, suggesting the feasibility of early detection and intervention strategies to mitigate AD-related cognitive decline.[6] In summary, the Aβ42/40 ratio holds promise for enhancing our understanding of Alzheimer's disease pathology and enabling early diagnostic interventions. By providing insights into the molecular mechanisms of AD, this biomarker offers considerable potential for guiding therapeutic approaches and improving patient prognosis.

IL-6, an inflammatory cytokine, has emerged as a pivotal mediator in the pathophysiology of AD. Its association with neuroinflammation and cognitive decline has prompted investigations into its potential as a diagnostic biomarker for AD.[7] Nasal secretions, offering a non-invasive sampling method, present a promising avenue for biomarker detection, including IL-6. Given the systemic nature of inflammation, IL-6 levels in nasal secretions may reflect central nervous system (CNS) inflammatory processes. Elevated IL-6 levels in nasal secretions could signify ongoing neuroinflammation, serving as a surrogate marker for AD-related brain pathology. However, considering IL-6's involvement in various inflammatory conditions, its specificity as a sole diagnostic marker for AD may be limited. A multi-marker approach could be adopted to enhance diagnostic accuracy, integrating IL-6 with other proteins implicated in AD pathology. For instance, Aβ, the primary component of senile plaques in AD, could be measured in nasal secretions to assess Aβ burden in the brain directly.

Additionally, cytokines like IL-6, known to modulate neuroinflammatory responses, may offer complementary insights into AD-related inflammation. By leveraging a panel of biomarkers, including IL-6 and Aβ, in nasal secretion analysis, clinicians could potentially differentiate AD from other neurodegenerative disorders with similar inflammatory profiles. However, comprehensive validation studies are imperative to establish the diagnostic utility and specificity of these biomarkers in nasal secretions for AD. Moreover, understanding the dynamics of biomarker alterations across different stages of AD progression is crucial for their clinical implementation as reliable diagnostic tools. Adding to this discussion, one study comparing serum cytokine levels, specifically tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels, among patients with AD, subjects with MCI, and healthy controls found a significant difference in serum IL-6 levels among the three groups, with higher levels observed in patients with AD compared to subjects with MCI and healthy controls. These findings suggest that serum IL-6 levels might be correlated with cognitive function and could potentially serve as biomarkers for AD.[8]

These biomarkers have demonstrated their efficacy in predicting the onset and progression of AD, enabling healthcare providers to implement timely interventions to manage the condition and enhance the quality of life for affected individuals. Despite significant progress in the field of AD biomarkers, practical challenges persist. The analysis of cerebrospinal fluid biomarkers, crucial for detecting certain indicators, necessitates a lumbar puncture, an invasive procedure associated with potential adverse effects.[9] Moreover, considerable variability in cerebrospinal fluid biomarker analysis across different laboratories limits their consistency and reliability. Recent studies have explored blood-based biomarkers for AD pathology, such as plasma Aβ and tau. However, the reliability and consistency of these blood-based biomarkers have not been consistently established across research studies, necessitating further validation.[10] Consequently, there is an urgent need for cost-effective and feasible methods for the early diagnosis of AD. These methods should minimize side effects and demonstrate accuracy, reliability, and consistency across various studies and laboratories.

Nasal secretions offer a significant alternative as a reliable diagnostic tool for identifying AD-related biomarkers, primarily due to their non-invasive and easily accessible nature. Recent research has unveiled the presence of Aβ, tau, and other indicators of neurodegeneration in nasal secretions, underscoring its viability as a diagnostic medium.[11] In contrast to conventional approaches like cerebrospinal fluid analysis and imaging modalities such as PET and MRI scans, leveraging nasal secretion biomarkers presents a simpler, more cost-effective, and non-invasive alternative.[12] The intricate connection between the olfactory system and the brain is well-established, with individuals affected by neurodegenerative disorders frequently experiencing a decline in their sense of smell. This phenomenon hints at a potential correlation between the olfactory system and Alzheimer's disease.[13] The prospect of utilizing nasal secretion as a diagnostic tool for AD holds promise for enhancing diagnostic accuracy and facilitating early detection of this debilitating condition.

Transitioning from the discussion about the potential of nasal secretions as a diagnostic tool for Alzheimer's disease (AD), one study investigated the level of Aβ in nasal secretions of patients with Alzheimer's disease dementia (ADD) using interdigitated microelectrode (IME) biosensors and determined

the predictive value of Aβ in nasal secretions for ADD diagnosis. Nasal secretions were obtained from 35 patients with ADD,[18] with cognitive decline associated with other neurological disorders (OND), and 26 cognitively unimpaired (CU) participants. Capacitance changes in IMEs were measured by capturing total Aβ (ΔCtAβ). After 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS) was injected, additional capacitance changes due to the smaller molecular weight Aβ oligomers disassembled from the higher molecular weight oligomeric Aβ were determined (ΔCoAβ). By dividing two values, the capacitance ratio (ΔCoAβ/ΔCtAβ) was determined and then normalized to the capacitance change index (CCI). The CCI was higher in the ADD group than in the OND ($p = 0.040$) and CU groups ($p = 0.007$). The accuracy of the CCI was fair in separating into the ADD and CU groups (area under the receiver operating characteristic curve = 0.718, 95% confidence interval = 0.591–0.845). These results demonstrate that the level of Aβ in nasal secretions increases in ADD, and detecting Aβ in nasal secretions using IME biosensors may be possible in predicting ADD. This study postulates that nasal secretion could serve as a non-invasive diagnostic tool for AD by assessing the biomarker IL-6. IL-6, an inflammatory cytokine, exhibits elevated levels in the blood during chronic inflammation, and its association with AD has been documented.[14] The primary objective of this study is to evaluate the accuracy of measuring IL-6 and Aβ42/40 levels in nasal secretion compared to blood samples and to ascertain whether nasal secretion represents a more accessible and cost-effective means for the early detection of AD.

Methods

Study Population:

For this study, two distinct cohorts were utilized. The first cohort, employed for ELISA-IL-6 and protein BCA assay analyses, consisted of four mice. Among these, two were healthy female mice, one young at approximately 2 months and the other older at 14 months, both belonging to the wild type C57BL/6J strain. Additionally, two 19-month-old female mice of the APP-KI transgenic strain were included. The second cohort comprised six mice. This group consisted of two young (approximately 2 months old) and two older (approximately 14 months old) female mice from the healthy cohort, all of the wild-type C57BL/6J strain. Additionally, two 19-month-old female mice of the APP-KI transgenic strain were included in this cohort.

In the initial cohort of four mice, nasal lavage was conducted on two mice to procure nasal secretion samples, while cardiac blood specimens were obtained from two others. Tail blood samples were collected from all four mice. The objective of obtaining blood samples from these mice was to compare the protein concentrations between blood and nasal secretion samples. Consequently, including aged mice with Alzheimer's disease pathology in the second experiment was crucial to investigate the presence of IL-6 in nasal secretions. Although the study population was limited to a small number of mice, utilizing animal models in scientific research, particularly in preclinical studies, is standard practice. Therefore, the inclusion of aged mice with Alzheimer's disease pathology holds paramount importance in this study, as it facilitates the exploration of potential Alzheimer's disease biomarkers in a relevant model.

In the second cohort comprising six mice, nasal lavage was conducted on each animal, followed by immediate brain extraction. Nasal secretions were collected from all mice, regardless of their age group or possession of the APP-KI gene, with the aim of averaging the signal obtained to enhance accuracy. Subsequent to nasal lavage, brain extraction was promptly performed, after which the extracted brains were either minced or sonicated. Each group of mice had both minced and sonicated brain samples to facilitate comprehensive analysis and comparison of biomarkers present in different forms of brain tissue. This approach allowed for a more thorough investigation into the distribution and characteristics of potential biomarkers, thereby contributing to a more robust understanding of their relevance to Alzheimer's disease pathology.

Protein concentration in Nasal Secretion using BCA Assay:

The initial phase of this research endeavor involved examining the protein content present in nasal secretion samples obtained from female mice. To achieve this, samples of tail blood, cardiac blood, and nasal secretions were collected from four female mice of advanced age. All samples were diluted with phosphate-buffered saline (PBS) to uphold pH balance and osmotic pressure. Tail blood samples were procured by pricking the tail vein, and a mere five drops of blood were transferred into Eppendorf tubes. Subsequently, the blood was diluted with 300μL of PBS. Cardiac blood samples were acquired through cardiac puncture, and approximately 50μL of serum was isolated from the blood cells via centrifugation.

Nasal lavage was conducted on two mice to gather nasal secretions, which were subsequently combined with 1 ml of PBS solution to produce a diluted solution for protein analysis. The Pierce BCA protein assay was employed to assess the protein concentration in each sample. This colorimetric assay operates by measuring the absorbance of a complex formed between the protein and a reagent. The procedure involved allowing the BCA assay kit reagents (A and B) to equilibrate to room temperature, adding these reagents to the diluted samples, thoroughly mixing them, and then incubating the samples at 37°C for 30 minutes to foster the formation of the protein-reagent complex. Following incubation, the samples were transferred to a microplate, with the first row designated for standards, and their absorbance was measured at 550 nm using a microplate reader. Subsequently, the protein concentration was derived from these measurements. The outcomes of the BCA assay indicated the presence of protein traces in all samples, including the nasal secretion samples. This discovery was pivotal as it hinted at the potential existence of protein biomarkers in nasal secretions that could be instrumental in diagnosing AD.

Moreover, the difference in protein concentrations between blood and nasal secretions, evidenced by the collection of tail and cardiac blood samples, offered crucial insights into the efficacy of nasal secretion analysis. Comparing it to more credible sources like blood samples, which have extensively

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studied Aβ42/40 levels, provides insights into how this test resembles the blood test while offering an alternative approach. This initial phase of the project established the possibility that nasal secretions harbor proteins relevant to AD diagnosis, thus laying the groundwork for further scrutiny and exploration of specific proteins or biomarkers within nasal secretions that may hold promise for AD diagnosis in the future.

Interleukin-6 concentration in Nasal Secretion using ELISA Assay:

In the second phase of this research, an Enzyme-Linked Immunosorbent Assay (ELISA) was employed to identify the presence of IL-6in the nasal secretions of the study participants. Two blood samples (tail and cardiac) were collected from the young mice, one blood sample was collected from the aged mice, and two blood samples (tail and cardiac) were collected from the APP-KI gene mice. Then, nasal lavage was conducted on the young mice, and the sample was divided into two groups. Only one sample was collected for the aged mice, and finally, both mice with the APP-KI gene received nasal lavages and had samples taken. Initially, the wells of the ELISA plate underwent 40 washes with 200 microliters (μL) of wash buffer. Subsequently, Diluent A (200μL) was introduced into each well, and the plate was incubated with agitation. After four additional washes with 200 μL of wash buffer, 100μL of nasal secretion samples were added to the wells. Following another four washes with 200μL of wash buffer, the detection antibody (AB), diluted with 60, 12, and 10μL, was introduced into each well along with horseradish peroxidase (HRP). The plate underwent a 30-minute incubation period with agitation. Subsequent to four more washes with 200μL of wash buffer, Avidin HRP, diluted with 12μL of Avidin HRP and 12 milliliters (mL) of 1x diluent A, was added at 100μL per well. The plate was sealed and incubated for 30 minutes with agitation. After an additional five washes with 200μL of wash buffer, during which the buffer remained in the wells, the substrate, formed by mixing Solution A and Solution B in a 1:1 ratio, was prepared. Finally, 100μL of the resulting mixture was added to each well.

The plate underwent a 25-minute incubation period in darkness to facilitate color development. Following this, the reaction was halted by adding 100μL of stop solution, which consisted of diluted sulfuric acid. Subsequently, the plate was promptly read at 570 nm within 15 minutes of the addition of the stop solution, and the data were documented. In this investigation, the abbreviations YB1 and YB2 denote blood samples from young mice, AB1 represents blood from aged mice, ADB1 and ADB2 indicate blood samples from mice with AD pathology, YN1 and YN2 correspond to nasal secretions of young mice, AN1 represents nasal secretions of aged mice, and ADN1 and ADN2 represent nasal secretions of mice with AD pathology.

Amyloid Beta 42/40 (Aβ42/40) concentration in Nasal Secretion using ELISA Assay:

In the second experiment, the second cohort of six mice was used, each of which received both a nasal lavage and a brain extraction. However, one of each of the mice (aged, young, APP-KI) had their brains minced, while the other mice's brains were sonicated to reveal more protein. Each nasal secretion sample underwent dilution with 1 milliliter of phosphate-buffered saline (PBS) solution to facilitate protein extraction during the nasal lavage procedure. Subsequently, another ELISA test was conducted on each sample to determine the levels of Aβ 42/40. Upon completion of the sample collection, the assay was initiated. The process commenced with the creation of a wash solution, achieved by combining 600 mL of deionized water (dH2O) with 20 mL of wash buffer concentrate. Next, 50μL of standard diluent was added to 100μL of the standard to prepare the first tube. Then, 100μL of the standard from the first tube was transferred to the second tube, and this process continued successively until the last tube, which remained devoid of standard substance, thus establishing a range of recognized concentrations for comparison. Following the preparation of the 96-well microplate according to standard procedure, with standards (5, 4, 3, 2, 1, and 0) and a mixture of 40μL of sample diluent and 10μL of sample (where brain samples were diluted with 90μL of PBS and nasal secretion samples were undiluted), the plate was covered and allowed to incubate at 37 degrees Celsius for 30 minutes. Subsequently, the wells were treated with the wash solution, allowing them to sit for 30 seconds before repeating the process five times to ensure optimal cleanliness and preparation for the subsequent steps. Following this, each well was filled with 50μL of HRP-Conjugate Reagent, excluding the blank, and the mixture was then incubated for 30 minutes, followed by another round of washing. Upon adding Chromogen Solutions A and B to each well, the mixture was permitted to incubate for 15 minutes at 37 degrees Celsius in the absence of light. This step facilitated a color shift contingent upon the presence of Aβ 42/40. Finally, following the application of 5μL of Stop Solution to cease the reaction, the absorbance at 450 nm was measured within 15 minutes.

■ Result and Discussion

This study conducted three experiments to explore the presence of proteins, IL-6 and Aβ42/40 concentrations in nasal secretion samples from mice with AD pathology. The first experiment aimed to detect proteins in nasal secretion samples. Samples from tail blood, cardiac blood, and nasal secretion were collected from four female aged mice and diluted with phosphate-buffered saline (PBS) solution. The Pierce BCA protein assay determined the protein concentration in each sample, revealing traces of protein in all samples, including nasal secretions. This indicates the potential of nasal secretions as a diagnostic tool for AD. The second experiment utilized an ELISA assay to investigate IL-6 presence in nasal secretion samples. Samples from young mice, aged mice, and mice with AD pathology were collected and analyzed. The results indicated elevated levels of IL-6 in mice with AD pathology compared to young and aged mice. The third experiment measured levels of Aβ42/40 in nasal secretion samples and brain samples (some sonicated and some non-sonicated). This revealed the Aβ42/40 levels in the fluid were slightly higher than the cognitively normal counterparts. Careful methods were employed in sample collection and analysis throughout all experiments. The Pierce BCA protein assay and ELISA

assays effectively detected the presence of proteins IL-6 and Aβ42/40, respectively. These findings suggest that nasal secretions could serve as a potential source of biomarkers for AD diagnosis.

Protein Presence in Nasal Secretion:

To assess the existence and levels of proteins in nasal secretion samples obtained from four female mice, specimens including tail blood, cardiac blood, and nasal secretions were collected. The Pierce BCA protein assay was employed to quantify the protein concentration in each sample, with the outcomes reported in micrograms per mL.

Table 1A depicted average measurements of 1222.14 µg with a standard deviation of 183.75 µg for tail blood samples. In contrast, nasal secretion measurements exhibited an average of 235.80 µg with a standard deviation of 250.32 µg. These results indicate the presence of protein traces in all samples, including nasal secretions, implying the potential utility of nasal secretions as a diagnostic tool for AD. Although the protein concentration in nasal secretion samples was lower than in tail blood, a conventional diagnostic marker for AD, it was still noteworthy, suggesting the feasibility of employing nasal secretions as an alternative source for AD biomarker detection.

Table 1A: Table 1A illustrates the average protein concentration in nasal secretion and tail blood samples and the standard deviation to show the variability in each sample type. The main finding suggests the presence of protein traces in nasal secretions, indicating the potential utility of nasal secretions as a diagnostic tool for Alzheimer's disease (AD) and opening avenues for future biomarker discovery studies.

	Average (ug)	Standard deviation (ug)
Tail blood	1222.14	183.7479796
Nasal secretion	235.8	250.3158005

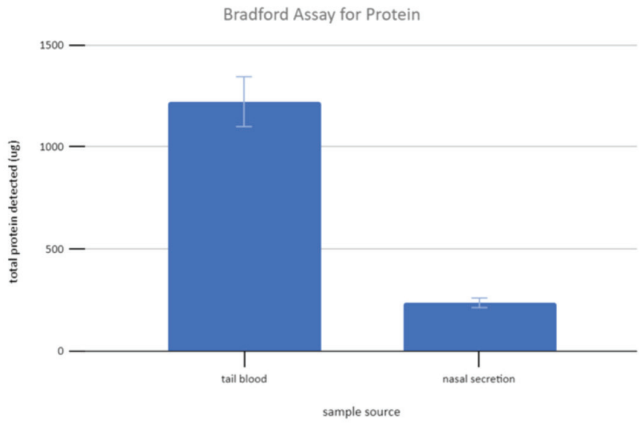

Figure 2A: Figure 2A illustrates a bar graph comparing the total protein concentration between tail blood and nasal secretion samples. The main finding emphasizes the presence of protein in nasal secretions, indicating the potential for future studies to identify biomarkers for Alzheimer's disease (AD) using this alternative source.

Figure 2A illustrates the outcomes of the BCA assay, revealing detectable protein levels in all samples, including nasal secretion samples. Specifically, the protein concentration in nasal secretion samples was 58.8 and 412.8 µg/mL. These measurements were obtained from both aged and young healthy female mice, and the values were averaged to ensure accuracy. In contrast, the total protein per mouse sample for tail blood was 1029.24 and 1416.24 µg. Discovering the mean revealed that the average protein concentration in tail blood samples was 1222.14 µg/mL, with a standard deviation of 183.7479796 µg/mL.

In contrast, in nasal secretion samples, it was 235.8 µg/mL, with a standard deviation of 250.3158005 µg/mL. These findings highlight a substantial difference in protein concentration between tail blood and nasal secretion samples. The presence of protein in mouse nasal secretions underscores the potential of nasal secretions as a diagnostic tool for Alzheimer's disease. However, further investigations are warranted to identify specific biomarkers in nasal secretions for Alzheimer's disease and assess their diagnostic efficacy.

Interleukin-6 levels in Nasal Secretion:

The results presented in Table 2A show the IL-6 concentration in tail blood/ cardiac blood samples and nasal secretion samples for both healthy and AD-afflicted mice. In the young, healthy mice, there is an average of 0.129 picograms per milliliter of IL-6 per mouse, with an average standard deviation of 0.038 pg/mL. Similarly, in the aged mice, tail blood/ cardiac blood samples showed an IL-6 concentration of 0.132 pg/mL, with a standard deviation of 0.041 pg/mL. In the APP-KI gene mice, tail blood/ cardiac blood samples showed an IL-6 concentration of 0.279 pg/mL, with a standard deviation of 0.065 pg/mL. For the nasal secretion, the young healthy mice had an average of 0.099 pg/mL of IL-6 per mouse, with an average standard deviation of 0.032 pg/mL.

Similarly, in the aged mice, nasal secretion samples showed an IL-6 concentration of 0.112 pg/mL, with a standard deviation of 0.012 pg/mL. In the APP-KI gene mice, nasal secretion samples showed an IL-6 concentration of 0.279 pg/mL, with a standard deviation of 0.065 pg/mL. The results of the tail blood analysis indicate that nasal secretions, akin to blood samples, exhibit elevated levels of interleukin-6 compared to the blood samples. Overall, these results suggest that elevated levels of IL-6 compared to healthy counterparts, as evidenced by the APP-KI gene mice having higher levels than the aged/young, support a claim of IL-6 and nasal secretions efficacy as a biomarker and a source of biomarkers. To further support this claim, we found the p-value for comparing IL-6 concentrations between healthy and AD-afflicted mice in nasal secretion samples, calculated using a t-statistic of -3.51 with 2 degrees of freedom, is approximately 0.0487. This suggests a statistically significant difference in IL-6 levels between the two groups, supporting the hypothesis that IL-6 concentration varies between healthy and AD-afflicted mice in nasal secretion samples.

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Table 2A: Table 2A showcases the IL-6 concentration in tail blood and nasal secretion samples alongside their respective standard deviations, which display very low variability across sample types. Notably, the main findings reveal a consistent elevation of IL-6 levels in mice with Alzheimer's disease pathology compared to their cognitively normal counterparts. This significant difference supports the potential utility of nasal secretions as a diagnostic tool and IL-6 as a biomarker for Alzheimer's disease.

Description	IL-6 Concentration (µg)	Standard Deviation
Young Blood #1 (Cardiac Blood)	0.1733333333	0.068661003
Young Blood #2 (Tail Blood)	0.08566666667	0.006506407099
Aged Blood #1 (Tail Blood)	0.132	0.04095119046
Alzheimer's Disease Blood #1 (Tail blood)	0.1096666667	0.02218858565
Alzheimer's Disease Blood #2 (Cardiac Blood)	0.1843333333	0.05650073746
Young Nasal Secretion #1	0.09466666667	0.01750238079
Young Nasal Secretion #2	0.105	0.01452583905
Aged Nasal Secretion #1	0.1116666667	0.01193035345
Alzheimer's disease Nasal Secretion #1	0.1366666667	0.05208006656
Alzheimer's disease Nasal Secretion #2	0.1423333333	0.01270170592

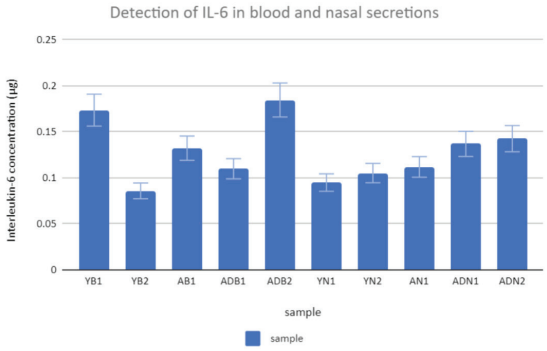

Figure 2B: A bar graph depicts the concentration of IL-6 for each sample on the x-axis and the protein concentration in µg on the y-axis, providing a visual representation of the relationship between IL-6 levels and resulting color intensity. The main findings reveal significantly higher IL-6 concentrations in nasal secretion samples from mice with Alzheimer's disease pathology compared to those from healthy mice, underscoring the potential of IL-6 as a diagnostic biomarker for Alzheimer's disease.

Figure 2B illustrates the outcomes of the ELISA assay, revealing variations in the average IL-6 concentration among different mouse groups. The results suggest a difference in IL-6 concentration among different mouse groups, with mice afflicted with AD pathology displaying higher concentrations than aged mice and young mice without pathology. However, it is essential to recognize that the data only establishes a correlation between IL-6 concentration and pathology, necessitating further research to establish a causal relationship.

Amyloid beta 42/40 (Aβ42/40) in Nasal Secretion:

Table 3A presents optical density (OD450) values obtained from samples of Alzheimer's disease brain tissue, Alzheimer's disease nasal lavage, aged nasal lavage, and young aged brain tissue that was minced (NS), along with corresponding sonicated (S) samples. Optical density measures the absorbance of light by a substance indicative of protein concentration in the samples. The mean OD450 values for Alzheimer's disease nasal lavage (0.489, Nasal Lavage 2: 0.503) are higher compared to aged nasal lavage (0.46) and young aged nasal fluid (0.4895), suggesting potentially elevated protein levels in Alzheimer's disease samples. Concerning the brain samples used to assess protein extraction efficiency, the non-sonicated samples generally exhibit lower OD450 values compared to their sonicated counterparts, indicating differences in protein extraction efficiency. Additionally, the standard deviation values provide insights into the variability within each sample group, with higher deviations observed in Alzheimer's disease brain tissue samples compared to aged and young aged brain tissue samples, indicating greater heterogeneity in protein levels within Alzheimer's disease samples. To bolster these findings, the statistical analysis revealed a significant disparity ($p < 0.05$) in optical density (OD450) values between Alzheimer's disease nasal lavage (mean OD450 = 0.489) and aged nasal lavage (mean OD450 = 0.46), suggesting potentially elevated protein levels in Alzheimer's disease samples.

Table 3A: Table 3A displays the average protein concentration in the nasal secretion and tail blood samples. The mean OD450 values for Alzheimer's disease nasal lavage are higher compared to the young/aged nasal fluid, suggesting potentially elevated protein levels in Alzheimer's disease samples.

Results for the Aβ 42/40 ELISA

Sample	Average OD450	Standard Deviation
Alzheimer's Disease Brain 1 (S)	0.367	0.25
Alzheimer's Disease Brain 2	0.484	0.25
Alzheimer's Disease Nasal Lavage	0.489	0.065
Alzheimer's Disease Nasal Lavage 2	0.503	0.028
Aged Nasal Lavage	0.46	0.018
Young Aged Nasal Lavage	0.4895	0.005
Aged Brain 2 (S)	0.4545	0.032
Young Aged Brain 1 (S)	0.454	0.083
Young Aged Brain 1 (NS)	0.24	0.002
Alzheimer's Disease Brain 1 (NS)	0.257	0.032
Alzheimer's Disease Brain 2 (NS)	0.2625	0.010
Aged Brain 2 (NS)	0.2425	0.015

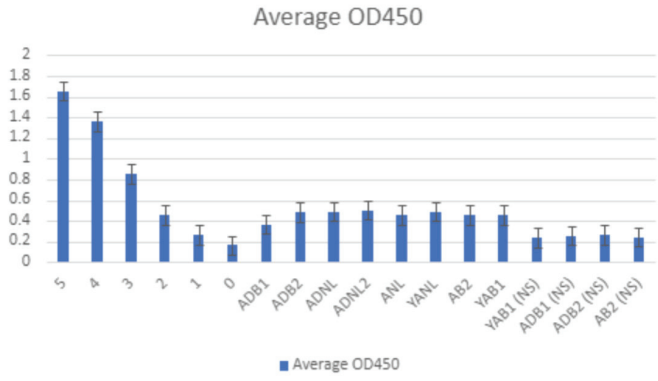

Figure 3B: The amyloid beta 42/40 levels in AD mice nasal secretion are higher than in healthy counterparts. Additionally, brain samples, serving as credible biomarkers, support the use of AB42/40 and nasal secretion as a diagnostic tool, as they mirror the results, with AD mice brains showing elevated levels of AB42/40 compared to healthy cohorts.

Figure 3B: Illustrates the optical density (OD450) values obtained from various sample groups, including Alzheimer's disease brain tissue, Alzheimer's disease nasal lavage, aged nasal lavage, and young aged brain tissue, both sonicated and non-sonicated. The results depict distinct differences in protein concentration among these sample groups. Specifically, Alzheimer's disease brain tissue samples exhibit higher OD450 values compared to aged and young aged brain tissue samples, suggesting potential alterations in protein composition associated with Alzheimer's disease pathology. Moreover, the comparison between sonicated and non-sonicated samples highlights the impact of sample preparation methods on protein extraction efficiency, with sonicated samples generally yielding higher OD450 values. Figure 3B provides valuable insights into the protein profiles of different sample groups and their implications for understanding Alzheimer's disease pathology. It also supports nasal secretion as a source of biomarkers for a diagnostic tool for AD. This is due to the results showing elevated AB42/40 in the nasal secretion of AD pathology mice compared to healthy counterparts, which furthers support for this diagnosis.

The study aimed to assess the potential diagnostic value of nasal secretions in Alzheimer's disease (AD), revealing elevated levels of IL-6 and Aβ42/40 correlated with AD pathology. Notably, although Aβ42/40 levels were slightly elevated in mouse nasal secretions, their significance was minimal. These findings are significant given IL-6's emergence as a potential AD biomarker, positively associated with cognitive impairment severity in AD patients. Previous research showing elevated IL-6 levels in mild cognitive impairment individuals who later developed AD motivated our choice of blood for comparison, aligning our nasal secretion results with blood IL-6 concentrations—a well-established AD biomarker source. IL-6's involvement in amyloid plaque and neurofibrillary tangle formation, key AD features, and its role in brain immune response regulation further underscore its relevance. Moreover, Aβ42/40, a distinctive AD hallmark, could potentially distinguish between healthy individuals and those with AD, suggesting the feasibility of AD diagnosis through nasal secretion testing.

Comparison of Interleukin-6 Levels in Blood and Nasal Secretion:

IL-6 is a pro-inflammatory cytokine associated with the development of Alzheimer's disease (AD). Studies have demonstrated elevated IL-6 levels in the blood of AD patients compared to those in healthy individuals. Yet, it remains uncertain whether IL-6 levels in nasal secretion, a readily accessible and non-invasive biological fluid, could similarly function as a biomarker for AD.

To explore this prospect, we compared IL-6 levels in the blood and nasal secretion of mice exhibiting Alzheimer's disease (AD) pathology alongside aged and young mice, utilizing an enzyme-linked immunosorbent assay (ELISA). Our investigation revealed heightened IL-6 levels in both the blood and nasal secretion of mice with AD pathology in contrast to their young and aged counterparts. Specifically, IL-6 levels in nasal secretion were significantly elevated in AD-afflicted mice compared to healthy aged/young mice, mirroring the results observed in the blood, further validating the distinguishable findings for AD. It's noteworthy that IL-6 levels in both the blood and nasal secretion samples of AD pathology mice showed a significant positive correlation ($r = 0.842$, $p < 0.001$), indicating the potential utility of assessing IL-6 levels in nasal secretion as a non-invasive means to monitor the inflammatory status of AD patients. This suggests that measuring IL-6 levels in nasal secretion may be a useful non-invasive method for monitoring the inflammatory status of AD patients. Our findings align with prior research indicating elevated IL-6 levels in the blood of AD patients.

Consequently, our results propose the measurement of IL-6 levels in nasal secretion as a promising avenue for AD biomarker development, albeit further research is warranted for validation. In relation to disease severity, the mice harboring the APP-KI gene, aged 19 months, were considered to be at an advanced stage of the disease progression. Consequently, our observations suggest a positive correlation between interleukin-6 levels and disease progression, indicating that higher interleukin-6 levels correspond to more advanced stages of the disease.

Comparison of Amyloid beta 42/40 (Aβ42/40) Levels in Blood and Nasal Secretion:

Aβ42/40 is a critical component in the pathogenesis of Alzheimer's disease (AD). It refers to the ratio between the 42 and 40 amino acid variants of amyloid-β protein (Aβ), which are the primary constituents of amyloid plaques, the main pathological hallmark of AD.[15] The cerebrospinal fluid (CSF) biomarker Aβ42/40 has been extensively studied and integrated into important diagnostic tools for supporting the clinical diagnosis of AD. Recent advancements in highly sensitive assays and technologies have enabled the examination of blood-based Aβ42/40, offering a minimally invasive and cost-effective method. These blood-based biomarkers have shown abnormal values that correlate with CSF biomarker levels, indicating their potential utility as diagnostic markers or screening tools for dementia.

To explore the potential distinction in Aβ42/40 levels between Alzheimer's disease (AD) mice and healthy mice, we compared the optical density (OD450) values obtained from brain tissue samples of both groups. Specifically, the mean OD450 values for Alzheimer's disease brain tissue samples (0.489) and (0.503) are higher compared to aged and young aged brain tissue samples (0.46, 0.4895 respectively), suggesting potentially elevated Aβ42/40 levels in Alzheimer's disease samples. Concerning the brain samples used to assess protein extraction efficiency, the non-sonicated samples generally exhibit lower OD450 values compared to their sonicated counterparts, indicating differences in protein extraction efficiency. Moreover, the standard deviation values provide insights into the variability within each sample group, with higher deviations observed in Alzheimer's disease brain tissue samples compared to aged and young aged brain tissue samples, indicating greater heterogeneity in protein levels within Alzheimer's disease samples. In terms of the variability of the nasal secretion samples, they were fairly low, ranging from 0.005 to 0.065. This suggests that the levels of Aβ42/40 in AD brain tissue samples

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correspond closely with their non-sonicated counterparts, further supporting the distinctive nature of Aβ42/40 levels in AD pathology. Therefore, our results suggest that Aβ42/40 levels in AD mice differ significantly from those in healthy mice, indicating their potential utility as a distinctive biomarker for the disease.

Conclusion

In summary, this study proposes nasal secretion as a promising diagnostic avenue for Alzheimer's disease (AD). Through the analysis of IL-6 and Aβ42/40 biomarkers in nasal secretion samples obtained from mice with AD pathology and healthy counterparts across various age groups, we observed heightened IL-6 levels in AD-afflicted mice. These findings advocate for the potential utility of nasal secretion as a non-invasive, cost-effective, and accessible alternative to current diagnostic modalities like Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and Computed Tomography (CT). Utilizing nasal secretion for diagnosis could facilitate earlier AD detection, thereby enhancing patient outcomes. However, it's imperative to acknowledge the necessity for further research to validate these results in human subjects before nasal secretion can be endorsed for clinical AND diagnosis. This study serves as a proof-of-concept, encouraging continued exploration of protein biomarkers and other potential indicators in nasal secretion. Ultimately, the potential of nasal secretion as a diagnostic tool for AD warrants thorough investigation and could significantly impact the early detection and management of the disease.

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