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RESEARCH ARTICLE

Differences in Microglial Colocalization with Amyloid-β in Patients with Dementia Using the SEA-AD Dataset

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ABSTRACT: Alzheimer's Disease (AD) is marked by cognitive decline, neurodegeneration, and the formation of amyloid-beta (Aβ) plaques and tau tangles. AD is the most common form of dementia, which is an umbrella term used to describe a decline in cognitive ability that affects daily life. This study is focused on differences in colocalized Aβ and microglia in the brains of 84 individuals, 42 diagnosed with dementia, and 42 without a diagnosis of dementia. Analyses of neuropathological data from the SEA-AD dataset found that there is a greater amount of colocalized 6e10 and Iba1 objects– markers for Aβ and microglia, respectively – in patients diagnosed with dementia, and less from patients with no diagnosis of dementia. In dementia, the degree of Iba1-6E10 colocalization varied across cortical layers, with more colocalized objects in layers 3 and 5/6. Notably, layer 3 showed the greatest increase compared to layer 5/6. This suggests that microglial colocalization of Aβ plaques is most pronounced in layer 3, offering insight into layer-specific microglial activity in Alzheimer's disease.

KEYWORDS: Biology, Neuroscience, Alzheimer's Disease, Neuropathology, Microglia.

Introduction

Alzheimer's disease (AD) is a disease marked by cognitive decline, neurodegeneration, and the presence of amyloid-beta (Aβ) plaques and formation of tau tangles. It is the most common form of dementia, an umbrella term used to describe a decline in cognitive ability that affects an individual's daily life. Microglia, which are phagocytic immune cells of the brain involved in regulatory processes critical for development, maintenance, and repair,[1] have an intriguing relationship with AD, and in turn, Aβ, that has yet to be entirely understood.[2,3]

Colocalization of microglia with Aβ plaques in AD has been previously associated with microglia's role in clearing and metabolizing the plaques, as was found by Van Olst *et al.*[4] Escariao *et al.*[5] conducted a similar analysis of colocalized Aβ and microglia in AD, but correlated it also to changes in global cognitive abilities. They found that higher expression of colocalized Aβ and microglia correlated with lower cognitive abilities, and therefore, the amount of colocalized objects was consistently higher in concentration in AD patients than in controls.

The present study aims to examine how colocalized microglia and Aβ in different cortical layers correspond with the incidence of dementia through analyzing quantitative neuropathological data from the open-source SEA-AD dataset.[6,7] The data were collected from the middle temporal gyrus (MTG) of 84 patients, evenly split between cohorts of patients presenting with and without the cognitive and behavioral deficits of dementia. The patients' Alzheimer's Disease Neuropathologic Change scores ranged from 9 with No AD, 12 Low, 12 Intermediate, and 42 High. The MTG is a region that is of great importance to cognitive functions such as language processing and semantic memory, and is also one of the first to be targeted in disease development. The identification of cortical layer-specific patterns of Aβ-microglia colocalization in the data can yield a greater understanding of which types of tissue and cells are implicated in the aggregation of these pathologies. This study hypothesized that there would be significant differences between the amount of colocalized microglia and Aβ between individuals with and without dementia.

Methods

The SEA-AD open-source database, containing detailed neuropathological, genomic, transcriptomic, and demographic data from post-mortem brain tissue samples of 84 donors, half of whom were affected by Alzheimer's Disease was used.[8]

SEA-AD Participant Recruitment:

The brain specimens used to create the SEA-AD database were obtained from the Adult Changes in Thought (ACT) Study and the University of Washington Alzheimer's Disease Research Center (ADRC) Clinical Core study.[8]

For the ACT study, participants were invited to enroll at age 65 by random selection from the patients of KPW Seattle and undergo study visits twice a year for physical and mental examinations. About 25% of ACT donations were from people with no MCI or dementia at their last evaluation; 30% met criteria for MCI, and 45% met criteria for dementia.[9]

Patients enrolled in the UW ADRC Clinical Core underwent annual study visits, including identical mental and physical exams to those conducted in the ACT study, as well as donations of biospecimens that included blood and serum, and family interviews.[10]

Individuals with a diagnosis of frontotemporal dementia, frontotemporal lobar degeneration, Down syndrome, amyotrophic lateral sclerosis, or other confounding degenerative disorder (not including Lewy Body Disease or VBI) were

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excluded from the database. Any donors who tested positive for COVID-19 were also excluded. Some aspects of donor metadata are present in Figure 1, and more information can be found on the websites of each respective study and within the SEA-AD dataset.[8]

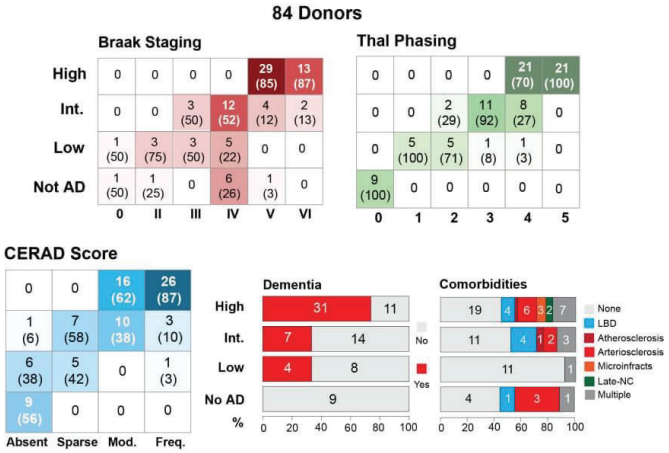

Figure 1: Donor metadata.[8] Braak stages and Thal phases are demonstrated to show the distribution of pathology, as well as cognitive abilities, as illustrated by the CERAD score and Dementia.

SEA-AD Tissue Collection, Imaging, and Quantification:

Approximately 30% of the combined ACT/UW ADRC cohort consented to brain donation upon death, and tissue collection was coordinated by the UW Biorepository and Integrated Neuropathology (BRaIN) lab. The collected brain tissue was preserved for different preparations, including fixed, frozen, and fresh, and certified neuropathologists performed full post-mortem neuropathological examination and diagnoses.[9,10]

Raw images were collected, stained, and quantified. Both raw and quantified images were made available to view in the database. The quantified images were used to collect pre-existing colocalization data used in this study. More detailed information exists on the websites of each respective study and within the SEA-AD dataset.[8]

Quantitative and Statistical Analyses:

To collect the specific data used in this study, the Quantitative Neuropathology file was utilized to obtain the data on the number of colocalized Iba1 and 6e10 in each donor. The file contains data from all 84 donors' MTG (middle temporal gyrus). Then, each patient's condition of "dementia" or "no dementia" was determined within the Donor Metadata file. A student's t-test was run across the gray matter (sums of colocalized objects in all 6 cortical layers) comparing donors with dementia to those who had no dementia. Afterwards, one-way ANOVAs were conducted across the dementia and no dementia groups separately to determine the variance across layers. Two-way ANOVA tests were then run to compare differences in the colocalized objects between dementia and no dementia between layers 3 vs 5/6 and layers 2 vs 4, as layer 3 and layer 5 are mainly composed of pyramidal cells, and layer 2 and layer 4 contain mainly stellate cells.[11] Post-hoc comparisons were corrected using Bonferroni's correction for multiple comparisons. Bar graphs represent the average of the data points in each layer, with error bars denoting the standard error of the mean. Statistics and graphs were generated using Prism version 10.5.0 (GraphPad; La Jolla, CA, US).

Results and Discussion

All Layers Analysis:

Comparison of colocalized objects across the two groups revealed that there was a significant effect of "group" overall across the cohort [F (1, 82) =2.728; p = 0.0042].

Comparisons Within Groups: Effect of MTG Cortical Layers:

Further analyses were conducted as standard one-way ANOVAs done separately within the dementia group and no dementia groups, (Figure 1), revealing a significantly larger number of colocalized Iba1 and 6e10 objects in layer 3 of donors with dementia when compared with layer 2 ($p < 0.001$), layer 4 ($p<0.001$), and layers 5/6 ($p<0.001$). This demonstrates that the number of colocalized Iba1/6e10 varies significantly across cortical layers in dementia [$F(3,123) = 20.38$; $p<0.001$]. Some of these observed differences were less significant when comparing between donors without dementia- layer 4 comparisons with layer 3 yielded a p of 0.017, and layers 5/6 had a p of 0.136. This indicates the amount of Iba1/6e10 colocalization varies less in donors with no dementia than it does in donors with dementia [$F\ 3, 123 = 9.159$; $p<0.001$].

Comparisons Between Groups: Dementia vs Controls Across Specific MTG Layers:

A standard two-way ANOVA was run comparing cortex layers 3 and 5/6, as well as the dementia/no dementia variable. The results of this analysis yielded significant differences between the dementia group of layer 3 and 5/6 ($p < 0.001$), but not between the no dementia groups ($p = 0.109$). $F(1, 82) = 3.002$ when comparing across layers and dementia.

Another standard two-way ANOVA was run between cortex layers 2 and 4 and the dementia/no dementia variable. This analysis demonstrated no significant differences between these layers. $p = 0.864$ for patients with dementia, and $p = 0.066$ for patients without dementia, $F(1, 82) = 2.068$).

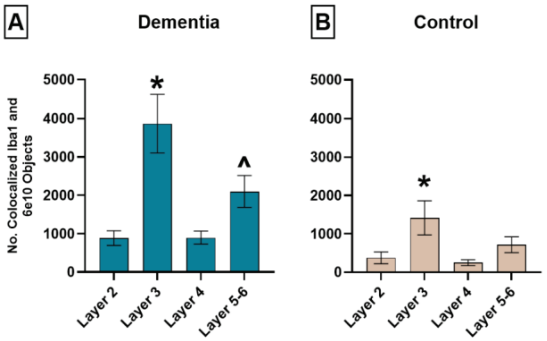

Figure 2: Effect of Cortical Layers on Number of Colocalized Iba1 and 6e10 Objects. A: Comparison of the averages of colocalized Iba1 and 6e10 objects in donors with dementia. There are significant differences between Layer 3 and the data from the other cortical layers analyzed. B: Comparison of the averages of colocalized Iba1 and 6e10 objects in donors without dementia. Layer 3 has significant differences when compared to the rest of the cortical layers. No other relations are shown to exist in this group.

Discussion:

It was hypothesized that there would be significant differences in the amounts of colocalized microglia and Aβ between conditions of dementia and no dementia, as well as across the cortical layers. The analyses conducted on this data have supported this hypothesis, as it was shown that there is generally a greater number of colocalized Iba1 and 6e10 objects in individuals with dementia across all layers of the cortex, as well as greater differences in the amounts of colocalized objects across the cortical layers in donors with dementia. After further analyzing and observing these analyses, the study aimed to further understand the effects of specific layers, especially given the significantly larger amount of colocalized microglia and Aβ specifically present in cortical layer 3 of patients with dementia.

Layer 3 has compositional similarities with layer 5- both being primarily composed of pyramidal cells.[12] Layer 2 and layer 4 also have this relationship, both being granular layers containing many stellate cells. Therefore, further analyses were conducted on layer 3 and layers 5/6, which found that there was not a significant difference between the number of colocalized microglia and Aβ in patients with no dementia, although there was a significant difference when comparing the same values in patients with dementia. This indicates that in dementia, microglial colocalization with Aβ increases most acutely in layer 3, despite its compositional similarities with other layers. This effect may be due to larger amyloid deposits or greater cell damage in the area, though the exact explanation for this finding will need to be looked into further in future studies.[13]

■ Conclusion

This study provides evidence that there are colocalized microglia in Aβ increase in dementia within cortical layer 3, providing a potential insight into the mechanism of the disease and the role that microglia play in either regulating or increasing neurodegeneration. However, questions have been raised regarding the reason why increased microglial colocalization with Aβ in dementia seems to occur mainly within layer 3, even when compared with layer 5, which also contains similar types of cells. It has been conjectured that this may potentially be due to increased Aβ deposits in layer 3 as a result of pathology that subsequently prompts a greater amount of microglial engulfment in this region of the cortex. Further research needs to be conducted to determine a cause for this effect, as well as the effect of various other factors on these values, such as the participants' individual cognitive abilities, birth sex, and other information.

The impacts of this study include furthering our current understanding of the mechanisms and pathology underlying AD, as well as the role that microglial engulfment of Aβ plaques plays in the progression of the disease. Previous studies have established that microglia can aid in clearing Aβ plaques through phagocytosis. That activation of microglia also leads to the secretion of neuroinflammatory substances that can cause worsening of AD symptoms, and this study's finding that microglia are most commonly colocalized in AD in cortical layer 3 provides a more specific "hotspot" location of increased microglial activity. Given this information, layer 3 may be subject to increased neuroinflammation, clearing of Aβ plaques, or any other number of effects that may be caused by increased microglial activity. This region can be targeted by studies or treatments aiming to better understand or utilize this relationship through innovations that can be developed to target this layer specifically.

Various limitations to this study should be acknowledged, including how the layers were grouped for analysis and how the statistics were handled. The SEA-AD dataset grouped cortical layers 5 and 6 together in the Quantitative Neuropathology file, which may have affected the specific analysis conducted between layers 3 and 5/6. Although layers 5 and 6 are occasionally grouped as "deep cortical layers" that primarily function as regions containing many efferent pathways, their cellular composition is different, which may have potentially confounded this analysis. The file also only contains data from the MTG, which means the results may have limited generalizability to the entire brain, especially in later stages of the disease, when pathology spreads to many different regions of the cortex.[14] In the future, similar tests to those conducted in this study may be applied to different datasets or subjected to more statistical analyses to corroborate these findings further.

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