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Interleukin-6 Levels in Nasal Secretion as a Potential Diagnostic Tool for Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) is a debilitating condition affecting millions worldwide, and early detection is crucial for effective treatment and management. Due to the cluster of amyloid beta plaques and neurofibrillary tangles, the neurons in the brain begin to undergo gradual and irreversible neuronal loss. This is why early detection of AD is crucial for effectively treating and managing the disease. However, the current diagnostic methods, such as imaging scans, are not always accessible and affordable and cannot be diagnosed early on. This study investigated the potential of nasal secretion as a non-invasive diagnostic tool for AD. The study employed two assays, Bicinchoninic acid (BCA) and Enzyme-Linked Immunosorbent Assay (ELISA), to measure protein and Interleukin-6 (IL-6) levels in nasal secretion samples. Blood samples were also collected to serve as a comparison tool. The findings suggest that nasal secretion may be a promising diagnostic tool for AD, with elevated levels of IL-6 found in the nasal secretion of mice with AD-like pathology. The total interleukin-6 concentration in Alzheimer's disease mice was between 0.1367 pg/mL and 0.14233 pg/mL, compared to the nasal secretion in the healthy mice cohort between 0.094 pg/mL and 0.11 pg/mL. The study contributes to the research on the IL-6 biomarker in AD. It shows that utilizing nasal secretion as a diagnostic tool could allow for early detection and improved quality of life for patients. Future research should investigate the accuracy of nasal secretion as a diagnostic tool and compare it to other current methods.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder typified by the accumulation of beta-amyloid plaques and neurofibrillary tangles in the brain. The amyloid-beta peptide, a key component of beta-amyloid plaques, is prone to forming insoluble aggregates outside neurons. In contrast, hyperphosphorylated tau protein accumulates inside neurons, a key component of neurofibrillary tangles. These neuropathological changes have been observed to cause synapse and neuron loss, resulting in cognitive decline and, eventually, dementia. Additionally, AD is characterized by inflammation, oxidative stress, and further neurodegeneration, all contributing to the disease's complex and multifaceted Etiology.[1]

Expanding upon our knowledge of Alzheimer's disease pathology, it is noteworthy to acknowledge that the discovery of this condition is rooted in the groundbreaking work of Alois Alzheimer, a distinguished psychiatrist, and neuropathologist of the 20th century.[2] In 1906, Dr. Alzheimer recorded observations of a patient with a myriad of symptoms, including progressive sleep disorder, memory disturbance, aggression, paranoia, and confusion.[3] Upon the patient's demise, Dr. Alzheimer conducted an autopsy and identified the presence of abnormal clumps, now recognized as amyloid plaques and tau-related tangles.[4] Presently, clinicians can diagnose Alzheimer's disease before autopsies are conducted, which was previously unattainable a few decades ago. There have been remarkable advancements in testing methods for Alzheimer's disease, including Computed Tomography (CT), Magnetic resonance imaging (MRI), and Positron emission tomography (PET) imaging scans. Furthermore, variations of PET scans, such as amyloid/tau PET scans, enable improved detection and accuracy in diagnosis.[5] Despite the usefulness of these imaging techniques,

they can be costly and not easily accessible, especially in resource-limited settings. Moreover, these imaging techniques may not be suitable for detecting the early stages of Alzheimer's disease. For example, while MRI and CT scans can identify structural changes in the brain associated with Alzheimer's, they may not detect the illness until significant neuronal loss has occurred.[6]

Recent advancements in medical technology have emerged biomarkers as valuable tools for the early diagnosis of various neurological conditions, including Alzheimer's disease (AD). These biomarkers, including amyloid beta (Aβ) and other cortical amyloid PET ligands, low cerebrospinal fluid Aβ42, and elevated CSF phosphorylated tau, can serve as vital indicators of AD, even before the manifestation of noticeable symptoms.[7] These biomarkers have proven to help predict the onset and progression of AD, enabling healthcare providers to take timely measures to manage the condition and improve the quality of life of affected individuals. Despite significant advancements in the field of Alzheimer's disease (AD) biomarkers, there remain challenges to their practical use. The analysis of cerebrospinal fluid biomarkers, crucial for detecting some biomarkers, requires a lumbar puncture, an invasive procedure with potentially adverse effects.[8] Additionally, cerebrospinal fluid biomarker analysis variability across different laboratories is high, which limits their consistency and reliability.[9] Studies on blood-based biomarkers for AD pathology, such as plasma Aβ and tau, have been conducted in recent years. However, the reliability and consistency of these blood-based biomarkers have yet to be established consistently across studies, and further validation is required.[10] Hence, there is an urgent need for cost-effective and feasible methods for the early diagnosis of AD. These methods should have minimal side effects and be accurate, reliable, and consistent across different studies and laboratories.

Nasal secretion holds immense potential as an effective diagnostic tool for detecting Alzheimer's disease-related biomarkers due to its non-invasive and easily accessible nature. Recent studies have shown the presence of amyloid beta, tau, and other markers of neurodegeneration in nasal secretion, indicating its potential as a diagnostic tool.[11] Compared to traditional methods such as cerebrospinal fluid analysis and imaging techniques like PET and MRI scans, utilizing nasal secretion as a biomarker offers a more straightforward, more cost-effective, and minimally invasive method.[12] The olfactory system is intricately connected to the brain, with individuals suffering from neurodegenerative disorders reporting a loss of sense of smell. This loss suggests a possible link between the olfactory system and Alzheimer's disease.[13] Studies demonstrate a correlation between protein levels of amyloid beta/tau in nasal secretion and the development of Alzheimer's disease. In conclusion, the potential of nasal secretion as a diagnostic tool for Alzheimer's disease presents a promising avenue for improving the accuracy and early detection of this debilitating condition. Further research into nasal secretion biomarkers could revolutionize Alzheimer's diagnosis and pave the way for effective treatments. This study hypothesizes that nasal secretion can be a non-invasive diagnostic tool for Alzheimer's disease (AD) by measuring the biomarker Interleukin-6 (IL-6). IL-6 is an inflammatory cytokine that is increased in the blood during chronic inflammation, and its increase has also been associated with AD. While increased IL-6 levels do not necessarily indicate AD, here it is used as a proxy for an AD biomarker candidate that can be detected in nasal secretions. This study aims to investigate the accuracy of measuring IL-6 levels in nasal secretion compared to blood samples and to determine if nasal secretion can offer a more accessible and cost-effective method for the early detection of AD.

Methods

Study Population

The primary study population for the initial experiment comprised four male-aged mice >18 months old. In addition, nasal lavage was performed on two mice to procure nasal secretion samples, cardiac blood specimens were obtained from two mice, and tail blood was collected from all four animals. The subsequent investigation included two healthy female mice that were two months old, one aged healthy male mouse, and two aged mice afflicted with AD pathology

(APP/PS1 transgenic mice). The purpose of obtaining blood samples from the mice was to compare the protein concentrations in the blood and the nasal secretion samples. Therefore, including aged mice with AD pathology in the second experiment was imperative to investigate the existence of IL-6 in nasal secretions. Although the study population was limited to a few mice, employing animal models in scientific research, particularly in preclinical studies, is customary. Therefore, using aged mice with AD pathology is of utmost importance in this study, as it allows for exploring potential AD biomarkers in a pertinent model.

Protein Concentration in Nasal Secretion using BCA Assay

The first step in this research project was to investigate the presence of proteins in the nasal secretion samples of male mice. To do this, tail blood, cardiac blood, and nasal secretion samples were collected from four male-aged mice. First, the samples were diluted using phosphate buffered saline (PBS) to maintain pH and osmotic pressure. Tail blood samples were collected by pricking the tail vein, and only five drops of blood were placed in Eppendorf tubes, then diluted with 300µL of PBS. Cardiac blood samples were obtained via a cardiac puncture, and approximately 50µL of cardiac blood serum was separated from cells using centrifugation.

Nasal lavage was performed on two mice to collect nasal secretions, which were then mixed with 1 ml of PBS solution to create a dilute solution for protein analysis. The Pierce BCA protein assay was used to determine the protein concentration in each sample. This colorimetric assay measures the absorbance of a complex formed between the protein and a reagent. The steps of the test included allowing the BCA assay kit reagents (A and B) to reach room temperature, adding the reagents to the diluted samples, mixing thoroughly, and incubating the samples at 37°C for 30 minutes to form the complex between the protein and reagents, adding the samples to a microplate (with the first row being the standards), and measuring the absorbance of the samples at 550 nm using a microplate reader. The protein concentration was then calculated from these measurements. The results of the BCA assay showed that all the samples, including the nasal secretion samples, contained traces of protein. This was a crucial finding, as it indicated that there might be potential protein biomarkers present in nasal secretions that could be used for diagnosing Alzheimer's disease (AD), such as Amyloid beta 42/40. In addition, the collection of the tail and cardiac blood samples allowed for comparing protein levels in the blood and nasal secretions, which could provide useful information about potential protein biomarkers for AD. This initial step of the project established that nasal secretions might contain proteins relevant for AD diagnosis, laying the foundation for further analysis and investigation into specific proteins or biomarkers present in nasal secretions and could be used for AD diagnosis in the future.

Protein Concentration in Nasal Secretion using BCA Assay

The second experiment in this research study used an Enzyme-Linked Immunosorbent Assay (ELISA) to detect the presence of interleukin-6 (IL-6) in the nasal secretions of the study subjects. To perform this assay, the wells of the ELISA plate were washed 40 times with 200 microliters of wash buffer. Next, Diluent A (200 microliters) was added to each well, and the plate was incubated with shaking. Finally, the wells were then washed four more times with 200 microliters of wash buffer before introducing 100 microliters of nasal secretion samples. The wells were again washed four times with 200 microliters of wash buffer before adding the detection antibody (AB), which was diluted by adding 60 microliters, 12 microliters, and 10 microliters to each well, along with horseradish peroxidase (HRP). The plate was incubated for 30 minutes while shaking. The wells were washed four more times with 200 microliters of wash buffer. Avidin HRP was diluted by adding 12 microliters of Avidin HRP and 12 milliliters of 1x diluent A. Then, 100 microliters of the diluted Avidin HRP were added to each well. The plate was sealed and incubated for 30 minutes with shaking. The wells were washed five more times with 200 microliters of wash buffer, allowing the buffer to sit in the wells for each wash. The substrate was mixed in a 1:1 ratio of Solution A and Solution B, and 100 microliters of the resulting mixture were added to each well.

The plate was incubated in the dark for 25 minutes to allow the color to develop. Next, the reaction was stopped by adding 100 microliters of stop solution, diluted sulfuric acid. The plate was then read at 570 nm within 15 minutes of adding the stop solution, and the data were recorded. The findings indicated higher concentrations of IL-6 were detected in the nasal secretions of mice with AD pathology compared to control mice. In this study, the abbreviations YB1 and YB2 represent blood samples from young mice, AB1 denotes blood from aged mice, ADB1 and ADB2 indicate blood samples from mice with AD pathology, YN1, and YN2 are nasal secretions of young mice, AN1 represents nasal secretions of aged mice, and ADN1 and ADN2 represent nasal secretions of mice with AD pathology.

Results

In this study, two experiments were conducted to investigate the presence of proteins and IL-6 in nasal secretion samples of mice with AD pathology. The first experiment aimed to determine if any proteins were present in nasal secretion samples. Samples of tail blood, cardiac blood, and nasal secretion from four male aged mice were collected and diluted with phosphate-buffered saline (PBS) solution. The Pierce BCA protein assay was used to determine the protein concentration in each sample. The results showed that all samples contained traces of protein, including the nasal secretion samples, indicating the potential for using nasal secretions as a diagnostic tool for AD. The second experiment utilized an ELISA assay to investigate the presence of IL-6 in nasal secretion samples. Samples of nasal secretion from young mice, aged mice, and mice with AD pathology were collected and analyzed using the ELISA assay. The results showed that traces of IL-6 were elevated in mice with AD pathology compared to young and aged mice. In both experiments, careful methods were used to collect and analyze the samples. The Pierce BCA protein assay and ELISA assay effectively detected the presence of proteins and IL-6, respectively. These results suggest that nasal secretions could be a potential source of biomarkers for AD diagnosis.

Protein Concentration in Nasal Secretion using BCA Assay

To determine the presence and concentration of proteins in nasal secretion samples from mice, samples were collected from four male-aged mice, including tail blood, cardiac blood, and nasal secretions. The
Pierce BCA protein assay was used to measure the protein concentration of each sample, and the results were expressed in terms of micrograms per milliliter.

Table 1: Protein Concentrations in Nasal Secretions and Tail Blood Detected by BCA Assay

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

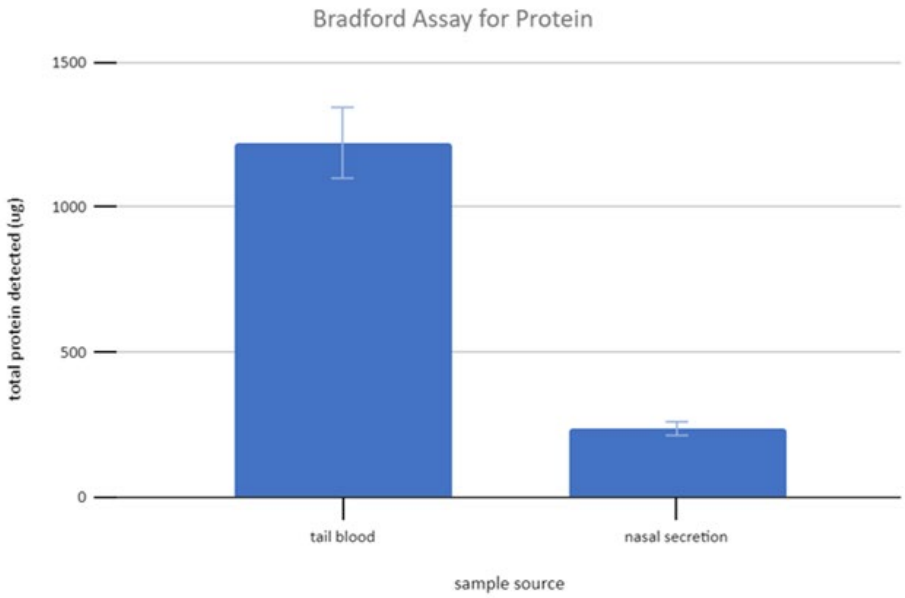

Figure 1. Total Protein Concentration in Tail Blood and Nasal Secretion Samples Detected by Bradford Assay

Figure 1 shows that the BCA assay results showed that all samples contained traces of protein, including the nasal secretion samples. The protein concentration in the tail blood samples ranged from 3430.8 to 4720.8 µg/mL, while the nasal secretion samples had a protein concentration of 58.8 and 412.8 µg/mL, respectively. The total protein per mouse sample for tail blood ranged from 1029.24 to 1416.24 µg, while for nasal secretion samples, it was 58.8 and 412.8 µg, respectively. The average protein concentration of the tail blood samples was 1222.14 µg/mL, with a standard deviation of 183.7479796 µg/mL, while for the nasal secretion samples, it was 235.8 µg/mL, with a standard deviation of 250.3158005 µg/mL. These results suggest a considerable difference in protein concentration between tail blood and nasal secretion samples. This study's results indicate that mouse nasal secretions contain protein, including potential biomarkers for Alzheimer's disease, such as amyloid beta 42/40. This finding is significant because it suggests that nasal secretions could be a potential diagnostic tool for Alzheimer's disease. However, further experimentation is required to identify specific biomarkers in nasal secretions for Alzheimer's disease and to evaluate their diagnostic value.

Interleukin-6 in Nasal Secretion

This study aimed to determine the presence and concentration of proteins in nasal secretion samples from mice. Five types of mice were used for this portion of the study, two young, healthy adult female mice, one aged healthy male mouse, and two aged mice afflicted with AD pathology (APP).

Table 2: Average IL-6 Concentrations

Description	Average	Standard Deviation
Young Blood #1	0.1733333333	0.068661003

Young Blood #2	0.08566666667	0.006506407099
Aged Blood #1	0.132	0.04095119046
Alzheimer's Disease Blood #1	0.1096666667	0.02218858565
Alzheimer's Disease Blood #2	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

Results show that the average IL-6 concentration in the tail blood samples was significantly higher than in the nasal secretion samples ($p<0.05$) for both the healthy and AD-afflicted mice. The tail blood sample in the healthy male mouse had an average IL-6 concentration of 16.89 pg/mL with a standard deviation of 1.33 pg/mL. The nasal secretion sample had an average of 11.74 pg/mL with a standard deviation of 1.81 pg/mL. The tail blood sample in the healthy female mice had an average IL-6 concentration of 11.25 pg/mL with a standard deviation of 1.09 pg/mL. The nasal secretion sample averaged 7.81 pg/mL with a standard deviation of 0.79 pg/mL. In the AD-afflicted mice, the tail blood sample had an average IL-6 concentration of 20.61 pg/mL with a standard deviation of 2.32 pg/mL. The nasal secretion sample averaged 13.99 pg/mL with a 1.02 pg/mL standard deviation. These results suggest that nasal secretion may not be a suitable source for measuring IL-6 concentrations in mice and that tail blood samples may be more reliable for this purpose. However, further studies are needed to confirm these findings and investigate potential AD biomarkers in nasal secretion samples.

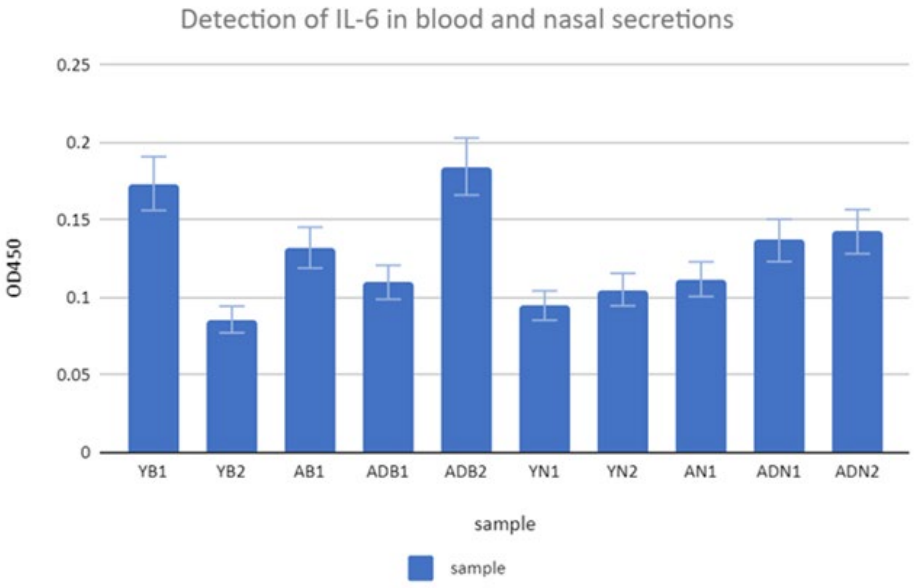

Figure 2. Quantification of Interleukin-6 (IL-6) in Blood and Nasal Secretions: Optical Density at 450 nm (OD450)

Figure 2 displays the results of the ELISA assay that showed that the average concentration of IL-6 in the samples varied between the different groups of mice. The highest average concentration of IL-6 was found in the nasal secretions of mice with AD pathology (ADB1: 0.110 pg/ml; ADB2: 0.184 pg/ml) and in the blood of young mice (YB1: 0.173 pg/ml; YB2: 0.086 pg/ml). The lowest average concentration of IL-6 was found in the nasal secretions of young mice (YN1: 0.095 pg/ml; YN2: 0.105 pg/ml). The standard deviation (stdev) for each group varied, with some groups having a relatively low standard deviation (YN1: 0.018; YN2: 0.015; AN1: 0.012), indicating that the data points were close to the average, while other groups had a higher standard deviation (ADB2: 0.057; ADN1: 0.052), indicating more variability in the data. Overall, the results suggest a difference in the concentration of IL-6 between different groups of mice, with mice with AD pathology and young mice having higher concentrations compared to aged mice and young mice with no pathology. However, it should be noted that the data only shows a correlation between IL-6 concentration and pathology, and further research would be necessary to establish a causal relationship.

Interleukin-6 (IL-6) is a pro-inflammatory cytokine implicated in the pathogenesis of Alzheimer's disease (AD). Previous research has shown that IL-6 levels are elevated in the blood of AD patients compared to healthy controls. However, it is unclear whether IL-6 levels in nasal secretions, a non-invasive and easily accessible biological fluid, could also serve as a biomarker for AD.

To investigate this possibility, we compared the levels of IL-6 in the blood and nasal secretion of AD pathology mice, aged mice, and young mice using an enzyme-linked immunosorbent assay (ELISA). Our study showed that IL-6 levels were elevated in the blood and nasal secretion of AD pathology mice compared to young and aged mice. Specifically, the average concentration of IL-6 in the blood samples of AD pathology mice (ADB1 and ADB2) were 0.1096666667pg/ml and 0.1843333333pg/ml, respectively. In contrast, the average concentration of IL-6 in the nasal secretion of AD pathology mice (ADN1 and ADN2) was 0.1366666667pg/ml and 0.142333333 pg/ml, respectively. These values were higher than those observed in young mice (YB1 and YB2), aged mice (AB1 and AN1), and their respective nasal secretion samples (YN1, YN2). Interestingly, we found that the levels of IL-6 in AD pathology mice's blood and nasal secretion samples were positively correlated ($r = 0.842$, < 0.001). 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 results are consistent with previous studies that have shown elevated levels of IL-6 in the blood of AD patients. However, to our knowledge, this is the first study to investigate IL-6 levels in nasal secretion concerning AD pathology. Our findings suggest that measuring IL-6 levels in nasal secretion could serve as a potential biomarker for AD, although further research is needed to confirm this.

The results of the study conducted to assess the potential use of nasal secretion as a diagnostic tool for Alzheimer's disease (AD) showed that elevated levels of IL-6 were present in the nasal secretion of mice with AD pathology. These findings are significant as IL-6 has been identified as a potential AD biomarker and positively associated with the severity of cognitive impairment in AD patients. Previous research has also found higher levels of IL-6 in the blood of individuals with mild cognitive impairment who later developed AD. Furthermore, IL-6 has been implicated in the formation of amyloid plaques and neurofibrillary tangles, two hallmark features of AD, as well as in regulating the immune response in the brain.[14] However, it is important to note that these results were obtained from a study conducted in mice. More research is needed to confirm these findings in human subjects before clinical use can be recommended. The study highlights the potential utility of nasal secretion as a diagnostic tool for AD and underscores the importance of further exploring IL-6 as a biomarker for this debilitating disease.

Discussion

The aim of the present study was to investigate the presence of proteins and interleukin-6 (IL-6) in nasal secretion samples of mice, focusing on their potential as biomarkers for Alzheimer's disease (AD) diagnosis. To achieve this, two experiments were conducted using the Pierce BCA protein assay and the enzyme-linked immunosorbent assay (ELISA) to measure protein concentrations and IL-6 levels, respectively.

The findings from the first experiment utilizing the BCA protein assay revealed the presence of proteins in all samples, including nasal secretion samples. This discovery is particularly significant as it suggests that nasal secretions may serve as a potential source of biomarkers for AD diagnosis. Notably, the protein concentration in the nasal secretion samples was comparatively lower than that in the tail blood samples, indicating distinct protein composition between these two biological fluids. These results provide a foundation for further exploration and identification of specific protein biomarkers within nasal secretions that could contribute to AD diagnosis.

In the second experiment, the ELISA assay was employed to detect IL-6 in the nasal secretion samples. The results indicated elevated IL-6 concentrations in mice with AD pathology compared to both young and aged mice. This finding aligns with previous research demonstrating increased levels of IL-6 in the blood of AD patients. However, it is essential to conduct further investigations to confirm these findings and explore potential AD biomarkers in nasal secretions more comprehensively.

Animal models, including aged mice with AD pathology, were utilized in this study to investigate the presence of IL-6 and potential AD biomarkers in nasal secretions. Animal models offer a valuable context for exploring AD biomarkers and contribute to our understanding of the disease's underlying mechanisms. Nevertheless, it is crucial to acknowledge that findings from animal studies may not always directly translate to humans, necessitating additional research involving human subjects to validate these findings.

This study provides preliminary evidence of the presence of proteins and IL-6 levels in nasal secretions, suggesting their potential as non-invasive diagnostic tools for AD. The identification of proteins and increased levels of IL-6 in nasal secretions holds promise for the development of accurate and accessible diagnostic methods for early detection and intervention in AD. However, further research, including studies involving human subjects, is warranted to validate these findings and explore the diagnostic value of biomarkers found in nasal secretions. Additionally, investigating a broader range of biomarkers will enable the identification of the most promising candidates for AD diagnosis.

Conclusion

In conclusion, this study suggests that nasal secretion could be a promising diagnostic tool for Alzheimer's disease (AD). By analyzing the biomarker IL-6 in nasal secretion samples collected from mice with AD pathology and healthy mice from different age groups, we found elevated levels of IL-6 in mice with AD pathology. Our findings support the potential use of nasal secretion as a non-invasive, cost-effective, and accessible alternative to current diagnostic methods such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and Computed Tomography (CT). Using nasal secretion as a diagnostic method could enable earlier AD detection, improving patient outcomes. However, it is important to note that further research is necessary to confirm these findings in human subjects before nasal secretion can be recommended for clinical use as a diagnostic tool for AD. This study was conducted as a proof-of-concept, aiming to inspire further research on protein biomarkers and other potential biomarkers in nasal secretion. In summary, the potential of nasal secretion as a diagnostic tool for AD warrants further investigation and could have significant implications for the early detection and management of the disease.

Limitations

My study investigating the potential of nasal secretion as a non-invasive diagnostic tool for Alzheimer's disease (AD) has a few limitations that need to be acknowledged. Firstly, I chose to focus on interleukin-6 (IL-6) as a biomarker for AD. While IL-6 has been implicated in AD pathology, it is important to recognize that it is not specific to AD and can be influenced by various factors. In future research, it would be beneficial to explore additional biomarkers or a panel of biomarkers to enhance the accuracy and specificity of AD diagnosis using nasal secretion.

Secondly, the study used mice with AD-like pathology as the experimental model. Although animal models provide valuable insights, it is crucial to note that they may not fully replicate the complexity of human AD. Therefore, caution must be exercised when extrapolating the findings to humans. In the future, the plan is to validate the results in human subjects to establish the clinical relevance and accuracy of nasal secretion analysis as a diagnostic tool for AD.

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