A Systematic Review on the Feasibility of Salivary Biomarkers for Alzheimer's Disease

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Research article

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Abstract

Background: Early AD diagnosis is critical for ameliorating prognosis and treatment. The analysis of CSF biomarkers yields accurate results, but it necessitates a lumbar puncture procedure. Screening for peripheral biomarkers in saliva is advantageous since this medium is noninvasive and inexpensive to obtain. The objective of this systematic review is to analyze saliva biomarker studies which aim to diagnose AD. Methods: Titles, abstracts, and reference lists for publications from February 2004 to March 2019 were screened for by searching Google Scholar and PubMed. The inclusion criteria involved published studies that consisted of both AD and control groups. Results: 77 studies were screened, and 13 publications fulfilled the inclusion criteria. These selected publications were scrutinized and included in this review. Numerous biomarkers were analyzed, including Aβ42, tau, and various salivary metabolites. Conclusion: Aβ42, tau, lactoferrin, and various metabolites might serve as a reliable biomarkers for AD diagnosis. However, these studies must be replicated with a large sample size. It is also important to standardize the analytical methods of measuring salivary biomarkers to establish coherence for the selection of valid AD biomarkers. Saliva composition can be affected by production rate, circadian rhythms, and oral health, so their cumulative effect on the accuracy of saliva testing requires further investigation. Keywords: Alzheimer, biomarker, saliva

Background

Alzheimer's Disease (AD) is the most common cause of dementia. Approximately 60% to 70% of dementia cases pertain to AD [7]. AD affects 46 million individuals, and 131.5 million people are projected to have AD by 2050 [1]. The prevalence of AD is most common among the senior demographic (65+). Its direct cause is unclear, but a multitude of neurodegenerative processes have been associated with the disease. The formation of intracellular amyloid plaques and neurofibrillary tau tangles constitute some of the neuropathological hallmarks of AD. These compounds are expressed several decades before AD can be clinically diagnosed.

There are several ongoing clinical trials that aim to halt the progression of AD in presymptomatic stages. Aducanumab and Solanezumab are monoclonal antibodies that have been investigated for their potency against soluble Aβ oligomers. After undergoing phase III trials, it was concluded that neither drug significantly affected cognitive decline [4,11]. This suggests that "plaque-busting" drugs may yield no significant effect if administered during the symptomatic phase of AD. Therefore, there is a pressing need to develop a simple and non-invasive test which can pinpoint the presence of AD in its presymptomatic phase so that treatment can mitigate its neurodegenerative effect.

Currently, an array of clinical approaches are implemented to determine the presence of AD. Utilizing a biomarker-driven test may also aid in establishing an early diagnosis. Biomarkers in CSF are used to

detect AD. Analyzing levels of Aβ42, tau, and phosphorylated tau in CSF is an accurate approach [5], but this method is both costly and invasive. Biomarkers in saliva are being explored as an alternative diagnostic approach.

Significantly altering the autonomic nervous system (ANS) function may have an effect on salivary production and composition [9]. The ANS is responsible for maintaining saliva secretion by innervating the glossopharyngeal cranial nerve and the facial cranial nerve. During presymptomatic AD, damage is induced to the nerve endings of the cholinergic system, which compromises ANS function. Saliva contains a plethora of biomarkers, as about 40% of diagnostic blood proteins are also found in saliva [13]. It features many advantages for diagnostic purposes compared to other bodily fluids. Saliva is both cheap and easy to obtain, as it can be collected in a non-invasive manner. These advantages facilitate the sampling process of this medium, which is useful for rapid disease screening. The objective of this review is to provide an overview of the literature pertaining to the utility of saliva as a medium for analyzing biomarkers that are specifically associated with AD. Developing accurate salivary diagnostics can facilitate early intervention of AD, which in turn may improve AD treatment.

Methods

A standard protocol was implemented for selection of publications in this review. PubMed and Google Scholar were used to conduct a literature search for publications from February 2004 to March 2019. Searches were conducted with the following keywords: *Alzheimer*, *biomarker*, *dementia*, *saliva*. Titles, abstracts, and reference lists were used to select pertinent publications. Studies included in this systematic review are original publications that analyze potential salivary AD biomarker candidates. Each study consisted of saliva samples from both AD subjects and control subjects. Subject metrics such as age, gender, and sample size for both AD and control groups were considered, along with biomarker type, technique of biomarker quantification, and statistical analysis.

Results

Of the 77 screened studies, 13 were selected, scrutinized, and included in this review. Several studies determined the potential utility of Aβ42 and tau as salivary biomarkers, but other compounds including acetylcholinesterase, lactoferrin, trehalose, and metabolites were investigated as well.

Acetylcholinesterase

Salivary acetylcholinesterase (AchE) levels were analyzed in two studies. Ellman's colorimetric method was implemented for both of the studies. Bakhtiari *et al*. tested saliva samples from 15 AD subjects and 15 control subjects. Higher levels of AchE was reported, but statistical significance was not established. Sayer *et al*. tested saliva samples from 47 volunteers (22 AD cases, 14 AD nonresponder cases, and 11 control cases). They found an overall negative correlation between age and AchE levels, as the r-value was -0.768 (with $p<0.001$). It was also reported that AD subjects had 73% lower levels of AchE in comparison to control subjects (with $p<0.005$) [22].

Aβ42

Salivary Aβ42 levels were analyzed in four studies, and all of them utilized an enzyme-linked immunosorbent-type assay (ELISA) with the exception of one. Bermejo-Pareja *et al*. tested 126 saliva samples from both AD and control cases, in addition to 51 saliva samples from Parkinson's patients. They concluded that salivary Aβ42 levels were significantly greater in patients suffering from mild to moderate AD, but not for patients with severe AD. Their results did not conclude a significant difference between Parkinson's patients and controls. Lee *et al*. analyzed the expression of Aβ42 in both saliva and other tissues. 37 volunteers participated in the study, including 27 non-AD and 7 AD cases. They reported a mean of 22.060.41 pg/mL of salivary Aβ42 for the non-AD cases and a mean of 59.076.33 pg/mL for the AD cases. Kim *et al*. utilized an immunoassay containing nanobeads to detect salivary Aβ42 levels for 45 individuals (28 AD cases and 17 normal controls). Their results concluded higher levels of salivary Aβ42 for the AD cases vs. control cases, but their study did not have a p-value. Sabbagh *et al*. analyzed salivary Aβ42 levels from 15 AD patients and 7 normal controls. They reported a mean of 21.1 0.3 pg/mL for the normal controls and a mean of 51.71.6 pg/mL for AD cases, with $p<0.001$.

Tau

Salivary tau levels were analyzed in three studies. Ashton *et al*. tested 213 saliva samples from both AD and control cases, in addition to 68 saliva samples from individuals with aMCI. This study used the single-molecule array (SIMOA) technique to analyze total tau levels. Increased t-tau levels in AD patients were observed, but statistical significance was not established. Shi *et al*. utilized an ELISA to analyze p-tau and t-tau levels. 59 volunteers participated in this study, which included 21 AD cases and 38 control cases. Increased p-tau/t-tau ratio levels were reported for AD patients, with $p<0.05$. Pekeles *et al*. obtained unstimulated saliva in order to analyze the p-tau/t-tau ratio at different phosphorylation sites (S396,S400,S404,T403,T404). This study implemented the Western Blot analysis method to quantify their findings. 337 volunteers participated throughout the two clinical studies conducted by Pekeles *et al*.,

including 87 AD subjects and 167 control subjects. Their first study included 55 aMCI subjects as well, and their second study included 16 FTD subjects and an additional 12 neurological patients that did not suffer from dementia. Their findings indicated a significantly higher p-tau/t-tau ratio at the S396 site for AD patients in comparison to the elderly control individuals. However, they reported no correlation between elevated salivary tau levels and both CSF tau and hippocampal volume. There was also significant variation for salivary tau levels in AD subjects, which may pose a limitation towards implementation of tau as a legitimate AD biomarker.

Trehalose and Lactoferrin

Both trehalose and lactoferrin levels in saliva were analyzed in two studies. Lau *et al*. utilized an extended gate ion-sensitive field-effect transistor (EG-ISFET) biosensor to analyze salivary trehalose. 60 saliva samples were tested, including 20 AD subjects, 20 PD subjects, and 20 control subjects. Higher salivary trehalose levels were found in the AD subjects, but statistical significance was not established. Carro *et al*. used an ELISA to detect salivary lactoferrin levels. The objective was to determine if decreased lactoferrin levels could serve as an indicator of AD. 365 individuals participated throughout the two clinical studies conducted by Carro *et al*., including 116 AD subjects, 59 aMCI subjects, and 131 control subjects. Their first study also included 59 aMCI subjects. Mass spectrometry was implemented to confirm that this protein could be detected in saliva before further experimentation. This study also analyzed salivary lactoferrin levels for aMCI and PD subjects. Carro *et al*. concluded (with $p<0.001$) significantly lower levels for both AD and aMCI subjects in comparison with the control subjects, but PD subjects had significantly higher levels in comparison with control subjects. 7.43 μg/mL was the established cutoff value between AD/MCI subjects and controls in this study.

Metabolites

Salivary metabolites were analyzed in two studies, which totaled 285 AD subjects, 35 aMCI subjects, and 263 control subjects. Huan *et al*. used liquid chromatography mass spectrometry (LC-MS) to assess the following metabolites: alanylphenylalanine, aminobytyric acid + H2, amino-dihydroxybenzene, choline-cytidine, glucosyl-galactosyl-hydroxylysine * (H2O), histidylphenylalanine, methylguanosine, phenylalanylphenylalanine, phenylalanylproline, and urocanic acid. Their work featured two clinical studies to further confirm their findings. Between AD and control subjects, there was a significant difference for the following metabolites (with $p<0.01$): choline-cytidine, histidylphenylalanine, methylguanosine, phenylalanylphenylalanine, phenylalanylproline, and urocanic acid. Between AD and aMCI subjects, there was a significant difference for the following metabolites (with $p<0.01$):

alanylphenylalanine, aminobytyric acid + H2, amino-dihydroxybenzene, glucosyl-galactosyl-hydroxylysine * (H2O), and phenylalanylproline. Q. Liang *et al*. implemented ultraperformance liquid chromatography mass spectrometry (UPLC-MS) to assess the following metabolites: inosine, ornithine, phenyllactic acid, and spinganine-1-phosphate. They concluded significantly elevated levels of spinganine-1-phosphate and ornithine for AD subjects in comparison to control subjects and significantly lower levels of inosine for AD subjects in comparison to control subjects (with $p<0.01$).

Discussion

This systematic review aims at providing a proper assessment on the literature addressing salivary AD biomarker candidates. The studies observing salivary AchE suggest that it may not serve as a reliable biomarker, despite overall decreased AchE with age [22]. Many other biological factors play a role in affecting overall AchE levels in both the brain and saliva, but significantly lower salivary AchE levels might prove to serve as a potential method of determining a compromised cholinergic system [2]. Salivary Aβ42 seems to be a reliable biomarker, as all four studies in this review analyzing salivary Aβ42 detected significant differences between AD subjects and control subjects. One study reported no significant differences when analyzing other isoforms of Aβ42, including Aβ40 [3]. Two studies concluded significant differences in p-tau and t-tau levels between AD subjects [17,12], but one of these studies reported significant variance in their data [12]. Statistical insignificance in salivary tau levels was determined in one study [18]. It was also reported that salivary tau expression was well characterized at the S396 phosphorylation site [12]. Carro *et al*.'s results show some validation of lactoferrin as a potential biomarker. Lactoferrin is present in several biological fluids and serves as part of the innate immune system. Some studies have suggested that certain pathogens may play a role in AD by compromising the function of the blood-brain barrier, thus enabling accelerated Aβ42 growth. This may justify the reason for lower lactoferrin levels for individuals with AD, but further studies are needed to confirm this. The disaccharide trehalose was analyzed as well as a multitude of metabolites. There seems to be a correlation between the expression of trehalose and metabolism of the Amyloid Precursor Protein (APP) [25]. There were no significant differences in levels of trehalose, but there were significant differences in levels of various metabolites between AD subjects and controls. Further verification is necessary to confirm the validity of these findings.

Several analysis techniques were implemented throughout these studies, so a standardization by which to investigate salivary biomarkers would provide a more coherent method of selecting future AD biomarkers. Many of these clinical studies featured a small sample size, so a large sample needs to be incorporated for future studies in order to establish reliable reference ranges for biomarker expression levels. Saliva production, circadian rhythms, and oral health are important factors which affect saliva composition. This necessitates further research into how these factors may affect the accuracy of saliva as a medium for AD diagnosis. The precise mechanisms by which these biomarkers become secreted in saliva is not understood. There is still a need to acquire insightful knowledge of the mechanisms by

which these biomarkers become secreted in saliva. Advancing the understanding of the pathophysiology of AD requires a thorough comprehension of the association between saliva and AD.

Conclusions

This systematic review intends to determine the feasibility of various salivary biomarkers in order to achieve an early diagnosis of AD. Subject metrics, biomarker type, and methods of biomarker analysis were examined to establish a solid answer on the viability of a saliva test. The purported data indicates that certain salivary compounds may serve as valid AD biomarkers, but a large sample size and a standardization of biomarker analysis techniques must be implemented to further assess the reproducibility of the studies included in this systematic review.

Declarations

Ethics Approval and Consent to Participate:

Not applicable

Consent for Publication:

Not applicable

Availability of Data and Materials:

All analyzed and generated data in this study, as well as supplemental information are included in this manuscript.

Competing Interests:

The author declares no potential conflicts of interest.

Funding:

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Authors' Contributions:

MB conducted the literature search, extracted the data, selected the studies for inclusion, performed the statistical analysis, and wrote the manuscript.

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Tables

Table 1: Summary of results and subjects involved in analyzing salivary AD biomarker levels

	Study	Result	Reference
esterase	● Method of quantification: Ellman's colorimetric method ● AD subjects: 15 (9 male, 6 female) ● Control subjects: 15 (7 male, 8 female) ● Mean age (AD): 78.4 ● Mean age (control): 71	● Higher AchE levels for AD, but statistically insignificant.	[2]
esterase	● Method of quantification: Ellman's colorimetric method ● AD subjects (AchE-1 therapy responsive): 22 (7 male, 15 female) ● AD (AchE-1 therapy unresponsive):14 (4 male, 10 female) ● Control subjects: 11 (6 male, 5 female) ● Mean age (AD responsive): 75 ● Mean age (AD unresponsive):75 ● Mean age (control): 71	● 73% lower AchE levels between AD and control ($p<0.005$), negative correlation between age and AchE levels ($r= -0.0.73$, $p<0.001$)	[22]
	● Method of	● Aβ42 p-value mild=0.043	[3]

	quantification: ELISA ● AD subjects: 70 (21 male, 49 female) ● PD subjects: 51 (26 male,25 female) ● Control subjects: 56 (17 male, 39 female) ● Mean age (AD): 77.2 ● Mean age (PD): 72.96 ● Mean age (control): 74.35	● Aβ42 p-value (control/AD)<0.05 ● Significantly higher Aβ42 levels for mild/moderate AD subjects vs control. Significantly higher Aβ40 levels for PD and control.	
	● Method of quantification: ELISA ● AD subjects: 10 (3 male, 7 female) ● Control subjects and PD subject: 27 (18 male,9 female) ● Mean age (AD): 70.1 ● Mean age (control and PD): 54.6	● Higher Aβ42 for AD vs. control ($p<0.001$). ● Non-AD mean: 22.06±0.41 pg/mL ● AD mean: 59.07±6.33 pg/mL	[15]
	● Method of quantification: Nanobead immunoassay ● AD subjects: 28 (age and gender not provided)	● Significant differences for Aβ42 levels ● Insignificant levels for Aβ40 ● No p-value provided during study	[6]

	● Control subjects: 17 (age and gender not provided)		
	● Method of quantification: ELISA ● AD subjects: 15 (7 male, 8 female) ● Control subjects: 7 (2 male, 5 female) ● Mean age (AD): 77.8±1.8 ● Mean age (control): 60.4±4.7	● Significant differences for Aβ42 vs. control ($p<0.001$) ● Non-AD mean: 21.1±0.3 pg/mL ● AD mean: 51.7±1.6 pg/mL	[16]
	● Method of quantification: SIMOA ● AD subjects: 53 (23 male, 30 female) ● aMCI subjects: 68 (33 male, 35 female) ● Control subjects: 160 (66 male, 94 female) ● Mean age (AD): 81.4±6.6 ● Mean age (aMCI): 79.8±7.4 ● Mean age (control):78.0±6.7	● Statistically insignificant differences for AD, aMCI, and control ($p=0.219$)	[18]
ɔ-tau, t-	● Method of quantification: ELISA ● AD subjects: 21 (10 male, 11	● Significance in the differences of p-tau/t-tau, p-tau, and t-tau for AD vs. control ($p<0.05$)	[17]

	female) ● Control subjects: 38 (19 male, 19 female) ● Mean age (AD): 68.8 ● Mean age (control): 69		
	● Method of quantification: Western Blot <u>Study 1</u> ● AD subjects: 46 (24 male, 22 female) ● aMCI subjects: 55 (23 male, 32 female) ● Control subjects: 47 (15 male, 32 female) ● Median age (AD): 80 ● Median age (aMCI): 78 ● Median age (control): 73 <u>Study 2</u> ● AD subjects: 41 (17 male, 24 female) ● FTD subjects: 16 (11 male, 5 female) ● Young controls: 76 (31 male, 45 female)	● Significant p-tau/t-tau ratio difference for AD vs. elderly control expressed at S396 ($p<0.05$). ● S396 sensitivity: 73% ● S396 specificity: 50% ● No correlation between salivary tau levels and CSF tau. ● No correlation between salivary tau levels and hippocampal volume.	[12]

	● Neurological subjects w/o dementia: 12 (5 male, 7 female) ● Older controls: 44 (14 male, 30 female) ● Mean age (AD): 80 ● Mean age (FTD): 71.5 ● Mean age (young controls): 32 ● Mean age (neurological subjects w/o dementia): 55 ● Mean age (older controls): 72		
	● Method of quantification: EG-ISFET biosensor ● AD subjects: 20 (8 male, 12 female) ● PD subjects: 20 (5 male, 15 female) ● Control subjects: 20 (9 male, 11 female) ● Mean age (AD): 72.5±7.68 ● Mean age (PD): 73±8.07 ● Mean age (control): 66.1±7.79	● Higher trehalose levels for AD subjects and PD subjects vs. control, but statistically insignificant	[10]

Study 1 ● AD subjects: 80 (31 male, 49 female) ● aMCI subjects: 44 (19 male, 25 female) ● PD subjects: 59 (27 male, 32 female) ● Control subjects: 91 (32 male, 59 female) ● Mean age (AD): 76.2±5.33 ● Mean age (aMCI): 75.16±5.13 ● Mean age (PD): 69.5±8.6 ● Mean age (control): 73.7±6.88 Study 2 ● AD subjects: 36 (13 male, 23 female) ● aMCI subjects: 15 (10 male, 5 female) ● Control subjects: 40 (15 male, 25 female) ● Mean age (AD): 80.67±8.67 ● Mean age (aMCI): 68.93±6.12	● Significantly lower levels of lactoferrin for AD and aMCI vs. control ($p<0.001$) ● PD subjects had significantly higher lactoferrin levels vs. controls ● Cutoff value between AD/aMCI and controls: 7.43 µg/mL	[8]

	● Mean age (control): 66.78±7.33		
	● Method of quantification: LC-MS <u>Study 1</u> ● AD subjects: 22 (6 male, 16 female) ● aMCI subjects: 25 (10 male, 15 female) ● Control subjects: 35 (13 male, 22 female) ● Mean age (AD): 77.09 ● Mean age (aMCI): 70.4 ● Mean age (control): 69.94 <u>Study 2</u> ● AD subjects: 7 (2 male, 5 female) ● aMCI subjects: 10 (5 male, 5 female) ● Control subjects: 10 (5 male, 5 female) ● Mean age (AD): 70.11 ● Mean age (aMCI): 71.5 ● Mean age (control): 71.4	● Significant differences of following metabolites for AD vs. control ($p<0.01$): ● Choline-cytidine ● Histidylphenylalanine ● Methylguanosine ● Phenylalanylphenylalanine ● Phenylalanylproline ● Urocanic acid ● Significant differences of following metabolites for AD vs. aMCI ($p<0.01$): ● Alanylphenylalanine ● aminobytyric acid + H2 ● Amino-dihydroxybenzene ● Glucosyl-galactosyl-hydroxylysine * (H2O) ● Phenylalanylproline	[24]

	● Method of quantification: UPLC-MS ● AD subjects: 256 (124 male, 132 female) ● Control subjects: 218 (102 male, 116 female) ● Mean age (AD): 78.6±6.80 ● Mean age (control): 77.9±5.60	● Significantly higher levels of spinganine-1-phosphate and ornithine for AD vs. control ($p<0.01$) ● Significantly lower levels of inosine for AD vs. control ($p<0.01$)	[20]

Figures

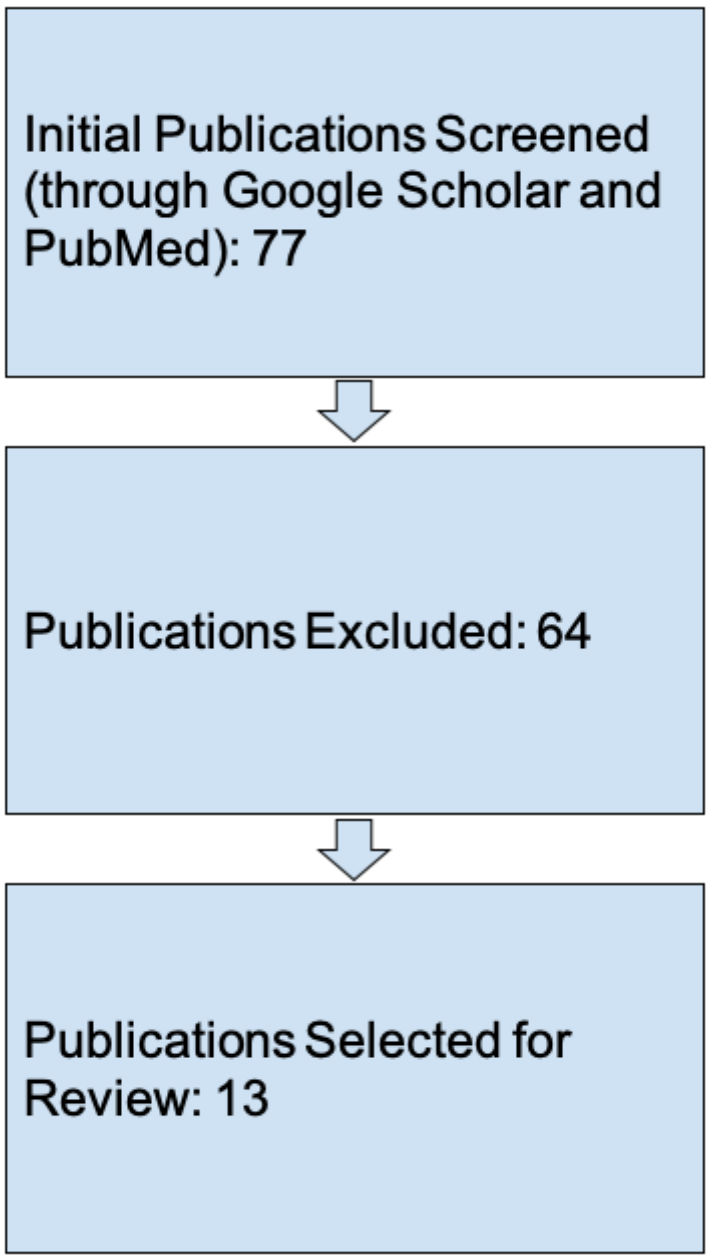

Figure 1

Publication Search and Selection Flowchart.

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