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Review Article

The role of salivary biomarkers in Alzheimer's disease: Advancing early detection and monitoring

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Abstract

Alzheimer's disease (AD), a progressive neurodegenerative disorder, poses significant challenges in early diagnosis and effective management. Recent advances highlight the potential of salivary biomarkers as a non-invasive, cost-effective tool for diagnosing and monitoring AD. Saliva, with its rich biochemical composition, offers an accessible medium for detecting various biomarkers, including amyloid-beta (Aβ) peptides, tau proteins, oxidative stress indicators, and inflammatory cytokines. This review explores the role of these biomarkers in reflecting the pathological processes of AD, emphasizing their correlation with cognitive decline and neurodegeneration. Additionally, emerging technologies such as proteomics and metabolomics are enhancing the sensitivity and specificity of salivary biomarker detection. However, challenges such as variability in salivary composition, standardization of collection methods, and the need for robust validation studies remain significant barriers to clinical implementation. By integrating insights from recent research, this review underscores the transformative potential of salivary biomarkers in advancing early AD detection and personalized therapeutic strategies.

Keywords: Alzheimer's disease, Salivary biomarkers, non-invasive diagnosis, Amyloid-beta, Neurodegeneration.

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1. Introduction

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative condition that predominantly affects older adults and is the most common cause of dementia, accounting for nearly two-thirds of cases worldwide.[1,2] Globally, an estimated 41 million people with dementia remain undiagnosed, with only about 25% of cases being clinically recognized.[3] AD exhibits complex pathobiology and diverse clinical manifestations, characterized by hallmark neuropathological features such as amyloid-beta (Aβ) plaques, formed by aggregated Aβ, and neurofibrillary tangles, composed of aggregated tau proteins.[4] These pathological changes contribute to synaptic and neuronal loss, neurotransmitter deficiencies, neuroinflammation, and reactive astrogliosis, ultimately leading to cognitive impairment.[5] Early diagnosis is essential for improving patient outcomes, facilitating timely interventions, and mitigating AD's societal and economic impact.[6,7] This review is mainly concerned with the salivary biomarkers of the most common Alzheimer's Disease (**Figure 1**).

The pathological progression of Alzheimer's disease (AD) is characterized by the accumulation of 42-amino acid amyloid-beta (Aβ) plaques (senile plaques), hyperphosphorylated tau protein (neurofibrillary tangles), and the activation of neuroinflammatory processes, as illustrated in.[8] Neurodegeneration begins years before clinical symptoms emerge, driving the development of early detection techniques for identifying these changes in preclinical stages.

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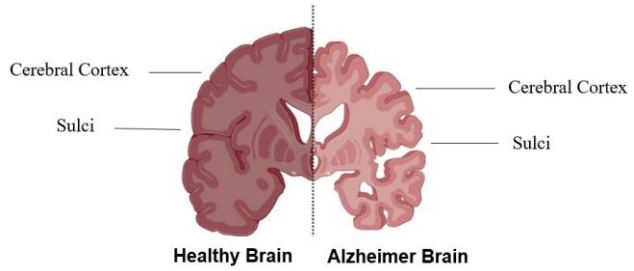

Figure 1: Comparison of a healthy brain and an Alzheimer's brain (Figure have been created using BioRender.com)

Key biomarkers of AD include amyloid beta, phosphorylated tau protein (p-Tau), and several neuroinflammation indicators are essential markers of the neurodegenerative process in AD. Notably, amyloid beta (Aβ) and phosphorylated tau (p-Tau), the primary proteins implicated in AD pathology, can be detected in saliva through multiple biological pathways. These proteins are released into the bloodstream when the blood-brain barrier (BBB) is compromised, which commonly occurs in AD due to neuroinflammation and vascular dysfunction. This breakdown of the BBB allows Aβ and p-Tau to leak into the peripheral circulation. Additionally, extracellular vesicles such as exosomes can facilitate the transport of these proteins across the BBB, enabling their entry into the bloodstream. Furthermore, degenerating neurons can directly release Aβ and p-Tau into interstitial fluid, which then makes its way into the bloodstream.[9]

Once in the bloodstream, Aβ and p-Tau can enter saliva through several pathways. One of the primary routes is passive diffusion across the blood-saliva barrier, where the proteins pass from the blood into the oral cavity. In addition, there may be direct release of Aβ and p-Tau from neurons into the oral cavity, or these proteins can be secreted via extracellular vesicles produced by the salivary glands. Emerging research indicates that the concentrations of Aβ and p-Tau in saliva correlate with their levels in cerebrospinal fluid (CSF) and blood, making saliva a promising, non-invasive medium for AD diagnostics. However, challenges such as reproducibility, sensitivity, and specificity of assays need to be addressed through further studies to confirm the clinical utility of these biomarkers.[10]

Saliva, an easily obtainable biofluid, offers a unique window into the physiological and pathological processes occurring within the body. With its rich repertoire of proteins, peptides, metabolites, and nucleic acids, saliva mirrors systemic health and disease states, including those of the central nervous system (CNS).[11] The oral-systemic connection, mediated by shared inflammatory pathways, vascular networks, and microbiota, further underscores the potential of salivary biomarkers in reflecting neurodegenerative processes like those seen in AD.[13,14]

The aim of this research is to provide an in-depth understanding of the current state of salivary biomarkers for the detection of AD, particularly within the context of an aging population. This review summarizes existing data supporting the use of salivary biomarkers for the identification of AD and related disorders, considering critical factors such as salivary production, composition, and collection in the elderly population.

2. Materials and Methods

A comprehensive keyword search was performed across four esteemed scientific databases: PubMed, Scopus, Web of Science, and Google Scholar. In the subsequent phase, a curated selection of keywords and key phrases was meticulously identified, leveraging both established knowledge and systematic data to ensure a thorough and exhaustive review of the literature. A range of relevant English terms, including synonyms and related concepts such as Alzheimer's disease (AD), salivary biomarkers, and biomarkers in saliva for AD, were incorporated. Titles, abstracts, and key terms from pertinent studies were systematically examined within these databases, with the search extending up to 2024.

2.1. Inclusion and exclusion criteria

Studies were included in the review if they met the following criteria: (i) involvement of Alzheimer's disease (AD) patients, (ii) investigation of salivary biomarkers specifically associated with AD, and (iii) publication in the English language. Exclusion criteria encompassed studies where (i) full-text access was unavailable and (ii) the publication type included letters, editorials, interviews, or systematic literature reviews.

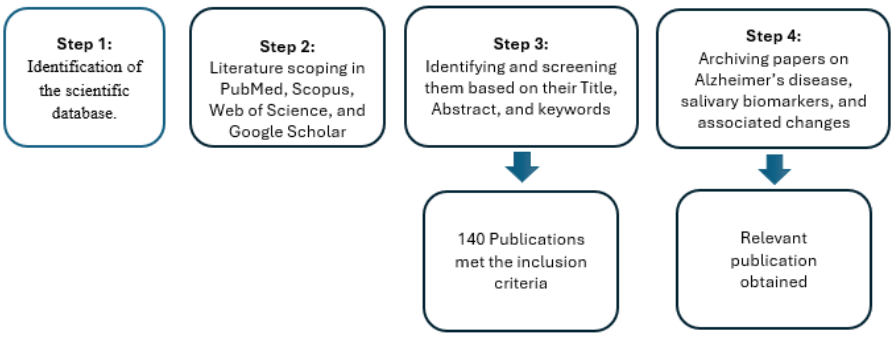

Figure 2: The steps followed in the search process for the literature review

2.2. Study selection

The relevance of each title and abstract was assessed based on predefined eligibility criteria. Inclusion and exclusion parameters were established prior to the screening process to ensure consistency. After an initial review of titles and abstracts, full-text articles were thoroughly examined to determine their suitability for inclusion in the study.

The next phase involved a comprehensive screening process based on titles, abstracts, and keywords. The selection primarily focused on peer-reviewed journal articles, conference papers, dissertations, and reports. In the fourth stage, the inclusion criteria were applied to the titles, resulting in the exclusion of 20 papers. Subsequently, 165 articles were selected from an initial pool of 200 after further refinement based on their abstracts. In the final phase, a thorough full-text review was conducted, leading to the selection of 140 articles that were either directly or indirectly relevant to the research focus.

The primary objective of this review is to provide an in-depth analysis of salivary biomarkers for Alzheimer's disease (AD). Additionally, this study examines the existing challenges that must be addressed before salivary biomarkers can be widely adopted for clinical diagnosis. Finally, it explores potential future strategies for leveraging salivary biomarkers in the early detection of AD.

3. Salivary Biomarkers and Their Correlation with Ad Pathology

Salivary biomarkers such as amyloid-β (Aβ42 and Aβ40), tau proteins (total tau and phosphorylated tau), and oxidative stress markers (e.g., malondialdehyde, 8-isoprostane) have shown promising correlations with AD pathology.[15,16] Elevated Aβ42 levels and altered Aβ42/Aβ40 ratios in saliva reflect amyloid plaque deposition, a hallmark of AD.[17] Similarly, tau protein levels in saliva align with neurofibrillary tangles observed in cerebrospinal fluid (CSF) and post-mortem brain tissue, providing a potential non-invasive proxy for tau pathology.[18] (**Figure 3**).

Inflammatory markers such as interleukins (IL-1β, IL-6) and tumor necrosis factor-alpha (TNF-α) in saliva highlight systemic and neuroinflammatory processes associated with AD.[19] These findings suggest that saliva may serve as a window into central nervous system (CNS) inflammation through oral-systemic interactions, such as gingival inflammation and blood-brain barrier dysfunction.[20]

Additionally, the discovery of salivary microRNAs (e.g., miR-107, miR-132) has introduced a novel layer of diagnostic potential.[12] These small, non-coding RNA molecules regulate gene expression and are implicated in synaptic dysfunction and amyloid processing in AD.[21,22]

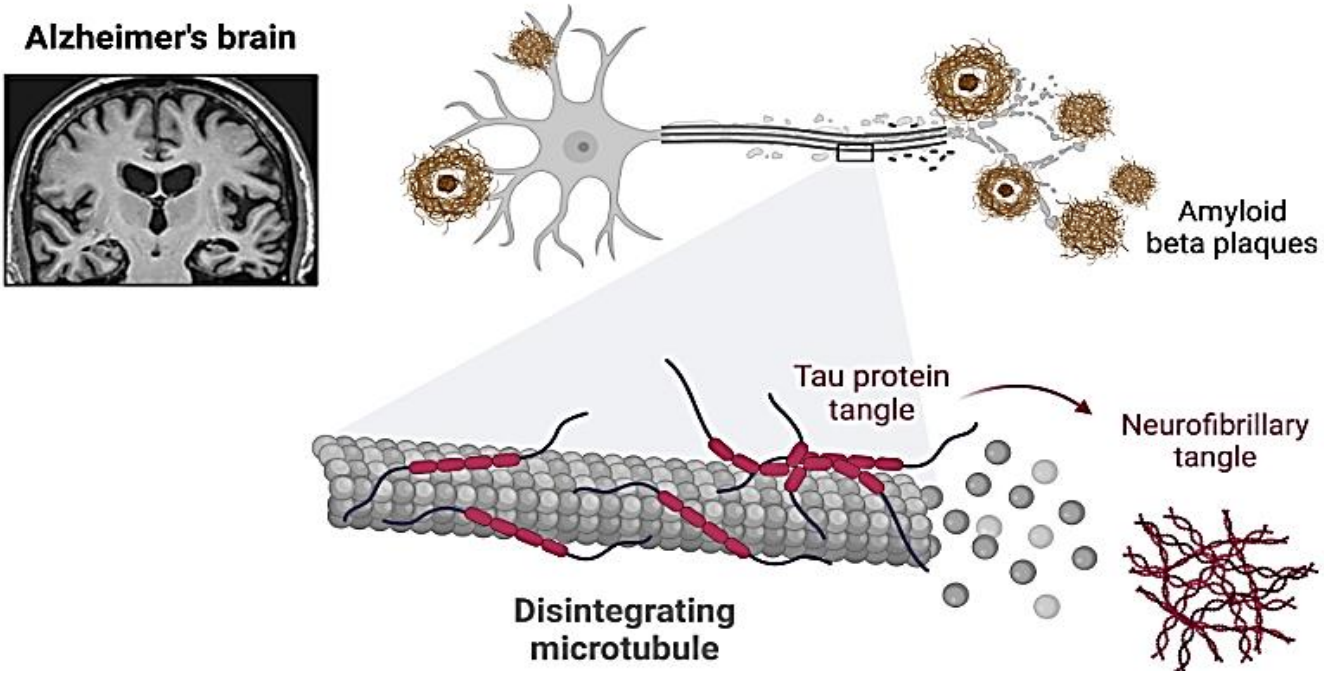

Figure 3: The pathology of tau and Aβ (Figure have been created using BioRender.com)

Table 1: Key salivary biomarkers and their significance in relationship with AD

Biomarkers	Relevance to AD Pathology	Biological Mechanism	Supporting Evidence
Salivary Amyloid-β (Aβ42)	Reflects amyloid plaque deposition	Amyloid-β accumulates in plaques in the brain, which can translocate into saliva	Elevated levels in AD patients[6]
Salivary Aβ42/Aβ40 Ratio	Indicator of amyloid pathology	Aβ42 and Aβ40 are both produced from amyloid precursor protein (APP), but Aβ42 aggregates more readily, contributing to plaque formation.	Reduced ratio in AD[23]
Salivary Total Tau (T-tau)	Associated with neuronal damage	Tau is a protein-stabilizing microtubule; in AD, tau becomes hyperphosphorylated and forms tangles.	Increased levels in saliva[10]
Salivary Phosphorylated Tau	Reflects neurofibrillary tangles	Phosphorylated tau is indicative of the dysfunction and aggregation that leads to neurofibrillary tangles.	Correlates with CSF tau[11]
Salivary Lactoferrin	Indicator of innate immune response and neuroinflammation.	Iron-binding glycoprotein with antimicrobial, anti-inflammatory, and immunomodulatory properties.	Reduced levels in AD patients compared to healthy controls, suggesting a potential non-invasive biomarker.[24]
Oxidative Stress Markers	Indicates systemic and neuronal oxidative damage	Oxidative stress damages cells and tissues, and is thought to contribute to neurodegeneration in AD.	Elevated markers like MDA[25]
Inflammatory Markers	Reflets Neuroinflammation (e.g., IL-1β, IL-6, TNF-α)	Inflammatory cytokines from systemic inflammation cross the blood-brain barrier and influence brain inflammation in AD.	Altered levels in AD patients[20]
MicroRNAs	Regulate gene expression related to synaptic dysfunction	miRNAs are non-coding RNAs that regulate gene expression, including genes related to neurodegenerative processes	Dysregulated miRNAs in AD[21]
Glial Fibrillary Acidic Protein (GFAP)	Reflects astrocyte activation and neuroinflammation	Increased GFAP expression indicates reactive Astro cytosis in AD pathology	Elevated levels in AD[26]

4. Existing Evidence on Salivary Biomarkers for AD-Related Disorders

4.1. Amyloid beta protein as a salivary biomarker for Alzheimer's disease

The accumulation of amyloid-beta (Aβ) plaques is a primary pathological hallmark of Alzheimer's disease (AD), occurring approximately 20 years before the onset of significant clinical symptoms. Salivary biomarkers associated with AD include Aβ1–40, Aβ1–42, and extracellular deposits of Aβ proteins found not only in the brain but also in peripheral tissues such as the skin, nasal mucosa, lacrimal glands, and lingual glands. Additionally, glandular salivary secretion has been linked to the diagnosis of familial amyloidotic polyneuropathy.[27,28]

A study by Lee et al. investigated the synthesis of Aβ1–42 across various organs, including the liver, spleen, kidneys, brain, intestines, and pancreas, in both healthy individuals and AD patients. The normal physiological range of Aβ1–42 in saliva is approximately 20 pg/mL; however, in AD patients and those at risk, this concentration was observed to double, reaching 40 pg/mL. Despite this increase, no statistically significant differences were identified across different stages of AD.[29]

Similarly, research by Sabbagh et al. corroborated these findings, demonstrating elevated salivary Aβ1–42 levels in AD patients compared to healthy controls. Their study, which involved 15 AD patients and 8 unaffected individuals, reported that Aβ1–42 concentrations were 2.45 times higher in AD patients than in the control group.[30]

Further investigations by Bermejo-Pareja et al. evaluated the salivary levels of Aβ1–42 and Aβ1–40 in a cohort of 70 AD patients, 51 Parkinson's disease (PD) patients, and 56 healthy controls. Although AD patients exhibited higher levels of Aβ1–42 compared to PD patients and controls, the difference was not statistically significant. Notably, salivary Aβ1–42 concentrations were markedly increased in individuals with mild to moderate AD when compared to those with severe AD or healthy individuals. Additionally, the study found no correlation between Aβ1–42 levels and known AD risk factors such as age or ApoE genotype, suggesting that salivary Aβ1–42 may serve as a distinguishing biomarker for AD rather than other neurodegenerative diseases.[31]

Kim et al. explored the association between salivary Aβ levels and AD severity in a study comparing 17 cognitively healthy individuals with 28 patients experiencing mild or severe cognitive impairment.[32] Unlike previous studies that utilized ELISA assays, this research employed a nanoparticle-based immunoassay, revealing significantly elevated Aβ1–42 levels in severe AD patients.[29] Interestingly, these findings contrasted with those of Bermejo-Pareja et al.,[33] who reported lower Aβ1–42 levels in individuals with advanced AD.[31]

McGeer et al. further analysed the progression of AD by stratifying participants into four groups based on postmortem immunohistochemical evaluations of Aβ1–42 accumulation. They observed that individuals at minimal risk for AD progression exhibited lower salivary Aβ1–42 levels than those at high risk. Additionally, salivary Aβ1–42 concentrations remained stable across a wide age range (16 to 92 years) in the low-risk control group, while AD patients displayed significantly elevated levels compared to high-risk controls. These findings suggest that salivary Aβ1–42 levels could serve as a potential diagnostic tool for AD and may even help predict disease progression.[33]

A separate study by Boschi et al. investigated the relationship between salivary and cerebrospinal fluid (CSF) Aβ1–42 levels among 100 participants, including 18 AD patients, 64 individuals with non-AD dementias, and 18 healthy controls. Their results demonstrated that AD patients had significantly higher mean salivary Aβ1–42 concentrations compared to both controls and individuals with other forms of dementia, further reinforcing the potential of salivary Aβ1–42 as a non-invasive biomarker for AD. These collective findings highlight the growing evidence supporting salivary Aβ1–42 as a promising biomarker for early AD detection. However, further large-scale, longitudinal studies are required to validate its clinical utility and establish standardized diagnostic thresholds.

4.2. Tau protein as a biomarker for Alzheimer's disease (AD)

Neurofibrillary tangles (NFT), a hallmark of Alzheimer's disease (AD), are primarily composed of aggregated and hyperphosphorylated tau proteins. First-generation PET ligands targeting tau have demonstrated increased retention in AD patients compared to controls, with uptake patterns correlating with disease progression. While much of the research on salivary biomarkers for AD has focused on amyloid-beta (Aβ1–42), investigations have also explored tau-related markers, including total tau (t-Tau), phosphorylated tau (p-Tau), and the t-Tau/p-Tau ratio.

The presence of tau proteins in various body fluids has been investigated as a potential diagnostic tool for AD, either independently or in combination with other biomarkers. Tau proteins, alongside Aβ and amyloid precursor protein (APP), are expressed in salivary epithelial cells. Their likely sources in saliva include acinar epithelial cells and neurons innervating the salivary glands. Notably, sublingual tau concentrations may reflect pathological changes in the brain and salivary glands, either directly or indirectly, in AD patients.

A study by Shi et al. utilized the Luminex assay to measure t-Tau, p-Tau, and Aβ1–42 levels in the saliva of 21 AD patients and 38 control individuals. The researchers further applied mass spectrometry, identifying five distinct tau peptides in saliva. While Aβ1–42 was undetectable via mass spectrometry, a significant increase in the t-Tau/p-Tau ratio was observed in AD patients. Interestingly, in contrast to the elevated levels of t-Tau and p-Tau in cerebrospinal fluid (CSF) seen in AD, salivary t-Tau levels remained stable or declined, whereas p-Tau levels were notably higher. The selective synthesis of p-Tau by salivary glands and the potential effects of salivary secretion stimulation may explain these elevated levels.

Pekeles et al. further examined the t-Tau/p-Tau ratio using Western blot analysis in saliva samples from 46 AD patients, 55 individuals with mild cognitive impairment (MCI), and 47 healthy controls. Their findings revealed a significantly higher t-Tau/p-Tau ratio in AD patients compared to MCI and healthy individuals. However, the

corresponding CSF data did not align, showing no significant differences in the p-Tau/t-Tau ratio across AD, MCI, and control groups. These results suggest that salivary tau biomarkers may provide independent diagnostic insights beyond CSF-based measures.[34]

Another study measured tau and phosphorylated tau-181 in saliva samples from 27 healthy individuals, 44 AD dementia patients, 45 individuals with MCI, and 31 patients with other forms of dementia. Using Lumipulse technology, researchers found a significant decrease in both total tau and phosphorylated tau-181 levels in AD patients. These findings further emphasize the potential of salivary tau biomarkers for AD diagnosis and disease monitoring.[35]

4.3. Salivary GFAP

A biomarker linked to neuroinflammation in Alzheimer's disease (AD) is a promising area of research, although it's still in the early stages. Studies have shown that certain biomarker levels are significantly altered in individuals with mild cognitive impairment (MCI) and AD, making them potential indicators for distinguishing between healthy individuals and those with MCI or AD.[36] Furthermore, these biomarkers exhibit strong correlations with other AD-related markers such as Aβ42, IL-1β, and caspase-8. However, further research is needed to fully evaluate their diagnostic and prognostic potential.

4.4. Salivary microRNA-485-3p

A research team from the Korean company Biorchestra has identified a potential biomarker for Alzheimer's disease (AD). They found that the concentration of miRNA-485-3p in salivary exosome-enriched extracellular vesicles (EE-EV) is linked to Aβ deposition in the brains of AD patients. Specifically, they observed a significant increase in miRNA-485-3p levels in the EE-EV of AD patients compared to healthy individuals. Moreover, the concentration of this miRNA in saliva was strongly correlated with brain Aβ deposition and demonstrated high diagnostic accuracy in predicting Aβ-PET positivity.

However, the mixed results surrounding salivary AD biomarkers highlight the urgent need for further research to standardize and validate testing methods, such as analytical techniques, sample collection protocols, collection timing, and stabilization procedures. Several factors, both pre- and post-analytical, have been identified as influencing the conflicting findings in the field.

In summary, salivary biomarkers like Aβ42, tau, pTau181, and lactoferrin show promise as reliable indicators for early AD detection, though additional studies are essential to confirm their role in diagnosis.[37]

5. Oxidative Stress and its Role in Alzheimer’s Disease

Oxidative stress occurs when reactive oxygen and nitrogen species (ROS/RNS) exceed the body's antioxidant defenses. Mitochondria, primarily through the electron transport chain, are the main ROS source. When superoxide anions react with nitric oxide, reactive nitrogen species like peroxynitrite form, leading to cellular damage.[38,39]

5.1. Impact on cellular components

DNA/RNA damage: Oxidative stress alters gene transcription and replication, producing markers like 8-hydroxydeoxyguanosine (8-OH-dG). RNA is particularly vulnerable due to its proximity to ROS sources.[60]

Lipid peroxidation: Unsaturated fatty acids undergo peroxidation, generating markers like isoprostanes, malondialdehyde (MDA), and 4-hydroxynonenal (HNE), which impair cell function.[41]

Protein oxidation: Proteins suffer from side-chain modifications, unfolding, and carbonyl formation, leading to dysfunction and aggregation.[42]

5.2. Mitochondrial damage and apoptosis

ROS disrupts mitochondrial function, triggering apoptosis via the ASK-1 pathway and redox-sensitive proteins like Trx-1 and p53.

5.3. Oxidative stress in Alzheimer’s disease (AD)

Oxidative stress plays a crucial role in AD pathogenesis, with elevated oxidative markers detected early in disease progression:

Lipid peroxidation: Increased levels of 4-hydroxyhexenal (HHE) and F2-isoprostanes contribute to mitochondrial dysfunction and tau pathology.[41]

DNA/RNA oxidation: Mitochondrial DNA is particularly vulnerable, exacerbating neuronal damage.

1. Protein oxidation: Elevated protein carbonyls and 3-nitrotyrosine (3-NT) are observed in AD-affected brain regions.[42,43]
2. Biomarkers in CSF and blood: While CSF markers correlate with cognitive decline, blood-based markers yield inconsistent results.

5.4. Oxidative stress as an early trigger in AD

Oxidative stress precedes amyloid-beta (Aβ) accumulation, suggesting it may initiate AD progression. The brain’s high oxygen consumption, PUFA content, and metal ions increase susceptibility, while limited antioxidant defenses offer inadequate protection.[44,45]

6. Other Biomarkers for Alzheimer's Disease (AD)

Recent research suggests that inflammation within the brain plays a crucial role in the development and progression of Alzheimer's disease (AD).[46] Evidence indicates that peripheral infections or inflammatory conditions may influence the level of inflammation in the central nervous system (CNS). Inflammatory markers associated with

inflammatory pathways, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), are currently being utilized as diagnostic tools to aid in the confirmation of AD.[47] However, since inflammation is a common factor in various diseases, these markers should be used in conjunction with other indicators to enhance diagnostic accuracy.

Additionally, diabetes mellitus has been found to be more prevalent in AD patients, suggesting a potential connection between the two conditions. In this context, salivary sugar levels have been proposed as possible diagnostic biomarkers for AD.[48] Lau et al. utilized two distinct types of cell-based biosensors to identify salivary glucose and trehalose levels in AD patients compared to non-AD individuals. The presence of specific salivary sugars may not only aid in diagnosing AD but could also be linked to the disease's progression. Although the exact origin of salivary trehalose remains unknown, these findings suggest that salivary sugars could offer valuable insights into AD diagnosis and pathophysiology, further supporting the need for comprehensive diagnostic approaches.[49]

7. Limitations and Key Challenges in Using Saliva Biomarkers

1. Standardization issues: One of the primary challenges in utilizing saliva biomarkers for diagnosis is the lack of standardized protocols for sample collection, preservation, and analysis. Variability in these procedures can result in inconsistent results, hindering the ability to draw reliable conclusions. As salivary biomarkers may change in concentration during disease progression, establishing a uniform approach to sample handling is essential for accurate diagnostics.
2. Biomarker variability: Salivary biomarker levels can vary significantly due to multiple factors, including the method of saliva collection, the timing of sample collection, and individual biological differences. Circadian rhythms, salivary gland activity, and the presence of underlying health conditions such as xerostomia or hypersialorrhea further contribute to the inconsistency in salivary content, complicating the use of saliva as a reliable biomarker source.[50,51]
3. Patient factors and noncompliance: The success of saliva-based diagnostics depends on patient compliance and the accurate collection of samples. Conditions associated with reduced or excessive saliva production, such as dry mouth (xerostomia) or excessive salivation (hypersialorrhea), can significantly affect the quality of the sample. Additionally, medications that alter salivation and systemic conditions like diabetes can also impact biomarker accuracy, presenting a challenge to the generalizability and precision of saliva-based diagnostics.[52]
4. External influences on saliva composition: Various environmental and lifestyle factors, including oral hygiene habits, microbial flora, and dietary influences, can affect the composition of saliva, introducing additional variability. These external factors must be considered to ensure that the biomarkers measured reflect the intended disease state, rather than confounding influences.[50]
5. Lack of validation across diverse populations: While salivary biomarkers show promise for diagnosing conditions such as Alzheimer's and Parkinson's diseases, there is a need for further validation across diverse patient groups. Research involving a broader range of disease stages and population demographics is essential to establish the reliability and clinical applicability of saliva biomarkers in routine diagnostics.[53]

8. Biological Plausibility and Oral-Systemic Connections

The oral cavity is intricately linked to systemic health, with saliva acting as a conduit for circulating biomarkers. The passage of amyloid-β, tau, and inflammatory molecules into saliva may occur via translocation from the bloodstream, leakage through compromised blood-brain barriers, or local production in salivary glands affected by systemic pathology.[54] The presence of oxidative stress markers and miRNAs further supports the hypothesis that saliva reflects both peripheral and CNS changes in AD.[55]

However, these connections also complicate biomarker interpretation, as saliva's composition is influenced by oral health conditions such as periodontitis, which independently modulates inflammatory and oxidative stress markers. Future studies must consider these confounding factors to improve biomarker specificity for AD.

9. Comparison with Other Diagnostic Modalities

While CSF biomarkers and PET imaging remain the gold standards for AD diagnosis, their invasive nature, high cost, and limited accessibility hinder widespread adoption.[56,57] Blood-based biomarkers have emerged as an alternative, but saliva offers additional benefits, such as non-invasive collection and potential integration with telemedicine platforms. Although salivary biomarkers are less studied than their blood counterparts, their diagnostic accuracy could be enhanced through the development of combinatorial biomarker panels.[58]

10. The Importance of Further Validation for Salivary Biomarkers

While salivary biomarkers demonstrate strong specificity and selectivity, their detection can be affected by the presence of various biological components in saliva.[59] Saliva composition can differ significantly between individuals due to factors such as matrix effects, viscosity, salivary flow rate, and diet, all of which must be accounted for in the development of reliable and precise diagnostic sensors.

Variability in saliva samples is particularly evident when testing across multiple individuals. To minimize external influences, participants in many studies are instructed to follow certain protocols, such as cleaning their teeth or refraining from eating certain foods before sample collection. These precautions aim to reduce the impact of food particles and large molecules, but they may not completely eliminate the inherent complexity of saliva that can affect test results.[60,61]

One alternative to traditional methods involves using polyethylene swabs to collect saliva, which has shown promise in reducing the complex nature of saliva and improving the ability of immunosensors to accurately measure biomarkers like cortisol.[62] This technique may prove more effective than conventional pre-treatment methods by providing better suppression of the saliva matrix, enabling more precise electrochemical analysis. However, further validation of these techniques is crucial to ensure that salivary biomarkers can be consistently and reliably used for diagnostic purposes.[63]

11. Future Outlook for Salivary Biomarkers

Research on salivary chemical biomarkers for Alzheimer's disease (AD) remains limited, with the majority of studies focusing on the clinical stages of the disease. Although salivary biomarkers are considered valuable for early AD detection, further investigation is necessary to establish their efficacy. While several salivary biomarkers have been explored, there is a need for more research to accurately differentiate individuals with AD from those suffering from other neurological disorders. To improve the precision and reliability of salivary biomarkers, the validation of combination biomarkers should be prioritized.

As new identification methods evolve, the validation of salivary biomarkers will become more specific and effective. Chronic diseases and medications can influence saliva production, leading to variability in biomarker levels between AD patients and healthy individuals. Therefore, salivary biomarker measurements must be adjusted for total salivary proteins to account for these differences. It is essential to establish standardized protocols for salivary biomarker analysis, including patient categorization based on disease stage, saliva collection methods, and protein-specific detection techniques.[64,65]

Despite recent progress in the identification and evaluation of biomarkers for early AD detection, the outcomes of current salivary biomarker research are still limited and require large-scale validation. Ongoing studies should focus on easily detectable variables in various body fluids that have known diagnostic value. Another key area of research is identifying biomarkers that can differentiate between various disease stages and monitor disease progression. Biomarkers that enable early detection of preclinical AD stages and predict disease progression from early signs to dementia hold particular promise. Additionally, reduced saliva production can alter the composition of salivary proteins, highlighting changes linked to dementia and other neurodegenerative disorders. The role of sialometric testing in Parkinson's disease (PD) is also a promising area for future research. Upcoming studies will likely expand on the mouth microbiome and the role of salivary exosomes in the progression of AD and related disorders.

12. Clinical Implications

Integrating salivary biomarkers into routine screening programs could revolutionize Alzheimer's disease (AD) diagnosis and management. Saliva-based diagnostic tools offer a non-invasive, cost-effective, and easily accessible method for early identification of at-risk individuals in primary care settings. This would allow for timely interventions, potentially altering the disease trajectory by enabling treatment before a significant cognitive decline occurs. Furthermore, salivary biomarkers could complement existing diagnostic modalities, such as neuroimaging and cerebrospinal fluid analysis, by providing a more comprehensive and holistic view of disease pathology and progression. These biomarkers can be used not only to detect the disease early but also to monitor its progression and tailor treatment plans accordingly. With the potential for widespread application, including at-home testing, salivary diagnostics could improve patient engagement, enhance adherence to treatments, and ultimately lead to better long-term outcomes. By integrating salivary biomarkers into clinical practice, AD diagnosis could become faster, more accurate, and more accessible, leading to significant advancements in the care and management of Alzheimer’s disease.

13. Conclusion

This review explores the potential of salivary biomarkers in the early detection of Alzheimer’s disease, highlighting their promise as non-invasive and cost-effective diagnostic tools. While research suggests that certain salivary biomarkers correlate with AD, large-scale, multi-center studies are needed to validate their reliability and sensitivity. If proven effective, these biomarkers could transform AD diagnosis and monitoring by providing a more accessible and patient-friendly alternative to traditional methods like lumbar punctures or PET scans. Additionally, combining salivary biomarkers with other diagnostic approaches may improve accuracy and support personalized treatment strategies. However, challenges remain in standardizing testing methods and understanding the complex biological mechanisms of AD. Future research should focus on identifying additional biomarkers, refining diagnostic criteria, and assessing their potential role in disease prevention and management. Successfully integrating salivary biomarkers into clinical practice could enhance patient care, advance therapeutic

development, and improve overall disease management for Alzheimer's disease.

14. Source of Funding

None.

15. Conflict of Interest

None.

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