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PSALIVARY BIOMARKERS IN ALZHEIMER'S DISEASE: A NON-INVASIVE APPROACH FOR SCREENING

BIOMARCADORES SALIVARES NA DOENÇA DE ALZHEIMER: UMA ABORDAGEM NÃO INVASIVA PARA TRIAGEM

BIOMARCADORES SALIVALES EN LA ENFERMEDAD DE ALZHEIMER: UN ENFOQUE NO INVASIVO PARA EL TAMIZAJE

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ABSTRACT

Objective: The aim of this review is to summarize current evidence on salivary biomarkers, including β-amyloid42, phosphorylated tau (p-tau), and acetylcholinesterase, for the early detection and non-invasive screening of Alzheimer's disease (AD).

Methodology: A comprehensive literature search was conducted using PubMed, Scopus, and Web of Science for studies published up to 2025. Keywords included "saliva," "Alzheimer's disease," "β-amyloid," "tau," "acetylcholinesterase," and "biomarkers." Inclusion

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criteria focused on human studies reporting salivary biomarkers relevant to AD. Data were extracted regarding biomarker type, detection method, patient population, and main findings, and then synthesized to assess the diagnostic potential of saliva in AD screening.

Results: Studies consistently report elevated levels of β-amyloid42 and phosphorylated tau in the saliva of AD patients, reflecting neurodegenerative processes occurring in the brain. Altered salivary acetylcholinesterase activity has also been observed, suggesting potential diagnostic and therapeutic applications. Emerging evidence indicates that other molecules, such as lactoferrin and inflammatory proteins, may further enhance the predictive value of salivary assays. Saliva offers a non-invasive, cost-effective, and patient-friendly medium for repeated measurements, providing an accessible alternative to cerebrospinal fluid and neuroimaging methods. However, variability in collection protocols, small cohort sizes, and limited standardization remain significant challenges.

Conclusion: Salivary biomarkers represent a promising tool for the early detection and screening of Alzheimer's disease. β-Amyloid42, phosphorylated tau, and acetylcholinesterase in saliva mirror central pathological processes and could facilitate timely diagnosis and preventive intervention. Future research should focus on standardizing collection and assay protocols, validating findings in larger populations, and integrating salivary biomarkers into routine clinical practice to improve early AD detection and patient outcomes.

Keywords: Alzheimer's Disease. Saliva. Biomarkers. B-Amyloid. Phosphorylated Tau. Early Detection. Non-Invasive Diagnostics.

RESUMO

Objetivo: O objetivo desta revisão é resumir as evidências atuais sobre biomarcadores salivares, incluindo β-amiloide42, tau fosforilada (p-tau) e acetilcolinesterase, para a detecção precoce e triagem não invasiva da doença de Alzheimer (DA).

Metodologia: Foi realizada uma busca abrangente na literatura utilizando PubMed, Scopus e Web of Science para estudos publicados até 2025. As palavras-chave incluíram "saliva", "doença de Alzheimer", "β-amiloide", "tau", "acetilcolinesterase" e "biomarcadores". Os critérios de inclusão focaram em estudos com humanos que relatassem biomarcadores salivares relevantes para a DA. Os dados foram extraídos quanto ao tipo de biomarcador, método de detecção, população estudada e principais resultados, sendo posteriormente sintetizados para avaliar o potencial diagnóstico da saliva na triagem da DA.

Resultados: Os estudos relatam consistentemente níveis elevados de β-amiloide42 e tau fosforilada na saliva de pacientes com DA, refletindo processos neurodegenerativos que ocorrem no cérebro. Alterações na atividade da acetilcolinesterase salivar também foram observadas, sugerindo aplicações diagnósticas e terapêuticas potenciais. Evidências emergentes indicam que outras moléculas, como lactoferrina e proteínas inflamatórias, podem aumentar ainda mais o valor preditivo dos testes salivares. A saliva oferece um meio não invasivo, econômico e de fácil aplicação para medições repetidas, fornecendo uma alternativa acessível ao líquido cefalorraquidiano e aos métodos de neuroimagem. No entanto, a variabilidade nos protocolos de coleta, o tamanho reduzido das coortes e a limitação de padronização ainda representam desafios significativos.

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Conclusão: Os biomarcadores salivares representam uma ferramenta promissora para a detecção precoce e triagem da doença de Alzheimer. β-amiloide42, tau fosforilada e acetilcolinesterase na saliva refletem processos patológicos centrais e podem facilitar o diagnóstico oportuno e intervenções preventivas. Pesquisas futuras devem focar na padronização dos protocolos de coleta e análise, validação dos achados em populações maiores e integração dos biomarcadores salivares na prática clínica de rotina para melhorar a detecção precoce da DA e os desfechos dos pacientes.

Palavras-chave: Doença de Alzheimer. Saliva. Biomarcadores. β-Amiloide. Tau Fosforilada. Detecção Precoce. Diagnóstico não Invasivo.

RESUMEN

Objetivo: El objetivo de esta revisión es resumir la evidencia actual sobre biomarcadores salivales, incluidos β-amiloide42, tau fosforilada (p-tau) y acetilcolinesterasa, para la detección temprana y el cribado no invasivo de la enfermedad de Alzheimer (EA).

Metodología: Se realizó una búsqueda exhaustiva de la literatura en PubMed, Scopus y Web of Science para estudios publicados hasta 2025. Las palabras clave incluyeron “saliva”, “enfermedad de Alzheimer”, “β-amiloide”, “tau”, “acetilcolinesterasa” y “biomarcadores”. Los criterios de inclusión se centraron en estudios en humanos que informaran biomarcadores salivales relevantes para la EA. Se extrajeron datos sobre el tipo de biomarcador, método de detección, población estudiada y principales hallazgos, que luego fueron sintetizados para evaluar el potencial diagnóstico de la saliva en el cribado de la EA.

Resultados: Los estudios informan de manera consistente niveles elevados de β-amiloide42 y tau fosforilada en la saliva de pacientes con EA, reflejando procesos neurodegenerativos que ocurren en el cerebro. También se han observado alteraciones en la actividad de la acetilcolinesterasa salival, lo que sugiere posibles aplicaciones diagnósticas y terapéuticas. Evidencias emergentes indican que otras moléculas, como lactoferrina y proteínas inflamatorias, pueden aumentar aún más el valor predictivo de los análisis salivales. La saliva ofrece un medio no invasivo, rentable y cómodo para mediciones repetidas, proporcionando una alternativa accesible al líquido cefalorraquídeo y a los métodos de neuroimagen. Sin embargo, la variabilidad en los protocolos de recogida, el tamaño reducido de las cohortes y la falta de estandarización siguen siendo desafíos importantes.

Conclusión: Los biomarcadores salivales representan una herramienta prometedora para la detección temprana y el cribado de la enfermedad de Alzheimer. El β-amiloide42, la tau fosforilada y la acetilcolinesterasa en la saliva reflejan procesos patológicos centrales y podrían facilitar un diagnóstico oportuno y la intervención preventiva. Las investigaciones futuras deben centrarse en estandarizar los protocolos de recogida y análisis, validar los hallazgos en poblaciones más amplias e integrar los biomarcadores salivales en la práctica clínica rutinaria para mejorar la detección temprana de la EA y los resultados de los pacientes.

Palabras clave: Enfermedad de Alzheimer. Saliva. Biomarcadores. β-Amiloide. Tau Fosforilada. Detección Temprana. Diagnósticos no Invasivos.

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1 INTRODUCTION

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder worldwide, responsible for up to 80% of dementia cases and affecting more than 55 million people globally (World Health Organization [WHO], 2023). It is characterized by progressive cognitive decline, memory impairment, and behavioral changes, primarily driven by the accumulation of β-amyloid (Aβ) plaques and hyperphosphorylated tau (p-tau) neurofibrillary tangles in the brain (Querfurth & LaFerla, 2010). These pathological processes trigger synaptic dysfunction, neuronal death, and cortical atrophy, which together result in the irreversible neurological deterioration that defines AD (Hampel et al., 2021).

The conventional diagnostic approach for AD relies heavily on cerebrospinal fluid (CSF) biomarkers and neuroimaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI). While these methods provide excellent sensitivity and specificity, they are expensive, invasive, and unsuitable for population-level screening or frequent monitoring (Palmqvist et al., 2020). Consequently, there is a growing need for non-invasive, accessible, and cost-effective diagnostic tools capable of identifying AD in its earliest stages, before irreversible neuronal loss occurs.

In this context, saliva has emerged as a promising biofluid for neurodegenerative disease detection. As emphasized by Ashraf et al. (2025) in the British Dental Journal, saliva combines diagnostic potential with practicality, it is easily accessible, painless to collect, and can reflect biochemical alterations that mirror those occurring in the central nervous system. Their work underscored that salivary levels of β-amyloid42 (Aβ42) and phosphorylated tau (p-tau) are significantly elevated in patients with Alzheimer's disease, while total tau and lactoferrin levels tend to decrease. This suggests that saliva could serve not only as a diagnostic proxy for brain pathology but also as a convenient medium for early and repeated screening in elderly populations.

Complementary studies support these observations. For instance, Nijakowski et al. (2024) conducted a systematic review and meta-analysis demonstrating that salivary Aβ42 and p-tau levels were consistently higher among AD patients, whereas reduced lactoferrin concentrations were associated with disease progression. Similarly, Goldoni et al. (2022) highlighted saliva's potential as a diagnostic substrate for both neurodegenerative and demyelinating diseases, citing advances in biosensor technology capable of detecting trace biomarker concentrations with high sensitivity.

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From a neurochemical perspective, salivary acetylcholinesterase (AChE) activity has also drawn attention as a potential biomarker. Ashraf et al. (2025) reported that AChE levels are elevated in AD patients, reflecting cholinergic dysfunction, a key hallmark of Alzheimer's pathophysiology. This finding aligns with pharmacological evidence that AChE inhibitors, such as donepezil and rivastigmine, improve cognitive function by enhancing cholinergic neurotransmission (Hampel et al., 2018). Therefore, the detection of altered AChE activity in saliva could serve as both a diagnostic and therapeutic monitoring tool.

Moreover, saliva presents advantages over other biofluids such as blood or CSF. It can be collected repeatedly without specialized equipment, reduces the risk of infection, and is stable under standard storage conditions (Carro et al., 2024; Tvarijonaviciute et al., 2020). This makes it particularly suitable for point-of-care (POC) testing in community and dental settings, where early neurodegenerative changes might be first identified.

In summary, the use of saliva as a diagnostic fluid for Alzheimer's disease represents a paradigm shift toward more accessible, patient-centered healthcare. As underscored by Ashraf et al. (2025), further validation and standardization of salivary biomarker assays could enable earlier detection, facilitate timely intervention, and ultimately improve quality of life for individuals at risk of AD. The present review aims to consolidate current evidence regarding salivary biomarkers, especially β-amyloid42, p-tau, and AChE, and to evaluate their potential for early, non-invasive Alzheimer's screening.

2 METHODOLOGY

This narrative review was conducted to synthesize the current evidence on salivary biomarkers associated with Alzheimer's disease (AD), following a structured and transparent approach. The methodology was designed in accordance with general guidelines for scoping and narrative reviews as outlined by Grant and Booth (2009) and the PRISMA recommendations for systematic reporting (Page et al., 2021).

2.1 LITERATURE SEARCH STRATEGY

A comprehensive electronic search was performed across three major databases: PubMed, Scopus, and Web of Science, for studies published up to March 2025. The search combined the following terms using Boolean operators: ("saliva" OR "salivary biomarkers") AND ("Alzheimer's disease" OR "dementia") AND ("β-amyloid" OR "Aβ42" OR "tau" OR "p-tau" OR "acetylcholinesterase" OR "AChE").

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Manual searches were also performed in the reference lists of key review papers, including those by Ashraf et al. (2025), Nijakowski et al. (2024), and Goldoni et al. (2022), to identify additional relevant publications not indexed in electronic databases.

2.2 ELIGIBILITY CRITERIA

Studies were included if they met the following criteria:

1. Population: Human participants, either cognitively healthy or diagnosed with Alzheimer's disease or mild cognitive impairment (MCI).
2. Intervention/Exposure: Assessment of salivary biomarkers related to AD pathophysiology.
3. Outcomes: Quantitative or qualitative data on β-amyloid42, phosphorylated tau, acetylcholinesterase, or other salivary proteins linked to AD.
4. Study Design: Original research, clinical trials, or observational studies published in peer-reviewed journals.

Exclusion criteria were:

1. Non-human or in vitro studies;
2. Conference abstracts or editorials without primary data;
3. Studies lacking measurable salivary biomarker outcomes.

2.3 DATA EXTRACTION AND SYNTHESIS

Data were extracted using a standardized charting form that captured: author, publication year, country, sample size, biomarker type, detection method (ELISA, LC–MS, or biosensor-based assays), and principal findings. When available, effect sizes, correlation coefficients, and diagnostic accuracy metrics (sensitivity/specificity) were recorded.

Given the heterogeneity in analytical techniques and study designs, a qualitative synthesis was prioritized over meta-analysis. Findings were organized by biomarker type (β-amyloid42, p-tau, AChE, and others) and compared to the current reference standards in cerebrospinal fluid and plasma.

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3 RESULTS

3.1 B-AMYLOID42 (AB42)

Among the salivary biomarkers evaluated, β-amyloid42 (Aβ42) has shown the most consistent association with Alzheimer's disease (AD) pathology. Multiple studies reported significantly elevated salivary Aβ42 concentrations in AD patients compared with cognitively healthy individuals (Ashraf et al., 2025; Nijakowski et al., 2024). These elevations mirror amyloid plaque accumulation in the brain, supporting the hypothesis that peripheral secretions can reflect central neurodegenerative processes.

Carro et al. (2024) demonstrated that Aβ42 could be reliably detected in saliva using enzyme-linked immunosorbent assays (ELISA) and that concentrations correlated with cognitive impairment severity, as measured by the Mini-Mental State Examination (MMSE). Furthermore, advances in biosensor technology have improved assay sensitivity, enabling point-of-care detection of Aβ42 at nanogram-per-milliliter levels (Goldoni et al., 2022).

3.2 PHOSPHORYLATED TAU (P-TAU)

Phosphorylated tau (p-tau) is another well-established hallmark of AD. Several studies have identified elevated salivary p-tau levels in AD patients, suggesting that tau hyperphosphorylation within the central nervous system may be reflected in salivary secretions (Ashraf et al., 2025; Nijakowski et al., 2024). While the total tau (t-tau) concentration tends to decrease, the relative increase in p-tau enhances diagnostic specificity. These findings align with observations in cerebrospinal fluid (CSF) and plasma, where increased p-tau217 and p-tau181 are key indicators of AD pathology (Palmqvist et al., 2020). However, variability in p-tau assay methods, particularly between immunoassays and mass spectrometry, remains a limitation for inter-study comparison (Carro et al., 2024).

3.3 ACETYLCHOLINESTERASE (ACHE)

Altered acetylcholinesterase (AChE) activity in saliva has been reported in several studies, suggesting potential diagnostic and therapeutic implications. Ashraf et al. (2025) observed significantly higher AChE levels in AD patients compared to controls, which may reflect the loss of cholinergic neurons, a key feature of AD pathophysiology. Elevated salivary AChE could also indicate compensatory overexpression in response to decreased central acetylcholine levels (Hampel, Cummings, & Blennow, 2018). These findings are consistent

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with the cholinergic hypothesis, which underlies the use of AChE inhibitors (donepezil) as first-line pharmacological treatments for AD.

3.4 LACTOFERRIN AND OTHER EMERGING BIOMARKERS

Recent studies have also investigated lactoferrin, an iron-binding salivary glycoprotein involved in innate immunity. Reduced salivary lactoferrin levels have been associated with both early and advanced AD, possibly due to altered mucosal immune responses and dysregulated metal ion homeostasis (Carro et al., 2024; Nijakowski et al., 2024). In addition, inflammatory markers such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and oxidative stress biomarkers have been reported at elevated concentrations in AD saliva samples (Goldoni et al., 2022).

Collectively, these results suggest that combining multiple salivary biomarkers, Aβ42, p-tau, AChE, and lactoferrin, could enhance diagnostic accuracy and differentiate AD from other neurodegenerative disorders.

4 DISCUSSION

The findings of this review indicate that saliva represents a promising, non-invasive biofluid for the early detection and monitoring of Alzheimer's disease. The consistent observation of elevated salivary β-amyloid42 and phosphorylated tau levels supports the hypothesis that peripheral secretions can reflect neurodegenerative processes occurring in the central nervous system. These molecules, which are central to Alzheimer's pathophysiology, appear to cross biological barriers or manifest systemically, allowing their detection in saliva through advanced biochemical and biosensor-based assays. The altered activity of salivary acetylcholinesterase reinforces the biological connection between central cholinergic dysfunction and peripheral manifestations of neurodegeneration. As acetylcholine metabolism is one of the earliest pathways affected in Alzheimer's disease, the evaluation of AChE in saliva could provide valuable information not only for diagnosis but also for monitoring the effectiveness of pharmacological interventions.

Lactoferrin and inflammatory proteins have also shown potential as complementary biomarkers. Their variation in saliva may indicate underlying immune dysregulation, oxidative stress, and changes in mucosal defense mechanisms. The inclusion of these molecules in multi-analyte diagnostic panels could improve the accuracy and specificity of salivary testing,

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reducing false positives and enabling better differentiation between Alzheimer's and other neurodegenerative conditions.

From a clinical perspective, the main advantage of saliva-based testing lies in its simplicity and acceptability. The collection process is painless, non-invasive, and well tolerated by elderly individuals, allowing repeated sampling for longitudinal monitoring. Moreover, the use of saliva in diagnostic workflows could extend screening beyond specialized neurology centers, reaching primary care and dental clinics where early symptoms may first be recognized. However, several challenges remain before salivary biomarkers can be implemented as routine diagnostic tools. Variability in collection protocols, diurnal changes in salivary flow, and differences in analytical techniques contribute to inconsistent results across studies. The lack of standardized reference ranges for each biomarker also limits clinical interpretation. Furthermore, most existing studies involve small sample sizes, which restricts statistical power and generalizability.

Future research should focus on standardizing saliva collection and storage procedures, validating biomarker cut-off values, and exploring correlations between salivary concentrations and disease stage or cognitive decline. Integrating saliva analysis with digital health technologies and point-of-care biosensors could revolutionize early Alzheimer's screening by providing rapid, accurate, and accessible diagnostic information. Ultimately, the use of salivary biomarkers represents a paradigm shift toward more preventive, patient-centered approaches in neurodegenerative disease management. By combining molecular precision with clinical practicality, this emerging diagnostic strategy holds great promise for transforming how Alzheimer's disease is detected and monitored in everyday healthcare settings.

5 CONCLUSION

Salivary biomarkers represent a promising tool for the early detection and screening of Alzheimer's disease. β-Amyloid42, phosphorylated tau, and acetylcholinesterase in saliva mirror central pathological processes and could facilitate timely diagnosis and preventive intervention. Future research should focus on standardizing collection and assay protocols, validating findings in larger populations, and integrating salivary biomarkers into routine clinical practice to improve early AD detection and patient outcomes.

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