Volume 4, Issue 5, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

SALIVARY BIOMARKERS FOR THE DIAGNOSIS OF ALZHEIMER'S DISEASE: A SYSTEMATIC REVIEW

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DOI: https://doi.org/10.5281/zenodo.20657988

Keywords
Alzheimer's disease; Saliva; Biomarkers; Amyloid-beta; Tau protein; Lactoferrin

Article History
Received: 03 April 2026
Accepted: 15 May 2026
Published: 30 May 2026

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Abstract

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder requiring early and accurate diagnosis. Conventional diagnostic methods such as cerebrospinal fluid (CSF) analysis and neuroimaging are invasive, costly, and not easily accessible. Saliva has emerged as a promising non-invasive alternative for biomarker detection.

Objective

To systematically review current evidence regarding salivary biomarkers for the diagnosis of Alzheimer's disease.

Methods

A systematic review was conducted in accordance with PRISMA guidelines. Electronic databases including PubMed, Scopus, Web of Science, and Google Scholar were searched for studies published up to March 2025. Studies investigating salivary biomarkers in human participants with Alzheimer's disease were included. Study selection and data extraction were independently performed by two reviewers. Due to heterogeneity in biomarker types, analytical methods, and study designs, a qualitative synthesis was conducted instead of meta-analysis.

Results

A total of 12 studies met the inclusion criteria. Identified salivary biomarkers included amyloid-beta (Aβ), tau proteins, microRNAs, lactoferrin, neurotransmitter metabolites, oxidative stress markers, and multi-omics signatures. Lactoferrin demonstrated particularly high diagnostic accuracy (AUC ~0.95), while salivary microRNA-485-3p and multi-omics approaches achieved AUC values approaching 0.92–0.96. Advanced Raman spectroscopy combined with machine learning demonstrated diagnostic accuracy approaching 99%. However, some biomarkers such as salivary total tau and acetylcholinesterase showed limited or inconsistent associations with Alzheimer's disease.

Conclusion

Salivary biomarkers demonstrate significant potential as non-invasive tools for Alzheimer's disease diagnosis. However, variability across studies and lack of methodological standardization currently limit clinical applicability. Further large-scale longitudinal studies are required to validate biomarker performance and establish standardized protocols.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia worldwide and represents a major global health challenge. Early diagnosis is essential for timely intervention, disease monitoring, and therapeutic planning. Current

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

diagnostic approaches primarily rely on neuroimaging techniques and cerebrospinal fluid (CSF) analysis; however, these methods are invasive, expensive, and often inaccessible in routine clinical settings.

In recent years, saliva has emerged as a promising diagnostic medium because of its non-invasive collection, cost-effectiveness, and ease of repeated sampling. Saliva contains numerous biological molecules including proteins, nucleic acids, enzymes, metabolites, and inflammatory mediators that may reflect systemic and neurological alterations associated with Alzheimer's disease.

Several studies have investigated salivary biomarkers including amyloid-beta (Aβ), tau proteins, lactoferrin, oxidative stress markers, neurotransmitter metabolites, and microRNAs. While many biomarkers have demonstrated promising diagnostic potential, findings remain heterogeneous due to variations in study design, sample collection techniques, analytical methods, and patient populations.

Therefore, this systematic review aims to evaluate existing evidence regarding salivary biomarkers for Alzheimer's disease diagnosis and assess their potential clinical utility as non-invasive diagnostic tools.

METHODS

Study Design

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search Strategy

A comprehensive literature search was performed using PubMed, Scopus, Web of Science, and Google Scholar for studies published up to March 2025.

The following keywords and search combinations were used:

- "Alzheimer's disease" AND "saliva" AND "biomarkers"
- "salivary amyloid beta"
- "salivary tau"
- "salivary lactoferrin Alzheimer's"
- "salivary microRNA Alzheimer's disease"

Boolean operators (AND, OR) were used to refine search results. Reference lists of selected studies were also manually screened to identify additional eligible articles.

Inclusion Criteria

Studies were included if they met the following criteria:

- Original human research studies
- Studies evaluating salivary biomarkers in Alzheimer's disease
- Studies comparing AD patients with healthy controls or other comparison groups
- Articles published in English
- Studies reporting quantitative or diagnostic performance data

Exclusion Criteria

The following studies were excluded:

- Review articles and meta-analyses
- Animal or in vitro studies
- Studies not involving saliva as a biomarker source
- Duplicate publications
- Studies lacking sufficient quantitative data

Study Selection Process

A total of 120 records were initially identified through database searching. After duplicate removal, 100 studies underwent title and abstract screening. Thirty full-text articles were assessed for eligibility, and 18 studies were excluded due to insufficient data or failure to meet inclusion criteria. Ultimately, 12 studies were included in the qualitative synthesis.

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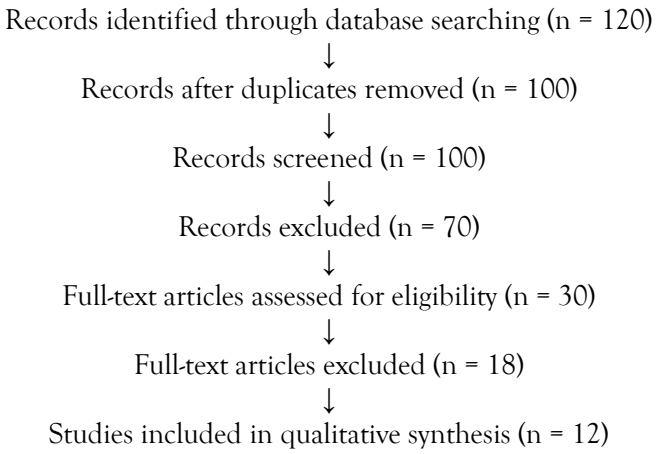
ISSN: (e) 3007-1607 (p) 3007-1593

FIGURE 1. PRISMA FLOW DIAGRAM



Data Extraction

Data extraction was independently performed by two reviewers using a standardized data collection form. Extracted information included:

- Author and publication year
- Study design
- Sample size
- Type of salivary biomarker
- Biomarker analysis method
- Diagnostic outcomes and key findings
- Sensitivity, specificity, and AUC values where available

Disagreements between reviewers were resolved through discussion and consensus.

Quality Assessment

Due to methodological heterogeneity among included studies, a formal meta-analysis was not performed. Potential sources of bias included small sample sizes, variability in saliva collection protocols, and differences in analytical techniques.

Data Synthesis

A qualitative synthesis was conducted. Findings were categorized into:

- Amyloid and tau biomarkers
- Molecular and genetic biomarkers
- Inflammatory and immune biomarkers
- Neurotransmitter and enzymatic biomarkers
- Advanced diagnostic technologies

RESULTS

A total of 12 studies met the inclusion criteria and were analyzed in this systematic review. Included studies investigated a wide spectrum of salivary biomarkers including amyloid-beta peptides, tau proteins, lactoferrin, microRNAs, oxidative stress markers, neurotransmitter metabolites, and multi-omics profiles.

Table 1. Characteristics of Included Studies

Author (Year)	Study Design	Sample Size (AD/Control)	Biomarker(s)	Method	Main Findings
Cui et al. (2022)	Case-control	30/30	Aβ40, Aβ42, t-tau, p-tau181	ELISA	Salivary Aβ42 elevated in AD; combined biomarkers AUC 92.11%

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Zalewska et al. (2021)	Case-control	25/25	Oxidative stress biomarkers, β-amyloid	Biochemical assays	Increased oxidative stress and reduced antioxidant activity in AD
Bakhtiari et al. (2017)	Cross-sectional	15/15	Acetylcholinesterase	Colorimetric assay	No significant association with AD
Ryu et al. (2023)	Observational	NR	miRNA-485-3p	qRT-PCR	Elevated in AD; associated with amyloid PET positivity; AUC ≈ 0.92
Peña-Bautista et al. (2020)	Case-control	NR	Neurotransmitter metabolites	LC-MS/MS	Diagnostic model AUC 0.806
François et al. (2024)	Case-control	20 AD, 20 MCI, 40 controls	Proteome, metabolome, microbiome	Multi-omics analysis	Diagnostic accuracy up to AUC 0.96
Ralbovsky et al. (2019)	Diagnostic study	NR	Raman spectral signatures	Raman spectroscopy + Machine Learning	Diagnostic accuracy 99–100%
Ashton et al. (2018)	Case-control	53 AD, 68 MCI, 160 controls	Total tau	Simoa assay	No significant diagnostic utility
Gleerup et al. (2021)	Observational	222 total	Lactoferrin	ELISA	No diagnostic value observed
Pekeles et al. (2019)	Case-control	NR	p-tau/t-tau ratio	Western blot	Elevated p-tau/t-tau ratio in AD; high variability
González-Sánchez et al. (2020)	Cross-sectional	NR	Lactoferrin	ELISA	Strong diagnostic performance; AUC ≈ 0.95
Bamford et al. (2025)	Observational	93	Aβ42, Aβ40, Aβ38	Immunoassay	Salivary Aβ levels correlated with cerebral amyloid burden

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Amyloid and Tau Biomarkers

Several studies evaluated classical Alzheimer's disease biomarkers including Aβ40, Aβ42, total tau, and phosphorylated tau proteins. Cui et al. (2022) reported significantly elevated salivary Aβ42 levels in patients with AD compared to controls, with combined biomarkers demonstrating diagnostic accuracy reaching 92.11%.

Similarly, Bamford et al. (2025) demonstrated that salivary amyloid-beta levels correlated with cerebral amyloid burden, supporting the biological relevance of salivary biomarkers.

In contrast, findings regarding tau proteins were inconsistent. Ashton et al. (2018) found no significant association between salivary total tau and Alzheimer's disease. However, Pekeles et al. (2019) reported elevated phosphorylated tau-to-total tau ratios in AD patients, although variability limited reliability.

Molecular and Genetic Biomarkers

Ryu et al. (2023) demonstrated significantly elevated salivary microRNA-485-3p levels in AD patients, with diagnostic accuracy approaching an AUC of 0.92.

François et al. (2024) utilized a multi-omics approach integrating proteomic, metabolomic, and microbiome analyses. Their combined biomarker model achieved AUC values up to 0.96.

Inflammatory and Immune Biomarkers

González-Sánchez et al. (2020) reported significantly decreased salivary lactoferrin levels in AD patients, demonstrating strong diagnostic performance with AUC values approaching 0.95.

Zalewska et al. (2021) identified increased oxidative stress markers and reduced antioxidant activity in AD patients, supporting the role of oxidative imbalance in disease pathogenesis.

Neurotransmitter and Enzymatic Biomarkers

Peña-Bautista et al. (2020) identified alterations in neurotransmitter-related metabolites including myo-inositol and acetylcholine, demonstrating moderate diagnostic value.

However, Bakhtiari et al. (2017) found no significant differences in salivary acetylcholinesterase activity between AD patients and controls.

Advanced Diagnostic Techniques

Ralbovsky et al. (2019) demonstrated exceptionally high diagnostic performance using Raman spectroscopy combined with machine learning algorithms, with reported diagnostic accuracy approaching 99–100%.

DISCUSSION

This systematic review highlights the growing interest in salivary biomarkers as non-invasive tools for Alzheimer's disease diagnosis. Several biomarkers demonstrated promising diagnostic potential, particularly lactoferrin, salivary microRNAs, and multi-omics signatures.

Among the biomarkers evaluated, lactoferrin demonstrated one of the strongest diagnostic performances. González-Sánchez et al. (2020) reported high sensitivity and specificity with AUC values approaching 0.95, indicating strong discriminatory capacity between AD patients and healthy controls.

MicroRNA-based biomarkers also demonstrated considerable promise. Ryu et al. (2023) reported that salivary microRNA-485-3p was strongly associated with amyloid deposition and achieved diagnostic performance approaching AUC 0.92.

Traditional Alzheimer's disease biomarkers such as amyloid-beta peptides also showed encouraging findings. Cui et al. (2022) and Bamford et al. (2025) demonstrated significant associations between salivary amyloid biomarkers and Alzheimer's pathology, supporting the biological relevance of saliva as a diagnostic medium.

However, findings related to tau proteins remained inconsistent. While Pekeles et al. (2019) observed elevated phosphorylated tau-to-total tau ratios in AD patients, Ashton et al. (2018) reported no significant association between salivary total tau and disease status. Differences in analytical sensitivity, saliva collection methods, and population characteristics may explain these discrepancies.

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The integration of advanced analytical technologies further strengthened diagnostic potential. François et al. (2024) demonstrated that combining proteomic, metabolomic, and microbiome data significantly improved diagnostic accuracy. Similarly, Ralbovsky et al. (2019) reported remarkably high diagnostic performance using Raman spectroscopy combined with machine learning.

Despite these promising findings, several limitations remain. Most studies included relatively small sample sizes and cross-sectional designs, limiting generalizability. Furthermore, variability in saliva collection protocols, storage conditions, and analytical methodologies contributes substantial heterogeneity across studies.

Another major limitation is the absence of standardized biomarker thresholds and validated clinical protocols. Some biomarkers, including salivary acetylcholinesterase, failed to demonstrate consistent diagnostic utility (Bakhtiari et al., 2017), emphasizing the need for further validation.

Future research should focus on large-scale longitudinal studies, standardization of saliva collection and processing methods, and validation of combined biomarker panels. Integration of artificial intelligence and multi-omics technologies may further improve diagnostic precision and facilitate early detection of Alzheimer's disease.

CONCLUSION

Salivary biomarkers represent a promising non-invasive approach for Alzheimer's disease diagnosis. Biomarkers including lactoferrin, salivary microRNAs, amyloid-beta peptides, and multi-omics signatures demonstrate particularly strong diagnostic potential.

However, methodological heterogeneity and lack of standardization currently limit clinical implementation. Further large-scale studies and standardized diagnostic protocols are necessary before salivary biomarkers can be routinely integrated into clinical practice.

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