REVIEW ARTICLE

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Salivary biomarkers: The early diagnosis of Alzheimer's disease

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Abstract

The precise identification of Alzheimer's disease and other prevalent neurodegenerative diseases remains a difficult issue that requires the development of early detection of the disease and inexpensive biomarkers that can replace the present cerebrospinal fluid and imaging biomarkers. Blood biomarkers, such as amyloid and neurofilament light, have been emphasized as an important and practical tool in a testing or examination procedure thanks to advancements in ultra-sensitive detection techniques. Although saliva is not currently being researched for neurodegenerative diseases, it is an important source of biomarkers that can be used for the identification of diseases and has some advantages over other biofluids. While this may be true for most people, getting saliva from elderly people presents some significant challenges. In this overview, we will first discuss how saliva is created and how aging-related illnesses may affect the amount and kind of saliva produced. The findings support the use of salivary amyloid protein, tau species, and novel biomarkers in the diagnosis of Alzheimer's disease.

KEYWORDS
Alzheimer's disease, biomarkers, early diagnosis, saliva

1 | INTRODUCTION

Neurodegenerative disorders are distinguished by a gradual deterioration of cells in the peripheral and central nervous systems (CNS), which eventually results in intellectual and neurological deficiencies.[1] Neural deterioration can be brought on by a variety of factors, including oxidative stress inflammation and neurological dysfunction.[2] Alzheimer's disease (AD) is one of the most prevalent neurological disorders among elderly people, with AD accounting for nearly 80% of all dementia cases.[3] Although Parkinson's disease (PD) primarily involves motor abnormalities, around 30% of all PD cases develop mental retardation.[4] AD is a neurological condition with a diverse etiology.[5] The most frequent reason for dementia is AD, and its prevalence is increasing around the globe.[6] Based on

various estimates, the total number of people suffering from AD will reach 131.5 million by 2050, with the newest case identified every 33s; thus, this is a globally significant disorder.[7] The key functions in the development of AD are the formation of clumps composed of 42-amino acid amyloid-beta (senile plaques) and hyperphosphorylated tau-protein (neurofibrillary tangles),[8] as well as the stimulation of neuroinflammatory processes as shown in Figure 1. Years before the first clinical symptoms manifested, the brain began to degenerate. The discovery encouraged the development of techniques for identifying aberrant changes in the early stages of preclinical studies. Amyloid protein ($A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$) and total Tau-protein (t-Tau), phosphorylated Tau-protein (p-Tau), and several neuroinflammation indicators are essential markers of the neurodegenerative process in AD[9] as shown in Figure 2. The ability to diagnose people

with Alzheimer's in the initial stages of AD makes it possible for researchers to investigate the pharmacological effects of potential AD treatments such as anti-amyloid, anti-tau, gamma-secretase inhibitors, and others.[10]

Neurodegenerative mechanisms associated with AD are progressing and cognitive deficits worsen over time.[11] Finding an indicator to facilitate preliminary evaluation and therapeutic monitoring is important because specific pathology associated with AD, such as the deposit of intracellular neurofibrillary tangles made of tau and amyloid plaques between neurons, develops decades before the disease manifests clinically.[12,13] The diagnostic and evaluation must be quick, affordable, and unobtrusive.[14] Cerebrospinal fluid (CSF) analysis is being used to aid in AD diagnosis, and it provides good diagnostic performance eventually in the illness's progression.[15] Most individuals can now be identified as having alterations to Tau, phosphorylated Tau, and amyloid beta ($A\beta_{1-42}$) levels in their CSF.[4] Though this biofluid assures excellent accuracy, the sample collection method is quite intrusive and necessitates hospitalization.[12] Furthermore, repeated measurements are difficult to obtain and costly to carry out.[16] Other diagnostic approaches, such as genomics, transcriptomics, proteomics, metabolomics, seretomics, and so on, have recently acquired popularity. These are noninvasive methods; for example saliva, blood, and urine[17] are used for the measurement of $A\beta$ species,[18] neurofilament light (NfL),[19] and phosphorylated tau on threonine 181 (p-Tau181).[20] The benefits and drawbacks of

various sample types are indicated in Table 1. Blood is the most feasible, easy, and cost-effective means of measuring biomarkers, but it is also the most stressful and fairly intrusive.[17] AD affects many body parts, especially the brain's nerve stem, hypothalamus, cerebral neocortex, insular cortex, and locus coeruleus,[21] which are all components of the autonomic nervous system (ANS).[22] Additionally, research has shown that AD damages nerve terminals in the cholinergic system, which controls the cardiovascular system and the autonomic nervous system, and that this alteration can already be noticed in the disease's early stages.[23] The primary salivary glands in the mouth—the submandibular, sublingual, and parotid glands—secrete saliva in response to cholinergic innervation from the face and glossopharyngeal cranial nerves, which are managed by the ANS.[24] As a result, abnormalities in the ANS, such as those seen in AD, may have an impact on saliva production and composition, and these changes in content may reflect neurological alterations in the CNS.[25] The majority of blood biomarkers have also been detected in saliva, according to research, and it has been suggested that blood proteins can enter saliva by passive diffusion, active transport, or microfiltration.[26] Tables 2 and 3 present AD-specific and other possible indicators extracted from CSF, blood, saliva, urine,[27] and tears.[28] The quantification of different salivary contents is commonly used in contemporary toxicological, hormonal problems, and viral illness tests, additionally to the development of novel medications.[29] Changes in the amounts of numerous compounds in saliva are
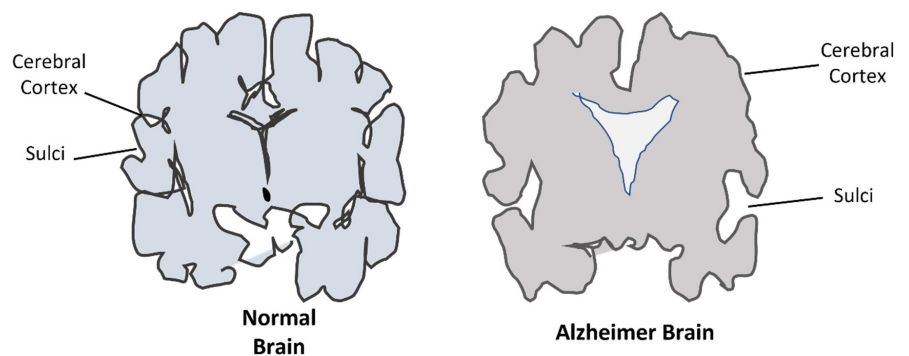

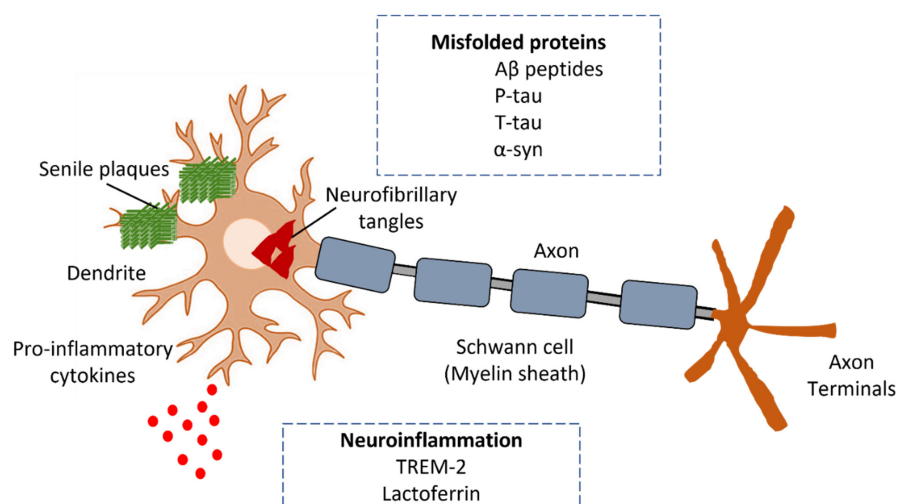FIGURE 1 The difference between a normal brain and an Alzheimer's brain.

FIGURE 2 Many types of saliva markers used to identify Alzheimer's disease.

TABLE 1 Summary of the potential advantages and drawbacks of biomarkers in cerebrospinal fluid (CSF), blood, saliva, and urine as biological fluids in the identification of Alzheimer's disease.

	CSF	Blood	Saliva	Urine
Advantages	High accuracy Biomarkers are in high-concentration Directly related to brain activity Consider as a gold standard	Repeatable and easy to employed Noninvasive collection of sample Inexpensive No hospitalization No clinical testing	Noninvasive Easily accessible Reproducible, simple, and convenient Lower stress during the collection of the sample Self-collective Largely available	Noninvasive Easily accessible Reproducible, simple, and convenient Lower stress during the collection of the sample Self-collective Largely available
Disadvantages	Technique for invasive data collecting Expensive Hospitalization and specialized personnel are required	Low concentration of specific biomarkers Nonspecific Less accurate Stressful collection Less available than saliva, tears and urine	Nonspecific Nonstandard Less accurate than blood and CSF Low concentration of biomarkers Lack of clear range for diagnosis	Less accurate than blood and CSF Low concentration of biomarkers Lack of clear range for diagnosis

thought to correlate with different levels of dysfunction and stage of neurodegeneration in the brain, especially in AD.[30] These metrics can also be used to predict the effects of treatment and efficiency. The goal of this study was to analyze and summarize current data from research on numerous saliva biomarkers to highlight modern trends and potential paths of early AD diagnoses.[31]

Saliva is a bodily fluid that is simple to acquire, and research has shown that the CNS excretes proteins into saliva[32] and is demonstrated as rich in biomarkers for the detection of different types of brain disease as shown in Figure 3. Salivary sampling has various benefits, including the following: it is minimally invasive free from anxiety, simple, and can be performed regularly at no physiological cost.[33] In addition, it is not dependent on any particular conditions of storage or training, and it is widely available.[34] Saliva can be extracted simply with the burr or activated in various ways that result in the collection of additional sample material.[31] Furthermore, saliva has a low to no risk of cross-infection or pathogen interaction, is less difficult to work with than blood, does not clot, and maintains its stability over prolonged periods.[35] These advantages, taken together, support saliva as a possibly beneficial instrument for point-of-care (POC) diagnostics and remote observation.[36] On the opposite ends of the spectrum, it has been demonstrated that the amounts of analyte in saliva are often lower than those of blood and that they can be influenced by a range of factors such as health conditions, stressful situations, and diurnal/circadian oscillations.[37] The multiplicity of procedures used for collecting, preserving, and analyzing saliva, as well as the lack of clear diagnostic ranges, now constrain the potential applications of saliva in the field of clinical investigation. Salivary analysis is typically carried out in centralized labs with specialized equipment that needs skilled workers.[38] These time-consuming processes are related to the pricey nature of salivary analysis, which further deters its use in clinical settings.[6]

Even though several peer-reviewed articles on this subject of biomarkers in saliva for AD have previously been reported, a publication synthesizing the most recent discoveries on targeting saliva markers for AD is still lacking. As a result, the goal of this research is to give the audience an in-depth understanding of what is the current state of the art in salivary biomarkers for the detection of AD, which is usually associated with an aging population. In this review, we summarize the existing data supporting the utilization of biomarkers in the saliva for the identification of AD and related disorders, taking into account critical factors of salivary production, composition, and collection in the elderly.

2 | MATERIALS AND METHODS

2.1 | Search strategy

This paper's literature review was conducted in five steps as presented in Figure 4. First, for the keywords search, Thomson Reuters Web of Science (WOS) and Scopus were recognized as the two most important and well-known scientific databases. Multiple keywords

TABLE 2 The comparison of the levels of Alzheimer's disease–specific biomarkers in cerebrospinal fluid (CSF), blood, saliva, and urine is presented in detail.

Biomarker	CSF	Blood	Saliva	Urine
$A\beta_{1-42}$	↓	Not constant	↑	↑
$A\beta_{1-40}$	Not constant	Not constant	Unchanged	No information
$A\beta_{1-38}$	Not constant	Not constant	No information	No information
$A\beta_{1-42}/A\beta_{1-40}$ ratio	↓	↓	No information	No information
$A\beta_{1-42}/A\beta_{1-38}$ ratio	↓	No information	No information	No information
t-Tau	↑	↑	↓	↑
p-Tau	↑	↑	↑	↑

TABLE 3 Detailed presentation of Alzheimer's disease nonspecific biomarkers and their level comparison in cerebrospinal fluid (CSF), blood, saliva, and urine.

Biomarker	CSF	Blood	Saliva	Urine
TREM2	↑	No change	No information	No information
YKL-40	↑	↑	No information	No information
IP-10	Not constant	Not constant	No information	No information
ICAM1	↑	No information	No information	No information
Neurogranin	↑	No change	No information	No information
SNAP-25	↑	No information	No information	No information
Synaptotagmin	↑	Limited data	No information	No information
Secretogranin-2	↓	No information	No information	No information
Neuronal pentraxin 1	↓	No information	No information	No information
Neurofascin	↓	No information	No information	No information
Myelin basic protein	↑	No information	No information	No information
BACE1	↑	↑	No information	No information
TDP-43	Lack of data	↑	No information	No information
Ferritin	↑	No change	No information	No information
VILIP-1	↑	↑	No information	No information
AchE activity	Lack of data	No information	No change	No information
Lactoferrin	Lack of data	No information	↓	No information
Metabolites (propionate, acetone)	Lack of data	Change	Change	No information
Inflammatory factors Trehalose	↑	↑	No information	No information
Alpha-synuclein	↑	No change	No information	No information
Submaxillary gland androgen-regulated protein 3B (SMR3B)	No information	No information	No information	No information
Statherin (STATH)	No information	No information	↓	No information
Histatin-1 (HTN1)	No information	No information	↓	No information
PRH1/2	No information	No information	↓	No information
AD7c-NTP	No information	No information	No information	Change

and key phrases were chosen in the next stage using methodical data and knowledge that was already available. Finally, several English keywords were chosen, including synonyms and phrases that were conceptually similar, such as AD, salivary biomarkers, and biomarkers in saliva for AD. Article titles, abstracts of various papers, and important key phrases were located in the aforementioned databases up until September 2023. A total of 155 and 224 articles were discovered in WOS and Scopus, respectively, based on the search using the chosen keywords.

2.2 | Inclusion and exclusion criteria

Publications were considered if they matched the subsequent requirements: (i) including AD patients; (ii) describing the salivary biomarkers specific for Alzheimer's disease; and (iii) writing in either English. We did not include studies conducted using the following criteria: (i) the whole text could not be obtained or was not accessible; and (ii) specific sorts of publications, such as letters, editorials, interviews, and (systematic) reviews of the literature.
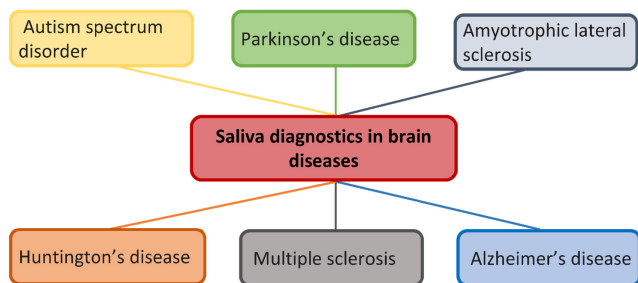
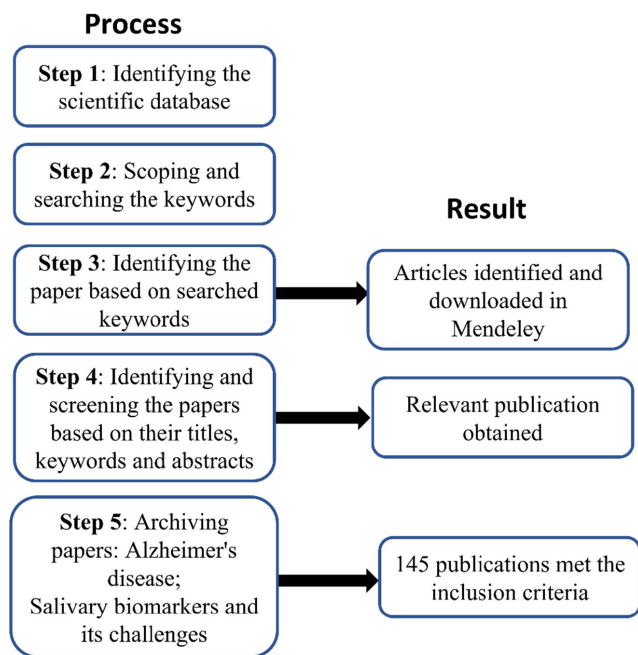**FIGURE 3** Saliva as a diagnostic tool for brain diseases.

FIGURE 4 The steps followed in the search process for the literature review.

2.3 | Study selection

The eligibility of each title and abstract that might be pertinent was checked. The criteria for inclusion and removal were all created beforehand. The full-text papers were checked once all of the titles and abstracts had been reviewed.

2.4 | Final selection of papers

The next step was an in-depth screening procedure based on the abstracts, titles, and keywords. Peer-reviewed journal articles, conference papers, dissertations, and reports were the main subjects of this inquiry. The fourth step involved applying the inclusion criteria to the titles, and 21 papers were disqualified. The remaining 165 articles were then selected from 203 by filtering them based on their abstracts. Finally, 145 articles that were both directly and indirectly related to the problem were chosen for detailed review analysis in

the fifth phase after reading the complete texts of the remaining articles. This review's major goal is to present in-depth data on the salivary biomarkers for AD. This study also covers the hurdles that must be overcome before salivary biomarkers may be used for clinical detection on a wide basis. Finally, the study proposes prospective approaches for using salivary biomarkers to detect AD.

3 | CURRENTLY AVAILABLE DATA ON SALIVARY BIOMARKERS FOR DISORDERS RELATED TO AD

3.1 | Studies on amyloid beta protein as a biomarker for AD

The deposit of Aβ plaques is the first marker of AD, starting 15 to 20 years before the beginning of serious complications. The specific biomarkers in saliva for AD include Aβ_{1-40}, Aβ_{1-42}, and the occurrence of Aβ protein deposits outside of the brain, such as in the skin, nasal mucosa, lacrimal, and lingual glands, as well as more typically in those areas.[39] In addition to these biopsies, familial amyloidotic polyneuropathy has been diagnosed using associated glandular salivary production.[40] Lee et al. conducted a study and stated that Aβ_{1-42} is frequently synthesized in the human body by all organs, including the human brain. They collected oral and cells from a variety of body parts, involving the liver, spleens, kidneys, brains, intestines, and pancreas of 27 healthy people and AD patients. Aβ_{1-42} has a normal range of 20 pg/mL; however, in those with AD or at risk of developing AD, the concentration has doubled to 40 pg/mL. When comparing different phases of the AD condition, the scientists could not discover statistically significant differences.[36] Sabbagh et al. used similar techniques as Lee et al. to collect salivary Aβ_{1-42} from 15 AD patients and 8 unaffected individuals in their investigation. Their results were consistent with Lee et al.'s results that showed that the Aβ_{1-42} levels increased in AD patients compared to healthy individuals. Furthermore, Aβ_{1-42} levels in AD patients were 2.45 times higher than in the control group.[41] Bermejo-Pareja et al. evaluated the amounts of salivary biomarkers of Aβ_{1-42} and Aβ_{1-40} of 70 individuals of AD, 51 PD subjects, and control groups of 56. The research team's attention was divided between determining the Aβ_{1-42} and Aβ_{1-40} concentrations and examining the connection between those values and the severity of AD. This study reported that the salivary levels of Aβ_{1-42} were higher in individuals with AD than in PD patients and healthy participants, but this difference was not statistically significant. Salivary Aβ_{1-42} levels significantly increased when patients with mild and moderate AD were compared to patients with severe AD and healthy individuals. Furthermore, Apo E genotype and age, two AD risk factors, were unrelated to the higher Aβ_{1-42} salivary levels in AD. Finally, the outcomes of the current research indicated that the amount of Aβ_{1-42} found in AD patients is distinct from other conditions such as PD.[42] The level of Aβ can be utilized to diagnose AD in its early stages and to distinguish it from other

neurodegenerative diseases. According to Kim et al.'s research, the severity of AD was correlated with salivary Aβ levels. In this study, concentrations of $A\beta_{1-42}$ and $A\beta_{1-40}$ were examined between 17 healthy persons and 28 people who had mild or severe cognitive impairment (MCI). Unlike past investigations, which used ELISA kits, the current study's researchers immunoassay based on nanoparticles. The results of this study showed that $A\beta_{1-42}$ levels were significantly greater in people with severe AD compared to healthy participants.[43] These results were not the same as those of the previous study by Bermejo-Pareja et al., which discovered that individuals with AD had significantly lower quantities of $A\beta_{1-42}$ than did those with severe AD.[42] McGeer et al. recognized that the members of the minimal levels control group of progression to AD risk had lower saliva $A\beta_{1-42}$ levels than the high-level control group of AD development risk when they studied the progression of AD. The study's four groups were divided according to a postmortem immunohistochemical evaluation of $A\beta_{1-42}$ build-up in the brains of AD patients. Different types of salivary biomarkers in AD are showed in Table 4.

Salivary $A\beta_{1-42}$ levels in the low-level group used as a control were impressively consistent between the ages of 16 and 92.

Salivary $A\beta_{1-42}$ levels were also elevated in AD patients compared to the high-level control group. These findings show that salivary $A\beta_{1-42}$ levels can be tested and used to diagnose AD, as well as perhaps predict the likelihood of future development.[58] The concentrations of saliva in AD patients are examined, as well as the link between salivary and CSF $A\beta_{1-42}$ levels in AD patients, patients with non-AD dementias, and controls in a different study by Boschi et al. One hundred participants were used, including 18 AD patients, 64 patients with dementias other than AD, and 18 controls. In comparison to controls and patients with dementias other than AD, AD patients had mean saliva $A\beta_{1-42}$ concentrations that were greater.[59]

3.2 | Studies on Tau protein as a biomarker for AD

The main component of neurofibrillary tangles (NFT) in AD is an aggregated and phosphorylated form of tau protein. Tau of the first-generation PET ligands exhibits increased retention in patients in comparison to controls, with uptake patterns that correspond to the histopathological stage. The majority of scientific studies have

TABLE 4 Saliva biomarkers in Alzheimer's disease (AD).

Potential biomarker	Method	Results	References
$A\beta_{1-42}$	ELISA assay	↑ $A\beta_{1-42}$ in AD	36
	ELISA assay	↑2.45 fold higher of $A\beta_{1-42}$ in AD	41
	ELISA assay	↑ $A\beta_{1-42}$ in AD	42
	ELISA assay	$A\beta_{1-42}$ ↑	44
	Luminex assay	$A\beta_{1-42}$ ↓	31
$A\beta_{1-40}$	Nanobead ELISA	No change	6
t-Tau	ELISA assay	t-Tau↓	31
	Lumipulse technology	t-Tau no change	45
	Simoa	No change in t-Tau	46
	Sandwich ELISA	No change in t-Tau	47
	ELISA assay	p-Tau/t-Tau ratio ↑	44
P-tau	Luminex ELISA	Increased p181/T-tau	23
	Western Blot	Increased s396/T-tau	48
Lactoferrin	ELISA assay	Lactoferrin ↓	49
	ELISA assay	Lactoferrin no change	50
Acetylcholinesterase (AChE)	Ellman colorimetric method	↓ AChE in AD	51
	Ellman colorimetric method	No change AChE in AD	52
	Ellman colorimetric method	↓ AChE in AD	53
	Ellman colorimetric method	No change AChE in AD	54
Saliva metabolomics	Fast ultra-HPLC coupled with TOF-MS	↓ inosine, 3-dehydrocarnitine, hypoxanthine in AD	55
	ELISA assay	↑ trehalose in AD vs. HS	47
	ELISA assay	SIRT-1, −3, −6 ↓	56
	ELISA assay	Proteomics: α-defensins, S100A8 and A9, Tb4, Hst-1, statherins, cystatin-B level altered	57

focused on measuring and identifying $A\beta_{1-42}$ in salivary biomarkers, although some scientists have also tried to test other indications like p-Tau, t-Tau, and the t-Tau/p-Tau ratio.[60] The presence of t-Tau and $A\beta_{1-42}$ proteins in different body fluids has been utilized singly or in conjunction with other markers to diagnose AD. Salivary epithelial cells express Tau proteins along with $A\beta$ and APP. The most likely origin of Tau proteins in saliva are the acinar epithelial cells and the neurons that innervate the salivary glands. Sublingual Tau concentrations indicate pathologic changes in the brain and salivary glands of AD patients in either a direct or indirect manner. Shi et al. measured the levels of t-Tau, p-Tau, and $A\beta_{1-42}$ in saliva from 21 patients with AD and 38 individuals within the control group using the Luminex assay. The researcher also used mass spectrometry to identify five distinct Tau peptides in saliva. The scientists discovered that while mass spectrometry did not allow for the detection of $A\beta_{1-42}$, there was an appreciable improvement in the t-Tau/p-Tau ratio in individuals with AD. Additionally, in contrast to rising CSF t-Tau and p-Tau levels in AD, salivary t-Tau levels are stable or decreasing. Within the same item, salivary p-Tau levels are significantly higher than t-Tau levels.[23] The selective synthesis of p-Tau by the salivary glands and the impact of salivary secretion inducement on the raised level of p-Tau are two potential explanations for the higher salivary p-Tau levels. Employing the methodology of Western blot analysis, Pekeles et al. investigated to calculate the t-Tau/pTau ratio. In this study, the researchers assessed the t-Tau/p-Tau ratio on numerous phosphorylated regions using saliva samples from 46 people with AD, 55 MCI individuals, and 47 healthy volunteers. The t-Tau/p-Tau ratio in individuals with AD was shown to be much higher than in MCI and those who were healthy. However, the data obtained from the CSF samples did not match. There were no noticeable variations in the p-Tau/t-Tau ratio when CSF was used to compare people with AD, MCI, and healthy individuals.[61] Pekeles et al. indicated a pointed improvement in the ratio of p-Tau/t-Tau in individuals with AD in comparison to healthy individuals and the findings of this study as determined by Western blot analysis. One limiting factor of this biomarker is that the ratio of p-tau/t-tau was not directly associated with the t-tau protein in CSF.[62] Another study determined saliva tau and phospho tau-181 in 27 healthy samples with 44 AD dementia, 45 with mild cognitive impairment, and 31 patients with dementia. Using Lumipulse technology they determined that the total Tau and phospho-Tau-181 were significantly decreased in AD patients.[45]

3.3 | Studies on lactoferrin as a biomarker for AD

In recent research, the proteins involved in the immune response are considered the major players in AD.[63] Saliva contains a lot of lactoferrin, a protein that is crucial for immunological responses and infection. Carro et al. employed salivary lactoferrin as a biomarker in the current research and selected four groups: AD patients, those with amnestic moderate to severe cognitive impairment (aMCI), sufferers of Parkinson's, and subjects who had neither dementia nor cognitive

decline. SDS-PAGE separation and mass spectrometry measurement were performed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the researchers discovered that both the aMCI and AD patient groups had lower levels of lactoferrin than the healthy volunteers. An ELISA test was used to confirm the findings and the researchers chose to look at lactoferrin levels in PD patients' saliva to determine if the low levels were caused by the disease. Salivary lactoferrin concentrations in PD patients were shown to be higher than in the healthy control group. It is worth mentioning that 78% of the people in the control group who started with lactoferrin levels of 7.43 g/mL ultimately developed AD within 5 years. The results of this study predict that lactoferrin is likely to be utilized as an accurate indicator to aid in the rapid detection of AD or aMCI people. Furthermore, salivary lactoferrin levels correlated positively with MMSE scores, negatively with $A\beta_{1-42}$ levels, and positively with t-Tau levels.[64]

3.4 | Studies on metabolomics as a biomarker for AD

Aside from the research on AD, tau-protein, lactoferrin, and acetylcholinesterase, the predictive importance of other biomarkers is being investigated. So far, several research on the salivary metabolome, as well as its changes in AD, have been reported. Saliva contains a lot of lactoferrin, a protein that is crucial for immunological responses and infection. Carro et al. employed salivary lactoferrin as a biomarker in a recent study and recruited four groups: those suffering from AD, those with amnestic mild memory loss, PD patients, and a control group who had neither dementia nor cognitive decline. Using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation and tandem mass spectrometry examination, the researchers discovered that both the aMCI and AD patient groups had lower levels of lactoferrin than the control group of healthy volunteers. The results were then confirmed by an ELISA analysis. One of the earliest studies, using the LC–MS method, discovered a substantial difference in the levels of 18 metabolites in MCI patients compared to a group of healthy adults. The content of taurine was properly measured.[65] Saliva metabolites are another putative AD biomarker and investigated using fast ultraperformance liquid chromatography-mass spectrometry (FUPLC-MS).[66] According to Liang et al., AD patients showed decreased levels of inosine, 3-dehydrocarnitine, and hypoxanthine and greater levels of sphinganine-1-phosphate, ornithine, and phenyl lactic acid. Liquid chromatography-mass spectrometry was used by Huan et al. to identify consistent differences between AD patients and healthy individuals in the salivary levels of methylguanosine, histidyl phenylalanine, choline-cytidine, and phenylalanyproline. Furthermore, there were changes in the amounts of phenylalanylproline and alanylphenylalanine in the saliva of AD and MCI patients.[65] Following that, higher amounts of trehalose were detected in AD patients in a study by Lau et al., but this study's results were not significantly different from the control group.[67]

3.5 | Studies on acetylcholinesterase as a biomarker for AD

Another promising marker for the diagnosis of AD is acetylcholinesterase (AChE). Acetylcholine (ACh), a neurotransmitter released into the synaptic cleft after a neural impulse, is broken down by the enzyme AChE. Its ability to diagnose AD is based on the fact that cholinergic neurodegeneration causes a drop in ACh concentration and that even in the early stages of the disease, there is a severe lack of cholinergic conductivity can be seen. Variations in AChE content have been suggested as a predictive indicator for AD. Based on this concept, investigations on the content of AChE in a range of kinds of biological fluids were undertaken to identify people with early AD.[68] Several research on the content of AChE in the CSF produced conflicting results. The time of CSF sample, nutrition, lumbar puncture circumstances, and the use of drugs are all relevant factors influencing AChE levels in the CSF.[69] Cognitive and learning impairment are directly correlated with an AD-related decline in cholinergic activity in AD patients. AChE levels are thought to indicate the health of neurons that carry information. In addition to the brain and CSF, AChE is found in tissue outside of the brain, skeletal muscles, and other body fluids such as blood and saliva. Furthermore, salivary AChE is a helpful diagnostic marker since cholinergic neurons are in charge of salivary production. Bakhtiari et al. claim that there were no statistically significant differences between the control group of 15 people and the 15 AD patients when salivary AChE activity was assessed by the Ellman colourimetric method.[51] The literature offers inconsistent findings of activity levels of AChE changes in regional body fluids associated with AD. Several research studies observed a decline in serum or CSF activity, whereas others found no difference. This is most likely owing to differences in sample methodologies and the likelihood of nonlinear variations in enzyme activity associated with illness progression.[14] Furthermore, the enzymes produced from peripheral tissues may not fully reflect the initial alterations that occur in AD patients' brains.[53]

3.6 | Other biomarkers

Currently, it has been proposed that inflammation inside the brain plays a critical role in the etiology and pathophysiology of AD.[70] Based on investigation, peripheral infection or inflammation may have an impact on the CNS's level of inflammation. Inflammatory variables associated with inflammatory pathways, such as IL-1 and TNF-α, are presently employed as diagnostic techniques to confirm AD.[71] However, an inflammatory response has been linked to several illnesses, thus it should be used in concert with other indicators of AD to ensure specificity. Previously, it was thought that excessive quantities of salivary sugar were related to the onset of diabetes mellitus. Numerous research studies have revealed a connection relationship between diabetes type 2 and the development

of AD, and diabetes mellitus was also found to be more prevalent in AD patients.[72] Salivary sugars may therefore be employed as diagnostic indicators for AD. Lau et al. employed two distinct types of cell-based biosensors to identify the secreted salivary glucose trehalose of the patients with AD from non-AD individuals.[47] Salivary sugars could be used to diagnose AD and may be associated with disease development, even if the origin of salivary trehalose remains unknown.

4 | MAIN CHALLENGES OF USING SALIVA BIOMARKERS

4.1 | Lack of standardization of conditions for saliva sampling

Technologies based on salivary biomarkers offer a noninvasive, straightforward, and affordable screening technique. The collection, preservation, and analysis of AD biomarkers do not have any established uniform methods. Over the past few decades, many medical professionals have struggled to diagnose AD.[73] In contrast to today's invasive and costly screening approaches, some scientists have begun to investigate saliva as an alternative diagnostic instrument for the detection of brain diseases such as AD. Additional validation on more varied and uniformly distributed samples at comparable illness stages is required since several indicators drastically alter their concentration as the disease develops. The current study's findings on the utilization of several salivary biomarkers for AD diagnosis showed significant variations. Although saliva is readily available, noninvasive, and a great testing tool, the usage of saliva diagnostic kits is still not standardized, making it difficult to reach a conclusive conclusion. The sensitivity and specificity of the markers must be established to standardize salivary marker studies and include the concentration of the markers in routine diagnostics.[74]

4.2 | Variability in the level of salivary biomarkers

The technique and kind of saliva collection are crucial in the research of salivary indicators. The number of salivary-specific peptides and markers shows how significantly salivary production activation changes the makeup of saliva.[75] Additionally, the circadian cycle and the time of sample collection can affect salivary content. The proportion of specific salivary glands changes along with the rise or fall in salivary gland activity, changing the amount of protein in saliva. Another issue with using saliva as a diagnostic tool is that patient noncompliance and clinical signs of AD and PD can make reliable saliva extraction. Xerostomia and hypersialorrhea, which both adversely affect saliva production, are common signs and symptoms of PD. There are differences in the consistency and content of saliva in those with xerostomia and hypersialorrhea. Salivary parameters like total protein content or salivary flow rate should be harmonized to prevent

the effects of hyposalivation. Different systemic drugs, such as narcotics, antihistamines, allergy drugs, chemotherapeutics, and painkillers, may change salivary quality and decrease the flow of saliva. Despite the similarity between saliva and serum, microorganisms, hygienic habits, and environmental factors all affect the regional changes in the mouth that are reflected in saliva.[76] Additional drawbacks include decreased levels of protein and high internal and inter-individual variation. In addition to saliva samples, other elements like working with, preparation, preservation, and analysis methods are important.[15] The value of saliva as a tool for AD and PD diagnosis is constrained by the lack of standards for these methods.

4.3 | The need of further validation of salivary biomarkers

Even though the salivary biomarkers have great selectivity and specificity, the presence of other biological components in saliva can readily interfere with their normal saliva.[77] The content of saliva varies from person to person, and this is a result of the saliva's matrix effects, viscosity, salivary flow rate, and the person's diet, all of which need to be taken into account while creating an appropriate and precise sensor. Additionally, there will be even more variances in each spit sample when examining the saliva of numerous donors.[78] The majority of subjects or participants are instructed to clean their teeth, rinse their mouths, or refrain from eating particular items prior to the collection of saliva in many studies involving the detection of analytes in saliva. These might reduce the amount of food particles or other large molecules that could affect the findings of a saliva test. The sensor was unable to pick up the analyte in the salivary sample since none of the pre-treatments for the saliva were able to sufficiently eliminate the matrix. Polyethylene Salivate Cortisol swabs were another alternative used in this investigation to process the saliva samples.[79] This option may be more effective at filtering the intricate structure of saliva and enabling immunosensors to measure cortisol levels in saliva. Swabs, in comparison to pretreatments, may be an effective means of suppressing the complex matrix of saliva, allowing electrochemical sensors to easily quantify analytes.[80]

4.4 | Validations in the presence of other types of disease

There is no agreement on the composition of synthetic saliva, and none of them replicate the rheological, chemical, and physical features of genuine mouth fluids.[81] In that regard, researchers usually decide which sort of simulated saliva is preferable based on the application, detecting principle, and potential interferences. Saliva-based biomarkers appear to be an essential tool for screening bigger populations on a regular basis.[82] However, more technological advancements and the identification of robust and selective sets of
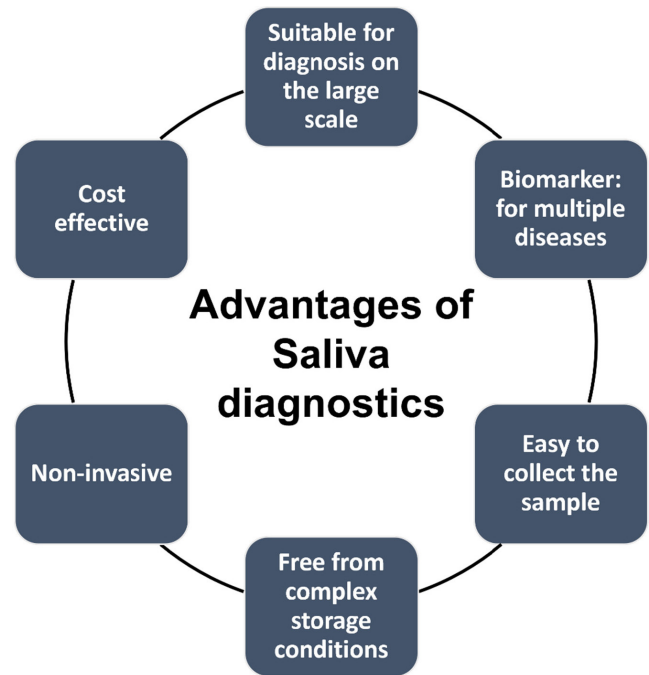

FIGURE 5 Advantages of saliva diagnostics.

salivary biomarkers are required before saliva may be used as a diagnostic tool in everyday practice.[83] Saliva is noninvasive, easy to handle, and has the option of self-collection fluid—these traits are important in a pandemic situation since they allow for reduced exposure to healthcare experts.[84]

5 | PERSPECTIVES FOR SALIVARY BIOMARKERS IN THE FUTURE

Research on the salivary chemical indicators of AD is currently scarce, with the majority of them concentrating on the clinical stage of the illness. Though salivary biomarkers are thought to be of considerable help in the early detection of AD, more research is needed in the future. Even though the above-described biomarkers in saliva have already been investigated, further research is still required to correctly distinguish between those with AD whose underlying cause may be AD and those who have additional diseases of the brain. To further enhance the accuracy and precision of saliva detection, combination biomarkers should be validated. Furthermore, when improved methods of identification are developed, salivary biomarker validation will become more specific and efficient, as shown in Figure 5. Chronic sickness or medicine frequently affects saliva output.[85] Due to varying levels of total salivary proteins produced, there may be variations in marker levels compared to individuals with AD and healthy participants. Therefore, measurements of salivary biomarkers must be adjusted for total salivary proteins. We need to establish established processes for methodologies for calculating salivary biomarkers, for example, the categorization of patients with distinct illness development, saliva testing methodologies, and

protein-specific detection techniques.[86] Considering substantial improvements in the identification and evaluation of biomarkers employed in the earlier detection of AD, the outcomes of salivary marker research are now restricted and need validation in research on a large scale. This section discusses broad, easily identifiable variables that can be detected in various body fluids and have known diagnostic values. The second area of study is the hunt for markers that can distinguish among the various illness stages and aid in monitoring and analyzing their progression. Biomarkers that predict disease development from the preclinical stages to the onset of dementia and that allow early detection of the preclinical stages of AD are particularly intriguing. Reduced production of saliva can alter the composition of salivary proteins, with an emphasis on alterations in concentrations associated with dementia and neurodegenerative disorders. Sialometric testing is especially important in PD. Future research will focus on the microbiome in the mouth cavity as well as salivary exosomes.

6 | CONCLUSION

Alzheimer's disease requires a multidisciplinary clinical diagnosis that takes considerable resources and time, and many people don't receive a good detection unless the illness has advanced past the point at which medications are most effective. For the creation of innovative therapies, it is essential to screen individuals for AD quickly and affordably. Many methods have emerged in recent years to evaluate the presence of biomarkers in saliva that are well correlated with the pathology and symptoms of known diseases, providing a secure and efficient way to screen patients and tailor their treatments. The most common diagnostic techniques, including neuroimaging, volumetric alterations in the brain, and detection of $A\beta$, total tau, and p-tau in CSF, can be replaced by these saliva-based diagnostics. Saliva has been investigated for its potential to be used as an investigation instrument for the fast detection and identification of AD. Saliva, as compared to CSF, is a body fluid that is simple to obtain, collected nonintrusively, and allows for the collection of numerous samples. Biomarkers in the saliva can be measured and utilized to identify AD, according to research. With the advancement in the study, standardization of gathering and measurement methodologies, and a greater number of samples, salivary biomarkers may become the gold standard for the early identification and diagnosis of AD.

CONFLICT OF INTEREST STATEMENT
The author proclaims no competing interests.

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