Received: 6 February 2025 | Revised: 23 October 2025 | Accepted: 10 January 2026

DOI: 10.1002/dad2.70268

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

REVIEW ARTICLE

Tears as a window to Alzheimer's disease: A systematic review of biomarkers for early detection

Inés López-Cuenca[1,2,3] | Rubén Masa-Castro[1] | Yael Hoz-Ruiz[1] | Lidia Sánchez-Puebla[1,2,4] | Lorena Elvira Hurtado[1,2] | Elena Salobrar-García[1,2,3] | José A. Matamoros[1,2,3] | José A. Fernández-Albarral[1,2,3] | Hector Leal-Lassalle[4] | Juan J. Salazar[1,2,3] | Ana I. Ramírez[1,2,3] | José M. Ramírez[1,2,4] | Rosa de Hoz[1,2,3]

[1]Ramon Castroviejo Institute for Ophthalmic Research, Complutense University of Madrid, Madrid, Spain

[2]Health Research Institute of the Hospital Clínico San Carlos (IdISSC), Madrid, Spain

[3]Department of Immunology, Ophthalmology and ENT, Faculty of Optics and Optometry, Complutense University of Madrid, Madrid, Spain

[4]Department of Immunology, Ophthalmology and ORL, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain

Correspondence
José M. Ramírez and Rosa de Hoz, Ramon Castroviejo Institute for Ophthalmic Research, Complutense University of Madrid, 28040 Madrid, Spain.
Email: ramirezs@med.ucm.es and rdehoz@med.ucm.es

Funding information
H2020-SC1-BHC-08-2020, Grant/Award Number: 101189689; Complutense University of Madrid, Grant/Award Numbers: CT82/20-CT83/20, CT58/21-CT59/21; FIB.HCSC-IDISSC, Grant/Award Number: 22TS-49-2024; Ministry of Science and Innovation (MCIN), State Research Agency (AEI), European Social Fund Plus (FSE+), Grant/Award Number: PRE2021-10011

Abstract

Alzheimer's disease (AD) is the leading cause of dementia, characterized by the accumulation of amyloid beta and tau proteins, leading to neuronal degeneration and brain atrophy. While cerebrospinal fluid and blood biomarkers have advanced early AD diagnosis, these methods are invasive and costly. This systematic review investigates tears as a non-invasive, accessible source of AD biomarkers. Using tears, directly linked to the central nervous system, we can effectively detect proteins, microRNA (miRNA), and extracellular vesicles (EVs), reflecting neurodegenerative processes. Tear collection is cost effective and minimally stressful, allowing continuous biomarker monitoring across disease stages. This review highlights recent findings on specific proteins, miRNA, and EVs in tears of patients with AD, and examines tear collection and analysis methods. The potential of these techniques for early, accessible disease detection is emphasized. Further research is needed to standardize methods and validate biomarkers in larger cohorts, positioning tears as a valuable tool for early AD diagnosis and management.

KEYWORDS
alzheimer's disease, biomarker, extracellular vesicles, microRNA, proteins, tears

Highlights

- Tears provide a non-invasive, cost-effective source of Alzheimer's disease (AD) biomarkers.
- Tear fluid can detect AD-specific proteins like amyloid beta and tau.
- Advanced tear collection methods improve biomarker monitoring.
- Recent studies show specific proteins and microRNA in tears of patients with AD.

Rubén Masa-Castro and Yael Hoz-Ruiz contributed equally to this work.

- Further research is needed to standardize methods and validate biomarkers.

1 | BACKGROUND

Alzheimer's disease (AD) is a heterogeneous, multifactorial, neurodegenerative disease that is the leading cause of dementia worldwide. This age-related neurodegenerative disorder affects > 50 million people worldwide and accounts for 60% to 70% of all dementia cases. The increase in the number of cases worldwide is related to the progressive aging of the population, as well as to the improvement in the diagnosis of this disease and the increased survival of patients. Histopathologically it is characterized by the abnormal aggregation of two proteins: extraneuronal amyloid beta (Aβ) in the form of plaques and intraneuronal hyperphosphorylated tau protein in the form of neurofibrillary tangles (NFTs), leading to neuronal death, which ultimately results in brain atrophy. In addition to the accumulation of these proteins, other risk factors involved in the development of this disease are genetic, environmental, vascular, and metabolic.[1]

It is known that brain structural changes and the concentration of biomarkers in cerebrospinal fluid (CSF) can appear even 20 years before cognitive symptoms appear;[2] this period is a key window that would allow early diagnosis and increase knowledge about the evolution of the pathophysiology of the disease. However, CSF biomarkers are obtained using expensive and invasive methods. In recent years, blood-based biomarkers have emerged as a valuable alternative, offering advantages such as easier collection, lower cost, and greater accessibility.[3] Despite these benefits, their clinical implementation faces several challenges. Studies have shown significant variability in pre-analytical factors—such as sample collection methods, processing times, tube types, and freeze–thaw cycles—which can affect the reliability of measured levels of biomarkers like Aβ_{42} and Aβ_{40}.[3] Moreover, the interpretation of plasma biomarker results is complicated by interindividual variability (ranging from ≈ 7% for Aβ_{40} to ≈ 39% for neurofilament light chain [NfL]) and the existence of gray zones in diagnostic thresholds, particularly in early disease stages.[4] These limitations highlight the need for the development of new, non-invasive biomarkers that provide stable and reliable information across all stages of AD, with particular relevance in its earliest phases.

The search for new biomarkers is necessary because both the study and monitoring of subjects at high risk for the development of AD are crucial. Additionally, developing reliable and sensitive tools for the early diagnosis of this disease would enable early intervention through drugs that slow its progression, preserve cognitive capacity, and improve our understanding of the neurodegenerative process.[5]

The retina is an extension of the central nervous system (CNS), providing a window through which neural and vascular changes in the brain can be observed.[6] The monitoring of retinal changes by optical coherence tomography (OCT) has emerged as a powerful diagnostic tool to identify potential biomarkers for the diagnosis and monitoring of various neurodegenerative diseases.[7]

Therefore, the visual system could be an important source of biomarkers for the early diagnosis of AD. One of the components of the visual system is tears, an interesting and important biological material, due to the simplicity of collection and the relation to the CNS;[8] tears are considered an intermediate fluid between CSF and serum.[9] Tears are produced by the lacrimal glands, by filtration of blood plasma and are made up of water, lipids, mucin, nucleotides, vitamins, electrolytes, and other components. One of its main components is proteins, with lactoferrin and lysozyme the main proteins. Potential biomarkers in tears include growth and neurotrophic factors, cytokines, cell adhesion molecules and immunoglobulins, sex hormones, proteases and protease inhibitors, matrix metalloproteinases, calcium binding proteins, glycoproteins, and—especially interesting—circulating microRNA (miRNA) and extracellular vesicles (EVs).[10]

Tears, as a biomarker, offer advantages including: the close relationship between the eye and the brain described above, that they can be collected using non-invasive and easily accessible techniques, and that they can be collected multiple times and thus allow monitoring of biomarker fluctuations. Furthermore, the technique is cost effective and minimally stressful for patients, regardless of their cognitive status, and tears can be collected in a short period of time.

Numerous studies have proposed tears as indicators of normal biological processes as well as pathogenic conditions due to advances in tear collection and analysis techniques.[11] Different studies have been carried out to study biomarkers in the tears of patients with AD. This study is justified by the growing interest in accessible, cost-effective diagnostic tools for AD, and contributes to the field by proposing tears as a viable medium for biomarker discovery. It lays the groundwork for future research aimed at clinical translation and integration of tear-based diagnostics into routine practice.

This systematic review summarizes the main molecular alterations identified in tear fluid from patients with AD, including changes in proteins and miRNA. These findings are discussed in relation to their potential as diagnostic biomarkers, based on their involvement in key pathological processes such as neurodegeneration, neuroinflammation, and amyloid/tau pathology.

2 | METHODS

2.1 | Search strategy

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement guidelines and was registered with Insplasy (INPLASY2024120034), DOI: 10.37766/inplasy2024.12.0034. We performed a search of the medical literature using the medical subject heading (MeSH) terms in PubMed and Scopus up to April 2024. The search terms were: "Alzheimer's disease," "tears," "biomarkers for Alzheimer's disease," "proteins," "microRNA," and "extracellular vesicles" as well as their possible combinations. The search string used in systematic review was: ("Alzheimer's disease" OR "Alzheimer

disease" OR "AD") AND ("tear" OR "tear fluid" OR "tear biomarkers") AND ("proteins" OR "microRNA" OR "extracellular vesicles").

2.2 | Study selection and screening process

All identified records were imported into Mendeley Reference Manager, Version 2.138.0 and duplicates were removed. Two independent reviewers (I.L.-C. and R.d.H.) screened titles and abstracts for relevance. Full texts of potentially eligible studies were then assessed independently by the same reviewers. Discrepancies were resolved through discussion or consultation with a third reviewer (J.M.R).

2.3 | Inclusion and exclusion criteria

The MeSH terms had to be in the title, in the abstract, or in the text of the article. The papers selected were written in English. All of them had to relate to the relationship between tears and AD. We also included articles defining the concept of tears as a biomarker in AD or generalizations about tears and AD as the main topic. These articles could be older than those focused on the review topic.

Clear inclusion and exclusion criteria were established to select the studies.

The inclusion criteria were: (1) studies investigating tears as a biomarker in AD, (2) publications in English, and (3) studies with complete and accessible data.

The exclusion criteria included: (1) duplicate studies, (2) publications without full text access, (3) studies with insufficient or irrelevant data, and (4) studies not focused on AD pathology

2.4 | Data extraction

Data were extracted independently by two reviewers using a standardized form. Extracted information included:

- Study type (e.g., observational, experimental)
- Sample size and population characteristics
- Diagnostic criteria used for AD/mild cognitive impairment (MCI)
- Tear collection method
- Analytical techniques (e.g., enzyme-linked immunosorbent assay [ELISA], liquid chromatography tandem mass spectrometry [LC-MS/MS], surface-enhanced Raman spectroscopy [SERS])
- Main findings and biomarkers identified

2.5 | Quality assessment

Although formal risk of bias tools (e.g., Quality Assessment of Diagnostic Accuracy Studies [QUADAS-2]) were not applied due to the heterogeneity of study designs, we qualitatively assessed the methodological quality of included studies based on:

- Sample size
- Diagnostic criteria clarity
- Standardization of tear collection and analysis
- Reporting of limitations

2.6 | Study selection process

The process of selecting studies was conducted in several stages. Initially, we identified records from various databases, including 1346 from PubMed and 1420 from Scopus, along with 2766 from specific registers. After this, we removed 854 duplicate records and 633 records for other reasons (irrelevant topic, language restriction, and insufficient data).

Next, we screened the titles and abstracts of the 1279 identified records, excluding 836 that did not meet the inclusion criteria. We then sought to retrieve 443 potentially relevant reports for further evaluation.

In the full-text report evaluation phase, we assessed the 443 reports to determine their eligibility. We excluded 60 articles that analyzed tears in other diseases, 184 articles that analyzed other fluids, and 104 articles not written in English.

Ultimately, we included a total of 95 studies in the systematic review (60 references in reference section and 35 references in Supporting Information).

Among the 60 main references, we distinguish between primary studies focused on tears as biomarkers in AD (9) and general background studies (51). These 9 studies were selected based on the following criteria:

- Use of quantitative tear biomarker analysis in patients with AD
- Inclusion of well-defined diagnostic criteria for AD or MCI
- Application of advanced analytical techniques (e.g., LC-MS/MS, ELISA, SERS)
- Studies with comparative data between patients with AD and controls

Figure 1 provides a summary of the study selection process; the PRISMA checklist and PRISMA diagram are available in the Supporting Information.

3 | RESULTS

3.1 | Tear extraction methods: tear collection and analysis

The structural and functional relationship between the eye and the brain positions tears as an alternative source of biomarkers for AD. Their stable composition, accessibility, and non-invasive sampling make them a promising biological material for detecting AD markers, integrating the advantages of CSF and blood, and facilitating early detection at the population level.[12] Additionally, tears

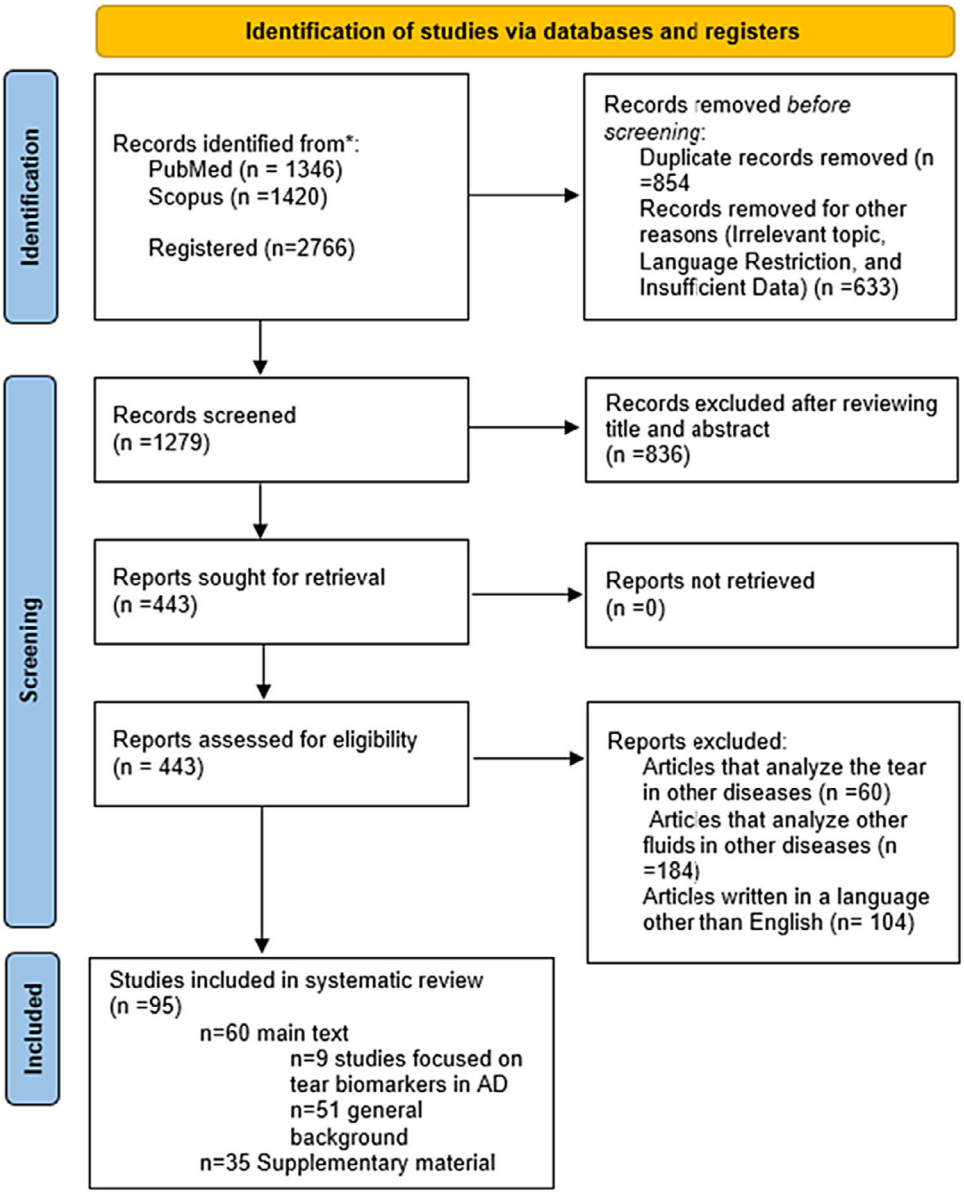


FIGURE 1 Flowchart study selection process according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. AD, Alzheimer's disease.

are easy to collect and can be stored for extended periods until analysis.[13]

3.1.1 | Collection methods

Tear collection includes the use of Schirmer strips, microsponge devices, microcapillary tubes, and micropipettes.[13] A summary of these methods is presented in Table 1.

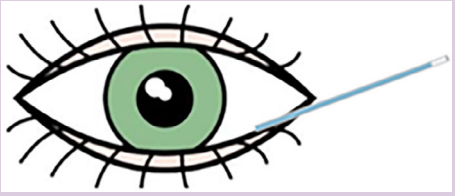
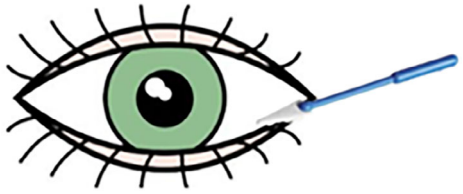
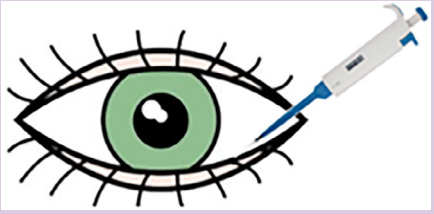
There is ongoing debate regarding the best method for tear sampling. Among the most used methods, due to their simplicity and accessibility, are capillary tubes and Schirmer strips, both considered useful, with no demonstrated superiority of one over the other.[14] Other authors have compared tear collection using capillary tubes and Schirmer strips, with the latter method resulting in a higher tear flow rate and greater protein concentration.[15]

The standard method for tear collection is the use of microcapillary tubes, which allow sample acquisition without the need for dilution or centrifugation. However, this procedure requires trained personnel.[15] In children, microsponges are preferred due to their high efficiency, reproducibility, and reduced discomfort during collection.[16]

Regarding patient preference, a 2014 study by Quah et al.[17] found that 74% of patients preferred tear collection using Schirmer strips over blood tests, making this method widely accepted in primary health screenings.

In patients with dry eye or hypolacrimation, the appropriate tear collection method is micropipette extraction, after cleaning of the ocular surface with sterile distilled water. This method allows the

TABLE 1 Summary of tear collection methods with their advantages and disadvantages.

Sample collection method	Description	Advantages	Disadvantages
Schirmer strips 	Sterilized filter paper strips with a printed graduated scale. They are placed on the tarsal conjunctiva of the lower eyelid and can be applied with or without anesthetics	Simple, highly available, and inexpensive	Not suitable for dry eye. Due to the contact of the strip with the tarsal conjunctiva, it can collect cells. Processing is needed after collection
Microcapillary tube 	A small tube that is placed in contact with the tear meniscus and has capillary action	Comfortable, shorter foreign body sensation, and allows easy processing after collection. No dilution is necessary	Not suitable for dry eye, there is a risk of reflex tearing, and it requires experience
Microsponges 	Sponges with a high absorption rate that are applied to the surface of the lower eyelid	A procedure that produces little discomfort, is very efficient, and has good reproducibility	Not suitable for dry eye, has risks of protein retention, and cannot accurately determine tear volume
Micropipette 	Pipettes that allow the collection of scarce tear samples	Suitable method for dry eye, comfortable and fast	Questionable reproducibility

collection of small amounts of fluid quickly and easily. However, it presents challenges in terms of reproducibility and the dilution required for analysis, which may alter results. As an alternative, punctal plugs can be used to block tear drainage and increase tear volume.[18]

Recently, neurostimulation of the lacrimal functional unit (LFU) has been used to enhance tear production by stimulating the corresponding nerves. Intranasal and extranasal devices, along with other electrical or chemical methods, are being developed and used as novel strategies to increase tear production.[19,20]

3.1.2 | Analysis methods

Advances in proteomics and lipidomics have enabled more precise analyses despite the limited volume of tear samples, improving the understanding of the role of tears in various diseases. After collection, techniques such as electrophoresis,[21] spectrophotometry,[22] ELISA,[23] microarrays,[21] and beads-based tests[24] are used for the analysis of the components of tear samples.

The initial studies conducted to investigate AD through tear fluid used the (untargeted) mass spectrometry method, which had certain disadvantages compared to those applied later, as it was less sensitive to the detection of low-abundance proteins.[25] In some cases, LC-MS/MS based on selected reaction monitoring (SRM) was used, which allowed for the assessment of the protein concentration of lipocalin-1, dermcidin, lysozyme-C, and lacritin in tear fluid, as well as the quantification of the fluid itself.[25] Other authors used an immunocytochemistry assay to determine the amount of $A\beta_{42}$ in the tears of two healthy subjects with a family history of AD.[26]

Another method to determine the concentrations of $A\beta_{40}$ and $A\beta_{42}$ in the tears of healthy individuals was through analysis using an electrochemical immunosensor, allowing for the detection of disease-specific proteins in a sample of just 5 µL. This approach revealed that $A\beta_{40}$ and $A\beta_{42}$ peptides could be up to 10 times higher in tear fluid than in blood, and that $A\beta$ levels might correlate with the age of healthy subjects.[27] Using the ELISA immunoassay method, $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ proteins were also measured in subjects with subjective cognitive decline, MCI, and mild dementia[28] and in mild to moderate dementia, with the determination of $A\beta_{1\text{-}42}$ using the same technique. In this latter study, the concentration of phosphorylated tau (p-tau) and amyloid precursor protein C-terminal fragment (APP-CTF) was also determined using Western blot.[29]

The study of the presence of miRNA was conducted using a genome-wide high-throughput quantitative polymerase chain reaction –based miRNA platform (OpenArray).[30] In this same study, to analyze which cellular processes might be overrepresented in tear proteins, a gene ontology analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID).[30]

Tear samples have recently been analyzed using a self-assembled nanoparticle-mediated amplified fluorogenic immunoassay (SNAFIA), composed of magnetic and fluorophore-loaded polymeric nanoparticles. This technique was used to detect adenylyl cyclase-associated protein 1 (CAP1) as a potential tear biomarker in individuals with AD. The SNAFIA assay exhibits a low detection limit (236 aM), excellent reliability ($R^2 = 0.991$), and a wide analytical range (0.320–1000 fM) for the quantification of CAP1 in tear fluid.[31]

Another analysis method is incorporated tear-exosomes analysis via rapid-isolation system (iTEARS), developed by Hu et al.[32] This method uses a negative pressure oscillation strategy for isolating EVs, demonstrating that EVs can be concentrated rapidly in < 5 minutes. Antibodies targeting their surface markers were used to quantify the EV signal immediately after isolation by immunodetection.[32]

3.2 | Determinations in tear fluid

3.2.1 | Proteins

In 2016, a study was conducted that collected tear samples from both healthy individuals and patients with AD. The results showed that patients with AD had a significantly higher tear flow rate (12 ± 2 µg/minute) compared to healthy controls (6 ± 2 µg/minute). Additionally, using quantitative proteomics, electrophoresis, and LC-MS, a notable increase in protein concentration was demonstrated in the tears of patients with AD (8.8 ± 2.9 µg/µL) compared to healthy controls (4.4 ± 1.4 µg/µL).[25] In the same work, elevated levels of dermcidin and reductions in lacritin, lysozyme-C, and lipocalin-1 were found in the tears of patients with AD, suggesting a possible dysfunction of the lacrimal glands associated with the disease. The authors hypothesized that patients with AD might be more susceptible to infections due to low levels of proteins related to ocular immune defense, although the increase in dermcidin could provide protection against infections. It is also suggested that the combination of the biomarkers lysozyme-C, lipocalin-1, lacritin, and dermcidin could have high diagnostic potential, with up to 81% sensitivity and 77% specificity in patients with AD.[25]

Kenny et al. studied the tears of patients with MCI and AD. In patients with AD, they identified the enrichment of specific proteins compared to the control group. Furthermore, the authors determined that eukaryotic translation initiation factor 4E (eIF4E) is a unique protein present only in samples from patients with AD.[30]

Gijs et al. have conducted various studies to analyze tear proteins related to AD. In 2019, these authors carried out a study focused on assessing the potential of tears as a source of biomarkers for the diagnosis of AD. They found a positive correlation between $A\beta_{42}$ and total tau (t-tau) in relation to the stage of AD. Furthermore, they observed that t-tau levels were significantly higher in patients with AD compared to those with MCI and subjective memory complaints. Additionally, the authors confirmed these findings by calculating the area under the receiver operating characteristic curve, yielding a tear discrimination capacity of 0.81 for t-tau levels and 0.72 for $A\beta_{42}$.[33]

In 2020, this same group analyzing tears reported a general increase in the levels of $A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, t-tau, and p-tau regardless of the degree of cognitive impairment or dementia compared to healthy controls. The only levels that varied according to the severity of the disease were the concentrations of t-tau, which increased significantly compared to healthy subjects. This study was the first paper to demonstrate the presence of $A\beta$ peptides and tau proteins in tear fluid.[34]

A year later, this same group conducted an observational study that included patients with subjective cognitive decline (SCD), MCI, AD, and healthy controls, in which the levels of $A\beta$ peptides ($A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$), t-tau, and p-tau were determined in tear fluid. Detectable levels of $A\beta_{40}$ and t-tau were found in > 94% of the samples across all study groups, with these levels increasing with the severity of the disease. However, $A\beta_{38}$, $A\beta_{42}$, and p-tau were only detected in 23% of the samples. In the control patients, p-tau was not detected, which increases the specificity and sensitivity for detecting AD when t-tau is found in CSF. However, $A\beta_{42}$ was detected in 78% of the analyzed control samples compared to patients with AD (< 18%). In the correlation analysis, the authors observed that CSF $A\beta_{42}$ and tear t-tau correlated negatively, as did CSF $A\beta_{42}$ and CSF t-tau. On the other hand, $A\beta_{42}$ and t-tau correlated positively in tear fluid, similar to what has been found in plasma.[28]

Regarding the presence of $A\beta$ proteins in tears, it was observed that two healthy patients with a family history of AD exhibited elevated levels of $A\beta_{42}$ protein. The presence of $A\beta_{42}$ in tear fluid was correlated with the presence of $A\beta_{42}$ retinal plaques, which were absent in the tears of healthy patients without AD antecedents. Thus, it was concluded that, as the patients were phenotypically healthy, the presence of $A\beta_{42}$ could be considered a method for the early diagnosis of AD.[26]

In relation to $A\beta$ levels, in 2023, the diagnostic role of $A\beta$ in tears was evaluated by correlating it with retinal and choroidal structures. For this study, the authors included patients with mild and moderate AD, MCI, and healthy controls. In patients with MCI and AD, $A\beta_{1\text{-}42}$ levels were detected in tears with a specificity of 93% and a sensitivity of

81%. Furthermore, the levels of this protein were significantly lower in the MCI and AD groups compared to the control group. However, no significant differences were observed in the tear concentrations of APP-CTF and p-tau regardless of the analyzed group. Statistically significant correlations were found between the choroidal thickness and $A\beta_{1\text{-}42}$ levels in tear fluid of patients with AD. This correlation could strengthen the diagnosis of AD.[29]

Among the functional proteins in tears is lactoferrin, a glycoprotein that performs numerous physiological functions, including iron binding/transfer, regulation of the immune response, and exhibiting antioxidant, anti-inflammatory, and anticancer properties, as well as neuroprotective effects.[35] Additionally, lactoferrin has been shown to bind to Aβ and is detected at high concentrations in neurons and glial cells (microglia and macroglia), Aβ senile plaques, and NFTs in the brains of individuals with AD compared to healthy controls.[36]

SERS analysis of tear fluids from individuals with AD and healthy controls clearly revealed changes in the spectra of these subjects, indicating conformational alterations in tear proteins. The spectral region associated with protein components exhibited the most significant changes, suggesting conformational alterations primarily attributed to lactoferrin and lysozyme. As previously mentioned, these substances are among the main components of tear fluid. Interval partial component analysis (i-PCA) of the spectra enabled the distinction between subjects with AD and those who were healthy or affected by MCI. This is the only study that analyzes lactoferrin in the tears of patients with AD.[37]

Given all these findings, it would be expected that lactoferrin levels could serve as a potential future tear-based biomarker for AD. These results are summarized in Table 2.

3.2.2 | MicroRNA

miRNA are small RNA molecules, typically 18 to 22 nucleotides long, that bind to messenger RNA (mRNA), leading to the inhibition of translation and gene expression. They are present in all eukaryotic cells. miRNA can be secreted into the extracellular environment and act as biomarkers for various diseases. Additionally, they may have significant functions in intercellular communication. These molecules are transcribed in the nucleus as primary miRNAs, where they are enzymatically processed and transported to the cytoplasm. In the cytoplasm, they undergo further processing by binding to proteins of the Argonaute family. The miRNAs bound to these proteins are stable and are present in biological fluids such as tears.[38] These miRNAs are involved in the regulation of astrocytes, microglia, the cerebrovascular system, and synaptic alterations associated with tau protein and Aβ, in addition to other functions.[39] It has been demonstrated that miRNA are involved in the pathological processes associated with AD, such as the phosphorylation of tau protein and the formation of amyloid precursor protein (APP) and Aβ.[40]

Numerous miRNAs have been detected in blood samples from patients with AD (see Supporting Information). To date, ≈ 300 distinct miRNAs have been isolated from tear fluid.[41] Among these, several are newly identified, and their regulatory functions remain largely uncharacterized.[16] In addition, a few studies have explored miRNA in tear samples. In research conducted by Kenny et al., elevated total levels of miRNA were found in the tears of patients with AD compared to MCI and control. Notably, miRNA-200b-5p emerged as a tear potential biomarker for AD, with its levels significantly higher in the tear samples of patients compared to MCI and controls. These results suggest that tear fluid analysis could serve as a promising diagnostic tool for AD.[30]

4 | DISCUSSION

The analysis of tear biomarkers represents a promising and non-invasive approach for the early detection and monitoring of AD, offering new perspectives in the fields of diagnosis and clinical research. However, despite the large number of studies, factors such as the unification of criteria, standardization of methodologies, and the determination of which biomarkers might be most effective for the early diagnosis of the disease should be considered.

4.1 | Most appropriate collection method for patients with neurodegenerative diseases

It is essential to establish a standard for the methods of sample collection, storage, processing, and analysis to facilitate comparisons between studies and ensure the consistency and reliability of the data obtained. Variations in results may be due to differences in sample collection, analytical processes, and storage methods. When collecting tears, it is crucial to avoid the activation of corneal nerves and reflex tearing, as this could significantly alter the composition of the tear fluid. Furthermore, external factors such as environmental conditions, the timing and duration of sampling, osmolarity, the use of artificial tears or contact lenses, and the application of topical and systemic medications can influence the composition of the tear fluid and affect the interpretation of results.[18] Circadian variations can also modify the composition of tear fluid, so it is important to collect samples at the same time of day to minimize these potential influences.

Additionally, sample handling should be standardized. After collection, samples must be transferred to dry ice to prevent protein degradation. Furthermore, protective or stabilizing solutions should not be used to prevent degradation, as they may interfere with the analysis of the sample. Finally, it is important that storage be done in aliquots and at temperatures of −80°C. Additionally, freezing/thawing cycles should be avoided to prevent any degradation or alteration of the samples.[42]

Patients with AD are typically older individuals, and it is well established that tear function diminishes with age. This may require multiple visits for elderly patients to obtain a sufficient sample. Among the methods analyzed in this review, it appears that the use of Schirmer strips is the most suitable. However, it should be noted that this method requires a collection time of ≈ 5 minutes and subsequent processing for sample extraction. Additionally, the collected volume is estimated

TABLE 2 Summary of studies analyzing protein determinations in tears of subjects with AD.

Study	Study type	Sample size	Diagnostic criteria	Biomarkers	Method of sample collection	Analytical method	Results	Limitations
Kalló et al. (2016)	Cross-sectional observational biomarker study	Exploratory analysis: 3 AD, 2 controls. Targeted proteomics validation: n = 37 (AD and controls)	AD (NINCDS-ADRDA); MMSE for controls; exclusion of confounding conditions	Lysozyme-C, lipocalin-1, lacritin, dermcidin	Microcapillary tube	Electrophoresis and LC-MS/MS.	Increased tear flow and protein concentration in AD. Combination of Lysozyme-C, Lipocalin-1, Lacritin, and Dermcidin showed 81% sensitivity and 77% specificity	Small sample size; variability in collection; lack of external validation; limited disease specificity; technical limitations; potential influence of comorbidities or medication
Gijs et al. (2019)	Observational, cross-sectional, exploratory biomarker study	25 patients (AD, MCI, SCI); 9 healthy controls	AD (NIA-AA / DSM-5, no biomarker confirmation). MCI (Petersen criteria)	$A\beta_{42}$ and t-tau	Schirmer strip	Immunoassay and mass spectrometry	Positive correlation between t-tau and $A\beta_{42}$ levels; t-tau significantly higher in AD vs. MCI	Small sample size
Kenny et al. (2019)	Cross-sectional observational study	9 AD, 8 MCI, 15 controls	AD: NIA-AA criteria; MCI: Petersen criteria; controls: neurologically healthy	MiRNA-200b-5p, eF4E	Schirmer strips	Mass spectrometry, SDS-PAGE, Western blot, and qPCR	Elevated RNA levels in AD; miRNA-200b-5p increased in AD; eIF4E present only in AD	Small sample; no independent validation; variability in collection; no longitudinal data; technical limitations; no comparison to standard biomarkers; uncontrolled confounders
Gijs et al. (2020)	Cross-sectional observational study	60 cognitive impairment patients (25 SCD, 24 MCI, 11 dementia), 9 controls	SCD: subjective complaints; MCI: NIA-AA criteria; dementia: DSM-5; MMSE ≥ 20; CDR 0–1; exclusion of neurological/psychiatric disorders	$A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, t-tau, p-tau	Schirmer strips	Mass spectrometry	Increased $A\beta$ and tau levels in all conditions except controls; t-tau elevated with disease severity; first demonstration of these biomarkers in tears	Small sample; low statistical power; clinical heterogeneity; low protein detection sensitivity; no comparative diagnostic discussion
Gijs et al. (2021)[26]	Observational, cross-sectional and correlational	23 SCD, 22 MCI, 11 dementia; 9 controls	Dementia: DSM-5; MCI: NIA-AA criteria	$A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, t-tau, p-tau	Schirmer strips (no anesthesia)	Multiplex immunoassay	$A\beta_{40}$ and t-tau detected in 94% of samples; p-tau absent in controls; t-tau 10× higher in tears than CSF	Small sample; tear dilution affects concentration; low sensitivity for low-abundance proteins
Del Prete et al. (2021)	Observational pilot/case-control study	3 healthy subjects (2 with AD family history, 1 without)	Based on phenotype and family history; $A\beta_{42}$ detection in tears	$A\beta_{42}$	Not reported	Immunocytosis emission assay	$A\beta_{42}$ detected in subjects with family history; association with retinal plaques and $A\beta_{42}$ expression.	Extremely small sample; no follow-up; no comparative diagnostics

(Continues)

TABLE 2 (Continued)

Study	Study type	Sample size	Diagnostic criteria	Biomarkers	Method of sample collection	Analytical method	Results	Limitations
Wang et al. (2021)[25]	Experimental feasibility study	50 healthy volunteers (ages 20-79)	Healthy individuals without chronic diseases	$A\beta_{40}$, $A\beta_{42}$	Schirmer strips	Electrochemical immunosensors	$A\beta$ levels in tears up to 10× higher than in blood; $A\beta$ concentration decreased with age	Homogeneous population; no standard comparisons; unclear age–$A\beta$ link; biosensor limitations; cross-sectional design
Gharbiya et al. (2023)	Cross-sectional cohort study	11 MCI due to AD; 10 mild-to-moderate AD; 14 controls	MCI: NIA-AA criteria, CDR = 0.5, MMSE > 24, amyloid PET+; AD: NIA-AA, CDR 1–2, MMSE 15–26, amyloid PET+; controls: CDR = 0, MMSE > 26, ADAS-Cog < 14	$A\beta_{42}$, APP-CTF, p-tau	Microsponge	ELISA	$A\beta_{42}$ lower in MCI/AD vs. controls; 93% specificity and 81% sensitivity; no differences in APP-CTF or p-tau	Small sample; no CSF comparison; no non-AD dementia group.
Cennamo et al. (2020)[37]	Cross-sectional observational study	17 AD, 7 MCI, 6 controls	AD & MCI: NIA-AA criteria; controls: healthy with no neurological disease or family history; all underwent ophthalmological exam and MMSE	Lysozyma and lactoferrin	Microcapillary tube	SERS	Spectral changes in AD vs. MCI and controls; conformational protein alterations	No specific biomarkers identified; small sample; no standard validation.

Abbreviations: $A\beta$, amyloid beta; AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale Cognitive Subscale; APP-CTF, amyloid precursor protein C-terminal fragment; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; eIF4E, eukaryotic translation initiation factor 4E; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography tandem mass spectrometry; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NIA-AA, National Institute on Aging–Alzheimer's Association; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association; PET, positron emission tomography; p-tau, phosphorylated tau; qPCR, quantitative polymerase chain reaction; SCD, subjective cognitive decline; SCI, subjective cognitive impairment; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SERS, surface-enhanced Raman spectroscopy; t-tau, total tau.

by weight after collection, and during processing, evaporation may complicate this measurement, as well as skew the concentration of solutes. It has also been demonstrated that this technique can induce an increase in reflex tear secretion due to its direct contact with the surface of the eye.[14] In addition, authors who have already used this technique in the study of tear proteins in patients with AD have postulated that the expression of certain proteins could be due to the collection method itself, because the Schirmer strip may include cells in addition to tears.[30]

Another suitable option is tear collection using microcapillaries, which, although it may be a slower method and subject to interruptions due to blinking, offers easier sample processing and analytically provides the most accurate analyte concentration in tears.[42]

Furthermore, due to the hypolacrimia that these patients may experience, because of both advanced age and the neurodegenerative pathology, it may be necessary to stimulate tear function beforehand or induce secretion with different volumes of saline solution. However, it is important to consider that these procedures could cause some dilution of the samples, thereby skewing the results.[43]

Finally, we have found only one published protocol designed for future analysis and collection of tear samples in AD: the TearAD study.[20] This highlights the need to standardize tear collection techniques in these patients. The TearAD study is a multicenter, longitudinal observational study with an approved design, involving cognitively healthy controls, and patients with SCD, MCI, and AD. Tear samples will be collected from these participants using Schirmer strips, and levels of $A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, t-tau, and p-tau will be determined through multiplex immunoassays. The study will analyze potential correlations between these proteins and hippocampal atrophy, as well as $A\beta$ positivity determined by positron emission tomography or CSF. It will also assess the level of concordance between $A\beta$ and tau protein levels in tear fluid, blood, and CSF.[20]

4.2 | More accurate methods of analysis

The study of tear composition in the search for new biomarkers is attracting interest in the field of medical diagnostics; however, technical challenges persist due to the limited volume of available samples and the complexity of their composition and analysis.[44]

So far, multiple tear proteins and lipids have been identified as potential biomarkers related to various diseases. In recent years, advancements in technologies such as proteomics, metabolomics, and lipidomics have significantly improved the understanding of the biochemical composition of tears. These techniques require special procedures for the collection, storage, and analysis of tear composition. However, they have enabled the identification of multiple biomarkers in various body fluids, including tears.[42]

The tear composition of patients with AD has been analyzed in nine studies, each presenting different sample analysis techniques. The methods used are summarized in Table 2. As can be observed, none of them uses the same analytical method, indicating that we are far from developing a standardized analytical method.

In the study by Kalló et al., tear samples were analyzed using electrophoresis and LC-MS/MS, and a targeted proteomics method based on SRM was developed and applied to examine quantitative changes in tear proteins. Due to the high protein concentration in tears, only those proteins for which at least two peptides could be detected with > 95% confidence was accepted.[25]

Raman spectroscopy has proven useful in the study of the protein composition of tears. However, this technique has limitations with respect to sensitivity, noise/signal level, and repeatability due to the non-homogeneous composition of the tear. To overcome these limitations, SERS has been developed, which shows improved results in the sensitivity and specificity of responses to spectral signals.[45]

One analysis method that has only been tested in healthy subjects is the determination of $A\beta$ using an electrochemical immunosensor. This method demonstrates high precision, being faster and more cost effective than other analytical techniques, positioning it as a potential test for early detection.[27]

Despite these results, the authors are aware of the limitations of their studies, suggesting that tests with higher sensitivity and precision are needed.[33]

4.3 | More diagnostic tear determination

There is no agreement regarding whether patients with AD exhibit an increase[25] or a decrease[30] in tear flow rate. Similarly, there appears to be no agreement on which proteins show differential expression or on the total protein concentration in the tears of patients with AD.

According to Kalló et al., the only protein that increases in the tears of patients with AD is dermcidin, an antimicrobial protein with a broad spectrum of activity. Tear proteins that decrease in these patients include lipocalin-1, lacritin, and lysozyme, which play a role in defense mechanisms and the chemical barrier of the eye. The balance between the increase of a broad-spectrum antimicrobial protein and the decrease of proteins involved in immune defense could explain why patients with AD do not suffer from a higher number of eye infections. Although the sample size is small (14 AD and 9 age-matched controls), the authors suggest that individuals with an increased tear flow rate and altered concentrations of these proteins should be studied to rule out AD.[25]

Thanks to the results of Kenny et al., we know that patients with AD have high concentrations of miRNA in their tears, in addition to a unique protein composition.[30] Although the exact reason for the increase in miRNA in AD is not known, it is known that miRNA expression changes rapidly in response to cellular disruptions common in the degenerative processes associated with AD.[46,47] These changes, along with the ocular alterations that occur in AD, could drive cellular responses that increase miRNA expression in the tears of patients with AD.[30]

Only the miRNA miR-200b-5p has been identified as a possible tear biomarker, as it was found only in the tear samples from patients with AD.[30] However, although the authors suggested that miRNA-200b-5p could be postulated as a good biomarker for AD, it does not seem

to make sense because its relationship to the eye and the CNS, both involved in AD, is questioned.

Interestingly, studies in an AD mouse model have also investigated miRNAs (see Supporting Information). Therefore, it would be important to determine which tear miRNA are closely related to the pathogenesis of the disease.

Although Li and collaborators consider it surprising that there is no difference in its concentration between healthy individuals and those with MCI, it is important to consider that MCI is a heterogeneous condition resulting from neurodegeneration during the prodromal stage of most neurodegenerative diseases, as well as cognitive impairments due to natural aging. Additionally, not all individuals with MCI will develop AD. Among the proteins that have differential expression in the tear fluid of patients with AD, eIF4E was found. These data are consistent with the overexpression of this protein in the brain of patients with AD.[48]

Cennamo et al.[37] also demonstrated that the tear emission spectrum of patients with AD shows different protein conformations, especially in lactoferrin and lysozyme, compared to patients with MCI and healthy subjects. This study focused on the assessment of secondary protein structures that appear to be altered in MCI and more markedly in AD. These alterations in lactoferrin levels have already been reported in previous studies in which salivary samples were analyzed in patients with AD.[49] The importance, therefore, of lactoferrin as a biomarker in AD lies in the relationship between this protein and the modulation of APP processing and its link to Aβ aggregation and neuroinflammation.[50]

The work of Gijs et al.[28] is the first to demonstrate the presence of Aβ peptides and tau proteins in tear fluid. In addition, these authors demonstrate how the presence of these proteins has a high capacity to differentiate patients with AD from controls. Likewise, the authors highlight the differences in t-tau levels between healthy subjects and those with dementia, MCI, and SCD, but do not find these differences when comparing these three pathological states. However, the fact that p-tau is not found in the tears of healthy subjects is interesting, as it could discriminate between healthy subjects and patients with some degree of dementia. Also, the authors warn about the processing of the original tear sample that must be diluted and the need for large sample volumes to perform the immunoassays, which makes it difficult to work with tear samples. The authors demonstrate that tear t-tau levels were elevated in patients with neurodegeneration, with a negative correlation between CSF Aβ_{42} protein and tear t-tau. This suggests that tear samples may mirror CSF samples in terms of t-tau, as well as the potential these proteins in tear fluid represent for both the diagnosis of disease severity and neurodegeneration. However, the cut-off levels for tear samples cannot yet be established.

In the Gharbiya et al. study, a reduction of A$\beta_{1\text{-}42}$ levels in the tears of patients with MCI and AD is demonstrated.[29] A possible explanation for the reduced level of A$\beta_{1\text{-}42}$ in tears could be a mechanism similar to what occurs in CSF, where the peptide becomes sequestered in the brain. In this case, however, the reduction would be due to sequestration within the lacrimal gland.[51–53] In addition, fragments of Aβ were found in the lacrimal glands and their acinar cells[54] could explain the low levels of A$\beta_{1\text{-}42}$ in tears.

4.4 | EVs: a future direction for tear-based biomarkers in AD

Although EVs have been extensively studied in other biological fluids such as CSF, blood, and saliva, and in various neurodegenerative and systemic diseases, their role in AD through tear fluid remains largely unexplored. The generic term EVs refers to lipid vesicles released from both the endosomal system (exosomes) and the plasma membrane (microvesicles or ectosomes). They are considered a novel intercellular communication system that facilitates the transfer of proteins, RNA, lipids, and metabolites. These vesicles can be shed from nearly all tissues and cells, and they play a critical role in transporting intercellular signals and effector molecules through circulating body fluids such as blood, urine, CSF, saliva, milk, and tears, making them significant sources for medical diagnosis and therapy. Tears have been identified as sources of EVs for liquid biopsies akin to blood samples.[55] Due to the lacrimal secretion process, tears carry rich bioinformation from other seemingly unrelated organs of the body.[8] Studies analyzing EVs in tears have demonstrated their value in highlighting their capacity to discern pathological changes in the ocular system, such as in dry eye disease and glaucoma and in changes in non-ocular tissues, such as in breast and prostate cancer and CNS diseases, such as multiple sclerosis[56] and Parkinson's disease.[57]

In the context of neurodegenerative diseases, it has been proposed that these organelles play a role in the propagation of misfolded proteins in the brain. Furthermore, it has been demonstrated that part of the communication between glial cells and neurons is mediated by EVs, and that their disruption has implications for the development of these diseases.[57] EVs perform functions in the CNS ranging from the removal of unwanted biomolecules to the propagation of pathogenic proteins associated with neurodegenerative diseases.[58] The proteomic characterization of EVs (exosomes and microvesicles) in preclinical models and patient samples has the potential to uncover novel proteins and molecular networks that influence normal physiology before traditional biomarkers of neurodegeneration manifest.[57]

The advantages of analyzing EVs compared to whole biofluids lie in the fact that the wide variety of proteins present in the latter can hinder the identification of biomarkers present in lower quantities.[59]

Although the first biological material to be explored in the search for exosomes should be CSF due to its proximity to the CNS, this technique is painful for the patient, causes significant anxiety in patients, has risks, and requires well-trained clinical personnel. However, there are studies demonstrating the presence of EVs in CSF and blood (see Supporting Information).

There is clear evidence that EVs can cross multiple biological barriers, as demonstrated by the presence of glial/neuron-derived EVs in CSF, blood, and tears.[41] Due to the lacrimal secretion process, tears carry rich bioinformation from other organs in the body that may appear unrelated at first glance.[8]

Exosomes have been observed in tears from healthy donors, with their presence confirmed using specific exosome receptor antibodies: CD63, CD9, and CD24. These exosomes contain both double-stranded genomic RNA and DNA.[60] Despite the extensive findings and our current knowledge, no previous study has investigated tear-derived EVs in patients with AD.

Given the non-invasive nature of tear collection and the rich bioinformational content of EVs, exploring their presence and diagnostic value in tear fluid could open new avenues for early detection and monitoring of AD.

We propose that future studies should focus on:

- Developing standardized protocols for EV isolation from tear fluid
- Characterizing the proteomic and transcriptomic profiles of tear-derived EVs in AD
- Comparing EV biomarkers across tear, blood, and CSF to assess concordance and diagnostic utility

4.5 | Limitations

This systematic review has certain limitations. Despite the existence of many studies investigating AD biomarkers in various body fluids (CSF, plasma, or blood), it is noteworthy that only a few of them have used tears. In addition, the reviewed studies also present several limitations. First, the small sample sizes limit the generalizability of the results. Additionally, variability in tear collection methods, such as the use of Schirmer strips, microcapillaries, microsponges, and micropipettes, introduces inconsistencies in the results. The lack of standardization in analysis methods also affects the comparability among studies. External factors such as age, disease stage, medication use, and environmental conditions can influence the composition of tears, affecting the results. Finally, many studies are cross-sectional, which limits the ability to establish causal relationships.

To advance in this area, it is essential to standardize tear collection and analysis methods. It is also necessary to conduct studies with larger and more diverse samples to validate the findings. Longitudinal studies would be valuable to observe changes in biomarkers over time and their relationship to disease progression. Additionally, new biomarkers in tears, such as EVs, should be explored as potential indicators of AD. Finally, it is crucial to develop clinical protocols for tear collection and analysis that can be used in medical practice for early diagnosis and monitoring of the disease.

5 | CONCLUSIONS

In conclusion, although the field of tear analysis appears highly promising for the study and early detection of AD, there remains a need to standardize methods for sample collection, preprocessing, and analysis. Additionally, studies should involve larger cohorts, and inclusion criteria must be unified. Cut-off points for the concentrations of various diagnostic proteins and miRNAs associated with the disease need to be established.

An unexplored approach in this neurodegenerative condition is the study and classification of EVs in the tears of these patients. Finally, at present, these determinations alone cannot yet be considered diagnostic for the disease. We believe that, when combined with other established ocular biomarkers, such as retinal thickness changes measured by OCT and vascular alterations, they could represent a significant step forward in the early diagnosis of AD.

AUTHOR CONTRIBUTIONS

Conceptualization: I.L.-C., J.M.R., and R.d.H.; methodology: I.L.-C., R.M.-C., Y.H.-R., L.S.-P., L.E.-H., E.S.-G., J.A.M., J.A.F.-A., H.L.-L., J.J.S., A.I.R., J.M.R., and R.d.H.; validation: I.L.-C., E.S.-G., A.I.R., J.M.R., and R.d.H.; formal analysis: I.L.-C., R.M.-C., Y.H.-R., L.S.-P., L.E.-H., E.S.-G., J.A.M., J.A.F.-A. H.L.-L., J.J.S., A.I.R., J.M.R., and R.d.H.; investigation: I.L.-C., (R.M.-C., Y.H.-R., L.S.-P., L.E.-H., E.S.-G., J.A.M., J.A.F.-A., H.L.-L., J.J.S., A.I.R., J.M.R., and R.d.H.; resources: J.J.S., A.I.R., J.M.R., and R.d.H.; data curation: I.L.-C., R.M.-C., Y.H.-R., and R.d.H.; writing—original draft preparation: I.L.-C., R.M.-C., Y.H.-R., J.M.R., and R.d.H.; writing—review and editing: I.L.-C., R.M.-C., Y.H.-R., L.S.-P., L.E.-H., E.S.-G. J.A.M., J.A.F.-A., H.L.-L., J.J.S., A.I.R., J.M.R., and R.d.H.; validation: I.L.-C., E.S.-G., A.I.R., J.M.R., and R.d.H.; visualization: I.L.-C., R.M.-C., Y.H.-R., J.M.R., and R.d.H.; supervision: I.L.-C., R.M.-C., Y.H.-R., J.M.R., and R.d.H.; project administration: J.J.S., A.I.R., J.M.R., and R.d.H.; funding acquisition: J.J.S., A.I.R., J.M.R., and R.d.H. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

L.S.-P. is currently supported by a predoctoral fellowship (CT82/20-CT83/20) from the Complutense University of Madrid. J.A.M. is currently supported by a predoctoral fellowship (CT58/21-CT59/21) from the Complutense University of Madrid. L.E.-H. is supported by the grant 101189689 funded by H2020-SC1-BHC-08-2020 and by the FIB.HCSC-IDISSC number 22TS-49-2024. H.L-L. is supported by the grant PRE2021-100112 funded by MCIN/AEI/10.13039/501100011033 and by the FSE+.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

ORCID

Inés López-Cuenca https://orcid.org/0000-0003-0511-515X
Rosa de Hoz https://orcid.org/0000-0002-1581-087X

REFERENCES

1. Abdul Manap AS, Almadodi R, Sultana S, et al. Alzheimer's disease: a review on the current trends of the effective diagnosis and therapeutics. *Front Aging Neurosci*. 2024;16:1429211. doi:10.3389/FNAGI.2024.1429211/BIBTEX
2. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. *Alzheimers Dement*. 2016;12:292-323. doi:10.1016/j.jalz.2016.02.002
3. Zeng X, Chen Y, Sehrawat A, et al. Alzheimer blood biomarkers: practical guidelines for study design, sample collection, processing, biobanking, measurement and result reporting. *Mol Neurodegener*. 2024;19(1):40. doi:10.1186/S13024-024-00711-1
4. Clemmensen FK, Gramkow MH, Simonsen AH, et al. Short-term variability of Alzheimer's disease plasma biomarkers in a mixed memory clinic cohort. *Alzheimers Res Ther*. 2025;17(1):26. doi:10.1186/S13195-024-01658-7
5. Hrelia P, Sita G, Ziche M, et al. Common protective strategies in neurodegenerative disease: focusing on risk factors to target the cellular redox system. *Oxid Med Cell Longev*. 2020;2020:8363245. doi:10.1155/2020/8363245
6. London A, Benhar I, Schwartz M. The retina as a window to the brain—from eye research to CNS disorders. *Nat Rev Neurol*. 2012;9:44-53. doi:10.1038/nrneurol.2012.227
7. Salobrar-García E, de Hoz R, Ramírez AI, et al. Changes in visual function and retinal structure in the progression of Alzheimer's disease. *PLoS One*. 2019;14:e0220535. doi:10.1371/journal.pone.0220535
8. Barmada A, Shippy SA. Tear analysis as the next routine body fluid test. *Eye*. 2020;34(10):1731-1733. doi:10.1038/s41433-020-0930-0
9. Cicalini I, Rossi C, Pieragostino D, et al. Integrated lipidomics and metabolomics analysis of tears in multiple sclerosis: an insight into diagnostic potential of lacrimal fluid. *Int J Mol Sci*. 2019;20(6):1265. doi:10.3390/IJMS20061265
10. Dor M, Eperon S, Lalive PH, et al. Investigation of the global protein content from healthy human tears. *Exp Eye Res*. 2019;179:64-74. doi:10.1016/J.EXER.2018.10.006
11. Król-Grzymała A, Sienkiewicz-Szłapka E, Fiedorowicz E, Rozmus D, Cieślińska A, Grzybowski A. Tear biomarkers in Alzheimer's and Parkinson's diseases, and multiple sclerosis: implications for diagnosis (systematic review). *Int J Mol Sci*. 2022;23:10123. doi:10.3390/IJMS231710123
12. Roda M, Ciavarella C, Giannaccare G, Versura P. Biomarkers in tears and ocular surface: a window for neurodegenerative diseases. *Eye Contact Lens*. 2020;46:S129-34. doi:10.1097/ICL.0000000000000663
13. Zhou L, Beuerman RW. The power of tears: how tear proteomics research could revolutionize the clinic. *Expert Rev Proteomics*. 2017;14:189-191. doi:10.1080/14789450.2017.1285703
14. Posa A, Bräuer L, Schicht M, Garreis F, Beileke S, Paulsen F. Schirmer strip vs. capillary tube method: non-invasive methods of obtaining proteins from tear fluid. *Ann Anat*. 2013;195:137-142. doi:10.1016/J.AANAT.2012.10.001
15. Bachhuber F, Huss A, Senel M, Tumani H. Diagnostic biomarkers in tear fluid: from sampling to preanalytical processing. *Sci Rep*. 2021;11:10064. doi:10.1038/S41598-021-89514-8
16. Kaštelan S, Braš M, Pjevač N, et al. Tear Biomarkers and Alzheimer's disease. *Int J Mol Sci*. 2023;24:13429. doi:10.3390/IJMS241713429
17. Quah JHM, Tong L, Barbier S. Patient acceptability of tear collection in the primary healthcare setting. *Optom Vis Sci*. 2014;91:452-458. doi:10.1097/OPX.0000000000000188
18. Tamhane M, Cabrera-Ghayouri S, Abelian G, Viswanath V. Review of biomarkers in ocular matrices: challenges and opportunities. *Pharm Res*. 2019;36:1-35. doi:10.1007/S11095-019-2569-8/TABLES/4
19. Erdinest N, Pincovich S, London N, Solomon A. Neurostimulation for dry eye disease. *Curr Opin Allergy Clin Immunol*. 2022;22:328-334. doi:10.1097/ACI.0000000000000841
20. van de Sande N, Ramakers IHGB, Visser PJ, et al. Tear biomarkers for Alzheimer's disease screening and diagnosis (the TearAD study): design and rationale of an observational longitudinal multicenter study. *BMC Neurol*. 2023;23(1):293. doi:10.1186/S12883-023-03335-Y
21. Soria J, Durán JA, Etxebarria J, et al. Tear proteome and protein network analyses reveal a novel pentamarker panel for tear film characterization in dry eye and meibomian gland dysfunction. *J Proteomics*. 2013;78:94-112. doi:10.1016/J.JPROT.2012.11.017
22. Nichols JJ, Green-Church KB. Mass spectrometry-based proteomic analyses in contact lens-related dry eye. *Cornea*. 2009;28:1109-1117. doi:10.1097/ICO.0B013E3181A2AD81
23. Seifert K, Gandia NC, Wilburn JK, et al. Tear lacritin levels by age, sex, and time of day in healthy adults. *Invest Ophthalmol Vis Sci*. 2012;53:6610-6616. doi:10.1167/IOVS.11-8729
24. Enríquez-de-Salamanca A, Castellanos E, Stern ME, et al. Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. *Mol Vis*. 2010;16:862.
25. Kalló G, Emri M, Varga Z, et al. Changes in the chemical barrier composition of tears in Alzheimer's disease reveal potential tear diagnostic biomarkers. *PLoS One*. 2016;11:e0158000. doi:10.1371/JOURNAL.PONE.0158000
26. Del Prete S, Marasco D, Sabetta R, et al. Tear liquid for predictive diagnosis of Alzheimer's disease. *Reports*. 2021;4(3):26. doi:10.3390/REPORTS4030026
27. Wang YR, Chuang HC, Tripathi A, et al. High-Sensitivity and trace-amount specimen electrochemical sensors for exploring the levels of β-Amyloid in human blood and tears. *Anal Chem*. 2021;93:8099-8106. doi:10.1021/ACS.ANALCHEM.0C04980/ASSET/IMAGES/LARGE/AC0C04980_0010.JPEG
28. Gijs M, Ramakers IHGB, Visser PJ, et al. Association of tear fluid amyloid and tau levels with disease severity and neurodegeneration. *Sci Rep*. 2021;11(1):22675. doi:10.1038/S41598-021-01993-X
29. Gharbiya M, Visioli G, Trebbastoni A, et al. Beta-Amyloid peptide in tears: an early diagnostic marker of Alzheimer's disease correlated with choroidal thickness. *Int J Mol Sci*. 2023;24:2590. doi:10.3390/IJMS24032590
30. Kenny A, Jiménez-Mateos EM, Zea-Sevilla MA, et al. Proteins and microRNAs are differentially expressed in tear fluid from patients with Alzheimer's disease. *Sci Rep*. 2019;9:1-14. doi:10.1038/s41598-019-51837-y
31. Lee S, Kim E, Moon CE, et al. Amplified fluorogenic immunoassay for early diagnosis and monitoring of Alzheimer's disease from tear fluid. *Nat Commun*. 2023;14:1-15. doi:10.1038/s41467-023-43995-5
32. Hu L, Zhang T, Ma H, et al. Discovering the secret of diseases by incorporated tear exosomes analysis via rapid-isolation system: iTEARS. *ACS Nano*. 2022;16:11720-11732. doi:10.1021/ACSNANO.2C02531/ASSET/IMAGES/LARGE/NN2C02531_0004.JPEG
33. Gijs M, Nuijts RM, Ramakers I, Verhey F, Webers CAB. Differences in tear protein biomarkers between patients with Alzheimer's disease and controls. *Invest Ophthalmol Vis Sci*. 2019;60:1744-1744.
34. Gijs M, Ramakers IHGB, Visser PJ, et al. Detection of amyloid-beta and tau in tear fluid of patients with Alzheimer's disease 2020. doi:10.21203/RS.3.RS-36499/V1
35. Zhang Y, Lu C, Zhang J. Lactoferrin and its detection methods: a review. *Nutrients*. 2021;13:2492. doi:10.3390/NU13082492
36. Kawamata T, Tooyama I, Yamada T, Walker DG, McGeer PL. Lactotransferrin immunocytochemistry in Alzheimer and normal human brain. *Am J Pathol*. 1993;142:1574.
37. Cennamo G, Montorio D, Morra VB, et al. Surface-enhanced Raman spectroscopy of tears: toward a diagnostic tool for neurodegenerative disease identification. *J Biomed Opt*. 2020;25:1. doi:10.1117/1.JBO.25.8.087002
38. Paprzycka O, Wieczorek J, Nowak I, Madej M, Strzalka-Mrozik B. Potential application of MicroRNAs and some other molecular

biomarkers in Alzheimer's disease. *Curr Iss Mol Biol*. 2024;46:5066-5084. doi:10.3390/CIMB46060304
39. Bell RD, Sagare AP, Friedman AE, et al. Diverse and composite roles of miRNA in non-neuronal cells and neuronal synapses in Alzheimer's disease. *Biomolecules*. 2022;12:1505. doi:10.3390/BIOM12101505
40. Klyucherev TO, Olszewski P, Shalimova AA, et al. Advances in the development of new biomarkers for Alzheimer's disease. *Transl Neurodegener*. 2022;11:1-24. doi:10.1186/S40035-022-00296-Z
41. Weber JA, Baxter DH, Zhang S, et al. The MicroRNA spectrum in 12 body fluids. *Clin Chem*. 2010;56:1733-1741. doi:10.1373/CLINCHEM.2010.147405
42. Rentka A, Koroskenyi K, Harsfalvi J, et al. Evaluation of commonly used tear sampling methods and their relevance in subsequent biochemical analysis. *Ann Clin Biochem*. 2017;54(5):521-529. doi:10.1177/0004563217695843
43. Small D, Hevy J, Tang-Liu D. Comparison of tear sampling techniques for pharmacokinetic analysis: ofloxacin concentrations in rabbit tears after sampling with schirmer tear strips, capillary tubes, or surgical sponges. *J Ocul Pharmacol Ther*. 2009;16(5):439-446. doi:10.1089/JOP.2000.16.439
44. Li K, Chen Z, Duan F, Liang J, Wu K. Quantification of tear proteins by SDS-PAGE with an internal standard protein: a new method with special reference to small volume tears. *Albrecht Von Graefes Arch Klin Exp Ophthalmol*. 2010;248:853-862. doi:10.1007/S00417-009-1275-3
45. Camerlingo C, Lisitskiy M, Lepore M, et al. Characterization of human tear fluid by means of surface-enhanced Raman spectroscopy. *Sensors*. 2019;19:1177. doi:10.3390/S19051177
46. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA*. 2007;104:1604-1609. doi:10.1073/PNAS.0610731104/SUPPL_FILE/10731FIG6.PDF
47. Nudelman AS, Dirocco DP, Lambert TJ, et al. Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. *Hippocampus*. 2010;20:492-498. doi:10.1002/HIPO.20646
48. Li X, An WL, Alafuzoff I, Soininen H, Winblad B, Pei JJ. Phosphorylated eukaryotic translation factor 4E is elevated in Alzheimer brain. *Neuroreport*. 2004;15:2237-2240. doi:10.1097/00001756-200410050-00019
49. Bermejo-Pareja F, del Ser T, Valentí M, de la Fuente M, Bartolome F, Carro E. Salivary lactoferrin as biomarker for Alzheimer's disease: brain-immunity interactions. *Alzheimers Dement*. 2020;16:1196-1204. doi:10.1002/alz.12107
50. Tsatsanis A, McCorkindale AN, Wong BX, et al. The acute phase protein lactoferrin is a key feature of Alzheimer's disease and predictor of Aβ burden through induction of APP amyloidogenic processing. *Mol Psychiatry*. 2021;26:5516. doi:10.1038/S41380-021-01248-1
51. Hampel H, Teipel SJ, Fuchsberger T, et al. Value of CSF β-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry*. 2003;9(7):705-710. doi:10.1038/sj.mp.4001473
52. Lim HJ, Park JE, Kim BC, et al. Comparison of two analytical platforms in cerebrospinal fluid biomarkers for the classification of Alzheimer's disease spectrum with amyloid PET imaging. *J Alzheimers Dis*. 2020;75:949-958. doi:10.3233/JAD-191331
53. Grimmer T, Riemenschneider M, Förstl H, et al. Beta amyloid in Alzheimer's disease: increased deposition in brain is reflected in reduced concentration in cerebrospinal fluid. *Biol Psychiatry*. 2009;65:927-934. doi:10.1016/J.BIOPSYCH.2009.01.027
54. Van Setten GB, Nilsson L, Hahne S, et al. Beta-amyloid protein protein precursor expression in lacrimal glands and tear fluid. *Invest Ophthalmol Vis Sci*. 1996;37:2585-2593.
55. Ponzini E, Santambrogio C, De Palma A, Mauri P, Tavazzi S, Grandori R. Mass spectrometry-based tear proteomics for noninvasive biomarker discovery. *Mass Spectrom Rev*. 2022;41:842-860. doi:10.1002/MAS.21691
56. Pieragostino D, Lanuti P, Cicalini I, et al. Proteomics characterization of extracellular vesicles sorted by flow cytometry reveals a disease-specific molecular cross-talk from cerebrospinal fluid and tears in multiple sclerosis. *J Proteomics*. 2019;204:103403. doi:10.1016/J.JPROT.2019.103403
57. Quiroz-Baez R, Hernández-Ortega K, Martínez-Martínez E. Insights into the proteomic profiling of extracellular vesicles for the identification of early biomarkers of neurodegeneration. *Front Neurol*. 2020;11:580030. doi:10.3389/fneur.2020.580030
58. Hill AF. Extracellular vesicles and neurodegenerative diseases. *J Neurosci Nurs*. 2019;39:9269. doi:10.1523/JNEUROSCI.0147-18.2019
59. Rosa-Fernandes L, Rocha VB, Carregari VC, Urbani A, Palmisano G. A perspective on extracellular vesicles proteomics. *Front Chem*. 2017;5:294384. doi:10.3389/FCHEM.2017.00102/BIBTEX
60. Grigor'eva AE, Tamkovich SN, Eremina AV, et al. Exosomes in tears of healthy individuals: isolation, identification, and characterization. *Biochem Mosc Suppl B Biomed Chem*. 2016;10:165-172. doi:10.1134/S1990750816020049

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: López-Cuenca I, Masa-Castro R, Hoz-Ruiz Y, et al. Tears as a window to Alzheimer's disease: A systematic review of biomarkers for early detection. *Alzheimer's Dement*. 2026;18:e70268. https://doi.org/10.1002/dad2.70268