Review

ANNALS OF MEDICINE & SURGERY

OPEN

Liquid clues: tear film biomarkers unravelling Alzheimer's mysteries

Amna Zaheer, MBBS[a], Insa Binte Anwar, MBBS[a], Abdul Haseeb, MBBS[b], Alisha Yadav, MBBS[c,*]

Introduction

Alzheimer's disease (AD), generally referred to as AD, is the most prevalent neurodegenerative condition that serves as the primary contributor to dementia cases. An estimated 44 million individuals globally are currently experiencing dementia, and this figure could potentially triple by the year 2050[1]. Symptoms initiate with mild memory difficulties and progress to cognitive impairment, hampering basic life activities. The development of this neurodegenerative condition is primarily marked by two key characteristics: the accretion of neurofibrillary tangles consisting of hyperphosphorylated tau proteins and amyloid beta (Aβ) plaques[2]. According to a hypothesis, the creation and placement of amyloid-β plaques in various brain regions trigger an inflammatory reaction because these plaques are perceived as foreign entities. This, in turn, results in neurodegeneration and the demise of cells[3]. Moreover, tau proteins preserve the structural integrity of the cytoskeleton by undergoing polymerization and supporting the stable assembly of microtubules; however, in AD this tau protein gets hyperphosphorylated and forms neurofibrillary tangles (NFTs) and gets stored in the cytosol, hence disrupting cell structure. These NFTs deposition leads to ineffective axonal transport, signal transduction and synaptic transmission; ultimately, the cell undergoes degeneration[4].

Main text

Currently, the biomarkers used for AD diagnosis are classified into two categories based on their method of analysis: biochemical cerebrospinal fluid (CSF) markers and biomarkers derived from imaging techniques[5]. CSF biomarkers are frequently utilized in clinical settings for the purpose of diagnosing AD. The concentrations of phospho-tau (p-tau) and amyloid-β peptide in CSF serve as particular bio indicators for AD because they signify the pathological neuronal damage associated with AD. Several studies highlighted the fact that abnormal levels of amyloid-β appeared many years before the occurrence of memory decline in Alzheimer's patients, making it the earliest marker to exist[6]. The association between genetic mutations, oxidative stress, and AD is intricate. Certain genetic variants, such as APOE ε4 and mutations in presenilin and APP genes, are linked to an increased risk of AD. These genetic influences can impact the production of Aβ and tau proteins, contributing to the characteristic plaques and tangles seen in AD pathology. Oxidative stress further complicates this relationship by promoting protein aggregation and cellular damage. The interplay between genetic factors and oxidative stress creates a complex web, influencing the clinical manifestations of AD and posing challenges in precisely delineating primary markers like Aβ and tau. Understanding these interactions is vital for unravelling the complexities of AD pathology and developing targeted therapeutic interventions[7]. Subsequently, there are Imaging Biomarkers to diagnose AD, comprising fluoro-D-glucose positron emission tomography (FDG-PET) and Pittsburgh Compound-B positron emission tomography (PiB-PET). PiB-PET utilizes Pittsburgh compound-B as a ligand for Aβ to examine the distribution and quantity of cerebral Aβ[8]. Additionally, by employing PET imaging with the 2-deoxy-2 [18F] fluoro-D-glucose tracer (FDG-PET), it is possible to gauge cerebral glucose metabolism, which serves as an indicator of both glial and neuronal functionality. In AD, there is a diminishment in FDG-PET signals, indicating decreased glucose metabolism and impaired synaptic function[9].

However, CSF collection for markers is quite invasive. Recent advances in AD biomarkers include tear markers, which are non-invasive. Tears are non-invasive biological fluid, easy to collect and can be stored for extended periods, certain amount of diseases can be assessed using tear markers in place of other biomarkers, including keratoconus, Parkinson disease, cystic fibrosis and thyroid disease[10]. Studies show that diagnostic biomarkers such as Aß42 and p-tau ,which are found in tears, are under investigation for their cost-effectiveness. However, as per an ongoing TearAD study, tear fluid diagnoses are marked as less invasive and cost-effective than other ways of biomarker testing[11].

HIGHLIGHTS

- Tear markers can be used as a potential biomarker for early detection of Alzheimer's disease (AD).
- Levels of Aβ1-42 in tears help in the diagnosis of AD, having 93% specificity and 81% sensitivity.
- Tear fluid biomarkers present challenges, including limited sample volume and the need for dilution, but offer opportunities for accessible, non-invasive, and cost-effective diagnostic tools for AD.

[a]Liaquat National Hospital and Medical College, [b]Jinnah Sindh Medical University, Karachi, Pakistan and [c]Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal. Tel.: +977 986 9133 010.
E-mail: alishayadavofficial207@gmail.com (A. Yadav).

Annals of Medicine & Surgery (2024) 86:3499–3502

Received 11 September 2023; Accepted 19 March 2024

Published online 3 April 2024

http://dx.doi.org/10.1097/MS9.0000000000002014

This delay has hampered AD treatment trials, emphasizing the urgent need for sensitive and practical biomarkers. While blood-based AD biomarkers have shown promising advances, they require further validation and guidelines for proper utilization. Blood-based markers offer cost-effective and less invasive options for AD diagnosis and monitoring, but their sensitivity is limited due to low concentrations and challenges related to detection. Interestingly, recent studies suggest that AD also presents in ocular structures, offering a potentially accessible screening avenue. Biomarkers discovered within ocular structures, including the retina, vitreous, cornea and tears, present promising alternatives for early AD diagnosis, prognosis, and management assessment, encompassing the notable advantages of non-invasiveness, accessibility, and alignment with central nervous system changes[12].

This article primarily revolves around biomarkers obtained from tears. Tears, which envelop the ocular surface, constitute a vital bio-fluid containing diverse molecules like nucleic acid, metabolites, lipids, vitamins, electrolytes and proteins. The Goblet conjunctival cells, lacrimal gland, and Meibomian glands contribute to tear fluid production, essential for maintaining eye health by eliminating debris, irritants, and supplying white blood cells for potential injury responses. Lacrimal gland protein secretion is intricately governed by parasympathetic and sympathetic innervation[13].

In a recent study by Wijesinghe and colleagues, the expression levels of ten microRNAs (miRNAs) were studied in transgenic APP-PS1 mice, non-carrier siblings, and wild-type controls at various stages of AD progression. The relative expression levels of miRNAs in APP-PS1 mice and non-carrier siblings appeared similar to age-matched and sex-matched wild-type controls, indicating a common pattern. However, distinctions in expression levels between APP-PS1 mice and non-carrier siblings could be attributed to the underlying molecular causes of AD. Notably, miRNAs associated with Aβ production and pro-inflammation exhibited significant up-regulation in tear fluids, correlating with disease progression as indicated by cortical Aβ load and reactive astrogliosis. This study underscores the potential translational utility of elevated tear fluid miRNAs as biomarkers for understanding the pathogenesis of AD[14].

The detection of Aβ42 in tear fluid has been verified by three separate groups of researchers. Del Prete and colleagues utilized an immunocytochemistry assay in tear smears from humans with a familial predisposition to AD, contrasting with its absence in tears from healthy individuals. Importantly, Aβ42 presence in tears included retinal plaques, hinting at its potential as a predictive marker for AD in cognitively normal donors[15].

In the 2016 study conducted by Wood and colleagues, 14 patients diagnosed with AD and nine cognitively healthy controls were included. The study revealed a higher tear flow rate in AD patients compared to controls, along with elevated total protein levels in the tears of AD patients. The researchers aim to validate these potential biomarkers in larger cohorts, assessing their specificity for AD or neurodegeneration. They envision a bedside test utilizing these markers as an initial screening tool, followed by thorough examinations for a definitive diagnosis. Eye-related complications, such as reduced retinal ganglion cells, thinning of the nerve cell layer, declining axon numbers, and amyloid deposition in the lens and retina, have been observed in AD. The study posits that alterations induced by AD in the eye may contribute to changes in tear production and the composition of tear proteins[16].

In a study by Kallo and colleagues, global changes in tear protein profiles were investigated using quantitative proteomics techniques. The research revealed alterations in tear flow rate, total tear protein concentration, and the composition of the chemical barrier specific to AD. A potential biomarker combination for AD, comprising lipocalin-1, dermcidin, lysozyme-C, and lacritin, demonstrated 81% sensitivity and 77% specificity. Tear fluid, containing antimicrobial and immunomodulatory proteins, plays a crucial role in the innate immune system's defense, responding to mechanical, microbial, and systemic challenges. The study suggested that inflammatory processes and increased brain protein oxidation are implicated in the pathophysiology of AD, with reactive oxygen species production and mitochondrial dysfunction potentially contributing to protein oxidation[17].

Sande and colleagues are conducting the TearAD study, whose protocol has been accepted and published. Their objective is to validate prior findings that indicated the presence of AD biomarkers in tear fluid and their correlation with disease severity and neurodegeneration. The study will involve 200 participants, encompassing cognitively healthy controls, individuals with subjective cognitive decline, those with mild cognitive impairment, and patients with AD dementia. Baseline examinations will be administered, followed by subsequent assessments at 1 and 2 years. The evaluations will encompass neuropsychological tests, ophthalmic examinations, MRI scans, and the collection of tear fluid. Schirmer strips will be used for tear fluid collection, and multiplex immunoassays will determine levels of AD biomarkers (Aβ38, Aβ40, Aβ42, t-tau, and p-tau). Additionally, blood samples will be collected. Retinal imaging will be conducted utilizing standard, hyperspectral, and ultra-wide field fundus cameras, along with optical coherence tomography imaging. The study's goal is to shed light on the potential diagnostic utility of tear biomarkers as a minimally invasive and cost-effective tool for AD screening and diagnosis. All collected data will be securely stored in the Castor EDC online database with restricted access, and subsequent exportation to IBM SPSS Statistics will facilitate statistical analysis. Tears in individuals with AD have not only been discerned from those in normal individuals, but several studies have established their potential as a distinguishing factor between AD and other neurodegenerative conditions. Recently, research revealed the presence of Aβ42, Aβ38, Aβ40, phosphorylated-tau (p-tau) and total-tau (t-tau) in tear fluid. Significantly, levels of p-tau were measurable exclusively in individuals with AD dementia, mild cognitive impairment (MCI), and subjective cognitive decline (SCD) but not in those with normal cognitive functioning. Furthermore, tear levels of t-tau showed a substantial increase in patients with AD dementia when compared to those with SCD. Based on research, levels of Aβ1-42 in tears have a 93% specificity and 81% sensitivity in identifying people with both AD and MCI[13].

Similarly, Gharbiya and colleagues supported the identification of Aβ42 and p-tau in the tears of people with MCI and AD dementia. Their research revealed notably reduced Aβ42 levels in AD dementia and MCI patients when compared to individuals with normal cognitive function[11]. Wang and colleagues showcased both Aβ42 and 40 peptides in tears from healthy individuals aged 20–79, utilizing an innovative electrochemical immune sensor. Interestingly, peptide levels in tear fluid

Table 1

Key findings from studies investigating biomarkers in tear fluid for Alzheimer's disease (AD) screening and diagnosis

References	Study design	Biomarker type	Methods used for biomarker assaying	Significance of results	Sensitivity/ specificity (if mentioned)
Wijesinghe *et al.*[14]	Cross-sectional	miRNAs (Aβ-associated)	Single tube TaqMan advanced miRNA assays	Elevated tear fluid miRNAs as potential biomarkers for AD pathogenesis	Not mentioned
Sande *et al.*[13]	Observational longitudinal multicenter study.	Aβ38, Aβ40, Aβ42, t-tau, p-tau	Multiplex immunoassays, retinal imaging, MRI, neuropsychological tests	Investigating diagnostic utility of tear biomarkers for AD screening	Aβ1-42: 93% specificity, 81% sensitivity
Del Prete *et al.*[15]	Case report	Aβ42	Immunocytochemistry assay	Aβ42 presence in tears, including retinal plaques, potential predictive marker for AD	Not mentioned
Wood *et al.*[16]	Cohort	Tear flow rate, total protein	SDS–PAGE and mass spectrometry,	Higher tear flow rate and elevated total protein levels in AD patients	81% sensitivity, 77% specificity
Kallo *et al.*[17]	Case-control study	Lipocalin-1, dermcidin, lysozyme-C, lacritin	Quantitative proteomics	Potential biomarker combination for AD due to high sensitivity and specificity	81% sensitivity, 77% specificity
Gharbiya *et al.*[11]	Cross-sectional	Aβ42, p-tau	spectral-domain optical coherence tomography (SD-OCT), PET, MRI imaging	Reduced Aβ42 levels in AD and MCI patients, identification in tears of individuals with cognitive impairment	93% specificity, 81% sensitivity
Wang *et al.*[18]	Experimental/Laboratory research	Aβ42, Aβ40	Electrochemical immune sensor	Higher peptide levels in tears than whole blood, age-related trends	Not mentioned
Gijs *et al.*[19]	Observational study	Aβ40, Aβ38, Aβ42, Tau, p-Tau	Multiplex immunoassay	Presence of classical AD biomarkers in tears, elevated levels in patients vs. healthy subjects	Not mentioned

Aβ, amyloid beta; MCI, mild cognitive impairment; PET, positron emission tomography.

surpassed those in whole blood by tenfold (10 vs. 1 pg/ml), displaying age-related trends[18]. Gijs and colleagues conducted extensive research on classical AD biomarkers in tear fluid from subjects with diverse cognitive impairments. Employing a multiplex immunoassay platform, they assessed Aβ and Tau protein levels. While Aβ40 was consistently detectable, Aβ38 and 42 were present in fewer than 23% of samples, mainly among the healthy group. Elevated peptide concentrations were noted in the groups of patients (ranging from a median of 17–1680 pg/ml) when compared to those in good health (with a median ranging from 4 to 60 pg/ml), but these variances did not reach statistical significance. A similar pattern emerged with Tau protein, detectable in 94% of samples at ng/ml levels, approximately ten times higher than CSF levels. However, differences between cognitively impaired patients and healthy subjects did not hold significance, except for elevated tears t-Tau levels in patients with greater neurodegeneration markers. Phosphorylated-Tau protein displayed remarkably low detectability (18%), identified in study groups only. In summary, while classical AD biomarkers can be evaluated in tear fluid, often exceeding levels in other biological materials, their predictive and diagnostic potential necessitates further exploration. However, constrained tear sample volume and the need for dilution present notable challenges in laboratory practice, impacting accurate quantification[19]. Apart from these factors, environmental factor also plays a vital role in tear biomarker stability, including humidity, warm temperature, contact lens and certain eye conditions like conjunctivitis, which lead to tear film instability, which leads to limitation of their use[20]. (Table 1)

Conclusion

AD presents a major health challenge worldwide due to its growing prevalence and severe consequences. Early diagnosis and effective management are essential, and this can be achieved by using biomarkers. Although traditional methods, like cerebrospinal fluid and imaging, are commonly used, recent studies have highlighted the potential of tear fluid as a non-invasive alternative. Tear fluid is a promising diagnostic medium that can provide access to central nervous system changes. Studies suggest that tears contain amyloid-β peptides and phosphorylated-tau proteins in individuals with AD and mild cognitive impairment. However, challenges such as sample volume limitations, dilution requirements, and environmental factors must be addressed when using tear fluid biomarkers[15,19,21]. However, challenges such as sample volume constraints, dilution requirements, and environmental factors need to be addressed to fully realize the diagnostic potential of tear biomarkers. Further research and validation efforts in this area are essential to offer hope for earlier and more accessible AD diagnosis and, ultimately, improved patient outcomes, necessitating the exploration of the effectiveness of biomarkers in tears for the diagnosis of AD in the future[17,20].

Ethical approval

This is a correspondence. Therefore, it didn't require ethical approval from the ethics committee.

Consent

Not applicable.

Source of funding

The study did not receive any grant from funding agencies in the public, commercial or not-for-profit sectors.

Author contribution

A.Z.: writing the paper, and concept. I.B.A.: writing the paper. A.H.: study concept and rechecking. A.Y.: proof reading.

Conflicts of interest disclosure

The author declares no conflicts of interest.

Research registration unique identifying number (UIN)

1. Name of the registry: Not applicable.
2. Unique Identifying number or registration ID:
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Alisha Yadav.

Data availability statement

Not applicable.

Provenance and peer review

Not commissioned, externally peer-reviewed.

References

[1] Klyucherev TO, Olszewski P, Shalimova AA, *et al*. Advances in the development of new biomarkers for Alzheimer's disease. Transl Neurodegener 2022;11:25.

[2] Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. F1000Research 2018;7:1161.

[3] Barage SH, Sonawane KD. Amyloid cascade hypothesis: pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides 2015; 52:1–18.

[4] Huang F, Wang M, Liu R, *et al*. CDT2-controlled cell cycle reentry regulates the pathogenesis of Alzheimer's disease. Alzheimers Dement 2019; 15:217–31.

[5] Robb MA, McInnes PM, Califf RM. Biomarkers and surrogate endpoints: developing common terminology and definitions. JAMA 2016; 315:1107–8.

[6] Dubois B, Hampel H, Feldman HH, *et al*. Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. Alzheimer Dement 2016;12(3):292–323.

[7] Cioffi F, Adam RHI, Broersen K. Molecular mechanisms and genetics of oxidative stress in Alzheimer's disease. J Alzheimers Dis 2019;72:981–1017.

[8] Lloret A, Esteve D, Lloret MA, *et al*. When does Alzheimer's disease really start? The role of biomarkers. Int J Mol Sci 2019;20:5536.

[9] Jagust W, Reed B, Mungas D, *et al*. What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia? Neurology 2007;69: 871–7.

[10] Kaštelan S, Braš M, Pjevač N, *et al*. Tear biomarkers and Alzheimer's disease. Int J Mol Sci 2023;24:13429.

[11] 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.

[12] Hussain A, Sheikh Z, Subramanian M. The eye as a diagnostic tool for Alzheimer's disease. Life (Basel) 2023;13:726.

[13] van de Sande N, Ramakers I, 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;23:293.

[14] Wijesinghe P, Xi J, Cui J, *et al*. MicroRNAs in tear fluids predict underlying molecular changes associated with Alzheimer's disease. Life Sci Alliance 2023;6:e202201757.

[15] Del Prete S, Marasco D, Sabetta R, *et al*. Tear liquid for predictive diagnosis of Alzheimer's disease. Reports 2021;4:26.

[16] Wood H. Could tear proteins be biomarkers for Alzheimer disease? Nat Rev Neurol 2016;12:432.

[17] Kalló G, *et al*. Changes in the chemical barrier composition of tears in Alzheimer's disease reveal potential tear diagnostic biomarkers. PloS one 2016;11:e0158000.

[18] Wang H-Y, Bakshi K, Frankfurt M, *et al*. Reducing amyloid-related Alzheimer's disease pathogenesis by a small molecule targeting filamin A. J Neurosci 2012;32:9773–84.

[19] Gijs M, Ramakers I, Visser PJ, *et al*. Association of tear fluid amyloid and tau levels with disease severity and neurodegeneration. Sci Rep 2021;11:22675.

[20] Król-Grzymała A, Sienkiewicz-Szłapka E, Fiedorowicz E, *et al*. Tear biomarkers in Alzheimer's and Parkinson's diseases, and multiple sclerosis: implications for diagnosis (Systematic Review). Int J Mol Sci 2022;23:10123.

[21] Roda M, Ciavarella C, Giannaccare G, *et al*. Biomarkers in tears and ocular surface: a window for neurodegenerative diseases. Eye Contact Lens 2020;46(Suppl 2):S129–34.