

Detection of amyloid-beta and tau in tear fluid of patients with Alzheimer's disease

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Abstract

Background There is growing interest in finding non-invasive alternatives to cerebrospinal fluid (CSF) that could serve as front-line diagnostics for Alzheimer's disease (AD). In this study, we investigated AD-specific biomarkers in tear fluid.

Methods Tear fluid was collected from 25 patients with subjective cognitive decline (SCD), 24 patients with mild cognitive impairment (MCI), 11 dementia patients and nine controls. Amyloid-beta peptides (AB38, AB40, AB42), t-tau and p-tau levels in tear fluid were determined using multiplex immunoassays.

Results Tear t-tau levels in dementia, MCI and SCD patients were higher than in HC ($p = 0.002$, $p = 0.002$ and $p = 0.013$, respectively). A negative correlation between AB42 and t-tau was found in both tear fluid and CSF. Levels of tear p-tau were detectable in patients but not in HC.

Conclusions This study shows for the first time presence of amyloid-beta peptides (AB38, AB40, AB42), t-tau and p-tau in tear fluid.

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in dementia and eventual death. It is the leading cause of dementia and the number of cases are projected to rise in the next few decades. Aggregation of amyloid-beta ($A\beta$) into oligomers, fibrils, and plaques together with the presence of hyperphosphorylated tau are central in the molecular pathogenesis of AD. Currently, these pathological biomarkers are detected through either cerebrospinal fluid (CSF) analysis, brain imaging or post-mortem tissue analysis. Low amyloid-beta-42 (AB42) and elevated total tau (t-tau) and phosphorylated tau (p-tau) concentrations in CSF are associated with AD. Though effective, the application of CSF is limited due to the invasive nature of a lumbar puncture. Given that the eye offers a direct window to cerebral pathology, there has been an increased interest in the use of ocular biomarkers.

Ocular biomarkers for AD have been identified on the functional, biological and biochemical level. Visual symptoms reported by AD patients include decreased visual acuity in low luminance, reduced visual contrast sensitivity, poor color discrimination and visual field loss (reviewed (1–3)). The most well-known examples of biological differences between AD patients and healthy controls are widespread retinal ganglion cell losses, retinal nerve fiber layer (RNFL) thinning and optic nerve degeneration (1–4). At the biochemical level, amyloid-beta plaques have been observed in postmortem retina samples of AD patients (5), although others have failed to reproduce these results (6). In addition, tau accumulation was identified in postmortem retina samples of AD patients (6). Previous studies also found soluble amyloid-beta in aqueous humor (7) and vitreous body (8) of cognitively healthy persons.

In 1996 Van Setten et al. (9) showed the presence of full-length amyloid-beta precursor protein (APP) in human tear fluid and the lacrimal gland (extraocular gland that produces tear fluid) of healthy persons. Kallo et al. (10) identified differences in the total tear protein composition in AD patients. Recently, Kenny

et al. (11) performed differential expression of tear fluid from AD patients and controls by mass spectrometry. Based on these studies, it is reasonable to assume presence of amyloid-beta and tau in tear fluid. No studies published thus far have reported the level of these AD-specific biomarkers in tear fluid.

In the present work, we investigated amyloid-beta peptides (AB38, AB40 and AB42), total-tau and p-tau in tear fluid in patients with cognitive impairment and healthy controls. We report differences in tear levels across diagnostic group, representing a proof-of-concept for the role of tear fluid in the diagnosis of Alzheimer's disease.

Methods

Participants

A total of 60 patients and nine cognitively healthy age-matched controls (HC) were enrolled in this study. Patients were recruited from the BioBank Alzheimer Center Limburg (BB-ACL), Maastricht, the Netherlands. Inclusion criteria were an MMSE score ≥ 20 and a CDR score from 0 to 1, thereby including patients across the whole clinical spectrum (i.e. from subjective cognitive disorder to (mild) dementia). Exclusion criteria at baseline were neurological diseases (such as Normal Pressure Hydrocephalus, Morbus Huntington, brain tumor, epilepsy, encephalitis, recent Transient Ischemic Attack (TIA) or cerebrovascular accident (CVA) (< 2 years), or TIA/CVA with concurrent (within three months) cognitive decline) and a history of psychiatric disorders (such as schizophrenia, bipolar disorder or psychotic problems, current major depressive disorder (within 12 months), or alcohol abuse). The local ethical committee approved the study protocol and this study followed the tenets of the current version of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical diagnosis and assessment

During their visit to the memory clinic, all patients underwent standardized clinical assessments including neuropsychological assessments, blood tests, MRI cerebrum and, from a subsample of patients, CSF sampling. A diagnosis of dementia was made according to regular DSM-5 criteria for neurocognitive disorders (12). A diagnosis of mild cognitive impairment (MCI) was based on the clinical National Institute on Ageing-Alzheimer's Association (NIA-AA) criteria, which entails that patients have impaired cognitive functioning but sufficiently preserved function performance such that a dementia diagnosis cannot be made (13). All other patients were classified as subjective cognitive decline (SCD), indicating the presence of a cognitive complaint initiation the visit to the memory clinic, but having no impairment in cognitive test performances and activities of daily living. The global cognitive status was evaluated using the Mini-Mental State Examination (MMSE (14)) and dementia severity was scored using the Clinical Dementia Rating (CDR (15)).

CSF collection and biochemical analyses

CSF was collected via a lumbar puncture. After collection, CSF was centrifuged at room temperature, aliquoted and stored at -80 °C until analysis. The levels of amyloid-beta-42 (AB42), total tau (t-tau) and

phosphorylated at threonine 181 tau (p-tau) were measured using commercially available single-parameter ELISA methods (respectively Innostest® beta-amyloid (1–42) and Innostest® hTAU-Ag; Innogenetics, Ghent, Belgium) at the department of Neurochemistry, Radboud University MC, Nijmegen the Netherlands. Diagnostic cutoff values were 500 pg/mL for AB42, 350 pg/mL for t-tau and 85 pg/mL for p-tau.

Tear fluid collection and biochemical analyses

Tear fluid was collected from the left and right eye via Schirmer tear strips (TEAR strips, Contacare Ophthalmics and diagnostics, Gujarat, India) without topical anesthesia. Care was taken not to touch the strip with fingertips. Before storage at -80 °C, the tear migration length (a marker for tear volume) was recorded. Tear fluid was extracted from the Schirmer strip by agitating small cut pieces of these strips in 60 µL PBS, Tween20 0.5% and cOmplete™ Protease Inhibitor Cocktail (Roche, Basel, Switzerland) at 4 °C for 1.5 hours (16, 17). Tear fluid was then eluted by centrifugation and stored at -80 °C until further use. Tear total protein content was determined using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce™, Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. Due to sample shortage, the protein content of tear samples from two healthy controls could not be determined. Tear levels of amyloid-beta peptides (AB38, AB40 and AB42) and t-tau and p-tau were determined using the Human Aβ triplex Ultra-Sensitive assay and the Phospho(Thr231)/Total Tau duplex assay (Meso Scale Discovery, Rockville, MD, USA) at the department of Internal Medicine, School for Cardiovascular Diseases (CARIM), Maastricht University Medical Center, Maastricht, the Netherlands. Assays were run in singlicate due to low sample volume. Biomarker levels of the left and right eye were combined and averaged as biological duplicates. Undetectable samples were labelled zero to allow statistical analysis. Raw data were corrected for total protein content and are thus expressed as pg/mg or U/mg.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 25 (Armonk, NY, USA) and graphs were created in GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Because of small and unbalanced sample sizes, non-parametrical tests were used to test statistical significance. Differences in continue variables (age, education level, MMSE and biomarker levels) across groups were analyzed using Kruskal-Wallis testing with Bonferroni post hoc correction for multiple comparison. Differences in categorical variables (sex and CDR) were analyzed using the Fisher exact test. Correlations were assessed using Spearman's correlation analysis and data were fitted with a linear regression line. For reasons of visibility, one extreme value for tear t-tau (4.14 pg/mg) was removed from the correlation graphs. Data in tables are presented as medians with interquartile range (IQR). As the group of dementia patients whose CSF was available for biomarker analysis contains only three individuals, the IQR cannot be computed for these variables.

Results

Participant characteristics

A total of 60 patients and nine cognitively healthy controls were included in the study. Twenty-five (42%) patients received a diagnosis of SCD, 24 (40%) patients had a diagnosis of MCI and 11 (18%) patients had a clinical diagnosis of dementia (Table 1). For healthy controls (HC), no information was available regarding their education level, CDR and MMSE scores. Dementia patients (median 74 years) and MCI patients (median 69.5 years) were older than SCD patients (median 60 years, $p < 0.001$ and $p = 0.010$ respectively) and dementia patients were older than healthy controls (median 60 years, $p = 0.044$). Sex and education level were comparable across diagnostic groups. CDR scores were not statistically different between diagnostic groups. Mini-Mental State Examination (MMSE) score of people with dementia (median 24) and MCI (median 27) was lower than that of SCD patients (median 29, $p = 0.001$ and $p = 0.012$ respectively).

Table 1
Participant characteristics

Participant characteristics	HC (n = 9)	SCD (n = 25)	MCI (n = 24)	Dem (n = 11)	p-value (overall)	p-value (groups)
Age, years, median (IQR)	60 (16)	60 (13)	69.5 (15)	74 (3)	< 0.001***	HC vs Dem 0.044* SCD vs MCI 0.010** SCD vs Dem < 0.001*** MCI vs Dem 0.648
Sex, male/female	4/5	15/10	13/11	6/5	0.891	-
Education level, median (IQR)	-	4 (3)	4 (3)	3 (3)	0.629	-
CDR, 0/0.5/1.0	-	2/22/1	0/23/1	0/8/3	0.086	-
MMSE, median (IQR)	-	29 (2)	27 (3)	24 (6)	< 0.001***	SCD vs MCI 0.012* SCD vs Dem 0.001*** MCI vs Dem 0.456
Differences between groups were analyzed by Kruskal-Wallis testing (age, education level, MMSE) including Bonferroni correction and Fisher's exact test (sex and CDR). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.						

CSF biomarker levels

CSF was available for clinical diagnostic purposes from 24 (40%) patients (10 SCD patients, 11 MCI patients and 3 patients with dementia) (Supplementary Table 1). It was not available from healthy controls. Within the subgroup of patients whose CSF was available, people with dementia (median 74 years) and MCI (median 69 years) were older than SCD patients (median 60 years, $p = 0.006$ and $p = 0.044$, respectively). Sex, education level, CDR and MMSE scores were comparable across diagnostic groups. Kruskal-Wallis tests showed that diagnostic groups differ significantly from each other in terms of CSF AB42 ($p = 0.042$), CSF t-tau ($p = 0.002$) and CSF p-tau ($p = 0.006$) levels. Although CSF AB42 levels in dementia patients (median 707 pg/mL) were lower than for MCI patients (median 747 pg/mL) and SCD patients (median 1241 pg/mL), these differences were not statistically significant. CSF t-tau levels were significantly increased in people with dementia (median 526 pg/mL) and MCI patients (median 604 pg/mL) compared to SCD patients (median 232.5 pg/mL, $p = 0.031$ and $p = 0.003$, respectively). The same trend was observed for CSF p-tau levels of people with dementia (median = 84 pg/mL) and MCI patients (median = 77 pg/mL) versus SCD patients (median = 44.5 pg/mL, $p = 0.04$ and $p = 0.017$, respectively). For these three CSF biomarkers, no significant differences were observed between MCI and dementia patients.

Tear fluid biomarker levels

Tear fluid was collected from both eyes of all 69 participants. Table 2 displays the number of samples with detectable levels of the three amyloid-beta peptides (38, 40 and 42), total-tau and p-tau. While AB40 and AB42 were detectable in more than 90% of tear fluid samples, AB38 was detectable in 39 out of 69 samples (57%) of tear fluid samples. Total-tau was well detectable (86%) whereas p-tau could be detected in 32 out of 69 samples (46%). Interestingly, tear p-tau was not detectable in tear samples from the HC group.

Table 2
Tear fluid biomarker detectable samples

Participant characteristics	HC (n = 9)	SCD (n = 25)	MCI (n = 24)	Dem (n = 11)	p-value (overall)	p-value (groups)
Age, years, median (IQR)	60 (16)	60 (13)	69.5 (15)	74 (3)	< 0.001***	HC vs Dem 0.044* SCD vs MCI 0.010** SCD vs Dem < 0.001*** MCI vs Dem 0.648
Sex, male/female	4/5	15/10	13/11	6/5	0.891	-
Education level, median (IQR)	-	4 (3)	4 (3)	3 (3)	0.629	-
CDR, 0/0.5/1.0	-	2/22/1	0/23/1	0/8/3	0.086	-
MMSE, median (IQR)	-	29 (2)	27 (3)	24 (6)	< 0.001***	SCD vs MCI 0.012* SCD vs Dem 0.001*** MCI vs Dem 0.456
Data are expressed as numbers and percentages (n, %).						

Table 3 and Fig. 1 show tear fluid biomarker levels across the groups. Tear volume (expressed in mm and corresponding to the tear migration length) of the HC group (median 29.25 mm) was higher than for SCD patients (median 15 mm, $p = 0.012$), MCI patients (14.75 mm, $p = 0.004$) and dementia patients (9.5 mm, $p = 0.001$) (Fig. 1A). Despite different tear volumes, no differences in total protein content across diagnostics groups were observed (Fig. 1B).

Table 3
Tear fluid biomarker levels

Tear fluid biomarker levels	HC (n = 7)	SCD (n = 25)	MCI (n = 24)	Dem (n = 11)	p-value (overall)	p-value (groups)
Tear volume (mm)	29.25 (6.75)	15 (12.56)	14.75 (12.69)	9.5 (9.5)	0.001 ***	HC vs SCD 0.012* HC vs MCI 0.004** HC vs Dem 0.001***
Total protein (mg/mL)	2.59 (0.90)	2.11 (0.60)	2.30 (1.35)	1.89 (0.82)	0.215	-
Tear AB38 (pg/mg)	0 (8.21)	2.58 (13.1)	3.56 (18.99)	0 (10.15)	0.530	-
Tear AB40 (pg/mg)	1.45 (1.3)	2.15 (2.01)	2.23 (1.71)	2.11 (1.73)	0.278	-
Tear AB42 (pg/mg)	0.41 (0.42)	0.56 (0.94)	0.87 (1.19)	0.84 (1.13)	0.274	-
Tear t-tau (pg/mg)	0 (0.04)	0.13 (0.35)	0.21 (0.30)	0.21 (0.73)	0.001 ***	HC vs SCD 0.013* HC vs MCI 0.002** HC vs Dem 0.002**
Tear p-tau (U/mg)	0 (0)	0.23 (1.01)	0 (0.65)	0.29 (1.29)	0.079 (incl. HC) 0.741 (excl. HC)	-

Data are expressed as median and IQR. Differences between groups were analyzed by Kruskal-Wallis testing including Bonferroni correction. * p < 0.05, ** p < 0.01, *** p < 0.001.

Tear fluid levels of AB38, AB40 and AB42 were higher in all patients compared to HC, but this difference was not statistically significant (Fig. 1C-E). Tear t-tau levels displayed statistically significant differences across diagnostic groups (p = 0.001). The level of tear t-tau in dementia patients (median 0.21 pg/mg), MCI patients (median 0.21 pg/mg) and SCD patients (median 0.13 pg/mg) were higher than in HC participants (median 0 pg/mg) (p = 0.002, p = 0.002 and p = 0.013, respectively) (Fig. 1F). Tear t-tau levels did not differ between the dementia, MCI and SCD groups. Tear fluid levels of p-tau did not differ between

groups (Fig. 1G). As p-tau was undetectable in the HC samples, Kruskal-Wallis testing was performed while including and excluding the HC group. In both cases, the difference between groups was not statistically significant ($p = 0.079$ including the HC group versus $p = 0.741$ excluding the HC group).

Correlations

Figure 2A shows that tear volume and tear protein content were positively correlated ($r = 0.597$, $p < 0.001$). We also observed that older participants had less tears, as tear volume was negatively correlated with age ($r = -0.272$, $p = 0.025$) (Fig. 2B). Age was not statistically significant correlated with tear biomarkers (Fig. 2C-E). MMSE was positively correlated with tear p-tau ($r = 0.261$, $p = 0.046$), but not with tear AB42 or tear t-tau (Fig. 2F-H).

Figure 3 presents inter-correlations of AB42, t-tau and p-tau between tear fluid and CSF. We found negative correlations between CSF AB42 and tear AB42 ($r = -0.373$, $p = 0.073$) and between CSF AB42 and tear t-tau ($r = -0.386$, $p = 0.062$) (Fig. 3A-B).

Intra-correlations of AB42, t-tau and p-tau within CSF and tear fluid are presented in Fig. 4. AB42 and t-tau were negatively correlated in tear fluid in the same way as in CSF (Fig. 4C-D). Tear AB42 and tear p-tau were significantly correlated ($r = 0.449$, $p < 0.001$) (Fig. 4B).

Discussion

To our knowledge, this is the first study that comprehensively investigated tear levels of amyloid-beta peptides and tau protein in patients with cognitive impairment compared to healthy controls. While AB40, AB42 and total-tau were well detectable, AB38 and p-tau were detectable in only half of the collected tear fluid samples. This result may be explained by the fact that AB38 levels are also (much) lower than AB40 levels in CSF (18) and than AB40 and AB42 levels in plasma (19). Similarly, p-tau levels in CSF are five to ten times lower than total-tau levels (18). Levels of p-tau were undetectable in tear fluid samples from healthy controls. This is an interesting finding since the determination of p-tau in CSF may increase the specificity and sensitivity in the detection of AD as opposed to total-tau (20). However, tests with higher sensitivity for p-tau are needed to confirm this observation in tear fluid. In all cases, highly sensitive assays are essential for tear fluid analysis due to limited sample volume.

The tear fluid level of t-tau in dementia, MCI and SCD patients was significantly higher than in HC participants. However, tear t-tau levels did not differ significantly between the dementia, MCI and SCD groups. One individual with dementia displayed a remarkable high tear t-tau level (4.14 pg/mg), while his CSF t-tau level was also higher than the threshold (350 pg/mL) as biomarker for neurodegeneration (21).

To the best of our knowledge, there have been no studies that used immunoassays of amyloid-beta and tau for tear fluid. Previous studies on tear fluid samples of AD patients (10, 11) used mass spectrometry and were therefore not able to detect amyloid-beta and tau, since mass spectrometry is less sensitive for low abundant proteins than the immunoassays we used in our study. Additionally, detection of amyloid and tau by mass spectrometry usually employs targeted methodologies using antibody immunoaffinity

enrichment. Kallo et al. (10) used mass spectrometry to compare ten priorly selected proteins in tear fluid samples from AD patients versus controls and observed differences for lipocalin-1, lactotransferrin, extracellular glycoprotein lacritin, lysozyme-C, prolactin inducible protein and dermcidin. Kenny et al. (11) used mass spectrometry on tear fluid from AD patients versus controls. They identified a panel of 12 proteins that were differentially expressed in tear fluid of AD patients compared to controls. Among these, elongation initiation factor 4E (eIF4E) was only detectable in AD samples (n = 5) and undetectable in samples from controls. EIF4E is involved in the initiation of protein synthesis by recognizing and binding the 7-methylguanosine-containing mRNA cap. The authors mention that they were not able to identify amyloid-beta or tau in their study and claim that this was probably due to their untargeted experimental set-up.

This investigation is also the first to evaluate both tear fluid and CSF. CSF was available from a subset of patients for clinical diagnostic purposes. We observed that AB42 and t-total were negatively correlated in tear fluid in the same way as in CSF. No significant correlations were observed between CSF p-tau and tear p-tau, however it must be noted that p-tau was analyzed in CSF using the p-tau-181 antibody and in tear fluid using the p-tau-231 antibody. Furthermore, p-tau levels in CSF are expressed in pg/mL while tear p-tau levels are expressed in U/mg.

We identified differences in tear volume between participants. Tear volume was lower in SCD, MCI and dementia patients compared to the HC group. This is in good agreement with previously published reports that demonstrate that neurodegenerative disease may be associated with abnormal tear function (22). For example, approximately 20% of patients with Sjögren's syndrome (an autoimmune disorder involving the exocrine gland) have CNS involvement including dementia (23). Another factor that might explain this difference in tear volume between groups is age. The frequency of dry eye syndrome is known to be gradually increased in middle and advanced ages (24). Nevertheless, since we correct our biomarker concentrations for the tear total protein content (that was comparable across groups), differences in tear volume do not influence the biomarker results.

We observed an average tear total protein content of approximately 3 mg/mL, which is in good agreement with previously published data (25). While Kallo et al. (10) identified a significant increase in the total protein content of tear samples from AD patients versus controls, in our study, and in line with previous reports (11), we did not observe such difference. The presence of ocular comorbidities (such as ocular inflammations or infections) may underline an increased total protein content. Tear protein concentrations (3–7 mg/mL) are generally lower than blood protein concentrations (60–80 mg/mL) but higher than CSF protein concentrations (0.15 to 0.45 mg/mL). While it is common for tear biomarkers to express their concentrations per unit protein (pg/mg protein), this is usually not done for CSF biomarkers. However, CSF protein concentrations vary greatly amongst individuals (26) and can be elevated after for example ischemic stroke (27).

This study is in line with others studies that identified tear biomarkers for different neurological conditions, such as TNF-alpha (28) and oligomeric alpha-synuclein (29) for Parkinson's disease and

alpha-1 anti-chymotrypsin for multiple sclerosis (30). Together, these studies support the hypothesis that tear fluid may predispose to mirror pathophysiological changes in the central nervous system. Furthermore, the fact that amyloid-beta and tau are detectable in tear fluid might reveal new fundamental insights in the disease molecular pathology. Retinal ganglion cells and retinal pigment epithelium have been identified as major sources of amyloid-beta synthesis (31). After secretion into the vitreous humor, amyloid-beta is subsequently transported to the aqueous humor. This follows a similar pattern observed in the central nervous system, where amyloid-beta is primarily synthesized in neurons but accumulate in CSF.

Finally, in this study we investigated tear fluid from patients that visit the memory clinic with the first complaint of cognitive impairment. We believe that this approach is clinically more relevant than comparing severely affected patients to healthy controls. In the end, it are patients with early symptoms that would benefit from future tear-based biomarker tests.

Limitations

We recognize that the commercially available immunoassays used in this study have not been validated for less common body fluids such as tear fluid. For future studies, such validation is highly recommended. In addition, immunoassays with higher sensitivity, such as the S-plex assays from Meso Scale Discovery or SimoA assays from Quanterix, may reduce the number of samples with undetectable biomarker levels. Unfortunately, ultrasensitive assays are most often not available as multiplex, while multiplexing is highly desired for biological samples with limited sample volumes such as tear fluid. One could think of using multiplex assays as first screener followed by fine-tuning of the results using ultrasensitive singleplex assays.

Conclusions

In summary, this preliminary study demonstrates the potential of tear fluid assays as a valuable addition to the armamentarium of diagnostic tools for AD. Advantages of tear sampling as compared to CSF sampling are its non-invasiveness, easiness and accessibility for the patient population with mental cognitive disorders. To be implemented as front-line diagnostics future studies are necessary to confirm, validate and quantify the disease-specific tear biomarkers for the various stages of cognitive disorders.

Abbreviations

HC, healthy controls; SCD, subjective cognitive disorder; MCI, mild cognitive impairment; Dem, dementia, CDR, clinical dementia rating, MMSE, Mini-Mental State Examination; CSF, cerebrospinal fluid; AB, amyloid-beta; IQR, interquartile range.

Declarations

Declarations

Ethics approval and consent to participate

The local ethical committee approved the study protocol and this study followed the tenets of the current version of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

MG performed data collection, processing, data analysis and data interpretation and drafted the manuscript. IR coordinated the study, performed data analysis, drafted and revised the manuscript for intellectual content. PJV and FV revised the manuscript for intellectual content.. MVDW performed data analysis and revised the manuscript for intellectual content. CS revised the manuscript for intellectual content. RN and CW provided overall study supervision and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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Figures

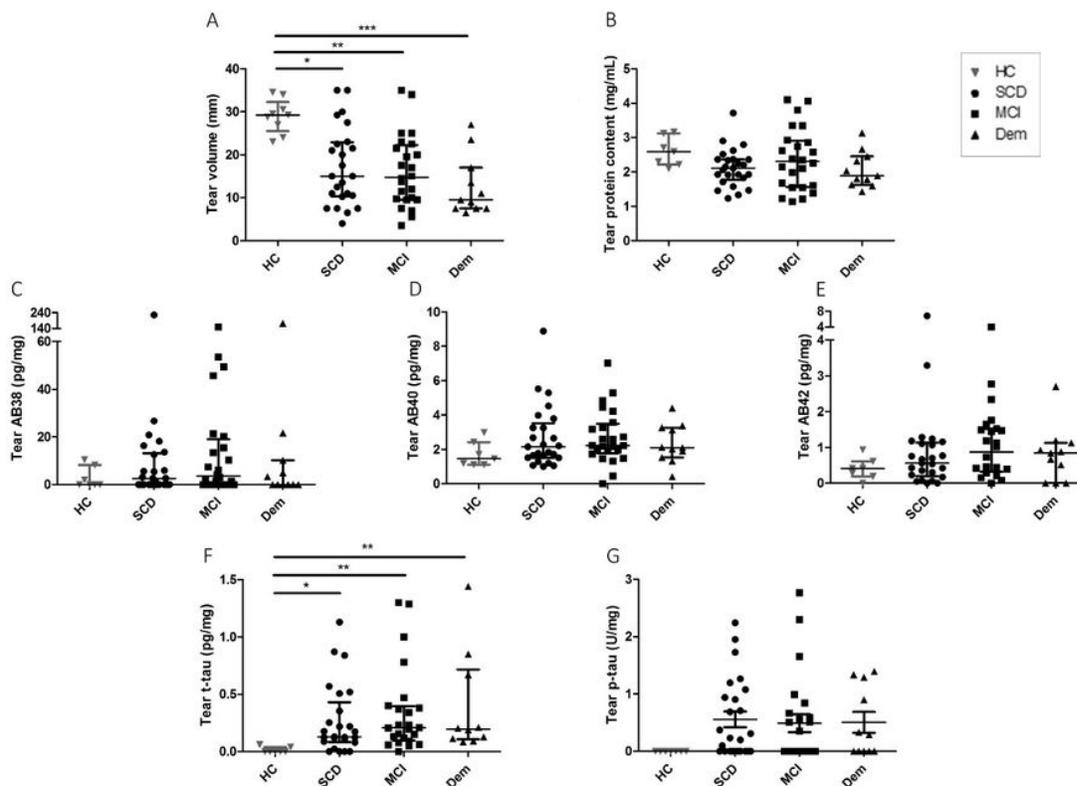


Figure 1

Tear fluid biomarker levels Evaluation of tear biomarker levels across groups (A) tear volume (B) tear total protein content (C) amyloid-beta 38 (D) amyloid-beta 40 (E) amyloid-beta 42 (F) total-tau (G) p-tau. Bars represent median and IQR. P-values were determined using Kruskal-Wallis testing including Bonferroni correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

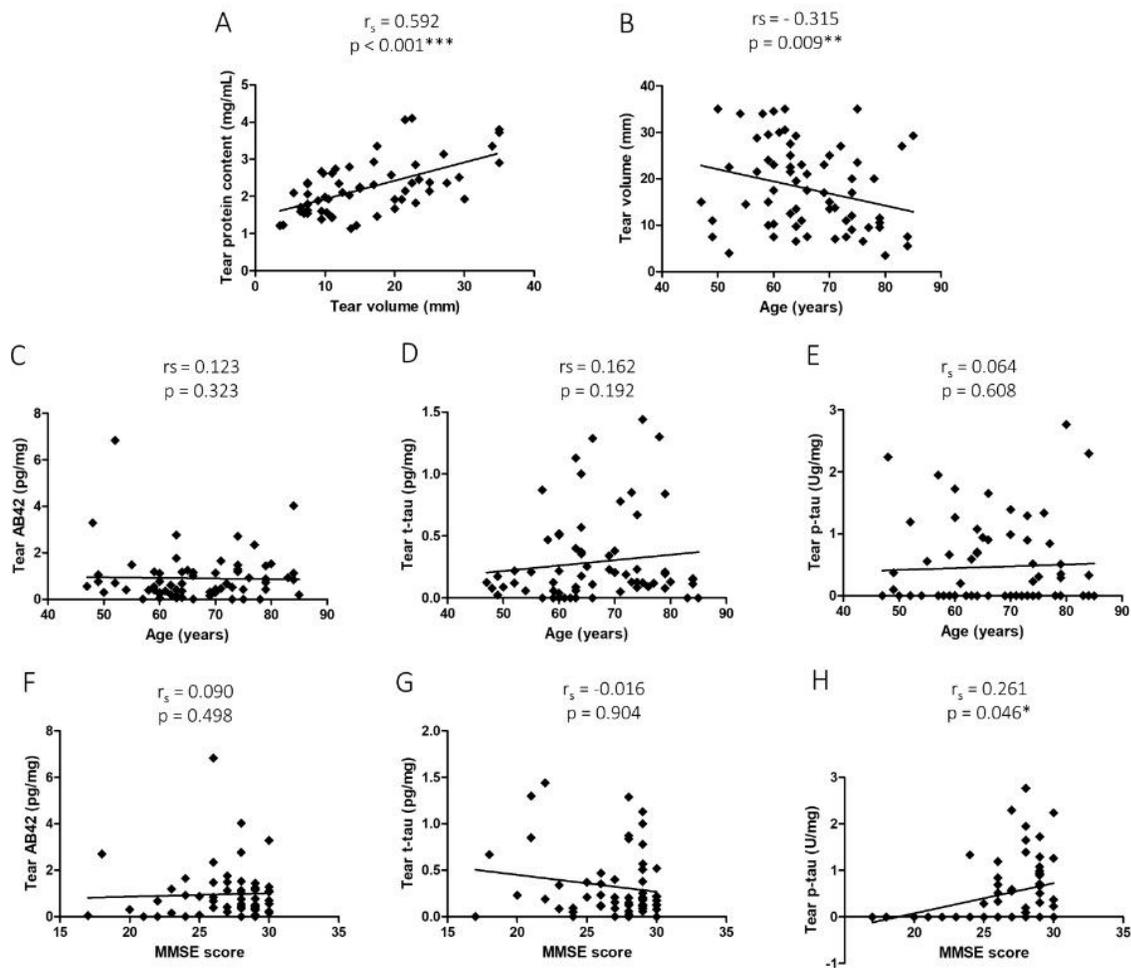


Figure 2

Correlation matrix between participant characteristics and tear fluid biomarkers. Correlation between tear volume and (A) tear total protein content and (B) age; Correlation between age and (C) tear amyloid-beta 42, (D) tear total-tau and (E) tear p-tau; Correlation between MMSE and (F) tear amyloid-beta 42, (G) tear total-tau and (H) tear p-tau. Results are presented as Spearman's correlation coefficients (r_s) and p-values (p). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

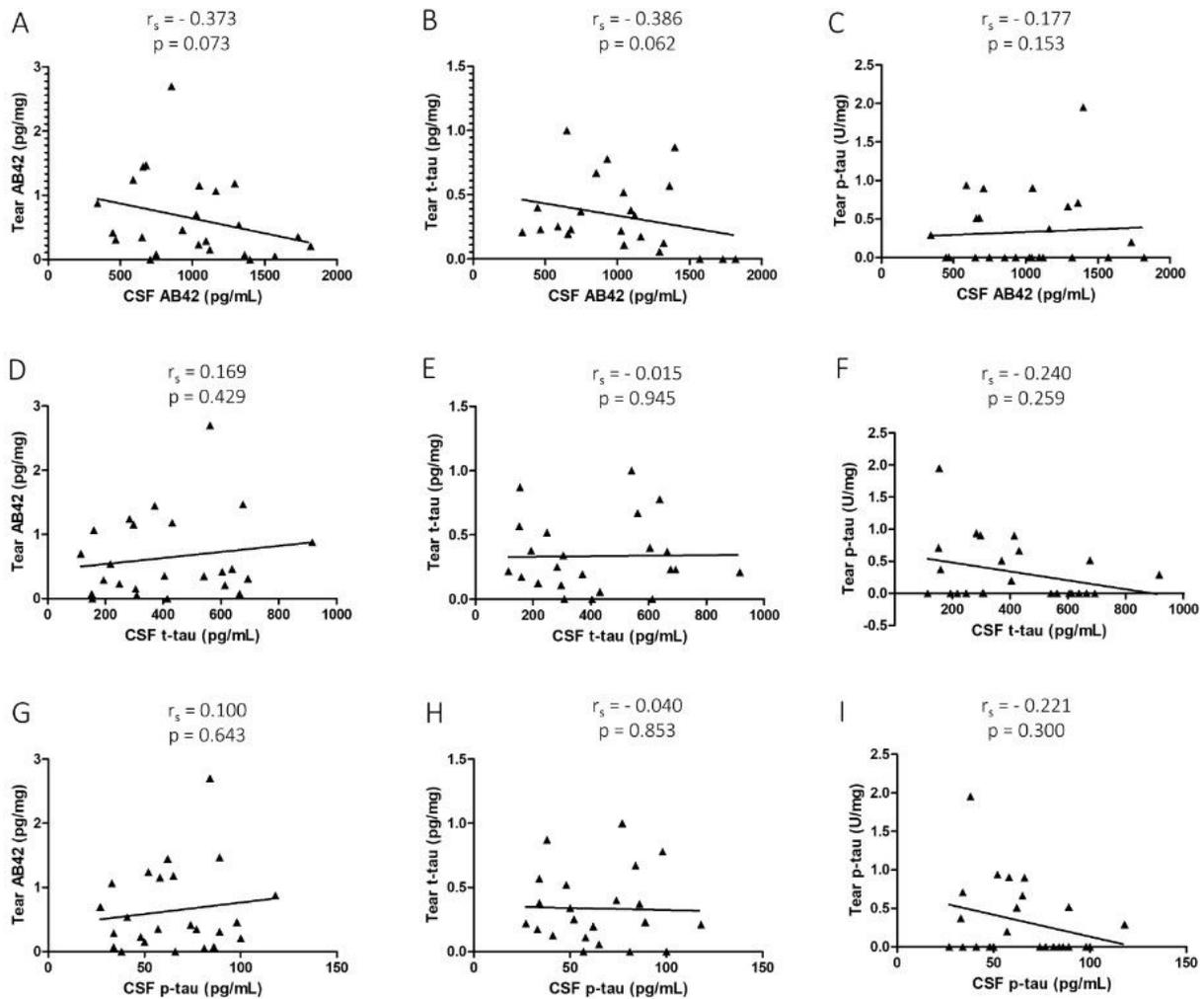


Figure 3

Correlation matrix between CSF and tear fluid biomarkers. Correlations between CSF biomarkers (x-axis) and tear fluid biomarkers (y-axis). Correlations of CSF amyloid-beta 42 (A-C), CSF total-tau (D-F) and CSF p-tau (G-I) with tear amyloid-beta 42 (A,D,G), tear total-tau (B,E,H) and tear p-tau (C,F,I). Results are presented as Spearman's correlation coefficients (r_s) and p-values (p).

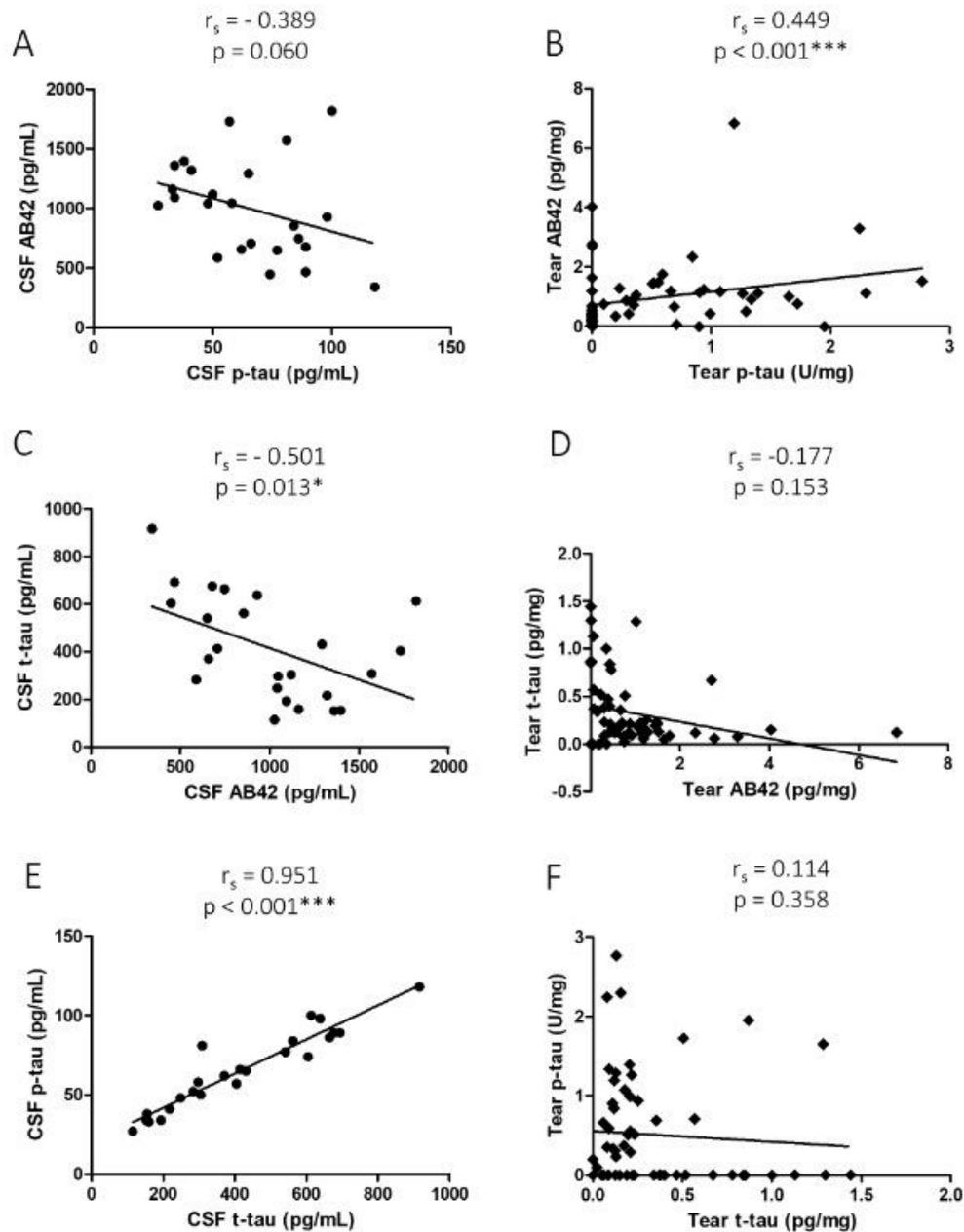


Figure 4

Correlation matrix within CSF and tear fluid biomarkers. Correlations of amyloid-beta 42, total-tau and p-tau within CSF (A, C, E) and tear fluid (B, D, F). Results are presented as Spearman's correlation coefficients (r_s) and p-values (p). * $p < 0.05$, *** $p < 0.001$.

Supplementary Files

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- [SupplementaryTable1.docx](#)