Case Report: Tear liquid for diagnosis of Alzheimer disease.

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Case Report

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Abstract

Introduction: The common approaches at the diagnosis of Alzheimer Disease(AD) is made with an analysis of the cerebrospinal fluid or the last techniques use the study of retinal fundus and the plaques formation, through OCT or more simply with a fundus camera. Tears' analysis is widely discussed in literature as an essential method to describe molecular and biochemical alterations in different diseases. The aim of our study was the identification with Immunocytochemistry of Amyloid Beta-42 in tears from patients with or without familiarity for Alzheimer disease, in order to make the diagnosis earlier and more accessible compared to other invasive methods. **Case presentation**: Our study was performed on tears from three phenotypically healthy subjects: two of them were Caucasian with Alzheimer familiarity (48 and 55 years old) and the other one was Asian without Alzheimer familiarity (45 years old) and affected by an adenoviral keratoconjunctivitis at the moment of the withdrawal. Tears samples were collected from eye fornix and were examinated by immunocytochemistry (ICC) assay using anti-Amyloid Beta X-42 antibody. Two out of three tears samples showed positive Amyloid Beta-42. **Conclusions**: Considering that our patients were phenotypically healthy, the identification of Amyloid Beta-42 by ICC could be a candidable method to make the diagnosis of the disease earlier and more accessible and available then other current and invasive methods and it could be candidate to screening method too.

Introduction

Alzheimer disease is a neurodegenerative pathology of the central nervous system. The diagnosis is currently performed according to the classical methods through bioptic withdrawal of brain tissue or withdrawal of cerebrospinal liquid, subjecting the patients under a significative physical stress and limiting the execution of the practice which cannot be extended to diagnostic screening. Human tears represent an exceptional biomaterial rich in information regarding the health status of eyes and, more generally, of whole body functionality. This is mainly due to the presence in tears of a large variety of organic components including proteins, lipids, metabolites, nucleic acids, and electrolytes, whose concentrations can be altered in pathologies of whole body too [1, 2]. An increasing attention is presently given to the analysis of this human body fluid. The small amounts of substances considered and the typical low concentration of organic compounds hamper the access to a direct analysis by biochemical methods, so the use of tears in diagnosis is in progress. We focused our attention on tears analysis [3], to detect the presence of Amyloid Beta-42 in order to hypothesize a less invasive method and more rapid diagnosis of Alzheimer disease. Native beta-amyloid is a transmembrane protein with a short cytoplasmic domain that undergoes proteolytic clevage by secretase on its N-terminal intraluminal domain; clevated protein found in the extracellular fluid and in tears [4]. Recent studies have demonstrated that Amyloid Beta-42 and Amyloid Beta-40 have different conformation and assembly states; the first one is associated with the formation of plaques and parenchymal damage in AD respect the classical vascular AD that associated to Amyloid Beta-40 [5]. We focused our study on Amyloid Beta-42. This plaques are formed from an alter composition of the chemical barrier, and the changes in the retinal vasculature and retinal morphology were detected in the eyes of patient with AD so it was

observed a relationship between beta-amyloid deposition in the retina, in brain and AD[6]. In that way the eyes are a good indicator for the study of AD and his progression [8]. In this context, the detection of Amyloid Beta-42 in tears could be a useful method for an early and less invasive diagnosis of AD [7].

Materials And Methods

Our study was performed on three phenotypically healthy subjects: two of them were Caucasian with Alzheimer familiarity (48 and 55 years old) and the other one was Asian without Alzheimer familiarity (45 years old) and affected by an adenoviral keratoconjunctivitis at the moment of the withdrawal. Patients were examinated with fundus camera to highlight retinal plaques as described in literature; only the Caucasian patient presented a strong alteration in the posterior chamber with numerous plaques on retina (fig. a 1 and 2). In this present work, human tear samples were examinated with ICC using anti-Amyloid Beta X-42 antibody; samples were stained with DAB if positive, no staining if negative. (Fig. B 1,2 and 3). NaCl solution was employed as negative control and protein Amyloid Beta-42 as positive control (Fig. C 1 and 2). Our test was not influenced by irritative and infective phenomena that can occur in tissue during the execution of withdrawal.

Tear's sample were dispensed on Thermo Scientific adhesion slides, dried at 76°C for 4 hours and hydrated with 100% ethanol for 3 minutes, 95% ethanol for 3 minutes, 70% ethanol for 3 minutes and distilled water for 3 minutes. The area of interest was marked using a PAP pen, which draws an hydrophobic barrier to prevent the waste of reagents by keeping liquid pooled in a small droplet. The endogenous peroxidases were neutralized using peroxidase block (3–4% v/v hydrogen peroxide) for 10 minutes, followed by protein block for 10 minutes (0.4% casein in phosphate-buffered saline, with stabilizers, surfactant and 0.2% bronidox L as preservative) to reduce non-specific binding of primary antibody and polymer. Samples were incubated with the primary antibody (anti-Amyloid Beta X-42, clone 12F4, a purified mouse monoclonal IgG1k in buffer containing 0.1 M Tris-Glycine pH 7.4, 150 mM NaCl with 0.05% sodium azide, Millipore) diluted 1:200 for 60 minutes. Post primary (rabbit anti-mouse IgG < 10µg/mL in 10% v/v animal serum in tris-buffered saline/0.09% Proclin 950) was incubated for 20 minutes, followed by Novolink Polymer (anti-rabbit Poly-HRP-IgG < 25µg/mL containing 10% v/v animal serum in tris-buffered saline/0.09% Proclin 950) for 20 minutes. To avoid the presence of residual reagent from the previous step, starting from peroxidase block, each step was interspersed with a washing with wash buffer (diluted 1:10, < 1%-2-Methyl-2H-Isothiazol-3-One) for 5 minutes. Peroxidase activity was developed with DAB working solution (DAB chromogen 1.74% v/v 3,3'-diaminobenzidine, in a stabilizer solution) diluted 1:20 in DAB substrate buffer and after 5 minutes the excess of reagent was washed with distilled water for 5 minutes. Samples were counterstained with hematoxylin for 30 seconds, washed again with running water for 5 minutes and distilled water for 3 minutes, and dehydrated with 70% ethanol for 3 minutes, 95% ethanol for 3 minutes and 100% ethanol for 3 minutes. Finally, they were covers lipped with synthetic mounting medium and results were interpreted using a light microscope.

Immunocytochemistry (ICC) allows the identification by light microscopy of an antigen and its location in cells through specific antigen-antibody reaction. In our study, we employed ICC indirect method: the

specific antigen was recognized by an unlabeled primary antibody which binds the secondary antibody (or post primary), conjugated to the horseradish peroxidase (HRP or polymer) which reacts with the substrate yielding a chromogenic development at the antigen site. Samples were counterstained with hematoxylin, covers lipped and results were interpreted at light microscopy.

Results

We observed that patient 1 and 2, with familiarity for Alzheimer, have Retinal plaques (figure A 1 and 2) and presence Amyloid Beta-42 residues on ICC samples (figure B 1 and 2), patient 3 not showed retinal plaques (figure A1) and not has Amyloid Beta-42 residues in ICC (figure B3). We have compared the samples 1, 2 and 3 with a negative control and positive control (1mg/5ml of Amyloid Beta-42) and we observe that the expression of Amyloid Beta-42 in sample 1 and 2 is comparable to positive control and the sample 3 is comparable with negative control. We observed that the appearance of retinal plaques were directly linked with the presence of residues of Amyloid Beta-42 in tears [9]. We can also note that the residues of beta 42 is not linked to expression of symptoms in the patients but at the appearance of the retinal plaques. The Amyloid Beta-42 residues in tears could have a predictive value in the diagnosis of AD.

Discussion And Conclusion

Recent studies have investigated the concentration of beta amyloid 42 and other potential biomarkers in tear fluid and blood of patients with Alzheimer's disease and other forms of dementia with controversial results [11]. In our study, for the first time, a high concentration of amyloid beta 42 was found in the tear fluid of the two healthy subjects tested with familiarity for Alzheimer's disease. This preliminary data suggests the possibility of being able to identify subjects with a genetic predisposition to the development of the disease early with non-invasive alternatives to cerebrospinal fluid that could serve as front-line diagnostics for Alzheimer's disease risk, before the development of any sign of the pathology. The negative test for beta amyloid 42 in the patient with adenoviral keratoconjunctivitis observed also suggests that inflammatory conditions do not cause the production of this substance and therefore are not responsible for false negative findings. Our results suggest that tear analysis may have a predictive role in the diagnosis of AD until 20 years before [1,7]; we detected the presence of the Beta-42 amyloid protein with DAB staining in the tears of patients with familiarity for AD. To demonstrate that the inflammatory process does not generate a false positivity, we tested a subject with viral conjunctivitis, who gave a negative response to the test. (Figure 3B) Our findings suggest that beta-42 amyloid expression is exclusively [7] linked to Alzheimer's disease. The closed relationship between the expression of retinal plaques and the expression of beta-42 amyloid residues in tears sets the stage for a larger study in order to verify the real predictive response of the test we propose.

Declarations

Funding: No funding was received for this study.

Conflict of Interest declaration: The patient consented to participate and have their clinical data published as a case report.

Compliance with Ethical Standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Hagan, S.; Martin, E.; Enríquez-de-Salamanca, A. Tear fluid biomarkers in ocular and systemic disease: Potential use for predictive, preventive and personalised medicine. EPMA J. 2016, 7, 15. [CrossRef] [PubMed]

1. Börger, M.; Funke, S.; Bähr, M.; Grus, F.; Lingor, P. Biomarker sources for Parkinson's disease: Time to shed tears? Basal Ganglia 2015, 5, 63–69.
2. Rusciano, G.; Zito, G.; Pesce, G.; Del Prete, S.; Cennamo, G.; Sasso, A. Assessment of conjunctival microvilli abnormality by micro-Raman analysis. J. Biophotonics 2016, 9, 551–559. [CrossRef]
3. Gysbert van Setten, Lars Nilsson, Solveig Hahne, Janet A. Jhonston, Anders Kvanta, Samuel E. Gandy, Jan Naslund, and Christer Nordstedt b-amyloid protein precursor expression in lacrimal glands and tears fluid. Investigative Opthalmology & visual Science, Dec. 1996, vol.37, No.13.
4. Xinke Yu, Eric Y Hayden, Ming Xia, Owen Liang, Lisa Cheah, David B Teplow, Ya-Hong XieSurface enhanced Raman spectroscopy distinguishes amyloid beta-protein isoforms and conformational states Protein Sci. 2018 Aug;27(8):1427-1438.doi: 10.1002/pro.3434. Epub 2018 Jul 10
5. Pade Colligris, Maria Jesus Perez de Lara, Basilio Colligris, Jesus Pintor Ocular Manifestations of Alzheimer's and Other Neurodegenerative Diseases: The Prospect of the Eye as a Tool for the Early Diagnosis of Alzheimer's Disease J Ophthalmol. 2018 Jul 30;2018:8538573. doi: 10.1155/2018/8538573. eCollection 2018.
6. C.Camerlingo[1], Mikhail Lisitskiy[1], Maria Lepore[2], Marianna Portaccio[3], Daniela Montorio[4], Salvatore Del Prete[5], Gilda Cennamo[6], Characterization of Human Tear Fluid by Means of Surface-Enhanced Raman Spectroscopy. Sensors (Basel). 2019 Mar 7;19(5):1177.doi: 10.3390/s19051177
7. Hart NJ, Koronyo Y, Black KL, Koronyo-Hamaoui M. Ocular indicators of Alzheimer's: exploring disease in the retina. Acta Neuropathol. 2016 Dec;132(6):767-787. doi: 10.1007/s00401-016-1613-6. Epub 2016 Sep 19.PMID: 27645291
8. Kalló G, Emri M, Varga Z, Ujhelyi B, Tőzsér J, Csutak A, Csősz É. Changes in the Chemical Barrier Composition of Tears in Alzheimer's Disease Reveal Potential Tear Diagnostic Biomarkers. PLoS One. 2016 Jun 21;11(6):e0158000. doi: 10.1371/journal.pone.0158000. eCollection 2016.PMID: 2732744
9. Roda, Matilde M.D.; Ciavarella, Carmen B.S.D.; Giannaccare, Giuseppe M.D., Ph.D.; Versura, Piera B.S.D. Biomarkers in Tears and Ocular Surface: A Window for Neurodegenerative Diseases Eye &

Contact Lens: Science & Clinical Practice: March 2020 - Volume 46 - Issue - p S129-S134
doi:10.1097/ICL.0000000000000663

10. Johan Sundelo¨ f, MD; Vilmantas Giedraitis, PhD; Michael C. Irizarry, MD; Johan Sundstro¨m, MD, PhD; Erik Ingelsson, MD, PhD; Elina Ro¨nnemaa, MD; Johan ¨Arnlo¨v, MD, PhD; Malin Degerman Gunnarsson, MD; Bradley T. Hyman, MD, PhD; Hans Basun, MD, PhD; Martin Ingelsson, MD, PhD; Lars Lannfelt, MD, PhD; Lena Kilander, MD, PhD. Plasma b Amyloid and the Risk of Alzheimer Disease and Dementia in Elderly Men A Prospective, Population-Based Cohort Study Arch Neurol . 2008;65(2):256-263

Figures

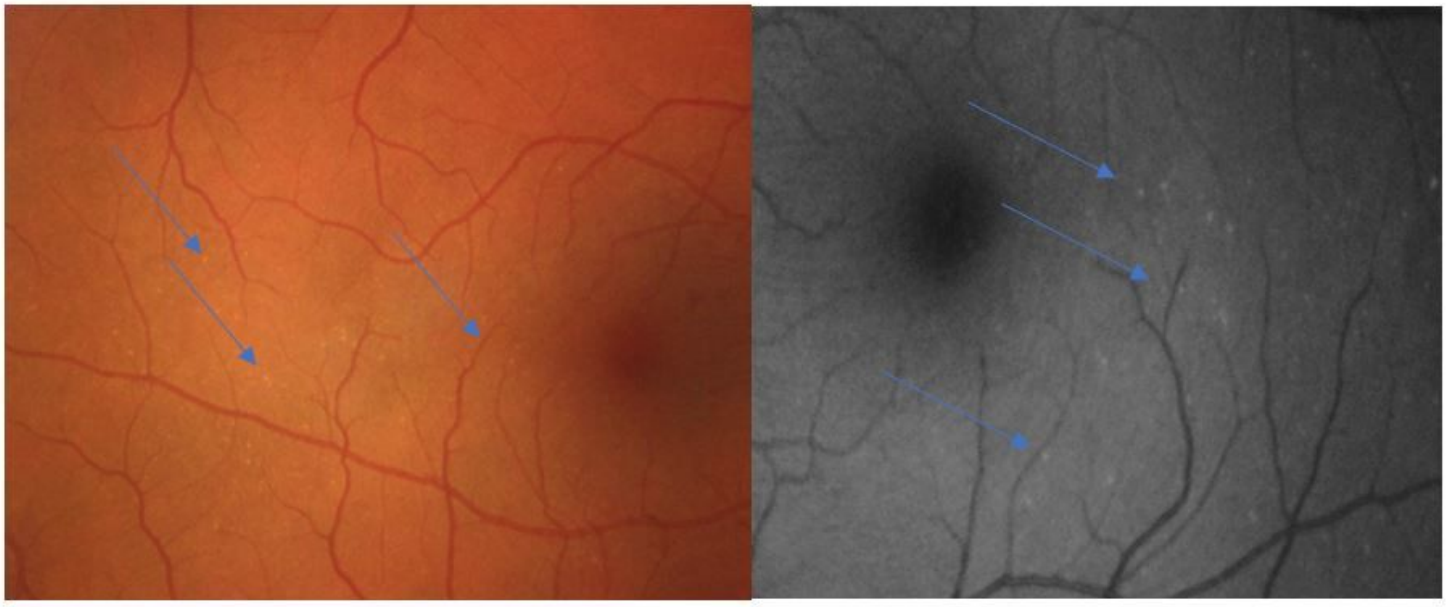

Figure 1

retinal plaques 1 patient

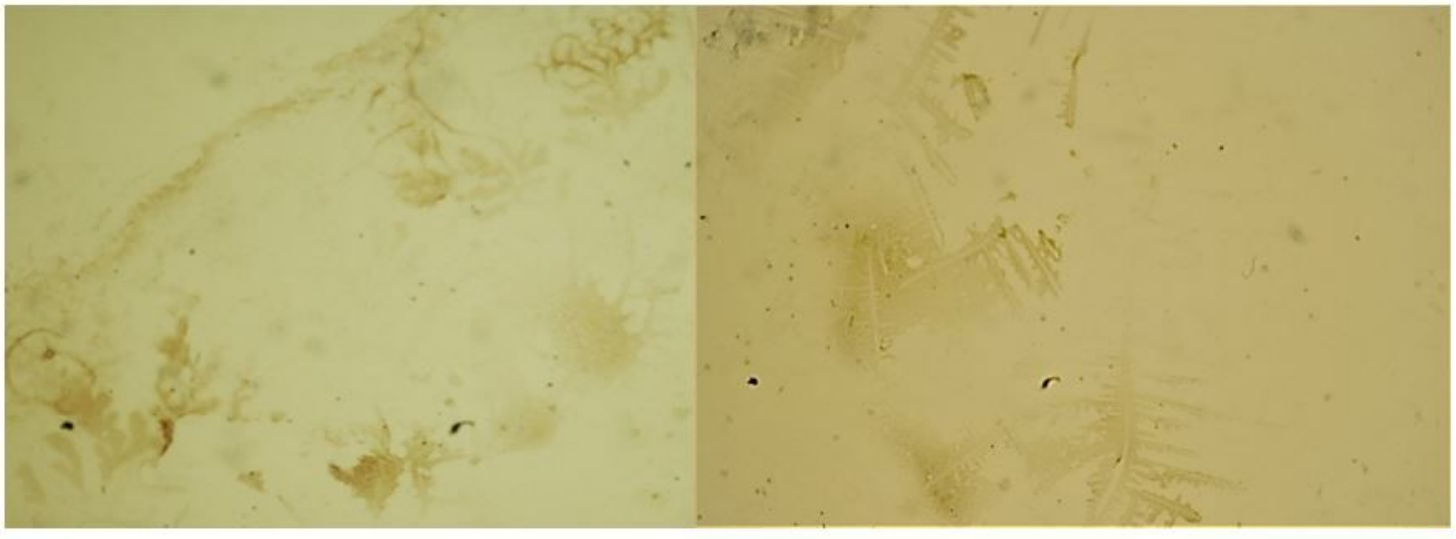

Figure 2

1,2 patients

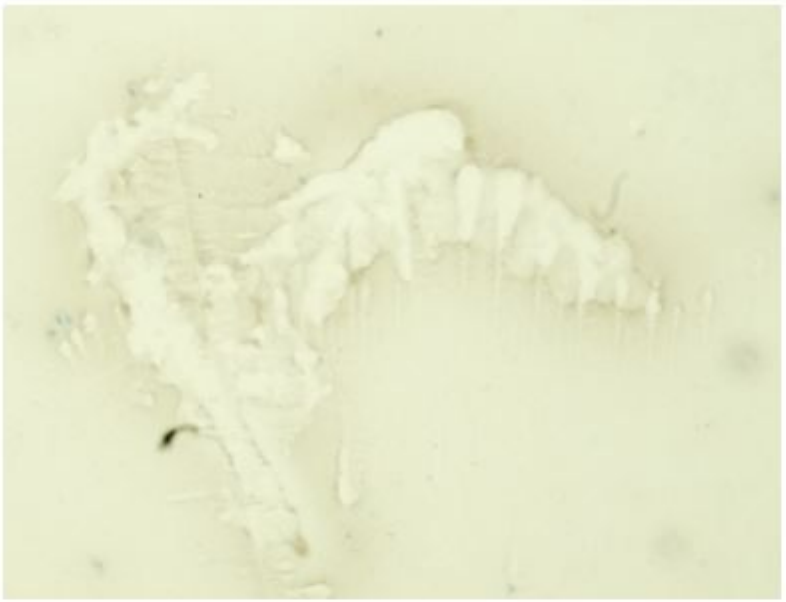

Figure C CN(1) patient 3 sane

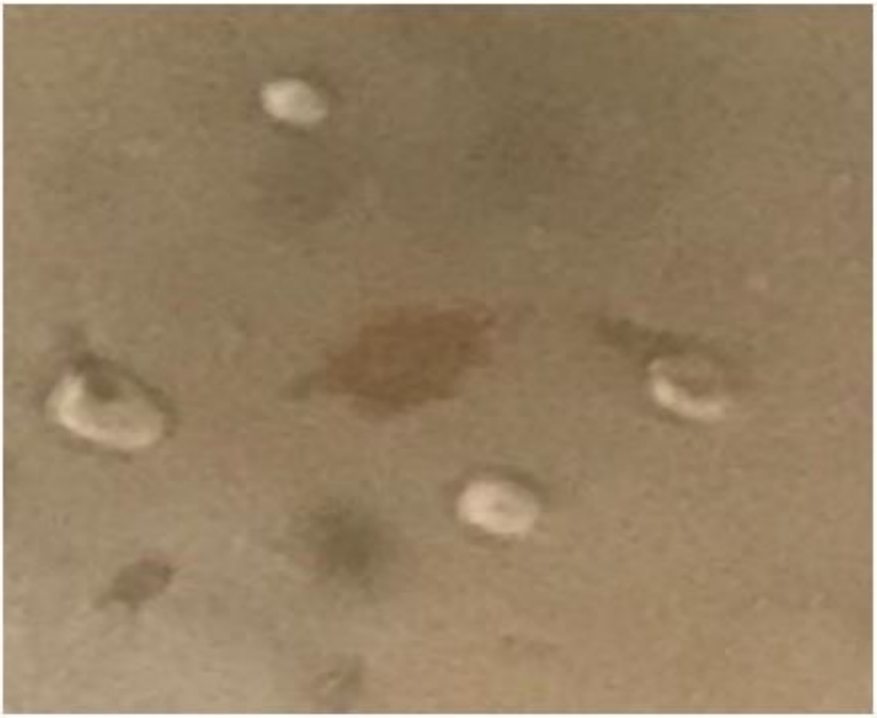

Figure 3

CN(1) patient 3 sane and CP (2) Beta Amiloide 5mg/ml

Supplementary Files

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