Biomarkers in Tears and Ocular Surface: A Window for Neurodegenerative Diseases

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Objectives: The purpose of this review is to briefly outline current scientific evidence on the potential role of tear analysis and ocular surface evaluation in diagnosis and monitoring of neurodegenerative diseases, especially Alzheimer disease, Parkinson disease, and glaucoma.

Methods: A systematic computerized search in the electronic databases PubMed, MEDLINE, and the Cochrane Collaborations was conducted to find eligible articles which their main topic was to investigate the tear and ocular surface in neurodegenerative diseases. After a first screening of titles and abstracts and a full-text review, 26 articles met the inclusion criteria (1 about the neurodegenerative diseases, 3 about the Alzheimer disease, 11 about the Parkinson disease, 11 about glaucoma, and 1 about amyotrophic lateral sclerosis).

Results: The ocular surface picture seems to be altered in the setting of neurodegenerative diseases with specific characteristics according to each disease. They seem to be associated with reduced corneal sensitivity and abnormal tear function, and each one presents the expression of specific biomarkers in tears.

Conclusions: The study of tears and ocular surface appears to be a new and noninvasive promising way to assist in the diagnosis and monitoring of neurodegenerative diseases.

Key Words: Ocular surface—Tears—Parkinson disease—Alzheimer disease—Glaucoma.

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Neurodegenerative diseases are a group of pathologies characterized by a progressive loss of functional neurons, often associated with structural and neuromorphological changes. The most studied diseases include Parkinson disease, Alzheimer disease, Friedreich ataxia, amyotrophic lateral sclerosis (ALS), Huntington disease, Lewy body disease, and spinal muscular atrophy. The number of patients suffering from neurodegenerative diseases is dramatically high, and the global incidence of these disorders is quickly growing. At present, the two most prevalent age-related neurodegenerative diseases are Alzheimer and Parkinson diseases.[1] The exact pathogenesis is still unknown, but it seems that they share some features such as atypical protein assembly and oligomerization, excitotoxicity, mitochondrial dysfunction and oxidative stress, neuroinflammation, reduced trophic support and neuroplasticity, and neuronal cell death.[2,3] Each neurodegenerative disease has its own signs and symptoms, but all are disabling diseases significantly affecting daily life.[4] Visual disturbance is one of the most frequent nonmotor abnormalities referred by patients suffering from Parkinson disease at early stages.[5] These dysfunctions are likely to be related to the progressive loss of dopamine and the accumulation of α-synuclein in the retina.[6] Visual dysfunctions also are reported by Alzheimer disease patients with associated nerve fiber layer thinning and degeneration of retinal ganglion cells.[7] The amyloid β-protein (Aβ) plaques and neurofibrillary tangles comprising hyperphosphorylated tau (pTau) proteins have been detected in the postmortem retinas from Alzheimer disease patients[8] although the accuracy of pathological Aβ detection for diagnosing Alzheimer disease has been recently questioned.[9] Because the accumulation starts years in advance before the clinical manifestation of the disease, research is now focused on the early detection of amyloid plaques in living Alzheimer disease patients, which could drive the identification of at-risk populations.[10] Actually, the eye as a whole can be considered a window toward systemic diseases because it is the unique place in the body where blood vessels and nerves can be directly and noninvasively observed by mean of high-tech imaging techniques. Although many studies focused on overall ocular findings in the setting of neurodegenerative diseases,[4–10] only few authors have investigated the possible changes occurring in the anterior segment of the globe, such as tear content and corneal sensitivity, which will be the focus of the present review.

Nowadays, magnetic resonance studies showing transsynaptic degeneration affecting the central areas of the visual system in patients with glaucoma confirmed that this can be considered a neurodegenerative disease such as Alzheimer and Parkinson diseases.[11,12] In all these diseases, age and family history are significant risk factors, and specific areas of the brain are damaged over time. The specific characteristic of glaucoma is that the area of the brain affected is the eye and optic nerve. Although most of the research lines in the setting of glaucoma are focused on the changes occurring at the level of retina and optic nerve, some authors also described changes in corneal sensitivity and tear content related to both the disease itself and the hypotensive medications.

The purpose of this review is to summarize the markers detected in tears and the ocular surface in the setting of neurodegenerative diseases, to further discuss whether they could be helpful for early diagnosis. The neurodegenerative diseases considered in this

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review will be either central nervous system-related (particularly Alzheimer and Parkinson diseases) and eye-related (glaucoma).

SEARCH STRATEGY

The present review was conducted by a systematic computerized search using following keywords and their abbreviation and MeSH synonyms in the electronic databases PubMed, MEDLINE, and the Cochrane Collaborations: ("tear" OR "cornea" OR "conjunctiva" OR "ocular surface") AND ("biomarkers" OR "confocal microscopy") AND ("neurodegenerative diseases" OR "glaucoma"). No language restriction was applied. In addition, references of selected retrieved articles were scanned manually to identify any additional studies. Two authors (M.R. and P.V.) independently performed assessments of titles and abstracts to identify potentially eligible studies for full-text reviews. The articles were considered eligible if their main topic was to investigate the ocular surface in patients with a neurodegenerative diseases or glaucoma in relation to their etiopathogenesis. Abstracts from conferences, letters, reviews, duplicate publications, and full texts without raw data available for retrieval were excluded. A total of 286 articles were initially identified. After screening of titles and abstracts, 90 articles were retrieved for full-text review. Finally, 26 articles that met the inclusion criteria were included in the review: 1 about neurodegenerative diseases, 2 about Alzheimer disease, 10 about Parkinson disease, 11 about glaucoma, and 1 about ALS (Fig. 1).

ALZHEIMER DISEASE

Alzheimer disease is one of the most common neurodegenerative diseases, affecting more than 25 million of people worldwide. The abnormal accumulation of misfolded proteins such as the β--amyloid (Aβ) protein, the microtubule-associated protein tau, and the α-synuclein are distinctive hallmarks of the disease. Alzheimer disease manifestations concern also the optical system, and Aβ deposits have been detected in the lens and in the retina.[7] Despite the technological advance in neuroimaging tools, the Alzheimer disease diagnosis is still confirmed by postmortem autopsy. The analysis of specific biomarkers in biological fluid has not yet established, and the introduction of proteomic techniques is addressed at improving Alzheimer disease detection with noninvasive strategies. At this regard, the analysis of tear composition and function and the evaluation of the expression of the proteolytic processing and isoforms of amyloid precursor protein (APP) in the cornea provide additional evidence that could add novel elements for the early diagnosis of Alzheimer disease. Dutescu et al.[13] was the first to investigate the expression of APP in retinal ganglion cells and inner nuclear layer cells, in lens, and corneal epithelia in Alzheimer transgenic mouse models and found

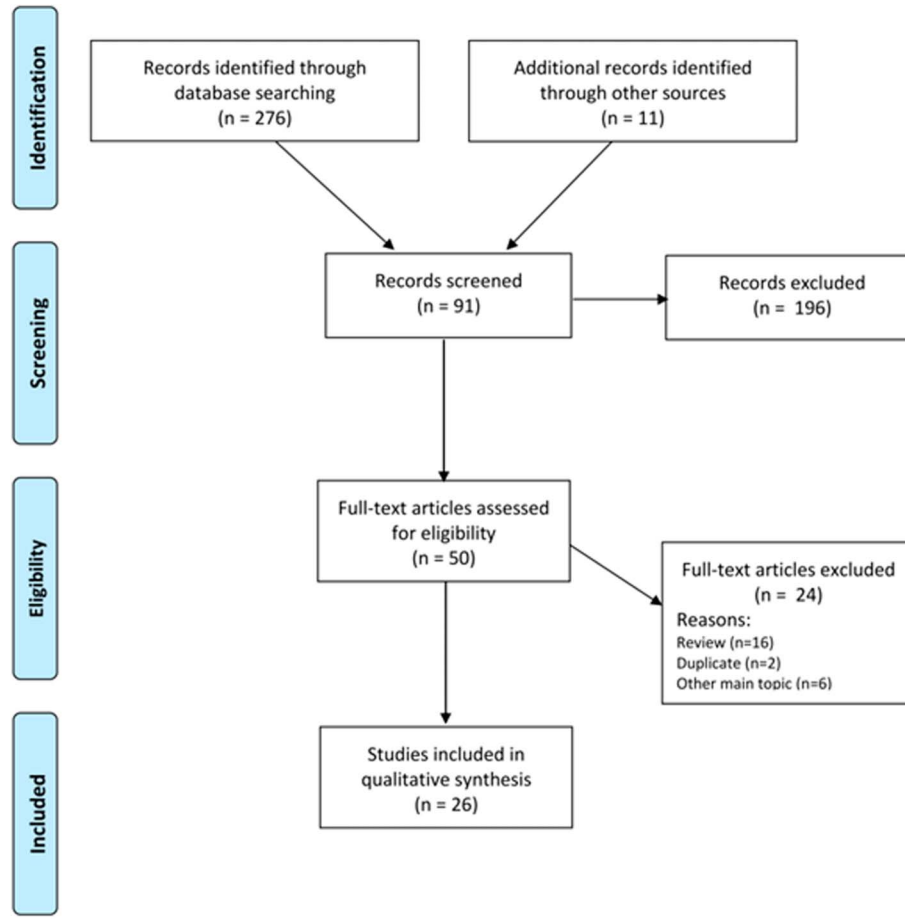

FIG. 1. Search strategy. full color online

a strong cytoplasmic expression of APP in the epithelial cell layers of the cornea. Aβ is released from APP through proteolytic processing by β-secretases and γ-secretases. Recently, Choi et al.[14] found that the human corneal fibroblasts and the corneal epithelium express APP and proteins involved in APP processing and Aβ degradation. As the expression of APP isoforms in the central nervous system could be reflected in other peripheral districts of the body,[15] the APP metabolism and Alzheimer disease–related proteins in corneal fibroblasts and corneal epithelium could be used to identify markers for Alzheimer disease diagnosis and progression. Moreover, like other neurodegenerative diseases, Alzheimer disease seems to be characterized by reduced corneal sensitivity, shorter tear break-up time (TBUT), and lower Schirmer test values.[4] Blinking plays an important role in the distribution and drainage of tears, helping the maintenance of the balance of the tear volume on the ocular surface. In Alzheimer disease, reduced corneal sensitivity and impaired tear function have been linked to abnormalities in the central outflow of cholinergic nerve fibers[16] and to severely impaired eye blink conditioning.[17] Other potential diagnostic elements in Alzheimer disease are tear rate and composition: Kalló et al. showed an increased tear flow rate (above 5 μL/min), an increased tear protein concentration, and an altered level of extracellular glycoprotein (lacritin, lipocalin-1, lysozyme-C, and dermicidin). Although this was a pilot study, it underlined the important role of tear's analysis in detecting systemic diseases.[18]

PARKINSON DISEASE

Parkinson disease is a neurodegenerative disease characterized by the progressive death of dopaminergic neurons and the accumulation of intracellular deposits of alpha-synuclein proteins.

Neuroinflammation, oxidative stress, and mitochondrial dysfunction are concomitant events leading to neuronal loss during Parkinson disease occurrence.[2] Currently, the diagnosis of Parkinson disease is complex and limited to clinical symptoms, whereas the analysis of biomarkers in biological fluids, such as blood and cerebrospinal fluid, has not fully determined. The analysis of inflammatory mediators in a more accessible fluid such as the tears could be promising for Parkinson disease detection and monitoring. At this purpose, it has been observed that tear volume is low in Parkinson disease patients and that its decrease was correlated with disease stage and severity.[19–23] A mass spectrometry study of tear proteome in Parkinson disease patients has identified several proteins differently regulated in comparison with the control group, mainly belonging to immunity, lipid metabolism, and oxidative stress pathways.[24] Furthermore, the TNF-alpha levels, measured by a multiplex immunohead assay, resulted significantly higher in Parkinson disease patients.[25] In addition, Parkinson disease patients have some peculiar ocular surface's changes related to the pathogenesis of the disease and, in particular, a reduced blinking rate with concomitant signs and symptoms of dry eye. A reduced blinking rate in patients with Parkinson disease has been reported in several studies, and this is considered a sign that supports Parkinson disease diagnosis. The reduced blinking rate seems to be associated with reduced nigrocollicular pathway activity resulting in greater inhibition of spontaneous blinking.[26] Abnormalities in tear secretion are frequently reported among patients with Parkinson disease: TBUT and Schirmer test scores were found to be significantly lower in patients with Parkinson disease than in controls, whereas corneal fluorescein staining and OSDI scores were higher.[19–23,25–27] The abnormality in each tear test seems significantly related to the Hoehn and Yahr score, the commonly used system for describing how the symptoms of Parkinson disease progress. Two studies showed corneal nerve changes in Parkinson disease using the noninvasive technique of in vivo corneal confocal microscopy: Misra et al.[28] found a significant reduction in subbasal corneal nerve density in Parkinson disease patients, with nerve density values reduced by approximately 50% when compared with healthy controls; Kass-Iliyya et al.[29] demonstrated that Parkinson disease patients had significantly reduced corneal nerve fiber density with increased corneal nerve branch density and corneal nerve fiber length compared with controls.

Hamm-Alvarez et al.[30] have recently demonstrated the expression of α-synuclein (α-Syn) in basal tears of Parkinson disease, in both its monomeric and oligomeric forms: α-SynTotal was decreased, whereas α-SynOligo and the ratio of α-SynOligo/α-SynTotal were increased in basal tears from Parkinson disease patients compared with healthy controls.

GLAUCOMA

The term glaucoma includes a group of progressive optic neuropathies characterized by degeneration of retinal ganglion cells and determining changes in the optic nerve head. Loss of ganglion cells is related to the level of intraocular pressure (IOP), but other factors may also play a role. The disease is commonly divided into two major subtypes, open-angle glaucoma and angle closure glaucoma. Data from population-based surveys indicate that one in 40 adults older than 40 years has glaucoma with loss of visual function, which equates to 60 million people worldwide being affected and 8.4 million being bilaterally blind. Even in developed countries, half of glaucoma cases are undiagnosed.

The diagnosis is made by a combination of high values of IOP, characteristic changes of the optic nerve, altered functional testing such as visual field, and structural imaging of the optic nerve head. Management is mainly aimed at reducing IOP.

The ocular surface's abnormalities in glaucoma patients are often associated with antiglaucomatous therapy that induces dry eye disease associated with decreased central sub-basal nerve fiber layer density,[31–34] morphological changes of meibomian glands,[35] and corneal and limbal inflammation with increased density of dendritic cells.[36–38] All these alterations are particularly pronounced in patients under therapy with prostaglandin analogue eye drops.

In eye drop–naive patients with open-angle glaucoma, the tear protein expression is a mixture of increased inflammatory protein associated with inflammatory response, free radical scavenging, cell-to-cell signaling, and interaction[39,40]; however, it is still under debate whether tear cytokines are increased or decreased in glaucoma patients as compared with controls. In fact, in some studies, multiplex cytokine analyses found several tear cytokines with increased values,[41] whereas in others, the mean concentrations of tear film cytokines were lower in the glaucoma group compared with controls.[42] Ghaffariyeh et al.[43] showed a reduced brain-derived neurotrophic factor level in tears of normal-tension glaucoma patients.

Mastropasqua et al.[36] observed in patients with primary congenital glaucoma (PCG) with the aid of scanning-slit corneal confocal

TABLE 1. *Tear and Ocular Surface Parameters of Neurodegenerative Disease*

Disease	Parameters	References
Alzheimer disease (AD)	Corneal sensitivity, TBUT, and Schirmer test	Örnek et al.[4]
	Expression of AD-related genes in corneal fibroblasts (APP, ADAM10, BACE1, BACE2, PSEN1, NCSTN, IDE, and NEP)	Choi et al.[14]
	Tear protein concentration, tear production rate, tear protein composition (Zn-α2 glycoprotein, PIP, LYZ, LTF, LPNA, LCN1, LACRT, IGLC: Ig λ chain C region, GAL3BP, and DCD)	Kalló et al.[18]
Parkinson disease (PD)	BR, TBUT, Schirmer test, and CCT	Aksoy et al.[19]
	Tear TNF-α, BR, and Schirmer test	Çomoğlu et al.[25]
	BR, Schirmer test, TBUT, corneal fluorescein staining, OSDI; corneal parameters evaluated by the Pentacam high-resolution rotating Scheimpflug imaging system	Demirci et al.[27]
	Corneal nerve parameters and IENFD	Kass-Iliyya et al.[29]
	Schirmer test	Kwon et al.[20]
	BR, CCS, and corneal parameters	Misra et al.[28]
	OSDI, tear film osmolarity, TBUT, and aqueous tear production	Nowacka et al.[21]
	BR, Schirmer test, TBUT, and tear osmolarity	Söğütlü Sarı et al.[22]
	BR, dry eye assessment questionnaire, meibomian gland evaluation, tear meniscus height, TBUT, fluorescein stain, rose bengal stain, Schirmer test, and phenol red thread test	Tamer et al.[23]
	Proteomic analysis of tear fluid	Boerger et al.[24]
	Oligomeric α-SynOligo and total α-SynTotal, CCL-2, DJ-1 gene, LF, and MMP-9 in basal tears	Hamm-Alvarez et al.[30]
Glaucoma	FGF, Eotaxin, G-CSF, GM-CSF, IFN-gamma, IL-10, IL-12, IL-13, IL-15, IL-17, IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MIP-1a, MIP-1b, PDGF-BB, RANTES, TNF-alpha, and VEGF in tear and aqueous humor originating from patients with glaucoma who underwent trabeculectomy	Csősz et al.[45]
	CCS, sub-basal nerve density and the total number of nerve fibers quantified by laser scanning confocal microscopy in PCG	Gatzioufas et al.[44]
	BDNF in tears of normal-tension glaucoma patients	Ghaffariyeh et al.[43]
	IFNγ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNFα in POAG	Gupta et al.[42]
	Dendritic cell distribution, morphology, and density in the entire cornea of medically controlled glaucoma patients	Mastropasqua et al.[38]
	POAG and PXG tear protein expression	Pieragostino et al.[39]
	TBUT Schirmer I test, ocular surface staining scores, OSDI, CCS, and SBNFLD by confocal microscopy in eyes with chronic glaucoma on long-term topical antiglaucoma therapy	Saini et al.[33]
	TBUT, Schirmer I test, OSDI, ocular surface staining, and corneal parameters by IVCM in patients on different long-term antiglaucoma therapies	Martone et al.[34]
	Morphologic changes of the corneoscleral limbus in glaucoma patients using LSCM and impression cytology	Mastropasqua et al.[36]
	Cytokine/MMP profile in tears in patients under prostaglandin analogues	Reddy et al.[41]
	OSDI, lid parallel conjunctival folds, Schirmer test, TBUT; corneal epithelial and Langherans cells densities	Marsovszky et al.[37]
Others	Corneal small fiber sensory nerve in ALS	Ferrari et al.[46]

ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; BR, blink rate; CCL-2, C-C motif chemokine ligand two; CCS, central corneal sensitivity; CCT, central corneal thickness; DCD, dermcidin; FGF, fibroblast growth factor; GAL3BP, galectin 3–binding protein; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IENFD, intraepidermal nerve fiber density; IFN-γ, interferon-gamma; IGLC, Ig λ chain C region; IL, interleukin; IP-10, interferon gamma-induced protein 10; IVCM, in vivo confocal microscopy; LACRT, extracellular glycoprotein lacritin; LCN1, lipocalin-1; LF, lactoferrin; LPNA, lipophilin A; LSCM, laser scanning confocal microscopy; LTF, lactotransferrin; LYZ, lysozyme-C; MCP-1, monocyte chemoattractant protein one; MIP-1a-b, macrophage inflammatory proteins; MMP-9, metalloproteinases nine; OSDI, ocular surface disease index; PCG, primary congenital glaucoma; PDGF-BB, platelet-derived growth factor; PIP, prolactin-inducible protein; POAG, primary open-angle glaucoma; PXG, pseudoexfoliative glaucoma; RANTES, regulated on activation, normal T cell expressed and secreted; SBNFLD, central sub-basal nerve fiber layer density; TBUT, tear break-up time; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor.

microscope that corneal sub-basal nerve plexus was clearly visualized, with evident thin and reflective nerve fibers and without abnormal branching; Gatzioufas et al.[44] investigated the morphology of corneal sub-basal nerve plexus in young patients with PCG and demonstrated that both the nerve density and the total number of nerve fibers were significantly decreased. Csosz et al.[45] suggested that the tear levels of IFN-y, granulocyte–macrophage colony-stimulating factor (GM-CSF), and IL-5 detected preoperatively may be predictive of appearance of late flap-related complications of trabeculectomy.

OTHERS NEURODEGENERATIVE DISEASES

Only one study analyzed corneal nerves by in vivo confocal microscopy in ALS, a disease characterized by progressive degeneration of upper and lower motor neurons, in the brain and spinal cord. Ferrari et al.[46] demonstrate for the first time a corneal small fiber sensory neuropathy in ALS patients. Studies on larger cohorts are needed to define better the corneal fibers' abnormalities in ALS and the possible correlation to the progression of the pathology.

CONCLUSION

The principal objective of this review was to evaluate the potential role of tear analysis and ocular surface evaluation in diagnosis and monitoring the neurodegenerative diseases. The eye shares many neural and vascular similarities, and many researchers are currently focusing on novel ocular biomarkers which could be helpful for an early diagnosis (Table 1). Tears are the liquid

component in the lachrymal function unit, an integrated system consisting of the cornea, conjunctiva, the main and accessory lacrimal glands, the meibomian glands, the lids, and the sensory and motor nerves that interconnect them. The lachrymal function unit plays a key role in regulating tear secretion and tear film distribution, with the aim to maintain the homeostasis of the ocular surface system. A condition of dry eye disease (DED) takes place whenever a failure occurs to any component of this functional unit.[47] Neurodegenerative diseases (namely Alzheimer disease, Parkinson disease, and ALS) may be associated with abnormal tear function: this is caused by the reduced blinking rate and the reduced corneal sensitivity in Parkinson and Alzheimer diseases; instead, in the setting of glaucoma, DED seems to be related to corneal inflammation, regardless of the use of hypotensive medications.

In conclusion, ocular surface picture seems to be altered in the setting of neurodegenerative diseases with specific characteristics according to each disease. Alzheimer disease patients show (1) a decrease of TBUT and Schirmer test values; (2) the expression of APP and proteins involved in APP processing and in Aβ degradation in corneal fibroblast; (3) an increased tear flow rate, an increased tear protein concentration, and an altered level of extracellular glycoprotein. Parkinson disease patients show (1) a reduced blinking rate due to the dysfunction of the dopaminergic pathway; (2) decreased corneal sensitivity due to corneal fibers abnormalities; (3) lower TBUT and Schirmer test and higher corneal fluorescein staining and OSDI values; (4) increased tear TNF-α values; and (5) the expression of specific-tear proteins identified by proteomic analysis. Amyotrophic lateral sclerosis patients show small corneal fiber sensory neuropathy. In glaucoma, the abnormalities of ocular surface seem to be associated with the topical therapy; in naive to treatment patients, a subclinical inflammation is present, but it is still unknown if a disease-specific cytokine expression may exist.

Overall, these studies provide a rationale for developing and validating noninvasive biomarkers in the ocular surface and, in particular, in tears, to facilitate the neurodegenerative disease early detection and prediction of progression. Of course, as a new area, further research is needed.

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