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REVIEW ARTICLE

Can We diagnose Keratoconus by evaluating Tear Sample?

[1]Burcu Nurözler Tabakcı, [2]Aylin Kılıç, [3]Mustafa Eliaçık

ABSTRACT

Keratoconus (KC) is the most common corneal ectatic disorder characterized by asymmetric cone-shaped corneal protrusion. The pathophysiology of KC is still unclear. For many years, KC has been considered as a noninflammatory degenerative disease. However, several studies suggested that inflammatory mediators play a role in KC pathogenesis. Tear film contains various mediators that reflect ocular surface diseases. Monitoring the changes in tear composition can be used as a diagnostic tool. In this review, the adequacy of tear markers in the diagnosis of KC was discussed.

Keywords: Biomarkers, Keratoconus, Tear fluid.

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INTRODUCTİON

Keratoconus is the most common ectatic corneal disorder. It is characterized by asymmetric, progressive corneal thinning and protrusion, leading to irregular astigmatism and visual impairment. The prevalence of KC is 1:2000 in the population. The onset of KC characteristically occurs in early adolescence and it progresses until the third or fourth decade of life.[1,2] The etiology of KC is not completely understood. Genetic, environmental, and biomechanical factors are thought to play a role in the pathogenesis. Atopy, ocular allergies, and eye rubbing are the proven risk factors for KC. For many years, KC has been considered as a noninflammatory disease. However, recent studies have suggested that inflammatory mediators are involved in KC pathogenesis.[3]

Clinical signs are well described, but vary according to the severity of the KC. It is difficult to diagnose KC with clinical signs in early stages. Corneal topography and tomography are important tools for diagnosis at every stage of KC. Soft and rigid-gas permeable contact lenses (RGP CLs), collagen cross-linking, intracorneal ring segments, and keratoplasty are the treatment options for patients in different stages.

[1]Ophthalmologist, [2,3]Associate Professor

[1-3]Department of Ophthalmology, Istanbul Medipol University Istanbul, Turkey

Corresponding Author: Burcu Nurözler Tabakcı, Ophthalmologist Department of Ophthalmology, Istanbul Medipol University Istanbul, Turkey, e-mail: burcunurtabakci@gmail.com

Tear Film

Tear film is a compound extracellular fluid that contains proteins/peptides, lipids, metabolites, and electrolytes with important functions to provide a healthy ocular surface. It is organized as a trilaminar structure consisting of an outer lipid layer, a middle aqueous layer, and an inner mucin layer. The lipid layer maintains a hydrophobic barrier and retards evaporation of the aqueous layer. The aqueous layer supplies oxygen and nutrients to the avascular corneal epithelium, washes away debris, provides antibacterial and antiviral defense, and regulates corneal and conjunctival cell function. The mucin layer is closest to the covers and converts the corneal epithelium from a hydrophobic layer to hydrophilic layer.[4]

Numerous studies have been conducted to understand the composition of tear film. The composition and function of tear fluid lipidome were analyzed using thin layer chromatographic, enzymatic, and mass spectrometric techniques. Rantamäki et al[5] showed that the lipidome of the tear fluid is not similar to meibomian gland secretions and the polar lipids are the most common lipid species followed by cholesteryl esters and triglycerides.

It is difficult to detect tear metabolites because of the small amounts in the tears. Chen et al[6] reported 60 metabolites including amino acids, amino alcohols, amino ketones, carbohydrates, carnitines, cyclic amines, nucleosides and nucleotides, peptides, phospholipids, purines, quaternary amines, and pyridoxal and tricarboxylic acids.

Predominant tear proteins like albumin, lysozyme, and lactoferrin are well known for their antimicrobial properties. Recent studies have focused on tear proteins extensively, and more than 1,500 proteins were defined in tear fluid.[7-10] Zhou et al[7] have identified a comprehensive tear protein list which can be used as a reference list for biomarker search of ocular and systemic diseases.

Biomarkers in Tear Fluid

Biomarkers are the indicators of biological processes. They can be used as a tool for identifying diseases or

monitoring pharmacologic responses in abnormal biological processes.[11] Human body fluids are important sources for the disease-specific biomarkers. Tear fluid is an accessible source for biomarker researches. Samples can be obtained sufficiently by using two different noninvasive techniques—capillary tube and Schirmer strip.[12] As tears contain numerous proteins and metabolites, proper storage of tear samples is essential for reliable results. Sitaramamma et al[13] showed that total protein concentration of tears decreased significantly when the samples are kept at room temperature for 4 to 8 hours. They also concluded that tear samples could be stored up to 4 months at –70°C without any change in protein composition.

Systemic and ocular diseases, such as diabetes mellitus,[14] Sjogren's syndrome,[15] glaucoma,[16,17] dry eye,[18,19] thyroid-associated orbitopathy[20] can alter the proteome patterns of tear fluid. Massingale et al[21] showed that interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, interferon (IFN)-γ, tumor necrosis factor (TNF)-α were all significantly increased in the tears of dry eye patients as compared with normal controls. Decreased levels of lactoferrin, lysozyme, and secretory immunoglobulin (sIgA) have been found to be associated with dry eye.[8]

Biomarkers for KC

Tear composition varies in ocular surface pathologies. Corneal ectasia in KC is associated with increased extracellular matrix disruption. Increased collagenase and gelatinase activities in cornea cultures and collagen degradation products in tears of KC have been reported.[22,23] Matrix metalloproteinases (MMPs) are a family of endopeptidases that are synthesized by corneal epithelial and stromal cells. Physiologically, the main function of MMPs is tissue remodeling, but pathologically, they cause extracellular matrix degredation.[24]

Lema and Durán[25] measured the tear concentrations of various cytokines (IL-4, IL-6, IL-10, and TNF-α), cell adhesion molecules [intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1] and MMP-9 by enzyme-linked immunosorbent assay (ELISA). They showed increased levels of IL-6, TNF-α, and MMP-9 in the tears of KC patients who had no clinical sign of inflammation and this overexpression was found to be related to the stage of KC. After this study, they investigated the effects of RGP CLs on tear composition and found that wearing RGP CLs induces overexpression of ICAM-1 and VCAM-1, in addition to proinflammatory cytokines (IL-6 and TNF-α) in KC patients.[26]

Pannebaker et al[27] reported the increased expression of MMP-1 in KC patients with and without RGP CL wear. No MMP-1 was found in the tears of healthy controls.

Jun et al[28] investigated a wide range of inflammatory cytokines including IL-1b, IL-4, IL-6, IL-10, IL-12, IL-13, IL-17, IFN-γ, chemokine C-C motif ligand 5 (CCL5), and TNF-α in tear samples of KC patients and healthy controls using multiplex immuno-bead assays. Their measurements for IL-6 and TNF-α were 5 to 10 times higher than the previously reported single ELISA measurements. After subtracting the effects of age and CL use, they determined significant decreases in IL-12, TNF-α, and CCL5 in KC compared with control tear fluids. They concluded that use of different antibody-based assays and diversity in patient population may cause variation in cytokine levels.

In most cases, KC is initially unilateral. The fellow eye may develop KC or remain unchanged. A previous study revealed that some cytokines, such as IL-6 and TNF-α are overexpressed in the tears of both eyes of unilateral KC patients, but MMP-9 levels increased only in the KC eyes.[29]

The concentration changes of tear film proteins, such as cystatin, lipocalin-1, lipophilin, and phospholipase A2, have been reported in KC patients. Cystatins are proteinase inhibitors. Decreased levels of cystatins in KC patients' tears are associated with the degradation of tear proteins, and that explains the decrease of tears' total protein concentration. Increased lipid peroxidation leads to elevated lipocalin-1 levels in the tears of KC patients. Decreased levels of lipophilin and phospholipase A2 can alter the tears' stability of KC patients. Increased levels of albumin in tears from KC patients indicate albumin exudation from conjunctival vessels.[30]

Another study showed that the amount of total protein and the concentrations of major tear proteins including lactoferrin and secretory IgA were significantly decreased in KC tears. These differences were not related to CL wear, age, gender, or atopy of subjects.[31] Similarly, decreased levels of lactoferrin, lysozyme, and sIgA have been reported in tears after photorefractive keratometry.[32] Secreted frizzled-related proteins (SFRPs) inhibit Wnt signaling pathways. You et al[33] showed that, tear SFRP1 was significantly decreased in KC, compared with healthy subjects but they concluded that tear SFRP1 levels alone do not provide an obvious biomarker for KC.

Balasubramanian et al[34] showed that the proteolytic activity in the tear of KC patients is 1.9 times higher and the total tear protein level was significantly reduced compared with healthy controls or patients who had undergone corneal collagen cross-linking. Significantly increased expressions of MMP-1, MMP-3, MMP-7, MMP-13, IL-4, IL-5, IL-6, IL-8, and TNF-α, and TNF-β have been demonstrated in tear of KC. The only cytokine that significantly increased in tears of KC is IL-6, compared with the collagen cross-linked group. The expression of

TNF-α was significantly increased in the cross-linked group compared with the healthy subjects. The activity of tear gelatinases and collagenases in the collagen cross-linked group was not significantly different compared with either KC or healthy subjects.

Sorkhabi et al[35] showed that patients with KC had significantly higher levels of the proinflammatory markers (IL-6, IL-1b, and IFN-γ), whereas significantly lower levels of the anti-inflammatory marker (IL-10) compared with controls.

Lysyl oxidase (LOX) is responsible for the formation of lysine-derived cross-links in extracellular matrix proteins. There are two contradictory reports in literature, suggesting reductions and increases of LOX expression in KC corneas. Shetty et al[36] demonstrated that the enzymatic activity of LOX in tears from KC patients is significantly reduced compared with healthy controls.

Gross cystic disease fluid protein-15 (GCDFP-15), also known as prolactin inducible protein (PIP), is a secretory glycoprotein in human tear fluids. Priyadarsini et al[37] reported that the intensity of GCDFP-15/PIP was significantly higher in control group compared with KC patients using both *in vivo* and *in vitro* systems. They defined GCDFP-15/PIP as a potential biomarker for KC.

Karamichos et al[38] have investigated the tear metabolite changes in KC. They have identified a total of 296 different metabolites of which >40 were significantly regulated between three groups (healthy controls, KC patients using RGP lenses, KC with no correction). The levels of metabolites related to citric acid cycle, urea cycle, and oxidative stress have shown significant alterations in KC patients' tears compared with healthy controls.

Oxidative stress is reported as a part of KC pathophysiology. Tear fluid antioxidants cysteine, ascorbic acid, glutathione, uric acid and tyrosine were analyzed.

In between the five antioxidants, tyrosine and glutathione showed significant decrease, while tyrosine and uric acid showed significant increase in KC compared with the healthy controls.[39]

CONCLUSİON

Tear fluid is an accessible source that contains numerous proteins and metabolites. Many inflammatory components have been detected in the tear analysis while trying to identify KC pathogenesis. Published data about tear components' changes of KC patients are limited and quite variable (Table 1). It has been shown that some cytokine levels in the tear are similarly altered in other ocular disorders. Despite large numbers of studies, a sensitive and specific marker in tear for KC has not been found yet. Using multiple biomarkers together instead of one can give more accurate results for diagnosis.

Table 1: Summary of tear biomarkers in KC

Reference	*Analysis techniques*	*Biomarkers*	*Expression*
Lema et al[25]	ELISA	IL-6	↑
		TNF-α	↑
		MMP-9	↑
Lema et al[26]	ELISA	IL-6	↑
		TNF-α	↑
		ICAM-1	↑
		VCAM-1	↑
Pannebaker et al[27]	LCMS	MMP-1	↑
Jun et al[28]	Multiplex immune-bead assay	IL-12	↓
		TNF-α	↓
		CCL5	↓
Acera et al[30]	2-DE/LCMS	Cystatins	↓
		Lipocalin-1	↑
		Lipophilin	↓
		Phospholipase A2	↓
		Albumin	↑
Balasubramanian et al[31]	ELISA	Lactoferrin	↓
		sIgA	↓
You et al[33]	ELISA	SFRP-1	↓
Balasubramanian et al[34]	Specific antibody array	MMP-1,3,7,13	↑
		IL-4,5,6,8	↑
		TNF-α,β	↑
Sorkhabi et al[35]	ELISA	IL-6,1b	↑
		IL-10	↓
		IFN-γ	↑
Shetty et al[36]	ELISA	LOX	↓
Priyadarsini et al[37]	LCMS	GCDFP-15/ PIP	↓

LCMS: Liquid chromatography–mass spectrometry; 2-DE: Two-dimensional gel electrophoresis

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