Biomarkers of Progression of Keratoconus

Several genes and proteins are involved in the onset and progression of this disease.

BY JESÚS MERAYO, MD, PhD, MBA; JOSÉ F. ALFONSO, MD; TATIANA SUÁREZ, PhD; AND ARANTXA ACERA, PhD

Keratoconus is a slowly progressive eye disease in which the normally round, dome-shaped cornea thins and begins to bulge into a cone-like shape. This cone shape is irregular and bends light as it enters the eye, causing distorted vision.

Early indications of keratoconus usually include blurring and distortion of vision. Currently, corneal topography is the most sensitive method for detecting early signs of keratoconus and following keratoconus progression based on morphologic analysis. However, in the early stages, these signs may barely be noticeable; therefore, it can be difficult to diagnose early keratoconus because many common symptoms can also be associated with other eye problems.

Keratoconus is a multifactorial disease in which several genes and proteins—both at the level of the stroma and the corneal epithelium—are involved in onset and progression. Alterations of the tear film have also been reported in patients with keratoconus, possibly as a result of corneal alteration in advanced disease. The study of alterations produced in the tear film proteome in keratoconus has become an area of high interest, as the identification of potential targets for early diagnosis and therapy is crucial to improve treatment of patients.

COMPARATIVE ANALYSIS

We performed a comparative analysis of samples collected from patients with varying degrees of keratoconus (mild to severe) and from healthy controls. The aim was to evaluate protein biomarkers that would allow categorization of patients from mild to severe clinical gradation (Figure 1). We hypothesized that some proteins would be progressively overexpressed, both in the cornea and tears, as the disease progresses, and others with higher normal levels in controls would be reduced in pathologic states.

Our multicenter study, which included 11 centers (10 in Spain and one in Colombia), aimed to study alterations of genes and/or proteins in the corneal stroma and epithelium and tear alterations at the proteome/peptidome level in order to identify potential biomarkers for keratoconus. In contrast to other investigations, our study did not evaluate only healthy patients versus those with keratoconus; we also classified keratoconus according to severity of disease and compared with healthy controls. A total of 173 patients were recruited for this study, including 46 healthy volunteers, 33 with mild keratoconus, 42 with moderate keratoconus, and 52 with severe keratoconus.

Representative samples of different natures, including the corneal epithelium and stroma, aqueous humor, and tears, were collected. Corneal and stromal epithelial samples were analyzed by genomic (gene expression arrays) and proteomic (2DE-DIGE) technologies. Aqueous humor samples were analyzed by label-free LC-MS/MS.
Corneal topography is the most sensitive method for detecting early signs of keratoconus and keratoconus gradation based on morphologic analysis. It can be difficult to diagnose keratoconus in the early stages because common keratoconic symptoms can also be associated with other eye problems. Keratoconus is a multifactorial disease in which several genes and proteins—both at the level of the stroma and the corneal epithelium—are involved in onset and progression. A comparative study found a progressive increase of expression in certain proteins that could eventually be used as diagnostic and therapeutic biomarkers for keratoconus.

**SUMMARY**

The results obtained from our studies were analyzed through bioinformatics and biostatistical techniques to determine their validity, select candidate biomarkers, and determine interactions and/or interconnections between proteins and genes through functional interaction network analyses.

This study confirmed our hypothesis and indicated that certain changes in protein expression are higher at the same time that the disease progresses (Figures 2A and 2C). We found a progressive deregulation of expression in corneal epithelium and stromal proteins, as shown in Figure 2, related to cytoskeleton organization, protein complex assembly, regulation of apoptosis, cell adhesion, regulation of actin cytoskeleton, phosphate and phosphorus metabolic processes, mitogen-activated protein kinase signaling, and fibroblast growth factor receptor signaling pathway between others; these could eventually be used as diagnostic and therapeutic biomarkers.

Jesús Merayo, MD, PhD, MBA, is an Associate Professor of Ophthalmology, University of Oviedo; Chief of the Ocular Surface & Inflammation Clinical Unit, Instituto Oftalmológico Fernández-Vega; and Research Director at Fundación de Investigación Oftalmológica in Spain. Dr. Merayo states that he is a shareholder in Biofaltmik and Ferrara & Hijos SL. He may be reached at e-mail: merayo@fio.as.

José F. Alfonso, MD, is Chief of the Cornea Surgery, Lens, and Refractive Unit at Instituto Oftalmológico Fernández-Vega. Dr. Alfonso states that he has no financial interest in the products or companies mentioned. He may be reached at e-mail: j.alfonso@fernandez-vega.com.

Tatiana Suárez, PhD, is the General Director and Scientific Coordinator of Biofaltmik. Dr. Suárez may be reached at e-mail: tatiana.suarez@biofaltmik.com.

Arantxa Acera, PhD, is the Clinical Relationships and Project Manager at Biofaltmik. Dr. Acera may be reached at e-mail: arantxa.acera@biofaltmik.com.