CHARACTERIZATION OF TEAR FLUID PROTEINS - DIAGNOSTIC TOOL IN SJOGREN'S SYNDROME

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Background: Tears are a complex solution in which the proteins are of special interest because of their possible role in the pathogenesis of inflammatory and autoimmune diseases. Their involvement in the protection of the external ocular surface was intense researched, but few proteins have been identified and demonstrated to offer a protective function. Mass spectrometry is a sensitive technique useful to determine with a high specificity and sensibility molecular weight of the proteins from biological fluids (1). Liquid chromatography electrospray ionization tandem mass spectrometry (ESI-LC/MS/MS) is a tool that can be performed to identify very low quantity of proteins in tear fluid.
Objectives: It's well know that rheumatoid diseases are frequently associated with quantitative and qualitative alterations of the tear film leading to sicca symptoms and/or secondary Sjogren's syndrome (s-SS). In this study we examined and characterized using ESI-LC/MS/MS technology the constituents of the tear film collected from the patients with primary Sjogren's syndrome (p-SS), s-SS, rheumatoid diseases without eye involvement and from healthy volunteers, trying to determine diseases biomarkers that can be used for an noninvasive diagnosis of SS.
Methods: Tear fluids were collected by Schirmer I method from 4 patients with p-SS, 4 patients with s-SS (2 patients had rheumatoid arthritis (RA) and 2 had systemic lupus erythematosus (LES)), 2 patients with RA without sicca symptoms, 2 patients with LES without sicca symptoms and 4 healthy volunteers. The filter papers were soaked with 100microL phosphate buffer solution. The samples were eluted by centrifugation and the total proteins concentrations were determined by Amidoblack method. The proteins were separated on sodium dodecyl sulfate-poliacrylamide gel (15%) under reduced conditions. The gel was colloidal Coomassie blue stained and the intensity and patterns of spots were analyzed and compared. Than spots were cut, incubated with trypsin and analyzed by ESI-LC/MS/MS. The components found were identificated searching through Swiss-Prot database, on the basis of minimum two different peptides detected (beyond the MASCOT's score).
Results: Gel analysis indicated a different proteins patterns between patients. Multiple protein and peptide components were detected in all tear fluids including lysozyme C precursor (16537Da), prolactin-inducible protein precursor (16572Da), tear prealbumin (19250Da), immunoglobulin alpha-1 chain C precursor (37655Da), serum albumin precursor (69367Da), lactotransferin precursor(78388 Da). In the samples from patients with p-SS were detected multiple protein changes with a potential of biomarkers. Also, the tears of the patients with RA with and without ocular involvement presented specific proteins with function still unknow.
Conclusion: Analysis of tear proteins by ESI-LC/MS/MS is a reproducible and noninvasive tool that can be used to identify potential biomarkers in SS and other rheumatic diseases. The identification of all tear proteins detected by mass spectrometry can provide information for a better understanding of the physiopathology of the ocular involvement in inflammatory and autoimmune diseases.
References: 1. Matsuo T, Seyama Y. Introduction to modern biological mass spectrometry. J Mass Spectrom 2000; 35:114-30.