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Hyaluronic Acid and Proteins Content in Tear Fluids with Adenovirus Keratoconjunctivitis Regatieri, C1; Coelho, B2; Barbosa, JB1; Freitas, D1; Belfort, R Jr1; Nader, HB2 and Martins, JR2,3.

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Purpose: The aim of this study was to analyze hyaluronic acid (HA) and proteins contents in the tear fluids of patients with adenoviral keratoconjunctivitis and normal donors.

Methods: Tear samples were obtained from 11 patients (22 eyes) affected by unilateral acute adenoviral conjunctivitis, diagnosed by clinical signs and positive for virus culture. Contralateral unaffected eyes from all patients were also analyzed. Fifteen normal subjects (30 eyes) without ocular or systemic diseases were used as control. To collect the tear fluid, Schirmer strips and microcaps (50 µL) were placed in the temporal side of each eye under the eyelid without any topical anesthesia. The strips were dried at room temperature and stored at -20o C until analysis. HA and protein were eluted from Schirmer strips before analysis. ELISA-like fluorometric assay (Martins et al, 2003) and colorimetric assay (Bradford, 1976) were employed to quantify HA and protein contents, respectively. Protein profile was analyzed using SDS-PAGE 3-20% under non-reduced conditions and coomassie blue-stained. **Results:** The mean of HA present in the tear was 96.7 ± 86.5 ng/mg protein (ranging from 13.7-337.0 ng/mg protein) in the affected eyes, 53.7 ± 32.0 ng/mg protein (ranging from 6.0-97.7 ng/mg protein) in the contralateral unaffected eyes ($p < 0.01$) and 18.9 ± 12.6 ng/mg protein (ranging from 3.2-45.0 ng/mg protein) in normal subjects ($p < 0.01$). Eight out 11 eyes had positive cultures for adenovirus, and in these cases, lacrimal HA increased to 117.8 ± 94.2 ng/mg protein. Two negative cultures showed HA of 13.7 and 47.8 ng/mg protein. We could observe an increase of total protein amounts in the affected eyes (2.0 ± 2.1 mg/mL) if compared to contralateral eyes (0.6 ± 0.5 mg/mL). An increased amount of 60 kDa protein was found in the eyes with conjunctivitis. Also, tear protein obtained from microcaps showed a higher amount of 15 kDa protein in the

affected and contralateral eyes. **Conclusion:** In this study, we found HA contents in the eyes with adenoviral keratoconjunctivitis that were statistically different when compared with contralateral unaffected eyes and normal subjects. Large amounts of total protein and distinct protein profile were, also, found. This new approach showed good correlation with gold standard test (viral culture) and could be useful as a preliminary test for detection of adenoviral conjunctivitis.

Key Words: hyaluronan; tear; conjunctivitis; adenovirus.