

TEAR CYTOKINES IN BASEDOW-GRAVES OPHTHALMOPATHY



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Background: Human tear is rich in cytokines. Even in healthy individuals the cytokine:total protein ratio is higher than that in serum. However, in Basedow-Graves disease an elevation of serum cytokine levels was found and also an altered cytokine response of the intra- and periorbital tissues was postulated. In our present study we focused on the measurement of IL-6, IL-8 and TNF- α levels and the amount of a lipocalin-type protein (orosomucoid) in tear samples of Graves patients.

Methods: Tear was collected by the Schirmer method from 35 patients and 20 control individuals without autoimmune and thyroid diseases. 80 % of them were females. The mean age was 45 (25-74) years. The total tear protein content was measured by the Bradford method and tear proteins were separated by SDS-PAGE electrophoresis. Tear orosomucoid was detected by chemiluminescent Western blot technique. Tear cytokine levels were quantified by an automated chemiluminescence immunoassay method (Immulite, Beckman-Coulter).

Results: TNF- α and IL-6 increased significantly (mean \pm SEM, $p < 0.01$) in tear samples of the patients (TNF- α : 513 \pm 57 vs. 217 \pm 27pg/mg, IL-6: 39 \pm 6 vs. 12 \pm 1.2 pg/mg protein, patients vs. control individuals, respectively). In contrast, IL-8 and orosomucoid levels were similar in the two groups.

Conclusions: In our opinion increased tear TNF- α and IL-6 levels are considered to be a new finding in Graves patients. Further studies are needed to decide if tear cytokines might be used in assessment of activity of this disease and/or to monitor the efficiency of treatment.

Patients

EOP group:

Patients diagnosed with endocrine ophthalmopathy

n = 26 (21 females, 5 men)
Mean age: 45 years

Control group:

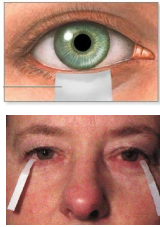
Healthy individuals without autoimmune and thyroid diseases

n = 20 (12 females, 8 men)
Mean Age: 50 years



Tear sampling: Schirmer method

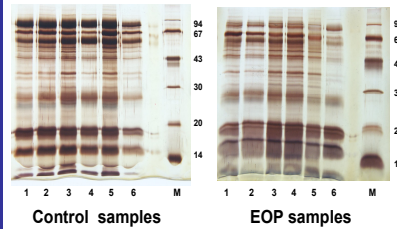
Filter paper



- Non invasive
- Simple
- Immediate

TEAR Proteins - SDS PAGE

1,8 μ g protein / lanes



Control samples

EOP samples

Methods

Analysis of tear proteins

- **Total protein:**
 - Bradford (CBB) – Quantitative measurement
 - SDS PAGE with silver intensification – protein pattern
- **Specific proteins:**
 - Western blotting/ECL \rightarrow AGP
 - Automatic \rightarrow Cytokins (Immulite, Beckman-Coulter)



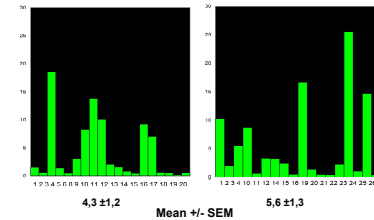
Western blotting: AGP

- **Detection: ECL**
- **Quantitative evaluation:**
 - Femtogram sensitivity
 - Internal standard (*) on base of calculated ECL ratios (individual samples/Std)

AGP(orosomucoid) – ECL ratios

CONTROL

EOP

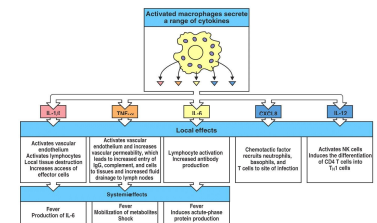


4,3 \pm 1,2

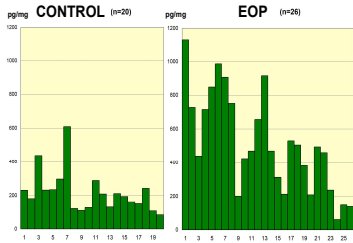
Mean \pm SEM

5,6 \pm 1,3

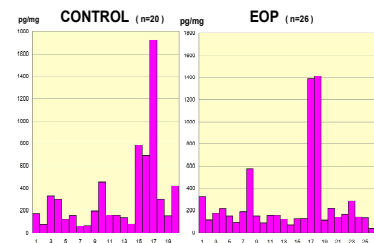
Cytokines - proinflammatory mediators



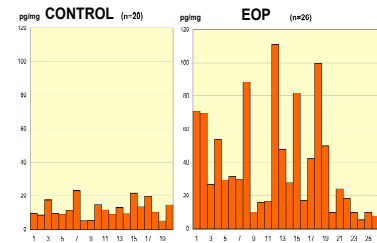
TNF- α pg/mg protein



IL-8 pg/mg protein



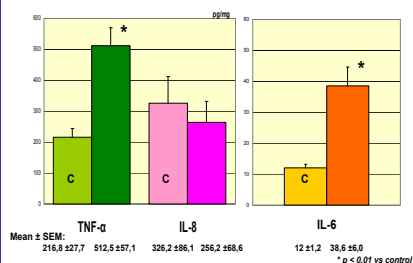
IL-6 pg/mg protein



TEAR CYTOKINES

CONTROL n=20

EOP n=26



Mean \pm SEM: TNF- α 216,8 227,7 512,5 457,1 IL-8 326,2 286,1 256,2 268,6 IL-6 12 \pm 1,2 38,6 48,0 *p < 0,01 vs control

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New findings

- Contents of some cytokines in the tear of EOP patients is different from those measured in healthy individuals.
- Increased tear TNF- α (having a primary role in the regulation of immune cells) and IL-6 levels (an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine secreted by T cells and macrophages) are considered to be a new finding in EOP patients.
- Mass spectrometric identification of some tear proteins is in progress to answer the question if tear as a non-invasive sample might be used for monitoring disease activity and/or effectiveness of treatment in EOP.