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 periocular 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 (25–71) 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 immunosassay method (Immulus, Beckman-Coulter).

Results: TNF-α and IL-6 increased significantly (mean ± SEM, p<0.01) in tear samples of the patients (TNF-α: 513.57 vs. 217.27 pg/mg, IL-6: 391.6 vs. 122.12 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.

**Tear sampling: Schirmer method**
- **Filter paper**
  - Non invasive
  - Simple
  - Immediate

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

**AGP(orosomucoid) – ECL ratios**

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>EOP</th>
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<tbody>
<tr>
<td>Mean</td>
<td>8.4±1.3</td>
<td>8.6±1.3</td>
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<tr>
<td>SEM</td>
<td></td>
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</tbody>
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**Cytokines - proinflammatory mediators**

**TNF-α pg/mg protein**

**IL-8 pg/mg protein**

**IL-6 pg/mg protein**

**Patients**
- **EOP group:**
  - Patients diagnosed with endocrine ophthalmopathy
  - n=26 (21 females, 5 males)
  - Mean age: 45 years
- **Control group:**
  - Healthy individuals without autoimmune and thyroid diseases
  - n=20 (12 females, 8 males)
  - Mean Age: 50 years

**Methods**
- **Total protein:**
  - Bradford (CBB) – Quantitative measurement
  - SDS PAGE with silver intensification – protein pattern
- **Specific proteins:**
  - Western blot/ECL + AGP
  - Automatic + Cytokins (Immulus, Beckman-Coulter)

**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.

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