EVALUATION OF THE USEFULNESS OF SERUM CYTOKINES IL-1β AND sFasL MEASUREMENTS IN THE DIAGNOSIS OF AUTOIMMUNE HYPOTHYROIDISM AND HYPERTHYROIDISM IN CHILDREN

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INTRODUCTION
Autoimmune thyroid disease (AID) is one of the most common organ-specific autoimmune disorders, of which Hashimoto’s thyroiditis (HT) and Graves’ disease (GD) are 2 of the most common clinical expressions. Cytokines play a crucial role in modulating immune responses in both these disorders. The apoptotic pathway is up-regulated in chronic autoimmune thyroiditis (cAIT) and destruction of the thyroid leads to hypothyroidism (hypothyroidism). This phenomenon is also present in Graves’ disease (GD) manifested with hyperthyroidism (hyperthyroidism). The role of soluble FasL (sFasL), a proteolytic product of FasL, is less clear in induction of apoptosis in both thyrocytes and lymphocytes. IL-1β is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis.

AIM OF THE STUDY
The aim of this study was to determine the relationship between the concentration of proinflammatory cytokines IL-1β and sFasL with immune thyroid factors in the serum of children with autoimmune thyroid disease (AID).

MATERIAL AND METHODS
1. Studied groups and analyzed markers: n = 45 children in 3 subgroups: n = 11 children with hypothyroidism (hypothyroidism group: n = 15); n = 11 children with hyperthyroidism (nearly healthy patients) and n = 15 healthy subjects as an euthyroid control.

The summary of the groups and descriptive statistics are presented in Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroidism (hypothyroidism)</th>
<th>Hyperthyroidism (hyperthyroidism)</th>
<th>Control group (n=15)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>12.2 ± 3.9</td>
<td>12.4 ± 4.9</td>
<td>10.5 ± 4.8</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>18.69 ± 0.45</td>
<td>18.25 ± 3.42</td>
<td>18.17 ± 3.50</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.3 (0.04)</td>
<td>-0.38 ± 0.05</td>
<td>-0.55 (1.29)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Cole’s Index</td>
<td>1.05 ± 0.22</td>
<td>0.95 ± 0.13</td>
<td>0.9 ± 0.70</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>TSH [mIU/L]</td>
<td>37.34 (19.06)</td>
<td>0.01 (0.1)</td>
<td>2.42 (1.52)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>FT3 [pg/mL]</td>
<td>4.34 (4.01)</td>
<td>1.24 (1.14)</td>
<td>1.03 ± 0.12</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>FT4 [pg/mL]</td>
<td>0.96 (0.87)</td>
<td>0.51 (0.50)</td>
<td>0.56 (0.56)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>T4 [µg/dL]</td>
<td>1.3 (1.50)</td>
<td>1.01 (1.23)</td>
<td>1.70 (1.84)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>TRAB [IU/L]</td>
<td>0.32 ± 0.11</td>
<td>0.12 ± 0.14</td>
<td>0.10 (0.25)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>IL-1β [pg/mL]</td>
<td>2.58 ± 1.80</td>
<td>2.16 (0.97)</td>
<td>1.70 ± 1.27</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>sFasL [ng/mL]</td>
<td>0.07 ± 0.11</td>
<td>0.09 ± 0.10</td>
<td>0.09 ± 0.10</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

Table 1. Descriptive statistics and significance of differences - hypothyroidism vs hyperthyroidism (ANOVA – analysis of variance, K-W – Kruskal-Wallis non-parametric test).

2. Inclusion criteria: clinical, hormonal and autoimmune: TRAB in GD: ATPO + ATG + ATPO + ATG in AITD.
3. Methods: thyroid hormones – MESA tests (Abbott, AxSYM); IL-1β and sFasL - ELISA tests (BenderMedSystem, Vienna, Austria), antibodies TRAB / ATPO / ATPO-RIA tests (Brahms, Berlin).
4. Serum concentrations of IL-1β and sFasL in patients with cAIT and GD; the control were evaluated at the beginning of disease (before treatment) by ELISA.
5. Statistical analysis was carried out in SPSS 17 GraphPad Prism 6. Shapiro-Wilk normality test, ANOVA (Kruskal-Wallis test) and Spearman’s rank correlation were used.

RESULTS
1. IL-1β concentration was significantly higher in cAIT (median [IQR]) 2.16 (0.87) pg/mL vs control 1.68 (1.04) pg/mL (p < 0.05) and in cAIT vs GD 1.39 (1.27) pg/mL (p < 0.01) (K-W test).
2. sFasL concentration was significantly higher in cAIT (median [IQR]) 0.26 (0.14) ng/mL vs control 0.06 (0.15) ng/mL (p < 0.01) and in cAIT vs GD 0.24 (0.09) ng/mL (p < 0.05) (K-W test).

Figure 1. Regulation of apoptosis in a thymocyte, based on Patricia L, Arning and James R. Baker Jr. „Short Analytic Review: Apoptosis and Thyroiditis“. Clinical Immunology and Immunopathology, Volume 82, Issue 3, June 1996, Pages 207–217, modified: W. Stacha, M. Niedziela, H. Mikos

Figure 2. Baxplot of IL-1β: hypov vs control (p < 0.005) hypov vs hyperp (p = 0.01) hypov vs control ns

Figure 3. Baxplot of sFasL: hypov vs control (p < 0.005) hypov vs hyperp (p = 0.05) hypov vs control ns

Figure 4. Positive nonparametric correlation in GD: IL-1β and ATPO

Figure 5. Positive nonparametric correlation in GD: sFasL and BMI SDS

Figure 6. ROC of IL-1β: cAIT vs control group (AUC = 0.77, p = 0.005, cut-off= 0.11 pg/mL, sens.: 72.7%, spec.: 86.4%)

Figure 7. ROC of sFasL: cAIT vs control group (AUC = 0.897, p < 0.001 cut-off= 0.341 mg/mL, sens.: 94.7%, spec.: 72.7%)

CONCLUSION
We suggest that both cytokines IL-1β and sFasL may be useful markers in the assessment of thyroid dysfunction of autoimmune hypothyroid and hyperthyroid children.

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