Serum cytokines in autoimmune and toxic multinodular thyroid diseases

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ABSTRACT

Objectives: Measuring serum cytokine levels in patients with Grave’s disease, Hashimoto’s thyroiditis, toxic multinodular goiter and compared them to a normal control group, to see if there is any correlation between the level of the serum cytokines and the pathophysiology of autoimmune and toxic multinodular thyroid diseases.

Methods: Serum INF-γ (Th1), IL-10 and IL-4 (Th2) and IL-6 cytokine levels were studied in 26 patients with Graves’ thyrotoxicosis, 27 patients with goitrous Hashimoto’s thyroiditis, 18 patients with toxic multinodular goiter and 40 normal controls.

Results: INF-γ was detected in all Graves’ and Hashimoto’s patients with mean values of 142.11±29.53 unit/ml and 85.70±25.86 unit/ml. The mean levels of INF-γ in Graves’ was significantly higher compared to Hashimoto’s (P<0.001). IL-10 was detected in all Graves’ patients with a mean value of 583.85±253.35 pg/ml and 19 out of 27 Hashimoto’s patients with a mean value of 332.63±213.20 pg/ml. The level of IL-10 was significantly higher in Graves’ than in Hashimoto’s (P<0.001). Both INF-γ and IL-10 were not detected in toxic multinodular goiter or normal individual. IL-4 was significantly elevated in Graves’ with a mean value of 132.4±44.52 but not in any of the other study groups. IL-6 was detected in the serum of 25 Graves’ with a mean value of 496.80±180.43 pg/ml, 10 Hashimoto’s with a mean value of 293.50±196.61 pg/ml and 10 toxic multinodular goiter patients with a mean value of 228.75±73.96 pg/ml but not in the serum of normal individuals. The levels of IL-6 was significantly elevated in autoimmune thyroid disease (Graves & Hashimoto’s) compared to toxic multinodular goiter (P<0.001); also IL-6 level was significantly higher in Graves’ than Hashimoto’s (P<0.001).

Conclusion: The immunopathology seen in both the Grave’s disease and goitrous Hashimoto’s thyroiditis groups that we studied are related in part to the presence and interplay of mixed Th1 and Th2 cytokines in which there is a down regulation of cytotoxic effect and production of high levels of autoantibodies. The raised level of IL-6 that we observed in autoimmune (Grave’s disease and Hashimoto’s thyroiditis) and toxic multinodular goiter could be an indication of a long standing inflammatory and destructive process of the diseases.

Keywords: INF-γ, IL-10, IL-4, IL-6, Grave’s disease, Hashimoto’s thyroiditis, Toxic multinodular goiter.

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Cytokines are hormone like proteins that enable immune cells to communicate and play an important role in the immune response. Although the role of cytokines in autoimmune thyroid diseases is not yet clearly understood, they appear to play a critical role in the pathogenesis of these diseases. The study of cytokines in autoimmune thyroid diseases has gained considerable interest in recent years, as it has been shown that cytokines are involved in the pathogenesis of these diseases.

Analysis of CD4 T-helper/inducer cell clones revealed two functional subpopulations based on their ability to produce cytokines. One population was characterized by the production of INF-γ, IL-10 and IL-4, while the other population produced IL-6. The study of these cytokines in autoimmune thyroid diseases has shown that INF-γ, IL-10 and IL-4 are involved in the pathogenesis of these diseases, while IL-6 is involved in the production of autoantibodies.

In conclusion, the study of cytokines in autoimmune thyroid diseases has shown that INF-γ, IL-10 and IL-4 are involved in the pathogenesis of these diseases, while IL-6 is involved in the production of autoantibodies. This information has important implications for the treatment of autoimmune thyroid diseases.
the immune response proinflammatory cytokines such as IL-1, IL-6, IL-8 and tumor necrosis factor-α (TNFα). The interaction between these cytokines and their immune regulation in the host plays an important role in the clinical and immunopathological presentation of many autoimmune diseases.

A mixed Th1 and Th2 cytokines mRNA were detected by PCR techniques in autoimmune thyroid disease (Grave's (GD) and Hashimoto's thyroiditis (HT)). But the definite role of these cytokines in the immunopathology and clinical manifestation was not clearly defined. As an example in GD the Th1 cytokines mRNA are predominantly expressed but the pathophysiology was mainly due to production of autoantibody to TSH receptor.

In the present study, we measured serum cytokine levels in patients with Grave's disease, Hashimoto's thyroiditis, toxic multinodular goiter (TMNG) and compared them to a normal control group, to see if there is any correlation between the level of the serum cytokines and the pathophysiology of autoimmune and toxic multinodular thyroid diseases.

IL-10 Assay. IL-10 was determined using IL-10 specific mAbx in microtiter plates. In brief, 96-well microtiter plates were coated overnight at 4°C with 100 ul (diluted 1:800 in 0.1m carbonate, Ph9.5) of anti-human IFN-γ (80-3959-02, Genzyme, Cambridge, MA). Wells were washed five times with phosphate-buffered saline (PBS) Ph7.3 with 0.05% Tween 20. The wells were blocked with 250 ul of 4% bovine serum albumin (BSA) in PBS, Ph7.3 for 2 hours at 37°C and 100 mL of serum samples and IFN-γ standards (25-750 unit/ml) were added to wells and incubated at 37°C for 2 hours, plates were washed five times with PBS-0.05% Tween and 100 ul of second antibody, anti-human IFN-γ-HRP conjugate (2.5 µg/ml) was added to wells and incubated for 30 minutes at 37°C the wells were washed five times with PBS-0.05% Tween and 100 ul of substrate reagent tetramethylbenzidine (KPL, USA) was added to all wells for 30 minutes at room temperature. The reaction was stopped by adding 100 ul of 2NH₄SO₄ and the optical density was measured at 450nm using a flow titertek microplate reader. The sensitivity of the IFN-γ test is 25 unit/ml.
at 4°C overnight. The plates were washed 4 times with wash buffer and 100 µl of serum samples and standards (45-3000 pg/ml) were added and incubated for 2 hours at room temperature. After that the plates were washed 4 times with washing buffer and 100 µl of ready to use rabbit anti-IL-4 antibody was added to each well and incubated for 2 hours at room temperature. The plates were washed 4 times with washing buffer and 100 µl of biotinylated goat anti-rabbit immunoglobulin antibody (1:6500) was added to all wells and incubated at room temperature for 45 minutes then washed 4 times with the wash buffer and 100 µl of streptavidin-conjugated horse radish peroxidase (1:7000) was added to each well and incubated for 40 minutes at room temperature followed by plate washing for 4 times and 100 µl of the substrate reagent (0-phenylendiamine-peroxide) was added to all wells and incubated for 5-10 minutes and the reaction was stopped by adding 100 µl of 1M H₂SO₄ to all wells. The plate was read at 492nm using a flow tiertek microplate reader and IL-4 values were obtained from the standard curve. The sensitivity of the IL-4 test is 45 pg/ml.

IL-6 Assay. IL-6 levels were measured in serum samples by a solid phase ELISA assay based on the antibody sandwich principle, using an Intertest ELISA kit purchased from Genzyme (Cambridge, MA, USA). The immunoassay was performed as follows: 100 µl of diluted serum samples (1:2) and standards (150-2500 pg/ml) were added to microtiter plates precoated with anti-IL-6 monoclonal antibody and incubated for 30 minutes at 37°C. The plates were washed 5 times with washing buffer and 100 µl of biotinylated polyclonal rabbit anti-IL-6 (ready to use) was added to all wells and incubated for 30 minutes at 37°C. The wells were washed 5 times with washing buffer and 100 µl of avidin-peroxidase conjugate (ready to use) was added to all wells and incubated for 15 minutes at 37°C. The wells were washed 5 times using washing buffer and 100 µl of substrate solution (tetramethyl benzidine-mg hydrogen peroxide) was added to all wells and incubated for 8 minutes and the color was stopped by adding 100 µl of 1M sulfuric acid. The absorbance was read at 450 using a flow tiertek microplate reader and IL-6 values were obtained through the standard curve. The sensitivity of the test was 150 pg/ml.

Statistical analysis. Experimental data were entered into a computer. Scattergrams and statistical analysis were performed by Prism™ version 2 (GraphPad software, Inc, San Diego, USA) and SPSS utilizing non-parametric analysis (ANOVA and Mann-Whitney) and one way ANOVA (Kruskal-Wallis test).

Results. Serum cytokines levels were measured by solid-phase sandwich ELISA assay. The levels of INF-γ, IL-10, IL-4 and IL-6 in all patients and normal control group were shown in Table 1. INF-γ was detected in all Graves’ and Hashimoto’s patients with mean values of 142.11±29.53 unit/ml and 85.70±25.86 unit/ml, but not in any of TMNG or normal individuals. The mean levels of INF-γ in Graves’ were significantly higher compared to Hashimoto’s (P<0.001) (Figure 1).

IL-10 was detected in all Graves’ patients with a mean value of 583.85±253.35 pg/ml and 19 out of 27 hashimoto’s patients with a mean value of 332.213.20 pg/ml. The level of IL-10 was significantly higher in Graves’ than in Hashimoto’s (P<0.001) (Figure 2). IL-4 was significantly elevated in Graves’ with a mean value of 132.40±44.52 pg/ml but not in any of the other study groups (Figure 3).

IL-6 was detected in the serum of 25 Graves’ with a mean value of 496.80±180.43 pg/ml, in 10 out of 27 Hashimoto’s patients with a mean value of 293.50196.61 pg/ml and in 10 out of 18 TMNG patients with a mean value of 228.75±73.96 pg/ml but not in the serum of normal individuals. The

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Graves’ (26)</th>
<th>Hashimoto’s (27)</th>
<th>TMNG (18)</th>
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<tbody>
<tr>
<td></td>
<td>No</td>
<td>Mean</td>
<td>SD</td>
<td>No</td>
</tr>
<tr>
<td>INF-γ unit/ml</td>
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<td>142.11</td>
<td>29.53</td>
<td>27</td>
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<tr>
<td>IL-10 pg/ml</td>
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<td>583.85</td>
<td>253.35</td>
<td>19</td>
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<tr>
<td>IL-4 pg/ml</td>
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<td>132.4</td>
<td>44.52</td>
<td>0</td>
</tr>
<tr>
<td>IL-6 pg/ml</td>
<td>25</td>
<td>496.80</td>
<td>180.43</td>
<td>10</td>
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</table>

No - Number of samples with detectable cytokine level
ND - None detectable serum cytokine level
TMNG - Toxic multinodular goiter
SD - Standard deviation
levels of IL-6 were significantly elevated in autoimmune thyroid disease (Graves and Hashimoto’s) compared to TMNG (P < 0.001). Also the level of IL-6 was significantly higher in Graves’ than in Hashimoto’s (P < 0.001) (Figure 4).

Discussion. In this study, serum Th1 (IFN-γ), Th2 (IL-10 and IL-4) cytokins and inflammatory cytokines (IL-6) were measured in Saudi patients with autoimmune and toxic multinodular thyroid disease and compared them with normal controls. It is apparent that autoimmune thyroid diseases (Graves’ and Hashimoto’s) were associated with high levels of Th1 (IFN-γ) and Th2 (IL-10 and IL-4) cytokines compared to TMNG and normal controls. In Graves’ the high level of serum IFN-γ, IL-10 and IL-4 indicated a mixed Th1/Th2 cytokines. This pattern may be reflected in a mixed intrathyroidal Th1/Th2 cells lymphocyte activity which may involve cell mediated tissue destruction and specific TSH-R antibodies production. This observation is in agreement with the analysis of intrathyroidal cytokine gene expression profiles which showed both types of Th1 (IFN-γ) and Th2 (IL-10 and IL-4) cells in the thyroid gland of patients with Graves’ hyperthyroidism with a predominance of Th1 CD4 helper cells. Similar results were also observed in the HT patient group of our study were all of the goitrous type with high titer of antimicrosomal antibodies and were all assessed for cytokine profiles before thyroxin therapy. These
patients had a mixed Th1 and Th2 cytokine profile with high IFN-γ and IL-10 levels. We did not detect IL-4 in serum of goitrous HT, however in a previous study marginally significant IL-4 levels were detected in PBMC cultures from chronic thyroiditis patients in response to phytohaemagglutinin (PHA) stimulation which decreased following therapy. Although the serum levels could not be compared to polyclonal mitogenic activation of PBMC, yet the difference between our observations could be due to variation in the type of patient studied. Fisfalen et al recently isolated T-cell clones (TCC) from the thyroid gland of HT and Graves' patients responding to thyroid antigens Thyroid Peroxidase (TPO), Thromboglobulin or recombinant TSH-receptor. The TCC were classified for their cytokines profiles into Th0 cells secreting IL-4, IL-5 and IFN-γ, Th1 secreting IFN-γ, and Th2 secreting IL-4 and or IL-5. IL-10 was secreted by all cell subset. They found that TCC from HT patients were predominantly of the Th1 like cells while TCC from GD were Th0 and Th1 like subsets.

We observed high IL-4 levels in the GD patient group compared to HT. This striking difference in IL-4 production between GD and HT could not be explained. High IL-4 levels were previously detected in peripheral blood mononuclear cell cultures (PBMC) from patients with Graves' and chronic thyroiditis during active disease which declined following therapy. The high IL-4 levels could as well indirectly add to the down regulation of the IFN-γ cytotoxic effect in both diseases. IL-6 was detected in autoimmune (GD and HT) and TMNG groups but not in the normal group. This may reflect an intrathyroidal inflammatory process induced by autoimmune and toxic Multinodular disease. The significantly elevated IL-6 level in Graves' compared to the other groups was probably related to long standing inflammatory process of Graves' ophthalmopathy.

The immunopathology seen in both the GD and goitrous HT groups we studied are related in part to the presence and interplay of mixed Th1 and Th2 cytokines in which there is a down regulation of cytotoxic effect and production of high levels of autoantibodies. The raised level of IL-6 that we observed in autoimmune (GD and HT) and TMNG could be an indication of a long standing inflammatory and destructive process of the diseases. Acknowledgments. I would like to thank Hashim W. Ghali and A. EL McKee for reviewing the manuscript and for valuable comments, and DR. M. Al-Jaradi for his valuable support and S. Raziauddin and R. Musa for the ELISA assays.

References

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