Cytokine profiling in active thyroid-associated ophthalmopathy and its relation to some hematological parameters

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Abstract
Thirty four patients having autoimmune hyperthyroid disease divided into two groups according to the presence of orbitopathy sign, were eighteen active thyroid associated G1-ophthalmopathy (TAO) (8 male and 10 female), and sixteen autoimmune thyroid without ophthalmopathy (10 male and 6 female), in addition to sixteen healthy control volunteers (7 male and 9 female); at a mean age of (29.27 ± 5.85 years), (32 ± 8 years) and (31.12 ± 8.55 years) respectively. Proper history with blood samples were collected from all subscribers for the estimation of some hematological, biochemical and immunological (IL-6) parameters in relation to thyroidal hormones. This study demonstrate a highly significant increase (P<0.01) in IL-6 level in patients without orbitopathy in comparison to TAO patients and healthy control group. While changes in the serum hormones level are the same in all patients. Also in this study we confirm the strong positive correlation between IL-6 and T3 in all the participants’ patients (TAO and TNAO).

Conclusion: Orbitopathy is one of the signs that may or may not involve in the prognosis of autoimmune thyroid disease depending on:
1. The type, stage and activity of the disease.
2. May be due to the involvement of loci other than the immune causes
3. May be advert to the importance of IL-6 in the immunity of the eyes before the appearance of ophthalmopathy complications specifically if IL-6 in turn act locally in the orbit to stimulate its own production.
4. Should be study the effect of T3 on the immune system both in health and in disease state.

Keywords: IL-6, autoimmune thyroid disease and thyroid associated orbitopathy

Introduction
The etiology of autoimmune thyroid disease is multifactorial involves genetic and environmental factors (1). Thyroid-associated ophthalmopathy (TAO) is an antibody-mediated autoimmunity, causing hyperthyroidism which may be associated with an inflammatory process in the orbit (2) due to self-reactive lymphocytes that escape immune tolerance. The interactions between orbital fibroblasts and lymphocytes lead to the expansion and remodeling of the orbital tissues, presenting the clinical manifestations of TAO (as shown in fig. 1) (3), characterizes by lid retraction, swelling, erythema of conjunctiva and proptosis; sight threatening complications as corneal ulceration due to

optic neuropathy may result in irreversible visual impairment or blindness if not treated\(^4\). Reactive T lymphocytes enhanced by circulating and local adhesion molecules is stimulated by cytokines. These cytokines stimulate the synthesis and secretion of glycosaminoglycans (GAGs), the key feature of the TAO-histopathology, by fibroblasts lead to the clinical manifestations of orbitopathy\(^5\).

Affected tissues exhibit immune responses elaborated by resident cells, including T helper cell bias, and cells recruited from the bone marrow through the release of cytokines and their expression to cytokine receptors; furthermore, chemoattractant cytokines in the thyroid and orbit may provoke mononuclear cell infiltration. Other cytokines driven cell activation and tissue remodeling that concerns the process of apoptosis\(^3\). Cytokines, especially interleukins (ILs), are pathologically involved\(^4\) and are key regulators of the immune and inflammatory responses\(^6\). Th2 cytokines, such as IL-6 is involved in humoral immunity to mediate differentiation of B cells\(^7\). The cause of the enhanced levels of IL-6 is feasible related to genetic factors, mainly gene polymorphisms\(^8\). Among numbers of signaling pathways CD40 and cognate ligand CD154 (CD40 ligand) was discovered on B cells induces IL-6 production\(^9\). In other experiments examine the prototypic CD4+ T cell–model of organ-specific autoimmune inflammation, and the regulatory effect of IL-6 in the acute-phase response\(^10\).

**Aim of the study:** This study was designed to examine the role of cytokine (IL-6) in the pathogenesis and complication (represented by orbitopathy) of autoimmune thyroid disease and to explore its relationship to the changes in different hematological and biochemical parameters.

![Diagram](image-url)
2. Subjects, Materials and Methods

2.1. Subjects and Methods
The blood samples were taken after getting official approval as well as the verbal consent of participants prior to their inclusion in the study. The sample size was small as this is a preliminary study; expanding sample size is warranted for more investigations in the future. Thirty four unrelated autoimmune thyroid patients divided into two groups: active thyroid associated orbitopathy G1 (TAO) and thyroid not associated orbitopathy (TNAO), in addition to sixteen healthy volunteers from the same ethnicity without any systemic or ocular disease were served as control group. The entire subscriber’s ages were between 18-47 years old. Five ml of venous blood was taken from the enrolled participants, 1 ml of a blood posit in EDTA tube for hematological tests and the remaining amount into gel tubes, left at room temperature (25 °C) for 15 minutes then centrifuged at 3000 rpm for 10 minutes in order to collect sera. Sera aliquots were placed in Eppendorf tubes and stored at-20 °C until used. This study was approved by the ethics committee, department of chemistry and biochemistry, College of Medicine, Al-Iraqia University, Baghdad, Iraq and the Iraqi ministry of health, Alnuaman teaching hospital approved this work as well.

2.2. Material
The laboratory investigations were performed using VIDUS instrument for hormonal assay (TT3, TT4 and TSH); IL-6 levels was measured in the sera for all participants using enzyme linked immunosorbent assay (ELISA) apparatus and CELL-DYN RUBY Analyzer for hematological assessment.

2.3. Statistical Analysis
The Statistical Analysis System- SAS* program was used to compare between control and patient groups in study parameters. Least Significant Difference-LSD test was used to compare between means (p value of 0.05 and 0.01 was considered to be statistically significant.) and estimate correlation coefficient between some variables in this study.


3. Results and Discussion
The body weight, height, age, gender and drug history were recorded from all subscribers in addition to the patient's history including duration of the disease (range from 1-36 months); clinical signs and symptoms (all have hand tremor, palpitation, 55% of patients with increase pulse rate, fatigability, heat intolerance, sweating, loss of weight associated with increase appetite) but all without skin changes. In this study measure serum level of IL-6 to follow its effect on the orbit in addition to other biochemical investigations.

Data in fig.(2) and table (1) show significant elevation (P<0.01) in IL-6 level in patients without orbitopathy (TNAO) in comparison to thyroid associated orbitopathy (TAO) and healthy control groups (considering mean± 1 standard deviation of IL-6 level in healthy control group as a cutoff value for IL-6 which is = 7.51 pg/ml). Our results revealed that TAO patients are in the active (inflammatory) phase presumably because IL-6 requirement restricted to the early stages of T cell activation were orbitopathy sign not yet started (3); and the natural course of TAO is an active (inflammatory) phase in the initial stage followed by an inactive (fibrotic) phase in the late stage (11). Our results is confirmed by Jie Shen and his
colleagues study findings that serum levels of the Th1 cytokines are potential biomarkers for active TAO, while serum levels of the Th2 cytokine such as IL-6 may be useful as a potential biomarker for inactive TAO\(^{(12)}\).

Other mechanism expect that IL-6 may not function primarily as an acute phase reactant, but may serve as inflammatory mediator in later disease\(^{(13)}\), and the development of orbitopathy through activation of orbital fibroblasts lead to the release of T cell chemoattractants\(^{(14)}\).

In this study we demonstrate a significant increase in the serum levels of thyroid hormones (T3 and T4) and a significant decrease in the TSH levels in the patient groups compared to healthy. These changes are same in all participated patients.

![Figure 2: IL-6 levels comparison among the studied groups](image-url)

Table (1): Comparison of different biochemical analysis among the studied groups

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>No. of participants</th>
<th>IL-6 (pg/ml)</th>
<th>T3 (nmol/L) Normal value = 0.92-2.33 nmol/L</th>
<th>T4 (nmol/L) Normal value = 60-120 nmol/L</th>
<th>TSH (mIU/ml) Normal value = 0.25-5.0 mIU/ml</th>
<th>Monocyte (%)</th>
<th>Lymphocyte (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active thyroid associated ophthalmopathy (TAO)</td>
<td>18</td>
<td>3.72 ± 0.71 b</td>
<td>6.40 ± 0.51 a</td>
<td>246.39 ± 16.06 a</td>
<td>0.135 ± 0.03 b</td>
<td>4.71 ± 0.26 a</td>
<td>36.74 ± 1.20 a</td>
</tr>
<tr>
<td>Thyroid not associated ophthalmopathy</td>
<td>16</td>
<td>10.03 ± 2.44 a</td>
<td>6.39 ± 0.40 a</td>
<td>229.50 ± 14.71 a</td>
<td>0.181 ± 0.04 b</td>
<td>4.27 ± 0.21 ab</td>
<td>38.91 ± 1.97 a</td>
</tr>
</tbody>
</table>
In addition we found a significant positive correlation of IL-6 with T3, TSH and negatively with monocyte % in TAO group; meanwhile a significant positive correlation of IL-6 with T3 hormone only is detected in TNAO patient, as revealed in table (2).

Table (2): Correlation coefficient-r among IL-6 and other parameters in autoimmune hyperthyroid patients with (TAO) and without orbitopathy (TNAO).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient-r with IL-6.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAO</td>
</tr>
<tr>
<td>T3</td>
<td>0.25</td>
</tr>
<tr>
<td>T4</td>
<td>-0.02</td>
</tr>
<tr>
<td>TSH</td>
<td>0.26</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>-0.35</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

* (P<0.05) , NS: Non-Significant

Even though in a study by Davies and his colleague found a statistical relation between elevated serum IL-6 concentrations and alterations in circulating thyroid hormone concentrations in non-thyroidal illness patients (15). These results can be explained on the basis of cytokine secretion profiles as a pro-inflammatory Th1 cells involve in the cellular response lead to the activation of macrophages and hence monocytes, or anti-inflammatory Th2 cells produce IL-4, 5, 6, 10 and 13 can inhibit the production of Th1 cytokines and associated with humoral response.

Therefore, the clinical manifestations of thyroid autoimmune phenotype largely depend on the balance of the immune response and the cytokine profile dominates in thyroid parenchymawhich is in fact a dynamic processarise under the influence of extrinsic factors locally in the thyroid gland and can develop in the same patient at different periods (16). It is feasible that the apparent discrepancies between our work and other studies could be due to the different genetic background of the individuals studied, the presence or absence of ophthalmopathy, and the stage of the disease of the patients.

A very significant differences (P<0.01) in the monocyte levels among the studied groups, were the level in active thyroid patients associated with orbitopathy significantly more than its level in TNAO although clinically is non-significant elevation as it is not deviated from the normal reference range, meanwhile they are considered as good factors for...
controlling and regulating immunity against foreign substances, as it is recruited to the affected site by the Th1 cells and this is confirmed by our results of negative significant correlation-r between IL-6 and monocyte% in TAO patients.

4. Conclusion

1. Orbitopathy is one of the signs that may or may not involve in the prognosis of autoimmune thyroid disease depending on the type, stage and activity of the disease.

2. The significant elevation of IL-6 concentration in the serum of hyperthyroid patients without orbitopathy in comparison to non-elevated serum level of IL-6 in active thyroid patients associated with ophthalmopathy may suggest involvement of loci other than the immune causes in the incidence of orbitopathy, or it may be advert to the importance of IL-6 in the immunity of the eyes before the appearance of ophthalmopathy complications specifically if IL-6 in turn act locally in the orbit to stimulate its own production. Altered actions, levels and complexity of cytokine in addition to the residential cells and professional immune cells recruited to the thyroid and orbit have been reported in TAO.

3. Also in this study we confirm the strong positive correlation between IL-6 and T3 in all the participants’ patients (TAO and TNAO) and that’s why should study the effect of T3 on the immune system in details.

References


[12] Jie Shen, Zhangfang Li, Wenting Li, Ying Ge, Min Xie, Meng Lv, Yanfei Fan, Zhi Chen, Defu Zhao, and Yajuan Han. Th1, Th2, and Th17 Cytokine Involvement in Thyroid Associated Ophthalmopathy. *Disease Markers.* 2015; volume 2015.


