Detection of interleukin 12 in serum of Iraqi children with type 1 diabetes

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Abstract

Introduction: Type 1 DM (T1DM), arising through interaction of immune, genetic and environmental factors, results from autoimmune destruction of insulin-producing β cells. Cytokines are critical to the function of both innate and adaptive immune responses. Interleukin-12 p40 production influences T cell response, and should therefore be important in T1DM pathogenesis. Objective: to study the relation between IL12 levels and etiopathogenesis for T1DM in Iraqi children. Material and methods: thirty T1DM children among those attending specialist center for endocrinology and diabetic, were included within the study. They were 15 males and 15 female. Thirty age and sex matched healthy children were serving as an impact group. All children were subjected to full history taking, physical examination, fasting blood glucose, glycated hemoglobin (HBA1C) and serum IL-12 levels assessed by ELISA. Results: Diabetic children had no significant higher IL12 levels than healthy children. While there was no effect of gender on IL12 levels, there was no significant increase in IL12 levels in newly diagnosed cases. Conclusion: In this study type1 diabetes not associated with elevation of IL-12 levels. However, serum IL-12 levels show study on large scale to give more information.

Keywords: health; serum; interleukin; type 1 diabetes

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Introduction

Diabetes mellitus type1 (T1DM) is characterized by the appearance of insulitis and therefore the presence of β cell autoantibodies [1]. Emerging evidence indicates that immune cell infiltrates comprised predominantly of CD8 and CD4 T cells, B cells, and macrophages are present in islets from newly diagnosed patients, implicating both innate and adaptive immune systems within the pro-inflammatory process resulting in islet cell death ⁵[2]⁵.Type 1 (insulin-dependent) DM may be a common chronic disease of childhood, and presents with acute, sometimes life-threatening, symptomatic hyperglycaemia. Children with diabetes present to paediatric services and are highly visible within the health system. This is often not the case in patients who are 15 to 29 years of age; symptoms are typically less acute, and both gestational and sort 2 diabetes are increasingly prevalent during this age group, compared with children ³⁴.Type 1 diabetes has traditionally been considered a disease of childhood, the bulk of cases present in adult life⁵[5].

Adult cases are characterized by a reduced frequency of HLA susceptibility haplotypes, as compared with children ³⁶⁷ and overall have less acute symptoms than children ³.Cytokines are reported to be involved in the immunopathology of several autoimmune diseases including type 1 diabetes .There is evidence that cytokines could have a direct role in β-cell death⁷.Interleukin-12
p40 production influences T cell response, may be therefore be important in T1DM pathogenesis. Interleukin-12 (IL-12) drives the differentiation of T lymphocytes towards the Th1 subset, characterized by production of cytokines resulting in cell mediated immunity. Additionally, IL-12 is very important in immunologic response to infections; however, it's been shown that in absence of infection, IL-12 induced autoreactive T cell responses might predispose to self-destructive immunity[9]. The significance of IL-12 in human autoimmunity isn't clear, and serum levels of IL12 in DM haven't been well established. Elevated levels of this cytokine are observed in most autoimmune diseases[7][10].

Materials and methods
The study population consisted of 30 patients with type 1 DM with age bracket (2-18years) recruited from the patient who attend to specialist center for endocrinology and diabetic disease. Type 1 diabetes was defined consistent with the standards of the planet Health Organization definition. Patients with micro-vascular complications, also as those with coexisting autoimmune, chronic, and acute inflammatory diseases were excluded from the study. All patients were treated with humanized insulin. At the time of sampling, a blood sugar level, biochemical measurement of fasting blood glucose (FBS), and glycosylated hemoglobin (HbA1c) were monitored. Additionally, 14 age and sex matched healthy children with no case history of diabetes served as an impact group. Ethical approval was obtained from the local research ethics; all subjects gave an informed written consent before the study.

Detection in Serum of IL-12 Levels
The samples were collected as 3ml of blood for each patient and then centrifuged to obtain serum. The serum stored at -4°C for the quantitative assessment of IL-12 using enzyme linked immunosorbent assay (ELISA) technique (Elabscience Human IL-12 EASIA kit). Samples were brought to room temperature and processed according to manufacturer’s description. Colour detection was read at 450 nm Correcting for optical imperfections was done at 630 nm. A standard curve was plotted and results were calculated.

Statistical analysis
All data were analyzed using the Statistical Package for Social Science (SPSS) 26 for Windows. All data were presented as mean ± standard deviation (SD). Student t test was used to compare mean values between groups. Comparing several means was done by one-way analysis of variance (Armonk,NY). Spearman rank correlation test was used for the assessment of correlation. The statistical significance was accepted as p value < 0.05.

The procedure
1. Standard working solution was added to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side-by-side (100 uL for each well). Add the samples to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Removal of liquid out of each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.
3. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3
6. Add 90 μL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 μL of Stop Solution to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

**Calculation:** The sample results were calculated by interpolation from a standard curve that was performed in the same assay as that for the sample using a curve fitting equation.

**Results**

Table 1 shows levels of HbA1c, Fasting blood sugar (FBS), the median and mean of IL-12 level of listed in table 2,3.

<table>
<thead>
<tr>
<th>Biochemical parameter data of the studied groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic children</td>
</tr>
<tr>
<td>HbA1c(%) mean</td>
</tr>
<tr>
<td>FBS mg/dl Mean</td>
</tr>
</tbody>
</table>

**Table 2: The IL-12 level median in diabetic patients group compared to control group.**

<table>
<thead>
<tr>
<th>IL level median ± SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>Control group</td>
</tr>
<tr>
<td>13.15 ± 2.80</td>
<td>15.60 ± 3.71</td>
</tr>
</tbody>
</table>

**Table 3: The IL-12 level mean in diabetic patients group compared to control group.**

<table>
<thead>
<tr>
<th>IL level mean ± SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients group</td>
<td>Control group</td>
</tr>
<tr>
<td>15.35 ± 2.80</td>
<td>17.95 ± 3.71</td>
</tr>
</tbody>
</table>

**Discussion**

Chronic inflammation in type 1 diabetes has been confirmed by many of studies [11][12]. Several proinflammatory cytokines were shown to be elevated in serum of diabetic patients with a new onset of diabetes or a longstanding disease[11][13][14]. IL-12 is additional one among cytokines that has been shown to exert strong proinflammatory activity, which synergize in action with other, cytokines as TNF-α or IL-1[15]. In the present study, patients with type 1 diabetes had no significant correlation between IL-12 serum level and diabetic type 1 as compared to the healthy subjects from the control group and this accept as true with [Skarsvik et al.2005], which may explained by many reasons one of these reasons is an impaired polarization of T cells towards a type 1 immune reaction in T1D[16].
Alternatively, our results may indicate a primary defect within the up-regulation of IL-12 level in T1D. Other explanations, enhanced secretion of other cytokines as IL-13 in the type 1 diabetes might be liable for down regulation gene of the IL-12 [17][18]. The polarization of T cells towards type 1 response, including IL-12 secretion, could also be emerges with age [19]. Our findings suggest that type 1 deviation is disturbed in patients with T1D. These results were unexpected within the light of this view of pathogenesis of T1D and polarization of islet-infiltrating T cells [16]. Our findings may reflect the polarization aberrancy related to metabolic disturbances in T1D Lohmann et al. found that reduced expression of CCR5 and IFN-c was normalized after diagnosis [20]. Furthermore, the extent of the IL-12 wasn’t related to the actual HbA1c levels of the patients In accordance with our results, Kukreja et al. also reported decreased IFN-c secretion in both newly diagnosed patients and in patients with longstanding T1D [21]. Accordingly, the probability that a decreased type 1 response of peripheral blood T cells is related to the regulatory defect resulting in clinical manifestation of autoimmune diseases should be considered in future studies.

Conclusion
In this study type 1 diabetes not related to elevation of IL-12 levels. However, serum IL-12 levels show study on large scale to offer more information. Future investigations including other cytokines will help clarify the role of interleukins in the etiopathogenesis of Th1 dependent diseases like diabetes.

Reference


