Cytokines in Egyptian Children with Type 1 Diabetes

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Abstract

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by progressive destruction of pancreatic beta cells by genetic and environmental factors which leads to an absolute dependence of insulin for survival and maintenance of health.

Objective: To investigate the role of serum IL-1\(\beta\), IL-2, IL-6, and TNF-\(\alpha\) in children with type 1 diabetes mellitus (T1DM) and diabetic ketoacidosis (DKA).

Design: Case-control study.

Patients and Methods: The study population consisted of 45 children with (T1DM) and 15 healthy controls. Children with T1DM were divided into three subgroups: (1) previously diagnosed patients (long standing T1DM) (n:15), (2) newly diagnosed patients with diabetic ketoacidosis (before treatment) (n:15), and (3) newly diagnosed patients with diabetic ketoacidosis (after treatment by 2 weeks) (n:15). Serum IL-1\(\beta\), IL-2, IL-6, and TNF-\(\alpha\) levels were measured in all subjects by enzyme linked immunosorbent assay.

Results: In comparison to control group, significant higher levels of IL-1\(\beta\), IL-2, IL-6 and TNF-\(\alpha\) were detected in all diabetic groups especially in newly diagnosed diabetics with diabetic ketoacidosis before treatment (group III).

Conclusion: Our data about elevated serum IL-1\(\beta\), IL-2, IL-6, and TNF-\(\alpha\) levels in newly diagnosed T1DM patients with DKA in comparison with longer standing cases supports an activation of systemic inflammatory process during early phases of T1DM which may be indicative of an ongoing \(\beta\)-cell destruction. Trials of anti cytokines might be tried in the management of newly diagnosed T1DM patients aiming to decrease \(\beta\)-cell destruction.

Key Words: Cytokines – Type 1 diabetes – Children.

Introduction

TYPE 1 diabetes mellitus (T1DM) is a multifactorial disease where genetic predisposition combines with environmental trigger(s) to induce the activation of a specific autoimmune destruction of pancreatic beta-cells [1]. The pathophysiological mechanism initiating this autoimmune response remains to be undetermined. Monocyte and type 1 T-cell-derived cytokines contribute to the pathogenesis of T1DM [2]. Chemokines play a central role in inflammatory processes by regulating leukocyte migration into sites of tissue damage [3]. Cytokines have been proposed as inducers of \(\beta\)-cell damage in human T1DM via the generation of nitric oxide (NO) [4]. The autoantigen insulin is responsible for stimulation in vitro of potentially hazardous memory lymphocytes to produce IL-6 and IL-10 [5]. A T helper 1 (TH1) subset of the T cells and their cytokine products (type 1 cytokines: IL-2, interferon (INF)-\(\gamma\), and tumor necrosis factor (TNF)-\(\beta\)) dominate over an immunoregulatory TH2 subset of T cells and their cytokine products (type 2 cytokines: IL-4, IL-5, and IL-13). There is an imbalance between TH1 and TH2 subsets. This allows type 1 cytokines to initiate a cascade of immune-inflammatory processes in the islet, which includes activating macrophages to produce proinflammatory cytokines. The pro inflammatory cytokines, IL-1\(\beta\), IL-6 and TNF-\(\alpha\) were found to have cytotoxic, cytostatic (inhibits insulin synthesis and secretion), or cytocidal actions to pancreatic islets [3,5,6].

The aim of this study is to investigate the role of serum concentrations of IL-1\(\beta\), IL-2, IL-6, and TNF-\(\alpha\) in children with controlled T1DM and in cases of DKA before and after treatment.

Patients and Methods

Setting: Abu el Reesh Hospital, Cairo University’s Specialized Pediatric Hospital in Cairo, Egypt. This tertiary care teaching facility is the largest pediatric hospital in Egypt with 500 beds, from September 2006 to March 2007.

Population and sampling: The study population consisted of 45 children with T1 DM and 15 healthy
controls (group I). Children with T1DM were divided into three subgroups: (a) group II: previously diagnosed patients (long standing T1DM) (n: 15), (b) group III: newly diagnosed patients with diabetic ketoacidosis (before treatment) (n: 15) and (c) group IV: newly diagnosed patients with diabetic ketoacidosis (after treatment) (n: 10) by 2 weeks.

Serum samples were collected from 30 children with T1DM (13 females, 17 males) at the pediatric diabetes clinics. Subjects with any diabetic complications such as nephropathy, neuropathy or retinopathy, acute or chronic diseases, or oral medication were excluded from this study.

All patients were treated with daily regular doses of insulin (1 IU/kg/d). Fifteen children without diabetes (8 females, 7 males) were recruited for the control group. The age range of the subjects was 2-11 years. Baseline features of the groups are presented in Table (1). The background information of subjects such as age, gender, body weight, daily dose of insulin injection and disease duration were recorded. Disease duration was defined in this study as the day of initial diagnosis of diabetes to the day of blood collection. Blood samples (five milliliters) were collected using standard venipuncture technique. Serum samples were separated immediately after centrifugation at +4°C, 4000 rpm for 10 minutes and stored at -20°C until analysis.

Percent concentration of HbA1c in whole blood was measured with Stanbio diagnostics HbA1c kits with autoanalyzer (Cobas Integra 800 Autoanalyzer, Roche Diagnostics, Germany). This assay based on the immunoturbidimetric determination of the stable glucose adduct to N-terminal group of the haemoglobin beta chain. Serum concentrations of cytokines such as IL-1β, IL-2, IL-6, and TNF-α were measured using commercially available enzyme linked immunosorbent assay kits (ELISA kits, Biosource Int, Calif).

Ethical approval from the Institutional Review Board of Abu el Reesh Hospital was obtained before conducting this study. Verbal consent was obtained from all study population.

Statistical methods:

Data is presented as mean ± SD. Comparison of variables was performed with the general linear model, student t test, or Mann-Whitney U test when necessary. Case-control differences in nominal data were evaluated with the χ² test. Statistical analysis was performed using SPSS for Windows (SPSS Advanced Statistics 7.5, SPSS, Chicago, Ill, 1997). The comparisons of serum cytokine between children with diabetes and healthy were determined by multivariate analysis of variance (MANOVA). A simple linear correlation analysis was processed by Pearson’s method to assess the correlation between age, HbA1c, and cytokine in healthy and diabetic subjects, respectively. Statistical significance was assumed at p<0.05.

Results

The characteristics of control subjects and patients with T1DM are shown in Table (1).

HbA1C levels were significantly higher in diabetic groups compared to control groups in this study (p<0.01). When diabetic groups were compared with each other, HbA1C% increased significantly (by 36.1%) in group III (p<0.01) and increased significantly (by 25.8%) in group IV (p<0.01) as compared to group II. On the other hand, no significant difference could be seen between group III and group IV (p>0.05).

A significant increase of IL-1β, IL-2, IL-6 and TNF-α was detected in all diabetic groups (groups II, III and IV) as compared to control group (p<0.01) as shown in Table (1) and Fig. (1).

When diabetic groups were compared with each other, TNF-α was found to be increased (by 310.9%) in group III (p<0.01) and also increased by 168.5% in group IV (p<0.01) as compared to group II. Also, a significant reduction of TNF-α was detected from 143.18 ±42.74 in group III to 93.55±22.63 pg/ml (by 34.6%) in group IV (p<0.01).

Regarding IL-1β, it increased significantly in group III (by 152.4%) in comparison to group II (p<0.01), whereas no significant difference was found between group IV and group II (p>0.05). Moreover, IL-1β was significantly decreased group III (by 42.2%) in comparison to group IV (p<0.01).

IL-2 was found to be increased significantly to (by 134.4%) in group III (p<0.01) and also increased significantly (by 49.9%) in group IV (p<0.01) as compared to group II. Also, a significant reduction of IL-2 was found in group III (by 36%) in comparison to group IV (p<0.01).

As for IL-6, it was found to be increased significantly (by 324.2%) in group III (p<0.01) and also increased significantly (by 201%) in group IV (p<0.01) as compared to group II. Also, a significant reduction was detected in group III (by 29%) in comparison to group IV (p<0.01).
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In the present study, there was a significant increase of HbA1C% in all diabetic groups as compared to the control group. In accordance, Gerstl et al. [13] reported that despite the much advancement in diabetes care over the past 25 yr, most children and adults with T1D fail to achieve target HbA1c. When diabetic groups were compared with each other, HbA1C% was significantly higher in recently diagnosed T1DM with DKA before treatment (group III) and after treatment (group IV) than long standing T1DM (group II). These results are in agreement with Goldstein et al. [14] who supported that the rate of synthesis of HbA1c is a function of the amount of glucose to which RBCs are exposed over their average 120-day life span and demonstrated that this relationship serves as a clinically useful index of mean glyce-

![Fig. (1): Comparison of plasma levels of random glucose (mg%), HbA1C %, TNF-α (Pg/ml), IL-1β (Pg/ml), IL-2 (Pg/ml), and IL-6 (Pg/ml) among the studied groups.](image)

Table (1): Characteristics of enrolled subjects (n=55).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I control (C)</th>
<th>Group II T1DM (LS-T1DM)</th>
<th>Group III newly diagnosed T1DM (ND-T1DM -before treatment)</th>
<th>Group IV newly diagnosed T1DM (ND-T1DM -after treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Age (y) mean±SD</td>
<td>6.4±3.8</td>
<td>7.2±3.3</td>
<td>5.2±3.14</td>
<td>6.5±2.61</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>1:2:1</td>
<td>1:3:1</td>
<td>1:2:1</td>
<td>1:2:1</td>
</tr>
<tr>
<td>Blood glucose* (mg/dl) mean±SD</td>
<td>106.32±13.56a*</td>
<td>17.39±34.32b</td>
<td>575.93±74.60c</td>
<td>168.00±32.59b</td>
</tr>
<tr>
<td>HbA1C%* mean±SD</td>
<td>5.38±0.77a</td>
<td>7.44±0.91b</td>
<td>10.26±1.03c</td>
<td>9.49±1.09c</td>
</tr>
<tr>
<td>TNF-α (Pg/ml)* mean±SD</td>
<td>11.35±4.18a</td>
<td>34.84±12.76b</td>
<td>143.18±42.74c</td>
<td>93.55±22.63d</td>
</tr>
<tr>
<td>IL-1β (Pg/ml)* mean±SD</td>
<td>40.46±16.62a</td>
<td>120.91±23.88b</td>
<td>305.16±123.00c</td>
<td>176.30±43.11b</td>
</tr>
<tr>
<td>IL-2 (Pg/ml)* mean±SD</td>
<td>20.54±6.35a</td>
<td>99.10±18.44b</td>
<td>232.28±65.06c</td>
<td>148.60±28.05d</td>
</tr>
<tr>
<td>IL-6 (Pg/ml)* mean±SD</td>
<td>16.72±7.56a</td>
<td>34.18±10.09b</td>
<td>145.01±49.43c</td>
<td>102.89±15.35d</td>
</tr>
</tbody>
</table>

*Values with identical lettering (a, b, c, d) showed no significant differences.

Discussion

Type 1 diabetes is an autoimmune disease that results from apoptotic death of the pancreatic beta cells by auto reactive T lymphocytes [7]. Macrophages, also involved in the autoimmune process as effector cells, release cytotoxic mediators such as IL-1 [8,9]. The proinflammatory cytokines IL-6 and TNF-α are common to both TH subsets in humans [3,10]. TNF-α and IL-6-mediated damage to micro- and macrovascular tissues, altered insulin secretion through direct or through stimulation of free fatty acid production, and altered glucose homeostasis are suggested [11,12].

In the present study, there was a significant increase of TNF-α in all diabetic groups as compared to control group. However, when diabetic groups were compared with each other, plasma TNF-α level was significantly higher in recently diagnosed T1DM with DKA before treatment and after treatment than long standing T1DM. These results are in agreement with those obtained by Abdel Shakour et al. [15] who concluded that, serum TNF-α level is significantly increased in type 1 diabetes and its level is positively correlated to blood glucose levels, HbA1c concentrations, duration of diabetes, and microalbuminuria. This could be through the resultant inflammatory process that could occur during the pathogenesis of type 1 diabetes itself or the pathogenesis of its complications. These changes could occur through the affection of serum nitric oxide levels. The present results are also similar to that obtained by Dogan et al. [16] who demonstrated elevated serum IL-
TNF-α in newly diagnosed T1DM patients in comparison with longer standing cases and supported an activation of systemic inflammatory process during early phases of T1DM which may be indicative of ongoing β-cell destruction. Persistence of significant difference between the cases with T1DM monitored for a long time and controls in terms of IL-1β, IL-2, IL-6, and TNF-α supports continuous activation during the late stages of diabetes. In contrast, Poulsen [21] found that TNF-α concentration in the previously diagnosed type 1 diabetes group was significantly lower than those of control group. However, they were in accordance in that they observed highest TNF-α level in recently diagnosed T1DM with DKA group. In addition to the contribution of TNF-α in β-cell death, TNF-α is also associated with insulin resistance which may explain the profound metabolic abnormalities observed in group III and group IV [17]. Zorena et al. [18] suggested that the early introduction of the TNF-α antagonists to the treatment of young patients with T1DM who show high serum activity of the TNF-α may prevent them from development of diabetic retinopathy.

In the present study, there was a significant increase of plasma IL-1β in all diabetic groups as compared to control group. However, when diabetic groups were compared with each other, plasma IL-1β was significantly higher in recently diagnosed T1DM with DKA before treatment, but after treatment of DKA, IL-1β level returns to the values of the long standing T1DM, as there was no significant difference between both levels. Our results are in agreement with results obtained by Dogan et al. [16] who demonstrated higher levels of IL-1β in all stages of diabetes. Also, Perez et al. [19] found high levels of IL-1beta and IL-2 in diabetic children with recent diagnosis of the disease. Melloul [20] suggested that inhibition of cytokine induced β-cells destruction could be a potential effective strategy for β-cell protection. Donath and Mandrup-Poul sen [21] studied the potential role of anakinra—a recombinant human IL-1β receptor antagonist—in the treatment of type 2 DM. The authors supported the principle that IL-1 antagonism has therapeutic potential in the treatment of DM but they suggested that autoimmune component of type 1DM could require combination therapy (e.g. with anti-CD3 antibodies).

In the present study, there was a significant increase of plasma IL-2 in all diabetic groups as compared to control group. However, when diabetic groups were compared with each other, plasma IL-2 was significantly higher in recently diagnosed T1DM with DKA before and after treatment, when compared to the long standing T1DM. Also, there was a significant reduction of plasma IL-2 in newly diagnosed T1DM with DKA after treatment when compared to DKA before treatment. These results are in agreement with a study performed by Perez et al. [19] and Hussain et al. [22] who found high levels of IL-1β, IL-2 IFN-γ and TNF-α in diabetic children with recent diagnosis of T1DM. In contrast with these findings, studies performed by Tomoda et al. [23] and Dogan et al. [16] found that IL-2 production by CD4-positive T lymphocytes was significantly decreased in diabetic children compared with the control children, especially in patients monitored for a long time with a diagnosis of T1DM. The authors supported that decreased IL-2 synthesis is specific for T1DM, not explainable solely as a consequence of poor metabolic control, but might be explained by an abnormal consumption or by the presence of increased sIL-2R levels or by a serum factor which interferes with IL-2 production.

In the present study, there was a significant increase of plasma IL-6 in DKA groups (before & after treatment), as compared to control group. However, there was no significant difference of IL-6 level between long standing T1DM and control group. Whereas, when diabetic groups were compared with each other, plasma IL-6 was significantly higher in recently diagnosed T1DM with DKA before and after treatment, when compared to the long standing T1DM. These results are in agreement with results obtained by Erbagci et al. [3] who found that elevated systemic IL-6 and TNF-α were limited to newly diagnosed cases with T1DM compared to cases with longer standing T1DM. Whereas, they found no significant difference in serum TNF-α and IL-6 levels between long standing type 1 diabetic and non diabetic groups, suggesting activation of the inflammatory immune response system at early stages of the disease.

However, in contrast with results of the current work were Geerlings et al. [24] and Dogan et al. [16] who found that IL-6 levels were found to be decreased statistically significantly in any group of children with T1DM especially in newly diagnosed cases when compared with healthy controls.

In the current study the plasma levels of all measured cytokines were significantly higher in newly diagnosed T1 DM patients with DKA when compared to both healthy controls and long standing T1DM patients. These results are in agreement with results obtained by Hoffman et al. [25] and Stentz et al. [26] who found elevations of plasma IL-10, IL-1beta, TNF-alpha, IL-6, IL-8 and IL-2.
levels in children with severe DKA (pH<7.2) prior to treatment. The authors strengthen the hypothesis that the metabolic crisis of DKA has differential effects on cellular activation and cytokine release.

In our study, the plasma levels of all measured cytokines (TNF-α, IL-1β, IL-2, and IL-6) were significantly reduced in newly diagnosed T1DM patients with DKA after treatment and return of blood glucose to the controlled T1DM values, compared to their levels before treatment of DKA. However, the cytokines levels didn’t return to the controlled T1DM levels.

These results are in agreement with results obtained by Das [27] and Das [28] who reported that Insulin/glucose-insulin-potassium (GIK) regimen suppresses the production of TNF-α, IL-6, IL-1, macrophage migration inhibitory factor and other pro-inflammatory cytokines, enhances the synthesis of endothelial nitric oxide (e NO), and anti-inflammatory cytokines IL-4 and IL-10. They supported that insulin (with or without glucose and potassium) therapy to maintain euglycemia suppresses the systemic inflammatory response, improves myocardial function.

Conclusion:

Our data about elevated serum IL-1β, IL-2, IL-6, and TNF-α levels in newly diagnosed T1DM patients with DKA in comparison with longer standing cases supports an activation of systemic inflammatory process during early phases of T1 DM which may be indicative of an ongoing β-cell destruction. Trials of anti cytokines might be tried in the management of newly diagnosed T1DM patients aiming to decrease β-cell destruction.

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