Original Article

Cytokines Profile in Newly Diagnosed Children with Type 1 Diabetes Mellitus

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Summary :

Background: Cytokines have long been implicated in the pathogenesis of autoimmune diabetes in a number of studies, and playing a role in the initiation of β-cell damaging process. The objective of this study is to gain more understanding about the role of cytokines in initiation of T1DM, through assessment of IFN-γ, IL-10 and IL-6 in diabetic patients.

Patients and methods: A total of 60 patients who were newly diagnosed as having T1DM (diagnosed less than five months) were included in the present study. Fifty apparently healthy control subjects were underwent the measurement of serum IFN-γ, IL-10 and IL-6 by ELISA.

Results: Higher serum levels of IFN-γ, IL-10, and Il-6 were observed in the investigated patients ≤10 years (75.60, 104.92, 147.6 pg/ml respectively) compared to controls (42.66, 57.01, 80.4 pg/ml respectively). The statistical analysis revealed a significant difference between patients and controls (P_1= 0.005, 0.003, 0.036 respectively). The mean levels of serum IFN-γ, IL-10, and Il-6 were also significantly elevated in >10 years old patients (70.78, 84.22, 171.8 pg/ml respectively) than controls (40.39, 59.50, 81.6 pg/ml respectively), (P_1= 0.006, 0.037, 0.04 respectively). A statistically difference of mean IL-10 concentration appears between patients in both age groups (P_2= 0.04). No significant differences appear in the mean serum concentrations of IFN-γ, and IL-6 between the two age groups (P_2=0.73, 0.07 respectively).

Conclusions: In children with diabetes, a significant elevation of serum levels of IFN-γ, IL-10 and IL-6 were observed.

Key Words: T1DM, IFN-γ, IL-10 and IL-6

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Introduction:

Type 1 diabetes mellitus (T1DM) is a chronic disease where insulin-producing beta cells in pancreatic langerhans islet are gradually destroyed. A disease afflicting 0.2% of the population. The process, which finally leads to complete β-cells loss and onset of clinical disease starts years before any clinical symptoms (1). It is considered to be resulted from a multifactorial process involving host genes, autoimmune responses and cytokines as well as environmental factors (2). Cytokines are important for coordinating the immune responses and a disturbance in the balance between autoreactivity and tolerance that can result in autoimmunity (3). Numerous studies have observed correlations between expression of proinflammatory cytokines in pancreatic islets of NOD mice and human (IL-1α, IL-1β, TNF-α, TNF-β, IFN-α, IL-2, IL-12, and IFN-γ), and the presence of destructive insulitis. Some of these proinflammatory cytokines (IL-1α, IL-1β, TNF-α, TNF-β, and IFN- γ) have well documented cytotoxic or cytostatic effects on pancreatic β-cells *in vitro*, but studies of transgenic mice expressing some of these cytokines in β-cells have known that they are not directly toxic to β-cells *in vivo*. This suggests that these cytokines participate in diabetogenesis by promoting the recruitment and activation of β-cells-toxic T cells, macrophages, and dendritic cells, rather than by killing β-cells or impairing their functions (2, 4, 5). Hussain and his colleagues, reported elevated levels of cytokines namely: IL-2, IFN-γ; TNF-α and IL-1α in recently diagnosed patients with T1DM, but no difference in the levels of IL-4 and IL-10 was recorded (6). Based on many studies, it was concluded that Th2 cytokines IL-4 and IL-10 protect from T1DM in NOD mice either by reverse T-cell unresponsiveness or via decreased Th1 cytokines IFN-γ to Th2 cytokine IL-4 ratio within T-cell infiltrated pancreatic islet (7). Other reports pointed against the anti-inflammatory action of Th2 cytokines. It was demonstrated that local production of IL-10 but not IL-4 accelerated autoimmune destruction of β-cells. In addition NOD mice were protected from development of diabetes by a neutralizing anti-IL-10 monoclonal antibodies (mAbs) but not anti-IL-4 mAbs, which were described to be ineffective in altering the course of Th2 autoimmune destruction of pancreatic beta islet cells (8). Another study found that IL-10 was essential for an early phase of diabetes in NOD mice via CD_8^+ T-cell pathway with out the participation of B-cells (9).

A study conducted by Targher *et al*., found that serum levels of IL-6 were elevated markedly in young T1DM patients without clinical evidence of microvascular and macrovascular complication versus healthy

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controls (10). In Iraq the diabetes prevalence is increasing compared with the rest of the world. Hence in order to gain more understanding about the role of cellular immune responses with initiation of T1DM, through the possible role of IFN-γ, IL-10 and IL-6 in diabetic patients.

Patients, Materials and Methods:

Sixty Iraqi T1DM children (28 males and 32 females) were subjected to this study. The patients were attending the National Diabetes Center at Al-Mustansiriya University during the period May 2004 to October 2005. Their ages range from 3 -17 years, and they were newly diagnosed of the disease (diagnosis was from one week up to five months). Diagnosis of Diabetes Mellitus and selection of patients was accomplished with the assistance of the consultant medical staff in the National Diabetes Center. All the patients were treated with daily replacement doses of insulin at the time of blood sampling. The patients were divided into two groups according to their ages in order to assess the aggressivness of immune responses: 36 children equal or less than 10 years and 24 children up to 10 years. For the purpose of comparisons, 50 healthy control subjects matched for age (4-17 years old) and sex (25 males and 25 females) were selected who have no history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group. Three milliliters of venous blood were drawn from each subject (patients and controls). The collected blood was displaced into plain test tubes, then the serum was separated by centrifugation at 2500 rpm for 10 min., divided into aliquots and kept at-20°C until used. Serum IFN-γ, IL-10 and IL-6 were measured by ELISA using human IFN-γ kit (Immunotech Beckman Coulter), human IL-10 kit (Mabtec), and human IL-6 kit (Mabtec).

Student t-test was used to measure the differences between the two means. The results were expressed as means ± standard error (SE).

Results:

Serum Level of hIFN-γ: Similar means of serum levels of IFN-γ were observed in the investigated patients ≤10 years and >10 years old who showed higher means (75.60 and 70.78 pg/ml respectively) than controls (42.66 and 40.39 pg/ml respectively). Out of 29 healthy controls in the age group >10 years old, only one of them had serum IFN-γ less than standard level (0.095 pg/ml). The statistical analysis revealed a significant difference between patients and controls (P_1= 0.005 and 0.006 respectively), while between patients no statistical difference appears (P_2= 0.73) table (1), figure (1).

Serum Level of hIL-10: Table (2) figure (1), demonstrated the mean serum levels of IL-10 in the studied groups. The mean value of serum IL-10 for patients group ≤10 years old was significantly higher than controls (104.92 *vs.* 57.01 pg/ml respectively, P_1= 0.003). Patients >10 years old showed also significant elevation in IL-10 serum levels (84.22 pg/ml) compared with controls (59.50 pg/ml) (P_1=0.037). A statistically difference of mean IL-10 concentration appears between patients in both age groups (P_2= 0.04).

Serum Level of hIL-6: The estimated levels of IL-6 in the sera of the patients were higher than control group (147.6 vs. 80.4 pg/ml respectively, P_1= 0.036) in ≤10 years old group table(3), figure (1). Out of 36 patients, 4 patients had serum levels of IL-6 out of standard level; three were less (0.097, 0.098 and 0.098 pg/ml), while the fourth one was high (2224.29 pg/ml) than the standard. In the same age group, out of 21 controls, 2 individuals had serum IL-6 levels less than standard (0.082 pg/ml).The mean levels of serum IL-6 were also significantly elevated in the patients >10 years old compared to controls (171.8 *vs.* 81.6 pg/ml respectively, P_1= 0.04). Out of 24 patients, 2 patients had serum IL-6 concentration less than standard (0.082 and 0.092 pg/ml) and another 2 patients had high levels (1410.86 and 1654.20 pg/ml) than standard. Concerning the healthy controls, 12 individuals had serum IL-6 level less than the standard levels. No significant differences appear in the serum IL-6 concentration between the two age groups of patients(P_2=0.70).

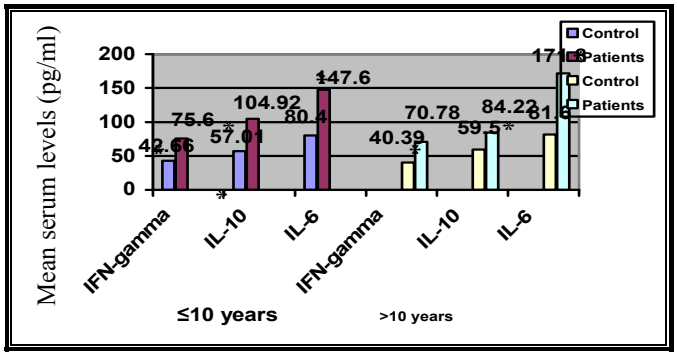

Figure - 1: Bar chart of mean serum levels of hIFN-γ, IL-10 and IL-6 in the healthy controls and T1DM patients

Table 1: Mean concentration of serum hIFN-γ in healthy subjects and T1DM patients groups.

Parameters	≤10 years							>10 years							P_2
	Groups	No.	Mean	SE	Min.	Max.	P_1	Groups	No.	Mean	SE	Min.	Max.	P_1	
hIFN-γ (pg/ml)	Controls	21	42.66	3.95	12.67	81.33	0.005 (S)	Controls	28	40.39	1.19	10.83	90.37	0.006 (S)	0.73 (NS)
	T1DM	36	75.60	10.3	30.1	345.5		T1DM	24	70.78	9.78	30.94	203.93		

P_1: T1DM patients *vs.* controls
P_2: T1DM patients ≤10 years *vs.* patients >10 years old.

Table 2: Mean concentration of serum hIL-10 in control and T1DM patients group.

Parameters	≤10 years							>10 years							P_2
	Groups	No.	Mean	SE	Min.	Max.	P_1	Groups	No.	Mean	SE	Min.	Max.	P_1	
hIL-10 (pg/ml)	Controls	21	57.01	9.92	20.97	97.62	0.003 (S)	Controls	29	59.50	12.6	20.43	81.37	0.037 (S)	0.04 (S)
	T1DM	36	104.92	8.81	57.63	360.0		T1DM	24	84.22	4.67	61.86	141.36		

P_1: T1DM patients *vs.* controls
P_2: T1DM patients ≤10 years *vs.* patients >10 years old.

Table 3: Mean concentration of serum hIL-6 in control and T1DM patients groups.

Parameters	≤10 years							>10 years							P_2
	Groups	No.	Mean	SE	Min.	Max.	P_1	Groups	No.	Mean	SE	Min.	Max.	P_1	
hIL-6 (pg/ml)	Controls	19	80.4	56.4	0.1	524.10	0.036 (S)	Controls	17	81.6	69.0	0.1	499.61	0.04 (S)	0.70 (NS)
	T1DM	32	147.6	45.0	0.1	1018.70		T1DM	20	171.8	80.2	0.1	981.30		

P_1: T1DM patients *vs.* controls
P_2: T1DM patients ≤10 years *vs.* patients >10 years old.

Discussion:

IFN-γ

The present data demonstrated that serum IFN-γ concentration were higher in patients with T1DM compared to its concentration in the healthy controls. These data were in common with other studies which stated that proinflammatory cytokines IFN-γ may play an important role in the pathogenesis of T1DM, and its concentration was higher in T1DM patients (11). Many studies largely support the concept that β-cell destructive insulitis is associated with increased expression of proinflammatory cytokines (IL-1, TNF-α and IFN-α) (6) and Th_1 cytokines (IFN-γ, TNF-β, IL-2) and IL-12 (12). Mechanically, proinflammatory and Th_1 cytokines including IFN-γ induced and accelerated β-cell destruction through direct and indirect mechanisms. Directly, Th_1 cytokines including IFN-γ exerted their effects primarily at the level of macrophages, enhancing infiltration of these cells in the islet, thus accelerating β-cells destruction through the release of performed de novo synthesized cytotoxic mediators (nitric oxide, oxygen radicals … etc.) (5), or induced T-cells infiltrate the islets (MHC class I restricted CD_8^+ T-cells) because IFN-γ and TNFup regulate expression of MHC class I, which in conjugation with autoreactive T-cells could bring about extensive tissue damage on rodents and human β-cells (12, 13). IFN-γ may render β-cells susceptible to T-cell mediated killing via induction of Fas (CD_{95}) receptor on their surface. Ligation of Fas receptors on β-cells by Fas ligand ($CD_{95}L$) on CD_4^+ and/or CD_8^+ T-cells has been postulated to be a mechanism of β-cell death by apoptosis in T1DM patients (4). Indirectly by several mechanisms as a result of their capacity to inhibit the production of Th_2 cytokines and Th_2 cell activity through induced activation and expansion of bystander autoreactive T-cell resulting in an increase in their overall proportion , and inhibited the production of soluble cytokines antagonists including the IL-1 receptor antagonist, which resulted in stimulation of IL-1 production by the macrophages, and in conjugation with continued autoantigenic stimulation, significant augmentation in the expression of IFN-γ and other Th_1 cytokines (14).

IL-10

The results of this study indicated a high level of serum IL-10 in T1DM patients compared to healthy controls. This result was encountered to many reports, which found that T1DM could be prevented by induction of Th_2 cells or by treatment with Th_2 cytokines which in turn blocked the production of Th_1 cytokines (15). In contrast, other reports pointed against the anti-inflammatory action of Th_2 cytokines. Th_2 cytokines (IL-10 but not IL-4) were shown to be involved in T1DM pathogenesis through facilitation of pancreatic mononuclear cells infiltration and producing intense and generalized pancreatitits and insulitis associated with islet cell necrosis in NOD mice (8). Another report conducted on NOD mice, found that serum levels of IFN-γ were initially low but increasingly reaching the highest levels at diabetes onset. In contrast an early peak of serum IL-10 level was observed initially, but continued loss of IL-10 until progression toward diabetes was observed, confirming the fact that IL-10 was essential for an early phase of diabetes (16). This promoted the conclusion that T1DM is a Th_1 and Th_2 mediated autoimmune disease. Functionally, Th_2 cytokines exert

their effects through direct or indirect mechanisms. In particular IL-10, may promote necrosis through occlusion of the microvasculature, thereby resulting in hypoxia and reducing the viability of the larger islets (17). IL-10 is a potent B-cell activator, enhances MHC class II expression on B-cells, thus promoting peri-insulitis and insulitis (2) or by altering the expression of endothelium-bound addressin, thereby stimulating accumulation of macrophages and B-cells (18), and because of its role as cytotoxic T-cell stimulatory factor, IL-10 may stimulate activated T-cells and its essential for an early phase of diabetes (9). In any event, Th_2 cytokines can no longer be viewed as "protective" of T1DM.

IL-6

The inflammatory cytokine IL-6 originally secreted from T-cells, B-cells and several non-lymphoid cells including macrophages, fibroblast endothelial cells and bone-marrow stromal cells (1). The present results indicated an elevated level of serum IL-6 in T1DM patients as compared to controls that added to the evidence that the disease is an immunoinflammatory disorder. This result is in common with other reports (10). IL-6 is a powerful inducer of hepatic acute phase protein (C-reactive protein), elevated C-reactive protein level detected in infants and young children before the onset of T1DM (19) may provide an additional marker for risk of progression to T1DM. IL-6 is a "co-stimulatory signal" for T-cell activation made by certain antigen presenting cells (APCs), and previously known as B-cell differentiation factor. It acts on most cells, but is particularly important in inducing B-cells to differentiate to antibody-forming cells (1). Mechanically, IL-6 may exert its effect by inducing a condition with increased energy expenditure in the islet, through elevated glucose oxidation and oxygen uptake accompanied by a partial inhibition of the glucose stimulated insulin release and lowering the islet cellular ATP contents (20).

Conclusions:

Cytokines are important in outcome of autoimmune diabetes, which were indicated by a significant elevation of serum levels of IFN-γ, IL-10 and IL-6 in the T1DM patients.

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