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The Evaluation of Serum IFN-γ and TGF-β in Some Patients with Type1 Diabetes mellitus in Al-diwanyah Province

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Abstract: The diabetes mellitus type one (T1DM) is considered as autoimmune disease due to the destruction of Langerhans beta cell, which is the insulin-producing cell as a result of the invasion by cells of the immune system with vital roles. There is evidence that interferone-γ IFN-γ is responsible for the T1DM disease developing, and so the transforming growth factor type beta (TGF-β) was known as an important regulator of the immune response. Throughout of this study gain more understanding about the two cytokines role in this disease and the correlation between them to provide immunotherapeutic strategies for T1DM. A total of 35 with T1DM (19 female, 16 male), in addition to (15) healthy control subjects undertook the measurement of serum IFN-γ and TGF-β by ELISA technique. The result explains higher mean serum levels of IFN-γ (684.867 ± 246.23 pg/ml) were observed in the investigated patients compared to healthy control (11.75 ± 4.56 pg/ml). Whereas observed decline in the serum TGF-β (302.2± 65.43 pg/ml) as compared to healthy control (1153 ± 186.35 pg/ml) at the level (p<0.01), and so the result explained a significant negative correlation between TGF_β and INF_γ concentration (p<0.05). The conclusion is that T1DM patients were detected with a significantly increase in INF_γ and decrease TGF_β serum level, and they have the antagonistic effect on the T1DM development.

Key words: IFN-γ, TGF-β, T1DM

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Introduction

The type one of diabetes mellitus (T1DM) is considered as an autoimmune dysfunction (1). Where the destruction of insulin-producing β_cells begins with cytotoxic T lymphocyte (CTL) infiltration and activation of macrophages, followed by cytokine release and the production of autoantibodies (1). The occurrence of the disease has increased yearly approximately ~3%, the percentage of children below 5 years is 10% of total disease occurrence (2). Well-thought-out results from a multifactorial process involving host genes, autoimmune responses, as well as environmental factors (such as viruses), contribute to the pathogenesis of T1DM (3). The islet environment contains an enormous group of inflammatory molecules, tumor necrosis factor-α [TNF-α] (4), and [IFN-γ] (5) are the predominant pro-inflammatory molecules. A number of studies had demonstrated vital associations between proinflammatory molecules and the impairment in endothelial tissue. The proinflammatory cytokine INF-γ is produced mainly by T helper cell (Th) and natural killer cell (NK), and this cytokine participates in the maturation and activation of T cytotoxic cell (Tc) lymphocytes, B cells, in addition to improves the cytotoxicity of Tc cells, NK cells, and

also considered as a strongest activator of macrophages (6). The INF-γ had cytotoxic activity (7), have been noticed on pancreas islet of T1DM patients (7).The TGF-β is an important cytokine with the different function to preserves the tolerance of peripheral through preventing the self-reactive T helper and T cytotoxic cells from proliferation and differentiation (8, 9).The TGF-β is considered as a significant inhibitor for multiplying and maturation of B lymphocyte and so preventing the manufacture of most antibody isotypes (10). It also serves as a co-stimulatory factor in the development of T cells with down-regulatory activities (11), and so it acts as a protector from auto-reactive T lymphocyte by preserving the normal existence of T regulatory cell [T reg] (12,13). The differentiation of both Treg and TH17 cells was regulated by TGF-β in a concentration dependent manner (14), in addition, the IL-10 and TGF-β were known as anti-inflammatory cytokines and the active secretion of them lead to immune suppressive functions due to the increase of iTregs (12,15). The IL-2, INF-γ and TNF-β are Th1 cytokines responsible for the progress of the T1DM disease, whereas IL-4, IL-10 and TGF-b are Th2 and Th3 cytokines act to repressed the type1 DM developments. However, the cytokines involvement in the pathogenesis of T1DM is complex. Since cytokines, such as INF-γ has participated in the progress of autoimmunity to the β-cell, it is essential to think through that a disproportion between pro-inflammatory (INF-γ) and anti-inflammatory (IL-4, IL-10, TGF-b) cytokines actions may approval to the initiation of autoimmunity and the continuing inflammation, which causing the complexity (16, 17). In this research, we systemically analysed the status of cytokines in patients with T1DM, in order to provide a new insight for prevention the disease or the improvement the strategies of T1DM immunotherapeutic.

Subject and Methods

The blood sample was collected from the 35 diabetes mellitus type1 patients (T1DM) (19 female, 16 male), their ages range between 9-17 years, in addition to 15 healthy as a control group. The period of study extended from November-2015 to March-2016.The patients were diagnosed as DMT1 by the medical staff at the Center of Diabetes and Endocrinology in Al-Diwanyah city. Blood samples (3 ml) were drawn from patients and healthy controls. The collected blood was moved into plain test tubes, then used centrifugation at 2500rpm for 10 min to get the serum, separated into aliquots and then stored at -20°C till used. Serum IFN-gamma and TGF-beta were detected by ELISA technique using human IFN-gamma and human TGF-beta kit (R&D system) by enclosed the plate with Capture Antibody over night, after washing using blocking buffer , then add 100µL of sample or standards after 2 hour washing the well,100µL of diluted detected Ab was additional to each well for 2 hour, then 100µL of diluted Streptavidin-HRP was added to each well for 20 minutes (avoided direct light), washing, next substrate solution was added (100 µL), then 50 µL of stop solution was supplementary to each wells and the optical density for plate was measured at 450 nm(18).

Statistical Analysis

The Least significant difference – LSD test was used to significant compare between the means of two cytokines (INF-γ and TGF-β) in this study (19).

Result and Discussion

Of the two cytokines measured in this study (INF-γ and TGF-β), the INF-γ showed an increase the concentration of serum level in investigated patients (684.867 pg/ml) as compared with healthy controls (11.75 pg/ml).The statistical analysis showed the patients had high significantly ($p<0.01$) increased IFN-γ concentrations compared to the healthy controls. Figure 1 and Table 1.

Table (1): Mean concentration of serum IFN-γ level in T1DM patients and healthy subject

Group	No.	Mean ± SE(pg\ml)
		IFN-γ
Healthy	15	11.75 ± 4.56
T1 DM	35	684.867 ± 246.23
P-Value	--	0.003**
LSD Value	--	111.36
** ($p<0.01$)		

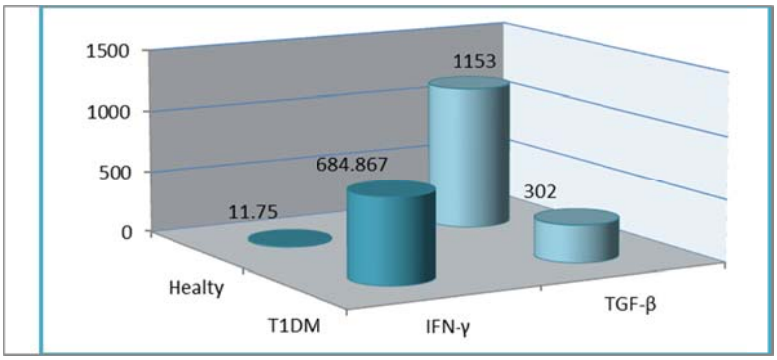

Figure (1): The means of IFN-γ and TGF-β concentration in the serum of healthy and T1DM patients.

Diabetes mellitus Type 1 is a chronic inflammatory disease. In general, is known as a Th1-type autoimmune illness resulted from attacking of a pancreatic beta cell by autoreactive T helper cells. The serum level of IFN-γ, which tested in this study is considered as a specific Th1 cytokine and noticed a high IFN-γ serum level in T1DM patients as compared to healthy control. A similar result was also published in a previous local study (20), as well as in and so in universal studies (21, 22). Several studies supported the idea that

damaging insulitis β-cell is related with high concentration of INF-γ, IL-2 and IL-12 in animal models, and pancreatic β-cell damage accompanies the inflammatory response (insulitis) within the islets (23),IFN-γ might show an essential role in the progressed of T1DM disease (22), by direct and indirect mechanisms. Directly, IFN-γ exerted their action by increased macrophages level in the islets, by improving infiltration of these cells, thus facilitate β-cells damaging by the discharge of performed cytotoxic mediators (oxygen radicals, nitric oxide) (24), or enhanced T-cells penetrate the islets (MHC classI restricted for CD8 + T-cells) because IFN-γ and TNF up-regulate the MHC classI expression, which linked with autoreactive T-cells could generate wide tissue damage on human and rodents β-cells (25, 26), so could consider the IFN-γ stimulus the processing and exhibition in target organs the self-antigens via dendritic cells (DCs) and macrophages (27). IFN-γ may introduce β-cells susceptible to killing by T-cell via stimulation of CD95 (Fas) receptor on their surface. The Fas receptors on β-cell Ligate to Fas ligand (CD95L) on the two type of T cell (CD+4 and CD+8) has been suggested to lose β-cell by apoptosis in T1DM patients (28). Systemic Fas-deficient The nonobese diabetic mice (NOD) mice did not progress diabetes or insulitis (29), through the progress of diabetes, the expression of Fas was increased, and so proposing the Fas displays a significant role in b-cell destruction(30). Indirectly by a number of mechanisms lead to inhibit the assembly of Th2 cytokines and Th2 cell action in addition to IFNγ act as an inhibitor for Th17 cells, by inducing the display of the peptide glutamate decarboxylase2(GAD2) (31), In addition to INF-alpha\gamma and IL-1β pathways, their stimulation related to the change in the responsiveness of the Toll-like receptor and improved the signaling of nuclear factor (NF)-ƙB for the monocytes and dendritic cells (DCs) of the patients recently detected with T1DM(32,33). These events lead to β-cells disorders and apoptosis which in turn lead to the beginning of disease clinically. While the second question in this study was about a TGF-β serum level in T1DM patients, the result estimated (302.2 pg/ml) for patients which a significantly decrease than a control group (1153 pg/ml) at a level (p<0.01) as described in Figure 1 and Table 2.

Table (2): Mean concentration of serum TGF-β level in T1DM patients and healthy subject

Group	No.	Mean ± SE(pg\ml)
		TGF-β
Healthy	15	1153 ± 186.353457
T1DM	35	302.2± 65.436653
P-Value	--	0.001**
LSD Value	--	295
** (p<0.01)		

The result clarify decrease the TGF-β serum level for T1DM patients in compared to healthy group, these results seem to be consistent with another researcher which found decrease serum level of TGF-b in patients with type 1 diabetes (34), the previously reported referred to the reduced in the level of gene expression and protein secretion of TGF-b in T1D (35). A TGF-b1 decrease is related specifically to the development of diabetes, These noted a relationship between the incidence, severity of islet inflammation and the decrease in serum concentration of TGF-β, due to the increase the activity of proinflammatory cytokines and so the insulitis damaging linked with the pre-diabetic stage, anywhere the decrease of the TGF-β level indicate a biomarker of insulitis (34), and it is considered an important marker for decoding the disease development through the silent stage of β-cell damage in human at threat with T1DM (34). The phosphorylation of Smad3 is stimulated by TGF-b and the gathering of Smad 3 and 4 in the nucleus was stimulated the TGF-b-responsive genes through Jak1 and Stat1 genes, while the INF-γ activation the proliferation of Smad7, which inhibit the collaboration of Smad3 with the TGF-b receptor(36), and so the STAT1 binds Smad3 and inhibits its function (36), and this had got clearly described for the negative significant correlation coefficient between IFN-γ and TGF-b, the IFN-γ is antagonistic action of TGF-β, where the TGF-b is an important cytokine with anti-inflammatory properties (37). Therefore this mechanism explains the result of correlation coefficients between the cytokines in this study, which introducing a significant (p<0.05) negative correlation (r = -0.55) between IFN-γ, and TGF-β in T1DM patients. The antagonistic effect between IFN-γ and TGF-β is also known due to the interface between Teff (Th1, which produced INF- γ) and T regulatory (Treg), which produced TGF-β via the IL-2/IL-2R interaction, which permitting the direct feedback between the Teff and Treg (38,39), also there are similarities in TCR for both Teffs and Tregs, which lead them to compete for same (auto) antigen, and then they response to antigen by stimulated and proliferate (38,39). So trigger this pathway to Teff production act to decrease the Treg account and so the secretion of TGF-β will be decrease.

In summary, the INF-γ and TGF-β have the antagonistic effect on the T1DM development so considerable interest in TGF-β as an anti-inflammatory and so therapeutic target to limited the expression and function of pro-inflammatory molecules IFN-γ.

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