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Serum IL-6 and TNF-α in Type 1 Diabetes Mellitus Patients in Mosul

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Abstract

The objective of the study is to evaluate the levels of IL-6 and TNF-α in sera of patients with type 1 diabetes mellitus (T1DM) compared to healthy people and correlate their levels with different factors. The results showed significant increase in the levels of IL-6 and TNF-α (P≤0.05) in T1DM compared to control group. The levels of IL-6 were not significant when compared with age groups, duration of the disease and level of HbA1c. On contrast the levels of TNF-α in T1DM patients was significantly increased (P≤0.05) when compared with age groups and duration of the disease but not significantly with different HbA1c.

Keywords: Type 1 Diabetes Mellitus; IL-6; TNF—α.

1. Introduction

Type 1 diabetes mellitus (T1DM) is considered as chronic inflammatory disease of pancreatic islets. It occurs as a result of destruction of beta cells of Langerhans which is responsible for insulin production. This destruction is due to autoantibodies, cytotoxic T cells and inflammatory mediators [1,2]. T1DM represents about 5-10% of diabetic cases and influences children and teenagers [3]. Cytokines are extracellular protein of low molecular weight serve as mediator of the immune response. They induce beta cells damage in human T1DM via generation of NO [4].

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IL-6 and TNF-α mediated damage to macrovascular tissues, altered insulin secretion and glucose homeostasis [5,6]. IL-6 and TNF-α are adipocyte secreting factors and their injection induce an increase in concentration of triglycerides and low density lipoprotein [7]. Cytokines may play important roles in the pathogenesis of T1DM and they are also regarded as markers of inflammatory response [8,9].The present study involves detection of IL-6 and TNF-α In T1DM patients in Mosul and correlates their concentrations with different factors.

2. Materials and Methods

2.1. Subject group

This study was carried out on 90 patients with T1DM (35 males and 55 females) and healthy control group (25 individual) with age range between 1-25 years. Data was obtained from subject group and control which include age, sex, duration of the disease, type of therapy, complications, family history of diabetes and residence.

2.2. Blood and serum collection

Five ml of venous blood was withdrawn from each patient and control subjects . One ml of blood was put in EDTA tube and the remaining blood samples were left to clot in a plain tube at 37˚C for 30 minutes, centrifuged at 3000 rpm for 5 minutes, and serum was separated and dispensed in 0.5 ml Eppendorf tubes and stored at -20˚C for subsequent specific laboratory investigations.

2.3. Serological and Chemical Tests

Elisa test was used to detect the concentration of IL-6 and TNF-α in serum. They were supplied by Wuhan Cundu Biological Company (China). The test is based on interaction between monoclonal antibodies against IL-6 and TNF-α which are coated in the microtiter plates with antigens in the serum. The interaction is detected by addition of HRP resulting in changing the color that can be read by Elisa reader at 450 nm (Rt-2100c microplate reader. The procedure of Elisa was done according to manufacturer instructions and the results were expressed in pg/ml .HbA1C depends on the measurement of hemoglobin in RBCs which carry sugar. In this test whole blood was used and the test was carried out according to the manufacturer instructions using On. Call. A1C Analyzer and kits (Acon. USA). And the results were expressed in percentages.

2.4. Statistical Analysis

The results were analyzed using SPSS. Results are expressed as mean ± standard deviation. Student t-test was used to compare between two groups, $P \leq 0.05$ was considered statistically significant. Linear regression analysis was used to study the correlation between parameters.

3. Results

The results of IL-6 levels in sera of T1DM patients showed clear increase (8.248 pg/ml) which is statistically significant ($P \leq 0.05$) when compared with control group which was 1.392 pg/ml (Table 1).

Table 1: Mean, standard deviation and correlation of IL-6 between type 1 diabetic mellitus.

IL-6	Mean	St.D	SE of Mean	Correlation	P	T - Value	P
T1DM	8.248	2.636	0.294	0.074	0.774	16.192**	0.000
Control	1.392	1.253	0.303				

**. Significant at the 0.01 level (2-tailed).

*. Significant at the 0.05 level (2-tailed).

IL-6 in T1DM when compared with different age groups showed no significant differences . The highest concentration of IL-6 was in age group >16 years (9.04 pg/ml) and the lowest (6.61 pg/ml) was in age group <5 years (Figure 1).

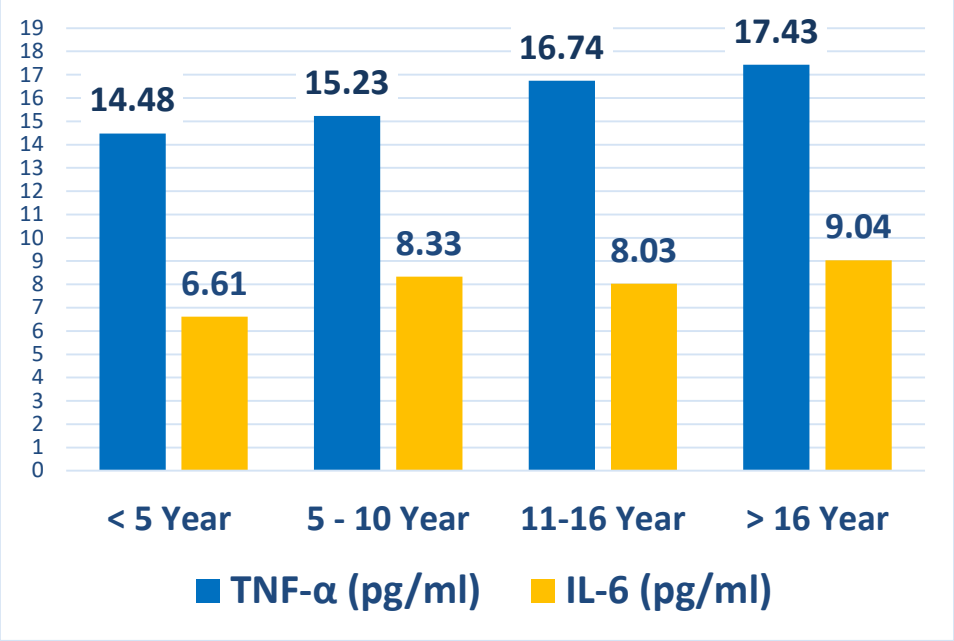

Figure 1: IL-6 and TNF-α levels in relation to age groups of type 1 diabetes mellitus

The correlation between IL-6 and duration of the disease, HbA1C, showed weak positive but not significant (Figures 2,3).

High level of IL-6 was observed when the duration of the disease was >7 years (9.03 pg/ml) and the lowest level at <5 years (6.61 pg/ml). Also high level of IL-6 was noticed at 14-17% HbA1C.

The result of TNF-α in T1DM patients is statistically significant (P≤0.05) compared with control group (Table 2).

Table 2: Table 1. Mean, standard deviation and correlation of TNF-α between type 1 diabetic mellitus.

TNF- α	Mean	St.D	SE of Mean	Correlation (r)	P r Correlation	T - Value	P T - Value
T1DM	16.371	2.847	0.828	0.016	0.952	16.955**	0.000
Control	2.472	2.951	0.762				

**. Significant at the 0.01 level (2-tailed).

*. Significant at the 0.05 level (2-tailed).

The mean of TNF-α levels in T1DM was 16.371 pg/ml which is higher than control group (2.472 pg/ml). Also positive correlation statistically significant (P≤0.05) was noticed with age groups and duration of the diseases (Figures 1,2), while no correlation exists with HbA1C (Figure 3).

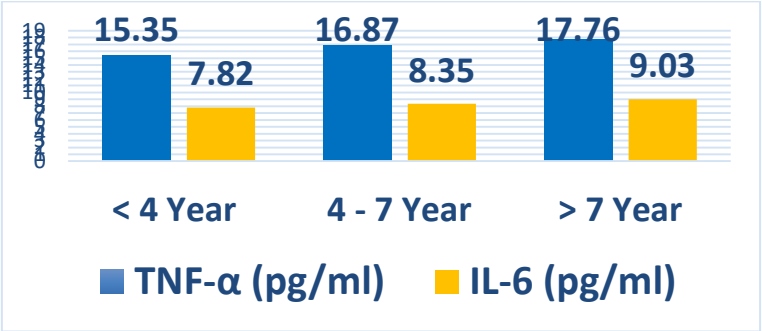

Figure 2: IL-6 and TNF-α levels in relation to duration of type 1 diabetes mellitus

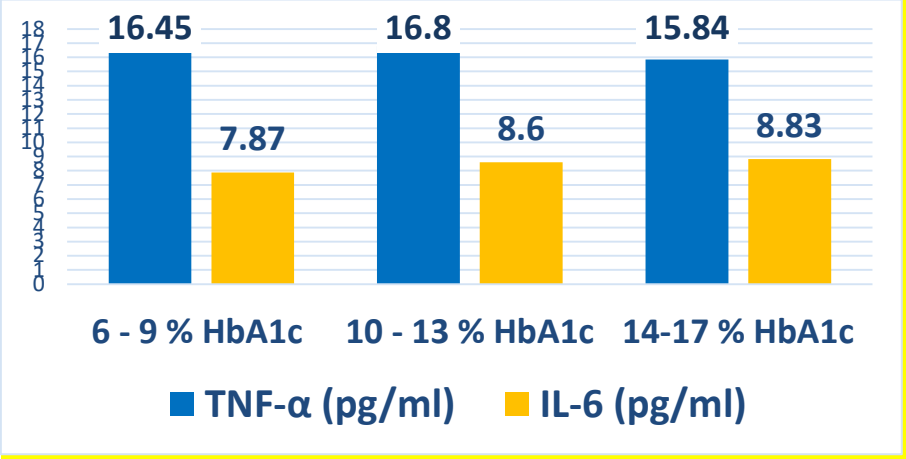

Figure 3: 1. IL-6 and TNF-α levels in relation to HbA1C in type 1 diabetes mellitus

The highest level of TNF-α was in age group >16 years (17.43 pg/ml) and the lowest was at age group <5 years 14.48. Also the highest level of TNF-α was observed at duration of the disease >7 years (17.76 pg/ml) and the lowest were at < 4 years (15.35 pg/ml).

4. Discussion

Cytokines play central role in the inflammatory process through regulation of leukocytes migration to damaged tissues [7]. Cytokines stimulate destruction of beta cells in diabetic patients through generation of NO [4].

IL-6 and TNF-α were selected as markers of inflammatory process as they alter insulin secretion through direct or through stimulation of free fatty acid production and altered glucose homeostasis [9]. IL-6 is important factor in the immune system and has important role in glucose metabolism [10,11]. Many studies showed the effect of IL-6 on secretion of insulin [12-15]. Cytokines have also a role in the development of cardiovascular disease [16].The current study showed significant increase of IL-6 mean in T1DM patients compared to control group. Its level increased with duration of the disease and age groups but not significant similar finding observed by others [17]. However, in some studies the level of IL-6 was high at beginning of the disease compared to those of long duration [18]. Other studies showed no difference in IL-6 [7,19] or even decreased [10]. There is a positive correlation between IL-6 and HbA1C but not significant, similar finding was observed [21]. This could be explained that hyperglycemia stimulates the monocytes to secrete high level of IL-6 [22] TNF-α stimulates macrophages and its level increases with duration of diabetes as well as physiological control of vascular adhesion molecules [23]. In this study, TNF-α was statistically significant in T1DM patients compared to control group as well as positive correlation with age groups and durations of the disease. Similar findings showed by others [24]. High level of TNF-α in all age groups and durations indicates persistent activation of immune inflammatory response [7]. However some studies showed increased level of TNF-α in early stage of the disease [7,25]. On contrast, other studies showed no change in the level of TNF-α during early and late stages of the disease [26]. No correlation was observed between TNF-α and HbA1C.

5. Conclusion

IL-6 and TNF-α were increased significantly in T1DM patients compared to control group and their correlations with HbA1C, age groups and duration of the disease were variable

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