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The some cytokines Levels (TGFβ1, IL-4 , IL-6, and IL-17) in sera Patients with Diabetes Mellitus Type1,Type 2 in Nassiriya city

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Astract:

The present study was carried out in the Labs of collage of education for pure science and Center for Diabetes and Endocrinology of the Health Directorate in Nassiriya city, during period from January 2017 to end July of the same year. The immune status investigates for patients with diabetes type I and type II by measuring the levels of cytokines (TGFβ1, IL4, IL6,IL17) in sera using a technique enzyme-linked immune Sorbent adsorptive (ELISA). The study included 88 subjects with (34) type I, (34) II diabetes and (20) were healthy control.

The statistical analysis showed that a high significant increase ($P \leq 0.05$) in serum IL6 and IL17 as the rate of concentration of IL-6 in patients (33.07 pg / ml) compared to the control group (4.68 pg/ml) with significant difference (0.00), while IL-17 concentration (113.66 pg / ml) for patients compared with the healthy control (43.67 pg / ml) with a significant difference (0.01) in type I patients. While a highly significant ($p<0.05$) of (TGFβ1 , IL-6, and IL-17) in patients with type 2 diabetes compared with the concentration in IL – 4 and a healthy control group. The highest concentration recorded was (132.26 , 24.69 and102.02 pg/ml) respectively, compared to the other groups . These results revealed that the excessive presence of cytokines might play a role in diabetic in Nassryain population.

Introduction:

Diabetes is currently one of the most prevalent diseases in the world, including the developed and developing countries, affecting the rich and the poor, young and old, men and women. Scientific studies have shown that approximately 5-8% of individuals have diabetes and many patients do not show symptoms of the disease and do not know that they have diabetes. Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterized by elevated blood glucose levels (hyperglycemia) that result from defects in the body's ability to produce and/or insufficiency of insulin action (Kharroubi and Darwish, 2015, Chala and Ali 2016).

Type 1 Diabetes Mellitus, A chronic systemic metabolic disorders characterized by destroyed pancreatic beta-cells and this typically leads to absolute insulin deficiency and abnormalities in the metabolism of fat and protein (Eisenbarth 2007).these metabolic abnormalities of patients are prone to ketoacidosis, microvascular complications, neuropathies, coma and death (Rohrer *et al*., 2015). Type 1 Diabetes Mellitus occurs at any age but is commonly diagnosed in children, adolescents and young adults (Reddy *et al*.,2013). Type 2 diabetes is a complex metabolic disorder , most common aetiological type (90-95% of cases) and is due to a progressive loss of insulin secretion on the background of insulin resistance (disorder of insulin action); it ranges from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance.(Smushkin and Vella . 2010 , Jemdsa , 2017) Millions of people around the world

have been diagnosed with Type II diabetes, and many more remain undiagnosed (Hagman 2016) .

The generation and maintenance immunological responses of the body is controlled by a network of small, soluble, intercellular regulatory proteins low molecular weigh less than 30 kDa (<200 amino acids) , cell signaling proteins mediating complex interaction from one cell to another and so mediate multiplicity of immunologic as well as non immunologic biological function , possess autocrine, paracrine, and juxtacrine effects with characteristic features, and they are called cytokines.(Stevens, 1995 ; Deverman and Patterson 2009, Ashif *et al* ., 2016). cytokines, are important players in the pathogenesis of autoimmune diseases through multiple ways, such as regulating inflammation and angiogenesis. (Braga 2016).In recent decades, several studies have shown the role of certain pro and/or anti-inflammatory cytokines in provide valuable information about the pathways involved in the regulation of T1D processes (Alnek *et al* ., 2015). and activation of the immune system in the pathogenesis of T2D (Gulati *et al* ., 2016) However, the mechanisms by which chronic inflammation is involved in T2D are not completely clear. In this study,we investigated the immune status of DM patients by studying the following immunological parameters by (TGFβ1,IL-4, IL-6, IL-17).

Materials & Methods:

This study was performed on 88 Iraqi patients with diabetes mellitus T1DM,T2DM patients, who attended the consultant clinic for diabetes mellitus in endocrine and diabetic center in Al-Nassiriya city in the period from the beginning of (January 2017 to end in July in the same year).

Blood samples were collected by venipuncture from 34 T1DM , 34 T2DM patients and 20 controls .Two milliliters were placed in a sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -20 C° freezing . These sera (68 patients and 20 controls) were used for estimating The concentration of interleukin (IL-TGFβ1,IL- 4 , IL-6 , and IL- 17).

ELISA (technique enzyme-linked immune Sorbent adsorptive) kit are employing the quantitative sandwich ,were based on similar principle according to the Elabscience company (China,E–EL-H0101).

Statistical analysis: Data were expressed as mean ± standard deviation (SD) or median (interquintile range). Differences between groups were tested with the Student's t-test.The values of $P < 0.05$ were considered significant.

Result:

Serum Interleukins (TGFβ1,IL-4,IL-6,IL17) concentration of patients of type 1:

The results of this study showed the presence of a significant increase ($P \leq 0.05$) in the concentrations of (IL-6,IL17) Table (1) as the concentration of IL-6 in patients (33.07 pg / ml) compared to the control group (4.68 pg/ml) with significant difference (0.00), while IL-17 concentration (113.66 pg / ml) for patients compared with the healthy control (43.67 pg / ml) with a significant difference (0.01) .While no significant difference in the concentrations of TGF β1 concentration (66.97 ± 2.9) and IL – 4 concentration (35.49 ± 16.47) for patients compared with the healthy control.

Table (1) Comparison of serum (TGFβ1, IL-4,IL-6, and IL-17) concentrations (pg/ml) of the D1M patient groups with healthy control group

Parameter	Subject	Sample	Mean ± SD	T-value	Df	P-value
TGF B1	Patients	34	66.97 ± 2.9	0.34	42	0.73
	Control	10	67.40 ± 4.7			
IL – 4	Patients	34	35.49 ± 16.47	0.74	42	0.467
	Control	10	31.37 ± 11.13			
IL – 6	Patients	34	33.07 ± 21.4	7.43	42	0.00
	Control	10	4.68 ± 3.2			
IL – 17	Patients	34	113.66 ± 88.9	2.46	42	0.01
	Control	10	43.67 ± 8.31			

(P<0.05). df : degree freedom

Serum Interleukins (TGFβ1,IL-4,IL-6,IL17) concentration of patients of type 2:

The statistical analysis showed ahigh significant ($p<0.05$) of (TGFβ1 , IL-6, and IL-17) in sera of patients with type 2 diabetes, compared with the average concentration in the sera IL – 4 and healthy control group The highest concentration recorded was (102.02, 24.69 and 132.26 pg/ml) respectively, compared to the other groups were recorded (40.43 ±6.04 and).

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Table (2) Comparison of serum (TGFβ1 , IL-4,IL-6, and IL-17) concentrations (pg/ ml) of the D2M patient groups with healthy controls group

Parameter	Subject	Sample	Mean ± SD	T-value	Df	P-value
TGF B1	Patients	34	102.02 ± 5.86	7.64	42	0.000
	Control	10	74.4 ± 18.56			
IL – 4	Patients	34	40.43 ±6.04	0.703	42	0.486
	Control	10	32.45 ± 9.95			
IL – 6	Patients	34	24.69 ± 13.7	6.92	42	0.00
	Control	10	6.27 ± 3.91			
IL – 17	Patients	34	132.26 ± 18.56	5.91	42	0.00
	Control	10	90.7 ± 22.6			

(P<0.05). df : degree freedom

Discussion:

Cytokines play important role in the development and activation of immune cells, since they act as cell-signaling molecules, especially in autoimmune diseases, including T_1D(Gomes, 2017) .

Transforming Growth Factor (TGF- β1) is a multifunctional cytokine that plays a role in several biological processes and it is the most abundantly expressed isoform, associated with susceptibility to various diseases(Cebinelli *et al.*, 2016).

The level of serum TGF-*β* was significantly decrease in diabetics type 1 in comparison with control table (1) . Different cell types such as dendritic cells and naturally occurring regulatory T cells (nTregs) can produce TGF-*β* under different immunological conditions. TGF-*β* is an essential cytokine for nTreg development and function (Yang and Zheng,2017) nTregs have a central role in prevention of T1D development and a major mechanism applied to delay T1D onset by nTregs is TGF-*β* secretion (Daneshmandi *et al.*,2017). Functional defect related to signaling pathways leading toTGF-*β* production in these cells or other TGF-*β* sources in T1D may be the reason for the reduced levels of the cytokine (Roohi *et al.*, 2014) .

These result don't agree with (Jakus *et al.*,2012; Zorena *et al.*, 2013) that found circulating increase in serum TGF-β of D_1M comparison with control.

Table (2) explain that the level of serum IL4 was non significantly higher in diabetics type 1 patients in comparison with control .The reports of IL-4 abnormal production in patients with DM are rare . However, These results agree with the results (Khazai *et al.*, 2007) which observed no significant difference in concentration of IL-4 in diabetics compared with healthy controls.

Interleukin (IL)-6 is a pleiotropic cytokine with a key impact on both immune regulation and non-immune events in most cell types and tissues outside the immune system (Jain *et al.*,2003). A vast number of epidemiological, genetic, rodent, and human in vivo and in vitro studies have investigated the putative role of action/lack of IL-6 in the pathogeneses underlying obesity, insulin resistance,β-cell destruction, type 1 diabetes, and type 2 diabetes (Kristiansen and Mandrup-Poulsen, 2005; Galassetti *et al* .,2006; Reis *et al*. 2012).

Some studies for IL-6 levels were found to be higher in newly diagnosed cases when compared with those monitored for a long time (Erbagci *et al*.,2001)

IL-6 increased more in children with T_1DM than in controls without diabetes after exercise and was greater in hyperglycemic children with T_1DM than in euglycemic children with T1DM (Galassetti *et al* .,2006). Reis *et al*. (2012) analyzed plasma samples of 42 T1DM patients and 24 healthy patients as a control group, finding higher circulating levels of IL-6 in DM1 patients than in the control group. Our results agree with all above studies and (Targher *et al.*, 2000;Choudhary and Ahlawat ,2008; Shelbaya *et al*.,2012; He *et al*., 2014) who found that serum IL-6 levels was higher in patients of diabetes type I than control group.

IL2,IL12,IFN-γ and TNF- α belong to type Th1 cytokines ,while IL4,IL5,IL6 and IL10 belong to type Th2 cytokines .Tow kinds of cytokines mutual antagonize each other to maintain a balance (Eyerich and Novak,2013). (Fisman *et al.*, 2008) reported that IL1,IL2 and IL6 actes as promotive inflammatory cytokines , while IL4 and IL10playes as promotive/anti - inflammatory cytokines in diabetes .In this study ,we found that serum levels of IL6 in peripheral blood from children with T1DM was significantly higher than those in control group.

The hypothesize that type Th1 inflammatory cytokines were excessively secreted in children with T1DM which led to islet damage and progression of disease . while type Th2 anti- inflammatory cytokines were elevated under regulation of internal environment and homeostasis (He *et al*., 2014) . On other hand, raising IL-17 levels in T1DM patiants compared with healthy group are in line with those previous of studies Bradshaw *et al.*, (2010); Honkanen, (2010) and Tuama

et al., 2014) all they found that the level of IL-17 in T1DM patients groups were a significant elevated in comparisons with healthy group .The explanation of this increased may possibly related to hyperglycemic state of diabetic subject which could induced the secretion of these cytokine by monocytes (Bradshaw *et al*., 2010). Serum Interleukins (IL-4,IL-6,IL17,TGFβ1) concentration patients of type 2

In type 2 patients our result explain that the levels of serum TGF- β1, IL6 and IL17 was higher significantly while no significantly increase in IL4 comparison with control .

The elevated levels of TGF-β_1 gene expression have been reported in patients with type 2 diabetes (Abbasi *et al.*,2012), these high of TGF-β_1 levels is the relative state of hyperglycemia that increases TGF-β_1 production from different cells. Type 2 diabetic patients had a higher blood glucose level than the normal controls, which may explain their higher serum TGF-b levels (Azar *et al.*, 2000) .

These result in line with Roohi *et al.*, 2014 ; Roopakala *et al.*, 2014 and Qiao *et al.* 2017 all they found that significant increase of serum TGF-β1 levels in Type 2 patients compared to healthy controls. Al-Dahhan *et al.*, 2015; Goyal *et al.*, 2015 ;Nazari *et al.*, 2017; Rodrigues *et al.*, 2017

IL-6 is the major cytokine produced by adipose tissue and their circulating level is increased in diabetics(Rajarajeswari *et al*.,2011).Elevated levels of IL-6, which is the main stimulator of the production of most acute-phase proteins ,increase the risk of diabetes(Al-Dahhan *et al.*, 2015). Goyal *et al.* (2015) found that insulin-induced down regulation of IL-6 in non-obese and obese diabetic patients. These increased in levels of IL6 may be result from the increased in IL-17 because that IL-17 is implicated in amplifying the immune response by triggering the production of proinflammatory cytokines such as IL-6, TNFα, and IL-1β, facilitating a link between T cell activation and inflammation (Aggarwal and Gurney, 2002). In addition, Th17 cells are known to secrete IL-6 and TNFα potentially (Tzartos *et al.*, 2008 ; Amadi-Obi *et al.*, 2007).

The exact role of IL-17 in the pathogenesis T_2DM has not been explored (Chen *et al.*, 2016), but some study showed that the serum concentration of IL-17 was significantly higher in the patients with T2DM than in the controls as Zareian *et al*.,(2014) and Chen *et al*.,(2016). These increased in level of IL-17 may be result from an increase number of Th17cell in D2M patient according to (Garidou *et al*., 2015) Which found that Th17 cells increased in T2DM patients and might be associated with dysregulated lipid metabolism. Th17cell, an important proinflammatory CD4+ T cell subtype secreting IL-17, has also been associated with T2DM(Zuniga *et al* ., 2010 ; Zhang *at el* ., 2014**)**.

In conclusion, we demonstrated that sera levels of (TGFβ1,IL6,IL17) are increased in patients with diagnosed type 1 , type 2 DM. Our proposed results (TGFβ1,IL6,IL17) might play the role in state of patients, And involved in causing Diabetes Mellitus.

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