Plant Archives Volume 20 No. 2, 2020 pp. 6616-6620 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DYNAMICS OF PRO-INFLAMMATORY CYTOKINES AND PROGRAMMED CELL DEATH IN TYPE 2 DIABETES PATIENTS IN DIYALA GOVERNORATE, IRAQ

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Abstract

Type II diabetes mellitus (T2DM) is characterized by chronic insulin resistance and a progressive decline in β-cell function, so diabetes is immunes dependent disease in which the changed patterns of expression of cytokine, anti-inflammatory factors as interleukin-22, interleukin-23 play an essential role in many infections. Therefore, Results of current study showed a high incidence of diabetes types II in the age group (50-59) years old and (60-69) years old as (NO.= 22.0, 22.0) and percentage as (36.66, 36.66)% respectively. A significant increase in level of IL-22 and FAS, but not IL-23, in diabetes patients compared to control (P<05). T2DM patients shows a significant reduction in serum level of FasL, if compared with control group. Also showed positive correlation between immunological Parameters.

Keywords: Type 2 Diabetes mellitus, Interleukine-22, Interleukine-23, Fas, FasL. 40%

Introduction

Diabetes mellitus (DM) is a major global health problem affecting approximately 451 million people. By 2045, this number is expected to increase to 693 million (Cho *et al*., 2018). Approximately 90-95% of these cases are type 2 diabetes mellitus (T2DM) (ADA, 2016). Overweight, obesity, a family history with T2DM and a sedentary life style are major causes of the current global Type 2 diabetes epidemic (Rawshani *et al*., 2020). Subclinical inflammation and the presence of almost all indicators of systemic inflammation have been observed in patients with T2DM. Inflammation has recently been suggested to be a crucial factor contributing to the development of this disease. This systemic inflammatory process is characterized by elevated circulating levels of pro inflammatory parameters, (Herder *et al*., 2013). Levels of Interluikine-22, Interluikine-23 are associated with the risk of developing type 2 diabetes, as (Gong *et al*., 2016) showed that IL-22 act as a double-edged sword in type 2 diabetes. Although, IL 22 is beneficial to the host in many infectious and inflammatory disorders, depending on the target tissue it can be pathogenic due to its inherent pro inflammatory properties, which are further enhanced when IL 22 is released together with other pro inflammatory cytokines, in particular IL 17 (Rutz *et al*., 2013). IL-23 plays a role in type 2 diabetes related inflammation (Vieira *et al*., 2011), IL-23 drives the development of autoreactive IL-17-producing T cells and promotes chronic inflammation dominated by IL-17, IL-6, IL-8 and tumor necrosis factor as well as neutrophils and monocytes (McKenzie *et al*., 2006). Apoptosis plays important roles in the pathophysiology of Type 2 diabetes mellitus (T2DM) Pancreatic β-cell apoptosis is a pathological feature that is common to T2DM., insulin resistance with visceral obesity leads to a glucose toxicity effect, which accelerates β-cell death by apoptosis and the reduction in β-cell mass (Mandrup-Poulsen, 2001). There are many studies that showed that the Fas/Fas ligand (FasL) system has an important role in the development of T2DM. (Cosson *et al*., 2005) pointed that Fas mediated apoptosis is involved in type 2 diabetes. (Blüher *et al*., 2014) showed that increased Fas expression may contribute to impaired insulin sensitivity and adipose tissue dysfunction in obesity. It was shown that increased Fas expression contributes to impaired insulin sensitivity and adipose tissue dysfunction in obesity, Thus Upon stimulation by mFasL, cells expressing Fas

Table 1: Comparison of the age distributions between the two groups

Group	Minimum	Maximum	Mean± SEM	P value
Patients	40	79	56.01±1.08	0.00**
Controls	33	62	45.39±1.76	
**Significant difference (P value < 0.01)				

receptor causes apoptosis by recruiting different adaptor proteins which cleavage procaspase-8 to caspase-8 the most upstream caspase in the Fas apoptotic pathway (Margaryan *et al*., 2018), therefore the aim of this study was designed to evaluate the serum level of Interleukin-22, Interleukin-23, also assess the role of Fas/ Fas ligand (FasL) system in the progression of type 2 diabetes mellitus (T2DM) in Iraqi diabetic patients and controls, Detection levels of immunological Parameters by using ELISA technique.

Materials and Methods

Subjects

This study included 88 participants, 28 healthy persons as a control group and 60 patients with T2DM (30 males and 30 females) with ages ranging from 40 to 79 years. The patients were selected from advisory clinic in Baquba Teaching Hospital in Baquba city during the period from the beginning of October 2019 to the beginning of January 2020.

Blood sampling

Five milliliters of venous blood was drawn by disposable syringe. placed in a sterile plane tube and allowed to clot; then, Serum was separated from the cells by centrifugation and then divided into small portions and kept frozen at -20°C until analysis. These sera were used for estimating IL-22,IL-23, sFas and sFasL.

Methods

IL-22, IL-23,sFas and sFasL were measured by sandwich enzyme linked immunosorbent assay kit which provided from Shanghai Company, china.

Principle of the assay

The microtiter plate provided in this kit has been pre-coated with specific antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then added Horseradish Peroxidase (HRP)-conjugated -anti-human immunoglobulin to each well and incubate. Finally, substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Calculate the concentrations of samples.

Statistical analysis

Data were processed and analyzed using the Statistical Package of the Social Science (SPSS 20). Descriptive formula variables were described in number and percentage formula and compared using Chi-square test. All results were expressed as mean ± standard error deviation. Quantitative variables were expressed as (mean ± SED) and compared using student t-test. The linear relationship between variables was assessed by Pearson's correlation coefficient (r). $P < 0.05$ was considered statistically significant.

Results and Discussion

Type 2 diabetes mellitus is the most common form of diabetes. It is characterized chronically elevated blood glucose concentrations, disorders of insulin action and insulin secretion (Mohamed *et al*., 2019). It is clear from table 1. The demographic data did reveal differences in age between the group (60 patients) with T2DM (56.01±1.08) and non-diabetic healthy controls (45.39±1.76). Moreover, the chi-square ($X2$) test did not show a significant difference in gender between the two groups (p=1.00), show a significant difference in age groups (p<0.01) (Table 2). The results of this study showed that the incidence of the disease was within a wide range of age groups, and the ages of the patients ranged between 40-79 years, the highest percentage of patients was recorded in the age groups (50-59) and (60-69) years. The prevalence of the metabolic syndrome rose with age reaching peak levels in the sixth decade and fifth decade is paralleled by similar increases in the prevalence of overweight and obesity (Park *et al*., 2003), key related factors in the development of visceral adiposity, insulin

Table 2: Distribution of the study group according to the percentages of sex and age groups.

Parameters		Groups		Total	P value
		Patients No. (%)	Controls No. (%)		
Gender	Male	30 (50%)	14 (50%)	44 (50%)	1.00
	Female	30 (50%)	14 (50%)	44 (50%)	
Total		60 (100%)	28 (100%)	88 (100%)	
		Patients		%	
Age groups (years)	40-49	12		(20.0%)	0.001**
	50-59	22		(36.66%)	
	60-69	22		(36.66%)	
	70-79	4		(6.66%)	
Total		60		(100%)	
**Significant difference (P value < 0.01)					

Table 3: Comparing immunological parameters with the two study groups.

Parameters	Group	Mean ± SEM	P value
Serum IL-22	Patients	175.85 ± 23.72	0.019*
	Control	108.31 ± 15.49	
Serum IL-23	Patients	66.28 ± 13.26	0.612NS
	Control	73.04 ± 19.63	
Serum Fas	Patients	1.77 ± 0.58	0.04*
	Control	1.09 ± 0.20	
Serum Fas L	Patients	8.07 ± 1.01	0.05*
	Control	10.41 ± 1.81	
*Significant difference (P ≤ 0.05) NS: No significant difference (P > 0.05)			

resistance, dyslipidemias, high blood pressure, and impaired glucose metabolism. In addition, aging per se is associated with evolution of insulin resistance, other hormonal alterations, and increases in visceral adipose tissue, (Akram, 2013) all of which are important in the pathogenesis of the Type 2 diabetes mellitus. Documented results were consistent with the findings of other authors (Jasim, 2015).

Table 3, shows the serum levels of some immunological parameters in two studied groups. The IL-22 and FAS levels show a high significant increase (P ≤0.05) for patients (175.85 ± 23.72) ng/l and (1.71 ± 0.58) ng/l, respectively compared to control (108.31 ± 15.49) ng/l and (1.09 ± 0.20) ng/l. levels of IL-23 showed nonsignificant decrease (P>0.05) for patients (66.28 ± 13.26) ng/l as compared to the controls, (73.04 ± 19.63) ng/l. Results of serum FasL in table 3 showed a significantly decrease (P≤ 0.05) in patients (8.07 ± 1.01) ng/dl compared to control (10.41 ± 1.81) ng/dl.

Recorded results revealed that the IL-22 is increased significantly (P ≤ 0.05) in T2DM patients while IL-23 is decreased non significantly (p > 0.05) when compared with the controls. These results agreed with several authors (Zhao *et al*., 2014; Dalmas *et al*., 2014; Gong *et al*., 2016), The probable reason for the elevating concentrations of serum IL-22 may be result Differentiation CD4+ T cells in adipose tissue into Th1, Th2, Th17. Th17 cells produce a series of cytokines such as IL-17 and IL-22 and are regulated by various cytokines (Gong *et al*., 2016) but Documented results were not consistent with the findings of other authors (Chen *et al*., 2012) who stated that serum of IL-22 showed lower in T2DM patients compared with controls, suggest the IL-22 could counteract the harmful effects of hyperglycemia on human islet cells and potentially support the strong protective effect of IL-22 on impaired islet function and survival., IL-22 suppresses oxidative and ER stress caused by cytokines or glucolipotoxicity in human beta cells (β-cell). Therefore, a decrease in the concentration of IL- 22 leads to Type 2 diabetes. The present study showed a nonsignificant decrease (p > 0.05) in the concentrations of IL-23 in T2DM patients compared with the controls. Similar to our findings (Gupta *et al*., 2017; Maboudi *et al*., 2019; Roohi *et al*., 2014) by evaluating different cytokines reported that no significant difference in the level of IL-23 among T2DM patients and controls, A low level of T- helper cells (Th17) expressing the IL-23 receptor may be responsible for a low of immune response in immune cell producing Interleukin-23, leading to a lower level of Interleukin-23 in diabetics group compared to the control group. Furthermore, High blood sugar (hyperglycemia), increased insulin, latent infections and pressure on T- helper cells (Th17) lead to decreased immune response and increased inflammation. The Fas/ FasL system is an important regulating system accountable for the activation of apoptosis in several cell types (Stoynev *et al*., 2014). The present study showed a significant decrease (P ≤ 0.05) in the concentrations of sFasL in T2DM compared with the controls. This decreasing may be explained that the higher Recorded results demonstrate that the serum sFas concentrations in participants with T2DM were significantly increased (P ≤ 0.05) compared to the controls. The reason of this increase is explained when the glucose levels are increasing (hyperglycemia). It would be caused an inflammation of β-cell and stimulating of elevating of Fas expression which correlates with β-cell inflammation and the islets become gradually sensitive to the apoptosis, The induction, transcription, and modulation of Fas of

Table 4: The relationship between immunological Parameters with the gender of patients and age groups.

Parameters	IL-22 Mean ± SEM	IL-23 Mean ± SEM	Fas Mean ± SEM	FasL Mean ± SEM
Male	179.59±38.11	69.78±21.95	2.11±1.01	8.58±1.59
Female	172.12±28.91	62.77±15.24	1.31±0.61	7.56±1.27
P value	0.537NS	0.388NS	0.178NS	0.501NS
Age group (years)	**IL-22 Mean ± SEM**	**IL-23 Mean ± SEM**	**Fas Mean ± SEM**	**FasL Mean ± SEM**
40-49	148.13±27.49	51.14±11.84	0.81±0.36	7.42±1.14
50-59	227.05±41.34	84.21±29.84	2.65±1.37	8.92±2.21
60-69	168.14±34.36	57.69±19.50	0.62±0.02	7.52±1.60
70-79	189.38±53.07	60.27±21.09	1.43±0.81	8.39±0.57
P value	0.870NS	0.786NS	0.645NS	0.932NS
NS: No significant difference (P > 0.05)				

Table 5: Correlation coefficient of immunological parameters in patients group.

Parameters	Correlation	IL-22	IL-23	FAS	FASL
IL-22	R		.894**	.745**	.851**
	P		.000	.000	.000
IL-23	R	.894**		.879**	.915**
	P	.000		.000	.000
FAS	R	.745**	.879**		.824**
	P	.000	.000		.000
FASL	R	.851**	.915**	.824**	
	P	.000	.000	.000	
R=Pearson Correlation, P=Probability,*=positive correlation.					

expression are initiated by a number of pro inflammatory cytokines such as interleukin IL 22, IL 1β, IFNγ, nitric oxide, and TNF α that cooperate with Fas to be the effector mechanisms of â cell destruction (Kumar *et al*., 2013). Documented results were in agreement with many studies worldwide, (Mahfouz *et al*., 2012) but they were not consistent with the findings of other authors, (Tankova, 2012) who stated that serum sFas showed nonsignificant differences in T2DM compared with controls. Further, the higher concentrations of glucose impair the islet function by disturbing the metabolism of glucose in the mitochondria of â cells and could induce apoptosis.

Table 4 revealed that IL-22, IL-23, sFasL and sFas levels did not show any significant difference among diabetic groups depending on gender and age groups.

The present study showed a statistically no significant difference (P>0.05) in the level of immune parameters with gender and age groups T2DM patients, Documented results were in agreement with many studies (Shen *et al*., 2018; Hamid and Shani, 2018). The lack of a statistically significant difference in level of immune parameters with gender and age groups may be due to the gender-based immune defense mechanisms, since males and females show the same immune cells to the immune response that occur in the patient's body, which may be somewhat similar regardless of the As the interaction within the patient's body leads to the activation of immune cells responsible for immunologic response in the serum of patients with diabetes, (Voskuhl, 2011).

Pearson correlation analysis revealed a significant positive correlation between immunological parameters as shown in table 5.

Conclusions

During the current study, there was significant increase in level of IL-22 and FAS, but not IL-23, in diabetes patients compared to control. T2DM patients shows a significant reduction in serum level of FasL. and no significance different between immunological Parameters with the gender of patients and age groups. Also showed positive correlation between immunological Parameters

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