Evaluation Of the Immunological Role Of Interleukins IL17, IL21,and CD4+,CD8+ T cells In Patients With Type 1 Diabetes In The City Of Baquba

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Abstract

Background: Diabetes Mellitus (DM) is a heterogeneous group of high blood sugar hyperglycemia and produces insulin, insulin action , or both. Diabetes is caused by the inability of beta cells in the pancreas. The production of these insulin cells is little or no. This is known as type 1 mellitus diabetes (Insulin-dependent T1DM) Insulin-dependent diabetes mellitus.

Objective: To evaluate the immune role of some of the immunological indicators of patients with type 1 diabetes by identifying the IL17 and IL21 concentrations, also The CD4 + and CD8 + T cells were identified by the Sandwich ELISA test.

Patients and Methods: Blood samples were collected from 70 patients with type 1 diabetes (diabetes mellitus type 1) after diagnosis and 20 samples of healthy people were collected as a control group, an the study has been done during the period between October 2016 and May 2017.

Results: The results of IL17 level showed a decrease of IL17 in patients with type 1 diabetes 114.31 ± 103.78 pg / ml compared to control group126.54±81.48pg / ml with no statistically significant difference (p> 0.05),and the results of IL21 level showed an crease of IL-21 in patients with type 1 diabetes , 209.40 ± 294.78 pg / ml compared with healthy 189.54 ± 274.82 pg / ml with no significant difference.The results showed a decrease in the concentration of CD4 + T cells in the group of patients 5.18±4.59 pg / ml compared to the healthy group whose concentration level was   5.52 ± 3.47 pg / ml.Also the results showed a high concentration of CD8+toxic T cells in the group of patients as it was 5.54 ± 7.39 pg / ml compared to the healthy group. The concentration of toxic T cells was 3.74 ± 3.57 pg / ml. There was no significant difference between the study groups for CD4 + and CD8 +.

Conclusion: A number of pro-inflammatory cytokines (IL17) and inflammatory cytokines (IL21) as well as cellular immunity of CD4+ and CD8+have an important role in the development of type 1 diabetes in children and affect the immune response in the body through immunodeficiency.

Key words: Cytokine,IL17,IL21,CD4+,CD8+, Diabetes Mellitus.

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Introduction

Diabetes Mellitus (DM) is a heterogeneous group of metabolic disorders characterized by hyperglycemia that results in an imbalance in the secretion of insulin, insulin

action, or both (1).The main cause of diabetes is the inability of beta cells to produce insulin is little or absent completely.This is known as type 1 mellitus diabetes (Insulin-dependent T1DM) Insulin-dependent diabetes mellitus (2). Type I, directly or indirectly, leads to the breakdown of insulin-producing beta cells (3).Some of these Symptoms make us suspect of diabetes or hyperglycemia are polydipsia, polyuria, and loss of weight (4). Diabetes - Type 1 is a serious disease and leads to death if not treated, and the risk is likely to be exposed to an increase in blood sugar and with the increased concentration of Ketoacidosis with loss of fluid body, leading to fainting or death if neglected or not addressed (5).Many studies have shown that T1DM is the type that affects children and adolescents, and is affected every year in four million people of the world with a material cost of up to 160 million US dollars, all of which are on treatment and medical care and the prevalence of this disease affects a certain percentage on the income and health of society(6). Cytokines, proinflammatory cytokines and inflammatory cytokines, play an important role in stimulating or increasing diabetes. Cellular immunity also has a clear role in the occurrence and development of type 1 diabetes, and this immunity is mediated by self-effective lymphocytic T cells(7). Some studies suggest that its pathogenes   is takes a protective pathway in which antibodies play a prominent role in the destruction of beta cells in the pancreas(8).Cellular autoimmune immunity also plays an important role in the development and development of diabetes-type 1 and this immunity is mediated by self-effective lymphocytic T cells(9).The aim of this study was evaluate the immune role of some immunological markers of patients with type 1 diabetes by identifying the IL17 and IL21 concentrations . The CD4 +and CD8 + T cells identified by the Sandwich ELISA   test.

Patients and Methods

Seventy blood samples of type 1 diabetic patients were collected after diagnosis by the diabetes specialist at the Batoul Hospital for Obstetrics and Gynecology, according to the criteria approved by the World Health Organization for the diagnosis of type 1 diabetic and 20 samples of healthy people were collected as a control group.The study has been during the period between October 2016 and May 2017.

  Measure the level of cytokines IL17, IL21,CD4+,CD8+ in the serum using the ELISA test: The level of concentration of immunological markers was measured by enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Interleukin , and add Interleukin to wells that are pre-coated with Interleukin monoclonal antibody and then incubate. After incubation, add anti antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing , and then add substrate A and B. The solution will turn blue and change to yellow with the effect of acid.

The shades of solution and the concentration of Human Interleukin are positively correlated(10).

Statistical Analysis

The statistical analysis of the current study data was carried out according to M13 and Graph pad prism, and the square (X2) was used to compare the percentages. For numerical variables, they were described using mean ± SD and the comparison between totals was done using a t-test between two sets of numbers or an ANOVA test when comparing more than two groups. And (T test) to compare between two groups and the level of $\alpha = 0.05$ was applied for the test (11).

Results

The study included (90) samples and were in two groups, namely, the first group of patients with type 1 diabetes, comprising (70) samples, Where the number of males (39) and the percentage (55.70%),and the number of females(31) and the proportion of females (44.30%). The control group was included (20) sample Where the number of males (16) and by a percentage (80.00%), and the number of females (4),and the proportion of females (20.00%).

Table (1): Distribution of the two studied groups for sex.

sex of study groups				
Sex		study groups		Total
		Patients	Control	
Male	Count	39	16	55
	%	55.70%	80.00%	61.10%
Female	Count	31	4	35
	%	44.30%	20.00%	38.90%
Total	Count	70	20	90
	%	100.00%	100.00%	100.00%
X^2	3.86			
Sig.	0.049*			

*There is a significant statistical difference p≤0.05

The results of this study showed that the average age of patients with type 1 diabetes (9.42± 3.34) for the range (1.00-16.00) years, while the mean age of control group (17.25±4.17)for the range of (5.00 - 25.00) years, with a significant difference Was very statistically significant among the study groups. The value of p-value (p <0.001).

Table (2): Distribution of the two studied groups for age.

Age of study groups					
study groups	N	Minimum	Maximum	Mean	Std. Deviation
Patients	70	1	16	9.4286	3.34738
Controls	20	5	25	17.25	4.17858
Total	90				
T	24.21				
Sig.	<0.001***				

*There is a very significant statistical difference

The results of the current study showed a decrease of IL-17 in patients with type 1 diabetes (114.31 ± 103.78) pg / ml Compared to control group (126.54±81.48) pg / ml with no statistically significant difference (p> 0.05).

Table (3): The level of IL-17 concentration for the two studied groups.

	GROUPS	N	Mean	Std. Deviation	Std. ErrorMean	T	Sig.
IL-17	Patients	70.00	114.31	103.78	12.40	-0.48	0.68 [NS]
	Control	20.00	126.54	81.48	18.22		

NS= There is no statistically significant difference p> 0.05

The results of this study showed a high concentration of interleukin IL-21 In the patient group It was (209.40±294.78)pg / ml Compared to the healthy group it was (189.54 ± 274.82)pg / ml with no significant differences between them as shown in the table (4).

Table(4): The level of concentration of IL-21 for the two studied groups.

	GROUPS	N	Mean	Std.Deviation	Std. Error Mea	T	Sig.
IL-21	Patients	70.00	209.40	294.78	35.23	0.27	0.78 [NS]
	Control	20.00	189.54	274.82	61.45		

NS= There is no statistically significant difference

The results of this study showed that the average concentration of T-cells was lower in the group of patients and was (5.18 ± 4.59pg/ml) than in the healthy group (5.52±3.47pg/ml) With no significant differences As shown in the table (5).

Table(5): CD4+ concentration level for the two studied groups.

	GROUPS	N	Mean	Std. Deviation	Std. Error Mean	T	Sig.
CD4^{+}	Patients	70.00	5.18	4.59	0.55	-0.31	0.75 [NS]
	Control	20.00	5.52	3.47	0.78		

NS= There is no statistically significant difference p> 0.05

The results of this study showed that the average concentration of CD8+cells was higher in the group of patients was (5.54 ± 7.39pg/ml) than in the healthy group was (3.74 ± 3.57pg/ml) With no significant differences,as shown in the table(6).

Table (6): The concentration level of CD8+ for the two studied groups.

	GROUPS	N	Mean	Std. Deviation	Std. Error Mean	T	Sig.
CD8+	Patients	70.00	5.54	7.39	0.88	1.05	0.29 [NS]
	Control	20.00	3.74	3.57	0.80		

NS= There is no statistically significant difference p> 0.05

Discussion

The results of the present study showed that the percentage of males infected with the disease more than the percentage of female infected with the disease with a significant difference of statistical significance between the sexes as the value of p-value($0.049*= p \leq 0.05$).

The cause of the sex hormone receptors is thought to have an effect on immune cell functions, and inflammatory(12). as shown in the table(1). The incidence of type 1 diabetes (T1DM), one of the most prevalent chronic diseases among children worldwide, has   increased(13).as   shown   in   the table(2),type 1 diabetes has the same risk of early artery disease as hypercholesterolemia, and   cardiovascular   disease   Diabetic nephropathy is the leading cause of early death   in   individuals   over   the   age   of 30(14).Recent studies have confirmed an increase in the rate of type 1 diabetes among children aged 5-10 years. This increase is due to environmental factors such as the living conditions of children and the impact of the environment in general such as a healthy diet and exercise(l5).

The results of this study were agreed with a study on type 1 diabetes (Abdel Nasser, 2016), where there was no significant

difference in the concentration of IL-17 prophylaxis among the patients and healthy group(16).While these results did not match the results of the study conducted by(Kikodze et al , 2014) for obtaining results with significant differences in the level of concentration with   IL-17 in patients with diabetes type 1(17). This IL-17 has a strong immune function in pancreatic islet cells and is associated with the development of type 1 diabetes. It may act as a vital indicator in the autoimmune   response   to   beta   cell destruction(18).Several   studies   have confirmed that there is a link between IL-17 and   other   cellular   anti-inflammatory compounds, which may mediate the harmful effect of pancreatic islet cells in humans and increase the programmed death of beta cells(19). The results of this study are consistent with (Ricardo, 2015) (20). The results suggest that increased production of this cytokines is cause of type 1 diabetes, has an important role in causing diabetes, which can be considered a potential therapeutic target ,The results of the current study show high level of interleukin IL-21 in patients with diabetes compared with the control group. The height of IL-21 may be due to the activation of T- helper cells, thus stimulating the immune response of the immune cells

produced by this cytokines a higher level compared to the control group. The increase in inflammatory cytokines in patients with diabetes indicates that the inflammatory stage may Obstruction of insulin function signals and then resistance to insulin, which leads to diabetes.

Type I diabetes is primarily an average T-cell disease, following early discoveries on autoimmune immunity in T1DM, there has been a marked expansion in the detection of antibody associated with T1DM as well as in the characterization of the molecular basis of the antigen of target proteins(21) .This finding coincided with Ifere (2009). that T lymphocytes have an important role to play in the resistance against the disease and that the defect in host defenses resulting from malfunction in the function of monocytes, neutrophils, and (Th) helper lymphocytes To impaired immune response in diabetics(22). A study of reduced regulatory T cells showed that IL21 production was sufficient to induce destruction of T cells(23). The increase in CD8+ may be due to the low duration of the disease in patients with type 1 diabetes , Several studies have shown the role of T cells in the development of type 1 diabetes, the cytoxic cells (CD8+ T) are self-effective and directed against beta-cell antigens. They also stimulate the different defense cells that attack beta cells. When effective T cells appear against beta cells, they restrict or are naturally inhibited by the mechanisms of immune regulation and immune immunity. The disease occurs when one or more immune regulation mechanisms

fail and allows self-effective T cells to be directed against beta cells cause the destruction of these cells and the disease appears(24). The high concentration of toxic cells may be due to an increase in the production of some,cytokines which can increase their spread due to of disorders in the immune response of the body (25).The results of this study were not consistent with (26). found that cellular immunity is affected by diabetes because a systemic disease affects the lymphatic organs.

Conclusion

1-The study showed that the percentage of males with type 1 Diabetes Mellitus type 1 was higher than that of females with significant difference between sexes.

2-The results of this study showed a significant difference of statistical significance between the study groups in terms of mean age of patients with type 1 diabetes where the age range of the group of patients (1-16) years while the age range of the control group of (5 - 25) years.

3-The IL17 showed low concentrations in patients with type-1 diabetes compared with control samples and had no positive association with the disease.

4- The IL21 showed high concentrations in patients with type 1 diabetes compared to control samples and had a positive correlation with the disease.

5- Assisted CD4 + cells showed low concentrations for patients with type 1 diabetes compared with control samples,and the CD8 + T cells showed higher

concentrations for patients with type 1 diabetes compared to control samples.

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