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Estimation the level of il-17a in a sample of type i diabetes mellitus patients

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ABSTRACT: *Type 1 diabetes mellitus (T1 DM) is a chronic autoimmune disease associated with selective destruction of insulin-producing pancreatic **β**-cells. Several features characterize type 1 DM as an autoimmune disease: presence of immuno-competent and accessory cells infiltrated pancreatic islets. This is a prospective study which was carried out to investigate the possible association of T1 DM with IL-17A.*

Sixty patients with Type 1 diabetes mellitus (T1 DM) who were diagnosed according to American Diabetes Association criteria (ADA) 1997 were selected from the Center of Endocrinology and Diabetes /AL-Kindey / Hospital/Baghdad. All patients were treated with daily replacement doses of insulin. Their age ranged 5- 40 years. As a control group, sixty apparently healthy volunteers whose their age (8- 40) years, and sex (Females: Males 1:1.31) were matched the patients' group. The study revealed that the high frequencies of T1 DM was at the age group 10-19 years while the lower frequency among age group <10 years old.

Regarding cytokine level, it was noticed that there was highly significant elevation in the mean of IL-17A in the sera of diabetic group (1312.06 Pg/ ml) in comparison with healthy control (332.28 pg/ml) (P=0.003).

Conclusion, there appears to be an important role for IL-17A in increasing the chance of enhancing the susceptibility for disease development or providing protection against it.

KEY WORDS: *Diabetes mellitus, IL-7A, ELISA*

I. INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. [1]. It was reported that Type 1D is caused by a deficiency in insulin secretion due to the loss of pancreatic β cells, and the disease requires life-long treatment with exogenous insulin. Without the body's own insulin production the body loses its ability to utilize carbohydrates as an energy source. Type 1D M is considered as an autoimmune disease, which is developed due to the T-cell-mediated destruction of β cells in the islets of Langerhans of the pancreas. In children with an active β-cell destruction process, autoantibodies against β-cell structures appear in the circulation [2]. It was observed that this process occurs in genetically susceptible subjects, and it is probably triggered by one or more environmental agents, and usually progresses over many months or years during which the subject is asymptomatic and euglyecemic [3].

The pathogenesis of T1D has been considered to result from a breakdown of immunologic tolerance towards β-cell antigens. Immunologic tolerance is maintained by two major phenomenon; central and peripheral tolerance. Peripheral tolerance is dictated by the fine balance between effectors' T cells and so called regulatory T cells. Defects in T-cell polarization and immune regulation have been associated with T1D. Recent advances in T cell research have provided new insights into the dynamics and regulation of T cell responses. Not only the balance between fully committed effectors' T cells and regulatory T cells, but also the plasticity between these phenotypes seems to play a role in autoimmunity and tolerance [4],[5].

A distinct and separate lineage of Th cells secreting the proinflammatory cytokine IL-17 has been recently described [6]. The discovery of these Th17 cells has had a major impact on our understanding of immune processes not readily explained by the Th1/Th2 paradigm. Th17 cells are intimately involved in promotion of autoimmunity [7]; in particular, rheumatoid arthritis [8], experimental autoimmune encephalomyelitis [9], and multiple sclerosis [10]. Moreover, there is preliminary evidence that IL-17 is expressed in the pancreas in the course of T1D in the murine model of T1D [11, 12]. Two recent articles found that transfer of islet-specific Th17 cells induced diabetes, but only after the cells converted to IFN-γ producing cells [13, 14].

II. MATERIALS AND METHODS

2.1. Patients Group

The study comprised sixty Iraqi Arab Patients (26 females and 34 males) with T 1 DM who was attending the center of Endocrinology and Diabetes/ AL-kindey Teaching Hospital/ Baghdad. All patients were selected on the basis of absolute dependency on insulin according to the revised criteria for diagnosis of DM as defined by the American Diabetes Association (ADA) (1997), The Expert Committee, (1997).

2.2. Control Group

Sixty apparently healthy volunteers whose their ethnic back ground, age, and sex were matched the patients group, consisted of unrelated non-diabetic individuals according to the laboratory finding of FPG value <6mml/L which was considered as control. All of them had negative family history of DM with age range was (5-40) years. All investigations were carried out for patients as well as the control group according to the study protocol. Venous blood sample which have been collected from each fasting subject and tested for the serological by enzymatic method (ELISA) and biochemical tests.

2.3. Methods

Reagents & Kits

A- Micro plate Elisa Kits For the quantitative determination of C- Peptide in human serum or EDTA, heparin or citrate plasma/Germany DRG.

B- Koma Bio Tech micro plate ELISA for the quantitative determination of cytokine in human serum, plasma, culture medium or other biological fluid/Koma BioTech Korea.

C- Fasting Plasma glucose test kit/spin react/Spain.

2.4 Statistical analysis

The suitable statistical methods were used in order to assess and analyze our results, by using SPSS version 13 (Statistical Package for Social Sciences**).**

III. RESULTS AND DISCUSSION

3.1. Demographical Picture of the Studied Groups

The demographical picture of the studied groups showed that the mean of diabetic patients was 16.32±0.85 (SE) years with M:F ration of 1:1.31 Family history revealed that (28.3%) of diabetic with a previous family history for diabetic as shown in Table 3.1.It s clear from this table that there is a significant difference in fasting plasma glucose level among diabetic patients (231.97±14.85 mg/ dL) in comparison with apparently healthy control group (80.57±1.85 mg/dL) (P< 0.001).

Table 3.1: Demographical picture of the studied groups

No.	Demographical Parameters	Diabetic Patients	Healthy Control	P value
1	Age (years)[Mean ±SE]	16.32 ±0.85	17.93 ±1.20	0.27 (NS)
2	Age of Disease Onset (years) (Mean)	11.38+5.79	-	
3	Males: Females Ratio	34: 26 = 1.31	36: 24=1.5	
4	Positivity of Family History [No (%)]	17 (28.3%)	-	
5	Fasting Plasma Glucose [mg/ dL]	231.97±14.85	80.57±1.85	<0.001
	Total number	**60**	**30**	

NS: Not Significant

3.2. Distribution of Patients & Healthy Control according to Age Groups

Distribution of patients and control groups according to age groups was listed in Table 4.2. In this table, it appears that most patients (73.33%) were at age group (10-19 years) no significant difference between this age group and the other groups (P=0.27).

Table 3.2: Distribution the diabetic patients and control groups according to age groups

Age Groups (Years)	Diabetic Patients No (%)	Healthy Control	P value
<10	5 (8.33)	2 (6.7)	**P= 0.27**
10-19	44 (73.33)	22 (73.3)	
20-29	7(11.67)	4 (13.3)	
≥30	4(6.67)	2(6.7)	
Total	60 (100.0)	30 (100.0)	

3.3. Level of Fasting Plasma Glucose among the Studied Groups:

The level of fasting plasma glucose was highly significantly altered among the patient's group in comparison with control group as shown in Table 3.3.

Table 3.3: Levels of FPG among diabetic and control group

	(FPG)mg/Dl	
	Study	**Control**
N	**60**	**30**
Minimum	**34.2**	**60**
Maximum	**491.4**	**99**
Mean	**231.974**	**80.5667**
Std. Error	**14.8502**	**1.8462**
Std. Deviation	**115.029**	**10.1121**
C.S.	**t=10.118 : P<0.001 HS**	

3.4: Estimation of IL-17A in the Sera of Diabetic Patients and Healthy Control Groups

Quantitation of IL-17A level in the sera of the studied groups revealed that there was highly significant difference between its level among patients (1312.06±697.03 pg/ ml) in comparison with control group 332.8±256.769 pg/ ml) (P=0.003). These data are represented in Table 3.4.

Table 3.4: Comparison of Interleukins -17A Level among the studied groups

Statistic Values	IL-17 A Pg/ ml		P value
	Diabetic Patients	Healthy control	
Mean	1312.06	332.8	**P=0.003 HS**
Standard Error	697.03	256.769	
Median	1322	226	
Maximum	3567	1116.4	
Minimum	230	210	
Range	3336.0	905	
Interquantile Range	790.0	100	
Geometric Mean	552.95	359.96	

IV. DISCUSSION

Sixty Iraqi Arab, diabetic patient (34 males and 26 females) were selected for this study with age ranged from (5-40) years. The highest incidence (73.33%) observed under the age group (10-19) years. This finding is in agreement with other Iraqi studies such as [14, 15, 16, 17] and with abroad studies [18, 19] who reported that the age of puberty represents the higher risk of developing T1DM than other age group. But this study disagrees with other Iraqi studies [20, 21]

To focus on sex variable of the studied group, this study revealed that the ratio of male: female in the patient group was 1:1.31 which is compatible with Iraqi studies [18, 20, 22, 23] and abroad studies reported by [24]. On the contrary, they incompatible with Iraqi studies [14, 18] and abroad studies [25, 26].

Statistically studied showed the level of IL-17A in patients group was elevated in comparisons with healthy group (1312 vs.332.8 pg /ml, P 0.003), which is agreement with a broad study [27,28].The explanation of this increased may possibly related to hyperglycemic state of diabetic subject which could induced the secretion of these cytokines by monocytes [28].

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