

Association of Interleukin-10 and Interleukin-17 Level with vitamin D Deficiency in Children with Type 1-diabetes Mellitus

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Abstract

Background: Diabetes mellitus is among the most prevalent metabolic illnesses, with high morbidity & mortality in the world. Its association with immunological disorders has been detected in many studies. The International Diabetes Federation (IDF) reported that 425 million people in the worldwide live with diabetes in 2017; and the number will probably rise to 629 million in 2045. The American Diabetes Association (ADA) distinguishes diabetes mellitus into the following four subtypes: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes mellitus, and certain kinds of diabetes related to other causes. Type 1 diabetes is the most common form of the disease. In this study, the association between the immunological markers IL-10 and IL-17 and vitamin D insufficiency in children with type 1 diabetes was examined. **Methods:** Overall, 51 samples were collected from patients both genders. The patients composed of (51), (23) of them are males and (28) females with age range 2-12 years old, there is seen in Endocrinology and Diabetes Center in Samawa. Those sample were diagnosed clinically by HBA1C to Diagnosis of the type diabetic by cobas c111 and evaluate IL-10 and IL-17 level by ELISA technique and evaluate vitamin- D by cobas e411 technique in diabetic patients. Further, groups consist of 50 samples were collected from apparently healthy individuals (25 males and 25 female) without any history of chronic disease. **Result:** Our results reveal that there was no significant difference in the mean age of the patient group compared to the control group, 8.43 ± 3.13 years versus 8.12 ± 2.69 years, respectively ($p = 0.594$). The percentage of males who were diagnosed with T1DM was 45.1 % while that of girls was 54.9 % and there was no significant difference in frequency distribution according to gender between type 1 diabetes mellitus group and control group ($P = 0.622$). There was no significant difference in mean age between boys & girls with type 1 DM ($P = 0.785$). The average HbA1c % of individuals with type 1 diabetes was significantly higher than that of the control group, 9.75 ± 0.80 % versus 4.64 ± 0.40 %, respectively ($P < 0.001$). In our research, the average level of vitamin D in the serum was significantly lower in patients with type 1 DM in comparison with control group, 11.14 ± 4.65 ng/ml versus 17.16 ± 6.39 ng/ml, respectively ($p < 0.001$). Diabetic patients with vitamin deficiency (< 20 ng/ml) accounted for 94.1 %, while control subjects accounted for 64 %, therefore, Patients diagnosed with T1D have a prevalence of vitamin D deficiency that is significantly higher than that of the control group ($p < 0.001$).

Keywords: Diabetes Mellitus, Vitamin D Deficiency, Cytokine, The Interleukin 17, The Interleukin 10, T1D.

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INTRODUCTION

Diabetes mentions to a group of metabolic illnesses that are characterized by the presence of hyperglycemia in the absence of treatment, is Etiopathology comprises problems in insulin secretion, insulin action, or both, as well as carbohydrate, lipid, and protein metabolic disorders [1]. Diabetes may manifest with symptoms such as dehydration, polyuria, blurred vision, and weight loss, yeast infections of the female genital tract are common among women, the most serious clinical signs of type 1 diabetes are

ketoacidosis, which can lead to dehydration, unconsciousness, and death in the absence of adequate treatment [2]. Cells in the pancreas are destroyed in all types of diabetes [3]. Numerous causes can result in the loss of function or full destruction of β -cells (these cells are not replaced, as the human pancreas does not appear to be able to regenerate β -cells as the pancreas ages) [4]. Hyperglycemia is the most prominent diabetic clinical symptom, nonetheless, insulin shortage or insulin resistance is also linked to abnormalities in lipid and protein metabolism, as well as mineral and electrolyte disorders

[5].For diabetes to develop, numerous causes can result in the loss of function or full destruction of β -cells must sustain extensive damage; the most prevalent causes are pancreatitis, trauma, and cancer, chronic pancreatitis can result in widespread inflammatory and fibrotic alterations in the pancreas, which can lead to diabetes. [6]. T1D is one of the most prevalent chronic illnesses affecting children, and it is brought on by the immune system killing insulin-producing cells in the pancreas. Its occurrence appears to vary geographically along a gradient in latitude, the opposite of the global distribution of ultraviolet B irradiation, which is necessary for the skin to produce vitamin D. Vitamin D insufficiency is more prevalent in type 1 diabetic individuals, according to research. [7].Typically, cytokines are glycoproteins or proteins with a molecular weight between 30 and 40 kDa, Leukocytes and other cell types respond to foreign stimuli by releasing cytokines [8]. It is possible for a specific cytokine to binds to receptors on the membrane of the cell that produced it, causing the cell to engage in autocrine activity, alternatively, the cytokine could bind to receptors on the membrane of a target cell that is located in close proximity to the cell that produced it, causing the target cell to engage in paracrine activity. Only in extremely unusual circumstances does the cytokine attach itself to cells that are further away and cause it to engage in [8].Numerous cytokines are interleukins, which suggests that they are secreted by certain leukocytes and work on other leukocytes. Interleukins 1–25 are identified [9].There are six members of the interleukin 17 (IL-17) cytokine family: IL-17A (also known as IL-17), IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. The biological function and control of IL-17A and IL-17F are best known [10].A recent research in which IL-1 and IL-6-secreting monocytes from diabetic patients stimulated IL-17 production in allogeneic memory T cells in vitro [11]. Implicitly suggested a role for Th17 immunity in human T1D, the interleukin IL-10 family of cytokines has crucial roles in the regulation of immune responses during host defense, as well as in autoimmune disorders, inflammatory illnesses, and malignancies. Other members of the IL-10 family specifically target non-immune compartments, like tissue epithelial cells, where they activate innate defense mechanisms to prevent microbial, viral, and fungal infections, preserve tissue integrity, and aid in tissue repair and regeneration [11].Vitamin D may be a secosteroid hormone, which is implicated in system modulation. It is supposed to influence the metabolism of calcium and phosphate and promote bone health. The extra skeletal effects of ergocalciferol have recently grabbed the focus of scientists [12] and are the subject of intensive research, Vitamin D is mostly synthesized in the skin of humans when they are exposed to sunlight. Nevertheless, a negligible amount of viosterol (20%) is received by diet [13].VDR mediates the impact of vitamin D on both the innate and adaptive systems[14].

MATERIALS AND METHODS

Patients and Control: There were a total of 51 patients seen at the Endocrinology and Diabetes Center in Samawa province. Of these patients, there were 23 males and 28 females who were between the ages of 2 and 12 years old. The other categories include fifty persons who were assessed to be in apparently health and who did not have a family history of any form of chronic illness. There were twenty-five boy and twenty-five girl in this group.

Cobas HBA1C Test: In order to separate the hemoglobin from the erythrocytes in the blood sample, it is first diluted and then combined with TRIS buffer. A portion of the substance is moved into a chamber where it is combined with sodium lauryl sulphate (also known as (SLS).

Immunological Methods

Enzyme-linked Immunosorbent Test: This kit (Shanghai YL Biont / China) use the enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to measure the Human Interleukin 17 (IL-17) and Interleukin 10 levels (IL-10). Add Interleukin 17(IL-17) and Interleukin 10(IL-10) to the wells that have been pre-coated with monoclonal antibodies to Interleukin 17(IL-17) and Interleukin 10(IL-10), and then incubate. After that, biotin-labeled anti-IL-17 and anti-IL-10 antibodies are combined with streptavidin-HRP to produce an immunological complex. After incubation and washing, remove any free enzymes. Add substrate A and substrate B. As a result, the acid will cause the solution to turn blue and then yellow. The concentrations of Human Interleukin 17 (IL-17) and Interleukin 10 (IL-10) are favorably associated with the solution colors.

Cobas Vitamin (D) Test: The purpose of this assay is to quantify 25-hydroxyvitamin D in human serum. This test is intended to assist in determining vitamin D sufficiency. The electro chemiluminescence binding test is compatible with cobas e411 immunoassay analyzers.

RESULT

Demographic characteristics of children with (T1DM) patients and Control Subjects:

Comparison of mean age and frequency distribution according to age between T1DM patient and control groups. The mean age of the patients and control groups did not differ statistically significantly, 8.43 ± 3.13 years versus 8.12 ± 2.69 years, respectively ($p = 0.594$).

Table 1: A comparison of the mean age and frequency distribution by age in the T1DM patient & the control group

Characteristic	T1DM No.= 51	Control group No.= 50	P
Age (years)			
Mean ±SD	8.43 ± 3.13	8.12 ±2.69	0.594 I
Range	2 -12	4 -12	NS
< 5, n (%)	7 (13.7 %)	4 (8.0 %)	0.291 C
5-10, n (%)	25 (49.0 %)	33 (66.0 %)	NS
> 10, n (%)	19 (37.3 %)	13 (26.0 %)	

Table 2: Comparison of the gender-based frequency distributions of patients with T1 DM & controls group

Gender	T1 DM No. = 51	Control group No. = 50	P
Male, n (%)	23 (45.1 %)	25 (50.0 %)	0.622 C
Female, n (%)	28 (54.9 %)	25 (50.0 %)	NS

The proportion of males with type 1 diabetes mellitus was 45.1 % while that of girls was 54.9 % and there was no significant difference in frequency distribution according to gender between type 1 diabetes mellitus group and control group (p = 0.622). Comparison of mean age between males and females with type 1 diabetes mellitus is shown in table 3.3. There was no significant difference in mean age between boys and girls with type 1 DM (p = 0.785). Comparison of mean HbA1c% between patients with type 1 diabetes mellitus and control group is shown in. The mean HbA1c% of patients with type 1 diabetes mellitus was significantly higher than that of control group, 9.75 ±0.80 % versus 4.64 ±0.40 %, respectively (p < 0.001). All control group members had HbA1c % level within normal range 4-5.6%; however, all patients with T1DM had HbA1c % level within diabetic range (≥ 6.5%). In our study, HbA1c % was higher in type 1 DM and all patients were poorly controlled since the level of HbA1c % was within diabetic range despite receiving insulin therapy. The mean serum vitamin – D in our study was significantly lower in T1D patients compared to the control group, 11.14 ±4.65 ng/ml versus 17.16 ±6.39 ng/ml, respectively (p < 0.001).

Table 3: Comparison of mean age between males and females with T1DM

Age (years)	male No. = 23	female No. = 28	P
Mean ±SD	8.57 ±2.92	8.32 ±3.35	0.785 I
Range	2 - 12	2 - 12	NS

Table 4: A comparison of the mean HbA1c% in T1D patients and the control group

HbA1c %	Type 1 DM No. = 51	Control group No. = 50	P
Mean ±SD	9.75 ±0.80	4.64 ±0.40	< 0.001 I **
Range	8 -12	4 -5.3	
4-5.6% (normal range)	0 (0.0 %)	50 (98.0 %)	< 0.001 C **
5.7-6.4% (pre-diabetic range)	0 (0.0 %)	0 (0.0 %)	
≥ 6.5% (Diabetic range)	51 (100.0 %)	0 (0.0 %)	

Table 5: Comparing the mean vitamin D serum concentrations of patients with T1D and the control group

Serum Vitamin D (ng/ml)	Type 1 DM No. = 51	Control group No. = 50	p
Mean ±SD	11.14 ±4.65	17.16 ±6.39	< 0.001 I **
Range	3 -22.7	4.9 -30.1	
Normal (≥ 20 ng/ml)	3 (5.9 %)	18 (36.0 %)	< 0.001 C **
Deficiency (< 20 ng/ml)	48 (94.1 %)	32 (64.0 %)	

Diabetic patients with vitamin D deficiency (< 20 ng/ml) accounted for 94.1 %, while control subjects accounted for 64 %, therefore, Vitamin D deficiency is significantly more common in type 1 diabetes patients than in the control group (p < 0.001). Patients in our study with type 1 diabetes showed vitamin d deficiency in significantly high rate, but the rate of vitamin deficiency in control group was also high.

The level of serum IL-10 of patients with type 1 DM was lower than that of control group, 20.90 (5.50) versus 24.50 (11.73), respectively (p < 0.001), figure 3.3. However, Serum IL-17 levels in patients with type 1 diabetes were greater than those in the control group (p < 0.001): 242.20 (140.90) versus 22.40 (12.20), respectively.

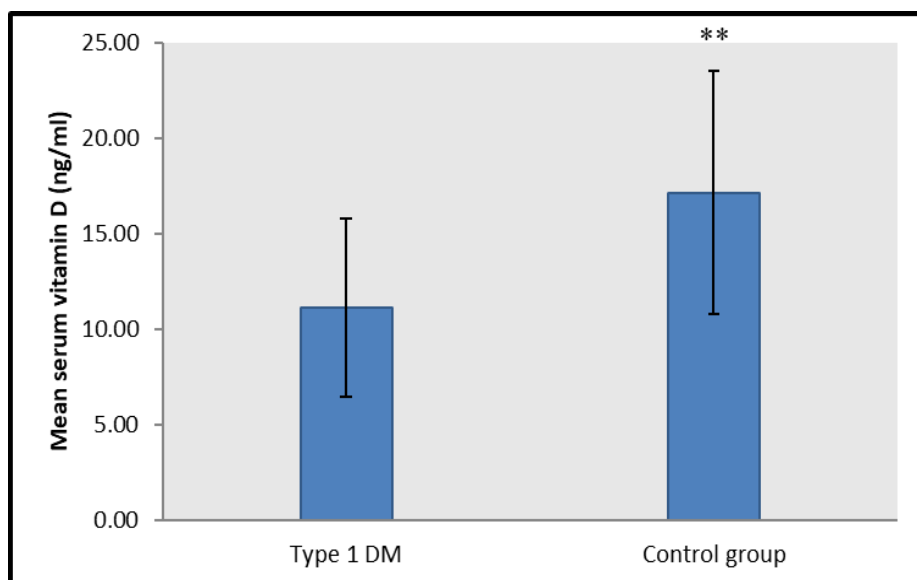


Figure 1: A bar chart comparing mean serum vitamin D levels in type 1 diabetes patients with the control

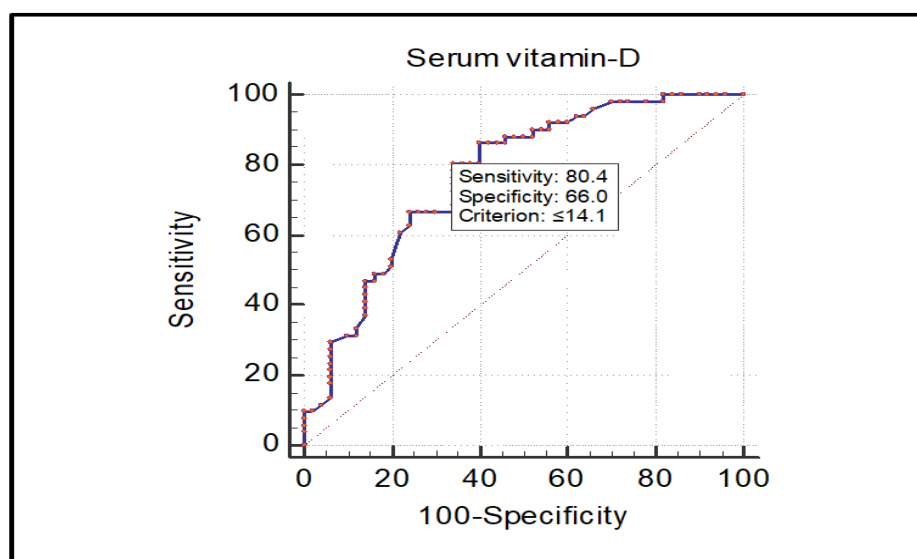


Figure 2: ROC curve analysis was used to determine the cut off value of serum vitamin D that can predict a diagnosis of type 1 diabetes mellitus.

Table 6: A comparison of blood median levels of IL-10 and IL-17 in type 1 diabetes patients and a control group

Characteristic	Type 1 DM No. = 51	Control group No. = 50	P
IL-10 level			
Median (IQR)	29.50 (11.60)	20.95 (5.70)	< 0.001 **
Range	25.00 -107.40	20.00 -152.20	NS
IL-17 level			
Median (IQR)	242.20 (140.90)	22.40 (12.20)	< 0.001 M **
Range	130.00- 320.00	20.00- 100.00	

DISCUSSION

In this study, the average age of children with type 1 diabetes was determined (8.43 ± 3.13 years) was lower than that reported by Almahfoodh et al., in 2017 in (15.3 ± 9 years) in a study done in Basra on 2536 patients diagnosed with type 1 diabetic mellitus; however, the mean age in another Iraqi study was comparable to ours, as the mean age of 60 patients diagnosed with type 1 DM in the Iraqi study was 9.8 ± 4.7 years (Mezher et al., 2011) [15]. In line with our study, Haleem in 2019, in Iraq, The prevalence rate of vitamin D deficiency was higher in children with type 1 diabetes than in the control group, and the mean blood vitamin D level of 50 children with type 1 DM was significantly lower than that of 50 control patients, 100 % versus 70 %. Therefore, we agree with Haleem (2019) [16]

in the high rate of serum vitamin d deficiency in both healthy children and diabetic children. In our study, thus, Patients with type 1 diabetes have higher than normal amounts of IL-10 in their serum. This result is consistent with what we consistent with (Gouda et al., 2018) [17]. These findings are in line with those of (Reis et al., 2012; He et al., 2014) [18], They discovered that the elevated IL-10 concentrations in T1DM patients may be the result of a compensatory mechanism that emerged in response to the surge in pro-inflammatory cytokines. In this way, the cytokines implicated in the pathophysiology of type 1 diabetes play a crucial role in the development of the condition. These cytokines have the ability to regulate inflammation in a pro-inflammatory or anti-inflammatory way, and they can do so either in a cascade or individually (Urcelay et al., 2004) [19], In addition, IL-10 ability to reduce inflammation has been established, which suggests that it may be useful in avoiding the development of diabetes.

CONCLUSION

Our study, is in line with the suggestion that there's a potential underlying relationship between vitamin D and glycemic control in diabetes. Therefore, it will be important to bear in mind of the actual fact that cholesterol deficiency is common in diabetes and treatment of the identical may help in improving the glycemic control. Vitamin D insufficiency and elevated levels of the immune proteins IL-17 and IL-10 are observed in children and adolescents with type 1 diabetes. The relationship between IL-10 and IL-17 can be used as a good diagnostic for the inflammatory state, and these inflammatory interleukins can be used as a biomarker in early screening for T1D, as well as a predictor of early diabetes complications, including nephropathy. IL-17 & IL-10 immune regulation and vitamin D supplementation could be viable targets for future T1D treatment research. Our study suggests that in children with T1D, there is a significant increase of IL-17 level as a major immune alteration and vitamin D deficiency induced inflammation of Langerhans' islets in the pancreas contributing to β -cell destruction in compared to unaffected children.

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