

Immunological Parameters Associated with Vitamin D3 and Ferritin Deficiency in Diabetic Patients

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Abstract

Objectives: The objectives of this research was to look into the relationship between Interleukin 10 (IL-10) , C-reactive protein, Deficiency of vitamin D3, ferritin and diabetes mellitus in diabetic patients who attended Ramadi Teaching Hospital and Fallujah Teaching Hospital – Anbar.

Method: The current study included 65 diabetic patients with deficiency of vitamin D3 (25 kinds 1 and 40 kinds 2). The diabetic patients' results were compared to 35 healthy individuals aged 16-69 years who served as a control group; ten milliliters of blood were drawn from each patient. The ELISA method was used to estimate vitamin D3 levels. The ferritin test is dependent on ferritin in the sample reacting with latex. Photometric analysis is used to determine ferritin levels. IL-10 in serum was measured using a commercially available kit and the ELISA method. Estimation of CRP based on the reaction of C reactive protein (CRP) and latex covalently bound antibodies against human CRP.

Results: Our findings revealed that all T1DM and T2DM participants had hypovitaminosis D3. Vitamin D3 revealed a non-significant difference between them but a significant difference between them and their controls. When compared to controls, ferritin levels in T1DM and T2DM patients were significantly lower. The study findings revealed that There was a significant ($p \leq 0.01$) decline in the level of Interleukin 10 in T1DM patients compared to healthy individuals (control) but no-significant differences between T1DM and T2DM, as well as a non- there is a significant difference in the amount of C-reactive protein in T1DM patients compared to T2DM. In the current study, we found a significant increase in C-reactive protein ($p \leq 0.01$) in both T1DM and T2DM when compared to controls.

Conclusion: Diabetes mellitus patients have low levels of vitamin D3 and ferritin. In this study, we found that the levels of Interleukin 10 in Patients with T1DM and T2DM had significantly lower than those in the control groups. While C-reactive protein levels in both T1DM and T2DM patients were significantly higher.

Keywords: Interleukin 10, C-reactive protein, Deficiency of vitamin D3, ferritin, diabetes mellitus.

Introduction

Today, diabetes mellitus is a common disease in the whole world, with a particular concern with its widely spread within the Arab countries. It is a chronic condition in which the blood glucose levels are high in blood (blood sugar), and this result over time in critical harm to the cardiovascular system, kidneys, eyes as well as nerves (1).

This disease affects individuals of various ages (adults and children), various economic conditions, various health status, and men and women. In addition, it is a disease that has enough capacity to damage the body (2). Current epidemiological studies have revealed the connection between vitamin D deficiency and diabetes mellitus, both kind 1 (T1DM) and kind 2 (T2DM). Furthermore, the impairment of glucose tolerance and diabetes has been found to upgrade in VD-deficient people. Deficiency of vitamin D, that might be a key factor for development of diabetes, is widespread in the whole world, vitamin D deficiency is estimated to affect one billion people worldwide. (3). After the detection of Vitamin D receptors (VDR) in the pancreas, the function of Vitamin D in diabetes became more apparent, skeletal muscle cells, adipose tissue, as well as immune cells that reveals a regulatory influence of Vitamin D on glucose homeostasis (4).

It is found that, Vitamin D is supposed to improve the body sensitivity toward insulin and therefore decrease the risk of insulin resistance, which often result in T2DM. Also, this vitamin is thought to might aid in regulating the insulin release from the pancreas as it can directly stimulate insulin synthesis and its release from pancreatic β -cells in addition to enhance the insulin receptor expression in peripheral tissues. Vitamin D also can indirectly give an ant-diabetic influence by working on cells of the immune system which produce pro-inflammatory cytokines as mediators concerning weight gain, systemic inflammation (contributing to insulin resistance), and autoimmune damage of pancreatic β -cells (5). The immune system as well as the inflammatory mechanisms was found to have a key role in the development and progression of diabetes specially kind 1 and kind 2 diabetes(6). In the study by Kopp et al. 2003, it was reported that, Chronic subclinical inflammation can be seen in elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6) and this is correlated to metabolic syndrome and cardiovascular diseases(7). So, there is relation between inflammation and insulin resistance which is bidirectional, meaning that any chronic inflammation enhances insulin resistance that, in turn, enhances inflammation(8).

IL-10 is a centrally functional cytokine that has strong anti-inflammatory characters. It is It's created by a variety of immune cells, including monocytes and

macrophages, and it's a potent inhibitor of pro-inflammatory cytokines and chemokines. (9).

Earlier studies have examined the IL-10 role within DM and it has been mostly in the direction of β -cells of the pancreas (10). In vitro studies have shown that IL-10 improves pancreatic β -cell function in response to glucose, Furthermore, IL-10 therapy has been found to hinder the onset of T1DM among non-obese diabetic mice and also prevent the return of the disease, deepening on the way and time of administration (11).

Materials and Methods

Patients Selection and Blood

Sampling:

The current study included 65 diabetic patients (25 kinds first and 40 kinds 2) with deficiency of vitamin D3, who attended Ramadi Teaching Hospital and Fallujah Teaching Hospital - Anbar during the period from September 2020 until January 2021. Their ages ranged between 16 and 70 years. Diabetes patients were diagnosed based on medical and laboratory reports as well. The results of the diabetic patients were parallel to 35 healthy individuals aged 16-69 years as a control group ten milliliters of blood samples were taken from each patient. Patient blood samples were obtained from the site of needle puncture in the patient's vein. The serum obtained was stored at -20 until the time of the assay. Venous blood samples were also taken from the control group using a disposable syringe and expelled to obtain blood serum.

Serum Levels of IL-10

The level of IL-10 was assessed in serum using commercially available kit (16.1.French, Cat No. 950.060.192). IL-10 ELISA is sandwich enzyme immunoassay for the quantitative measurement of IL-10 in pre-coated micro plate wells with monoclonal anti-IL-10 antibody.

CRP

The reaction between C reactive protein (CRP) and latex covalently bound antibodies against human CRP is the basis for this CRP test. CRP values are determined photometrically.

Determination of Serum Vitamin D^{(product code:7725-300(96T))}

Serum vitamin D was measured by Kit providing From (Monobind-U.S.A) Company using Competitive ELISA:

❖ Principle of ELISA

1. Incubation of unlabeled antibody with the sample antigen.
2. Addition of the complex to the 96 well plates which coated with the same antigen in the incubated complex.

3. Washing of the unbound antibody away from the plate.
4. The presence of more antigens in the sample leads to decrease antibodies binding to the antigens in the well, so the method is termed competitive.
5. Then, a secondary antibody conjugated with the enzyme is added to the well.
6. Finally, a substrate is added and enzymes extract a chromogenic or fluorescent response.

Ferritin

This Ferritin test is based upon the reactions between Ferritin in the sample and latex covalently bound rabbit antihuman Ferritin antibodies. Ferritin values are determined photometrically.

Results and Discussion

Immunity

The data of this study showed that there was significant decrease ($p \leq 0.01$) in level of Interleukin 10 in T1DM patients (17.05 ± 0.490 Pg/ml) compared with healthy individuals (control) but no-significant differences between T1DM and T2DM, whereas the level of C-reactive protein in T1DM patients (1.78 ± 0.067 mg/dl) was non-significant difference with T2DM (3.18 ± 0.092 mg/dl).

As shown in table [4.2], both T1DM and T2DM had a significant increase ($p \leq 0.01$) in C-reactive protein as compared to controls.

Cytokines are immune system hormones that enable immune cells to communicate with each other and control the overall immune response, One of them is IL-10, which is generated by the majority cells of innate and adaptive immune response. **(12)**. IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29 are members of the IL-10 family of cytokines. They perform a variety of biological functions, including maintaining epithelial layers integrity and fostering innate immune responses to pathogenic infections, promoting tissue healing in injuries, suppressing proinflammatory responses, and limiting excessive tissue disturbances **(13)**.

C-reactive protein (CRP) levels in the blood of healthy people are quite scarce,, but they can spike dramatically in response to tissue damage and inflammation caused by trauma and infectious and non-infectious diseases. As a result, inpatients' CRP plasma

is routinely monitored to determine the seriousness of their initial illness and injury, as well as their subsequent reaction to therapy and return to health. **(14)**.

In this study, we demonstrated that the levels of Interleukin 10 in T1DM and T2DM patients were significantly decreased comparing with the normal groups, Although C-reactive protein levels were significantly increased in both T1DM and T2DM patients. Other researchers also showed same results such as **(15)** who indicated that the serum levels of IL-10 in T2DM patients compared to controls. Based on this fact, it may be concluded that low serum levels of IL-10 can be considered as a risk factor of T2DM.

C-reactive protein (CRP) is a plasma protein that is pentameric and highly conserved during the acute phase of inflammation. Increased levels of inflammatory cytokines, particularly interleukin-6, cause transcriptional induction of the CRP gene, which occurs primarily in hepatocytes in the liver (IL-6) **(16)** It was found that people and experimental animals with T2DM have inadequate IL-10 secretion and that IL-10-592 gene polymorphism is closely related to T2DM onset **(17)**.

Obesity, hypertension, heavy drinking, smoking, and a lack of physical activity have all been linked to chronic inflammation with elevated CRP levels. **(18)**. Drugs for treating T2DM have recently been found to reduce serum CRP levels, according to reports. It's still unclear if high CRP levels cause T2DM. **(19)**.

Increased CRP levels are linked to an increase in microalbuminuria in patients with kind 1 or kind 2 diabetes. according to may evidence, Inflammatory and metabolic factors associated with diabetes, such as high glucose, adipokines, and modified lipoproteins, are linked to blood levels of CRP and can cause its production. **(20)**.

Diabetes kind 1 is now identified as a chronic immunoinflammatory condition. It's not unexpected that elevated CRP levels were discovered because it's an inflammatory disorder affecting both the innate and adaptive immune systems. **(21)**.

Vitamin D3 and ferritin

As the normal value of Vitamin D ranges from 30 to 70 ng/dL, our results showed that all T1DM and T2DM participants were suffering from hypovitaminosis D3. Most of those with deficiency of vitamin D3 were in the T2DM group the mean of T1DM and T2DM (15.15 ± 0.477 ng/ml), (13.90 ± 0.298 ng/ml) respectively for Vitamin D3 showed that there is non-significant difference between them but significant difference with their controls as shown in table [1].

Vitamin D is a steroid hormone that has been associated to the prevention of kind 2 diabetes mellitus (DM). Vitamin D regulates adipogenesis during adipocyte differentiation, induces insulin production, protects pancreatic B cells, and reduces insulin resistance in muscles, all of which are important factors in the development of kind 2 diabetes. (22).

The preservation of calcium and phosphorus homeostasis is vitamin D's most well-known feature. Vitamin D deficiency causes secondary hyperparathyroidism, which causes increased calcium release from the skeleton, resulting in osteopenia or osteoporosis. This is important because bone demineralization is a symptom of osteoarthritis (Charcot foot) in diabetic patients. (23).

Categories	Mean $\pm$ SE	
	Interleukin 10 (Pg/ml)	C.R.P (mg/dl)
Control No.15 T1DM	27.00 ± 0.793 A	0.41 ± 0.019 A
Patient No.25 T1DM	17.05 ± 0.490 B	1.78 ± 0.067 B
Control No.20 T2DM	29.00 ± 0.548 A	0.38 ± 0.022 A
Patient No.40 T2DM	16.28 ± 0.622 B	3.18 ± 0.092 B
Various Letters: Mean there is significant difference at $p \leq 0.01$		

Table 4.2: Mean $\pm$ SE for Interleukin 10 and C.R.P in study group

A research examining the relationship between vitamin D3 serum and kind 2 diabetes mellitus discovered that the means vitamin D3 serum level in kind 2 diabetes mellitus was significantly lower than in controls ($p=0.007$). When compared to people with average vitamin D levels, people with deficiency of vitamin D3 had 3.4 times the risk of developing kind 2 diabetes. The negative association ($p=0.026$) between vitamin D3 serum and kind 2 diabetes mellitus was found to be significant ($r= -0.351$) in a correlation test. According to the results, vitamin D deficiency is related to kind 2 diabetes mellitus. (24). Collecting evidence proposes that vitamin D deficiency increases the risk of kind 2 diabetes (T2DM). Vitamin D Deficiency is connected with

both resistant against insulin and beta cell dysfunction (25). In kind 1 diabetes mellitus (T1DM), a similar connection has been discovered. (26).

The effect of vitamin D on glucose and insulin metabolism is due to a lot of processes. According to some animal studies, vitamin D can directly stimulate pancreatic insulin secretion. (27).

Diabetic ketoacidosis and vitamin D deficiency are related. Vitamin D levels were slightly reduced in diabetic ketoacidosis, which led to the development of diabetic ketoacidosis. These findings suggest the examination of vitamin D. These results indicate that patients with diabetes should have their vitamin D levels tested and vitamin D supplements provided to those who are deficient in order to avoid diabetic ketoacidosis. (28).

As shown in table [4.3] there is a significant decrease in mean of Ferritin ($p \leq 0.01$) in T1DM patients (48.20 ± 0.439 mg/dl) and T2DM patients (81.56 ± 0.563 mg/dl) comparing with their controls (48.20 ± 0.439 mg/dl) and (72.32 ± 0.670 mg/dl) respectively.

In terms of insulin resistance, vitamin D helps to prevent the development of diabetes by increasing the expression of the insulin receptor, which helps to maintain the insulin signaling pathway. Vitamin D plays a key role in regulating the secretion of adipokines, which are crucial for maintaining glucose and lipid homeostasis. It reduces the intake of unnecessary metabolites by controlling the development of leptin, which suppresses appetite. Vitamin D also reduces inflammation, which is a significant factor in the inducing of insulin resistance. (29).

Table 4.3: Mean $\pm$ SE for Vitamin D3 and Ferritin in study group

Categories	Mean $\pm$ SE	
	Vitamin D3 ng/ml)(	Ferritin mg/dl)(
Control No.15 T1DM	37.93 ± 0.700 A	40.00 ± 0.516 A
Patient No.25 T1DM	15.15 ± 0.477 B	48.20 ± 0.439 B
Control No.20 T2DM	37.40 ± 0.716 A	72.32 ± 0.670 A
Patient No.40 T2DM	13.90 ± 0.298 B	81.56 ± 0.563 B
Various Letters: Means there is significant difference at $p \leq 0.01$		

Conclusion

Vitamin D3 and ferritin levels were observed to be decreased in T1DM and T2DM patients in our study. It is possible to conclude that deficiency of vitamin D3 and ferritin deficiency are linked to diabetes mellitus. our study discovered that the levels of Interleukin 10 in T1DM and T2DM patients were significantly decreased, on the other hand the level of C-reactive protein was significantly increased in both T1DM and T2DM patients.

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