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# Investigation of Vitamin D Levels in Iraqi Patients with Type 2 Diabetes Mellitus: A case- control study

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**Abstract**

**Objective:** Vitamin D deficiency is common in most regions of the worldwide. Its deficiency or insufficiency is implicated in cardiac disease, infectious diseases, bone disorders, autoimmune and metabolic diseases, such as type 2 diabetes mellitus (T2DM). This study aims to investigate the association between vitamin D levels and development of T2DM in Iraqi population.

**Methods:** A case-control study was conducted with 200 T2DM patients and 200 healthy control groups. Standard methods were used to determine vitamin D, insulin, and fasting blood glucose (FBG) levels. A homeostatic model assessment (HOMA-IR) was used to evaluate insulin resistance (IR).

**Results:** The patients' body mass index (BMI), fasting blood glucose, vitamin D levels, insulin levels, and insulin resistance were all significantly higher in patients with T2DM than in the healthy control group ($P<0.05$). Vitamin D deficiency and insufficiency were observed to be (20.5%, 28%) in the healthy control group and (37%, 35.5%) in the T2DM group, respectively. Individuals with vitamin D deficiency (less than 20 ng/ml) had a three-fold increased risk of T2DM, whereas those with vitamin D insufficiency (20–30 ng/ml) had a two-fold increased the risk. Vitamin D levels were negatively correlated with BMI, FBG, insulin levels, and insulin resistance (IR).

**Conclusions:** Deficiency and insufficiency of serum levels of vitamin D were found to be a risk factors for the development of T2DM in the Iraqi population and direct the development of insulin resistance.

**Keywords:** T2DM; Vitamin D; Insulin Resistance; Vitamin D insufficiency

## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex chronic metabolic disease that has become a serious healthcare issue [1]. According to the World Health Organization's (WHO) most current 2016 data, 422 million individuals worldwide have diabetes mellitus, with the number expected to rise to 642 million by the end of 2040 [2]. The global prevalence of diabetes among individuals over the age of 18 has increased from 4.7% in 1980 to 8.5% in 2014. T2DM accounts for 90% of cases, with no sex predominance [3]. T2DM is indicated by pancreatic $\beta$-cell dysfunction, systemic inflammation, hyperglycemia due to defects in insulin secretion, insulin sensitivity, or both [1].

Colecalciferol, often known as vitamin D3 or calcitriol, is a steroid hormone that is involved in a variety of cellular and molecular functions, including calcium and magnesium metabolism and, as a result, appropriate bone mineralization [4,5]. Vitamin D is synthesized in the skin of mammals under the effect of ultraviolet light on some provitamins such as 7-dehydrocholesterol. This process begins with the rapid production of previtamin D3, which is subsequently transformed to the active form of vitamin D3 over time [6]. The liver and kidneys complete subsequent steps [7]. Vitamin D3 is converted to 25-hydroxycolecalciferol (calcidiol) in the liver by 25-hydroxylase, which is subsequently metabolized to calcitriol (1,25-dihydroxycolecalciferol) or in the proximal renal tubules by the 1-hydroxylase [6]. Vitamin D level more than 75 nmol/l (30 ng/ml) is considered typical for vitamin D, whereas, deficiency is considered as a serum vitamin D levels is less than 20 ng/mL (50 nmol/L). In other hand, 50–75nmol/l (20–30 ng/ml) is defined as vitamin D insufficiency [8].

Vitamin D is related to the pathogenesis of metabolic syndrome and T2DM, such as reduced β-cell function and insulin sensitivity, through direct activation of vitamin D receptors or indirect effects by calcium

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homeostasis modulation [9]. Although there is increasing evidence to link vitamin D deficiency to diabetes, little is known about the effects of vitamin D status on glycemic control and vice versa [3].

Vitamin D deficiency is now recognized as a global public health issue. In 2008, it was reported that one billion people were suffering from vitamin D deficiency or insufficiency, and the number is rising as days pass on [10]. In addition to bone metabolism, recent data suggests that vitamin D is involved in a number of other mechanisms. Its role implicated in impaired glucose metabolism and type 2 diabetes mellitus [3,11]. Vitamin D deficiency, according to some authors, may lead to glucose intolerance, abnormal insulin secretion, and T2DM, either directly by vitamin D receptor (VDR) activation or indirectly by calcemic hormones and inflammation [12,13]. In this study, we examined the association between vitamin D levels and T2DM in the Iraqi population and compared it to a healthy control group. Also, we examined the correlation between serum levels of vitamin D and clinical with some anthropometric parameters.

## 2.Materials and methods

### 2.1 Study subjects

A case-control study was carried out in the Diabetic Center of Al-Sader Teaching Hospital, Al-Najaf, from February 1, 2021, to June 25, 2021. A total number of 400 individuals were included, 200 patients with T2DM as a patient group and 200 healthy individuals without type 2 diabetes mellitus from Al-Najaf province was used as control group. The patients and controls were matched for age, ethnicity, and gender.

The American Diabetes Association criteria were used to diagnose T2DM patients by a specialist physician [14]. The control group consisted of individuals who seemed to have no prior history of T2DM and included relatives of non-diabetic patients from non-diabetic centers. Patients with T2DM who are taking oral hypoglycemic drugs and have a proven history of T2DM. Pregnant and breastfeeding women, participants using vitamin D or calcium supplements, subjects with liver diseases, renal diseases, cancer, pigment abnormalities, and other chronic medical diseases were all excluded from both the control and T2DM groups.

Biochemical assessments of this study was conducted in the laboratory of the biochemical department, faculty of medicine, university of Kufa. The research protocol was approved by the ethics committee of the university of Kufa and patients gave informed written consent.

### 2.2 Measurements

The analysis of serum vitamin D levels, and clinical parameters such as fasting blood glucose (FBG) and insulin levels were computed during the initial consultation and examination by ELISA and spectrophotometer, respectively. Serum concentrations of vitamin D and insulin were measured by electrochemiluminescence immunoassay (ECLIA) method on Roche Hitachi E170 (Roche/Hitachi MODULAR Analytics Combination Systems, Roche Diagnostics, USA). FBG was measured by spectrophotometer. Insulin resistance (IR) was calculated from the following equation: IR = Fasting insulin (mU/l) × Fasting glucose (mmol/l) / 22.5.

According to the American association of clinical endocrinology, vitamin D status in T2DM patients has been classified using the serum circulating 25(OH) D3 level, as measured by a reliable assay, as follows: A vitamin D levels of more than 75 nmol/l (30 ng/ml) is considered normal, whereas a 25(OH)D3 concentration of 50-75 nmol/l (20–30 ng/ml) is considered insufficient, and a 25(OH)D3 levels of less than 50 nmol/l (20 ng/ml) is considered deficient.

### 2.3 Statistical analysis

All the analyses were carried out by using SPSS software (ver. 21.0). All data were expressed as means ± standard deviation (SD). The data was checked for normality using the Kolmogor3ov-Smirnov test for Gaussian distribution, and descriptive statistics were obtained. For comparison of parameters with normal distribution, parametric tests were used (Student´s t-test and ANOVA). Pearson's correlation coefficients were used to analyze the relationships between variables. A statistically significant p-value was defined as less than 0.05.

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## 3. Results

### 3.1 Characteristics of studied groups

The anthropometric and clinical features of the participants were shown in Table 1. This study included a total of 200 patients with T2DM from the diabetic center and 200 healthy controls during a five-month period. There was no statistically significant difference in age or gender between the two studied groups (P > 0.05). Mean BMI, FBG, insulin levels, and IR were significantly higher in patients with T2DM than healthy control groups (P<0.05). While there were significant differences in vitamin D status in patients compared with the healthy control group (P< 0.05).

### 3.2 Vitamin D status and type 2 diabetes mellitus

Distribution of vitamin D status indicated significant increases of carriers of the insufficient levels (OR: 2.37, CI 95%: 1.47- 3.83, P = 0.0001) and deficient levels (OR: 3.38, CI 95%: 2.04 – 5.58, P = 0.0001) in patients with T2DM when compared with those of the normal levels, suggesting a risk factor for developing T2DM by 2 and 3 for insufficient and deficient, respectively (Table 2).Variations in FBG, insulin and the insulin resistance values seemed to be dependent on the vitamin D status distribution in patients group as shown in figure 1.

### 3.3 Relationships between vitamin D status and clinical parameters

The correlation between serum levels of vitamin D and clinical with some anthropometric parameters were shown in figure 2. Figures 2a, 2b, 2c, and 2d show a significant negative correlation between serum levels of vitamin D and FBG (r =-0.296, P = 0.001), BMI (r =-0.402, P = 0.001), insulin levels (r =-0.296, P = 0.001), and insulin resistance (r =-0.402, P = 0.032) among 200 patients with T2DM.

## 4. Discussion

The finding of this study vitamin D status revealed that insufficient vitamin D levels raised the risk of T2DM by 2 times when compared to normal group. On the other hand, vitamin D deficiency increased the risk of T2DM by three times. These findings strongly suggest that vitamin D may play an important role in the pathogenesis of T2DM in Iraqi individuals. These results could provide an understanding of the mechanisms by which T2DM develops in the Iraqi population. As a consequence, it may lead to improved protection, diagnosis, and treatment methods for the Iraqi population. Vitamin D increases the insulin exocytosis directly or indirectly by activating calcium-dependent endopeptidases, as it is the key regulator of calcium homeostasis [15]. The current study added to the evidences that vitamin D insufficiency and deficiency are more common in T2DM patients, as we found that 37 % and 35.5 % of total T2DM patients were vitamin D deficient and insufficient, respectively. Our results are attributed to the fact that vitamin D deficiency is associated to decreased insulin sensitivity, insulin resistance, and impaired β-cell function, all of which are major issues in T2DM [3,16]. Our findings comes consistent with those of Salih et al and Hetta et al. studies, who demonstrated that the prevalence of vitamin D deficiency / insufficiency was (60% and 45%), (32.7% and 40.6%), respectively, in T2DM patients [15]. Our analyses indicated a significant negative association between serum vitamin D levels and BMI, which is consistent with previous studies [15,17].

These findings could be explained by the circumstance individuals with a high BMI had reduced amounts of exercise and sunlight exposure, as well as adipose tissue sequestration of 25-hydroxyvitamin D [18].

In our investigation, we found a significant inverse correlation between serum levels of vitamin D and insulin resistance, which agrees with Chiu et al. and Zhang et al. findings in T2DM patients with severe vitamin D deficiency [19,20]. Our findings were attributed to the fact that vitamin D plays an important role in the regulation of glucose-insulin homeostasis. The active form of vitamin D [1,25 (OH) 2D] attaches to the vitamin D receptors (VDRs) in the cell and stimulates the retinoid X receptors (VDR-RXR) complex. This complex interacts with a vitamin D transcription factor in the human insulin receptor gene's in promoter region, improving insulin sensitivity for glucose transport. The role of 1,25(OH)2D in the regulation of intracellular calcium (through the cell membranes in insulin target tissues) and activation of peroxisome proliferator-activated receptor delta (PPAR- δ), a signaling cascade that mediates fatty acid metabolism in adipose tissue and skeletal muscle, may explain its potential effect on improving insulin sensitivity (IS) [21,22]. Previous studies on the correlation between vitamin D deficiency and IR, IS, and

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T2DM have been inconsistent, with many studies finding a significant association [23, 20], whereas no difference with previous [24, 25].

The main finding of our study highlights the relationship between vitamin D status and T2DM in Iraqi population. In the Iraqi population, very few studies have been conducted related to vitamin D and T2DM. Thus, the current study provides a baseline for future studies that will investigate the relationship between vitamin D levels and T2DM.

**●Abbreviation List**

T2DM: Type 2 diabetes mellitus
DM: Diabetes mellitus
FBG: fasting blood glucose
Vit. D: Vitamin D
HOMA-IR: homeostatic model assessment
BMI: Body mass index
VDR: Vitamin D receptor
ECLIA: Electrochemiluminescence immunoassay
RXR: Retinoid X receptors
PPAR- δ: Peroxisome proliferator-activated receptor delta

**●Competing interests**

The authors declare that they have no competing interests.

**●Authors' contributions**

Fieldwork and statistical analyses were done by FFESA.

**●Acknowledgments**

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**Table 1: Anthropometric and clinical characteristics of studied groups**

| | **Controls (n=200)** | **T2DM (n=200)** | **P-Value** |
|---|---|---|---|
| **Anthropometric Parameters** | | | |
| Males (%) | 87 (44%) | 91 (45%) | - |
| Females (%) | 113 (56%) | 109 (56%) | - |
| Age (years) | 48.02 ± 4.44 | 49.16 ± 5.61 | 0.113 |
| BMI (kg/m$^2$) | 24.77 ± 4.01 | 26.62 ± 4.21 | 0.001* |
| **Clinical Parameters:** | | | |

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| | | | |
|---|---|---|---|
| FBG (mg/dL) | 86.80 ± 9.44 | 207.35 ± 60.77 | 0.000* |
| Insulin levels (mU/L) | 16.33 ± 4.61 | 28.07 ± 1.22 | 0.000* |
| Insulin resistance (IR) | 4.1 ± 1.01 | 20.07 ± 4.29 | 0.000* |
| Vitamin D levels (ng/mL) | 31.12 ± 5.04 | 20.44 ± 9.35 | 0.000* |

BMI, FBG, insulin levels, insulin resistance, and vitamin D levels are statistically significant in patients compared with the control group. P* < 0.05 = significant

**Table 2: The distribution of vitamin D status among studied groups**

| Vitamin D status | Controls (n=200) | T2DM (n=200) | P-Value | OR | 95% CI |
|---|---|---|---|---|---|
| Normal > 30 ng/mL | 103 (51.5%) | 55 (27.5%) | - | - | - |
| Insufficient (20–30 ng/mL) | 56 (28%) | 71 (35.5%) | 0.000* | 2.37 | 1.47- 3.83 |
| Deficient < 20 ng/ mL | 41 (20.5%) | 74 (37%) | 0.000* | 3.38 | 2.04 – 5.58 |

Insufficient and deficient levels of vitamin D increase the risk of T2DM by 2 and 3 folds, respectively. P* < 0.05 = significant

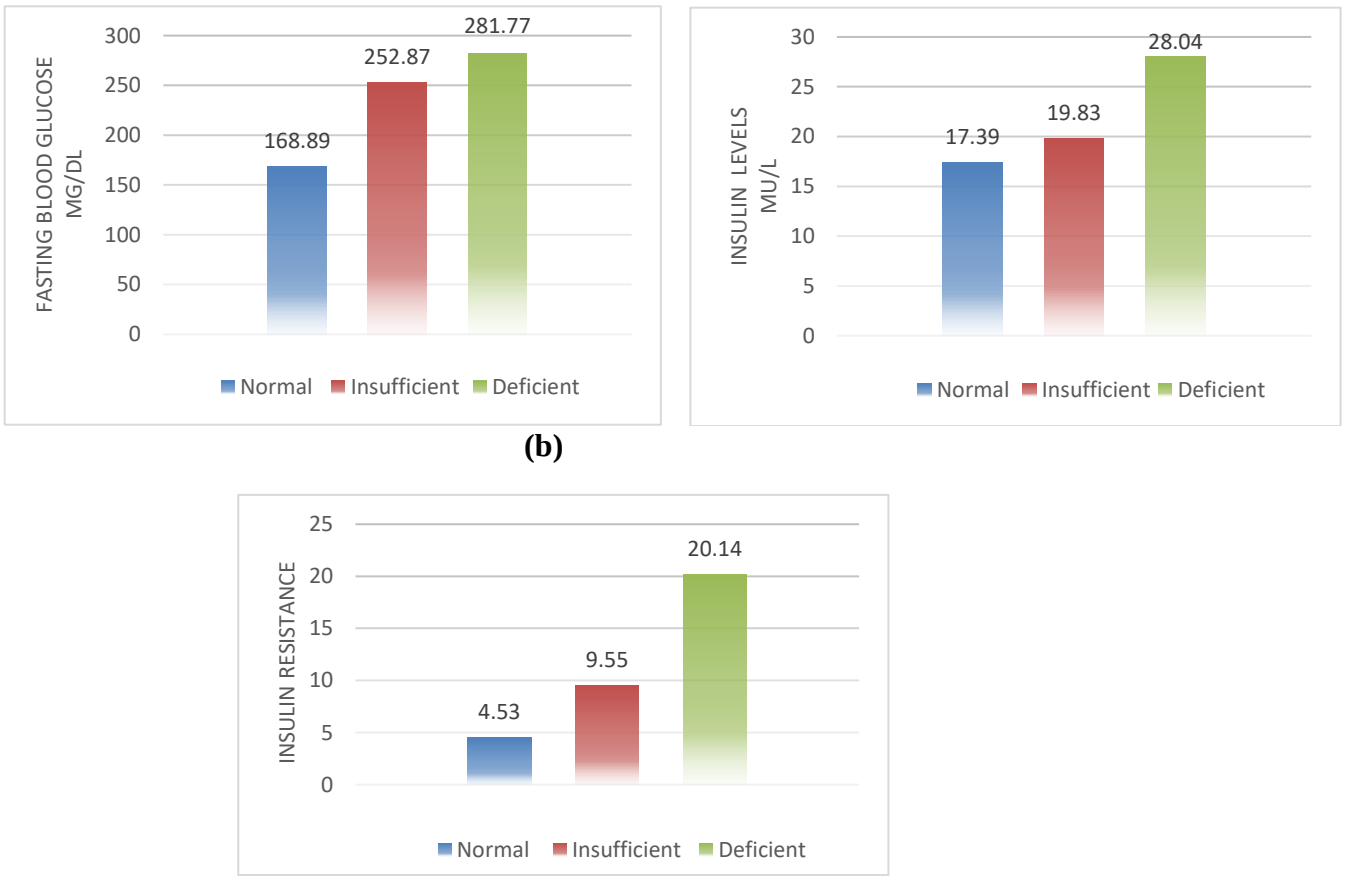


Fig.1: Distribution of clinical parameters (a) FBG (b) insulin levels (c) insulin resistance in patient group based on vitamin D status, fasting blood glucose, insulin levels, and insulin resistance were significantly higher in deficient status compared with insufficient and normal status

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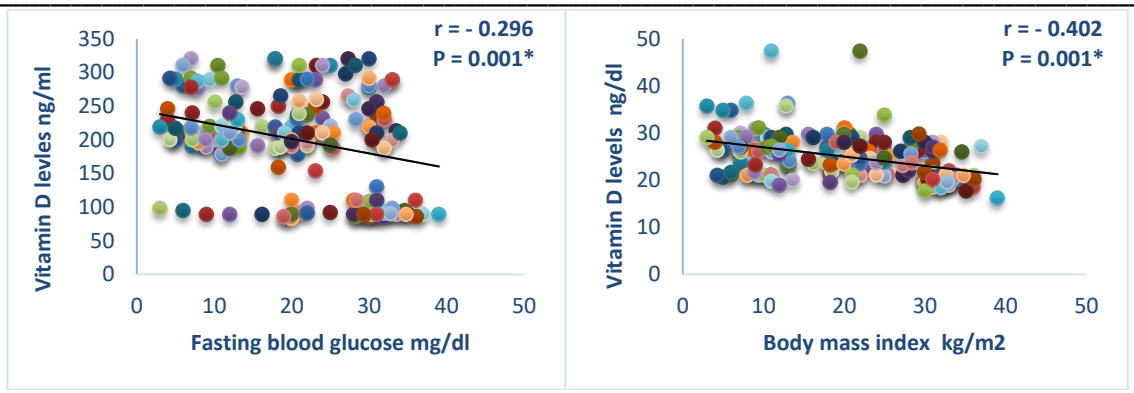


Fig.2: Relationship between serum level of vitamin D and (a) FBG (b) BMI (c) Insulin levels (d) insulin resistance(IR) in patients with T2DM. Each of the following has a significant negative correlation with serum vitamin D levels: (a) FBG (r = - 0.296, P= 0.001), (b) BMI (r = - 0.402, P = 0.001), (c) insulin levels (r =- 0.473, P = 0.001), (d) insulin resistance (r =- 0.152. P= 0.032*). $P^{*} < 0.05$ = significant