Evaluation of Serum Levels of Vitamin D in Patients with Type 2 Diabetes Mellitus on Metformin Therapy: A Cross-Sectional Controlled Study

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Abstract
BACKGROUND Vitamin D is well-known for its primary dominant role in maintaining calcium and phosphate concentrations in blood, yet it is associated with the stimulation of insulin secretion from pancreatic beta cells.OBJECTIVE The aim of the study is to measure serum level of vitamin D and to investigate its relationship with other parameters in type 2 diabetes mellitus patients.SUBJECTS AND METHODS: This is a cross-sectional controlled study was carried out during the period from December 2021 to the end of March 2021 at private internal medicine clinics in Tikrit city, Iraq. The study included 90 participants, 60 type 2 diabetes mellitus patients on metformin therapy designated as (metformin group) and 30 type 2 diabetes mellitus without metformin therapy considered as (control group). Blood samples were assessed for serum vitamin D using Cl-900i Chemiluminescence Immunoassay Analyzer and glycated hemoglobin HbA1c using AFIAS Analyzer in all metformin and control group.RESULTS:The study showed no significant difference in serum vitamin D and HbA1c levels between metformin and control group. Highly Significant negative correlations were found between vitamin D and diabetes mellitus (DM) duration and HbA1c ($P < 0.01$, $P < 0.001$, respectively). Significant negative correlations were found between vitamin D and metformin dose ($P < 0.05$, respectively). CONCLUSION: The results showed that type 2 diabetic patients had low vitamin D levels and strongly validate the role of vitamin D deficiency in etiology of type 2 diabetes mellitus. Correction of vitamin D may help to improve disease outcome.
Keywords: Type 2 Diabetes mellitus (T2DM), Vitamin D, HbA1c.

1. Introduction

Diabetes mellitus (DM) is regarded as a serious health issue, and its effects are significant cause of morbidities and fatalities worldwide [1]. The most common kind of diabetes, type 2 diabetes mellitus (T2DM), primarily affects adults and responsible for more than 90% of diabetes cases globally [2, 3]. It is defined by characteristic hyperglycemia that arises from defective insulin receptors responsiveness coupled with dysregulated insulin secretion from pancreatic beta cells [3, 4]. The hallmark of T2DM is the emergence of insulin resistance, a state of insulin inability to regulate blood glucose [5]. The development of T2DM is associated with long-term problems [6].

Vitamin D, also known as calciferol, is secosteriod hormone that is lipid soluble having hormone-like properties. Calcium and phosphate balance depend on the hormone calcitriol, which is the active form of vitamin D, known as 1,25-dihydroxy vitamin D (1,25$(OH)_2$D) as well, is produced mostly in the kidney [1, 7]. Vitamin D Receptors are expressed in various tissues including pancreas, therefore; Vitamin D is associated with stimulation of insulin secretion [8] either directly by acting on pancreatic beta cells or indirectly by regulating calcium concentration, extracellularly [9].

By influencing insulin sensitivity, cell function, or both, vitamin D has been shown to play a significant role and be a risk factor in the emergence of insulin resistance and the pathogenesis of T2DM [10, 11]. Many studies have explored the correlation between vitamin D and T2DM and found an inverse relation between vitamin D and HbA1c in T2DM patients in individuals without history of DM [12, 13, 14].

The aim of the study is to measure serum level of vitamin D and to investigate its relationship with other parameters in type 2 diabetes mellitus patients.

2. Subjects and Methods

This is a cross-sectional controlled study was carried out during the period from December 2021 to the end of March 2021 at private internal medicine clinics in Tikrit city, Iraq. The study included 90 participants, 60 T2DM patients on metformin therapy designated as (metformin group) and 30 T2DM without metformin therapy considered as (control group). Investigations included serum vitamin D, glycated hemoglobin Ac (HbA1c), BMI (body mass index), DM duration and metformin dose. Serum vitamin D levels were measured by Cl-900i Chemiluminescence Immunoassay Analyzer, expressed in units of (ng/ml) and HbA1c levels by AFIAS Analyzer, expressed in units of percentage (%).

The included diabetic patients aged (20-79 years old) men and women, not receiving metformin (for control group), early diagnosed T2DM, receiving metformin for at least 6 months (for metformin group). Exclusion criteria are those with type 1 diabetes mellitus, liver and pancreatic disease, documented diagnosed reasons for malabsorption (gastrointestinal disturbances), oral and/or intramuscular vitamin D supplements, consuming alcohol and pregnant women.

The study was approved by the Scientific Committee at

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Tikrit University - College of Medicine, and the agreement of the attendance to Salah al-Din General for collecting the samples from the patients was approved via the Directorate of Salah al-Din Health. Each patient was educated about the research's purpose of study, filled out questionnaire and signed a consent form to participate in the study.

Metformin and control groups were subjected to the following biochemical investigations: HbA1c was considered as (normal range = 4.5-6.5 %), and serum vitamin D levels were categorized into: (Deficient < 20 ng/ml), (Insufficient = 20-30 ng/ml), (Sufficient > 30 ng/ml). All patients signed an informed consent to take part in the study and the study was approved by ethical committee of Tikrit University, College of Medicine. All data were presented as mean and standard deviation (SD). Statistical analysis was implemented with correlation analysis and t-test. A p value of less than 0.05 was regarded significant. Analysis was performed by IBM SPSS Statistics for Windows version 23.0.

3. Results

The (mean ± SD) age of metformin group was (51.13 ± 10.76 years), whereas in control group was (49.80 ± 11.29 years). The clinical characteristics of diabetic patients' BMI, DM duration and metformin dose for metformin group were shown in (Table 1) and (Figure 1).

Table 1: Clinical characteristics of studied groups.

Parameter	Metformin Group (Mean ± SD)	Control Group (Mean ± SD)	P value
Age (years)	51.13 ± 10.76	49.80 ± 11.29	0.12
DM Duration (years)	4.90 ± 2.67	2.23 ± 1.54	< 0.05
BMI (kg/m^2)	29.10 ± 4.86	27.19 ± 6.09	0.37
Metformin Dose (mg/day)	1337.50 ± 516.55	-	-

The study showed no significant difference in mean serum vitamin D and HbA1c levels between metformin and control group (P > 0.05) as demonstrated in (Table 2) and (Figure 1).

Table 2: Comparison between metformin and control group regarding vitamin D and HbA1c levels.

Parameter	Metformin Group (Mean ± SD)	Control Group (Mean ± SD)	P value
Vitamin D (ng/ml)	19.84 ± 5.69	21.15 ± 7.36	0.092
HbA1c (%)	8.12 ± 1.14	8.78 ± 1.76	0.25

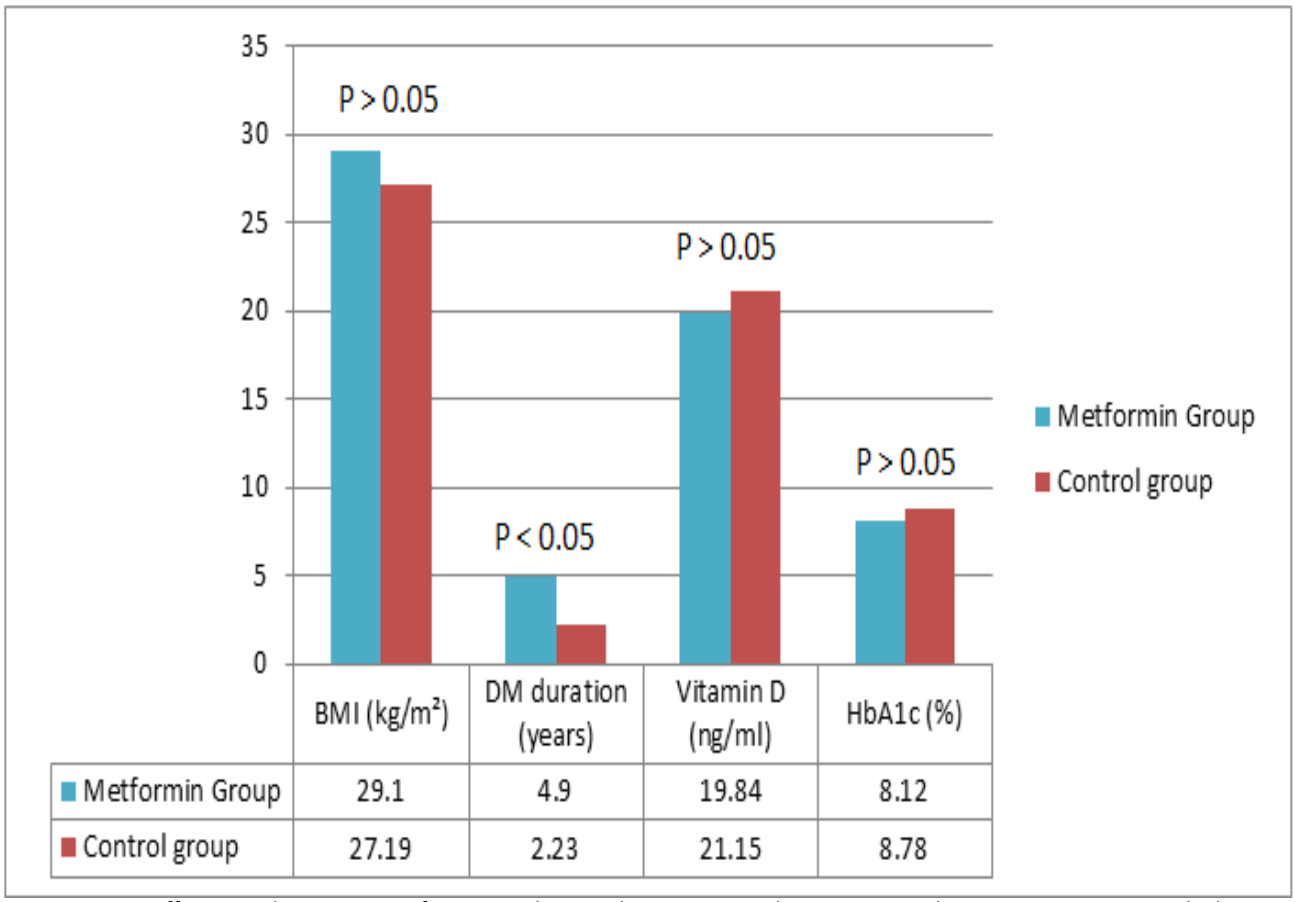

Figure 1: Differences between metformin and control group regarding BMI, DM duration, Vitamin D and HbA1c level.

Correlations were carried out between vitamin D levels and different parameters. Highly significant negative correlations were found between vitamin D and DM duration (r = -0.277; P < 0.01), as illustrated in (Figure 2) and HbA1c (r = -0.635; p < 0.01), as depicted in (Figure 3). Significant negative correlations were found between vitamin D and metformin dose (r = -0.304; P < 0.05), as shown in (Figure 4).

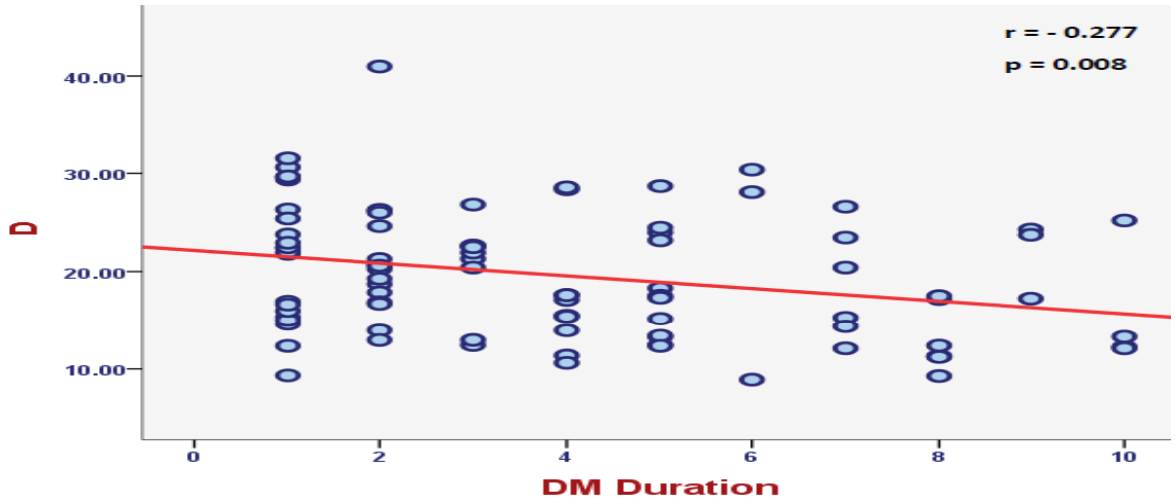

Figure 2: Correlation of vitamin D (ng/mL) with DM duration (years) in T2DM patients.

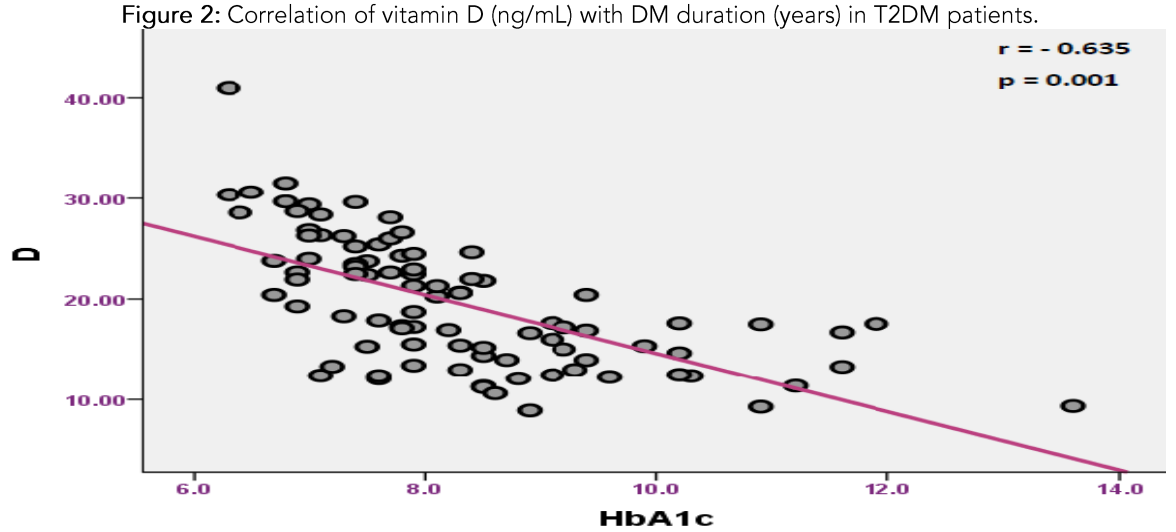

Figure 3: Correlation of vitamin D (ng/mL) with HbA1c (%) in T2DM patients.

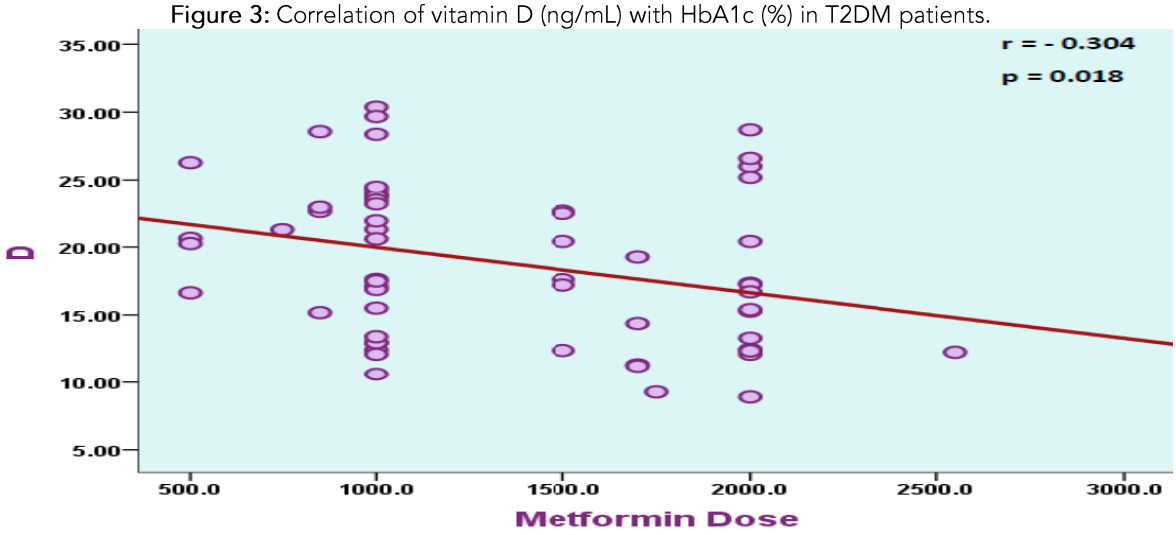

Figure 4: Correlation of vitamin D (ng/mL) with metformin dose (mg/day) in T2DM patients.

4. Discussion

Vitamin D levels did not differ significantly between metformin and control group (P > 0.05), as shown in (Table 2) and (Figure 1). Such finding is in agreement with Out M *et al.* [15] whom mentioned that during 16 months, metformin had no impact on serum D levels compared to placebo of T2DM individuals. Moreover, other study stated no statistically significant difference in serum vitamin D levels between users and nonusers of metformin [16]. It is important to mention that the time of sample collection during the winter season and daytime work hours and the fact the women enrolled wear an Islamic Abayah and Hijab which covers a vast surface area [17] minimizes the exposure to sunlight possibly explain the low levels in both groups and insignificant decrease between them. In contrast, Masliy [18] reported that T2DM patients using metformin had a higher frequency of vitamin D deficiency than those in the healthy group. The noteworthy reasons of this comparable variation may be related to hormonal changes, methodological differences, age and nutritional practices that have a big impact on vitamin D.

As for HbA1c results, there was no significant difference between metformin and control groups (P > 0.05), as demonstrated in (Table 2) and (Figure 1). Consistently, Sahin et al. [19] showed that after (6 weeks) of metformin

use it decreased HbA1c levels in patients with newly diagnosed diabetes. Perhaps, diabetic patients upon their new diagnosis are more commitment to therapy, improved diet, respond better and have still preserved pancreatic function. In disagreement, Neda et al. [20] who conducted a study on (60) T2DM patients observed that in the metformin group, HbA1c levels were significantly greater than in the non-metformin group.

In this study, there was highly significant negative relation between vitamin D and DM duration ($P < 0.01$), depicted in (Figure 2). In coherence, Habibi et al. [21] observed statistically significant inverse relationship between vitamin D and length of illness was found in a study conducted on (1764) T2DM patients compared to (2914) healthy subjects. Also, in case control study [22], found that vitamin D insufficiency and deficiency were the highest in T2DM patient group vs. control group. Vitamin D role in the pathogenesis of T2DM is a topic of debate. Besides, the long duration of DM might result in progressive beta cell dysfunction and affect vitamin D receptors in pancreas and liver, which might affect vitamin D uptake and utilization.

Another highly significant correlation between vitamin D and HbA1c ($P < 0.001$), as shown in (Figure 3). Along with this finding, Mahmoud et al. [23] demonstrated that the level of vitamin D and HbA1c were significantly inversely correlated in their investigation. Also, AlHweishel et al. [12] demonstrated a negative correlation between vitamin D and HbA1c levels. In comparison to the insufficiency group's mean HbA1c was (7.23%) lower than the deficiency group's mean HbA1c that was (8.06%). However, no statistical significant difference between vitamin D levels was found in other study [24]. Poor dietary practices and insufficient sun exposure during the winter months are further possible explanations for the disparate results, variable laboratory measures and the different patient sample sizes. The inverse relationship between vitamin D and HbA1c reveals a link between hypovitaminosis D and T2DM's chronically impaired glucose metabolism and insulin resistance [10, 11].

Lastly, a significant inverse relation between vitamin D and metformin dose in T2DM patients, as illustrated in (Figure 4). In consistent, Alvaerez et al. [25,26] observed that diabetic patients taking metformin had low vitamin D levels (27 %) and that (16%) were deficient vitamin D levels. Also, a study mentioned that levels of vitamin D in metformin group (66.7%) were markedly lower compared to the control group (7.69%) [18]. Possibly the metformin associated GIT issues may reduce vitamin D absorption with consequent bowel and microbiome alternations as well as inadequate sun exposure in winter conditions at which the sample collection was performed. While a randomized placebo-controlled trial concluded that metformin at a dose of (850 mg / 3 times a day) for (52 months) had no noticeable effect on serum vitamin D levels [15]. The different patient sample sizes, poor dietary, metformin dose differences might explain the different outcomes.

5. Conclusion

The results showed that type 2 diabetic patients had low vitamin D levels and strongly validate the role of vitamin D deficiency in etiology of type 2 diabetes mellitus. Correction of vitamin D may help to improve disease outcome.

AUTHOR'S CONTRIBUTIONS

Yasameen Nasih Tawfeeq: Student
Nihad Najres Hilal: Supervisor
Zaidan Jayeed Zaidan: Supervisor

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