

# Association of Vitamin D and B12 in Glycemic status among Iraqi Patients with Type 2 Diabetes

Nidhal Khaleefa Ahmed<sup>1</sup><sup>\*</sup>

<sup>1</sup>Ministry of Education, Baghdad, Iraq

\*Corresponding Author: Nidhal Khaleefa Ahmed

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**ABSTRACT:** The purpose of this study was to assess the correlation between vitamin D, vitamin B12, and glycemic control (HbA1c) in individuals with type 2 diabetes. For this investigation, a total of seventy volunteers were enlisted. Standard biochemical techniques were used to assess serum levels of vitamin D, vitamin B12, and HbA1c, and SPSS was used for statistical analysis. The average vitamin B12 level was  $363.89 \pm 122.17$  ng/mL, the average vitamin D concentration was  $26.64 \pm 12.12$  ng/mL, and the average HbA1c level was  $5.32 \pm 0.81\%$ . Vitamin D and HbA1c showed a weak negative connection ( $r = -0.28$ ,  $p = 0.048$ ), indicating that lower vitamin D levels might be linked to worse glycemic management. HbA1c and vitamin B12 ( $p = 0.75$ ) and vitamin D and vitamin B12 ( $p = 0.86$ ) did not significantly correlate. The findings indicate that Vit-D may play a minor but significant role in glucose regulation, whereas Vit-B12 appears to have no direct influence on glycemic status in this cohort. Further studies with increased sample magnitude recommended to substantiate these preliminary data.



**Keywords:** Type 2 Diabetes, Vitamin D, Vitamin B12, HbA1c

## 1. INTRODUCTION

Diabetes mellitus is a chronic metabolic ailment characterized by hyperglycemia due to compromise insulin release or resistance (1). Insulin regulates blood sugar by promoting glucose uptake into cells (2). In insulin resistance, cells need more insulin, causing the pancreas to increase insulin production, which can lead to persistent hyperglycemia (3). The Greek physician Aretaeus (130–200 CE) is credited with coining the term "diabetes," which means "flowing through," after observing important signs such as persistent thirst, weight loss, and frequent urine. The pancreatic tissues now known as the Islets of Langerhans were discovered by Paul Langerhans (1847–1888), and Thomas Cawley later described the first known case. Banting and John Macleod received the Nobel Prize in 1923 for discovering insulin (4).

### 1.1 CLASSIFICATION OF DIABETES (5)

The American Diabetes Association (ADA) (1) divides diabetes into several major groups.

- Type-1: This form of diabetes results from an autoimmune process that leads to destruction of the pancreatic islets'  $\beta$ -cells, which usually leaves the body completely insulin-deficient.
- Type-2: A metabolic disease that typically coexists with underlying insulin resistance and is typified by a progressive decrease in  $\beta$ -cell insulin production.
- Gestational Diabetes Mellitus (GDM): In people who did not have overt diabetes before to conception, this form of the disease is initially detected in the second or third trimester of pregnancy.
- Additional Particular Diabetes Types: These include neonatal diabetes and maturity-onset diabetes of the young (MODY), diseases affecting the exocrine pancreas, and diabetes caused by medications or diseases like glucocorticoid therapy, HIV/AIDS treatments, or organ transplantation. Glycated hemoglobin

## 1.2 GLYCATED HEMOGLOBIN

Glycated hemoglobin, commonly referred to as HbA1c, serves as an indicator of a patient's mean blood glucose levels over the prior 60-90-day period. It reflects roughly the last three months of glycemic status and is widely used as a standard measure for evaluating long-term glycemic control(6, 7). Glycated hemoglobin of 6.5% used for diagnosis of diabetes(8). Human hemoglobin is composed mainly of HbA, which represents about 97% of the total and consists of four polypeptide chains (2 $\alpha$  and 2 $\beta$ ). Smaller fractions include HbA<sub>2</sub>, accounting for roughly 2.5% and formed from (2 $\alpha$  and 2 $\delta$ ) chains, and HbF, which makes up about 0.5% and contains (2 $\alpha$  and 2 $\gamma$ ) chains. In chromatographic studies, several minor hemoglobin components derived from HbA1 can also be detected, such as HbA1a and HbA1b(9). The glycation of glucose non-enzymatically with the amino terminus of the Hb,  $\beta$ -chain, generating an initial Schiff base. 120-day lifespan of the RBC during this period the hemoglobin glycation occur. Because of this stable and time-dependent formation, HbA1c serves as a reliable biomarker for evaluating average blood glucose levels over preceding weeks(10) see figure 1.

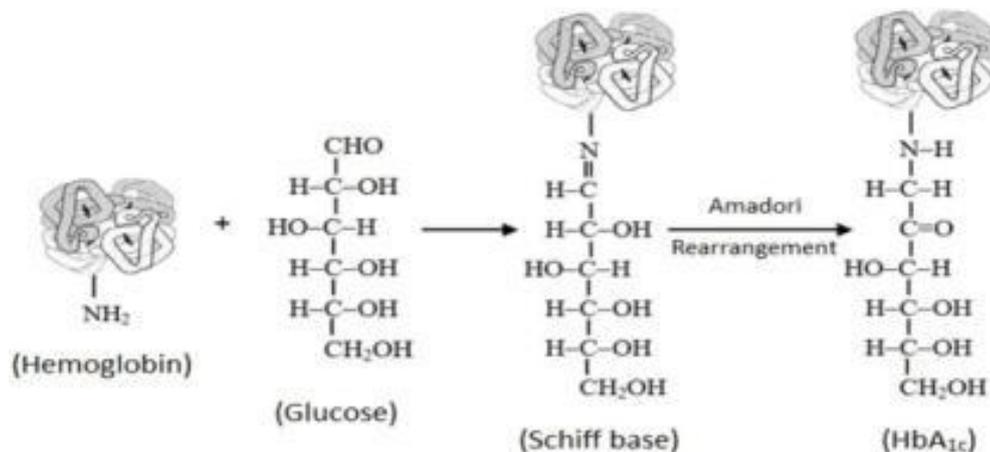


FIGURE 1. - Formation of HbA1c (9)

Individuals with prediabetes and for tracking long-term glycemic control in patients who already have elevated blood glucose levels were subjected to biochemical analysis ( HbA1c) levels ( 11).

Nutritional elements like vitamins influence glucose metabolism. Vitamin D and Vitamin B12 have improvement insulin function, beyond genetic and metabolic factors(12). Type-2 diabetes, metabolic syndrome, and insulin resistance are all linked to low vitamin D levels (13).

Vitamin D affects the pancreas, muscles, and immune cells by enhancing insulin secretion and improving tissue insulin sensitivity(14). Deficiency disrupts calcium balance and insulin release, promoting insulin resistance and low levels are linked to elevated HbA1c, fasting glucose, and fasting insulin, indicating impaired glucose control(15).

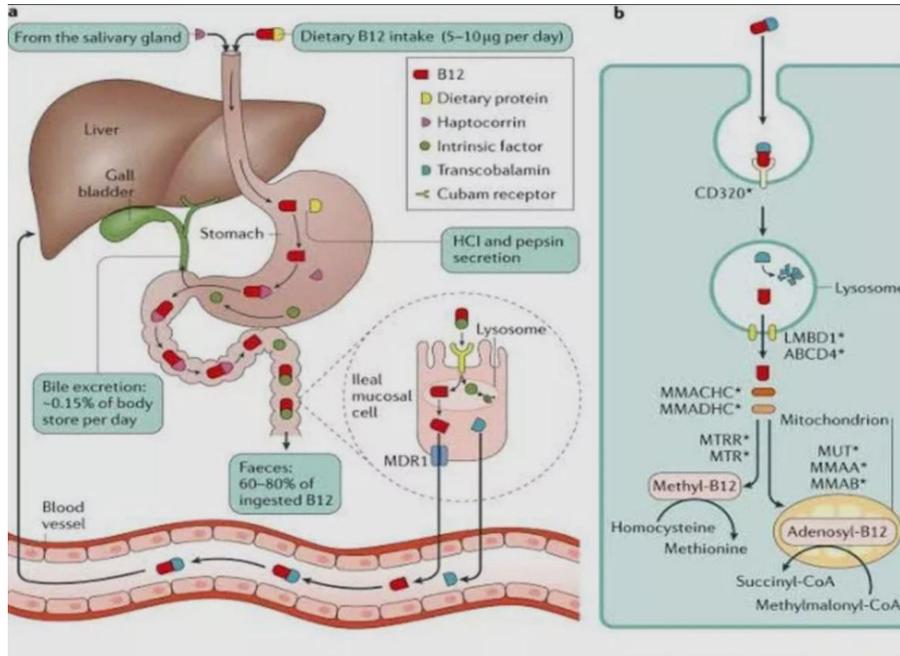
Correcting vitamin D deficiency through supplementation can improve insulin sensitivity and reduce metabolic disturbance, especially in deficient individuals(16). Despite abundant sunlight, deficiency is widespread, particularly in the western Asia, women limited sun exposure lead to produces few of vitamin D(17). Additionally, a number of studies have revealed few vit. D raises the risk of type-1 and type-2 diabetes(18).

Numerous research has shown the effect of vit-B12 on glycemic control in diabetic patients, even though there is strong indication that vit-D may have a role in controlling glucose metabolism and lowering HbA1c levels (22 The main reason for this interest is because people on metformin, most often prescribed drug for type-2 diabetes, frequently show a decrease in vit-B12 levels(19).

It has been shown that metformin reduces the gastrointestinal absorption of vit-B12, increasing the risk of deficiency and its related neurological and metabolic problems, which may have an associated neurological and metabolic complications, which may indirectly influence glucose regulation(20-22).

However, metformin use seems to have a major role in the impact of vitamin B12 on HbA1c (23). Patients on metformin, the group most likely to have lower B12 levels, were not included in our study (24). This greatly reduced the likelihood of finding any discernible B12 status changes or substantial correlations with HbA1c(19). In this research did not show a significant relationship between vit-B12 who are not receiving metformin therapy, but rather a phenomenon linked to treatment-related factors and supported the interpretation that the impact of vitamin B12 on glucose regulation is a phenomenon associated with treatment-related factors rather than an independent effect in patients not receiving metformin therapy(20).

Dietary vit-B12 initially binds to R protein (haptocorrin) secreted by the salivary glands. In the duodenum, pancreatic secretions and an alkaline environment degrade this protein, releasing vit- B12, which subsequently binds to intrinsic factor (IF), a glycoprotein produced in the stomach and resistant to digestive enzymes(21). This complex protects vit-B12 from utilization by intestinal bacteria and facilitates its transport to the ileum, where the vit-B12–IF complex binds to cubilin receptors on enterocytes. Following absorption, vit-B12 enters the circulation within 2–3 hours, bound to transcobalamin II(22) see figure 2.



**FIGURE 2. - Absorption and enterohepatic circulation of Vit- B12(23)**

The vit-D and vit-B12 have role in glucose metabolism and considering that metformin users were excluded from this study, an important question arises: Do vit-D and vit-B12 levels independently influence HbA1c in patients with type-2 D.M? The aim of the current study was to assess any possible correlations of vit-D and B12 and glycemic status, as determined by HbA1c, in people with type-2 diabetes.

## 2. METHOD

70 patients included in this study to assess the link between vit-D and vit-B12, and HbA1c in individuals with T2DM. Every laboratory technique was carried out at a medical research facility that has been certified.

Each participant was divided into age and sex-based subgroups for descriptive analysis. The age range of the participants was 30–70 years. The BMI was calculated for all participants to assess obesity status using the following formula:

BMI=dividing a person’s weight in kilograms by the square of their height in meters.

### 2.1 BIOCHEMICAL MEASUREMENTS

Specimen blood (5 mL) were grouped from all participants via venipuncture after an overnight fast. Blood was drawn using sterile plastic syringes while the patient was seated.

The samples were transferred into plain tubes, and separated serum and stored at –20°C until analysis.

HbA1c levels were measured using a standardized immunoassay method.

Serum concentrations of Vit-D(25-hydroxyvitamin-D) and Vit-B12 were determined using the Sandwich-ELISA technique.

Prior to participation, each participant provided written informed consent, and all procedures were carried out in compliance with ethical standards.

#### CRITERIA FOR EXCLUSION:

- Metformin-treated patients
- gastrectomy
- terminal ileal resection
- and K/C/O pernicious anemia

**INCLUSION CRITERIA:**

- Diabetes Mellitus cases in people aged 30 to 75
- Pregnancy

**3. RESULTS**

A total of 70 were included in the present study. Serum Vit-D and Vit-B12 levels were measured, and HbA1c percentage was estimated for all participants.

**Table 1. - Mean, SD and Range of HbA1c, Vit-D and Vit-B12**

Parameters	No.	Mean	SD	Range (Min. – Max.)
HbA1c (%)	70	5.323	0.805	4.00 – 7.80
Vitamin D (ng/ml)	70	26.64	12.12	7.60 – 52.96
Vitamin B12 (ng/ml)	70	363.89	122.17	155.67 – 591.00

**Table 2. - Vit-D Distribution**

Vit-D (ng/ml)	No	Percentage (%)
< 20	22	31.43
≥20	48	68.57
Total	70	100%
P-value	---	0.0019

\*\* (P≤0.01): Highly significant.

**Table 3. - Effect of Vit-D in HbA1c and Vit-B12 in sample study**

	Mean ±SD	
Vitamin D (ng/ml)	HbA1C (%)	Vitamin B12 (ng/ml)
< 20	5.38 ±0.69	342.54 ±125.61
≥20	5.30 ±0.86	373.68 ±120.62
T-test	0.4164 NS	NS
P-value	0.7049	0.3257

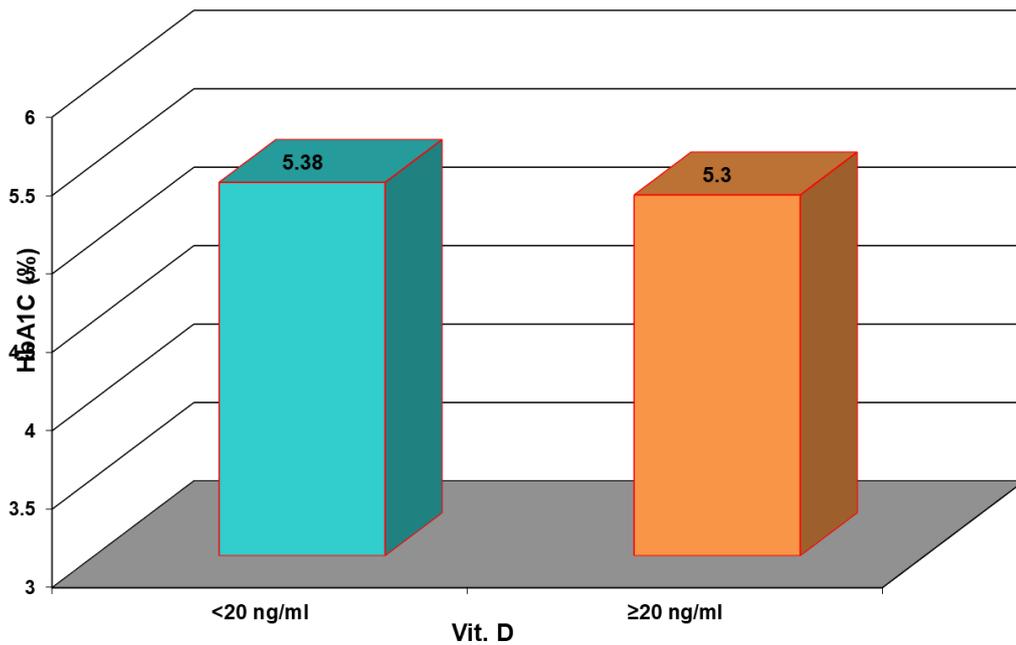
NS: Non-Significant.

**Table 4. - Correlation coefficient between VitD in HbA1c and Vit- B12 in sample study**

Parameters	Correlation coefficient-r	P-value
HbA1c and Vitamin D	-0.28 *	0.0484 *
HbA1c and Vitamin B12	-0.04 NS	0.7547
Vitamin D and Vitamin B12	0.02 NS	0.8623

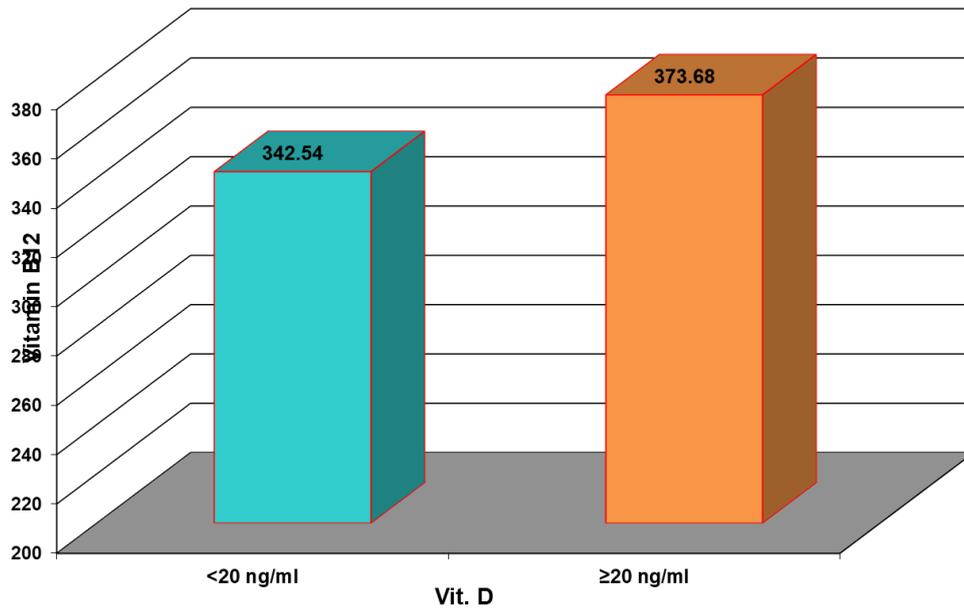
\* (P≤0.05), NS: Non-Significant

This table show the percentage distribution of study participants by serum Vitamin D levels (ng/ml) i. According to the analysis, the majority of the sample (68.57%) had level of vit-D 20 ng/ml or greater, despite this, deficiency was observed in 31.43% of the sample, with levels falling below 20 ng/ml see figure3,4.



**FIGURE 3. – Effect vitamin D in HbA1c**

This figure illustrates demonstrate a slight inverse trend between vitamin D levels and HbA1c, whereby vitamin D deficiency was linked with a modest increased in glycated hemoglobin values. Although this difference was minimal.



**FIGURE 4. – Effect vitamin D in B12**

The bar chart compares mean vitamin B12 concentrations according to vitamin D status, showing higher B12 levels among participants with sufficient vitamin D (≥20 ng/ml) than among those with vit-D deficiency (<20 ng/ml).

#### 4. DISCUSSION

The study investigated the relationship between glycemic control in patients with type-2 DM and their serum level of vit-D and vit-B12. This study suggests a potential linked between changes in these micronutrients and the

population's general metabolic control and glucose metabolism. The results of this study show that a significant percentage of the sample had few levels of vit-D, with about 31.43% of participants having levels below 20 ng/ml.

This proportion was highly statistically significant ( $P = 0.0019$ ), confirming the widespread prevalence of Vitamin D deficiency among the subjects. The strong prevalence observed in this study aligns with the literature, which suggested the reduced vitamin D levels to metabolic disturbances, particularly impaired glucose absorption and decreased insulin sensitivity, both of which may contribute to dysregulated glycemic control (27, 28). The study results showed that the mean HbA1c did not differ significantly among the two groups ( $<20$  vs.  $\geq 20$  ng/ml), with values of 5.38 and 5.30, respectively ( $P = 0.4164$ ). This lack of significance aligns with several studies that reported no direct impact of vit-D on HbA1c, such as the findings of Chen et al. However, it contrasts with other studies that have demonstrated a significant reduction in Nikooyeh et al (24).

Despite the absence of an observable difference in mean HbA1c levels between the two vit-D groups, the correlation test revealed a weak but significant inverse association between vit-D and HbA1c ( $r = -0.28$ ,  $P = 0.0484$ ). This correlation indicates that lower vit-D levels may be linked with a slight increase in HbA1c, supporting the biological hypothesis that vit-D contributes to glycemic regulation by enhancing pancreatic  $\beta$ -cell function, modulating calcium channels, and improving insulin sensitivity through several cellular and molecular mechanisms (25). Furthermore, vit-D exerts a regulatory effect on insulin transcription by binding to the vitD response element (VDRE), which is situated within the promoter sequence of the insulin gene (26, 27), and previous studies have confirmed that insulin secretion is impaired in the lack of functional vit-D receptors (28).

This finding aligns with studies reporting an association among low vit-D levels and impaired glucose regulation, such as those by Nikooyeh et al, Lemieux et al. (2019), and Babarawi (2020), all of which demonstrated that increasing vitamin D levels may improve glycemic control and insulin sensitivity (29, 30).

Analysis of our data revealed that patients with type-2 diabetes in this cohort exhibited normal vit-B12 levels ( $363.89 \pm 122.17$  ng/ml), despite elevated HbA1c along with vit-D deficiency was observed in some participants. Vit-B12 contributes to cellular protection against oxidative stress and has an important role in carbohydrate and lipid metabolism and (31). It is also essential for key enzymatic reactions, including the isomerization of Methylmalonyl CoA to Succinic CoA and the biosynthesis of methionine, which is necessary for myelin formation and proper nerve function (32). In the current study, vit-B12 concentrations did not show a significant association with higher HbA1c levels or with vit-D deficiency, suggesting that B12 may not play a direct role in regulating blood glucose, in contrast to the effects observed with vit-D (33). These findings are consistent with previous research reporting no direct correlation between HbA1c and vit-B12 levels in patients not treated with metformin (34).

## 5. CONCLUSION

Higher vitamin D concentrations were somewhat associated with better glycemic management, according to this study, which showed a slight opposing relationship among blood vit-D levels and HbA1c. The intensity of this association, however, indicates that vit-D may not have a significant independent impact on long-term glucose management. On the other hand, the research participants' vitamin B12 levels stayed within normal ranges, suggesting that changes in glycemic status within this cohort were unlikely to be significantly influenced by B12 insufficiency. Notably, a high incidence of vit-D deficiency was vit-D may function as a supportive or modulatory factor, rather than a primary determinant, in maintaining optimal glycemic control.

## FUNDING

None

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest

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