ALKUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Vitamin B12 Deficiency in Type 2 Diabetes Mellitus: Prevalence, Risk Factors, and the Influence of Metformin

Intisar R. Sharba[1]
Baneen Ali Abdulrahman[2]
Dhamya Kadhim Sarhan[3]
Fatima K. Kazim[4]
[1,3,4]Department of Biology, Faculty of Science, University of Kufa, Kufa, Iraq.
[2]University of Jabir Ibn Hayyan for Medical and Pharmaceutical Sciences, Iraq

Article history
Received: 15/7/2025
Revised: 14/8/2025
Accepted: 25/8/2025

*Corresponding Author:
Dhamyak.eleiwi@uokufa.edu.iq

Abstract:
Vitamin B12 deficiency represents a clinically relevant concern in patients with type 2 diabetes mellitus (T2DM), as it may exacerbate neuropathic, hematologic, and cognitive complications. This study aimed to assess serum vitamin B12 levels in T2DM patients and to identify independent clinical roles and long-term therapeutic predictors of deficiency. A case-control design was implemented at Al-Sadder Hospital in Najaf, Iraq, between January and March 2025, involving 60 diabetic patients and 20 healthy individuals. Vitamin B12 levels in serum were measured using chemiluminescent immunoassay, and glycemic regulation was evaluated through HbA1c and random blood glucose. Different types of treatment were written down, with a focus on metformin monotherapy and insulin-based protocols. In the diabetic group, mean blood vitamin B12 levels were much lower than in the control group (395.48 ± 147.36 pg/ml vs. 211.53 ± 95.48 pg/ml, $P < 0.0001$), and 70% of diabetic patients were found to be vitamin B12 deficient. Age ≥ 40 years (OR = 7.33, 95% CI: 2.15–24.99, P = 0.001), BMI ≥ 25 kg/m² (OR = 5.03, 95% CI: 1.54–16.43, P = 0.008), 5 years or more of diabetes (OR = 0.64, 95% CI: 1.70–18.66, P = 0.005), and a HbA1c level > 5.6% (OR = 7.50, 95% CI: 2.11–26.69, P = 0.002) were all strong indicators of vitamin D deficiency. A higher risk was linked to taking metformin by itself (odds ratio = 8.50, 95% confidence interval (CI): 2.44–29.56, p=0.001). But hypertension wasn't forecast very well (odds ratio = 2.94, 95% CI: 0.92–9.36, p=0.068). Strong negative correlations were found between vitamin B12 levels and age (r = -0.67), length of diabetes (r = -0.69), body mass index (r = -0.44), glycated hemoglobin (HbA1c = -0.49), and random blood glucose (r = -0.39). These results indicated that vitamin B12 insufficiency in type 2 diabetes is caused by more than one thing. They also stress the importance of regular screening, especially for people who are taking metformin or who don't have adequate metabolic control.

Keywords: Vitamin B12, T2DM, Metformin, and Glycemic Control

1. Introduction

People with type 2 diabetes (T2DM), a long-lasting metabolic disease that is becoming more common around the world [1], often don't know they are deficient in vitamin B12. Taking metformin for a long time is a popular way to treat type 2 diabetes, which can cause a lot of problems. Vitamin B12 shortage is more likely to happen if you take this medicine [2]. Vitamin B12 is needed for the brain to work, for making red blood cells, and for making DNA. It can hurt your brain and blood if you don't get enough of it. For example, it can make diabetic neuropathy and anemia worse [3]. A new study shows that people with type 2 diabetes often don't get enough vitamin B12. The rates vary by group, but they are between 18% and more than 28%. This is especially

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

true for people who have been taking a lot of metformin for a long time [4, 5]. The risk goes up even more if you are older, have had diabetes for a long time, eat a vegan diet, or have other health problems like hypothyroidism or folate deficiency. The chance of an iron shortage was found to be strongly linked to race, how well diabetes was controlled, and the total amount of metformin taken [6, 7]. Notably, not getting enough vitamin B12 can make problems like peripheral neuropathy, anemia, and cognitive damage worse in people with T2DM and lower their quality of life [4, 8, 9]. Despite how important this issue is from a medical point of view, not all T2DM patients are routinely screened for vitamin B12 deficiency. This means that some patients may not be diagnosed, which can lead to complications that could have been avoided [6, 10]. Regular monitoring of vitamin B12 levels is becoming more and more recommended, especially for high-risk groups like the elderly, people who have had diabetes for a long time, vegetarians, and people who are on long-term or high-dose metformin. [11]. Early diagnosis and the right supplements can help keep major problems from happening and make things better for patients [12, 13]. The study's goals are to look into the link between long-term metformin therapy and vitamin B12 deficiency in people with type 2 diabetes mellitus by finding out how common it is, what clinical factors can be used to predict it, and what this means for early detection and regular screening in groups that are more likely to get it.

2. Methodology

Subject

Between January 1, 2025, and March 15, 2025, 80 people took part in this case-control study. The diabetic group was made up of 60 people from the Diabetic Center at Al-Sadder Hospital in Al-Kufa, Iraq, who had been identified with type 2 diabetes mellitus (T2DM). Twenty healthy people with no history of diabetes were chosen from the general population in Al-Kufa through outpatient clinics and community marketing. They were in the control group. Everyone who took part was at least 25 years old and was getting routine care. Different diabetic patients were given different treatments. Some were only given metformin, while others were given both metformin and insulin. This change in therapy was recorded and looked at in the study to see if it might have an effect on serum vitamin B12 levels. People who had pernicious anemia, had bariatric surgery, or had Crohn's disease were not included in the study because they might have been affected by other causes. People who were pure vegans, had late-onset autoimmune diabetes (LADA), or were currently taking vitamin B12 pills were also not allowed to participate. In line with the Declaration of Helsinki, all subjects gave their written consent after being fully informed. The work was approved by the Institutional Review Board of Kufa University as being ethical.

Laboratory and Clinical Data Collection

All subjects filled out an organized questionnaire and had their medical papers looked over to get information about their medical background and sociodemographic information. This included details about the person's height, weight, age, sex, present health problems, and any diabetes medicines they were taking. To get serum for testing in the lab, 5 mL of venous blood was taken from each subject and spun at 3000 cycles per minute.

Measurement of HbA1c and Vitamin B12

All of the subjects had their blood vitamin B12 and glycosylated hemoglobin (HbA1c) levels checked. If the tests were done within three months of registration, data from electronic medical records that were already stored were used. The COBAS INTEGRA® 400 Plus device (Roche, Switzerland) was used to measure HbA1c using a turbidimetric inhibition immunoassay. A chemiluminescent paramagnetic microparticle immunoassay on the UniCel DxI 600 detector (Beckman Coulter, USA) was used to measure the amount of vitamin B12 in the blood. Participants were put into two groups based on these measurements: those whose blood levels were below 200 pg/mL (not enough vitamin B12) and those whose levels were above 200 pg/mL (not enough vitamin B12), which is in line with established standards [14].

Statistical Analyses

All statistical analyses in this study were performed using SPSS version 28. Continuous variables are presented as either the mean with standard deviation (SD) or the median with the interquartile range (IQR), depending on their distribution. Independent t-tests or Mann-Whitney U tests were used for pairwise comparisons, specifically between patients and controls, individuals with and without vitamin B12 deficiency, and those receiving monotherapy versus dual combination therapy (metformin vs. metformin + insulin). Categorical variables are summarized as counts and percentages and were compared using chi-square tests. The correlation between patients' clinical characteristics and their vitamin B12 levels was evaluated using either Pearson's or Spearman's rho, and these relationships are illustrated in scatter plots. To

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

determine independent risk factors for vitamin B12 deficiency, a multivariate logistic regression model was constructed, and the results are reported as odds ratios (OR) with corresponding 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant for all tests [15].

3. Results and Discussion

Demographic and Clinical Features of the studied subject

Table 1 shows that the study had 60 people with type 2 diabetes mellitus (T2DM) and 20 healthy controls. People with T2DM had a mean age of 45.93 ± 11.38 years, while people without T2DM had a mean age of 44.40 ± 12.27 years ($p = 0.608$, not significant). Among T2DM patients, 21 (35.0%) were younger than 40 years and 39 (65.0%) were 40 years or older, compared to 8 (40.0%) and 12 (60.0%) in the control group, respectively ($X^2 = 0.535$, $p = 0.465$, not significant). Regarding sex, 39 (60.0%) T2DM patients were male and 21 (40.0%) were female, compared to 10 (50.0%) males and 10 (50.0%) females among controls ($X^2 = 0.487$, $p = 0.485$, not significant). The mean duration of diabetes among T2DM patients was 7.12 ± 3.31 years, with 37 (61.7%) having diabetes for more than 5 years and 23 (38.3%) for less than 5 years ($X^2 = 8.733$, $p = 0.020$). Hypertension was present in 33 (55.0%) T2DM patients compared to 5 (25.0%) controls, while 27 (45.0%) T2DM patients and 15 (75.0%) controls had no hypertension ($X^2 = 10.58$, $p = 0.002$). The mean BMI of T2DM patients was 26.64 ± 2.79 kg/m² and 24.82 ± 2.93 kg/m² for controls ($p = 0.014$); 39 (65.0%) T2DM patients had BMI ≥25 kg/m² and 21 (35.0%) had BMI <25 kg/m², compared to 11 (55.0%) and 9 (45.0%) in controls ($X^2 = 4.089$, $p = 0.043$). The mean random blood sugar (RBS) in T2DM patients was 228.07 ± 85.43 mg/dl versus 78.80 ± 11.67 mg/dl in controls ($p = 0.0001$). HbA1C was 8.07 ± 2.12% in T2DM patients and 4.78 ± 0.38% in controls ($p = 0.0001$), with 44 (73.3%) T2DM patients having HbA1C >5.6% compared to 2 (10.0%) controls, and 16 (26.7%) T2DM patients with HbA1C <5.6% versus 18 (90.0%) controls ($X^2 = 24.62$, $p = 0.0001$). Mean serum Vitamin B12 in T2DM patients was 211.53 ± 95.48 pg/ml and 395.48 ± 147.36 pg/ml in controls ($p = 0.0001$). Vitamin B12 deficiency was observed in 42 (70.0%) T2DM patients compared to 4 (20.0%) controls, while 18 (30.0%) T2DM patients and 16 (80.0%) controls had no deficiency ($X^2 = 15.35$, $p = 0.0001$). Regarding treatment, 44 (73.3%) T2DM patients were on metformin alone, and 16 (26.7%) were on a combination of metformin and insulin ($X^2 = 13.07$, $p = 0.0001$).

The Clinical Factors and Their Impact on Vitamin B12 Levels

The findings show a strong and important link between Vitamin B12 amounts and a number of health issues. Figure 1. Patients older than 40 had much lower B12 levels (174.10±42.10 pg/ml) than those younger than 40 (298.89±123.65 pg/ml), which was a very big difference (p=0.001). In the same way, people who were overweight or obese (≥25 kg/m2) had significantly lower B12 levels than people who were a healthy weight (<25 kg/m2), with a p-value of 0.034. Diabetes length was also very important; people who had diabetes for 5 years or more had much lower B12 levels (172.55±44.76 pg/ml) than people who had diabetes for less time (289.50±118.83 pg/ml), a result with a very strong p-value (0.0001). A p-value of 0.0001 showed that people with high blood pressure had lower B12 levels (171.05±44.55 pg/ml) than people who did not have high blood pressure (292.50±115.78 pg/ml). When it came to treatment, people who took Metformin by itself had much lower B12 levels (188.30±77.17 pg/ml) than people who took Insulin and Metformin together (258.00±110.44 pg/ml), which was a big difference (p=0.017). Low B12 levels were also linked to not being able to handle blood sugar well, which was shown by an HbA1c level of 5.6% or higher (180.66±53.68 pg/ml). Only sex didn't have a big effect; the average B12 levels for men (218.50±98.59 pg/ml) and women (201.08±89.49 pg/ml) were not statistically different (p=0.490).

Correlation Analysis Between Clinical and Biochemical Parameters in Patients with T2DM

The correlation results in Heatmap Figure 2. Age was strongly and positively correlated with duration of diabetes mellitus ($r = 0.853$, $p < 0.001$), RBS ($r = 0.65$, $p < 0.001$), and BMI ($r = 0.69$, $p < 0.001$), and moderately correlated with HbA1c ($r = 0.53$, $p < 0.001$). Age was strongly and negatively correlated with vitamin B12 ($r = -0.67$, $p < 0.001$). BMI showed moderate positive correlations with duration of DM ($r = 0.53$, $p < 0.001$) and weak positive correlations with RBS ($r = 0.34$, $p = 0.008$) and HbA1c ($r = 0.32$, $p = 0.013$). BMI exhibited a moderate negative correlation with vitamin B12 ($r = -0.44$, $p < 0.001$). Duration of DM was strongly and positively correlated with RBS ($r = 0.61$, $p < 0.001$) and moderately correlated with HbA1c ($r = 0.56$, $p < 0.001$), while showing a strong negative correlation with vitamin B12 ($r = -0.69$, $p < 0.001$). HbA1c was strongly and positively correlated with RBS ($r = 0.63$, $p < 0.001$) and moderately and negatively correlated with vitamin B12

Dhamya and Intisar, *Journal Name* 2025, Volume (17) Number (3): Page Numbers 110-119
DOI: https://doi.org/10.36320/ajb/v17.i3.21730

(r = -0.49, p < 0.001). RBS showed a moderate negative correlation with vitamin B12 (r = -0.39, p = 0.002).

Distribution and Predictors of Vitamin B12 Deficiency in T2DM: A Multivariate Analysis

The table presents the distribution of vitamin B12 deficiency according to several categorical variables among the study participants Table 2. For age, participants aged 40 or above had 33 cases (78.6%) with B12 deficiency and 6 without (33.3%), while those under 40 had 9 cases (21.4%) with deficiency and 12 without (66.7%). The likelihood ratio for age was 11.134 with a p-value of 0.001. Regarding sex, 24 males (57.1%) had a deficiency compared to 12 (66.7%) without, and 18 females (42.9%) had a deficiency versus 6 (33.3%) without; the likelihood ratio was 0.483 with a p-value of 0.487. For BMI, participants with BMI ≥25 kg/m² had 32 cases (76.2%) with deficiency and 7 without (38.9%), whereas those with BMI <25 kg/m² had 10 cases (23.8%) with deficiency and 11 without (61.1%), with a likelihood ratio of 7.531 and a p=0.006. Duration of diabetes of 5 years or more showed 31 cases (73.8%) with deficiency and 6 without (33.3%), compared to 11 cases (26.2%) with deficiency and 12 without (66.7%) for duration less than 5 years, with a likelihood ratio of 8.663 and p=0.003. Regarding hypertension, 25 participants (59.5%) with hypertension had deficiency compared to 6 without (33.3%), while 17 participants (40.5%) without hypertension had deficiency and 12 without (66.7%), likelihood ratio 3.505, p-value 0.061. For HBA1C, participants with values >5.6% had 36 cases (85.7%) with deficiency and 8 without (44.4%), whereas those with <5.6% had 6 cases (14.3%) with deficiency and 10 without (55.6%), likelihood ratio 10.409, p-value 0.001. Treatment, 34 participants (81.0%) on Metformin alone had deficiency, and 6 without (33.3%), while 8 participants (19.0%) on Insulin plus Metformin combination had deficiency and 12 without (66.7%), with a likelihood ratio of 12.567 and a p-value of 0.0001. The logistic regression analysis examined the association between several clinical and demographic factors and vitamin B12 deficiency. Participants aged 40 years or older had significantly higher odds of deficiency, with an OR of 7.33 (95% CI: 2.15–24.99, p = 0.001) compared with those under 40. Male sex was associated with a lower odds ratio of 0.667 (95% CI: 0.21–2.12), although this was not statistically significant (p = 0.491). Patients with a BMI of 25 kg/m² or higher had increased odds of deficiency (OR 5.03, 95% CI: 1.54–16.43, p = 0.008). Similarly, a duration of diabetes of five years or more was associated with higher odds (OR 5.64, 95% CI: 1.70–18.66, p = 0.005). Hypertension showed a trend toward increased odds (OR 2.94, 95% CI: 0.92–9.36), but this did not reach statistical significance (p = 0.068). Elevated HBA1C levels above 5.6% were strongly associated with deficiency (OR 7.50, 95% CI: 2.11–26.69, p = 0.002). Regarding treatment, patients taking Metformin alone had substantially higher odds of vitamin B12 deficiency compared with those on an insulin plus Metformin (OR 8.50, 95% CI: 2.44–29.56, p = 0.001), as shown in the forest plot Figure 3.

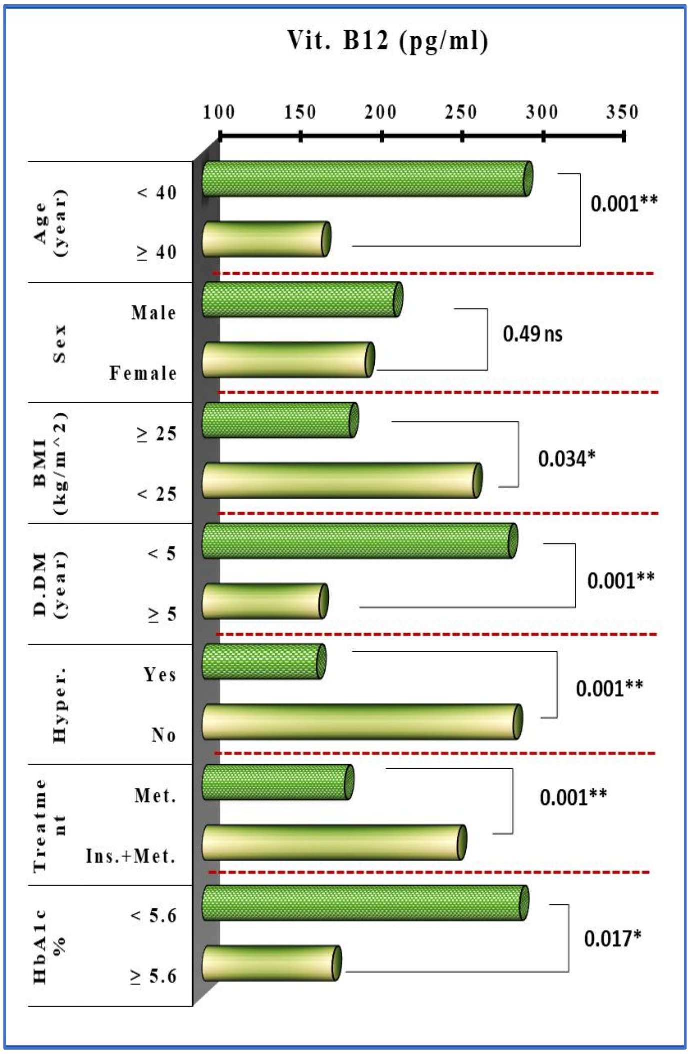

Figure 1. Vitamin B12 Deficiency Association with Clinical Features in T2DM Patients

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

Table 1. General demographics and Clinical Characterizes in Diabetic Mellitus Type 2 and Control Groups

Characteristics		Groups		
		T2DM N=60	**Healthy Controls N=20**	**p-value**
		N %	N %	
Age (year)	Mean ± SD	45.93±11.38	44.40±12.27	0.608 # Ns
	Less than 40	21(35.0%)	8 (40.0%)	X2=0.535 0.465 Ns
	More than 40	39 (65.0%)	12(60.0%)	
Sex	Male	39 (60.0%)	10 (50.0%)	X2=0.487 0.485 Ns
	Female	21 (40.0%)	10 (50.0%)	
Duration of DM (years)	Mean ± SD	7.12±3.31	-	NA
	More than 5	37 (61.7%)	-	X2= 8.733 0.020*
	Less than 5	23 (38.3%)		
Hypertension	Yes	33 (55.0%)	5 (25.0%)	X2= 10.58 0.002*
	NO	27 (45.0%)	15 (75.0%)	
BMI (kg/m^2)	Mean ± SD	26.64±2.79	24.82±2.93	0.014* #
	≥25 kg/m²	39 (65.0%)	11 (55.0%)	X2=4.089 0.043*
	<25 kg/m²	21(35.0%)	9 (45.0%)	
RBS (mg/dl)	Mean ± SD	228.07±85.43	78.80±11.67	0.0001**
HbA1C %	Mean ± SD	8.07±2.12	4.78±0.38	0.0001**
	< 5.6 %	16 (26.7%)	18 (90.0%)	X2= 24.62 0.0001**
	> 5.6 %	44 (73.3%)	2 (10.0%)	
Vit. B12 (pg/ml)	Mean ± SD	211.53±95.48	395.48±147.36	0.0001**
Vit. B12 Deficiency	Yes	42 (70.0%)	4 (20.0%)	X2= 15.35 0.0001**
	No	18 (30.0%)	16 (80.0%)	
Treatment Type	Metformin	44 (73.3%)	-	X2=13.07 0.0001**
	Metformin + Insulin	16 (26.7%)	-	

Significant differences at p-value*<0.05, **<0.01. X^2= Chi-Square. NA: Non-applicable. #: Independent t-test between two groups.

Table 2. Distribution of Factors Associated with Vitamin B12 Deficiency in T2DM Patients

		Vitamin B12 Deficiency			
Variable	**Category**	**Yes N (%)**	**No N (%)**	**Likelihood Ratio**	**P-value**
Age (year)	≥ 40	33 (78.6%)	6 (33.3%)	11.134	0.001*
	< 40	9 (21.4%)	12 (66.7%)		
Sex	Male	24 (57.1%)	12 (66.7%)	0.483	0.487 ns
	Female	18 (42.9%)	6 (33.3%)		
BMI (kg/m²)	≥25	32 (76.2%)	7 (38.9%)	7.531	0.006*
	<25	10 (23.8%)	11 (61.1%)		
Duration DM	≥ 5	31 (73.8%)	6 (33.3%)	8.663	0.003*
	< 5	11 (26.2%)	12 (66.7%)		
Hypertension	Yes	25 (59.5%)	6 (33.3%)	3.505	0.061 ns
	No	17 (40.5%)	12 (66.7%)		
HBA1C %	> 5.6	36 (85.7%)	8 (44.4%)	10.409	0.001*
	< 5.6	6 (14.3%)	10 (55.6%)		
Treatment	Met.	34 (81.0%)	6 (33.3%)	12.567	0.0001**

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

Ins.+ Met.	8 (19.0%)	12 (66.7%)

Significant differences at p-value*<0.05, **<0.01.

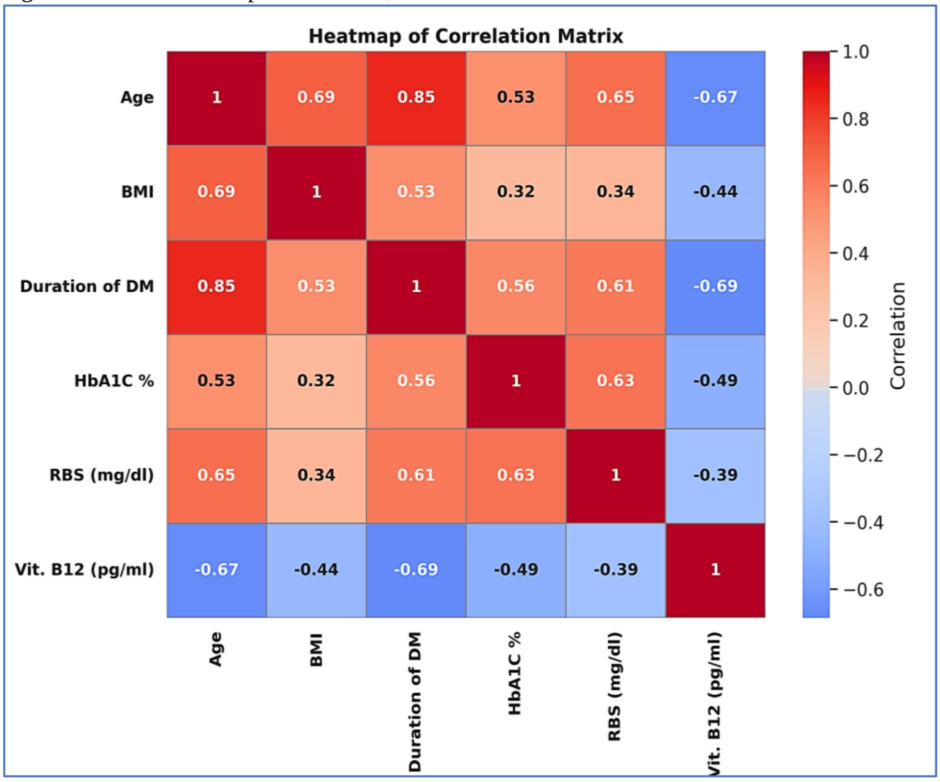

Figure 2: Heatmap of Clinical and Biochemical Parameter Correlation Analysis in T2DM Patients

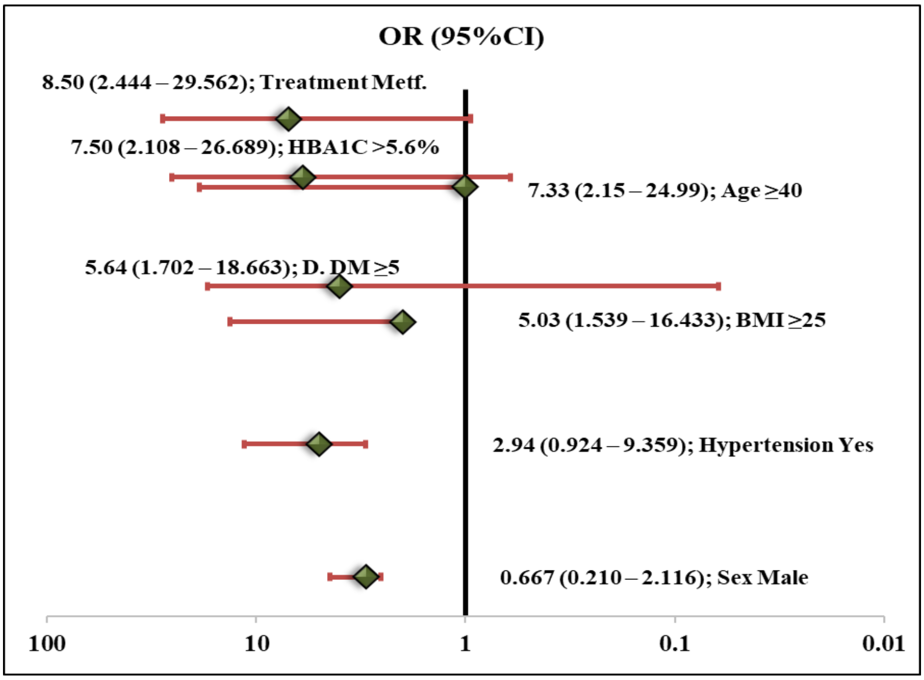

Figure 3. Forest plot to Logistic Regression Analysis of Risk Factors Associated with Vitamin B12 Deficiency

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

General Characteristics of T2DM Compared with Healthy Controls

This study clarifies the important demographic, clinical, and biochemical characteristics of patients with Type 2 Diabetes Mellitus (T2DM) and identifies predictors of vitamin B12 deficiency in this population. The T2DM and control groups were well-matched by age and gender, which helped reduce confounding biases and improve the reliability of our comparisons. In line with recognized research linking obesity to the pathophysiology of type 2 diabetes, the T2DM cohort notably had a significantly higher BMI [16]. Hyperglycemia is crucial for the advancement of diabetes, as seen by the T2DM group's elevated HbA1c and random blood glucose levels [17,18]. A key finding was the significantly reduced vitamin B12 levels in T2DM patients, highlighting the need for routine monitoring of this deficiency in diabetic populations [19].

Clinical Factors Associated with the Risk of Vitamin B12 Deficiency

The study found significant correlations between vitamin B12 insufficiency and metformin monotherapy, poor glycemic control, longer diabetes duration, and hypertension in patients with T2DM. The association between B12 insufficiency and hypertension is consistent with research suggesting that shared endothelial dysfunction or dietary variables may be common in cardiometabolic illnesses [20]. Similarly, the correlation between B12 deficiency and a prolonged duration of diabetes supports evidence suggesting that cumulative, long-term metformin use reduces B12 absorption over time [21]. Hypotheses suggesting persistent hyperglycemia aggravates deficiency through oxidative stress or renal dysfunction are supported by the inverse connection between HbA1c and B12 levels ($r = -0.521$) [22, 23]. The relationship between metformin monotherapy and B12 deficiency is also noteworthy and consistent with other findings on its malabsorptive effects [24]. Interestingly, the decreased prevalence of B12 insufficiency in individuals receiving combined insulin-metformin therapy implies protective mechanisms that warrant further investigation.

The strong correlations between age and metabolic parameters (BMI, diabetes duration, HbA1c) align with longitudinal studies demonstrating age-related declines in insulin sensitivity and beta-cell function [25, 26]. The association between BMI and glycemic markers (HbA1c: $r = 0.394$; RBS: $r = 0.457$) mirrors findings that adiposity-driven insulin resistance worsens glucose homeostasis [27]. Furthermore, the multivariate predictors of B12 deficiency—age >40 years, diabetes duration >5, and HbA1c >5.6% resonate with large-scale studies highlighting these factors as cumulative risks [28, 29].

Contrary to some reports, sex and treatment type (metformin alone vs. combination therapy) did not independently predict B12 deficiency. This finding contrasts with studies that identify female and metformin monotherapy as risk factors [31], which could be due to differences in our sample's demographics or supplementation practices. Similarly, while BMI showed a non-significant trend, larger studies have reported modest associations between obesity and B12 deficiency, possibly mediated by adiposity-related inflammation [27, 31]. These discrepancies may arise from variations in population characteristics, dietary habits, or study design. Overall, our study found that age, length of the disease, glycemic control, and medication use all have an impact on vitamin B12 insufficiency in T2DM. Key findings support the existing body of knowledge on metabolic factors and the function of metformin, but differences in BMI and gender point to the necessity of population-specific studies.

Conclusion

In conclusion, clinical and vitamin B12 deficiency are common in T2DM patients and are associated with older age, longer illness duration, poor glycemic control, and hypertension. While BMI exhibited a non-significant trend toward increased risk, it was not found to be an independent predictor of insufficiency in this group. Interestingly, metformin monotherapy had no significant effect on B12 status, which needs additional examination. Based on these findings, we advocate routine vitamin B12 testing in at-risk adults, especially older patients with chronic diabetes or poor glycemic control. Proactive intervention, including screening and tailored supplements, can reduce deficiency-related health risks and enhance overall metabolic health in this population.

Acknowledgement

I would like to thank all workers in the Diabetic and Endocrinology Unit of Al-Sadder Teaching Hospital in Iraq, Al-Najaf Province, and my thanks and appreciation to all who participated in this study.

DOI: https://doi.org/10.36320/ajb/v17.i3.21730

Funding Information

No financial support was received for this manuscript.

Author's Contributions

Intisar Razzaq Sharba led the study, overseeing the planning, supervision, and statistical analysis of the research. She also drafted the initial manuscript and was responsible for figure design. Baneen Ali Abdulrahman and Dhamya K. Sarhan contributed to the interpretation of results, assisted with laboratory analyses, and supported the preparation of tables and figures.
Fatima K. Kazim (undergraduate student) was responsible for sample collection, performing the experimental procedures, and drafting the initial version of the manuscript. All authors reviewed the results, contributed to the discussion, and approved the final version of the manuscript.

Ethics

This investigation was carried out with permission from the University of Kufa medical ethics committee (2024). Consent was given verbally by each participant.

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