ORIGINAL ARTICLE

Hidden Deficiency: How Metformin Use Drives Vitamin B12 Depletion in Type 2 Diabetes

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is commonly managed with metformin, an effective oral hypoglycemic agent. However, prolonged metformin use has been linked to vitamin B12 deficiency, a condition that, if left untreated, can lead to serious complications. Limited research on this issue in certain populations underscores the importance of understanding metformin's impact on vitamin B12 levels.

Objective: The study aims to assess the prevalence of vitamin B12 deficiency in T2DM patients on long-term metformin therapy and to identify its associated factors.

Methodology: A cross-sectional study was conducted at Chandka Medical College, Larkana for one year (January 2023 to December 2023) with 200 T2DM patients treated with metformin for one year. Participants were interviewed regarding socio-demographics, lifestyle, and clinical history, with particular attention to metformin dose and treatment duration. Blood samples were collected to measure serum vitamin B12 levels, and data was analyzed using SPSS 24.0. A p-value of ≤0.05 was considered statistically significant.

Results: The mean age of participants was 43.75 years, with an age distribution consisting of 41-50 years (42.5%), followed by 31-40 years (35%). The majority of patients were females (56%). The prevalence of vitamin B12 deficiency was 40%. In multivariable regression analysis, female gender (aOR=1.82, 95% CI: 1.10-3.05) and increasing diabetes duration (aOR=2.08, 955 CI: 1.20-3.45) were found to be associated with b12 deficiency. No significant relationship of B12 deficiency was found with metformin dosage or duration.

Conclusion: This study highlights a substantial prevalence of vitamin B12 deficiency among T2DM patients on metformin, with female gender and longer diabetes duration identified as a notable risk factor. Regular monitoring of vitamin B12 levels in metformin-treated patients, particularly females and those with prolonged diabetes, is recommended to prevent potential complications.

Keywords: *Type 2 diabetes mellitus, metformin, vitamin B12 deficiency, glycemic control, risk factors, observational study.*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is diagnosed by insulin resistance of the body with a lack of sufficient insulin production that triggers chronic hyperglycemia, or high blood sugar levels [1]. Among the available drugs meant to treat this, biguanides, especially metformin are most used as the initial oral drug interference in the management of T2DM, mainly administered along with healthy lifestyle changes such as diet and exercise [2]. The medication works by increasing the body's sensitivity to insulin, thereby reducing insulin resistance. It can be used as a monotherapy or as a combination with other oral antidiabetic agents to enhance glycemic control [3]. However, metformin's side effects may onset with the first dose, and it may vary with the dose or frequency of administration. Where most gastrointestinal problems, including nausea and diarrhea, are the common side effects of metformin treatment, another related but lesser-known side effect is the risk of vitamin B12 deficiency, which also may remain unappreciated in actual clinical practice [4, 5].

Vitamin B12 is a water-soluble nutrient. The richest sources of vitamin B12 include meats, fish, poultry, eggs, and dairy products. It has a daily recommended adult intake of 2.4 micrograms and participates in many important physiological functions [6, 7]. Among those functions are the production of red blood cells or hematopoiesis, DNA synthesis, and neurologic function management. Severe health conditions such as megaloblastic anemia, digestive disorders, and neurological conditions like peripheral neuropathy could occur if there is a shortage of this essential vitamin [8-10]. Several clinical studies have documented that the serum levels of vitamin B12 are frequently decreased in patients with long-term treatment of metformin [11-14]. Observational research has also documented that higher doses of metformin, longer exposure duration, and concomitant therapy with proton pump inhibitors or

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histamine H2-antagonists increase the risk of vitamin B12 deficiency [12]. For example, a meta-analysis that examined T2DM patients under metformin therapy found significant reductions in vitamin B12 during this course of treatment compared to controls not receiving this drug [15].

Studies show that the serum vitamin B12 concentration is reduced by approximately 14% to 30% when metformin is used [13, 14]. Because T2DM is prevalent globally in most regions and complications may be given credit because of metformin-related vitamin B12 deficiency, it is one such area that needs to be taken more seriously. This is particularly true for a country like Pakistan, wherein a minimal amount of research is done on this subject, and therefore, further knowledge of such correlation has to be established to enhance patient care in this regard. Therefore, the current study seeks to assess the prevalence rate of vitamin B12 deficiency among patients diagnosed with T2DM, on metformin therapy. Additionally, the present study seeks to identify factors for vitamin B12 deficiency among T2DM patients treated with metformin.

METHODOLOGY

This was a cross-sectional study, conducted at medicine clinics, Chandka Medical College, SMBBMU, Larkana, Pakistan for one year from January 2023 to December 2023. After obtaining the ethics clearance from the institutional review board (SMBBMU/ERB/2022-16), recruitment of participants from outpatient medical clinics was started. All patients were given written, informed consent before data collection was initiated.

The study enrolled T2DM patients on treatment with metformin for at least one year. Patients older than 30 years, both males and females, were selectively included. Patients diagnosed with diabetes for less than a year, those on insulin, PPIs, and H2 receptor blockers, and less than one year of disease duration were excluded from the study. Other exclusion criteria included pregnant women, secondary diabetes, T1DM, alcohol users, patients with gastrointestinal diseases such as inflammatory bowel disease, or those who have had any surgical procedure like gastrectomy or bariatric surgery. Any patient with an incomplete medical history or having serious comorbidities, or a patient on vitamin B12 supplements in any form; that is oral and intramuscular were excluded.

A total sample size of 200 was determined based on a previous study [16]. The calculation was performed using the WHO sample size calculator, assuming a 95% confidence interval level and a 5% margin of error. Non-probability consecutive sampling was employed to ensure that every eligible participant available during the study period was included.

Interviews with participants were face-to-face and comprehensive. At the time of the interviews, a structured data collection tool, adapted from a validated Food Frequency Questionnaire (FFQ), was used with a total of 32 items [13]. The first component focused on socio-demographic information, comprising seven questions covering age, gender, education, marital status, residence, smoking status, and occupation. The second component, comprising nine questions, assessed clinical history through items on comorbidities, including hypertension, diabetes duration, and metformin usage details. The third component, comprising thirteen questions, explored lifestyle habits, consisting of questions, including food frequency related to vitamin B12 intake from sources such as dairy, meats, seafood, and fortified foods. The questionnaire included both categorical and continuous variables. Socio-demographic and clinical history responses were recorded as distinct categories, while lifestyle habits, including dietary frequency of vitamin B12 intake, were scored numerically. For instance, food frequency was categorized on a Likert scale (*e.g.*, daily = 5, weekly = 4, monthly = 3, rarely = 2, never = 1). The cumulative scores for dietary intake provided a questionnaire measure of vitamin B12 consumption, enabling comparison across groups.

Samples of blood were drawn from every patient and forwarded to the diagnostic laboratory for serum vitamin B12 levels. Samples forwarded were then subjected to standardized procedures for vitamin B12 assays, which will thus allow proper categorization of the patients as either having a deficiency or normal vitamin B12 levels (Normal levels ≥300 pg/mL; Borderline: 200-300 pg/mL; Deficiency <200 pg/mL). Patients in borderline range were not included because borderline levels, while acknowledged as an intermediate state, often do not represent a distinct clinical entity, but rather a transitional zone that is treated variably in practice. The clinical features of patients in each of the two groups were compared to find any possible associations between metformin treatment and vitamin B12 deficiency after grouping.

The statistics in the three variables were determined by using the software of SPSS 24.0. For categorical variables like gender, comorbidities, and smoking status, the frequency and percentage were used, while for continuous variables like age, metformin dosage, and levels of vitamin B12, mean ± SD was used. Continuous variables between groups were compared using an appropriate version of the student's t-test, while Pearson's chi-square test was used to assess the relationship between categorical variables. A binary logistic regression analysis with the manual purposeful selection method was also performed to examine associations between vitamin B12 deficiency with socio-demographics, metformin dosage, and other clinical variables. Odds ratios (OR) were calculated to determine the strength of such associations. The calibration of the final model was assessed using the Hosmer and

Lemeshow goodness-of-fit test, and its discrimination was assessed by the area under the receiver operator characteristic (ROC) curve. A p-value of ≤0.05 was considered statistically significant.

RESULTS

The average age of participants was 43.75±5.62 years, with a majority being females (56%) and predominantly urban residents (75%). Smoking status was reported by 28% of participants, and 40% had a history of hypertension. Detailed demographic distributions are presented in Table **1**.

Vitamin B12 deficiency was observed in 40% of the participants. The prevalence of vitamin B12 deficiency was 40% with a significantly higher rate observed in female as compared to male patients (65% vs. 35%, p=0.002). Patients with a diabetes duration of more than two years were more likely to have vitamin B12 deficiency (p=0.014). There was no significant difference in the prevalence of deficiency based on metformin dosage or duration, hypertension, or smoking status (**Table 2**).

Logistic regression analysis revealed that female gender and longer diabetes duration were significantly associated with an increased risk of vitamin B12 deficiency. Unadjusted odds showed that females had

Table 1: Socio-demographic characteristics of patients (n=200).

Parameter	n(%)	Vitamin B12 Deficient n(%)	Normal Vitamin B12 n(%)	p-value
Age (in years)				
31-40	70 (35)	32 (45.71)	38 (54.28)	0.242
41-50	85 (42.5)	36 (42.35)	49 (57.64)	0.512
51-60	45 (22.5)	16 (33.55)	29 (64.44)	0.196
Gender				
Male	88 (44)	28 (31.81)	60 (68.18)	*0.002
Female	112 (56)	52 (46.42)	60 (53.57)	
Education				
Not educated	40 (20)	18 (45)	22 (55)	0.472
Primary education	50 (25)	21 (42)	29 (58)	0.984
Secondary education	65 (32.5)	25 (38.46)	40 (61.53)	0.765
Higher education	45 (22.25)	17 (37.77)	28 (62.22)	0.714
Marital Status				
Married	160 (80)	64 (40)	96 (60)	0.461
Single	40 (20)	16 (40)	24 (60)	
Residence				
Urban	150 (75)	66 (44)	84 (56)	0.682
Rural	50 (25)	20 (40)	30 (60)	
Smoking				
Yes	56 (28)	22 (39.28)	34 (60.71)	0.622
No	144 (72)	58 (40.27)	86 (59.72)	
Occupation				
Employed	70 (35)	33 (47.14)	37 (52.85)	0.598
Unemployed	48 (24)	21 (43.75)	27 (56.25)	0.821
Retired	55 (27.5)	20 (36.36)	35 (63.63)	0.513
Student	27 (13.5)	10 (37.03)	17 (62.96)	0.738

*Significant at p<0.05

Table 2: Clinical and medication variables by Vitamin B12 status (n=200).

Parameter	n(%)	Vitamin B12 Deficient n(%)	Vitamin B12 Deficient n(%)	p-value
Duration of Metformin (years)				
≤1	94 (47)	34 (36.17)	60 (63.82)	0.241
>1	106 (53)	48 (45.28)	58 (54.71)	
Dosage of Metformin (mg/day)				
≤1000	95 (47.5)	38 (40)	57 (60)	0.589
>1000	105 (52.5)	40 (39.09)	65 (61.9)	
Hypertension	80 (40)	34 (42.5)	46 (57.5)	0.728
Duration of T2DM (in years)				
1-2	72 (36)	24 (33.33)	48 (66.66)	*0.014
>2	128 (64)	56 (42.75)	72 (56.25)	

*Significant at p<0.05

Table 3: Logistic regression for risk factors of vitamin B12 deficiency (n=200).

Parameter	Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio (95% CI)	p-value
Female Gender	1.984 (1.215-2.8540	*0.002	1.82 (1.10-3.05)	*0.024
Duration of T2DM	2.15 (1.325-3.124)	0.014	2.08 (1.20-3.45)	0.016
Hypertension	1.064 (0.623-1.631)	0.728	1.15 (0.65-1.95)	0.553
Metformin Dosage	0.924 (0.675-1.482)	0.589	0.88 (0.61-1.42)	0.635
Metformin Duration	0.864 (0.522-1.342)	0.241	0.91 (0.56-1.47)	0.461
Smoking	0.921 (0.572-1.568)	0.682	0.94 (0.59-1.61)	0.721
Hosmer-Lemeshow Test	X^2= 8.5	0.30	-	-

CI: Confidence interval, *Significant at p<0.05

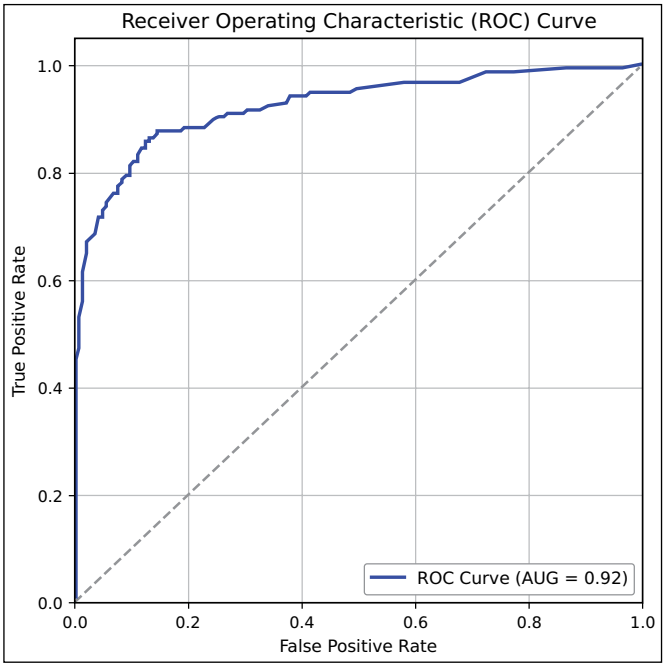

Fig. (1): Calibration and discrimination of the model by ROC curve.

nearly twice the risk (OR = 1.984; 95% CI: 1.325-3.124). After adjustment, these factors remained significant, with females (aOR = 1.82, 95% CI: 1.10 - 3.05 and longer diabetes duration (aOR = 2.08; 95% CI: 1.20-3.45) showing strong associations with vitamin B12 deficiency. Other variables such as metformin dose, duration of metformin use, smoking, and hypertension, did not exhibit significant associations (**Table 3**). The model's calibration was assessed using the Hosmer-Lemeshow test, and its discrimination was evaluated by calculating the area under the ROC curve, demonstrating the model's reliability in predicting vitamin B12 deficiency (**Fig. 1, Table 3**).

DISCUSSION

This study reported a high prevalence of vitamin B12 deficiency, with 40% of T2DM subjects on metformin having serum levels below 200 pg/mL. This value is similar to that found in the literature, where the prevalence ranged from 4 to 51% among users of metformin [9-14]. However, the prevalence of vitamin B12 deficiency remained undetermined due to limited population-based data. The results of this study also agree with those of Iftikhar *et al.* (31%) and Khan *et al.* (30.7%) [14, 16]. A study conducted by Shahwan *et al.* reported a higher prevalence rate of 48% [17]. Another study that found prevalence rates was conducted by Malla *et al.,* at a rate of 50.95% [18]. The other studies had very low rates; some were reported by Damião *et al.,* at 22.5% [19]. Still in Brazil, Chapman *et al.* reported a rate of 21.5%, and Almatrafi SB *et al.* reported a rate of 17.5% [13, 20]. Differences in prevalence rates across studies can stem from various factors, including variations in methodological approaches, differences in cutoff values of vitamin B12 deficiency, and cultural or geographic influences on dietary intakes. For instance, geographical variations in dietary habits, where higher intakes of products from animal sources that are rich in vitamin B12, account for the variation of deficiency rates in populations. Not least, different definitions of deficiency may be applied in different studies, leading to reported prevalence differences. A further limitation is a reliance on other diagnostic tests, serum homocysteine levels, and methylmalonic acid, which are more sensitive for the determination of vitamin B12 status. Metformin is mainly prescribed as an initial oral hypoglycemic agent, and the dose is carefully increased step-wise to cope with gastrointestinal adverse effects of diarrhea, nausea, flatulence, indigestion, vomiting, or abdominal pain. A severe deficiency of vitamin B12 can cause major anemia and even other problems in gastrointestinal or neurological spheres. Vitamin B12 deficiency was mainly observed in middle-aged patients; most of them were 41-50 years of age, with a gender disparity of 65% being female. According to Almatrafi *et al.* and Damião *et al.,* female gender has been associated with vitamin B12 deficiency and even reported similar findings [13, 19]. Studies by Chapman *et al.* and Tan *et al.* identified female sex and middle-aged groups (40-55 years) as the most significant predictors of vitamin B12 deficiency among T2DM patients on prolonged metformin therapy [20, 21]. These findings align with the current study, where a significant gender disparity and a higher prevalence in middle-aged females were observed. Variations in prevalence across studies may be attributed to differences in dietary practices, genetic predispositions, and healthcare access between populations.

Of note, 41.1% (39 patients) of deficient patients had been on metformin for one to two years, whereas 58.9% (56 patients) had received it for longer than two years. There are observations aligned with results from various studies, demonstrating that the risk associated with vitamin B12 deficiency is the time spent on metformin treatment [22-25]. However, the dosage of metformin was not significantly different between deficient and non-deficient groups (1410 ± 340 mg), and also that the duration of therapy did not show any significant difference between deficient and non-deficient groups (2.15 ± 0.55 years; p=0.448 and p=0.589, respectively). The absence of a significant association between metformin dosage or treatment duration and vitamin B12 deficiency suggests that other factors, such as dietary supplementation, absorption variability, or genetic influences, may play a larger role in determining deficiency risk. Additionally, the small sample size and lack of detailed dietary data could have limited the detection of potential associations. Hypertension was not a risk factor in this study p=0.55, and other comorbidities were also insignificantly connected to vitamin B12 deficiency; results are in contrast with some other reports indicating that chronic diseases are risk factors for nutrient deficiencies. Hence, this lack of correlation can further support the idea of the fact that managing hypertension does not inherently alleviate the risk of vitamin B12 deficiency in T2DM patients on metformin. Among the patients, 35.8% had low vitamin B12 intake, although the levels of serum vitamin B12 did not show significant association with these patients. This finding is in line with studies done by other studies, which explained that few correlations were not significant due to various dietary as well as cultural influences [12, 25]. Although low dietary intake indeed has a link with increased risk of deficiency, other factors such as malabsorption syndromes, gastrointestinal health, and lifestyle-related factors may play an important role in determining vitamin B12 status.

Additionally, the period that patients experienced T2DM was statistically significant as vitamin B12 levels were significantly different depending on the period of T2DM experience (p=0.016) because a high prevalence of deficiency happened in patients whose history of diabetes extends more than two years. This aligns with the published work that details a connection of more considerable T2DM duration with inducing vitamin B12 deficiency [12,17-19]. This also highlights the need for the regular screening of vitamin B12 levels of T2DM

patients on metformin medications, particularly those diagnosed with diabetes for a long time and women.

Some strengths of this study consist of the consideration given to risk factors for the development of vitamin B12 deficiency and the statistics on metformin therapy. Although the calibration of the model was poor with the p-value of Hosmer and Lemeshow goodness-of-fit test 0.30, the discrimination of the model was high with AUC 0.92. A standardized food frequency questionnaire was administered to elicit dietary appraisals of vitamin B12 intake. However, several limitations ought to be acknowledged. The sample size is rather small, and participants were recruited from only one hospital, which can limit the generalizability of findings. Baseline vitamin B12 levels before metformin therapy are lacking and limit our insights into the causal relation between metformin use and vitamin B12 deficiency. Finally, the study did not use more sensitive methods of deficiency assessment, such as serum homocysteine and methylmalonic acid concentrations. Such levels are key to the proper assessment of vitamin B12 status. Additionally, more possible biases could be recall bias, selection bias, confounding factors, geographic and cultural variability, and cross-sectional design.

Future research should focus on longitudinal studies involving larger and more diverse populations to better understand the interplay between metformin dosage, dietary habits, genetic predisposition, and vitamin B12 metabolism. Incorporating sensitive diagnostic tools, such as serum homocysteine or methylmalonic acid levels, would provide a more accurate assessment of vitamin B12 status. In future studies, longitudinal follow-up for vitamin B12 levels, combined with nutritional evaluation, and gastrointestinal health monitoring, will provide more comprehensive insights into risk factors associated with vitamin B12 deficiency in patients with T2DM on metformin. Exploring lifestyle interventions like diet modification and supplementation may also help in managing vitamin B12 deficiency in this population.

CONCLUSION

The study identifies a high prevalence of vitamin B12 deficiency among T2DM patients on metformin, particularly in females and those with prolonged diabetes. These findings underscore the need for regular monitoring of vitamin B12 levels and proactive nutritional management to prevent complications. Future research is needed to explore underlying mechanisms and effective interventions.

ETHICS APPROVAL

Ethical approval was obtained from the Institutional Review Board (No. SMBBMU/ERB/2022-16). All procedures performed in studies involving human participants were following the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

All patients gave written, informed consent before data collection was initiated.

AVAILABILITY OF DATA

The data that supports the findings are available in the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTION

Rizwan Ali Talpur: Conceptualization, Writing, Original Draft Preparation, Formal Analysis, Review & Editing, Supervision.

Shahzad Ali Jiskani: Data Collection, Formal Analysis, Writing, Review & Editing.

Muhammad Ishaque Bhatti: Analysis, Writing, Review & Editing, Visualization.

Anam Shaikh: Writing, Review & Editing, Analysis.

Jawed Iqbal: Writing, Review & Editing, References.

Rida Qureshi: Writing, References, Formal Analysis.

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