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PREVALENCE AND CLINICAL PREDICTORS OF VITAMIN B12 DEFICIENCY AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS RECEIVING LONG-TERM METFORMIN THERAPY

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ABSTRACT

Background: Long-term metformin therapy may increase the risk of vitamin B12 deficiency in patients with type 2 diabetes mellitus. The objective is to determine the prevalence and clinical predictors of vitamin B12 deficiency among long-term metformin users.

Materials and Methods: A hospital-based cross-sectional analytical study was conducted at Pakistan Institute of Medical Sciences, Islamabad, from January 2024 to December 2025 among 360 adults with type 2 diabetes receiving metformin for ≥2 years. Demographic, clinical, dietary, treatment, and laboratory data were analyzed using multivariable logistic regression.

Results: Vitamin B12 deficiency was found in 116 (32.22%) patients (95% CI: 27.42–37.32%), while 67 (18.61%) had borderline and 177 (49.17%) adequate levels. Deficient patients had lower serum B12 (157.40 ± 28.60 vs. 384.70 ± 112.50 pg/mL; p<0.001), longer metformin use (9.80 ± 4.10 vs. 7.30 ± 3.80 years; p<0.001), and higher daily dose (1650 ± 540 vs. 1420 ± 480 mg/day; p<0.001). Independent predictors included metformin duration >10 years (AOR=2.31; p=0.003), dose >2000 mg/day (AOR=2.18; p=0.041), peripheral neuropathy (AOR=2.07; p=0.005), low dietary B12 intake (AOR=1.91; p=0.009), age ≥60 years (AOR=1.82; p=0.025), diabetes duration >10 years (AOR=1.74; p=0.039), and acid-suppressive medication use (AOR=1.61; p=0.046).

Conclusion: Vitamin B12 deficiency is common among long-term metformin users, highlighting the need for regular assessment and early identification of high-risk patients.

Keywords: Vitamin B12 deficiency; Type 2 diabetes mellitus; Metformin; Peripheral neuropathy; Risk factors; Vitamin B12 screening.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease, wherein hyperglycemia and gradual metabolic dysfunction are present, necessitating long-term pharmacologic therapy for glycemic control and minimizing microvascular and macrovascular complications.[1,2] Metformin continues to be one of the main options for managing T2DM due to its proven glucose-lowering effects, its good safety profile, limited hypoglycaemic risk, and low cost.[3] Although it is widely used and long term exposure is a common occurrence, evidence has emerged showing that long-term metformin exposure can result in clinically relevant adverse effects involving B12 deficiency.[4] Metformin may lead to

biochemical vitamin B12 deficiency over time by causing changes in the way the vitamin is handled in the intestines. This risk may be more prominent over the course of treatment and with longer metformin use.[5]

Vitamin B12 is a micronutrient that plays a crucial role in DNA synthesis, erythropoiesis, myelin formation and normal neurological function.[6] Deficiency may lead to hematological, neurological and neurocognitive disorders such as megaloblastic anemia, fatigue, peripheral neuropathy, paresthesia, imbalance and cognitive dysfunction.[7] The symptoms of B12 deficiency are sometimes similar to those of diabetic complications, thus making the diagnosis of B12 deficiency a matter of concern in patients with T2DM.[8] For example, peripheral neuropathy might be solely due to diabetes, and a B12 deficiency which is present might not be identified. If this potentially reversible cause is not recognized, it may lead to continued neurologic complications from diabetes even with the correct management. Therefore, patients on long-term metformin treatment are an important population to consider vitamin B12 status in.[9]

Besides metformin therapy, several demographic, clinical, nutritional, and treatment-related factors contribute to the occurrence of vitamin B12 deficiency in patients who take the drug.[10] Risk factors include increasing age, prolonged metformin use, higher level of metformin exposure, insufficient dietary vitamin B12 intake, intestinal diseases that impair vitamin B12 absorption and concurrent use of other drugs that interfere with vitamin B12 absorption.[11] Anemia, peripheral neuropathy, renal dysfunction, and other metabolic features can also be clues of clinical relevance regarding a higher probability of deficiency. The magnitude and distribution of these risk factors, however, could differ across populations due to variations in dietary habits, access to health care services, and treatment patterns and baseline patient characteristics.[12]

This is especially important in Pakistan, where metformin is used extensively in primary, secondary and tertiary care settings, and is particularly used long term in patients with T2DM due to its burden.[13] But, there is limited evidence from clinical population in Pakistan about the prevalence of vitamin B12 deficiency in patients on long-term metformin therapy and importantly independent clinical characteristics predicting the vitamin B12 deficiency. In addition, the ability to identify patients at high risk based on easily obtained demographic, metabolic, treatment history, and clinical data may hold special promise in diabetes care. Therefore, systematic assessment of vitamin B12 status and associated clinical and treatment-related factors can yield population-specific evidence of the burden of vitamin B12 deficiency and factors associated with its occurrence among long-term metformin users. This is especially relevant given the fact that vitamin B12 deficiency can be clinically asymptomatic, as symptoms can mimic those of typical complications of T2DM.

Research Objective: To determine the prevalence of vitamin B12 deficiency and identify its clinical predictors among patients with type 2 diabetes mellitus receiving long-term metformin therapy.

MATERIALS AND METHODS

Study Design and Setting: A cross sectional analytical study was conducted at PIMS, Islamabad, Pakistan between January 2024 to December 2025 in the hospital setting. Patients included in the study were adult patients with type 2 diabetes mellitus (T2DM) who were on long-term metformin treatment.

Study Population: Eligible patients were age ≥18 years with documented T2DM who had been on metformin for a minimum of 2 years. All patients taking metformin alone or with other antidiabetic drugs were recruited. Patients who had previously been diagnosed with a B12 deficiency and prescribed replacement therapy, patients who became pregnant, patients who were unable to give informed consent, and patients who received regular vitamin B12 supplementation, major gastrointestinal surgery, bariatric surgery, or disorders that affect the ability to absorb or metabolize vitamin B12 were excluded.

Sample Size and Sampling: The sample size formula for one population proportion was used, $n = Z^2p(1-p)/d^2$, where the prevalence of vitamin B12 deficiency is expected to be 30%, the desired confidence level is 95%, and the margin of error is 5%. A sample size of 323 was calculated and after the potential for 10% incomplete or missing data, 360 participants were recruited. The number of participants was determined by consecutive sampling until the required number was reached.

Data Collection and Clinical Assessment: A structured and pretested questionnaire was used for data collection on demographic and clinical data. The data were age, sex, BMI, duration of diabetes, duration and daily dose of metformin, other antidiabetic and concomitant medications, dietary characteristics, gastrointestinal history and symptoms suggestive of vitamin B12 deficiency. The amount of metformin was documented according to the patient's medical records and treatment regimen as the total daily dose (mg/day). Prescribed doses were determined based on FDA-approved metformin labeling that recommends starting at 500 mg once daily and titrating as needed, to a maximum recommended dose of 2,000 mg per day, based on glycemic response and tolerability. For analysis, metformin exposure was categorized as ≤1,000 mg/day, 1,001–2,000 mg/day, and >2,000 mg/day. Standardised clinical and laboratory parameters were used to assess peripheral neuropathy and anaemia.

Laboratory Assessment: Serum samples were drawn from the veins for levels of vitamin B12, hemoglobin, HbA1c, serum creatinine and eGFR.

The validated laboratory method was used to measure the Vitamin B12 concentration at PIMS. Serum vitamin B12 < 200 pg/mL was defined as vitamin B12 deficiency and serum vitamin B12 of 200-300 pg/mL as borderline and recorded separately. In equivocal cases, the presence of methylmalonic acid or of homocysteine was taken into consideration, if present.

Study Variables: The major outcome was vitamin B12 deficiency. Factors that were considered as potential predictors were: age, sex, duration of diabetes, BMI, duration and dose of metformin use, dietary intake, anemia, peripheral neuropathy, renal function, glycemic control, and other medications that might impact vitamin B12 levels.

Statistical Analysis: Data were analyzed with the help of suitable statistical software. Means with standard deviations or medians with interquartile ranges were used for continuous variables and frequencies and percentages were used for categorical ones. A 95% confidence interval was determined for the prevalence of vitamin B12 deficiency. The independent t-test was used to test differences between groups for continuous variables while the chi-square test was used for categorical variables. Multivariable binary logistic regression was used to determine independent predictors of a vitamin B12 deficiency. Reported are adjusted odds ratios (AORs) with 95% confidence intervals (CIs). The clinically important variables and variables of relevance were considered for the multivariable model, based on their identification during initial analysis. Multicollinearity and model adequacy was evaluated. A two-sided $p < 0.05$ was considered statistically significant.

Ethical Considerations: All ethical approvals were granted by the respective institutional review committee (IRC) of PIMS, Islamabad. All participants gave informed written consent. The confidentiality of the data was respected during data collection, data analysis and reporting, and participants were notified that there was no impact on their medical care with their withdrawal.

RESULTS

[Table 1] presents the sociodemographic and clinical characteristics of participants according to vitamin B12 status. Patients with vitamin B12 deficiency had a significantly higher mean age than non-deficient patients (58.70 ± 11.20 vs. 53.40 ± 10.60 years; t=4.27, p<0.001) and longer diabetes duration (10.10 ± 4.80 vs. 8.20 ± 4.40 years; t=3.60, p<0.001). Significant associations were observed for age ≥60 years (44.83% vs. 25.00%; χ^2=14.35, p<0.001), diabetes duration >10 years (44.83% vs. 26.64%; χ^2=11.86, p<0.001), low dietary B12 intake (52.59% vs. 33.61%; χ^2=11.83, p<0.001), anemia (34.48% vs. 18.03%; χ^2=11.89, p<0.001), peripheral neuropathy (44.83% vs. 23.36%; χ^2=17.16, p<0.001), gastrointestinal disorders (23.28% vs. 13.93%; χ^2=4.88, p=0.027), renal dysfunction (20.69% vs. 9.43%; χ^2=8.79, p=0.003), and acid-suppressive medication use (46.55% vs. 31.97%; χ^2=7.20, p=0.007).

Table 1: Sociodemographic and clinical characteristics of patients according to vitamin B12 status (n=360)

Variable	B12 deficient (n=116)	B12 non-deficient (n=244)	Statistical test	Test value	*p*-value
Age, years	58.70 ± 11.20	53.40 ± 10.60	Independent t test	t = 4.27	<0.001
Age ≥60 years	52 (44.83%)	61 (25.00%)	Chi-square	χ^2 = 14.35	<0.001
Male sex	54 (46.55%)	140 (57.38%)	Chi-square	χ^2 = 3.71	0.054
BMI, kg/m²	28.40 ± 4.70	27.90 ± 4.50	Independent t test	t = 0.96	0.340
BMI ≥30 kg/m²	41 (35.34%)	72 (29.51%)	Chi-square	χ^2 = 1.24	0.265
Duration of diabetes, years	10.10 ± 4.80	8.20 ± 4.40	Independent t test	t = 3.60	<0.001
Diabetes duration >10 years	52 (44.83%)	65 (26.64%)	Chi-square	χ^2 = 11.86	<0.001
Low dietary B12 intake	61 (52.59%)	82 (33.61%)	Chi-square	χ^2 = 11.83	<0.001
Anemia	40 (34.48%)	44 (18.03%)	Chi-square	χ^2 = 11.89	<0.001
Peripheral neuropathy	52 (44.83%)	57 (23.36%)	Chi-square	χ^2 = 17.16	<0.001
Gastrointestinal disorder	27 (23.28%)	34 (13.93%)	Chi-square	χ^2 = 4.88	0.027
Renal dysfunction	24 (20.69%)	23 (9.43%)	Chi-square	χ^2 = 8.79	0.003
Acid-suppressive medication use	54 (46.55%)	78 (31.97%)	Chi-square	χ^2 = 7.20	0.007

Table 2: Metformin exposure and glycemic characteristics according to vitamin B12 status (n=360)

Variable	B12 deficient (n=116)	B12 non-deficient (n=244)	Statistical test	Test value	*p*-value
Metformin duration, years	9.80 ± 4.10	7.30 ± 3.80	Independent t test	t = 5.53	<0.001
Metformin duration >10 years	45 (38.79%)	50 (20.49%)	Chi-square	χ^2 = 13.56	<0.001
Daily metformin dose, mg/day	1,650 ± 540	1,420 ± 480	Independent t test	t = 3.91	<0.001
≤1000 mg/day	24 (20.69%)	80 (32.79%)	Chi-square	χ^2 = 9.43	0.009
1001–2000 mg/day	73 (62.93%)	148 (60.66%)			
>2000 mg/day	19 (16.38%)	16 (6.56%)			
Metformin monotherapy	38 (32.76%)	88 (36.07%)	Chi-square	χ^2 = 0.38	0.537
HbA1c, %	8.10 ± 1.90	7.80 ± 1.80	Independent t test	t = 1.42	0.156
HbA1c ≥9.0%	34 (29.31%)	55 (22.54%)	Chi-square	χ^2 = 2.04	0.153

[Table 2] shows the association of metformin exposure and glycemic characteristics with vitamin B12 status. Participants with deficiency had significantly longer metformin exposure (9.80 ± 4.10

vs. 7.30 ± 3.80 years; t=5.53, p<0.001) and a higher mean daily metformin dose (1,650 ± 540 vs. 1,420 ± 480 mg/day; t=3.91, p<0.001). Metformin use for >10 years was significantly more frequent among deficient participants (38.79% vs. 20.49%; χ^2=13.56, p<0.001), as was a dose >2000 mg/day (16.38% vs. 6.56%; χ^2=9.43, p=0.009). Metformin monotherapy did not differ significantly between groups (32.76% vs. 36.07%; χ^2=0.38, p=0.537), and HbA1c was also not significantly different (8.10 ± 1.90% vs. 7.80 ± 1.80%; t=1.42, p=0.156).

[Table 3] summarizes the laboratory findings according to vitamin B12 status. Mean serum vitamin B12 concentration was substantially lower among deficient participants than non-deficient participants (157.40 ± 28.60 vs. 384.70 ± 112.50 pg/mL; t=−29.61, p<0.001). Mean hemoglobin was also significantly lower in the deficient group (11.80 ± 1.70 vs. 12.60 ± 1.60 g/dL; t=−4.25, p<0.001). Serum creatinine was higher (1.19 ± 0.42 vs. 1.07 ± 0.31 mg/dL; t=2.74, p=0.007), while eGFR was lower (68.40 ± 21.70 vs. 76.20 ± 19.80 mL/min/1.73 m²; t=−3.28, p=0.001) among deficient participants. Renal dysfunction (eGFR <60 mL/min/1.73 m²) was also more frequent in the deficient group (20.69% vs. 9.43%; χ^2=8.79, p=0.003).

Table 3: Laboratory characteristics according to vitamin B12 status (n=360)

Laboratory parameter	B12 deficient (n=116)	B12 non-deficient (n=244)	Statistical test	Test value	*p*-value
Serum vitamin B12, pg/mL	157.40 ± 28.60	384.70 ± 112.50	Independent t test	t = −29.61	<0.001
Hemoglobin, g/dL	11.80 ± 1.70	12.60 ± 1.60	Independent t test	t = −4.25	<0.001
Anemia	40 (34.48%)	44 (18.03%)	Chi-square	χ^2 = 11.89	<0.001
Serum creatinine, mg/dL	1.19 ± 0.42	1.07 ± 0.31	Independent t test	t = 2.74	0.007
eGFR, mL/min/1.73 m²	68.40 ± 21.70	76.20 ± 19.80	Independent t test	t = −3.28	0.001
eGFR <60 mL/min/1.73 m²	24 (20.69%)	23 (9.43%)	Chi-square	χ^2 = 8.79	0.003

[Table 4] demonstrates the prevalence and distribution of vitamin B12 status among the 360 participants. Vitamin B12 deficiency (<200 pg/mL) was identified in 116 (32.22%) participants, with a 95% CI of 27.42–37.32%. A further 67 (18.61%) participants had borderline vitamin B12 concentrations of 200–300 pg/mL, whereas 177 (49.17%) had adequate concentrations (>300 pg/mL). Thus, approximately one-third of patients receiving long-term metformin therapy had biochemical vitamin B12 deficiency.

Table 4: Prevalence and distribution of vitamin B12 status among study participants (n=360)

Vitamin B12 status	Number of Patients (n)	Percentage (%)	95% CI
Deficient (<200 pg/mL)	116	32.22	27.42–37.32
Borderline (200–300 pg/mL)	67	18.61	14.78–23.02
Adequate (>300 pg/mL)	177	49.17	43.86–54.48
Total	360	100.00	—

[Table 5] summarizes the categorical comparisons between vitamin B12-deficient and non-deficient participants. Significant associations were observed for age ≥60 years (χ^2=14.35, p<0.001), diabetes duration >10 years (χ^2=11.86, p<0.001), metformin duration >10 years (χ^2=13.56, p<0.001), metformin dose >2000 mg/day (χ^2=7.61, p=0.006), low dietary B12 intake (χ^2=11.83, p<0.001), anemia (χ^2=11.89, p<0.001), peripheral neuropathy (χ^2=17.16, p<0.001), gastrointestinal disorders (χ^2=4.88, p=0.027), renal dysfunction (χ^2=8.79, p=0.003), and acid-suppressive medication use (χ^2=7.20, p=0.007). HbA1c ≥9.0% was not significantly associated with deficiency (χ^2=2.04, p=0.153).

Table 5: Comparison of categorical clinical and treatment-related factors between vitamin B12-deficient and non-deficient participants

Variable	B12 deficient n (%)	B12 non-deficient n (%)	χ^2 value	*p*-value
Age ≥60 years	52 (44.83%)	61 (25.00%)	14.35	<0.001
Diabetes duration >10 years	52 (44.83%)	65 (26.64%)	11.86	<0.001
Metformin duration >10 years	45 (38.79%)	50 (20.49%)	13.56	<0.001
Metformin dose >2000 mg/day	19 (16.38%)	16 (6.56%)	7.61	0.006
Low dietary B12 intake	61 (52.59%)	82 (33.61%)	11.83	<0.001
Anemia	40 (34.48%)	44 (18.03%)	11.89	<0.001
Peripheral neuropathy	52 (44.83%)	57 (23.36%)	17.16	<0.001
Gastrointestinal disorder	27 (23.28%)	34 (13.93%)	4.88	0.027
Renal dysfunction	24 (20.69%)	23 (9.43%)	8.79	0.003
Acid-suppressive medication use	54 (46.55%)	78 (31.97%)	7.20	0.007
HbA1c ≥9.0%	34 (29.31%)	55 (22.54%)	2.04	0.153

[Table 6] presents the multivariable binary logistic regression analysis of independent predictors of vitamin B12 deficiency. Metformin duration >10 years was independently associated with higher odds of deficiency (AOR=2.31, 95% CI: 1.34–3.98; Wald χ^2=8.72, p=0.003), followed by peripheral neuropathy (AOR=2.07, 95% CI: 1.25–3.43; Wald χ^2=7.88, p=0.005), metformin dose >2000 mg/day

(AOR=2.18, 95% CI: 1.03–4.61; Wald χ^2=4.18, p=0.041), low dietary B12 intake (AOR=1.91, 95% CI: 1.18–3.10; Wald χ^2=6.78, p=0.009), age ≥60 years (AOR=1.82, 95% CI: 1.08–3.07; Wald χ^2=5.03, p=0.025), diabetes duration >10 years (AOR=1.74, 95% CI: 1.03–2.95; Wald χ^2=4.26, p=0.039), and acid-suppressive medication use (AOR=1.61, 95% CI: 1.01–2.58; Wald χ^2=3.98, p=0.046). Anemia, gastrointestinal disorders, renal dysfunction, female sex, and HbA1c ≥9.0% were not independently significant.

Table 6: Multivariable binary logistic regression analysis of independent predictors of vitamin B12 deficiency

Predictor	β coefficient	AOR	95% CI	Wald χ^2	*p*-value
Age ≥60 years	0.599	1.82	1.08–3.07	5.03	0.025
Female sex	0.191	1.21	0.76–1.93	0.65	0.418
Diabetes duration >10 years	0.554	1.74	1.03–2.95	4.26	0.039
Metformin duration >10 years	0.837	2.31	1.34–3.98	8.72	0.003
Metformin dose >2000 mg/day	0.779	2.18	1.03–4.61	4.18	0.041
Low dietary B12 intake	0.647	1.91	1.18–3.10	6.78	0.009
Anemia	0.519	1.68	0.98–2.88	3.58	0.058
Peripheral neuropathy	0.727	2.07	1.25–3.43	7.88	0.005
Gastrointestinal disorder	0.445	1.56	0.82–2.97	1.83	0.176
Renal dysfunction	0.637	1.89	0.94–3.80	3.19	0.074
Acid-suppressive medication use	0.476	1.61	1.01–2.58	3.98	0.046
HbA1c ≥9.0%	0.166	1.18	0.70–1.98	0.38	0.536

[Table 7] presents the assessment of the multivariable model. The Hosmer–Lemeshow goodness-of-fit test showed acceptable model fit (χ^2=6.84, p=0.554). The Nagelkerke R^2 was 0.286, indicating that the model explained approximately 28.60% of the variation in vitamin B12 deficiency. The maximum VIF was 2.41 and minimum tolerance was 0.415, indicating no important multicollinearity. The overall model classification accuracy was 72.50%.

Table 7: Assessment of multicollinearity and adequacy of the multivariable logistic regression model

Model assessment	Statistic	Value	Interpretation
Hosmer–Lemeshow goodness-of-fit	χ^2	6.84	Acceptable model fit
Hosmer–Lemeshow test	*p*-value	0.554	Acceptable fit
Nagelkerke R^2	R^2	0.286	28.60% variation explained
Maximum variance inflation factor	VIF	2.41	No important multicollinearity
Minimum tolerance	Tolerance	0.415	No important multicollinearity
Overall classification accuracy	%	72.50	Acceptable classification

DISCUSSION

In the present study, vitamin B12 deficiency was identified in 116 of 360 participants (32.22%; 95% CI: 27.42–37.32%), while 67 (18.61%) had borderline concentrations and 177 (49.17%) had adequate levels. This prevalence is similar to that observed in patients with T2DM who took metformin in Khyber Pakhtunkhwa, Pakistan (29.66%).[14] but was based on lower deficiency level of 150 pg/mL. Likewise, a study in Pakistan from Rahim Yar Khan showed that 36.54% of the metformin-treated patients were deficient.[15] There is also plenty of international evidence showing that the burden is significant: a large study showed biochemical deficiency in 22.2%, while a long-term metformin study showed higher rates of biochemical deficiency over time.[16] Some discrepancies between studies could be attributed to different definitions of deficiencies, varying duration of metformin treatment, varying diet habits and varying population traits. However, the current vitamin B12 deficiency prevalence of almost one-third shows that vitamin B12 deficiency is a major clinical problem in long-term metformin users.

The present study also showed that there were significant differences in the demographic and diabetes-related parameters between the deficient and non-deficient groups. Deficient patients were older (58.70 ± 11.20 vs. 53.40 ± 10.60 years; p<0.001) and had longer diabetes duration (10.10 ± 4.80 vs. 8.20 ± 4.40 years; p<0.001); age ≥60 years occurred in 44.83% vs. 25.00% (p<0.001) and diabetes duration >10 years in 44.83% vs. 26.64% (p<0.001). These results are similar to those of other studies which have found increased age and duration of diabetes as two factors that are associated with reduced B12 level. Time since T2DM has been shown to be a significant risk factor for B12 deficiency in a recent study conducted in Pakistan.[15] and a study conducted in Vietnam also reported an association between the duration of T2DM and B12 deficiency.[15] The number of years of diabetes (duration >10 years; AOR=1.74, 95% CI: 1.03–2.95; p=0.039) and age ≥60 years (AOR=1.82, 95% CI: 1.08–3.07; p=0.025) were other factors associated with deficiency in multivariable analysis, suggesting that these variables may identify patients who need more frequent biochemical monitoring.

One of the strongest relationships was observed with the exposure of metformin, and vitamin B12 deficiency. Deficient participants had longer metformin use (9.80 ± 4.10 vs. 7.30 ± 3.80 years; p<0.001) and a higher daily dose (1,650 ± 540 vs.

1,420 ± 480 mg/day; p<0.001). Metformin use >10 years occurred in 38.79% vs. 20.49% (p<0.001), while doses >2000 mg/day occurred in 16.38% vs. 6.56% (p=0.006). Importantly, both remained independent predictors: metformin duration >10 years (AOR=2.31, 95% CI: 1.34–3.98; p=0.003) and dose >2000 mg/day (AOR=2.18, 95% CI: 1.03–4.61; p=0.041). The results are consistent with Kim et al., (2019), who reported a dose-dependent relationship with dose ≥2000 mg/day being significantly more likely to be deficient, and Khattab et al., (2023) who showed that greater metformin cumulative exposure was associated with increased odds of deficiency.[16] A randomized trial found a 19% decrease in serum B12 levels during long-term metformin therapy vs placebo, lending further support to the biological plausibility.[17]

These nutritional and clinical factors also made a significant contribution to the pattern observed. Participants with B12 deficiency had low dietary B12 intake 52.59% compared to 33.61% of non-deficient participants (p<0.001) and this remained independently associated with B12 deficiency (AOR=1.91, 95% CI: 1.18–3.10; p=0.009). Peripheral neuropathy was observed in 44.83% vs. 23.36% (p<0.001) and was independently associated with deficiency (AOR=2.07, 95% CI: 1.25–3.43; p=0.005). These results have clinical significance, since B12 deficiencies can have neurological symptoms similar to diabetic neuropathy. However, the findings are not completely congruent, because Ahmed et al. (2016) did not observe any significant relationship between B12 deficiency and neuropathy, whereas others noted that patients with lower B12 levels exposed to metformin had more neuropathy.[18] Thus, the correlation observed in the present study should not be interpreted as causal, because of the crosssectional design, but rather as an important clinical indicator.

The significance of the deficiency was further demonstrated in the laboratory. Mean serum B12 was markedly lower among deficient participants (157.40 ± 28.60 vs. 384.70 ± 112.50 pg/mL; p<0.001), accompanied by lower hemoglobin (11.80 ± 1.70 vs. 12.60 ± 1.60 g/dL; p<0.001), higher creatinine (1.19 ± 0.42 vs. 1.07 ± 0.31 mg/dL; p=0.007), and lower eGFR (68.40 ± 21.70 vs. 76.20 ± 19.80 mL/min/1.73 m²; p=0.001) (Table 3). Anemia occurred in 34.48% vs. 18.03% (p<0.001) and renal dysfunction in 20.69% vs. 9.43% (p=0.003). However, neither anemia (AOR=1.68; p=0.058) nor renal dysfunction (AOR=1.89; p=0.074) were independently significant, suggesting that these crude associations can be explained partly by age, diabetes duration, and/or metformin use, and other correlated factors. Deficiency was also found to be independently associated with acid-suppressive medication use (46.55% vs. 31.97%; AOR=1.61, 95% CI: 1.01–2.58; p=0.046), in line with literature indicating that the use of acid-suppressive medication may exacerbate metformin-associated B12 deficiency.[19]

In overall, the present study findings showed that long-term use of metformin is associated with Vitamin B12 deficiency in about one-third (32.22%) of patients and is independently associated with older age, longer duration of diabetes, long duration of metformin use, low dietary intake of Vitamin B12, peripheral neuropathy, and use of acid-suppressive drugs. In the regression model, the Hosmer–Lemeshow χ^2=6.84, p=0.554, there was no important multicollinearity (VIF maximum=2.41; tolerance minimum=0.415) and the classification accuracy was 72.50%. This is similar to the systematic evidence of decreased B12 status with metformin and to dose- and time-dependent effects.[20] Clinically the results strongly endorse regular monitoring of B12 levels in long-term metformin users, especially in those with long-term or high dose exposure and/or neurological side effects. However, this was a cross sectional study and prospective studies are required to determine whether B12 deficiency is a cause of neuropathy and it will also help to see if correcting B12 deficiency will enhance blood and nerve parameters.

Strengths And Limitations: The analytical evaluation of vitamin B12 deficiency in a well-defined population of 360 adults with T2DM who had received long-term metformin therapy was a key strength of this study, with systematic assessment of demographic, clinical, nutritional, treatment-related and laboratory characteristics. Standardized biochemical assessment, categorical comparisons, and multivariable logistic regression were used to identify independent predictors, and to control for potential confounding. The model showed a good fit, no important multicollinearity and a classification accuracy of 72.50%, which increased the reliability of the results. Nonetheless, some shortcomings need to be taken into account. The cross-sectional design does not allow for the determination of temporal and causal relationships between exposure to metformin and Vitamin B12 deficiency, especially with respect to peripheral neuropathy. The study was done in a single tertiary-care hospital, which may not be representative of other Pakistani population and community settings. Some clinical data and dietary information was collected using questionnaires and could thus suffer from recall or reporting bias. The main biochemical markers were serum vitamin B12; if present, this was used on top of serum folate, whereas the use of methylmalonic acid or homocysteine was only considered as a second level of confirmation of borderline or functional deficiency. Additionally, there is still the possibility of unrecognized confounding from nutritional, genetic or drug effects.

CONCLUSION

Patients with T2DM on long-term treatment with metformin were prone to vitamin B12 deficiency. Important and independent variables associated with

deficiency were older age, duration of diabetes more than 10 years, use of metformin, higher exposure to metformin, low dietary intake of B12, presence of peripheral neuropathy, and use of acid suppressive medication. The results of these studies raise clinical awareness and the need for periodic B12 monitoring in patients on long-term metformin therapy, especially those with known risk factors or neurological symptoms. Early diagnosis and treatment of the deficiency could help to avert potentially reversible hematological and neurologic complications.

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