Original Article
DOI: https://doi.org/10.70749/ijbr.v3i7.2902

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Association Between Uses of Metformin for Type II Diabetes Mellitus and Vitamin B12 Deficiency

Ayesha Naveed[1], Tariq Mahmood[2]

[1-2]Department of General Medicine, Combined Military Hospital Kohat, KPK, Pakistan

ARTICLE INFO

Keywords: Diabetes Mellitus, Type 2; Metformin; Vitamin B 12 Deficiency; Cross-Sectional Studies

Correspondence to: Ayesha Naveed, Department of General Medicine, Combined Military Hospital Kohat, KPK, Pakistan.
Email:ayeshanaveedayesha999@gmail.com

Declaration
Authors' Contribution: Both authors equally contributed to the study and approved the final manuscript.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 23-05-2025 Revised: 25-06-2025
Accepted: 03-07-2025 Published: 07-07-2025

ABSTRACT

Background: Type 2 diabetes mellitus is a major metabolic disorder that is usually managed by metformin as a first-line treatment. The long-term use of metformin has been known to cause a reduction in the blood level of vitamin B12. This can lead to anemia and neuropathy, which could be mistaken for diabetic neuropathy. **Objective:** To determine the frequency of vitamin B12 deficiency in patients with type 2 diabetes mellitus taking metformin. **Design of the study:** Cross sectional study. **Duration and Place of Study:** This study was conducted from 31 January 2025 to 20 May 2025 at the Department of General Medicine, Combined Military Hospital Kohat. **Methodology:** The study included a total of 160 subjects in the age group of 30-70 years, diagnosed with type 2 diabetes mellitus and undergoing metformin treatment for a minimum of two years. Vitamin B12 deficiency was defined as a level of less than 300 pg/mL. The analysis was done using SPSS software version 22. The quantitative values are presented as mean and standard deviation. After stratification of the subjects, Fisher's exact test was done. A value of ≤0.05 was considered significant. **Results:** Mean age was 50.16 ± 12.65 years. Vitamin B12 deficiency was found in 11 patients (6.90%). No significant association was found between deficiency and age, gender, duration of diabetes, body mass index, smoking, hypertension, education, residence or socioeconomic status ($p > 0.05$). **Conclusion:** Vitamin B12 deficiency was present in patients using metformin, but no significant association was observed with studied demographic and clinical factors.

INTRODUCTION

Type 2 Diabetes Mellitus is a metabolic disorder that is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency.[1] This disorder is associated with obesity, lack of exercise, and genetics. The complications that can arise from the hyperglycemia of Type 2 Diabetes Mellitus include retinopathy, nephropathy, neuropathy, and heart disease.[2] The management of Type 2 Diabetes Mellitus is done through modifications, such as exercise, diet, and the use of medications such as insulin and oral hypoglycemic agents.[3] The main goal of the management of Type 2 Diabetes Mellitus is to control the hyperglycemia and the complications that can arise from it.[4]

Metformin is the drug of choice for the treatment of Type II diabetes mellitus and belongs to the biguanide class of drugs.[5] The drug primarily works by decreasing hepatic glucose output and increasing the sensitivity of peripheral tissues.[5] The drug does not usually cause hypoglycemia and is safe, effective, and cost-efficient. The drug is especially useful for those who are overweight and have diabetes. It has been found that metformin has certain benefits for the heart as well.[6] The side effects of metformin are nausea, diarrhea and in a few cases, lactic acidosis. The drug is widely used for the treatment of type II diabetes.[7]

Prolonged administration of metformin has been associated with vitamin B12 deficiency.[8] Vitamin B12 plays an important role in erythropoiesis as well as maintaining normal neurological functions.[9] It has been proposed that metformin may interfere with vitamin B12 absorption from the terminal part of the ileum, leading to a progressive fall in serum vitamin B12 concentrations.[10] Vitamin B12 deficiency may present as megaloblastic anemia, fatigue, paresthesias and neuropathy which may be confused with diabetic neuropathy.[11] Several studies have shown an increased incidence of vitamin B12 deficiency in individuals receiving prolonged metformin therapy.[12] It is therefore important to monitor vitamin B12 concentrations in diabetic patients receiving long-term metformin therapy.

Type 2 Diabetes Mellitus has shown a high prevalence rate in the population of concern, and metformin is commonly used as an initial drug for long-term therapy. Although metformin is considered safe and effective, the link between vitamin B12 deficiency and metformin has not

been given adequate consideration in clinical practice. Vitamin B12 deficiency may be asymptomatic or may manifest as nonspecific symptoms. Vitamin B12-associated neuropathy may be confused with diabetic neuropathy. There is a lack of local literature regarding the prevalence of vitamin B12 deficiency among metformin users in the region.

METHODOLOGY

This cross sectional study was conducted from 31 January 2025 to 20 May 2025 at the General Medicine Department of CMH Kohat. Ethical approval was obtained from the institutional review committee of the hospital before starting the research work, vide File Number E-2005/A/06. The sample size was calculated by using WHO sample size calculator keeping 95% confidence level, 3% absolute precision and 3.9% previously reported frequency of vitamin B12 deficiency.[13] The calculated sample size was 160 patients. Eligibility criteria included both Male & Female patients, age 30–70 Years, having type II Diabetes and taking metformin alone for at least 2 years. Diabetes Mellitus was taken as present if patient was already on anti-diabetic medication, or FPG level ≥ 126 mg/dL or HBA1c ≥ 6.5%. Metformin use was considered as oral intake of 500 mg twice daily with meal for minimum duration of 2 years. Patients having history of gastrectomy, surgery involving small intestine, IBD, Pernicious Anemia, history of Cancer, acute coronary syndrome within last 3 months, Liver Cirrhosis (AST > 3 Times normal), CKD (GFR < 30 ml/min) and folate deficiency were excluded from study. Hypertension was taken when BP was ≥140/90 mmHg or patient was on anti-hypertensive medication. Smoking was considered positive if patient had active smoking history. CKD was considered when GFR was < 30 ml/min. Liver Cirrhosis was considered when AST was more than 3 Times normal range.

All the participants who were part of the study were given laboratory tests at the beginning of the study, including blood sugar (BSF), blood sugar random (BSR), vitamin B12, folic acid, liver function tests (LFTs), renal function tests (RFTs), blood electrolytes, HbA1c, and lipid profile. Blood samples were collected using aseptic methods. After the data collection and laboratory tests, vitamin B12 levels were checked. Vitamin B12 levels were considered normal if the levels were more than 300 pg/mL, while levels less than 300 pg/mL were considered low.

All the analysis was done using SPSS software version 22.0. Descriptive analysis was done for quantitative variables. The quantitative variables included age, BMI, serum vitamin B12 level and duration of diabetes. These quantitative variables were analyzed using means and standard deviations or median and interquartile ranges. The categorical variables included gender, vitamin B12 deficiency, hypertension, smoking status, educational status, place of residence, and socioeconomic status. These categorical variables were analyzed using frequencies and percentages. To adjust for potential effect modifiers of age, gender, BMI, duration of diabetes, hypertension, smoking status, educational status, place of residence, and socioeconomic status, stratification was done. After stratification, the chi-square test of significance and Fisher's exact test of significance were performed. The level of significance was set at ≤0.05.

RESULTS

The mean age of the patients was 50.16 ± 12.65 years, with a mean duration of T2DM of 7.64 ± 3.74 years. The mean BMI was recorded as 28.19 ± 3.07 kg/m^2, and the mean serum vitamin B12 level was 486.08 ± 132.85 pg/mL. Regarding the gender distribution, 96 patients was male which constitute 60.0% of the total sample, while 64 patients was female making up 40.0%. In terms of education, 77 patients (48.1%) was literate and 83 patients (51.9%) was illiterate. For the residence, 84 patients (52.5%) was belong to rural areas and 76 patients (47.5%) was from urban areas. The socioeconomic status shows that 80 patients (50.0%) was from low socioeconomic group, 61 patients (38.1%) was from middle group, and 19 patients (11.9%) was from high socioeconomic group. With regards to smoking, 33 patients (20.6%) was smokers and 127 patients (79.4%) was non-smokers. Hypertension was present in 79 patients (49.4%) whereas 81 patients (50.6%) did not had hypertension (Table 1).

Table 1

Patient Demographics

Demographics		Mean ± SD
Age (years)		50.16 ± 12.65
Duration of T2DM (years)		7.64 ± 3.74
BMI (Kg/m^2)		28.19 ± 3.07
Serum B12 (pg/mL)		486.08 ± 132.85
Gender	Male n (%)	96 (60.0%)
	Female n (%)	64 (40.0%)
Education	Literate n (%)	77 (48.1%)
	Illiterate n (%)	83 (51.9%)
Residence	Rural n (%)	84 (52.5%)
	Urban n (%)	76 (47.5%)
Socioeconomic Status	Low n (%)	80 (50.0%)
	Middle n (%)	61 (38.1%)
	High n (%)	19 (11.9%)
Smoking	Yes n (%)	33 (20.6%)
	No n (%)	127 (79.4%)
Hypertension	Yes n (%)	79 (49.4%)
	No n (%)	81 (50.6%)

Regarding the frequency of vitamin B12 deficiency among the study participants, a total of 11 patients was found to have vitamin B12 deficiency, which represent 6.90% of the whole sample, whereas 149 patients (93.10%) was not deficient (Table 2).

Table 2

Frequency of Vitamin B12 Deficiency Among Patients Using Metformin for Type II Diabetes Mellitus

Vitamin B12 Deficiency	Frequency	% age
Yes	11	6.90%
No	149	93.10%
Total	160	100%

When vitamin B12 deficiency was analyzed across demographic and clinical variables, no statistically significant associations were observed. Deficiency was noted in 4 patients (5.0%) aged ≤50 years and 7 patients (8.8%) aged >50 years (p=0.534). Among males, 6 (6.3%) were deficient compared to 5 females (7.8%) (p=0.756).

Regarding duration of T2DM, deficiency was present in 1 patient (2.2%) with ≤5 years and 10 patients (8.8%) with >5 years duration (p=0.180). In terms of BMI, 2 patients (6.7%) with BMI ≤25 and 9 patients (6.9%) with BMI >25 had deficiency (p=1.000). Deficiency was observed in 6 literate (7.8%) and 5 illiterate patients (6.0%) (p=0.760), 6 rural (7.1%) and 5 urban residents (6.6%) (p=1.000), and 6 (7.5%) from low, 5 (8.2%) from middle, and none from high socioeconomic status (p=0.512). Among smokers, 3 (9.1%) were deficient versus 8 (6.3%) non-smokers (p=0.698). Hypertension was present in 5 deficient patients (6.3%) compared to 6 without hypertension (7.4%) (p=1.000). (Table 3).

Table 3

Association of Vitamin B12 Deficiency with Demographic Factors

Demographic Factors	Subgroups	Vit B12 Deficiency		p-value
		Yes n(%)	No n(%)	
Age (years)	≤50	4 (5.0%)	76 (95.0%)	0.534*
	>50	7 (8.8%)	73 (91.3%)	
Gender	Male	6 (6.3%)	90 (93.8%)	0.756*
	Female	5 (7.8%)	59 (92.2%)	
Duration of T2DM (years)	≤5	1 (2.2%)	45 (97.8%)	0.180*
	>5	10 (8.8%)	104 (91.2%)	
BMI (Kg/m^2)	≤25	2 (6.7%)	28 (93.3%)	1.000*
	>25	9 (6.9%)	121 (93.1%)	
Education	Literate	6 (7.8%)	71 (92.2%)	0.760*
	Illiterate	5 (6.0%)	78 (94.0%)	
Residence	Rural	6 (7.1%)	78 (92.9%)	1.000*
	Urban	5 (6.6%)	71 (93.4%)	
Socioeconomic Status	Low	6 (7.5%)	74 (92.5%)	0.512*
	Middle	5 (8.2%)	56 (91.8%)	
	High	0 (0.0%)	19 (100.0%)	
Smoking	Yes	3 (9.1%)	30 (90.9%)	0.698*
	No	8 (6.3%)	119 (93.7%)	
Hypertension	Yes	5 (6.3%)	74 (93.7%)	1.000*
	No	6 (7.4%)	75 (92.6%)	

*Fischer Exact Test

DISCUSSION

The overall prevalence of vitamin B12 deficiency in the present study was found to be 6.90% (n = 11), suggesting that the prevalence rate is low in the study population. This low prevalence rate may be due to the longer period required for vitamin B12 depletion to occur after the start of metformin therapy, since vitamin B12 has a large reserve in the liver, where it is depleted gradually. Furthermore, the mean duration of diabetes in the present study population was found to be 7.64 years, which may not be sufficient to induce significant vitamin B12 depletion. The duration of type 2 diabetes mellitus in the present study population was found to have a trend toward increased vitamin B12 deficiency in the population with diabetes for more than 5 years, with 10 individuals being vitamin B12 deficient compared to only 1 individual in the population with diabetes for ≤5 years, even though the p-value of 0.180 was not statistically significant. This is because the longer the period for which the patient is on metformin therapy, the longer the calcium-dependent membrane receptors in the terminal ileum will be inhibited, where the intrinsic complex of vitamin B12 absorption occurs, leading to gradual depletion of vitamin B12 reserves in the body.

The frequency of vitamin B12 deficiency in the present study was found to be 6.90% (11/160), which is relatively lower as compared to many other studies conducted on similar topic. Yadav *et al.* [14] reported a deficiency prevalence of 25.7% and Gundu *et al.* [15] found 26.36% deficiency among metformin-treated T2DM patients, both of which is considerably higher than what was observed in present study. Similarly, Asghar *et al.* [16] reported a much higher prevalence of 36.54%, and Mir *et al.* [17] found deficiency in 29.29% of their patients. Ko *et al.* [18] reported 9.5% prevalence which is somewhat closer to present findings, while Galtier *et al.* [19] found 12.5% deficiency in metformin-treated group. This variation in prevalence across different studies may be because of differences in the cutoff values used for defining vitamin B12 deficiency, differences in study population characteristics, mean duration of metformin use, and daily dose of metformin. In the present study, the mean duration of T2DM was only 7.64 ± 3.74 years and the sample may have included patients on relatively lower doses of metformin, which could explain the lower prevalence observed. Additionally, it is well established that metformin cause B12 deficiency *via* interference with calcium-dependent absorption of intrinsic factor-B12 complex in terminal ileum, and this mechanism require prolonged exposure to produce clinically significant depletion. Regarding the association of vitamin B12 deficiency with duration of diabetes, the present study show a non-significant trend where patients with >5 years duration had more deficiency 10 (8.8%) compared to those with ≤5 years 1 (2.2%), with p-value of 0.180. This finding is in agreement with several studies. Ko *et al.* [18] demonstrated a highly significant association between longer metformin duration and deficiency, where prevalence increased from 4.4% for <4 years to 56.6% for ≥10 years (p<0.001). Yadav *et al.* [14] also found significant association with metformin duration >5 years (p<0.01), and Phan *et al.* [20] showed that patients with deficiency had significantly longer metformin use duration (p=0.044). Sharan *et al.* [21] similarly reported lower mean B12 levels in patients with >10 years of metformin use compared to ≤10 years (p=0.006). The non-significant result in present study despite showing same directional trend is likely because of smaller sample size and shorter mean disease duration, which may not have been sufficient to demonstrate statistically significant depletion.

For the association with age, the present study found slightly higher deficiency in patients aged >50 years 7 (8.8%) compared to those ≤50 years 4 (5.0%), but this was not statistically significant (p=0.534). This is comparable to findings of Galtier *et al.* [19] where older age was found to be the only independent predictor of vitamin B12 deficiency (OR 1.09, p<0.001) among metformin-treated patients, and deficient patients were significantly older (66.7 ± 7.7 vs. 60.6 ± 9.1 years). The biological reason is that older patients already have reduced gastric acid secretion and decreased intrinsic factor production, which when combine with metformin-induced impairment of ileal absorption, further reduce the B12 levels. The lack of significance in present study may be attributed to the relatively younger mean age of the study population (50.16 ± 12.65 years) and small number of deficient cases. Regarding gender, the present study did not found any

significant association with vitamin B12 deficiency, where deficiency was seen in 6 males (6.3%) and 5 females (7.8%) with p-value of 0.756. Asghar *et al.* [16] however reported that deficiency was significantly higher in females (69.5% vs. 30.5% males; p=0.0035), while Elsaier *et al.* [22] found that males had significantly higher serum B12 levels than females (p=0.001). The absence of gender-based difference in present study may be because of the smaller sample and fewer deficient cases, which limit the statistical power to detect such associations. No significant association was found between vitamin B12 deficiency and BMI, education, residence, socioeconomic status, smoking, or hypertension in the present study, which is broadly consistent with findings of Asghar *et al.* [16] and Elsaier *et al.* [22] who also did not find significant associations with residence and smoking related variables. Phan *et al.* [20] however found hypertension to be more prevalent in deficient patients (87.5% vs. 68.1%; p=0.031), which was not replicate in present study where hypertension showed no significant association (p=1.000), possibly because of the different patient profiles and smaller sample size in the present study.

The current study has a number of limitations. It was carried out in a single center and had a relatively low number of patients, totaling 160. This could limit the study in its generalization. The cross-sectional nature of the study cannot confirm a cause-and-effect relationship between metformin and vitamin B12 deficiency. The study did not collect information about the daily and total cumulative dose of metformin and its relationship with B12 deficiency. This could be a limitation because the higher the dose of metformin taken, the higher the risk of B12 deficiency. The study did not collect information about the intake of vitamin B12 and supplements. This could be a potential confounding factor in the study.

CONCLUSION

The study found that vitamin B12 deficiency was present in patients with type 2 diabetes mellitus. It was not found to be significantly associated with age, gender, duration of diabetes, body mass index, literacy level, place of residence, socioeconomic status, smoking, and hypertension.

Disclaimer: Nothing to declare.

Acknowledgment: We thank the department medical team for their support. Their proper documentation and careful management of patient data help a lot in completing this research work.

REFERENCES

1. Westman EC. Type 2 diabetes mellitus: a pathophysiologic perspective. *Front Nutr.* 2021;8:707371. https://doi.org/10.3389/fnut.2021.707371
2. Kulkarni A, Thool AR, Daigavane S. Understanding the clinical relationship between diabetic retinopathy, nephropathy, and neuropathy: a comprehensive review. *Cureus.* 2024;16(3):e56674. https://doi.org/10.7759/cureus.56674
3. Yang T, Wang H, Li C, Duan H. Mechanisms of drugs in the treatment of type 2 diabetes mellitus. *Chin Med J (Engl).* 2023;136(4):394-396. https://doi.org/10.1097/CM9.0000000000002356
4. Gieroba B, Kryska A, Sroka-Bartnicka A. Type 2 diabetes mellitus - conventional therapies and future perspectives in innovative treatment. *Biochem Biophys Rep.* 2025; 42:102037. https://doi.org/10.1016/j.bbrep.2025.102037
5. Dutta S, Shah RB, Singhal S, Dutta SB, Bansal S, Sinha S, *et al.* Metformin: a review of potential mechanism and therapeutic utility beyond diabetes. *Drug Des Devel Ther.* 2023;17:1907-1932. https://doi.org/10.2147/DDDT.S409373
6. Chen C, Yuan S, Zhao X, Qiao M, Li S, He N, *et al.* Metformin protects cardiovascular health in people with diabetes. *Front Cardiovasc Med.* 2022;9:949113. https://doi.org/10.3389/fcvm.2022.949113
7. Dutta P, Kumar Y, Babu AT, Giri Ravindran S, Salam A, Rai B, *et al.* Tirzepatide: a promising drug for type 2 diabetes and beyond. *Cureus.* 2023;15(5):e38379. https://doi.org/10.7759/cureus.38379
8. Sayedali E, Yalin AE, Yalin S. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes. *World J Diabetes.* 2023;14(5):585-593. https://doi.org/10.4239/wjd.v14.i5.585
9. de Carvalho LP, Akamine N, Di Pietro MS, França CN, Oliveira R, Lopes RD. Vitamin B12 deficiency and its impact on healthcare: a population-level analysis and call for action. *Front Nutr.* 2026;12:1701661. https://doi.org/10.3389/fnut.2025.1701661
10. Ramzan NUH, Shahjahan K, Dhillon RA, Khan NTA, Hashmat MB, Anwer MU, *et al.* Vitamin B12 deficiency in patients taking metformin: pathogenesis and recommendations. *Cureus.* 2024;16(9):e68550. https://doi.org/10.7759/cureus.68550
11. Alvarez M, Prieto AE, Portilla N, Moya D, Rincon O, Guzman I. Metformin-induced vitamin B12 deficiency: an underdiagnosed cause of diabetic neuropathy. *World J Diabetes.* 2025;16(7):107514. https://doi.org/10.4239/wjd.v16.i7.107514
12. Infante M, Leoni M, Caprio M, Fabbri A. Long-term metformin therapy and vitamin B12 deficiency: an association to bear in mind. *World J Diabetes.* 2021;12(7):916-931. https://doi.org/10.4239/wjd.v12.i7.916
13. Miyan Z, Waris N, MIBD. Association of vitamin B12 deficiency in people with type 2 diabetes on metformin and without metformin: a multicenter study, Karachi, Pakistan. *BMJ Open Diabetes Res Care.* 2020;8(1):e001151. https://doi.org/10.1136/bmjdrc-2019-001151
14. Yadav A, Khare RK, Sandeep VVS, Ahmed R, Parmar V. A study on vitamin B12 deficiency in non-vegetarian diabetic patients on long-term metformin therapy at a tertiary care centre. *J Cardiovasc Dis Res.* 2024;15(10):209-214.
15. Gundu V, Chintha VS. A cross sectional study to estimate the prevalence of vitamin B12 deficiency among type 2 diabetes cases on metformin therapy: a one year study. *Int J Adv Med.* 2021;8(5):657-661. https://doi.org/10.18203/2349-3933.ijam20212138
16. Asghar S, Tanvir H, Riaz A, Ejaz MH, Akram M, Chowdhury Evan AM, *et al.* Prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin therapy: a cross-sectional study. *Cureus.* 2024;16(10):e72184. https://doi.org/10.7759/cureus.72184
17. Mir IA. Vitamin B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes. *Diabetes Manag.* 2022;12(3):345-349. https://doi.org/10.4172/2161-0487.1000672

18. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, *et al.* Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *J Korean Med Sci.* 2014;29(7):965-972. https://doi.org/10.3346/jkms.2014.29.7.965
19. Galtier F, Gastaldi G, Mura T, Sultan A, Piot C, Cristol JP, *et al.* Clinical consequences of metformin-associated vitamin B12 deficiency among patients with type 2 diabetes. *JSM Nutr Disord.* 2017;1(1):1002.
20. Phan HH, Nguyen ATT, Do MD. Association between vitamin B12 deficiency and metformin use in type 2 diabetic patients: a cross-sectional study in a South Vietnam tertiary hospital. *BMJ Open.* 2025;15:e100672. https://doi.org/10.1136/bmjopen-2025-100672
21. Sharan N, Kumari V, Kumari R, Gupta S. Vitamin B12 levels in type 2 diabetes patients taking metformin alone for more than six months. *Int J Acad Med Pharm.* 2023;5(1):419-424. https://doi.org/10.47009/jamp.2023.5.1.87
22. Elsaier A, Elamami A, Zaid I. Vitamin B12 deficiency in patients with type 2 diabetes on metformin therapy. *Ibnosina J Med BS.* 2017;9(3):72-75. https://doi.org/10.4103/1947-489x.210114