

Correlation of Vitamin B12 Deficiency with Diabetic Neuropathy in Type II Diabetics on Long-term Metformin Therapy

Maryam Nadeem¹, Maryam Saqib², Shoaib Muhammad Daniyal³, Syeda Mah-e-Noor Zahra⁴,
Muhammad Saroosh Jamil⁵, Jazib Andleeb⁶

Department of Pharmacology¹, Rahbar Medical and Dental College, Lahore, Shifa College of Medicine², Islamabad, Department of Cardiology³, Services Hospital, Lahore, Department of Pharmacology⁴, Akhtar Saeed Medical and Dental College, Lahore, Department of Pathology⁵, Rahbar Medical and Dental College, Lahore, Department of Physiology⁶, CMH Institute of Medical Sciences, Bahawalpur.

Abstract

Background: Vitamin B12 deficiency is a well-documented side effect of long-term metformin use in patients with diabetes. However, its potential relationship with diabetic neuropathy remains underexplored in the local diabetic population.

Objective: This study aimed to determine the correlation between vitamin B12 deficiency and diabetic neuropathy in type II diabetics on long-term metformin therapy.

Study type, settings & duration: This cross-sectional analytical study was conducted at Rahbar Medical College/ Punjab Rangers Teaching Hospital Lahore from May to November 2024.

Methodology: Total 150 type II diabetics taking metformin >12 months were included. Vitamin B12 levels estimated using chemiluminescence immunoassay and were considered deficient. Diabetic neuropathy was diagnosed by using Michigan Neuropathy Screening Instrument.

Results: Out of total 150 type II diabetics, 80 (53.3%) were women and 70 (46.7%) were men. Overall mean age was 63±11 years. Among total 42 (28%) patients with vitamin B12 level below normal (≤ 300 pg/mL), 10 (6.7%) were deficient and 32 (21.3%) were inadequate. Diabetic neuropathy was diagnosed in 32 (21.3%) patients. Of them, total 22 (68.7%) patients had vitamin B12 level below normal (≤ 300 pg/mL), that included 10 (31.3%) deficient and 12 (37.5%) inadequate. Duration of metformin use and B12 levels were not associated [coefficient: 0.70, 95% CI: -0.40, 1.71]. However, diabetic neuropathy showed a strong negative association with B12 levels [coefficient: -115.6, 95% CI: -164.1, -67.0].

Conclusion: There is a strong link between low vitamin B12 levels and diabetic neuropathy in long-term metformin users, suggesting B12 deficiency as a modifiable risk factor. Regular B12 monitoring may help prevent neurological complications.

Key words: Diabetes, metformin, neuropathy, vitamin B12.

Introduction

Vitamin B12 deficiency is a common side effect in patients with long-term use of metformin, as first reported in 1969 and then several subsequent studies.¹ An average of 5-40% patients using metformin develop a B12 deficiency depending upon their demographics.² However, the etiology of this deficiency in association with metformin use is vague, literature suggests mechanisms like inhibition of B12-Intrinsic factor complex preventing absorption and small intestinal bacterial overgrowth.³ Some studies have also reported the competitive inactivation of B12 absorption and

changes in levels of intrinsic factor and cubilin as cause of this deficiency.⁴

Corresponding Author:

Maryam Nadeem

Department of Pharmacology
Rahbar Medical and Dental College, Lahore.
Email: drm95435@gmail.com

Received: 07 June 2024, **Accepted:** 16 June 2025

Published: 02 July 2025

Authors Contribution

MN & MS conceptualized the project. SMNZ & MSJ did the data collection. SMD did the literature search. MS & SMNZ performed the statistical analysis. Drafting, revision & writing of manuscript were done by MN & JA.

Copyright © 2025 The Author(s). This is an Open Access article under the CC BY-NC 4.0 license.

Deficiency of vitamin B12 causes various neurological and cognitive disorders such as neuropathy, spinal cord subacute combined degeneration, dementia, demyelination and delirium. These disorders can be seen as manifestation of neuropathy in diabetes patients using metformin long-term.⁵

The diagnostic gold standard for diabetic neuropathy in chronic users of metformin is unknown, but multiple methods with comparable sensitivity and specificity are used as alternative among which, electrodiagnostic methods reported high diagnostic accuracy. Michigan Neuropathy Screening Instrument is the best examination to diagnose diabetic neuropathy.⁶

Since the diabetic neuropathy has been frequently reported in metformin users in literature, American Diabetes Association⁷ and American College of Endocrinology⁸ recommend assessing B12 inadequacy in such patients during routine examination. However, limited studies have comprehensively analyzed this association while considering key risk factors such as gender and dosage levels. This study aimed to determine the correlation between vitamin B12 deficiency and diabetic neuropathy in type II diabetics on long-term metformin therapy.

Methodology

This cross-sectional analytical study was conducted at Rahbar Medical College/ Punjab Rangers Teaching Hospital Lahore from May to November 2024. Type II diabetics taking metformin >12 months, aged >40 years, and of both genders were included using consecutive sampling technique. Sample size of 150 was calculated keeping 20% estimated incidence.⁹ Patients with history of GI tract surgery, malabsorptive syndrome and patients who were taking B12 supplements or multivitamins were excluded from the study. All patients provided their informed consent for their data being used for research.

Patients' data including demographics, age, sex, diabetes diagnosis time, metformin use duration, and metformin dose were recorded. Chemiluminescence immunoassay was performed to measure vitamin B12 levels where <200 pg/mL was considered as deficient, 200 to 300 pg/mL as inadequate and >300 pg/mL as normal.¹⁰ Diabetic neuropathy was diagnosed by using Michigan Neuropathy Screening Instrument (MNSI) followed by physical exam. If the MNSI questionnaire indicates sufficient proof of diabetic neuropathy (score ≥ 7), and physical examination >2 score was

considered as positive test as it has shown 80% sensitivity and 95% specificity previously.¹¹

All data were analyzed by using Statistical Package for Social Sciences (SPSS) version 23. Continuous variables were presented as Mean $\pm$ SD and categorical variables as frequency (percentage). Vitamin B12 levels (deficiency and inadequacy) were further stratified for age and gender and percentage with 95% CI were reported. Spearman's rank correlation coefficient was determined to evaluate the correlation between continuous vitamin B12 variables and duration and dose of metformin, time of diabetes diagnosis and diagnostic scores of diabetic neuropathy. A probability value ≤ 0.05 was considered significant.

The ethical approval of the study was obtained from the Ethics Review Committee of the Punjab Rangers Teaching Hospital Lahore vide letter no. 28/2021.

Results

Among 150 type II diabetics, 80 (53.3%) were women and 70 (46.7%) were men. Overall mean age was 63 $\pm$ 11 years. Diabetic neuropathy was diagnosed in 32 (21.3%) patients (Table-1).

Table 1: Patients' demographic and clinical features

Characteristics	N=150
Females	80 (53.3%)
Age (years)	63 $\pm$ 11
Neuropathy	32 (21.3%)
Vitamin B12 level	409 $\pm$ 153
Duration of metformin use (months)	107 $\pm$ 89
Metformin dose (mg)	1530 $\pm$ 615
Duration of diabetes diagnosis (years)	7 $\pm$ 6.5
MNSI 1	6 $\pm$ 3.8

Overall, 42 (28%) patients had vitamin B12 level below normal (≤ 300 pg/mL). Of them, 10 (6.7%) were vitamin B12 deficient and 32 (21.3%) were inadequate. Gender-wise stratification revealed that 11.3% men and 5.3% women had B12 deficiency (Table-2).

Table 2: Stratification of vitamin B12 deficiency by age and gender

	B12 Levels <200 pg/mL	B12 Levels 200-300 pg/mL
All patients	6.7% (8-13%)	21.3% (16-28%)
Men	11.3% (7-21%)	22% (15-35%)
Women	5.3% (2-11%)	19% (13-28%)
Age <70 years	6% (3-11%)	21% (15-30%)
Age >70 years	11% (6-23%)	21% (14-25%)

Table 3: Spearman's rank correlation analysis

	Model A			Model B		
	Spearman's correlation coefficient	95% Confidence Interval	Probability	Spearman's correlation coefficient	95% Confidence Interval	Probability
Metformin dose (mg)	-0.05	-0.09, -0.030	0.001	-0.056	-0.10, -0.027	0.001
Gender female	48.2	2.0-94.2	0.036	53.4	10.0-101.9	0.020
Diabetic neuropathy (yes)	-115.6	-164.1, -67.0	0.0	-109.3	-161.0, -60.7	0.0
Age (years)	-0.90	-3.0, 1.2	0.370	-	-	-
Duration of diabetes diagnosis (years)	-4.8	-15.4, 6.7	0.380	-	-	-
Duration of metformin use (months)	0.70	-0.40, 1.71	0.218	-	-	-

Duration of metformin use and B12 levels were not associated [coefficient: 0.70, 95% CI: -0.40, 1.71]. However, diabetic neuropathy showed a strong negative association with B12 levels [coefficient: -115.6, 95% CI: -164.1, -67.0] (Table-3). Out of total 150 patients, 32 (21.3%) were diagnosed with diabetic neuropathy. Of them, total 22 (68.7%) patients had vitamin B12 level below normal (≤ 300 pg/mL), that included 10 (31.3% deficient and 12 (37.5%) inadequate (Table-4).

Table 4: Diabetic neuropathy in different vitamin B12 levels

B12 levels	Diabetic Neuropathy		
	Positive (n=32)	Negative (n=118)	Total (n=150)
Deficiency	10 (31.3%)	0 (0.0%)	10 (6.7%)
Inadequacy	12 (37.5%)	20 (16.9%)	32 (21.3%)
Normal	10 (31.3%)	98 (83.1%)	108 (72.0%)

Discussion

According to our results almost 6.7% of the population had low B12 levels which significantly lower than the prevalence of 28-41% in other studies.^{12,13} This difference can be explained by exclusion of high-risk patients like those with malabsorptive syndrome that can lead to anemia and those with prior prescription for B12. Secondly, different cut-off values for B12 deficiency classification can also account for this difference.

A recent meta-analysis concluded metformin as a predictor of B12 deficiency in diabetes patients.¹⁴ The results of our study are similar to previous studies which define arbitrary equivalent cut off points and reported the incidence of B12 deficiency in 5-40% study population.^{15,16} In our study the male patients who were administered high dose of metformin and those with neuropathy had significantly low B12 levels and most of them had low or borderline levels. Previous literature has

also reported an inverse relationship between dose of metformin and B12 levels.¹⁷

Only 28% of our patients were diagnosed with diabetic neuropathy which was lower than the prevalence reported previously in diabetes patients i.e 40-50%.¹⁸ We observed that low B12 levels were significantly associated to development of diabetic neuropathy. Several studies have been conducted to evaluate the associations between deficiency of B12 and diabetic neuropathy but they yielded inconclusive results as they were descriptive analyses.¹⁹ Gupta et al. reported a significant association between duration of metformin administration and diagnosis of diabetic neuropathy and an inverse relationship between B12 levels and metformin duration.²⁰ A review study found that neuropathy could be improved by vitamin B12 supplements and methylcobalamin by reducing pain and paresthesia but they did not affect the electrophysiological measurements.²¹

In contrast to this a recent meta-analysis did not report any association between neuropathy and B12 deficiency but the results were inconclusive due to lack of evidence.¹ Another study was conducted where diabetic neuropathy patients were administered B12 supplements but the results regarding improvement of neuropathy by the supplementation were inconclusive.²² On the other hand, folic acid supplements have been reported to improve symptoms of diabetic neuropathy.²³

In the present study, B12 levels were significantly different in men unlike previous data.²⁴ As a result, diabetic neuropathy was also more common in men but no physiological etiology was discovered for this association. Not much data is available regarding stratification of neuropathy with respect to gender. One study we found reported conflicting results to our study and showed high incidence of B12 deficiency in females.²⁵ The possible explanation of prevalence of B12 deficiency in men could be the older age of males as compared to females.

The present study has certain limitations. Neuropathy was diagnosed using methods with variable diagnostic accuracy, such as MNSI or clinical history, which may influence the accuracy of the results; however, bias in MNSI is limited to different levels of B12 (low, normal, borderline). Preexisting neuropathy prior to metformin use was not assessed. Furthermore, a causal correlation between B12 deficiency and neuropathy could not be established.

There is a strong link between low vitamin B12 levels and diabetic neuropathy. Additionally, male gender and increased doses of metformin being the risk factor for low B12 levels.

Conflict of interest: None declared.

References

1. 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.
2. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. *Medicine* 2019; 98(46): e17918.
3. Shivaprasad C, Gautham K, Ramdas B, Gopaldatta KS, Nishchitha K. Metformin Usage Index and assessment of vitamin B12 deficiency among metformin and non-metformin users with type 2 diabetes mellitus. *Acta Diabetol* 2020; 57(9): 1073-80.
4. Owhin SO, Adaja TM, Fasipe OJ, Akhideno PE, Kalejaiye OO, Kehinde MO. Prevalence of vitamin B12 deficiency among metformin-treated type 2 diabetic patients in a tertiary institution, South-South Nigeria. *SAGE Open Med* 2019; 7: 2050312119853433.
5. Alvarez M, Sierra OR, Saavedra G, Moreno S. Vitamin B12 deficiency and diabetic neuropathy in patients taking metformin: a cross-sectional study. *Endocr Connect* 2019; 8(10): 1324-9.
6. Viswanathan V, Ahmed Khan B, Nachimuthu S, Kumpatla S. Precision of Michigan neuropathy screening instrument (MNSI) tool for the diagnosis of diabetic peripheral neuropathy among people with type 2 diabetes—a study from South India. *Int J Low Extrem Wounds* 2023; 15347346231163209.
7. American Diabetes Association. 4. Comprehensive medical evaluation and assessment of comorbidities: standards of medical care in diabetes-2019. *Diabetes Care* 2019; 42: S34-45.
8. Grunberger G, Sherr J, Allende M, Blevins T, Bode B, Handelsman Y, et al. American Association of Clinical Endocrinology clinical practice guideline: the use of advanced technology in the management of persons with diabetes mellitus. *Endocr Pract* 2021; 27(6): 505-37.
9. Miyan Z, Waris N. 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.
10. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, et al. Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. *J Clin Endocrinol Metabol* 2016; 101(4): 1754-61.
11. Carmichael J, Fadavi H, Ishibashi F, Shore AC, Tavakoli M. Advances in screening, early diagnosis and accurate staging of diabetic neuropathy. *Front Endocrinol* 2021; 12: 671257.
12. Almatrafi SB, Bakr E-SH, Almatrafi AA, Altayeb MM. Prevalence of vitamin B12 deficiency and its association with metformin-treated type 2 diabetic patients: a cross sectional study. *Human Nutr Metabol* 2022; 27: 200138.
13. Marar O, Senturk S, Agha A, Thompson C, Smith D. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin. *R Coll Surg Irel Stud Med J* 2011; 4(1): 16-20.
14. Yang W, Cai X, Wu H, Ji L. Associations between metformin use and vitamin B12 levels, anemia, and neuropathy in patients with diabetes: a meta-analysis. *J Diabetes* 2019; 11(9): 729-43.
15. Khattab R, Albannawi M, Alhajj Mohammed D, Alkubaish Z, Althani R, Altheeb L, et al. Metformin-induced vitamin B12 deficiency among type 2 diabetes mellitus patients: A systematic review. *Curr Diabetes Rev* 2023; 19(4): e180422203716.
16. Gao L, Ran X, Liu X, Shen X, Chen S, Liu F, et al. The effects of daily dose and treatment duration of metformin on the prevalence of vitamin B12 deficiency and peripheral neuropathy in Chinese patients with type 2 diabetes mellitus: A multicenter cross-sectional study. *J Diabetes* 2023; 15(9): 765-76.
17. Al Saeed RR, Baraja MA. Vitamin B12 deficiency in patients with type 2 diabetes mellitus using metformin and the associated factors in Saudi Arabia. *Saudi Med J* 2021; 42(2): 161.
18. Alam MS, Kamrul-Hasan A, Kalam ST. Serum vitamin B12 status of patients with type 2 diabetes mellitus on metformin: a single-center cross-sectional study from Bangladesh. *J Family Med Primary Care* 2021; 10(6): 2225-9.
19. Krishnan GD, Zakaria MH, Yahaya N. Prevalence of vitamin B12 deficiency and its associated factors among patients with type 2 diabetes mellitus on metformin from a district in Malaysia. *J ASEAN Fed Endocr Soci* 2020; 35(2): 163.
20. Gupta K, Jain A, Rohatgi A. An observational study of vitamin b12 levels and peripheral neuropathy profile in patients of diabetes mellitus on metformin therapy. *Diabetes Metab Syndr* 2018; 12(1): 51-8.
21. Kakarlapudi Y, Kondabolu SK, Tehseen Z, Khemani V, Nousherwani MD, Saleem F, et al. Effect of metformin on vitamin B12 deficiency in patients with type 2 diabetes mellitus and factors associated with it: a meta-analysis. *Cureus* 2022; 14(12): e32277.
22. Lata Kanyal M, Mujawar A. Status of vitamin b12 in type 2 diabetes mellitus patients taking metformin based oral hypoglycemic agent-a cross sectional study. *Indian J Basic Applied Med Res* 2019; 1(9): 18-26.

23. Mottaghi T, Khorvash F, Maracy M, Bellissimo N, Askari G. Effect of folic acid supplementation on nerve conduction velocity in diabetic polyneuropathy patients. *Neurol Res* 2019; 41(4): 364-8.
 24. Fakkar NFH, Marzouk D, Allam MF, Fouad MM, Aboromia MM, Gadallah M. Association between vitamin B12 level and clinical peripheral neuropathy in type 2 diabetic patients on metformin therapy. *Egypt J Neurol Psychiatry Neurosurg* 2022; 58(1): 46.
 25. Al-Hamdi A, Al-Gahhafi M, Al-Roshdi S, Jaju S, Al-Mamari A, Al Mahrezi AM. Vitamin B12 deficiency in diabetic patients on metformin therapy: a cross-sectional study from Oman. *Sultan Qaboos Univ Med J* 2020; 20(1): e90.
-