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Original Research Article

RELATIONSHIP BETWEEN VITAMIN B12 DEFICIENCY AND PERIPHERAL NEUROPATHY IN METFORMIN-TREATED DIABETES MELLITUS: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background: Metformin remains the first-line oral agent for type 2 diabetes mellitus (T2DM) in India, yet long-term use has been linked to vitamin B12 malabsorption and a consequent risk of peripheral neuropathy. Data from semi-urban Eastern India are scarce. The objective is to estimate the prevalence of vitamin B12 deficiency and to examine its relationship with peripheral neuropathy in metformin-treated T2DM patients in Durgapur, West Bengal.

Materials and Methods: A prospective observational study was conducted over six months (February–July 2025) among 20 adults with T2DM aged 28–48 years who had received metformin for at least 12 months. Serum vitamin B12 was measured, and peripheral neuropathy was assessed using the Michigan Neuropathy Screening Instrument (MNSI). Demographic, glycaemic, and metformin exposure variables were recorded. Associations were examined using appropriate non-parametric tests.

Results: Vitamin B12 deficiency (<200 pg/mL) was present in 9/20 participants (45.0%). Peripheral neuropathy (MNSI score >2) was observed in 11/20 participants (55.0%). Among B12-deficient participants, 7/9 (77.8%) had neuropathy, compared with 4/11 (36.4%) among B12-sufficient participants ($p = 0.087$). Mean metformin duration was longer in the deficient group (6.8 ± 2.1 years vs 4.3 ± 1.9 years; $p = 0.012$). Mean daily metformin dose was also higher in the deficient group (1722 ± 363 mg vs 1318 ± 405 mg; $p = 0.031$). No participant had received routine B12 screening or supplementation.

Conclusion: Vitamin B12 deficiency was highly prevalent in this metformin-treated cohort and showed a clinically meaningful, though statistically borderline, association with peripheral neuropathy. Duration and dose of metformin were significantly associated with deficiency. Opportunistic B12 screening should be considered in metformin-treated patients with neuropathic symptoms in this setting.

Keywords: Metformin; Vitamin B12 deficiency; Peripheral neuropathy; Type 2 diabetes mellitus; India.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder of escalating public health importance in India, where the estimated prevalence among adults aged 18–69 years is approximately 9.3%. Peripheral neuropathy is one of the most common complications of T2DM and contributes substantially to morbidity, falls, foot ulceration, and reduced quality of life in Indian patients.[1] Metformin remains the most widely prescribed first-line oral glucose-lowering agent worldwide because of its efficacy, favourable safety profile, and low cost. In India, where vegetarian dietary patterns are common and metformin use is extensive, the coexistence of pharmacological and nutritional risk factors for vitamin B12 insufficiency creates a particularly vulnerable clinical scenario.[2]

The relationship between metformin and vitamin B12 has been recognised for more than half a century, yet it continues to be under-appreciated in routine diabetes care.[3] Metformin is thought to interfere with the calcium-dependent binding of the intrinsic factor–vitamin B12 complex to the cubam receptor in the terminal ileum, and it may also alter small intestinal motility, promote bacterial overgrowth, and reduce hepatic vitamin B12 stores.[4] These mechanisms collectively produce a state of functional malabsorption that can gradually deplete serum vitamin B12 over years of treatment.[5] The neurological consequences of vitamin B12 deficiency are clinically important because they overlap substantially with diabetic peripheral neuropathy.[6] Vitamin B12 is essential for axonal myelination and for methionine synthesis in both the central and peripheral nervous systems. Deficiency can produce distal symmetrical neuropathy, autonomic dysfunction, and subacute combined degeneration of the spinal cord, and it may accelerate the progression of pre-existing diabetic neuropathy.[7] Because the symptoms are indistinguishable from those of hyperglycaemia-related nerve injury, metformin-induced B12 deficiency is frequently misattributed to "worsening diabetes" and remains untreated.[8]

The evidence base linking metformin to B12 deficiency and neuropathy has strengthened considerably in the past five years.[9] A large analysis of the NIH All of Us database, published in 2025, reported that long-term metformin users had a 67% higher likelihood of vitamin B12 deficiency than non-users and a 38% higher likelihood than short-term users, with peripheral neuropathy prevalence 39% higher than in short-term users.[10] A dose-dependent relationship has also been demonstrated, with higher daily metformin doses associated with greater B12 deficiency prevalence, although the effect on neuropathy risk is less consistent.[11] A Chinese multicentre study further showed that both daily dose and treatment duration influence B12 deficiency and neuropathy prevalence.[12] In India, prevalence estimates vary widely from 15.7% in a mixed cohort to 32% in Eastern Indian patients with established neuropathy and 45%–52% in some clinic-based samples reflecting differences in dietary patterns, B12 cut-offs, and study populations.[13] The MHRA has classified B12 deficiency as a common adverse effect of metformin, and recent reviews have emphasised the need for greater clinical awareness.[14]

Despite this growing literature, prospective data from semi-urban Eastern India remain limited. Community- and clinic-based Indian studies have documented a high burden of peripheral neuropathy, but few have simultaneously measured B12 status in metformin-treated patients in this region.[15] The present study was therefore designed to estimate the prevalence of vitamin B12 deficiency in metformin-treated T2DM patients in Durgapur, West Bengal, and to examine its relationship with peripheral neuropathy using a simple, reproducible screening instrument suitable for resource-constrained settings.

MATERIALS AND METHODS

Study design and setting: This was a prospective observational study conducted in the Department of Medicine of a tertiary care teaching hospital in Durgapur, West Bengal, over six months from February 2025 to July 2025. The study was approved by the institutional ethics committee prior to commencement, and written informed consent was obtained from every participant in accordance with the Declaration of Helsinki.

Study population and eligibility: Adults aged 28–48 years with a physician-diagnosed history of T2DM who had been receiving metformin continuously for at least 12 months were eligible for inclusion. Patients were excluded if they had type 1 diabetes, gestational diabetes, chronic kidney disease stage 4 or 5 (estimated glomerular filtration rate <30 mL/min/1.73 m²), chronic liver disease, known pernicious anaemia, prior gastric or ileal resection, active malignancy, alcohol dependence, or had received vitamin B12 supplementation (oral or parenteral) within the preceding six months. Patients with thyroid disease, HIV infection, or exposure to other drugs known to affect B12 status (including proton pump inhibitors and histamine H2 receptor antagonists) were also excluded to reduce confounding.

A total of 20 participants were enrolled by consecutive sampling from the diabetes outpatient clinic. The sample size was determined by the feasibility of completing comprehensive clinical and biochemical assessments within the six-month study window; it was not powered for formal hypothesis testing but was considered adequate for a descriptive exploratory analysis.

Clinical assessment: At the baseline visit, a structured questionnaire was used to record age, sex, body mass index, duration of diabetes, duration of metformin therapy, total daily metformin dose, concomitant glucose-lowering medications, dietary pattern (vegetarian or mixed), and socio-demographic details. Blood pressure and anthropometric measurements were obtained using standard techniques. Peripheral neuropathy was assessed using the Michigan Neuropathy Screening Instrument (MNSI), a validated and widely used tool comprising a 15-item symptom questionnaire and a brief physical examination of the feet (appearance, ulceration, ankle reflexes, and vibration perception at the great toe). An MNSI examination score >2 was defined as the presence of peripheral neuropathy, consistent with previously published thresholds. All assessments were performed by a single trained investigator to minimise inter-observer variability.

Biochemical measurements: A venous blood sample was collected after an overnight fast of at least eight hours. Serum vitamin B12 was measured by electrochemiluminescence immunoassay on a fully

automated analyser. Vitamin B12 deficiency was defined as a serum concentration <200 pg/mL, and borderline insufficiency as 200–300 pg/mL, in accordance with commonly used clinical thresholds. Fasting plasma glucose, glycated haemoglobin (HbA1c), serum creatinine, and a complete blood count were measured using standard laboratory methods. Macrocytosis was defined as mean corpuscular volume >100 fL.

Statistical analysis: Data were entered into Microsoft Excel and analysed using SPSS version 26.0 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation or median with interquartile range, depending on distribution. Categorical variables were expressed as frequencies and percentages. Between-group comparisons for continuous variables were performed using the Mann–Whitney U test, and categorical variables were compared using Fisher's exact test because of the small sample size. A two-sided p value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics: The study cohort comprised 20 participants (12 men and 8 women) with a mean age of 38.6 ± 5.9 years. The mean duration of diabetes was 7.1 ± 2.8 years, and the mean duration of metformin therapy was 5.5 ± 2.3 years. The mean total daily metformin dose was 1500 ± 437 mg; 11 participants (55.0%) were receiving ≥1500 mg/day, and 9 (45.0%) were receiving <1500 mg/day. Fourteen participants (70.0%) followed a vegetarian diet, and six (30.0%) followed a mixed diet. The mean HbA1c was 8.4 ± 1.3%, indicating suboptimal glycaemic control in a substantial proportion of the cohort. Baseline characteristics are summarised in [Table 1].

Vitamin B12 status: The mean serum vitamin B12 concentration was 231.4 ± 88.7 pg/mL. Vitamin B12 deficiency (<200 pg/mL) was present in 9 participants (45.0%). A further 4 participants (20.0%) had borderline insufficiency (200–300 pg/mL), leaving only 7 participants (35.0%) with adequate B12 levels (>300 pg/mL). The distribution of B12 status categories is shown in [Figure 1]. Macrocytosis (MCV >100 fL) was observed in 3 of the 9 deficient participants (33.3%) and in none of the B12-sufficient participants.

Peripheral neuropathy: Peripheral neuropathy, defined as an MNSI examination score >2, was present in 11 participants (55.0%). The mean MNSI score was 2.6 ± 1.5. Among the 9 B12-deficient participants, 7 (77.8%) had neuropathy, compared with 4 of the 11 B12-sufficient participants (36.4%). This difference did not reach conventional statistical significance in this small sample (Fisher's exact test, p = 0.087), but the direction and magnitude of the association were consistent with the hypothesised relationship. The cross-tabulation is presented in [Table 2].

Metformin exposure and B12 deficiency: Comparisons between B12-deficient and B12-sufficient participants are shown in Table 3. Participants with deficiency had a significantly longer mean duration of metformin therapy (6.8 ± 2.1 years vs 4.3 ± 1.9 years; p = 0.012) and a significantly higher mean daily metformin dose (1722 ± 363 mg vs 1318 ± 405 mg; p = 0.031). Age, duration of diabetes, HbA1c, and dietary pattern did not differ significantly between the groups, although the deficient group had a numerically higher proportion of vegetarians (88.9% vs 54.5%; p = 0.155).

Dose- and duration-related trends: When participants were stratified by metformin dose category, B12 deficiency was present in 7 of 11 participants (63.6%) receiving ≥1500 mg/day, compared with 2 of 9 (22.2%) receiving <1500 mg/day (p = 0.087). Similarly, when stratified by metformin duration, deficiency was present in 7 of 10 participants (70.0%) with ≥5 years of exposure, compared with 2 of 10 (20.0%) with <5 years of exposure (p = 0.070). These trends are illustrated in [Figure 2 and 3], respectively.

The relationship between neuropathy prevalence and combined B12 and metformin exposure is shown in [Figure 4]. Neuropathy was most frequent among participants who were both B12-deficient and long-term metformin users.

Table 1: Baseline characteristics of the study participants (n = 20)

Variable	Value
Age (years), mean ± SD	38.6 ± 5.9
Sex, n (%)	
Male	12 (60.0)
Female	8 (40.0)
Duration of diabetes (years), mean ± SD	7.1 ± 2.8
Duration of metformin therapy (years), mean ± SD	5.5 ± 2.3
Daily metformin dose (mg), mean ± SD	1500 ± 437
Dose category, n (%)	
<1500 mg/day	9 (45.0)
≥1500 mg/day	11 (55.0)
Dietary pattern, n (%)	
Vegetarian	14 (70.0)
Mixed	6 (30.0)
HbA1c (%), mean ± SD	8.4 ± 1.3
Serum vitamin B12 (pg/mL), mean ± SD	231.4 ± 88.7
MNSI examination score, mean ± SD	2.6 ± 1.5

Table 2: Vitamin B12 status and peripheral neuropathy (n = 20)

Vitamin B12	Neuropathy present (MNSI >2)	Neuropathy absent (MNSI ≤2)
B12 deficient (<200 pg/mL)	7 (77.8%)	2 (22.2%)
B12 sufficient (≥200 pg/mL)	4 (36.4%)	7 (63.6%)

Fisher's exact test, p = 0.087.

Table 3: Comparison of clinical and biochemical parameters by vitamin B12 status

Variable	B12 deficient (n = 9)	B12 sufficient (n = 11)	p value
Age (years)	39.2 ± 6.4	38.1 ± 5.6	0.683
Male sex, n (%)	6 (66.7)	6 (54.5)	0.670
Duration of diabetes (years)	8.1 ± 2.6	6.3 ± 2.8	0.152
Duration of metformin (years)	6.8 ± 2.1	4.3 ± 1.9	0.012
Daily metformin dose (mg)	1722 ± 363	1318 ± 405	0.031
Vegetarian diet, n (%)	8 (88.9)	6 (54.5)	0.155
HbA1c (%)	8.7 ± 1.2	8.1 ± 1.3	0.287
MNSI score	3.2 ± 1.4	2.1 ± 1.4	0.087

Values are mean ± SD or n (%). p values from Mann–Whitney U test or Fisher's exact test.

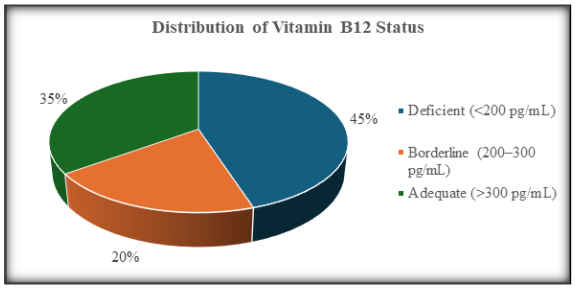


Figure 1: Distribution of vitamin B12 status among metformin-treated participants (n = 20).

Pie chart illustrating the proportion of participants in each B12 category. Deficient: <200 pg/mL; borderline: 200–300 pg/mL; adequate: >300 pg/mL.

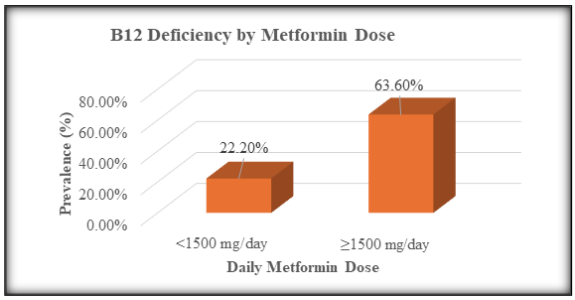


Figure 2: Prevalence of vitamin B12 deficiency by daily metformin dose category.

Bar chart comparing the prevalence of B12 deficiency (<200 pg/mL) between participants receiving <1500 mg/day and those receiving ≥1500 mg/day. Fisher's exact test, p = 0.087.

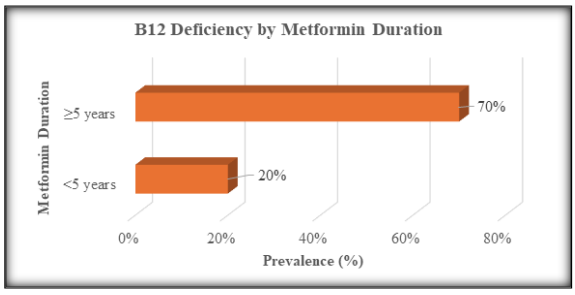


Figure 3: Prevalence of vitamin B12 deficiency by duration of metformin therapy.

Bar chart comparing the prevalence of B12 deficiency (<200 pg/mL) between participants with <5 years and ≥5 years of metformin exposure. Fisher's exact test, p = 0.070.

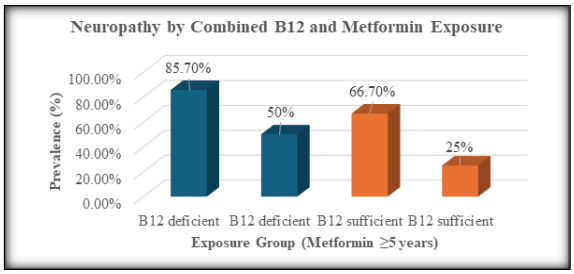


Figure 4: Peripheral neuropathy prevalence by combined vitamin B12 and metformin exposure status.

Bar chart showing the proportion of participants with MNSI examination score >2 in each combined exposure category. Neuropathy was most frequent among B12-deficient participants with ≥5 years of metformin exposure.

DISCUSSION

This prospective observational study provides contemporary data on the interplay between metformin, vitamin B12, and peripheral neuropathy in a semi-urban Eastern Indian population. Three principal findings emerge. First, vitamin B12 deficiency was highly prevalent, affecting nearly half of the cohort. Second, neuropathy was more common among B12-deficient participants, although the association did not achieve conventional statistical significance in this small sample. Third, longer duration and higher dose of metformin were significantly associated with deficiency, reinforcing the dose–duration relationship reported in larger studies.[12] The 45% prevalence of B12 deficiency observed here is consistent with the upper range of Indian estimates.[16] A 2023 study from Eastern India reported a prevalence of 32% among metformin-treated T2DM patients with established peripheral neuropathy, while other Indian series have documented rates between 25.7% and 52%

depending on the cut-off and population studied.[17] The predominance of vegetarian dietary patterns in our cohort is likely to have contributed to this high prevalence, as plant-based diets contain negligible amounts of bioavailable vitamin B12 and Indian vegetarian populations are recognised to be at particular risk.[18] Recent reviews have also highlighted that B12 deficit status is common among Indian T2DM patients and is often overlooked in routine practice.[19] The fact that nearly one-fifth of participants had borderline B12 levels further underscores the burden of subclinical insufficiency.[20]

The association between B12 deficiency and neuropathy in our study merits careful interpretation. The absolute difference was substantial, 77.8% versus 36.4%, and the direction is consistent with biological plausibility and with the findings of the All of Us analysis, which demonstrated a 39% higher prevalence of peripheral neuropathy among long-term metformin users compared with short-term users.[10] Metformin-induced B12 deficiency has been proposed as a cause or worsening factor for distal symmetrical, autonomic, and cardiac neuropathy in diabetes, and longitudinal cohort data suggest a bidirectional relationship between diabetic peripheral neuropathy and B12 deficiency . However, the p value of 0.087 reflects the limited statistical power of a 20-patient sample rather than a true absence of effect. A Chinese multicentre study similarly found a numerically higher prevalence of peripheral neuropathy in B12-deficient patients that did not reach significance, suggesting that neuropathy risk is multifactorial and that hyperglycaemia, diabetes duration, and other metabolic insults interact with B12 status over time.[12] The significantly longer metformin duration in our deficient group is clinically important because it implies that the cumulative risk of both deficiency and neuropathy rises with continued treatment.[20]

The dose-related finding of 63.6% deficiency among those receiving ≥1500 mg/day versus 22.2% among those receiving lower doses aligns with the dose-dependent pattern reported in several recent investigations.[21] The Chinese multicentre study identified ≥1500 mg/day as an important threshold for metformin-associated B12 deficiency, although it did not find a corresponding increase in neuropathy risk at that dose.[12] A dose-dependent relationship between metformin use and diabetic peripheral neuropathy risk has also been reported in large cohort analyses,[11] while other studies from Beijing have examined metformin treatment and neuropathy risk with variable findings.[22] Taken together, these observations suggest that B12 deficiency and neuropathy are related but not perfectly coupled outcomes, and that neuropathy in metformin-treated patients may reflect the combined burden of glycaemic injury and B12 insufficiency rather than B12 deficiency alone.[11] The complete absence of B12 screening or supplementation in our cohort is a notable care gap. The MHRA has recommended monitoring of vitamin B12 in patients at risk who are treated with metformin,[14] and recent clinical reviews have emphasised the long-term effects of metformin beyond glucose lowering.[23] Supplementation is inexpensive and can reverse haematological and, in some cases, neurological manifestations if initiated before irreversible axonal loss occurs; different supplementation methods have been reviewed in the context of metformin-induced B12 deficiency.[24] In the Indian context, where vegetarian diets are widespread, and metformin is used almost universally as first-line therapy, opportunistic screening at the time of neuropathy assessment would be a low-cost, high-yield intervention.[17]

CONCLUSION

In this six-month prospective observational study of 20 metformin-treated T2DM patients in Durgapur, West Bengal, vitamin B12 deficiency was present in 45% of participants, and peripheral neuropathy was present in 55%. B12 deficiency was significantly associated with longer duration and higher dose of metformin therapy, and neuropathy was more frequent among B12-deficient participants, although the latter association did not reach statistical significance in this small sample. No participant had been screened or supplemented for B12 during metformin therapy. These findings support the case for routine B12 surveillance in metformin-treated patients with neuropathic symptoms in similar semi-urban Indian settings, and they highlight the need for larger prospective studies with sufficient power to clarify the independent contribution of B12 deficiency to neuropathy risk.

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