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Research Article

Vitamin B12 Status, Metformin Exposure, and Peripheral Neuropathy in Type 2 Diabetes: A Hospital-Based Cross-Sectional Study

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ABSTRACT
Background: Long-term metformin use can deplete vitamin B12, but how often this occurs in Indian clinic populations, and whether the resulting deficiency accounts for neuropathy beyond hyperglycaemia, is not settled.
Methods: In a hospital-based cross-sectional study, 60 adults with type 2 diabetes treated with oral glucose-lowering therapy for at least 6 months underwent chemiluminescent immunoassay for fasting serum vitamin B12, glycated haemoglobin, and estimated glomerular filtration rate, together with the Michigan Neuropathy Screening Instrument and bilateral hallux biothesiometry.
Results: Suboptimal vitamin B12 (≤300 pg/mL) was present in half the cohort, including overt deficiency in about one in five. Peripheral neuropathy was found in 55% and increased from normal through borderline to deficient B12 categories. After adjustment, B12 category and glycated haemoglobin were independent correlates of neuropathy. Metformin duration and daily dose, not duration of diabetes, accounted for most of the variation in serum B12. The association between longer metformin use and neuropathy was largely attenuated once serum B12 was included.
Conclusion: Borderline as well as overt B12 depletion marked a higher neuropathy burden. Periodic B12 testing in metformin-treated patients should not wait for advanced disease or for treatment lasting many years.

Keywords: Type 2 Diabetes Mellitus, Metformin, Vitamin B12 Deficiency, Peripheral Neuropathy, Biothesiometry.

INTRODUCTION

India has one of the largest populations of adults living with diabetes, estimated at 89.8 million in 2024, with an age-standardised prevalence of 10.5%.[1] Distal symmetric polyneuropathy is among the most frequent chronic complications. It is a diagnosis of exclusion: glucose-mediated nerve injury, entrapment, alcohol, uraemia, and nutritional deficiency can produce a similar distal sensory picture.[2] Guidelines recommend structured annual screening rather than reliance on symptoms alone, and an elevated vibration perception threshold is an established marker of ulcer risk.[3,4]

Metformin remains a foundation of oral therapy, yet randomised and long-term observational evidence shows that it lowers circulating vitamin B12 in a manner related to both dose and duration.[5–8] The best supported mechanism is interference with calcium-dependent binding of the intrinsic-factor–cobalamin complex to the ileal cubilin receptor.[9] The clinical difficulty is that the resulting neuropathy resembles diabetic neuropathy and may be added to it.[10–12] Reported deficiency rates vary widely with the cut-off and the population, from well below 10% in some trial cohorts to roughly one-third in clinic series, including Indian patients on metformin.[13,14]

Two practical questions follow. First, is the borderline range of 200–300 pg/mL already associated with measurable nerve injury, or only values below 200 pg/mL? Second, is the link between metformin and neuropathy statistically accounted for by lower B12, or does it persist after B12 is taken into account? This study addressed both questions in adults with type 2 diabetes attending a teaching-hospital medicine service, using a uniform clinical score and quantitative biothesiometry.

METHODS

Design and participants

This was a hospital-based cross-sectional observational study in the outpatient and inpatient general medicine clinics of S.S. Institute of Medical Sciences and Research Centre, Davangere, between 2 January and 29

June 2025. Sixty adults with confirmed type 2 diabetes, aged 18–75 years, who had taken oral glucose-lowering therapy for at least 6 months, were evaluated. Exclusion criteria were an estimated glomerular filtration rate below 30 mL/min/1.73 m², current or prior vitamin B12 supplementation, known malabsorption, and neuropathy attributed to a non-diabetic cause. All 60 records met these boundaries.

Measurements

Fasting venous blood was assayed by chemiluminescent immunoassay for serum vitamin B12. Glycated haemoglobin and estimated glomerular filtration rate were recorded at the same visit. Vitamin B12 status followed the protocol cut-offs: deficient, below 200 pg/mL; borderline, 200–300 pg/mL; and normal, above 300 pg/mL. Values at or below 300 pg/mL were termed suboptimal.

Neurological assessment used the clinical portion of the Michigan Neuropathy Screening Instrument and digital biothesiometry at both halluces.[15] Peripheral neuropathy required an instrument score above 2.0 together with a bilateral mean vibration perception threshold of at least 15 V. These two rules agreed in every participant. Severe sensory loss was defined as a bilateral mean threshold of at least 25 V, the range previously linked to a high risk of foot ulceration.[4] Distal numbness, tingling, burning pain, and loss of protective sensation were recorded, as were Achilles reflexes graded as normal, diminished, or absent. Metformin use, daily dose, and duration were taken from the clinical record. Cumulative exposure was expressed in gram-years.

Statistical analysis

Continuous variables were tested for normality with the Shapiro–Wilk test. Because several distributions were skewed, means with standard deviations are presented alongside medians and interquartile ranges, and each parametric test is paired with a rank-based counterpart. Prevalence estimates use Wilson score intervals. Ordered B12 categories were tested with the chi-square test and the Cochran–Armitage trend test. Group comparisons used Welch tests, analysis of variance, Mann–Whitney tests, and Kruskal–Wallis tests as appropriate.

Adjusted associations with neuropathy were estimated by multivariable logistic regression, including age, diabetes duration, glycated haemoglobin, and estimated glomerular filtration rate. Metformin duration was then entered with and without serum B12 to test statistical mediation, following the Baron and Kenny sequence and a Sobel test.[16] Ordinary least-squares models related metformin dose and duration to serum B12, and serum B12 to neurological scores. Receiver-operating-characteristic curves identified screening thresholds by the Youden index. Analyses were two-sided, with $p < 0.05$ considered statistically significant. No imputation was required.

RESULTS

The cohort was middle-aged, had longstanding diabetes, and had generally poor glycaemic control, with kidney function above the exclusion limit (Table 1). Most participants were taking metformin. Only estimated glomerular filtration rate was approximately normally distributed. B12 category did not differ by sex or by glycated haemoglobin band. Combined depletion was greatest in the sixth decade, and overt deficiency was more common after more than 10 years of diabetes than at or before 10 years. Metformin use, higher daily dose, and longer treatment were associated with worse B12 status. Overt deficiency occurred only among metformin users.

Table 1. Cohort characteristics and vitamin B12 prevalence (N = 60).

Characteristic	Result
Age, years	54.0 ± 11.9; median 55 (IQR 43–62)
Women	32 (53.3%)
Diabetes duration, years	10.0 ± 7.1; median 8 (IQR 4–16)
HbA1c, %	9.05 ± 1.52; median 9.3 (IQR 7.7–10.1)
eGFR, mL/min/1.73 m²	78.8 ± 15.0; median 79.6 (IQR 68.5–89.3)
Metformin users	49 (81.7%)
Dose among users, mg/day	1163 ± 449; median 1000 (IQR 1000–1500)
Duration among users, months	59 ± 61; median 41 (IQR 15–74)
Serum B12, pg/mL	292 ± 119; median 306 (IQR 222–388)
MNSI score	3.07 ± 2.17; median 2.95 (IQR 1.18–4.92)

Bilateral mean VPT, V	19.9 ± 9.7; median 20.2 (IQR 10.7–28.1)
B12 deficient, <200 pg/mL	13 (21.7%; 95% CI 13.1–33.6)
B12 borderline, 200–300 pg/mL	17 (28.3%; 95% CI 18.5–40.8)
B12 normal, >300 pg/mL	30 (50.0%; 95% CI 37.7–62.3)
Peripheral neuropathy	33 (55.0%)

B12 category versus sex, p = 0.47; versus age band, p = 0.027; versus HbA1c band, p = 0.42; versus metformin use, p = 0.010. Overt deficiency was 37.5% after more than 10 years of diabetes and 11.1% at or before 10 years (p = 0.015). All 13 deficient participants were metformin users. CI = Wilson score interval; eGFR = estimated glomerular filtration rate; IQR = interquartile range; MNSI = Michigan Neuropathy Screening Instrument; VPT = vibration perception threshold.

Peripheral neuropathy increased monotonically from normal through borderline to deficient B12 categories, as did severe loss of vibration sense (Figure 1).

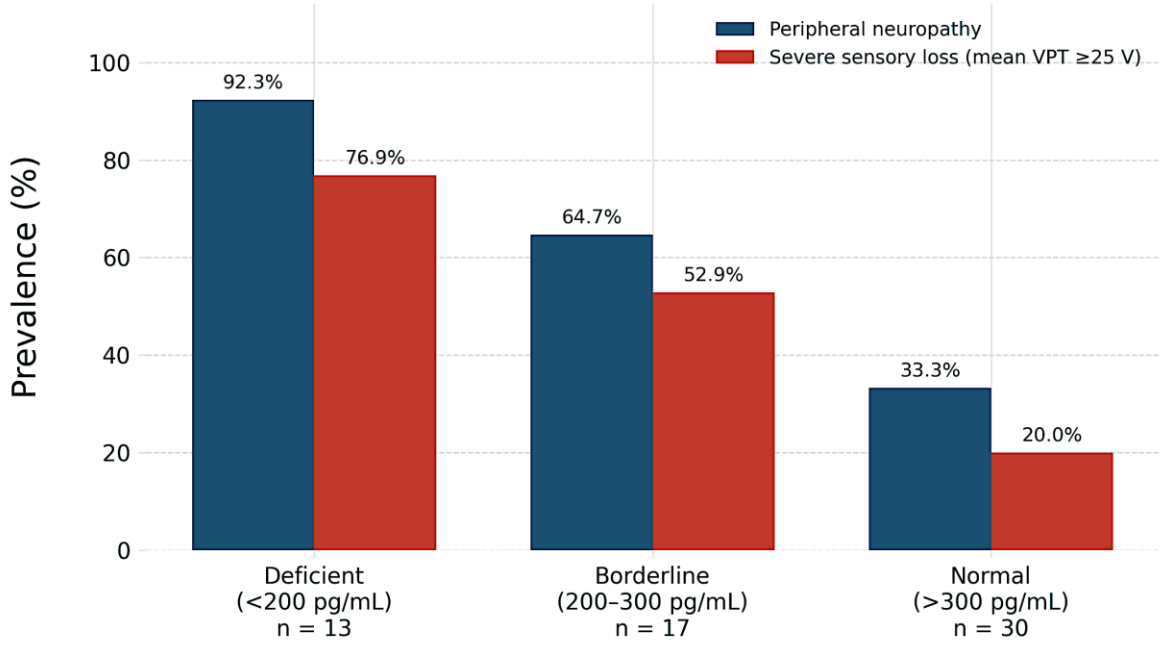


Figure 1. Prevalence of peripheral neuropathy and of severe sensory loss (bilateral mean vibration perception threshold ≥25 V) across vitamin B12 categories. Neuropathy required a Michigan Neuropathy Screening Instrument score above 2.0 and a bilateral mean threshold of at least 15 V. The ordered trend in neuropathy was significant (Cochran–Armitage p = 0.00025).

The three B12 groups were similar in age, glycated haemoglobin, and estimated glomerular filtration rate. Clinical scores and vibration thresholds were already higher in the borderline range than in the normal range, and distal numbness, burning pain, and ankle areflexia became more common as B12 fell (Table 2). On unadjusted comparison, neuropathy-positive participants had lower serum B12 and longer metformin exposure, whereas age, diabetes duration, glycated haemoglobin, and estimated glomerular filtration rate did not differ significantly.

Table 2. Neurological findings by vitamin B12 category.

Finding	Deficient (n = 13)	Borderline (n = 17)	Normal (n = 30)	p
Age, years	53.8 ± 11.7	53.7 ± 8.9	54.3 ± 13.7	0.98
HbA1c, %	8.68 ± 1.81	8.86 ± 1.35	9.32 ± 1.48	0.38
eGFR, mL/min/1.73 m²	73.9 ± 14.9	81.3 ± 11.2	79.5 ± 16.8	0.39

Metformin duration, months	132 ± 76	39 ± 26	18 ± 20	<0.001
MNSI score	4.39 ± 1.80	3.72 ± 2.37	2.12 ± 1.79	0.0015
Bilateral mean VPT, V	27.3 ± 6.4	21.2 ± 9.7	15.8 ± 9.0	0.0008
Distal numbness, %	84.6	41.2	36.7	0.012
Burning pain, %	69.2	64.7	30.0	0.017
Any absent ankle reflex, %	76.9	52.9	26.7	0.007
Unadjusted OR vs normal (95% CI)	24.0 (2.72–212)	3.67 (1.05–12.8)	1.00	—

Values are mean ± SD unless noted. Group p values are from analysis of variance or chi-square tests; rank tests agreed wherever the parametric test was significant. Prevalence of neuropathy and of severe sensory loss is shown in Figure 1 and is not repeated here. OR = odds ratio.

Metformin users had lower serum B12 than non-users despite similar age, diabetes duration, glycated haemoglobin, and kidney function. Mean B12 fell across daily-dose tiers, with the clearest drop at 1000 mg/day and above, and fell further as duration lengthened (Figure 2).

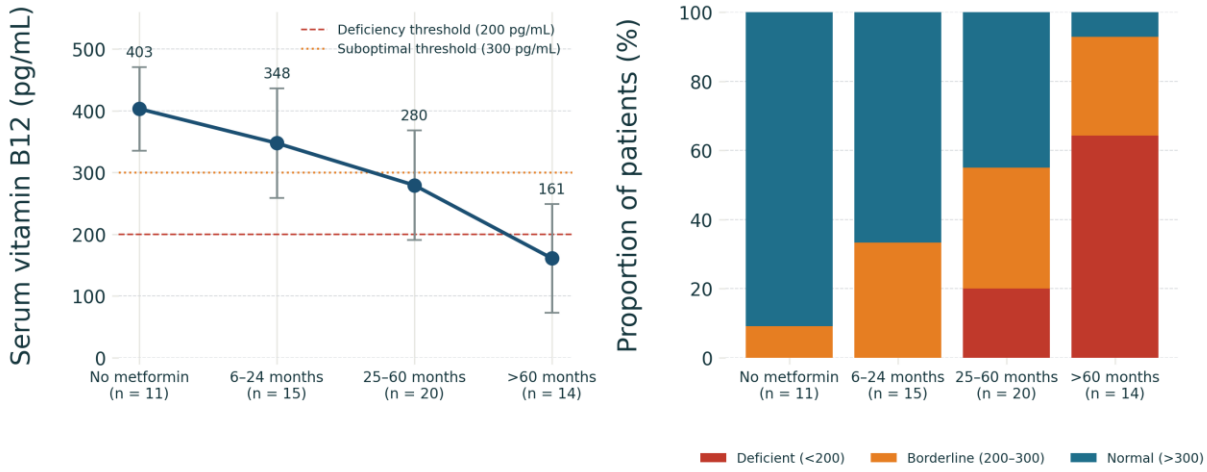


Figure 2. Metformin duration and vitamin B12. (A) Mean serum B12 (± SD) by duration tier, with reference lines at 200 and 300 pg/mL. (B) Proportion deficient, borderline, and normal in the same tiers. Overt deficiency was absent before 25 months and predominated after 60 months.

In the linear model, metformin duration and daily dose were independently associated with lower B12, whereas diabetes duration was not once those two exposures were included. After adjustment, both deficient and borderline status remained associated with neuropathy, as did a higher glycated haemoglobin. Age, diabetes duration, and estimated glomerular filtration rate did not. Serum B12 was also the strongest independent correlate of the clinical score and of the bilateral vibration threshold. The categorical odds ratio for overt deficiency is large because nearly all deficient participants had neuropathy; the continuous model is the more stable estimate. Longer metformin use predicted neuropathy when B12 was omitted. After serum B12 was added, that association lost significance, while B12 remained associated with neuropathy. This is statistical mediation in cross-sectional data, not evidence that metformin cause's neuropathy only through B12. Screening thresholds for prevalent neuropathy and for suboptimal or overt deficiency are listed in Table 3.

Table 3. Adjusted associations and screening thresholds.

Model	Estimate (95% CI)	p
Neuropathy: B12 deficient vs normal	aOR 82.9 (5.35–1283)	0.0016
Neuropathy: B12 borderline vs normal	aOR 7.16 (1.53–33.5)	0.012
Neuropathy: HbA1c, per 1%	aOR 2.00 (1.19–3.37)	0.009

Neuropathy: B12, per 50 pg/mL decrease	aOR 1.81 (1.28–2.57)	0.0009
Metformin duration per year, without B12	aOR 1.32 (1.06–1.63)	0.012
Metformin duration per year, with B12	aOR 1.08 (0.83–1.40)	0.57
Serum B12: metformin, per month	−1.29 pg/mL (−1.75 to −0.83)	<0.001
Serum B12: dose, per 100 mg/day	−5.6 pg/mL (−9.2 to −1.9)	0.003
B12 ≤322 pg/mL for neuropathy	AUC 0.76; sens. 75.8%, spec. 74.1%	<0.001
Metformin ≥41 months for B12 ≤300	AUC 0.85; sens. 73.3%, spec. 90.0%	<0.001
Metformin ≥89 months for B12 <200	AUC 0.93; sens. 69.2%, spec. 100%	<0.001
≥3.5 gram-years for B12 <200	AUC 0.92; sens. 92.3%, spec. 76.6%	<0.001

Logistic models for B12 category also included age, diabetes duration, HbA1c, and eGFR (AUC 0.83). Age, diabetes duration, and eGFR were not significant. Adding serum B12 reduced the metformin-duration coefficient by 72.7% (Sobel p = 0.029). The six-predictor linear model for serum B12 had $R^2 = 0.62$; diabetes duration was not significant after dose and duration (p = 0.88). aOR = adjusted odds ratio; AUC = area under the receiver-operating-characteristic curve.

DISCUSSION

This single-centre cohort shows a high burden of suboptimal vitamin B12, a graded relationship between lower B12 and neuropathy that includes the borderline range, and a metformin signal carried by dose and duration rather than by how long diabetes has been present. The pattern agrees with trial evidence that metformin lowers B12,[5,6] with dose- and duration-response analyses,[7] and with meta-analyses reporting about a two-fold or greater increase in biochemical deficiency.[8,17] Clinic prevalence here is higher than in the Diabetes Prevention Program Outcomes Study. That programme enrolled people at high risk of diabetes, used a lower deficiency cut-off, and accumulated exposure more slowly than a medicine-ward population already living with diabetes.[6] Population surveys likewise find a smaller absolute difference than hospital series.[18]

Borderline status was not an inert laboratory category. Clinical scores and vibration thresholds were already worse than in participants with normal B12, and burning pain was common in both the borderline and deficient groups, whereas distal numbness and areflexia were most marked in overt deficiency. That combination fits a mixed large- and small-fibre picture and argues against waiting for a value below 200 pg/mL before taking a low-normal result seriously. Other clinic studies have linked metformin-associated B12 depletion to more severe neuropathy scores, although not every cross-sectional sample finds a neuropathy association after adjustment.[10,14,19] Differences in neuropathy definition, cumulative dose, and whether borderline values are pooled with normal values likely explain part of that disagreement.

Glycaemia and cobalamin behaved as separate pathways. B12 category was unrelated to glycated haemoglobin, yet a higher glycated haemoglobin independently raised the odds of neuropathy once B12 was in the model. Glucose optimisation therefore remains necessary,[2] and a recent glycated haemoglobin in the target range does not remove the need for B12 testing. Conversely, a long diabetes history should not be assumed to explain a low B12 result. Diabetes duration lost its association with B12 after metformin dose and duration were included.

The mediation analysis should be read narrowly. It shows that, in these data, the statistical path from longer metformin use to prevalent neuropathy runs largely through lower serum B12. It does not prove that B12 depletion is the only mechanism, and it cannot establish time order. Diet, vegetarian practice, and acid-suppressing drugs were not measured and could lie on the same path.[12] Even so, the finding matches the clinical concern that metformin-related cobalamin deficiency can be mistaken for, or added to, diabetic neuropathy.[11,12]

The adjusted odds ratio for overt deficiency is imprecise. With few deficient participants and almost complete separation, the point estimate is inflated. The direction is supported by the ordered trend, the continuous B12 model, and the vibration-threshold regression. Those, rather than the extreme categorical odds ratio, should guide interpretation.

Screening can be more specific than a general suggestion of periodic testing.[3,12] In this sample, biochemical insufficiency was already identifiable after a few years of metformin, and overt deficiency became likely only with longer exposure, particularly beyond five years and at doses of 1000 mg/day or more. That

chronology matches the pharmacology of a vitamin with large hepatic stores and a low daily requirement: deficiency is delayed, then accelerates.[12] A practical approach is a baseline measurement when metformin is started or when neuropathy is first recognised, repeat testing within about four years, and earlier testing if the dose is at least 1500 mg/day, anaemia appears, or new sensory symptoms develop. Serum B12 alone misclassifies some patients. Methylmalonic acid or holotranscobalamin is appropriate when the result is borderline and the clinical picture is uncertain.[12] Replacement corrects serum levels, but trials do not yet agree on how reliably symptoms improve, so treatment should be framed as correction of a deficiency rather than as proven disease-modifying therapy for diabetic neuropathy.[20]

Several limits apply. The sample is small and drawn from one teaching hospital, so estimates are imprecise and may not generalise to primary care. The design is cross-sectional. Five deficient results sat at the assay floor of 80 pg/mL, which can attenuate correlations. Nerve-conduction studies and functional B12 markers were not obtained. Agreement between the clinical score and biothesiometry is reassuring but is not a reference standard. Diet, proton-pump inhibitor use, and non-metformin drug exposure were not modelled. Participants with severe kidney disease and those already taking B12 were excluded, which reduces some confounding but also means the prevalence is not that of an unselected diabetic population. Multiple comparisons were made. The main inferences rest on pre-specified trends and adjusted models rather than on every pairwise test.

CONCLUSION

In adults with type 2 diabetes attending this centre, suboptimal vitamin B12 was as common as a normal level. Neuropathy and severe loss of vibration sense increased as B12 fell, including across the borderline range, and this association remained after adjustment for age, diabetes duration, glycated haemoglobin, and kidney function. Metformin dose and duration, not the duration of diabetes itself, accounted for the lower B12, and lower B12 statistically accounted for most of the association between longer metformin use and neuropathy. B12 status should be checked during metformin therapy, especially after a few years, at higher doses, and whenever a distal sensory neuropathy is found.

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