RESEARCH PAPER

Retrospective Analysis Of Metformin Use And Its Association With Vitamin B12 Deficiency In Patients With Type 2 Diabetes Mellitus

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ABSTRACT

Background: Metformin, the cornerstone pharmacotherapy for type 2 diabetes mellitus (T2DM), has been associated with impaired absorption of vitamin B12. Long-term deficiency may precipitate peripheral neuropathy, megaloblastic anaemia, and cognitive decline. Despite its clinical importance, systematic monitoring of vitamin B12 in metformin-treated patients remains inconsistent in routine practice.

Objectives: To determine the prevalence of vitamin B12 deficiency in T2DM patients receiving metformin, and to evaluate the association between deficiency and duration of use, daily dosage, and clinical manifestations.

Methods: This retrospective cohort study analysed medical records of 312 T2DM patients on metformin therapy at a tertiary care centre from December 2023 to January 2026. Serum vitamin B12 levels were categorised as deficient (<200 pg/mL), insufficient (200-300 pg/mL), and normal (>300 pg/mL). Association with duration and dose was examined using ANOVA, chi-square tests, and binary logistic regression.

Results: Vitamin B12 deficiency was identified in 94 patients (30.1%) and insufficiency in 79 patients (25.3%). Mean serum B12 declined significantly with increasing duration: 389.4 pg/mL (<1 year) to 196.8 pg/mL (>5 years) ($p<0.001$). Higher daily doses (>1500 mg/day) were independently associated with deficiency (OR 2.34; 95% CI 1.45-3.78). Neurological symptoms were present in 54.3% of deficient patients, with peripheral neuropathy being most common (40.4%).

Conclusion: Vitamin B12 deficiency is highly prevalent among long-term metformin users and follows a dose- and duration-dependent pattern. Routine periodic screening and supplementation should be integrated into standard diabetic care protocols.

Keywords: *Metformin; Vitamin B12 deficiency; Type 2 diabetes mellitus; Cobalamin; Peripheral neuropathy; Screening*

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1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) represents one of the most significant global public health challenges of the twenty-first century. The International Diabetes Federation estimates that 537 million adults worldwide are living with diabetes as of 2021, a figure projected to exceed 783 million by 2045.[1] In South Asia, including India, the burden is disproportionately high, driven by genetic susceptibility, lifestyle transitions, and rapid urbanisation.[2,37] The management of T2DM requires sustained pharmacotherapy, and metformin (1,1-dimethylbiguanide) has remained the foundational first-line agent recommended by the American Diabetes Association, European Association for the Study of Diabetes, and most national guidelines for over four decades.[2,3,24]

Metformin exerts its primary antihyperglycaemic effects through the inhibition of hepatic gluconeogenesis, enhancement of peripheral insulin sensitivity, and modest promotion of intestinal glucose utilisation.[3] Its favourable safety profile, low cost, weight neutrality, and emerging pleiotropic benefits have reinforced its pre-eminent position in diabetes management. However, a clinically significant but historically underappreciated adverse consequence of long-term metformin use is the impairment of vitamin B12 (cobalamin) absorption.

The association between biguanides and vitamin B12 malabsorption was first described by Adams and colleagues in 1983, when they demonstrated reduced ileal

uptake of the vitamin B12-intrinsic factor complex in patients receiving biguanide therapy.[29] The proposed mechanism involves metformin's interference with calcium-dependent endocytosis at the ileal brush border, disrupting the binding of the intrinsic factor-cobalamin complex to its cubilin receptor.[8,29] Bauman et al. elegantly demonstrated that supplemental calcium intake could reverse this metformin-induced malabsorption, strengthening the mechanistic hypothesis.[8]

Vitamin B12 is an essential water-soluble micronutrient that plays a critical role in DNA synthesis, neurological function, erythropoiesis, and one-carbon metabolism through its co-enzymatic activity in the methylmalonyl-CoA mutase and methionine synthase pathways.[33,36] Deficiency manifests across a spectrum, ranging from asymptomatic biochemical depletion to debilitating neuropsychiatric disorders, including subacute combined degeneration of the spinal cord, peripheral neuropathy, and cognitive impairment - even in the absence of frank haematological abnormalities such as macrocytic anaemia.[21,31]

The reported prevalence of biochemical vitamin B12 deficiency in metformin-treated T2DM patients varies considerably across studies, ranging from 5.8% to 52%, depending on the definition of deficiency, population demographics, dietary habits, duration of therapy, and dose used.[5,6,11,15] The landmark randomised controlled trial by de Jager et al. demonstrated that long-term metformin use was associated with a 19% reduction in serum B12 concentration after 4.3 years, with a 7.2% absolute increase in the risk of biochemical deficiency compared to placebo.[5] Using data from the National Health and Nutrition Examination Survey (NHANES) 1999-2006, Reinstatler and colleagues found that metformin use was associated with significantly higher odds of biochemical B12 deficiency, and that this risk escalated with higher cumulative doses.[6]

A particularly insidious aspect of metformin-associated B12 deficiency is its clinical overlap with diabetic peripheral neuropathy. Both conditions can present with distal symmetrical sensorimotor neuropathy, paraesthesia, and ataxia, potentially leading to the erroneous attribution of neurological symptoms solely to diabetes while an underlying, correctable nutritional deficiency is left untreated.[7,23] Furthermore, elevated homocysteine concentrations arising from B12 depletion may independently increase cardiovascular risk in this already high-risk population.[9,38]

Despite this body of evidence, structured monitoring of vitamin B12 in metformin-treated patients is not uniformly implemented in clinical practice.[40] The ADA recommends periodic measurement of serum B12 in patients on long-term metformin therapy, particularly those with anaemia or peripheral neuropathy, yet real-world adherence to this recommendation remains suboptimal.[2,17] Data from Indian tertiary care centres specifically examining this relationship across dose and duration strata are limited. This retrospective cohort study was designed to determine the prevalence of vitamin B12 deficiency in T2DM patients receiving metformin, to evaluate the dose- and duration-dependent nature of this association, and to document the clinical manifestations attributable to deficiency in this cohort.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a retrospective cohort study conducted at the Department of General Medicine, Sree Balaji Medical College and Hospital, India. Medical records from December 2023 to January 2026 were reviewed systematically. As this was a retrospective analysis of existing records, the requirement for individual informed consent was waived.

2.2 Study Population

A total of 312 adult patients with confirmed T2DM diagnosis (as per American Diabetes Association criteria)[2] who were receiving metformin monotherapy or metformin in combination with other oral antidiabetic agents for at least three consecutive months were included. The study excluded patients who: (i) were receiving vitamin B12 or folate supplementation at the time of blood sampling; (ii) had declared strict vegetarian or vegan dietary practices without supplementation; (iii) had documented malabsorption disorders (including coeliac disease, Crohn's disease, atrophic gastritis); (iv) had a history of bariatric or gastric surgery; (v) had severely impaired renal function (estimated glomerular filtration rate <30 mL/min/1.73 m2); or (vi) had chronic alcoholism or active haematological disorders.[11,13]

2.3 Data Collection

Data were extracted from electronic medical records using a standardised data abstraction form. Variables collected included: age, sex, body mass index (BMI), duration of T2DM diagnosis, duration of metformin therapy (stratified as <1 year, 1-3 years, 3-5 years, >5 years), daily metformin dose (low: <1000 mg/day; moderate: 1000-1500 mg/day; high: >1500 mg/day), HbA1c, complete blood count, serum creatinine, serum vitamin B12, and documented clinical symptoms including peripheral neuropathy, fatigue, and cognitive complaints. Serum vitamin B12 was measured by electrochemiluminescence immunoassay (ECLIA) on the Roche cobas e601 analyser.[27]

2.4 Definitions

Vitamin B12 status was classified based on established biochemical thresholds: deficient (<200 pg/mL), insufficient/borderline (200-300 pg/mL), and normal (>300 pg/mL).[22,23] Macrocytic anaemia was defined as haemoglobin <12 g/dL in women or <13 g/dL in men, with mean corpuscular volume >100 fL. Peripheral neuropathy

was defined by documented clinical assessment (vibration sense, monofilament testing, ankle reflex) or nerve conduction study findings in the medical record.[21]

2.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 26.0. Continuous variables are expressed as mean ± standard deviation (SD); categorical variables as frequency and percentage. Differences in mean serum B12 across duration and dose groups were assessed by one-way ANOVA with Tukey's post-hoc correction. Pearson's correlation coefficient was used to examine the linear relationship between metformin dose, duration, and serum B12. Binary logistic regression was performed to identify independent predictors of B12 deficiency, adjusting for age, sex, BMI, and HbA1c. Chi-square test was used for categorical comparisons. A two-tailed p-value of <0.05 was considered statistically significant.[11,13]

3. RESULTS

3.1 Demographic and Clinical Characteristics

A total of 312 patients met the inclusion criteria. The mean age of the cohort was 54.3 ± 9.8 years. Male patients comprised 53.8% (n=168) of the study population. The mean duration of diabetes diagnosis was 6.2 ± 4.1 years, while the mean duration of metformin use was 3.4 ± 2.3 years. The mean HbA1c was 8.1 ± 1.4%, and mean BMI was 27.4 ± 4.2 kg/m2. Baseline demographic and clinical data are summarised in Table 1.

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants (n = 312)

Characteristic	Value
Age (years), mean ± SD	54.3 ± 9.8
Sex: Male, n (%)	168 (53.8%)
Sex: Female, n (%)	144 (46.2%)
BMI (kg/m2), mean ± SD	27.4 ± 4.2
Duration of T2DM diagnosis (years), mean ± SD	6.2 ± 4.1
Duration of metformin use (years), mean ± SD	3.4 ± 2.3
Daily metformin dose (mg/day), mean ± SD	1,342 ± 614
HbA1c (%), mean ± SD	8.1 ± 1.4
Serum creatinine (mg/dL), mean ± SD	0.89 ± 0.21
Mean serum B12 (pg/mL), mean ± SD	291.4 ± 108.7
Concurrent oral antidiabetic agents, n (%)	187 (59.9%)
Hypertension (comorbidity), n (%)	146 (46.8%)
Dyslipidaemia (comorbidity), n (%)	132 (42.3%)

SD = Standard Deviation; BMI = Body Mass Index; T2DM = Type 2 Diabetes Mellitus; HbA1c = Glycated Haemoglobin

3.2 Distribution of Vitamin B12 Status

Of the 312 patients, 94 (30.1%) were classified as vitamin B12 deficient (<200 pg/mL), 79 (25.3%) as insufficient (200-300 pg/mL), and 139 (44.6%) had normal serum B12 levels (>300 pg/mL). Collectively, 55.4% of patients demonstrated suboptimal vitamin B12 status. Figure 1 illustrates the distribution of B12 categories across the cohort.

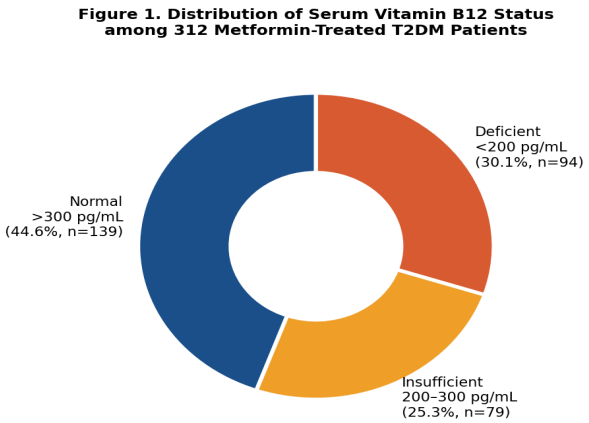

Figure 1. Distribution of Serum Vitamin B12 Status Among 312 Metformin-Treated T2DM Patients

3.3 Association with Duration of Metformin Use

When stratified by duration of metformin therapy, a clear inverse relationship was observed between duration of use and mean serum vitamin B12 concentration (p<0.001, one-way ANOVA). In patients using metformin for less than one year, the mean serum B12 was 389.4 ± 78.3 pg/mL, with a deficiency prevalence of 10.3% (8/78). This declined progressively: mean B12 was 312.6 ± 91.2 pg/mL in the 1-3 year group (deficiency 23.9%), 248.3 ± 84.7 pg/mL in the 3-5 year group (deficiency 39.0%), and 196.8 ± 77.5 pg/mL in those using metformin for more than five years (deficiency 55.4%). Post-hoc analysis demonstrated significant pairwise differences between all adjacent duration groups (all p<0.05). Details are presented in Table 2 and Figures 2 and 3.

Table 2. Serum Vitamin B12 Levels and Deficiency Prevalence by Duration of Metformin Use

Duration Group	n (%)	Mean B12 (pg/mL ± SD)	Deficient n (%)	Insufficient n (%)	Normal n (%)	p-value
<1 year	78 (25.0%)	389.4 ± 78.3	8 (10.3%)	14 (17.9%)	56 (71.8%)	Reference
1-3 years	96 (30.8%)	312.6 ± 91.2	23 (23.9%)	26 (27.1%)	47 (49.0%)	<0.01
3-5 years	82 (26.3%)	248.3 ± 84.7	32 (39.0%)	24 (29.3%)	26 (31.7%)	<0.001
>5 years	56 (17.9%)	196.8 ± 77.5	31 (55.4%)	15 (26.8%)	10 (17.8%)	<0.001
Total	**312 (100%)**	**291.4 ± 108.7**	**94 (30.1%)**	**79 (25.3%)**	**139 (44.6%)**	**-**

p-values calculated by one-way ANOVA with Tukey's post-hoc correction vs. <1 year reference group. SD = Standard Deviation.

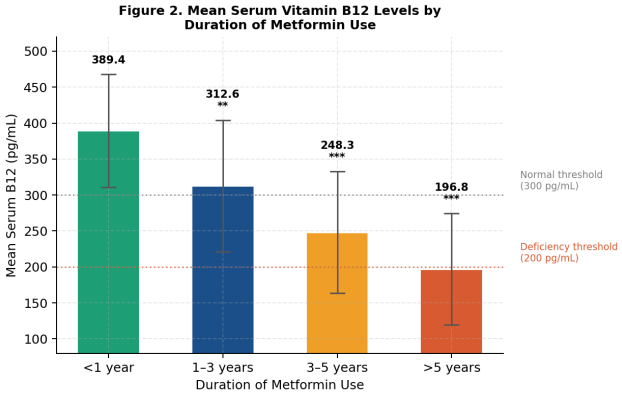

Figure 2. Mean Serum Vitamin B12 Levels (pg/mL) by Duration of Metformin Use. Error bars represent ± 1 SD. **p<0.01; ***p<0.001 vs. <1 year group.

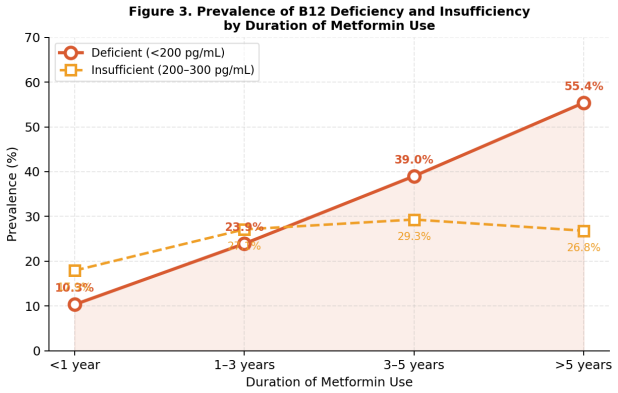

Figure 3. Prevalence of Vitamin B12 Deficiency (%) by Duration of Metformin Use

3.4 Association with Daily Dose of Metformin

A statistically significant dose-dependent decline in serum B12 was observed across dose strata (p<0.001). Patients on low daily doses (<1000 mg/day; n=94) had a mean serum B12 of 348.7 ± 85.3 pg/mL and a B12 deficiency prevalence of 18.1%. In the moderate dose group (1000-1500 mg/day; n=128), mean B12 was 289.4 ± 92.1 pg/mL (deficiency 32.8%). In the high dose group (>1500 mg/day; n=90), mean B12 was 241.2 ± 88.7 pg/mL (deficiency 38.9%), as detailed in Table 3 and Figure 4.

Table 3. Serum Vitamin B12 and Deficiency Prevalence by Daily Metformin Dose

Dose Category	n (%)	Mean B12 (pg/mL ± SD)	Deficient n (%)	Insufficient n (%)	Normal n (%)
Low (<1000 mg/day)	94 (30.1%)	348.7 ± 85.3	17 (18.1%)	22 (23.4%)	55 (58.5%)
Moderate (1000-1500 mg/day)	128 (41.0%)	289.4 ± 92.1	42 (32.8%)	34 (26.6%)	52 (40.6%)
High (>1500 mg/day)	90 (28.9%)	241.2 ± 88.7	35 (38.9%)	23 (25.6%)	32 (35.5%)
Total	**312 (100%)**	**291.4 ± 108.7**	**94 (30.1%)**	**79 (25.3%)**	**139 (44.6%)**

One-way ANOVA: F = 19.32, p<0.001. Tukey's post-hoc: Low vs. High p<0.001; Moderate vs. High p=0.018; Low vs. Moderate p=0.004.

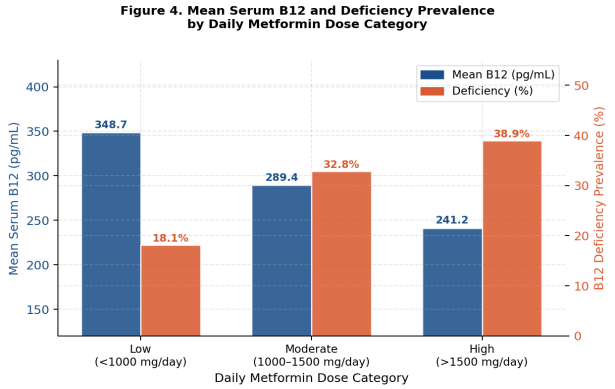

Figure 4. Mean Serum B12 and Deficiency Prevalence by Daily Metformin Dose Category

3.5 Clinical Manifestations

Among the 94 patients with B12 deficiency, 51 (54.3%) had at least one clinically documented symptom attributable to cobalamin deficiency. Peripheral neuropathy was the most frequent finding, identified in 38 patients (40.4%), followed by fatigue and generalised weakness in 29 (30.9%), and cognitive impairment or memory decline in 12 (12.8%). Macrocytic anaemia was present in 28 patients (29.8% of the deficient group). Importantly, 6 patients (6.4%) had been previously attributed their neuropathy solely to diabetic peripheral neuropathy without prior B12 evaluation (Table 4).

Table 4. Clinical Manifestations Among Vitamin B12 Deficient Patients (n = 94)

Clinical Feature	n	% of Deficient (n=94)
Peripheral neuropathy (distal paraesthesia / numbness)	38	40.4%
Fatigue / generalised weakness	29	30.9%
Macrocytic anaemia (Hb low + MCV >100 fL)	28	29.8%
Cognitive impairment / memory decline	12	12.8%
Glossitis / angular cheilitis	9	9.6%
Gait disturbance / ataxia	6	6.4%
No documented symptoms (asymptomatic)	43	45.7%

Patients may have more than one clinical feature; percentages do not sum to 100. MCV = Mean Corpuscular Volume; Hb = Haemoglobin.

3.6 Logistic Regression Analysis

Binary logistic regression identified duration of metformin use (OR 1.42 per year; 95% CI 1.18-1.71; p<0.001) and high daily dose (>1500 mg/day) (OR 2.34; 95% CI 1.45-3.78; p=0.001) as independent significant predictors of B12 deficiency after adjusting for age, sex, BMI, and HbA1c. Female sex showed a trend towards higher odds (OR 1.56; 95% CI 0.98-2.47; p=0.062) but did not reach statistical significance. Age and HbA1c were not independently associated with deficiency in this model.

Table 5. Binary Logistic Regression - Predictors of Vitamin B12 Deficiency

Predictor	Odds Ratio	95% CI	p-value
Duration of metformin use (per year)	1.42	1.18 - 1.71	<0.001
High dose (>1500 mg/day) vs. low dose	2.34	1.45 - 3.78	0.001
Moderate dose (1000-1500 mg) vs. low dose	1.68	1.07 - 2.64	0.024
Female sex	1.56	0.98 - 2.47	0.062
Age (per year)	1.03	0.99 - 1.06	0.118
BMI (per kg/m2)	0.97	0.91 - 1.04	0.392
HbA1c (%)	1.09	0.93 - 1.28	0.284

Model fit: Nagelkerke R2 = 0.31; Hosmer-Lemeshow test p=0.52 (good fit). CI = Confidence Interval; BMI = Body Mass Index.

4. DISCUSSION

This retrospective cohort study of 312 T2DM patients receiving metformin demonstrated a substantial prevalence of biochemical vitamin B12 deficiency (30.1%), with a further 25.3% showing insufficient levels, meaning over half the cohort had suboptimal cobalamin status. These findings are consistent with and reinforced by the broader literature on this subject. The landmark RCT by de Jager et al. reported that 4.3 years of metformin use resulted in a progressive decline in mean serum B12 and a 7.2% absolute increase in deficiency risk compared to placebo.[5] Similarly, using large cross-sectional NHANES data, Reinstatler and colleagues reported that metformin use was associated with significantly lower serum B12 and higher odds of biochemical deficiency.[6] Our observed prevalence of 30.1% falls within the broad range of 10-40% consistently reported in systematic reviews and meta-analyses.[11,13,18]

The mechanistic basis of metformin-induced B12 malabsorption is well characterised. In the terminal ileum, the absorption of the intrinsic factor-cobalamin complex is mediated through a calcium-dependent receptor-mediated

process involving the cubilin-amnionless receptor complex.[8,29] Metformin is thought to competitively antagonise calcium ions at this membrane transporter, thereby impairing complex internalisation.[8] The reversal of this malabsorption through calcium supplementation, as demonstrated by Bauman et al., provides compelling mechanistic evidence for this pathway.[8] Additional mechanisms postulated include alterations in the gastrointestinal microbiome, reduced secretion of intrinsic factor, and changes in intestinal motility caused by metformin.[34]

The duration-dependent pattern of declining B12 in our study is particularly instructive. The mean serum B12 fell from 389.4 pg/mL in patients treated for less than one year to 196.8 pg/mL in those treated for more than five years - a near 50% decline representing a trajectory into the deficient range. This temporal progression aligns with the findings of Aroda et al. from the Diabetes Prevention Program Outcomes Study (DPPOS), which demonstrated that metformin-associated B12 deficiency and its clinical consequences, including peripheral neuropathy, increased significantly with cumulative exposure over more than ten years.[17] Wulfele and colleagues similarly documented progressive increases in homocysteine concentration alongside declining B12, implicating worsening functional cobalamin deficiency with continued therapy.[9]

The dose-response relationship observed in our cohort, with high daily doses (>1500 mg/day) conferring an OR of 2.34 for deficiency relative to low doses, corroborates findings from Kim et al. and Yang et al., who reported significant dose-dependent associations in their respective analyses.[25,26] These findings carry practical clinical implications: in patients who require higher metformin doses for glycaemic control - particularly those who are overweight or who have significant insulin resistance - the threshold for B12 monitoring should be lowered and the interval shortened.

A particularly clinically relevant finding in our study was that 40.4% of deficient patients had peripheral neuropathy, and that in 6.4% of cases, neuropathy had previously been attributed solely to diabetes without prior B12 evaluation. This diagnostic conflation is well recognised in the literature. As Lindenbaum et al. demonstrated, neuropsychiatric manifestations of cobalamin deficiency may occur without overt haematological features such as macrocytic anaemia or elevated MCV, potentially delaying diagnosis.[21] Given that metformin is itself used in patients who already bear a high neuropathy risk from T2DM, disentangling the contributions of cobalamin deficiency and diabetic peripheral neuropathy requires active screening rather than passive symptom-based detection.[7,23]

Notably, 45.7% of deficient patients in our cohort were entirely asymptomatic at the time of identification. This reinforces the case for proactive biochemical screening independent of symptom burden. Current ADA guidance advocates periodic serum B12 monitoring in long-term metformin users, particularly those with anaemia or peripheral neuropathy, yet global and national adoption of this recommendation in routine diabetic follow-up remains inconsistent.[2,40] Mazokopakis and Starakis have proposed a structured approach: baseline B12 measurement followed by annual or biennial monitoring in high-risk patients (those on high doses or with therapy duration exceeding three years).[23]

The role of dietary pattern in mediating this risk deserves comment. While we excluded declared strict vegetarians, borderline dietary B12 intake - common in semi-vegetarian populations in South Asia - may synergistically compound metformin-induced malabsorption.[32] The interaction between dietary cobalamin intake, intrinsic factor sufficiency, gastric acidity, and metformin dose likely accounts for part of the heterogeneity in deficiency rates across studies conducted in different geographic and demographic contexts.

Our study has several limitations inherent to its retrospective design. First, dietary B12 intake was not quantified. Second, serum B12 measurements may not fully reflect tissue-level cobalamin adequacy; functional biomarkers such as methylmalonic acid (MMA) and holotranscobalamin II, which are more sensitive indicators of cellular deficiency, were not uniformly available in the records reviewed.[27,36] Third, concurrent use of proton pump inhibitors - an additional recognised cause of B12 malabsorption - was not systematically recorded, and residual confounding from this source cannot be excluded. Finally, the single-centre nature of this study limits generalisability to broader populations. Strengths include the relatively large sample size, five-year study span, availability of complete metformin dosing data, and comprehensive clinical records allowing symptom documentation.

5. CONCLUSION

This retrospective cohort study demonstrates that vitamin B12 deficiency is highly prevalent among T2DM patients receiving metformin therapy, affecting nearly one in three patients, with a further one in four showing insufficient levels. The deficiency follows a clear dose- and duration-dependent pattern, with patients on high doses (>1500 mg/day) and long-term users (>5 years) being at greatest risk. The majority of deficient patients manifested clinically meaningful features - most prominently peripheral neuropathy - highlighting the importance of B12 deficiency as an aggravating and potentially misattributed contributor to neurological morbidity in this population.

These findings support the integration of routine, periodic serum vitamin B12 screening into standard protocols for

T2DM management, particularly for patients on long-term or high-dose metformin. Based on the evidence, a pragmatic recommendation would be to obtain a baseline B12 level at initiation of metformin and repeat annually in patients on doses exceeding 1000 mg/day or those with therapy duration beyond three years. Prompt supplementation with intramuscular or oral high-dose cyanocobalamin in deficient patients is warranted to prevent progressive neurological and haematological sequelae. Proactive identification and correction of vitamin B12 deficiency in metformin-treated patients represents a simple, low-cost intervention that can meaningfully reduce preventable morbidity in this large and growing patient population.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas, 10th edition. Brussels: International Diabetes Federation; 2021.
2. American Diabetes Association Professional Practice Committee. Standards of Care in Diabetes - 2023. Diabetes Care. 2023;46(Suppl 1):S1-S291.
3. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab. 2014;20(6):953-66.
4. Caspary WF, Zavada I, Reimold W, Deuticke U, Emrich D, Willms B. Alteration of bile acid metabolism and vitamin B12 absorption in diabetics on biguanides. Diabetologia. 1977;13(3):187-93.
5. de Jager J, Kooy A, Lehert P, Wulfele MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ. 2010;340:c2181.
6. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr. Association of biochemical B12 deficiency with metformin therapy and vitamin B-12 supplements: the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care. 2012;35(2):327-33.
7. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B12 deficiency in patients receiving metformin. Arch Intern Med. 2006;166(18):1975-9.
8. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227-31.
9. Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, et al. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med. 2003;254(5):455-63.
10. Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? J Diabetes Metab Disord. 2013;12(1):17.
11. Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes: a systematic review and meta-analysis. Diabetes Metab. 2016;42(5):316-27.
12. Ahmed MA. Metformin and vitamin B12 deficiency: where do we stand? J Pharm Pharm Sci. 2016;19(3):382-98.
13. Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med. 2015;10(1):93-102.
14. Beulens JW, Hart HE, Kuijpers CC, Chin Paw MJ, Bots ML, Stehouwer CD, et al. Risk of long-term deficiency of vitamin B12 in relation to metformin use and low dietary intake. Eur J Intern Med. 2015;26(7):525-31.
15. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med. 2009;22(5):528-34.
16. Kos E, Liszek MJ, Emanuele MA, Durazo-Arvizu R, Camacho P. Effect of metformin therapy on vitamin D and vitamin B12 levels in patients with type 2 diabetes mellitus. Endocr Pract. 2012;18(2):179-84.
17. 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 Metab. 2016;101(4):1754-61.
18. Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. PLoS One. 2014;9(6):e100379.
19. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. J Med Assoc Thai. 2004;87(7):780-7.
20. Filioussi K, Bonovas S, Katsaros T. Should we screen diabetic patients using biguanides for megaloblastic anaemia? Aust Fam Physician. 2003;32(5):383-4.
21. Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the

absence of anemia or macrocytosis. N Engl J Med. 1988;318(26):1720-8.
22. Andres E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. Arch Intern Med. 2002;162(19):2251-2.
23. Mazokopakis EE, Starakis IK. Recommendations for diagnosis and management of metformin-induced vitamin B12 deficiency. Diabetes Res Clin Pract. 2012;97(3):359-67.
24. Saenz A, Fernandez-Esteban I, Mataix A, Ausejo M, Roque M, Moher D. Metformin monotherapy for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2005;(3):CD002966.
25. 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 (Baltimore). 2019;98(46):e17918.
26. 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.
27. Obeid R, Jung J, Falk J, Herrmann W, Geisel J, Friesenhahn-Ochs B, et al. Serum vitamin B12 not reflecting vitamin B12 status in patients with type 2 diabetes. Biochimie. 2013;95(5):1056-61.
28. Stover PJ. Vitamin B12 and older adults. Curr Opin Clin Nutr Metab Care. 2010;13(1):24-7.
29. Adams JF, Clark JS, Ireland JT, Kesson CM, Watson WS. Malabsorption of vitamin B12 and intrinsic factor secretion during biguanide therapy. Diabetologia. 1983;24(1):16-8.
30. Shane B, Stokstad EL. Vitamin B12-folate interrelationships. Annu Rev Nutr. 1985;5:115-41.
31. Greibe E, Miller JW, Foutouhi SH, Green R, Nexo E. Metformin increases liver accumulation of vitamin B12 - an experimental study in rats. Biochimie. 2013;95(5):1062-5.
32. Pawlak R, Lester SE, Babatunde T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. Eur J Clin Nutr. 2014;68(5):541-8.
33. Bazzano LA, Reynolds K, Holder KN, He J. Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. JAMA. 2006;296(22):2720-6.
34. World Health Organization. Global report on diabetes. Geneva: WHO; 2016.
35. Boyle JP, Thompson TJ, Gregg EW, Barker LE, Williamson DF. Projection of the year 2050 burden of diabetes in the US adult population. Popul Health Metr. 2010;8:29.
36. Guldal A, Semin I. Family physicians' perspectives on vitamin B12 deficiency screening and management among metformin-treated patients: a qualitative study. BMC Fam Pract. 2016;17:22.
37. Stover PJ. Physiology of folate and vitamin B12 in health and disease. Nutr Rev. 2004;62(6 Pt 2):S3-12.
38. Nexo E, Hoffmann-Lucke E. Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility. Am J Clin Nutr. 2011;94(1):359S-365S.