



ONE-YEAR INCIDENCE AND DETERMINANTS OF VITAMIN B12 DEFICIENCY IN NEWLY DIAGNOSED TYPE 2 DIABETES MELLITUS PATIENTS INITIATED ON METFORMIN: A PROSPECTIVE COHORT STUDY

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Abstract

Background: Metformin is the first-line therapy for type 2 diabetes mellitus and is widely prescribed at diagnosis. Long-term use of metformin has been linked to vitamin B12 deficiency, but prospective Indian data on early incidence after initiation of therapy are limited.

Objectives: To determine the one-year incidence of vitamin B12 deficiency among newly diagnosed type 2 diabetes mellitus patients with normal baseline vitamin B12 levels who were initiated on metformin.

Methods: This prospective observational cohort study was conducted at a tertiary care teaching hospital in India. A total of 112 newly diagnosed type 2 diabetes mellitus patients with normal baseline serum vitamin B12 levels were enrolled at the time of initiation of metformin therapy and followed for one year. Participants were reviewed at three-month intervals, and serum vitamin B12 levels were measured at each follow-up visit. Baseline demographic, clinical, haematological, and biochemical parameters were recorded.

Results: Over one year, 21 participants (18.75%) developed incident vitamin B12 deficiency. Participants who developed deficiency were significantly older and more likely to follow a vegetarian diet, receive higher daily doses of metformin, have lower baseline haemoglobin levels, and use proton pump inhibitors. On multivariable analysis, increasing age, vegetarian diet, metformin dose ≥ 1500 mg/day, lower baseline haemoglobin, and proton pump inhibitor use were independently associated with vitamin B12 deficiency.

Conclusions: Vitamin B12 deficiency can develop within the first year of metformin therapy in newly diagnosed type 2 diabetes mellitus patients. Early and risk-based monitoring of vitamin B12 levels should be considered, particularly in patients with identifiable risk factors.

Keywords: Vitamin B12 deficiency; Metformin; Type 2 diabetes mellitus; Incidence; Prospective cohort; India

Introduction

Type 2 diabetes mellitus is a major public health problem in India, with a steadily rising incidence and earlier age of onset(1). Metformin is recommended as the first-line oral antidiabetic drug and is widely prescribed at the time of diagnosis(2). Owing to its proven efficacy, safety, and low cost, many patients continue metformin for prolonged periods. However, long-term metformin therapy has been associated with reduced absorption of vitamin B12, leading to biochemical deficiency in a proportion of users(3). Vitamin B12 plays an essential role in haematopoiesis and neurological function. Deficiency may result in anaemia, peripheral neuropathy, and cognitive symptoms, which can overlap with or worsen diabetic complications(3). Indian populations are already vulnerable to vitamin B12 deficiency due to dietary habits, particularly vegetarian diets. Despite this, vitamin B12 status is not routinely monitored at the initiation of metformin therapy, and deficiency often remains unrecognised until symptoms develop.

Most available studies have assessed the prevalence of vitamin B12 deficiency in patients on long-term metformin therapy(3). There is limited prospective data from India examining the **incidence** of vitamin B12 deficiency after starting metformin, particularly among newly diagnosed type 2 diabetes patients with normal baseline vitamin B12 levels. Estimating the incidence over a defined follow-up period provides clearer evidence on the temporal relationship between metformin initiation and vitamin B12 deficiency. Such data are important to guide recommendations on baseline testing, follow-up intervals, and targeted screening strategies in routine clinical practice in Indian settings.

Aim:To determine the **incidence of vitamin B12 deficiency** among newly diagnosed type 2 diabetes mellitus patients with normal baseline vitamin B12 levels who are initiated on metformin therapy.

Material and Methods

This study was conducted as a prospective observational cohort study. Newly diagnosed type 2 diabetes mellitus patients with normal baseline vitamin B12 levels were enrolled at the time of initiation of metformin therapy and were followed longitudinally for one year. The study was carried out in the Department of Pathology and Department of General Medicine of a tertiary care teaching hospital in India. The hospital caters to a large outpatient population and provides comprehensive diagnostic and laboratory services, enabling systematic enrolment and follow-up of newly diagnosed diabetes patients. Ethical approval for the study was obtained after submission of the detailed study protocol, participant information sheet, and informed consent documents to the Institutional Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki and the Indian Council of Medical Research ethical guidelines. This study was conducted over a span of 18 months the year 2023 to 2025. Participants were followed for a total duration of 12 months from the date of initiation of metformin therapy. Study Outcome: Incidence of vitamin B12 deficiency over one year among newly diagnosed type 2 diabetes mellitus patients initiated on metformin, assessed using serum vitamin B12 levels at follow-up. Change in mean serum vitamin B12 levels from baseline to the end of one year. Occurrence of haematological abnormalities suggestive of vitamin B12 deficiency during follow-up. The exposure of interest was initiation and continued use of metformin therapy in newly diagnosed type 2 diabetes mellitus patients. Metformin was prescribed as per standard clinical practice, and information regarding dose and duration of therapy during follow-up was recorded. The study included newly diagnosed type 2 diabetes mellitus patients who were initiated on metformin therapy and had normal serum vitamin B12 levels at baseline. These participants were selected to allow assessment of new-onset vitamin B12 deficiency following exposure to metformin.

Inclusion criteria

1. Newly diagnosed type 2 diabetes mellitus
2. Age ≥ 18 years
3. Initiation of metformin therapy
4. Normal baseline serum vitamin B12 levels

5. Willingness to provide informed consent and comply with follow-up

Exclusion criteria

1. Baseline vitamin B12 deficiency or borderline levels
2. Prior vitamin B12 supplementation
3. Known malabsorption disorders
4. Pregnancy
5. History of gastric or intestinal surgery affecting absorption

All eligible individuals attending the facility during recruitment who consented were enrolled. This approach yielded a final sample size of **148 participants**. A non-random, consecutive sampling method was employed. Potential participants were initially screened by the treating physician at the time of diagnosis of type 2 diabetes mellitus. Eligibility was assessed using predefined inclusion and exclusion criteria. Baseline vitamin B12 levels were reviewed, and eligible patients were referred to the Principal Investigator for enrolment. Written informed consent was obtained by the Principal Investigator using a pre-approved consent form in the language preferred by the participant. Written consent was obtained after clarification of queries, in the presence of a witness.

Data Collection Procedure: Adults attending the medicine outpatient department were first evaluated by the treating physician for a new diagnosis of type 2 diabetes mellitus as per the routine diagnostic criteria followed at the institute. Patients who were newly diagnosed were referred to the Principal Investigator for further screening. Eligibility was assessed using the predefined inclusion and exclusion criteria. A baseline clinical evaluation was then completed. This included detailed history taking, assessment of dietary pattern and place of residence, and anthropometric measurements. Body weight and height were measured using standard calibrated equipment, and body mass index was calculated accordingly. Baseline laboratory investigations were obtained prior to initiation of metformin therapy. These included complete blood count, iron profile, serum folic acid, and serum vitamin B12 levels. Only patients with serum vitamin B12 values within the institutional reference range at baseline were considered eligible for enrolment. Following enrolment, metformin therapy was initiated by the treating physician as part of standard clinical care. Details regarding the starting dose and any planned dose titration were recorded from the prescription records. Participants were followed up at regular intervals of three months from baseline, with scheduled visits at 3, 6, 9, and 12 months. Follow-up dates were provided at the time of enrolment, and reminder calls were made before each visit to improve adherence. At every follow-up visit, interim clinical history was reviewed by the Principal Investigator, with specific attention to symptoms suggestive of vitamin B12 deficiency, changes in dietary pattern, and the occurrence of any intercurrent illness. Antidiabetic medications were reviewed at each visit. Continuation of metformin therapy was confirmed, the daily dose was documented, and any interruptions, dose modifications, or discontinuation were recorded along with the reasons.

Concomitant medications known to affect vitamin B12 status, particularly proton pump inhibitors and H2 receptor blockers, were specifically enquired about and documented at each follow-up visit. Anthropometric measurements were repeated where required, and glycaemic profile parameters were recorded from contemporaneous clinical records. Serum vitamin B12 levels were measured at every follow-up visit at 3, 6, 9, and 12 months using the hospital laboratory services. The results were recorded in the data collection proforma on the same day after report availability.

For participants in whom serum vitamin B12 levels fell below the institutional cut-off for deficiency at any follow-up visit, the date of first detection was recorded and considered as the time point of incident vitamin B12 deficiency. This information was used for estimation of incidence over the one-year follow-up period. Complete blood count parameters were reviewed during follow-up as per protocol to identify haematological changes associated with vitamin B12 deficiency. Where iron profile and folate reports were available as part of routine care, these were also documented. Participants were monitored for any adverse events during the study period. Any untoward events

identified during follow-up were documented and managed according to institutional standard protocols and reported to the Institutional Ethics Committee as required.

Statistical Analysis: Categorical variables were summarised using frequencies and percentages, while continuous variables were described using mean and standard deviation or median and interquartile range as appropriate. The one-year cumulative incidence of vitamin B12 deficiency was calculated with 95% confidence intervals. Changes in serum vitamin B12 levels over time were analysed using appropriate paired statistical tests. Both statistical and graphical analyses were performed using **Stata version 17.0**.

Results

A total of 148 newly diagnosed patients with type 2 diabetes mellitus who had normal baseline serum vitamin B12 levels and were initiated on metformin were enrolled in the present study. A total of 36 participants were lost to follow during the 12 months follow and remaining 112 participants were included in the analysis. The baseline demographic, anthropometric, and glycaemic characteristics of the study population are summarised in **Table 1**.

Variable	Category / Measure	Value
Age (years)	Mean $\pm$ SD	52.4 $\pm$ 9.6
	Range	32 – 72
Gender	Male	68 (60.7%)
	Female	44 (39.3%)
Body Mass Index (kg/m²)	Mean $\pm$ SD	26.8 $\pm$ 3.4
	Normal (18.5–22.9)	24 (21.4%)
	Overweight (23.0–24.9)	31 (27.7%)
	Obese ($\geq$ 25.0)	57 (50.9%)
Residence	Urban	71 (63.4%)
	Rural	41 (36.6%)
Fasting Plasma Glucose (mg/dL)	Mean $\pm$ SD	156.2 $\pm$ 28.5
Post-prandial Plasma Glucose (mg/dL)	Mean $\pm$ SD	238.6 $\pm$ 42.7
HbA1c (%)	Mean $\pm$ SD	8.1 $\pm$ 1.2

Parameter	Unit	Mean $\pm$ SD	Range
Haemoglobin	g/dL	13.2 $\pm$ 1.6	9.8 – 16.8
Total RBC count	$\times 10^6/\mu\text{L}$	4.72 $\pm$ 0.54	3.6 – 5.9
Total leukocyte count	cells/mm ³	7,420 $\pm$ 1,860	3,900 – 12,600
Platelet count	$\times 10^3/\mu\text{L}$	254 $\pm$ 68	138 – 410
Mean corpuscular volume (MCV)	fL	87.6 $\pm$ 6.4	72 – 101
Mean corpuscular haemoglobin (MCH)	pg	28.4 $\pm$ 2.6	22.1 – 33.9
Mean corpuscular haemoglobin concentration (MCHC)	g/dL	32.6 $\pm$ 1.4	29.4 – 35.8
Red cell distribution width (RDW)	%	13.8 $\pm$ 1.2	11.4 – 17.2
Serum iron	$\mu\text{g/dL}$	86.5 $\pm$ 24.1	42 – 148
Total iron binding capacity (TIBC)	$\mu\text{g/dL}$	318.4 $\pm$ 52.7	210 – 428
Serum ferritin	ng/mL	112.6 $\pm$ 68.3	22 – 356
Serum folic acid	ng/mL	9.4 $\pm$ 3.1	3.6 – 18.2

Serum vitamin B12	pg/mL	468.7 ± 112.5	302 – 812
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Baseline haematological and micronutrient parameters are presented in **Table 2**. The mean haemoglobin concentration was 13.2 ± 1.6 g/dL, and most red cell indices, including mean corpuscular volume and red cell distribution width, were within reference ranges. The mean baseline serum vitamin B12 level was 468.7 ± 112.5 pg/mL, confirming that all participants met the eligibility criterion of normal vitamin B12 status at study entry.

During the one-year follow-up, a gradual increase in incident vitamin B12 deficiency was observed across successive visits. At the first follow-up visit at 3 months, 4 participants (3.6%) developed vitamin B12 deficiency. By 6 months, an additional 6 participants were identified, increasing the cumulative incidence to 10 cases (8.9%). At the 9-month follow-up, a further 5 new cases were detected, raising the cumulative number of deficient participants to 15 (13.4%). By the end of 12 months, 6 additional participants developed vitamin B12 deficiency, resulting in a total of 21 incident cases and a cumulative one-year incidence of 18.75%.

A comparison of participants who developed vitamin B12 deficiency with those who did not is shown in **Table 3**.

Table 3: Incidence of vitamin B12 deficiency by participants characteristics (n = 112)				
Variable	Category	Vitamin B12 Deficiency (n = 21)	No Deficiency (n = 91)	P value
Age (years)	Mean ± SD	56.8 ± 8.4	51.3 ± 9.7	0.02
Gender	Male	14 (66.7%)	54 (59.3%)	0.54
	Female	7 (33.3%)	37 (40.7%)	
BMI (kg/m ²)	Mean ± SD	27.6 ± 3.1	26.5 ± 3.5	0.18
Residence	Urban	15 (71.4%)	56 (61.5%)	0.41
	Rural	6 (28.6%)	35 (38.5%)	
Dietary pattern	Vegetarian	13 (61.9%)	32 (35.2%)	0.03
	Mixed diet	8 (38.1%)	59 (64.8%)	
Baseline HbA1c (%)	Mean ± SD	8.5 ± 1.1	8.0 ± 1.2	0.07
Metformin daily dose (mg)	≥1500 mg	16 (76.2%)	43 (47.3%)	0.02
	<1500 mg	5 (23.8%)	48 (52.7%)	
Duration of metformin use	12 months (continuous)	21 (100%)	91 (100%)	–
Baseline haemoglobin (g/dL)	Mean ± SD	12.6 ± 1.4	13.4 ± 1.6	0.04
Baseline MCV (fL)	Mean ± SD	89.8 ± 6.2	87.1 ± 6.4	0.09
Proton pump inhibitor use	Yes	9 (42.9%)	18 (19.8%)	0.03
	No	12 (57.1%)	73 (80.2%)	

The multivariable logistic regression analysis identifying independent predictors of incident vitamin B12 deficiency is presented in **Table 4**. After adjustment for relevant demographic, clinical, and treatment-related variables, increasing age was independently associated with a higher risk of developing vitamin B12 deficiency, with each additional year conferring a modest but significant increase in risk. Dietary pattern remained a significant determinant, with participants adhering to a vegetarian diet showing nearly threefold higher odds of deficiency compared with those consuming a mixed diet. Higher metformin exposure also retained significance in the adjusted model, as patients receiving a daily dose of ≥1500 mg had a significantly increased likelihood of developing vitamin B12 deficiency during follow-up.

Table 4. Multivariable logistic regression analysis of factors associated with incident vitamin B12 deficiency

Predictor variable	Adjusted Odds Ratio (AOR)	95% Confidence Interval	P value
Age (per 1-year increase)	1.06	1.01 – 1.12	0.02
Female sex	0.82	0.31 – 2.14	0.69
BMI (per 1 kg/m ² increase)	1.05	0.93 – 1.18	0.42
Urban residence	1.38	0.48 – 3.94	0.55
Vegetarian diet	2.74	1.03 – 7.29	0.04
Baseline HbA1c (per 1% increase)	1.21	0.88 – 1.67	0.24
Metformin dose $\geq$ 1500 mg/day	3.16	1.14 – 8.74	0.03
Baseline haemoglobin (per 1 g/dL increase)	0.72	0.54 – 0.97	0.03
Proton pump inhibitor use	2.89	1.01 – 8.27	0.048

Table 5. Haematological and peripheral smear findings among participants who developed vitamin B12 deficiency during follow-up (n = 21)

Parameter	Baseline (Mean $\pm$ SD)	At time of B12 deficiency detection (Mean $\pm$ SD)	P value
Haemoglobin (g/dL)	12.6 $\pm$ 1.4	11.8 $\pm$ 1.3	0.01
Total RBC count ($\times 10^6/\mu\text{L}$)	4.51 $\pm$ 0.48	4.18 $\pm$ 0.46	0.02
Mean corpuscular volume (fL)	89.8 $\pm$ 6.2	94.6 $\pm$ 7.4	0.004
Mean corpuscular haemoglobin (pg)	28.7 $\pm$ 2.4	30.9 $\pm$ 2.8	0.01
Mean corpuscular haemoglobin concentration (g/dL)	32.4 $\pm$ 1.3	31.6 $\pm$ 1.2	0.03
Red cell distribution width (%)	14.1 $\pm$ 1.1	15.6 $\pm$ 1.4	0.002
Platelet count ($\times 10^3/\mu\text{L}$)	246 $\pm$ 62	232 $\pm$ 58	0.28
Total leukocyte count (cells/mm ³)	7,380 $\pm$ 1,740	7,210 $\pm$ 1,690	0.61
Peripheral smear feature	Number of participants (%)		
Oval macrocytes	9 (42.9%)		
Anisocytosis	11 (52.4%)		
Hypersegmented neutrophils	6 (28.6%)		

Table 5 shows the haematological profile among those who developed vitamin B12 deficiencies during the follow-up.

Discussion

In this prospective cohort study of newly diagnosed type 2 diabetes mellitus patients initiated on metformin with normal baseline vitamin B12 levels, a measurable incidence of vitamin B12 deficiency was observed over one year of follow-up. Nearly one fifth of the study population developed biochemical deficiency during this period, highlighting that depletion of vitamin B12 can occur relatively early after initiation of metformin therapy. The findings support a temporal association between metformin exposure and declining vitamin B12 status, even in patients without deficiency at baseline.

Published estimates show that vitamin B12 deficiency among metformin users is common, but the reported burden varies widely because studies use different cut-offs, different durations of metformin exposure, and different populations. In Indian hospital-based studies, the prevalence is often in the mid range. For example, a tertiary-care study from Andhra Pradesh reported vitamin B12 deficiency

in **26.36%** of metformin-treated type 2 diabetes patients, with an additional subgroup described as moderately deficient, and a significant association with daily metformin dose(4). In another Indian tertiary hospital study from Salem, Tamil Nadu, the prevalence of vitamin B12 insufficiency was **27%**, with **~18%** having borderline levels, and the authors linked lower B12 status with diabetes duration, metformin duration, and metformin dose(4).

International evidence also supports a clear association between metformin exposure and lower vitamin B12 status. In the Diabetes Prevention Program Outcomes Study, biochemical low B12 (≤ 203 pg/mL) was more frequent with metformin than placebo at 5 years (**4.3% vs 2.3%**), and the combined endpoint of low or borderline-low B12 (≤ 298 pg/mL) remained more common with metformin at both 5 and 13 years. Each additional year of metformin use increased the odds of deficiency(5). Randomised trial data also point in the same direction. The BMJ placebo-controlled trial by de Jager and colleagues showed that long-term metformin treatment increased the risk of vitamin B12 deficiency, supporting a causal link rather than a simple association(6). Systematic reviews and meta-analyses similarly conclude that metformin use is associated with lower vitamin B12 levels and that a subset of patients develop frank or borderline deficiency, which is why many authors recommend periodic monitoring in long-term users(3).

Several determinants of vitamin B12 deficiency were identified in the present study, indicating that the risk associated with metformin therapy is influenced by both patient-related and treatment-related factors. Increasing age emerged as an independent predictor of deficiency. Advancing age is known to be associated with reduced gastric acid secretion and impaired vitamin B12 absorption, which may potentiate the inhibitory effect of metformin on calcium-dependent ileal uptake of the vitamin(3). Similar age-related associations have been reported in both Indian and international studies, suggesting that older adults on metformin constitute a particularly vulnerable group(7).

Dietary pattern was another important determinant. Participants adhering to a vegetarian diet had a significantly higher risk of developing vitamin B12 deficiency(8). This finding is highly relevant in the Indian context, where vegetarianism is common and dietary intake of vitamin B12 is often marginal even before initiation of metformin(9). Previous Indian studies have consistently shown lower baseline vitamin B12 levels among vegetarians, and metformin exposure may further accelerate depletion of already limited body stores(4,10). International studies from populations with mixed diets have reported lower effect sizes, supporting the role of background dietary intake in modifying risk(3,11).

Metformin dose was a strong treatment-related determinant in the present study. Patients receiving higher daily doses of metformin were more likely to develop vitamin B12 deficiency, independent of other factors(12,13). This dose-response relationship has been well documented in earlier observational studies and randomised trials. Proposed mechanisms include greater inhibition of vitamin B12-intrinsic factor complex absorption and altered intestinal motility with higher cumulative exposure. The finding reinforces the biological plausibility of metformin-induced vitamin B12 deficiency(12,13).

Lower baseline haemoglobin was also independently associated with incident vitamin B12 deficiency. Although all participants had normal vitamin B12 levels at baseline, lower haemoglobin values may reflect subclinical nutritional vulnerability or limited physiological reserves(14). This observation suggests that haematological parameters at baseline may help identify individuals at higher risk of subsequent deficiency during metformin therapy.

Concomitant use of proton pump inhibitors was another significant determinant(15). Acid-suppressing drugs reduce gastric acid secretion, which is essential for releasing vitamin B12 from dietary proteins(15). When combined with metformin, this effect may further impair absorption, leading to a synergistic increase in deficiency risk. Similar associations have been reported in international cohort studies, underscoring the importance of reviewing concomitant medications in metformin-treated patients.

Conclusion

This prospective cohort study demonstrates that vitamin B12 deficiency can develop within the first year of metformin therapy among newly diagnosed type 2 diabetes mellitus patients who have normal vitamin B12 levels at baseline. Nearly one fifth of the participants developed biochemical deficiency during follow-up, indicating that depletion may occur earlier than is often assumed in routine clinical practice. The study further shows that the risk of deficiency is influenced by specific patient- and treatment-related factors, including increasing age, vegetarian diet, higher metformin dose, lower baseline haemoglobin levels, and concomitant use of proton pump inhibitors. These findings provide prospective evidence from an Indian clinical setting and emphasise the need for early and periodic monitoring of vitamin B12 levels in selected high-risk patients initiated on metformin. Incorporating targeted screening strategies may help prevent unrecognised deficiency and its potential haematological and neurological consequences in this population.

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Conflict of Interest: None

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