RESEARCH PAPER

Effect of Cobalamin Levels in Patients with T2DM Undergoing Metformin Therapy

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ABSTRACT

Low vitamin B12 concentrations are being documented more often in people living with T2DM. Metformin, still the first choice for glycaemic control, appears to be a major contributor to this trend. Although the link between metformin use and reduced Cobalamin levels is well supported, the extent to which dose, duration of therapy and individual patient factors shape this relationship varies across studies. Interpretation is made more difficult by the absence of a single, universally accepted cutoff for defining deficiency, which leads to wide differences in reported prevalence.

Long-standing Cobalamin deficiency can result in neurological and haematological problems, making periodic evaluation of vitamin B12 status important for those at higher risk. Individuals following vegan diets while using metformin are particularly susceptible because their dietary intake of Cobalamin is limited. Lifestyle approaches that help maintain metabolic balance may also reduce the chance of developing nutritional deficiencies during extended treatment.

Keywords: Metformin, blood glucose monitoring, Vitamin B12, Neurological symptoms.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a long-standing metabolic disorder marked by insulin resistance and disrupted glucose regulation. Its growing prevalence has made sustained glycaemic control central to preventing complications such as cardiovascular disease, renal failure and neuropathy. Treatment usually begins with lifestyle adjustment, but most patients require pharmacotherapy over time. Several drug classes are available, each acting on a different point in glucose homeostasis. Selection often depends on comorbid conditions, tolerability and expected glycaemic response.

Metformin is generally used at the outset because it lowers hepatic glucose output and improves peripheral insulin sensitivity without causing weight gain or hypoglycaemia. When glucose levels remain elevated despite metformin, clinicians turn to agents with complementary actions, such as sulfonylureas that stimulate endogenous insulin release, thiazolidinediones that enhance insulin sensitivity, DPP-4 inhibitors that increase incretin activity, SGLT-2 inhibitors that promote renal glucose loss or GLP-1 receptor agonists that combine glucose lowering with appetite suppression. Some individuals eventually need insulin therapy once beta-cell function declines. Insulin dosing varies with daily routines and metabolic demands, making close monitoring essential to prevent hypoglycaemia.

Vitamin B12 is required for red blood cell production, DNA synthesis and neurological integrity. Deficiency may present with anaemia, paraesthesia or cognitive

changes, which is particularly relevant in people with diabetes who already face neuropathic risk. The definition of vitamin B12 deficiency differs between assays, although serum cobalamin levels below 148 pmol/l (200 ng/l), especially with elevated homocysteine or methylmalonic acid, indicate impaired status. Two coenzyme forms, methyl-cobalamin and adenosyl-cobalamin, support methionine synthase and methylmalonyl-CoA mutase respectively, both of which handle essential one-carbon and mitochondrial reactions. Low intake, malabsorption and intrinsic factor deficiency remain the common causes of deficiency. Prevalence increases with age, and restrictive diets raise the risk further.

Metformin's long history in T2DM care reflects its strong glucose-lowering effect, favourable cardiovascular profile and low incidence of serious adverse events when renal function is stable. Its non-glycaemic actions include improved endothelial function, reduced oxidative stress, modulation of lipid levels and lower accumulation of advanced glycation products. It has also been investigated in various insulin-resistant states and as a possible antitumour agent. Although older reports linked biguanides to lactic acidosis, current evidence shows that the risk with metformin is minimal when contraindications are followed.16

A recurring concern with prolonged therapy is its association with reduced vitamin B12 levels.1 Evidence indicates that metformin interferes with cobalamin uptake in the terminal ileum, possibly by altering intestinal motility, modifying the gut microbiota or affecting cellular mechanisms involved in active transport.2,3 This interaction matters clinically because many individuals remain on metformin for years, making subtle declines in B12 levels easy to miss unless monitored.

METHODOLOGY

The study followed a cross-sectional design and was carried out in the Diabetic Outpatient Department of Sree Balaji Medical College Hospital. All procedures were performed within the same clinical unit to maintain consistency in recruitment and sample handling.

Participant Selection

Outpatient records were reviewed to identify individuals with Type 2 Diabetes Mellitus who had been using metformin for more than five years. Fifty patients (N=50) who met the eligibility criteria were included. Men and women aged 35 years and above were considered. Patients with incomplete documentation or conditions known to alter cobalamin metabolism were not taken forward for analysis.

Sample Collection and Processing

After confirming eligibility, blood samples were collected from each participant under aseptic conditions. Samples were transported without delay to the Central Biochemistry Laboratory for analysis. All measurements adhered to the routine laboratory quality-control procedures followed by the institution.

Biochemical Analysis

Random Blood Sugar was estimated using the GOD-POD method on the MINDRAY BS 600M automated system. Serum cobalamin was measured by Chemiluminescent Immunoassay (CLIA) using the MINDRAY CL 960i platform. Each batch of assays was run with internal controls in place to maintain analytical reliability.

Data Classification

Cobalamin values were grouped into predefined categories to allow comparison of patterns within the study population. Metformin exposure was treated as long-term therapy based on continuous documented use exceeding five years.

Statistical analysis

The relationship between cobalamin status and prolonged metformin therapy was assessed using the Chi-Square test (χ^2). The test was applied to the observed frequency distribution and odds ratios with 95 percent confidence intervals were calculated. A p-value below 0.05 was taken to indicate statistical significance.

RESULT

A total of 50 participants aged 35 years and above were included, all of whom had been using metformin for more than five years. Serum cobalamin levels were assessed to determine whether long-term therapy affected their nutritional status.

Thirty of the fifty individuals (60 percent) showed altered cobalamin values, with several presenting a clear reduction. The remaining twenty participants (40 percent) had concentrations within the expected range.

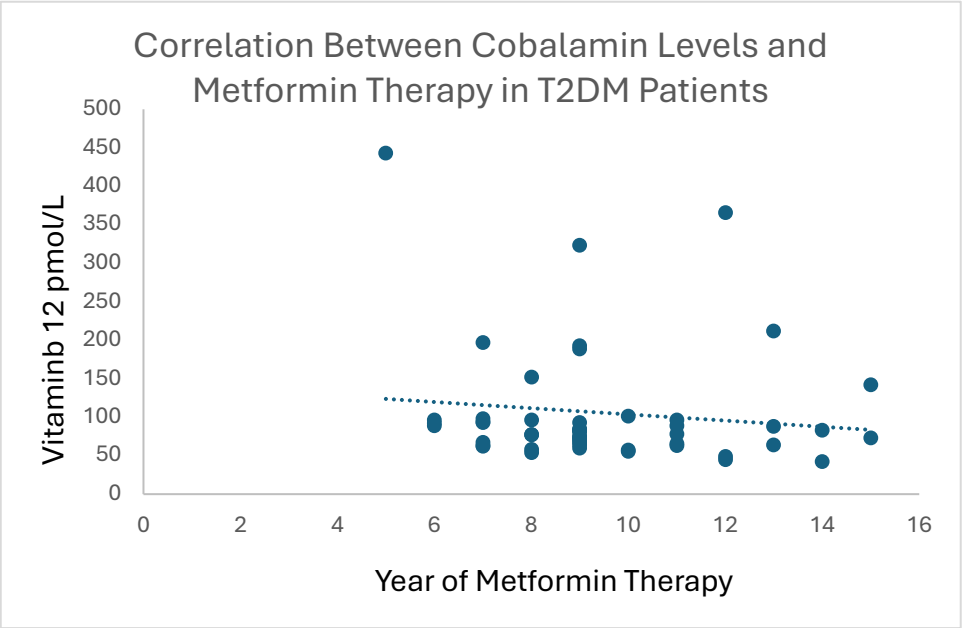

Fig: 1 Correlation Between Cobalamin Levels Metformin Therapy in T2DM Patients

Statistical Outcome

The association between cobalamin status and prolonged metformin use was examined using the Chi-Square test. The calculated value was $\chi^2 = 5.357$. The corresponding p-value was 0.02 and the odds ratio with its 95 percent confidence interval supported the same direction of effect. These findings indicate a statistically significant reduction in cobalamin levels among individuals receiving metformin for extended periods.

DISCUSSION:

The association between metformin and reduced Cobalamin absorption has been described since the late 1960s. Early work pointed to impaired uptake of the vitamin in people taking metformin for T2DM. A systematic review covering studies published up to 2013 noted that most observational data (59 percent) reported lower vitamin B12 concentrations in metformin users than in nonusers.5,6 The same review included four intervention trials and pooled analysis showed a mean drop of 57 pmol/L after treatment periods ranging from six weeks to three months (weighted mean difference –57.1; 95 percent CI –35.5 to –78.8). Because the included studies differed considerably (I^2 72 percent), the authors advised caution when interpreting the pooled estimate.5

Research published in the years following that review has continued to support the link between metformin use and declining serum Cobalamin levels. Much of this work has focused on whether dose and treatment duration contribute to the degree of deficiency.12 A large cross-sectional analysis from Korea found that every 1 mg increase in daily metformin dose corresponded to a reduction of 0.142 pg/mL in serum vitamin B12 (95 percent CI –0.169 to –0.114). When compared with individuals using less than 1000 mg per day, those in the 1500–2000 mg and at least 2000 mg groups had markedly higher odds of deficiency, with odds ratios of 3.34 (95 percent CI 1.95 to 5.75) and 8.67 (95 percent CI 4.68 to 16.06). A separate study reported a similar pattern, noting an odds ratio of 3.8 for deficiency at 2000 mg versus 1000 mg (95 percent CI 1.82 to 7.92). Other analyses, however, found no dose effect after adjusting for confounding variables, indicating that dose-response findings are not fully consistent across the literature.

The influence of treatment duration remains equally uncertain. A large retrospective cohort involving 13,489 adults estimated that vitamin B12 deficiency appeared, on average, 5.3 years after starting metformin. A post hoc evaluation of a randomized trial reported that each additional year of metformin therapy increased the odds of deficiency by 13 percent (OR 1.13; 95 percent CI 1.06 to 1.20). In contrast, a Dutch cross-sectional study of 298 participants showed no association between duration and deficiency (OR 0.98; 95 percent CI 0.87 to 1.11). Another cross-sectional analysis also found no link between metformin exposure and serum Cobalamin (β –0.14; 95 percent CI –0.44 to 0.16) or holotranscobalamin (β 0.003; 95 percent CI –0.09 to 0.09).

Earlier studies emphasized dose and duration separately, leaving little clarity on whether the combined exposure

better explains the risk. A recent observational study addressed this using a Metformin Usage Index (MUI), which multiplies daily dose (in milligrams) by duration of therapy (in years) and divides by 1,000.10 After adjustment for several clinical variables, the MUI remained the strongest predictor of Cobalamin deficiency. Higher values corresponded to substantially greater risk. Individuals with an MUI above 15 had the greatest odds of deficiency (OR 6.7; 95 percent CI 4.4 to 10), followed by those with an MUI above 10 (OR 5.1; 95 percent CI 3.1 to 8.5). The odds were much lower in those with an MUI under 5 (OR 1.37; 95 percent CI 0.9 to 2.2).10,11 These results indicate that cumulative exposure may capture deficiency risk more reliably than dose or duration on their own and may help identify individuals who would benefit from early testing or supplementation.

Several explanations have been proposed to describe how metformin lowers circulating vitamin B12, although none fully account for the entire effect. The mechanism most frequently cited involves interference with calcium-dependent absorption of the intrinsic factor (IF)–Cobalamin complex in the ileum. Uptake through the cubilin receptor requires calcium and metformin appears to impair this process, reducing the amount of vitamin B12 that enters circulation.

Altered intestinal motility offers another possible pathway. Metformin increases transit time in the small intestine,15 which can shift the composition and distribution of the gut microbiota. These changes may predispose individuals to small intestinal bacterial overgrowth (SIBO). Bacterial populations in the proximal and mid-intestine consume vitamin B12 and generate metabolites that disrupt the interaction between the IF-Cobalamin complex and its receptors on the ileal mucosa.A This competition limits the availability of absorbable Cobalamin.

Reduced intrinsic factor secretion has also been mentioned as a contributing factor. If gastric production of IF falls, reduced levels of vitamin B12 reaches the ileum in a form suitable for receptor-mediated uptake. Some authors have proposed that metformin may alter intracellular handling of Cobalamin, shifting more of the vitamin toward hepatic storage and lowering the amount detectable in serum.

Although many studies show that metformin use is associated with lower serum Cobalamin levels in people with T2DM, the patterns relating to dose and duration are not entirely consistent.10 These differences stem from several methodological problems. Cutoff values for deficiency vary widely because there is no universally accepted diagnostic threshold. Immunoassays differ in calibration, making comparisons across studies difficult. Some investigations lacked a non-metformin comparison group, while others had insufficient sample sizes to demonstrate clear associations. Variations in demographic characteristics, clinical profiles and study designs add further heterogeneity.

Despite these limitations, the overall body of evidence supports an inverse relationship between metformin exposure and serum vitamin B12 concentrations. Deficiency is clinically relevant because low Cobalamin can lead to anaemia, elevated methylmalonic acid (MMA), hyperhomocysteinemia and neurological symptoms. These risks support the need for periodic assessment of Cobalamin status, especially in groups already prone to deficiency, including older adults and individuals following vegan diets.12

Age adds another layer to the problem. T2DM commonly affects older adults and aging itself reduces vitamin B12 absorption due to gastric atrophy, decreased acid production and a greater likelihood of bacterial overgrowth. These factors may compound the effects of long-term metformin therapy. More research is needed to define the dietary intake required to offset these combined influences at different metformin dosages.

Recent work points to the MUI as a practical way to capture cumulative exposure and identify individuals at higher risk.18 In the absence of a single gold-standard biomarker for assessing Cobalamin status, incorporating the MUI into routine screening can help clinicians identify patients who would benefit from early testing or supplementation. Our findings reinforce the value of periodic Cobalamin measurement in people with diabetes, allowing timely detection of deficiency and prevention of neurological complications.

CONCLUSION

Individuals with type 2 diabetes mellitus who were receiving metformin in this study showed lower serum Cobalamin levels. Since diabetes already places patients at risk for a range of metabolic, macrovascular and microvascular problems, a further drop in vitamin B12 can add to their vulnerability. Metformin remains a widely used oral hypoglycaemic drug and long-term therapy may gradually reduce Cobalamin stores. Supplementing vitamin B12 in patients on prolonged metformin treatment could help prevent or delay neurological problems linked to deficiency.

Regular evaluation of vitamin B12 status in people with T2DM may therefore be useful, along with their routine laboratory investigations. Low Cobalamin levels, when left unrecognized, can contribute to neuropathic symptoms such as numbness, tingling, burning

sensations or weakness. In someone who already has diabetic neuropathy, an additional B12 deficiency may worsen the condition and increase the risk of lasting nerve injury.

Early identification of declining vitamin B12 levels allows timely supplementation and may help protect neurological function. Incorporating periodic B12 testing into the care of individuals taking metformin can support safer long-term diabetes management and reduce the likelihood of avoidable nerve-related complications.

REFERENCES

1. De Jager J, Kooy A, Lehert P, et al. Long term treatment with metformin in patients with T2DMand risk of vitamin B-12 deficiency: randomized placebo controlled trial. BMJ. 2010;340:c2181.
2. Andre`s E, Noel E, Goichot B. Metformin-associated vitamin B12 deficiency. Arch Intern Med. 2002;162:2251–2252.
3. Gilligan MA. Metformin and vitamin B12 deficiency. Arch Intern Med. 2002;162:484–485.
4. Kanaley JA, Colberg SR, Corcoran MH, et al. Exercise/physical activity in individuals with type 2 diabetes: a consensus statement from the American College of Sports Medicine. Med Sci Sports Exerc. 2022;54:353–368.
5. Sundus Fituri, Zoha Akbar and Vijay Ganji. Impact of metformin treatment on cobalamin status in persons with type 2 diabetes. Nutrition Reviews. 2023;82(4):553–560.
6. Rodrıguez-Gutierrez R, Montes-Villarreal J, Rodrıguez-Velver KV, et al. Metformin use and vitamin B12 deficiency: untangling the association. Am J Med Sci. 2017;354:165–171.
7. Andre`s E, Goichot B, Schlienger JL. Food Cobalamin malabsorption: ausual cause of vitamin B12 deficiency. Arch Intern Med. 2000;160:2061–2062.
8. Bauman WA, Shaw S, Jayatilleke E, et al. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23:1227–1231.
9. Raizada N, Jyotsna VP, Sreenivas V, et al. Serum vitamin B12 levels in T2DMpatients on metformin compared to those never on metformin: a crosssectional study. Indian J Endocrinol Metab. 2017;21:424–428.
10. Horakova O, Kroupova P, Bardova K, et al. Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. Sci Rep. 2019;9:6156.
11. 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 Nov;98(46):e17918.
12. Sato Y, Ouchi K, Funase Y, Yamauchi K, Aizawa T. Relationship between metformin use, vitamin B12 deficiency, hyperhomocysteinemia and vascular complications in patients with type 2 diabetes. Endocr J. 2013;60(12):1275-80.
13. Baig FA, Khan S, Rizwan A. Frequency of Vitamin B12 Deficiency in Type 2 Diabetic Patients Taking Metformin. Cureus. 2022 Mar7;14(3):e22924.
14. Sayedali E, Yalin AE, Yalin S. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes. World J Diabetes. 2023 May 15;14(5):585-593.
15. Asghar S, Tanvir H, Riaz A, Ejaz MH, Akram M, Chowdhury Evan AM, Shahid S. Prevalence of Vitamin B12 Deficiency in Patients With T2DM on Metformin Therapy: A Cross-Sectional Study. Cureus. 2024 Oct 23;16(10):e72184.
16. Hostalek U, Gwilt M, Hildemann S. Therapeutic Use of Metformin in Prediabetes and Diabetes Prevention. Drugs. 2015 Jul;75(10):1071-94.
17. Sanchez-Rangel E, Inzucchi SE. Metformin: clinical use in type 2 diabetes. Diabetologia. 2017 Sep;60(9):1586-1593.
18. Kao KT, Sabin MA. T2DM in children and adolescents. Aust Fam Physician. 2016 Jun;45(6):401-6.
19. Shipton MJ, Thachil J. Vitamin B12 deficiency - A 21st century perspective . Clin Med (Lond). 2015 Apr;15(2):145-50.