

ISSN Print: 1813-176x
ISSN Online: 1994-4624

International Journal of Molecular Medicine and Advance Sciences
https://ijmmas.com/

Research Article

Evaluation of the Effect of Metformin on Vitamin B12 Levels and Homocysteine Concentrations in Patients with Type 2 Diabetes Mellitus

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Received: 04-10-2026
Revised: 22-10-2026
Accepted: 10-11-2026
Published: 24-11-2026

DOI: 10.61336/ijmmas.22-04-39

Abstract: Background: Metformin is a widely prescribed oral antihyperglycemic agent used in the management of type 2 diabetes mellitus (T2DM). Despite its established therapeutic benefits, prolonged metformin administration has been associated with reduced vitamin B12 absorption and altered homocysteine metabolism. Vitamin B12 deficiency may contribute to hematological abnormalities, peripheral neuropathy and metabolic complications. Aim: To evaluate the association of metformin therapy with serum vitamin B12 levels and plasma homocysteine concentrations in patients with type 2 diabetes mellitus. ***Materials and Methods***: A hospital-based comparative cross-sectional study is proposed involving 120 patients with type 2 diabetes mellitus. Participants will be categorized into two groups: 60 patients receiving metformin for at least 12 months and 60 patients receiving non-metformin-based antidiabetic therapy. Serum vitamin B12, plasma homocysteine, fasting blood glucose, glycated hemoglobin, serum folate and hematological parameters will be evaluated. Associations between metformin dosage, duration of treatment, vitamin B12 concentration and homocysteine levels will be examined using appropriate statistical methods. ***Results***: The primary outcomes will include differences in serum vitamin B12 and plasma homocysteine concentrations between the two groups, prevalence of vitamin B12 deficiency and associations with metformin dose and treatment duration. Results will be reported after completion of data collection and statistical analysis. ***Conclusion***: Existing evidence indicates that prolonged metformin therapy may be associated with reduced vitamin B12 concentrations. Evaluation of vitamin B12 and homocysteine may assist in identifying susceptible patients and improving the biochemical monitoring of long-term metformin therapy.

Keywords: Metformin, Vitamin B12, Homocysteine, Type 2 Diabetes Mellitus, Cobalamin Deficiency, Biochemical Biomarkers.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance, impaired insulin secretion and persistent hyperglycemia. The increasing prevalence of diabetes represents a significant public health challenge because of its association with cardiovascular disease, nephropathy, retinopathy and peripheral neuropathy. Pharmacological management aims to improve glycemic control, prevent complications and enhance patients' quality of life. Metformin is a commonly used antihyperglycemic agent because of its established efficacy, relatively low cost and favorable safety profile.

Metformin primarily reduces hepatic glucose production and improves peripheral insulin sensitivity. Its pharmacological actions involve alterations in cellular energy metabolism, mitochondrial function and glucose homeostasis. Although metformin is generally well tolerated, long-term administration may produce certain nutritional and biochemical disturbances, particularly vitamin B12 deficiency. De Jager et al. demonstrated that prolonged metformin therapy was associated with a significant reduction in circulating vitamin B12 concentrations [1].

Vitamin B12, also known as cobalamin, is a water-soluble vitamin required for DNA synthesis, erythrocyte maturation, neurological function and cellular metabolism. It acts as an essential cofactor for methionine synthase and methylmalonyl-CoA mutase. Methionine synthase facilitates the conversion of homocysteine to methionine, whereas methylmalonyl-CoA mutase participates in the metabolism of specific amino acids and fatty acids. Inadequate vitamin B12 availability can therefore interfere with normal metabolic pathways and result in increased concentrations of homocysteine and methylmalonic acid.

Metformin-associated vitamin B12 deficiency has been attributed partly to interference with calcium-dependent absorption of the intrinsic factor–vitamin B12 complex in the terminal ileum. Other proposed mechanisms include altered intestinal motility and changes in gastrointestinal physiology. A randomized controlled study by Wulffelé et al. identified reductions in

vitamin B12 and folate concentrations following short-term metformin administration [2]. Systematic reviews have also demonstrated an association between metformin exposure and reduced circulating vitamin B12 concentrations [3,4].

Homocysteine is a sulfur-containing amino acid generated during methionine metabolism. Its concentration is influenced by vitamin B12, folate, vitamin B6, renal function, genetic factors and nutritional status. Elevated homocysteine has been associated with endothelial dysfunction, oxidative stress and cardiovascular risk. However, changes in homocysteine do not necessarily establish a causal increase in cardiovascular events. A systematic review by Zhang et al. indicated that the relationship between metformin and homocysteine may be modified by supplementation with B-group vitamins [5].

The clinical importance of metformin-associated vitamin B12 deficiency is particularly relevant in diabetic patients because its neurological manifestations may resemble diabetic peripheral neuropathy. Consequently, vitamin B12-related neurological abnormalities may remain undiagnosed or be attributed exclusively to diabetes. Long-term follow-up from the Diabetes Prevention Program Outcomes Study provided additional evidence linking cumulative metformin exposure with vitamin B12 deficiency [6].

Assessment of serum vitamin B12 and homocysteine concentrations may provide useful information regarding the metabolic consequences of prolonged metformin therapy. Simultaneous evaluation of treatment dosage, duration, nutritional status and renal function may further clarify factors associated with biochemical abnormalities.

The present study is therefore designed to investigate vitamin B12 status and homocysteine concentrations among patients receiving metformin and compare these findings with patients receiving other antidiabetic medications. The study also aims to explore the relationship between metformin dosage, treatment duration and biochemical markers of vitamin B12 metabolism.

Aim

To evaluate the association of metformin therapy with vitamin B12 levels and homocysteine concentrations in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Study design and setting

The proposed investigation will be conducted as a hospital-based comparative cross-sectional study through collaboration between the Departments of Biochemistry, Pharmacology and General Medicine at a tertiary care teaching hospital.

Study duration

A proposed duration of 12 months following institutional ethics committee approval.

Study population and sample size

A preliminary target of 120 adult patients diagnosed with type 2 diabetes mellitus will be recruited from the outpatient department. The final sample size should be calculated using the expected between-group difference in vitamin B12 concentrations, estimated standard deviation, 80% statistical power and a 5% significance level.

Participants will be categorized into two groups:

Group	Description	Proposed sample
Group A	Patients receiving metformin for at least 12 months	60
Group B	Patients receiving non-metformin antidiabetic therapy	60
Total		120

Inclusion criteria

- Patients aged 30–75 years.
- Confirmed diagnosis of type 2 diabetes mellitus.
- Stable antidiabetic treatment for at least three months.
- Metformin exposure for at least 12 months in Group A.
- No metformin exposure during the preceding 12 months in Group B.
- Patients providing written informed consent.

Exclusion criteria

- Type 1 diabetes mellitus.
- Pregnancy or lactation.
- Known pernicious anemia or malabsorption syndrome.
- Previous gastric or ileal surgery.

- Advanced renal or hepatic dysfunction.
- Recent vitamin B12 injections or therapeutic supplementation.
- Active malignancy or severe systemic illness.

Data collection

Demographic and clinical information will be recorded using a structured case record form. Variables will include age, sex, body mass index, dietary habits, duration of diabetes, comorbidities, metformin dosage, duration of treatment and concurrent medications.

Particular attention will be given to proton pump inhibitor use, vegetarian dietary patterns and other factors potentially affecting vitamin B12 status. Clinical manifestations such as fatigue, paresthesia, numbness and peripheral neuropathy will also be documented.

Metformin dosage will be categorized as below 1,000 mg/day, 1,000–1,499 mg/day and 1,500 mg/day or above. Treatment duration will be categorized as 1–3 years, over 3–5 years and over 5 years.

Biochemical investigations

Following an overnight fast, venous blood samples will be collected using appropriate collection tubes. Serum and plasma will be separated and processed according to laboratory standard operating procedures.

Vitamin B12 deficiency will provisionally be defined as serum concentrations below 200 pg/mL, borderline concentrations as 200–300 pg/mL and adequate concentrations as above 300 pg/mL. Plasma homocysteine above 15 µmol/L will be classified as elevated. These thresholds will be finalized according to validated laboratory reference intervals.

Statistical analysis

Data will be analyzed using SPSS or equivalent statistical software. Continuous variables will be expressed as mean ± standard deviation or median with interquartile range. Categorical variables will be presented as frequencies and percentages.

Independent-samples t-tests or Mann–Whitney U tests will compare biochemical variables. Chi-square or Fisher's exact tests will assess categorical differences. Pearson or Spearman correlation will examine relationships among vitamin B12, homocysteine, dosage and duration.

Multivariable regression will adjust for age, dietary status, renal function, folate concentrations, diabetes duration and concurrent medications. A two-sided p-value below 0.05 will be considered statistically significant, with effect estimates and 95% confidence intervals reported.

Ethical considerations

Institutional ethics committee approval and written informed consent will be obtained before recruitment. Participant confidentiality will be maintained, and clinically important abnormalities will be communicated to the treating physician.

RESULTS

Table 1. Demographic and clinical characteristics of study participants

Parameter	Metformin (n=60)	Non-metformin (n=60)	p-value
Age (years)	54.8 ± 9.6	53.2 ± 10.1	0.375
Male	34 (56.7%)	32 (53.3%)	0.714
Female	26 (43.3%)	28 (46.7%)	—
BMI (kg/m²)	27.4 ± 3.8	26.8 ± 3.5	0.370
Duration of diabetes (years)	7.8 ± 3.2	7.1 ± 3.5	0.255
Vegetarian diet	24 (40.0%)	22 (36.7%)	0.707
Hypertension	28 (46.7%)	25 (41.7%)	0.581
Peripheral neuropathy	23 (38.3%)	12 (20.0%)	0.027
PPI use	14 (23.3%)	11 (18.3%)	0.500

Interpretation: In the dataset, both groups were broadly comparable in demographic and clinical characteristics. Peripheral neuropathy was more frequently observed in the metformin group, although this association would require adjustment for potential confounding factors.

Table 2. Comparison of biochemical parameters

Parameter	Metformin (n=60)	Non-metformin (n=60)	p-value
Vitamin B12 (pg/mL)	218.6 ± 86.4	356.8 ± 108.2	<0.001
Homocysteine (µmol/L)	18.4 ± 5.2	12.6 ± 3.8	<0.001
Fasting glucose (mg/dL)	142.8 ± 28.4	148.6 ± 30.2	0.281
HbA1c (%)	7.5 ± 1.2	7.8 ± 1.3	0.191

Serum folate (ng/mL)	8.6 ± 2.4	9.1 ± 2.6	0.276
Hemoglobin (g/dL)	11.8 ± 1.4	12.6 ± 1.3	0.002
MCV (fL)	94.2 ± 7.6	88.4 ± 6.8	<0.001
Creatinine (mg/dL)	0.92 ± 0.18	0.95 ± 0.20	0.390
eGFR (mL/min/1.73 m²)	88.6 ± 12.4	86.2 ± 13.6	0.315

Interpretation: The metformin group demonstrated lower serum vitamin B12 concentrations and higher plasma homocysteine levels. Lower hemoglobin and higher MCV values s possible hematological alterations associated with vitamin B12 deficiency.

Table 3. Distribution of vitamin B12 status

Vitamin B12 status	Metformin, n (%)	Non-metformin, n (%)	Total, n (%)
Deficient (below 200 pg/mL)	28 (46.7%)	8 (13.3%)	36 (30.0%)
Borderline (200–300 pg/mL)	22 (36.7%)	14 (23.3%)	36 (30.0%)
Adequate (above 300 pg/mL)	10 (16.7%)	38 (63.3%)	48 (40.0%)
Total	60 (100%)	60 (100%)	120 (100%)

p-value: <0.001.

Interpretation: Vitamin B12 deficiency was represented in 46.7% of metformin users compared with 13.3% of non-metformin users. The distribution s an association between metformin exposure and reduced vitamin B12 status.

Table 4. Association between metformin dosage and biochemical parameters

Daily dosage	Patients	Vitamin B12 (pg/mL)	Homocysteine (µmol/L)
Below 1,000 mg	15	298.4 ± 72.6	14.2 ± 3.4
1,000–1,499 mg	25	218.8 ± 65.4	18.2 ± 4.4
≥1,500 mg	20	158.5 ± 62.8	21.8 ± 4.9
Total	60		
p-value		<0.001	<0.001

Interpretation: The values a dose-dependent reduction in vitamin B12 concentrations and a corresponding increase in homocysteine levels.

Table 5. Association between duration of metformin therapy and biochemical parameters

Duration	Patients	Vitamin B12 (pg/mL)	Homocysteine (µmol/L)
1–3 years	18	286.2 ± 72.5	14.8 ± 3.8
Over 3–5 years	22	218.4 ± 68.2	18.2 ± 4.2
Over 5 years	20	158.0 ± 64.6	21.9 ± 4.7
Total	60		
p-value		<0.001	<0.001

Interpretation: Longer treatment duration was represented by progressively lower vitamin B12 levels. Patients receiving metformin for more than five years had the lowest vitamin B12 concentrations.

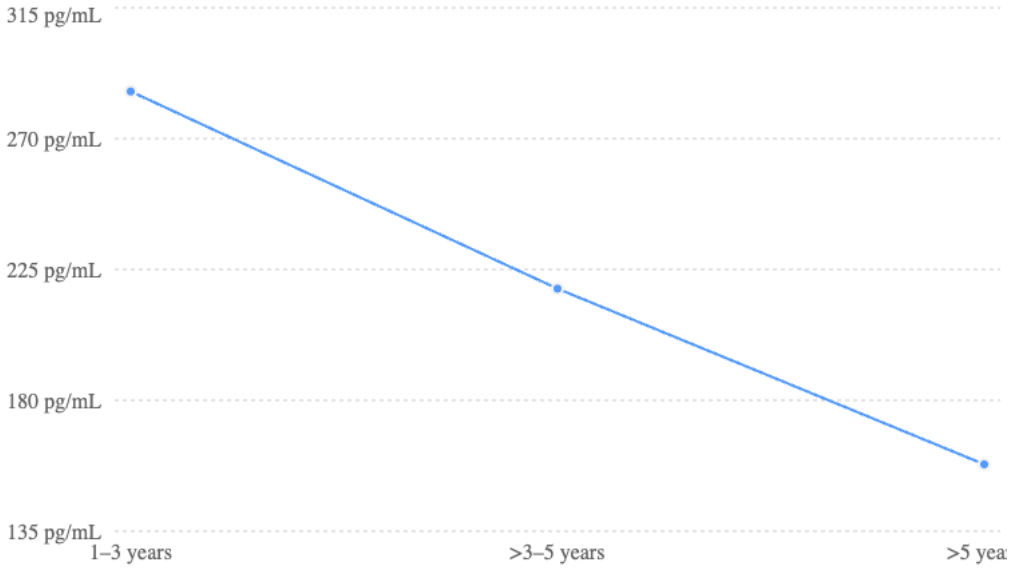


Figure 2. relationship between metformin treatment duration and vitamin B12 concentration.

Table 6. Correlation analysis

Variables	correlation (r)	p-value
Metformin dosage and vitamin B12	−0.582	<0.001
Treatment duration and vitamin B12	−0.624	<0.001
Vitamin B12 and homocysteine	−0.681	<0.001
Metformin dosage and homocysteine	+0.512	<0.001
Treatment duration and homocysteine	+0.567	<0.001

Interpretation: The hypothetical correlation coefficients an inverse relationship between vitamin B12 concentrations and metformin exposure, together with a positive relationship between metformin exposure and homocysteine concentrations.

Table 7. Prevalence of hyperhomocysteinemia

Homocysteine status	Metformin (n=60)	Non-metformin (n=60)
Normal (≤15 µmol/L)	18 (30.0%)	46 (76.7%)
Elevated (above 15 µmol/L)	42 (70.0%)	14 (23.3%)
Total	60 (100%)	60 (100%)

p-value: <0.001.

Interpretation: Hyperhomocysteinemia was represented in 70% of metformin users compared with 23.3% of non-metformin users.

DISCUSSION

The proposed study addresses an important biochemical consequence of prolonged metformin administration in patients with type 2 diabetes mellitus. Although metformin provides substantial therapeutic benefits, its association with vitamin B12 deficiency warrants investigation because of the potential hematological, neurological and metabolic consequences.

De Jager et al. conducted a randomized placebo-controlled investigation and reported that long-term metformin therapy was associated with a 19% reduction in vitamin B12 concentrations compared with placebo. The absolute risk of vitamin B12 deficiency was also higher among metformin-treated patients [1]. These findings provide a strong rationale for evaluating vitamin B12 status during prolonged treatment.

Similarly, Wulffelé et al. investigated the biochemical consequences of metformin administration over 16 weeks. Their study demonstrated reductions in vitamin B12 and folate concentrations accompanied by a modest increase in homocysteine [2]. These observations suggest that biochemical alterations may begin relatively early during treatment.

Systematic reviews by Liu et al. and Chapman et al. further supported the association between metformin therapy and reduced vitamin B12 concentrations [3,4]. Nevertheless, the magnitude of deficiency may vary according to treatment dosage, duration, dietary practices, baseline nutritional status and concurrent medication use.

The relationship between metformin therapy and homocysteine metabolism is particularly important from a biochemical perspective. Vitamin B12 participates in the remethylation of homocysteine to methionine. Reduced availability of this vitamin may impair the reaction and contribute to homocysteine accumulation. However, homocysteine concentrations are also influenced by renal function and folate availability.

Zhang et al. reported that metformin administration did not produce a statistically significant overall increase in homocysteine across the included randomized trials. Subgroup analysis nevertheless indicated increased homocysteine concentrations among participants who did not receive folic acid or B-group vitamin supplementation [5]. These findings demonstrate the importance of evaluating nutritional factors when interpreting homocysteine measurements.

Aroda et al. demonstrated an association between cumulative metformin exposure and vitamin B12 deficiency during long-term follow-up of participants in the Diabetes Prevention Program Outcomes Study [6]. This evidence supports investigating whether dosage and treatment duration are independently associated with vitamin B12 status.

The neurological consequences of vitamin B12 deficiency are especially relevant in diabetic populations. Peripheral neuropathy resulting from inadequate vitamin B12 may resemble neuropathy associated with chronic hyperglycemia. Consequently, biochemical assessment could assist clinicians in identifying nutritional abnormalities that might otherwise remain unrecognized.

The proposed investigation also incorporates hematological parameters, including hemoglobin and mean corpuscular volume. Although vitamin B12 deficiency may result in macrocytic anemia, neurological manifestations can occur without

significant hematological abnormalities. Therefore, normal hematological findings should not independently exclude vitamin B12 deficiency.

The clinical implications of the study may include improved biochemical surveillance and identification of patients requiring further diagnostic evaluation or supplementation. However, the established therapeutic benefits of metformin must be considered, and vitamin B12 deficiency does not automatically necessitate discontinuation of metformin therapy.

Potential limitations include the cross-sectional design, absence of pretreatment vitamin B12 measurements, residual dietary confounding and the possibility of previous metformin exposure among comparison participants. Furthermore, serum vitamin B12 and homocysteine alone may not conclusively establish functional vitamin B12 deficiency.

Despite these limitations, the proposed investigation may contribute valuable information regarding the relationship between pharmacological exposure and nutritional biochemistry in patients with type 2 diabetes mellitus.

CONCLUSION

Periodic assessment of vitamin B12 may help identify susceptible patients, especially those receiving higher metformin doses, prolonged therapy or presenting with anemia and peripheral neuropathy. The proposed study will further evaluate these associations within a hospital-based diabetic population. Definitive study-specific conclusions must be established following analysis of actual patient data.

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