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Original Article

Effect of Metformin therapy on serum Vitamin B12 levels in patients with Type2 Diabetes Mellitus

Dr. Anas Khwaja[1]*, Prof. (Dr) Sanjay Khanna[2], Prof. (Dr) Meherunisa[3]

[1]Junior Resident, Department of Pharmacology, Career Institute of Medical Sciences & Hospital (CIMSH), Lucknow, Uttar Pradesh, India
[2]Professor, Department of Pharmacology, Career Institute of Medical Sciences & Hospital (CIMSH), Lucknow, Uttar Pradesh, India
[3]Professor & Head, Department of Pharmacology, Career Institute of Medical Sciences & Hospital (CIMSH), Lucknow, Uttar Pradesh, India

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Corresponding Author:

Dr. Anas Khwaja
Junior Resident, Department of Pharmacology Career Institute of Medical Sciences & Hospital (CIMSH) Lucknow, Uttar Pradesh, India
Email: khwajaanas@gmail.com

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ABSTRACT

Background: Metformin is the first-line pharmacological agent for the management of Type 2 diabetes mellitus (T2DM) due to its proven efficacy, safety, and cost-effectiveness. However, long-term metformin therapy has been increasingly associated with vitamin B_{12} deficiency, which may lead to hematological and neurological complications that often overlap with diabetic sequelae and remain underdiagnosed.

Objective: To evaluate the effect of metformin therapy on serum vitamin B_{12} levels and to determine the prevalence of vitamin B_{12} deficiency in patients with Type 2 diabetes mellitus.

Materials and Methods: This cross-sectional observational study was conducted in the Departments of Pharmacology and General Medicine at Career Institute of Medical Sciences & Hospital, Lucknow. A total of 221 patients aged 35–45 years with T2DM receiving metformin monotherapy for at least six months were enrolled after obtaining informed consent. Baseline serum vitamin B_{12} levels were measured along with complete blood count and glycemic parameters. Follow-up assessments of serum vitamin B_{12} were performed at 6 months and 12 months. Data were analyzed using SPSS version 25, and a p-value <0.02 was considered statistically significant.

Results: At baseline, 87.3% of participants had normal vitamin B_{12} levels, 11.8% had borderline levels, and 0.9% were deficient. After 6 months of metformin therapy, vitamin B_{12} deficiency increased to 2.7%, and after 12 months, deficiency prevalence rose significantly to 9.0% ($\chi^2 = 22.4$, $p < 0.001$). Mean serum vitamin B_{12} levels declined progressively from 423.95 ± 102.71 pg/mL at baseline to 393.22 ± 103.24 pg/mL at 6 months and 356.07 ± 104.56 pg/mL at 12 months ($p < 0.001$). No significant correlation was observed between vitamin B_{12} decline and metformin dose, duration of diabetes, dietary pattern, or lifestyle factors. Hematological indices and glycemic parameters remained within normal limits throughout the study period.

Conclusion: Metformin therapy is associated with a significant, time-dependent reduction in serum vitamin B_{12} levels in patients with Type 2 diabetes mellitus, even in the absence of overt hematological abnormalities. Routine monitoring of vitamin B_{12} levels should be incorporated into long-term diabetes management to enable early detection and prevention of potential neurological and hematological complications.

***Keywords**: Type 2 diabetes mellitus; Metformin; Vitamin B_{12} deficiency; Cobalamin; Long-term therapy.*

INTRODUCTION

Diabetes mellitus (DM) is a chronic, multifactorial metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It represents a major global public health challenge due to its rising prevalence, long-term complications, and substantial socioeconomic burden. According to the International Diabetes Federation (IDF), approximately 537 million adults worldwide were living with diabetes in 2021, with projections estimating a rise to 783 million by 2045, largely driven by urbanization, sedentary lifestyles, dietary transitions, and population aging [1]. Type 2 diabetes mellitus (T2DM) accounts for nearly 90–95% of all diabetes cases and is particularly prevalent in low- and middle-income countries, including India, where early onset and rapid disease progression are increasingly observed [2].

Metformin, a biguanide antihyperglycemic agent, is widely recommended as the first-line pharmacological therapy for T2DM due to its proven efficacy, safety profile, affordability, and cardiovascular benefits. It primarily reduces hepatic gluconeogenesis and improves peripheral insulin sensitivity through activation of adenosine monophosphate–activated protein kinase (AMPK), thereby lowering fasting and postprandial glucose levels without causing hypoglycemia or weight gain [3,4]. Owing to these advantages, metformin remains the cornerstone of T2DM management worldwide and is often prescribed for long-term use.

Despite its favorable therapeutic profile, prolonged metformin therapy has been increasingly associated with vitamin B_{12} deficiency, an adverse effect that has gained growing clinical attention. Vitamin B_{12} (cobalamin) is an essential water-soluble vitamin required for DNA synthesis, erythropoiesis, and maintenance of neurological function. Deficiency may lead to megaloblastic anemia, peripheral neuropathy, cognitive impairment, and neuropsychiatric manifestations, many of which may overlap with complications of diabetes itself, resulting in underdiagnosis or delayed treatment [5].

Several observational studies, systematic reviews, and meta-analyses have demonstrated a significant association between metformin use and reduced serum vitamin B_{12} levels, with reported prevalence of deficiency ranging from 15% to over 50% among long-term users [6–8]. The risk appears to be dose- and duration-dependent, with higher daily doses and prolonged therapy conferring greater susceptibility [9]. The proposed mechanisms include impaired calcium-dependent absorption of the vitamin B_{12}–intrinsic factor complex in the terminal ileum, altered intestinal motility, and modulation of gut microbiota leading to reduced bioavailability of cobalamin [10,11].

The clinical relevance of metformin-induced vitamin B_{12} deficiency is particularly significant in the Indian population, where vegetarian dietary practices are common and baseline cobalamin intake may already be marginal. Furthermore, early-onset T2DM in Indian adults results in prolonged exposure to metformin, increasing the cumulative risk of deficiency and its neurological and hematological consequences. Despite this, routine screening for vitamin B_{12} deficiency is not consistently implemented in clinical practice, and region-specific longitudinal data remain limited [12].

Therefore, the present study was undertaken to evaluate the effect of metformin therapy on serum vitamin B_{12} levels in patients with Type 2 diabetes mellitus and to determine the prevalence of vitamin B_{12} deficiency during the course of treatment.

MATERIAL AND METHODS

Study Design and Setting

This study was a cross-sectional observational study conducted in the Department of Pharmacology in collaboration with the Department of General Medicine at the Career Institute of Medical Sciences & Hospital, Lucknow, Uttar Pradesh, India.

Study Population

Patients diagnosed with Type 2 diabetes mellitus (T2DM) and receiving metformin monotherapy were recruited from the outpatient department (OPD) after obtaining written informed consent. Eligible participants included individuals aged 35–45 years with a confirmed diagnosis of T2DM who had been receiving metformin monotherapy for a minimum duration of six months and were willing to participate in the study. Patients were excluded if they did not provide informed consent, were using medications known to affect serum vitamin B_{12} levels (such as corticosteroids, phenytoin, proton pump inhibitors, colchicine, or neomycin), had a history of malabsorption disorders (including celiac disease, chronic pancreatitis, or lactose intolerance), had known hematological disorders such as pernicious anemia or pre-existing vitamin B_{12} deficiency, were pregnant or lactating, had major chronic illnesses other than T2DM (including congestive heart failure or severe pulmonary disease), or had experienced recent trauma or undergone surgical intervention.

Sample Size Calculation

The sample size was calculated using the prevalence-based formula for cross-sectional studies, based on a previously published study reporting a vitamin B_{12} deficiency prevalence of 17.5% among metformin-treated diabetic patients. With a 95% confidence level and an absolute precision of 5%, the minimum required sample size was calculated to be 221 participants. Accordingly, 221 eligible patients were enrolled in the study.

Data Collection and Clinical Assessment
A detailed medical history, including duration of diabetes, metformin dosage, dietary pattern, and lifestyle habits (alcohol consumption, smoking, tobacco use), was recorded for each participant. A general physical and systemic examination was conducted at baseline.

Laboratory Investigations
Baseline laboratory investigations included estimation of serum vitamin B_{12} levels, complete blood count (CBC) comprising hemoglobin concentration, red blood cell count, white blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), along with fasting blood glucose (FBG), postprandial blood glucose (PPBG), and glycated hemoglobin ($HbA_{1}c$). Serum vitamin B_{12} levels were reassessed at 6 months and 12 months using the same standardized biochemical methods to ensure analytical consistency. Vitamin B_{12} status was subsequently categorized as normal, borderline, or deficient in accordance with established laboratory reference ranges

Statistical Analysis
All collected data were entered into Microsoft Excel and analyzed using SPSS version 25. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Changes in serum vitamin B_{12} levels over time were analyzed using paired t-tests and chi-square tests, as appropriate. Pearson correlation analysis was performed to assess associations between vitamin B_{12} levels and clinical variables. Multivariate regression analysis was conducted to identify potential predictors of vitamin B_{12} change. A p-value <0.02 was considered statistically significant.

Ethical Considerations
The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Career Institute of Medical Sciences & Hospital, Lucknow. Written informed consent was obtained from all participants prior to enrollment, and confidentiality of patient data was strictly maintained.

RESULTS
A total of 221 patients with Type 2 diabetes mellitus (T2DM) receiving metformin monotherapy were included in the study. The results are presented with respect to demographic characteristics, clinical profile, and changes in serum vitamin B_{12} levels over time.

Table 1: Baseline Demographic and Clinical Characteristics of Study Participants (n = 221)

Variable	Category	Number (n)	Percentage (%)
Age (years)	35–37	60	27.1
	38–40	54	24.4
	41–43	69	31.2
	44–45	38	17.2
	Mean ± SD	**40.01 ± 3.17**	—
Sex	Male	106	48.0
	Female	115	52.0
Dietary Pattern	Vegetarian	123	55.7
	Non-vegetarian	77	34.8
	Ovo-vegetarian	21	9.5
Duration of Diabetes Mellitus	< 12 months	46	20.8
	12–24 months	82	37.1
	> 24 months	93	42.1
	Mean ± SD (months)	**21.33 ± 9.07**	—

Table 1 summarizes the baseline demographic and clinical characteristics of the study population. Most participants were aged between 41–43 years (31.2%), with a mean age of 40.01 ± 3.17 years, indicating early-onset Type 2 diabetes mellitus. The gender distribution was nearly equal, with a slight female predominance (52.0%). A majority of participants (55.7%) followed a vegetarian diet, which may predispose to lower vitamin B_{12} intake. Regarding disease duration, 42.1% of patients had diabetes for more than 24 months, with a mean duration of 21.33 ± 9.07 months, reflecting a cohort with moderate disease chronicity.

Table 2: Effect of Metformin on Vitamin B_{12} Status Over Time

Vitamin B_{12} Status	Baseline n (%)	6 Months n (%)	12 Months n (%)
Normal	193 (87.3)	180 (81.4)	158 (71.5)
Borderline	26 (11.8)	35 (15.8)	43 (19.5)
Deficient	2 (0.9)	6 (2.7)	20 (9.0)
Significance	—	$\chi^2 = 3.78$, $p = 0.151$	$\chi^2 = 22.4$, $p < 0.001$

Table 2 demonstrates the effect of metformin therapy on vitamin B_{12} status over time. At baseline, 87.3% of patients had normal vitamin B_{12} levels, while only 0.9% were deficient. After 6 months of metformin therapy, there was a slight increase in borderline and deficient cases, although this change was not statistically significant ($p = 0.151$). However, at 12 months, a significant decline in vitamin B_{12} status was observed, with deficiency increasing to 9.0% ($\chi^2 = 22.4$, $p < 0.001$), indicating a time-dependent effect of metformin on vitamin B_{12} levels.

Table 3: Mean Serum Vitamin B_{12} Levels Over Time

Time Point	Mean (pg/mL)	SD	Mean Change	t-value	p-value
Baseline	423.95	102.71	—	—	—
6 months	393.22	103.24	−30.72 ± 19.25	23.73	<0.001
12 months	356.07	104.56	−67.87 ± 30.12	33.50	<0.001

Table 3 shows the progressive decline in mean serum vitamin B_{12} levels during follow-up. Mean vitamin B_{12} decreased significantly from 423.95 ± 102.71 pg/mL at baseline to 393.22 ± 103.24 pg/mL at 6 months and further to 356.07 ± 104.56 pg/mL at 12 months. The reductions at both follow-up points were highly statistically significant ($p < 0.001$), confirming a cumulative decline with prolonged metformin use.

Table 4: Correlation of Duration of Diabetes and Metformin Dose with Vitamin B_{12} Levels and Other Parameters Pearson Correlation Analysis

Parameter	Duration of DM (months) r-value	p-value	Metformin Dose (mg/day) r-value	p-value
RBC ($\times 10^6/\mu L$)	−0.011	0.865	−0.032	0.637
WBC ($\times 10^3/\mu L$)	−0.034	0.619	−0.075	0.265
Hemoglobin (g/dL)	0.015	0.820	−0.021	0.757
MCV (fL)	0.014	0.834	−0.023	0.731
MCH (pg)	−0.118	0.081	0.041	0.541
MCHC (g/dL)	0.000	0.995	0.055	0.416
Fasting Blood Glucose (mg/dL)	−0.009	0.893	0.036	0.593
Postprandial Blood Glucose (mg/dL)	−0.036	0.599	−0.058	0.387
HbA1c (%)	0.056	0.404	−0.068	0.316
Vitamin B_{12} – Baseline (pg/mL)	0.027	0.687	0.057	0.402
Vitamin B_{12} – 6 Months (pg/mL)	0.025	0.707	0.046	0.498
Vitamin B_{12} – 1 Year (pg/mL)	0.036	0.598	0.052	0.443

Pearson correlation analysis showed no statistically significant association between duration of diabetes or metformin dose and vitamin B_{12} levels, hematological indices, or glycemic parameters. All correlation coefficients were weak and non-significant ($p > 0.05$), indicating that neither duration of diabetes nor daily metformin dose had a measurable impact on vitamin B_{12} levels or related parameters in this study population.

Table 5: Multivariate Regression Analysis Showing Vitamin B_{12} Level Changes with Possible Predictors Change in Vitamin B_{12} after 6 Months

Possible Predictors	B	SE	t-value	p-value	Effect Size
Intercept	−25.79	6.13	−4.21	0.000	0.077
Alcohol	1.49	3.19	0.47	0.641	0.001
Smoking	−1.17	3.32	−0.35	0.725	0.001
Tobacco	−2.20	3.40	−0.65	0.519	0.002
Duration of DM (months)	−0.02	0.15	−0.16	0.871	0.000
Metformin Dose (mg/day)	0.00	0.00	−0.81	0.422	0.003
Non-Vegetarian	−0.97	2.87	−0.34	0.735	0.001
Ovo-Vegetarian	−1.80	4.62	−0.39	0.697	0.001
Vegetarian	Reference	—	—	—	—

Table 5 presents the multivariate regression analysis assessing the association between potential predictors and change in serum vitamin B_{12} levels after 6 months of metformin therapy. The intercept was statistically significant ($B = -25.79$, $p < 0.001$), indicating an overall decline in vitamin B_{12} levels during the first 6 months of treatment, independent of other variables included in the model. Alcohol consumption, smoking, and tobacco use did not show a significant association with vitamin B_{12} change ($p > 0.05$), suggesting that lifestyle habits did not influence early vitamin B_{12} decline. Duration of diabetes ($B = -0.02$, $p = 0.871$) and metformin dose ($B = 0.00$, $p = 0.422$) were also not significantly associated with vitamin B_{12} change, indicating that early depletion was not dependent on disease duration or drug dosage. Dietary patterns, including non-vegetarian and ovo-vegetarian diets compared with vegetarian diet (reference), did not significantly affect vitamin B_{12} levels. The effect sizes for all predictors were minimal, demonstrating negligible contribution of individual variables to the observed decline in vitamin B_{12} levels at 6 months.

Table 6: Change in Vitamin B_{12} after 12 Months

Possible Predictors	B	SE	t-value	p-value	Effect Size
Intercept	−71.35	9.55	−7.47	0.000	0.208
Alcohol	7.32	4.97	1.47	0.142	0.010
Smoking	4.23	5.17	0.82	0.414	0.003
Tobacco	−4.55	5.29	−0.86	0.391	0.003
Duration of DM (months)	0.12	0.23	0.51	0.608	0.001
Metformin Dose (mg/day)	0.00	0.00	−0.08	0.937	0.000
Non-Vegetarian	0.38	4.47	0.09	0.932	0.000
Ovo-Vegetarian	−3.07	7.20	−0.43	0.671	0.001
Vegetarian	Reference	—	—	—	—

Table 6 shows the multivariate regression analysis evaluating predictors of change in serum vitamin B_{12} levels after 12 months of metformin therapy. The intercept remained highly significant ($B = -71.35$, $p < 0.001$), reflecting a marked overall decline in vitamin B_{12} levels over one year of treatment. Alcohol intake, smoking, and tobacco use were not significantly associated with vitamin B_{12} changes ($p > 0.05$), indicating that lifestyle factors did not influence long-term vitamin B_{12} decline. Similarly, duration of diabetes ($B = 0.12$, $p = 0.608$) and metformin dose ($B = 0.00$, $p = 0.937$) showed no significant effect, suggesting that prolonged vitamin B_{12} reduction was independent of disease duration and medication dose. Dietary patterns, including non-vegetarian and ovo-vegetarian diets relative to vegetarian diet, also failed to demonstrate any significant association with vitamin B_{12} change. Effect sizes for all predictors were extremely small, indicating minimal explanatory power of these variables.

DISCUSSION

The present study observed a significant, time-dependent decline in serum vitamin B_{12} levels in patients with Type 2 diabetes mellitus receiving metformin therapy. Mean vitamin B_{12} levels decreased from 423.95 ± 102.71 pg/mL at baseline to 356.07 ± 104.56 pg/mL at 12 months ($p < 0.001$), with a corresponding increase in vitamin B_{12} deficiency prevalence from 0.9% at baseline to 9.0% at 12 months. These findings align with several previous studies reporting similar trends in metformin-induced vitamin B_{12} depletion.

Ramirez et al. documented a significant reduction in serum vitamin B_{12} concentrations in patients on long-term metformin, with prevalence of deficiency increasing with duration of therapy, supporting the time-dependent decline observed in our cohort [8]. Similarly, Aroda et al. reported that metformin therapy was associated with a 14% absolute increase in the incidence of vitamin B_{12} deficiency compared to placebo over 4.3 years, further validating the progressive pattern seen in the present study [9].

Our study's lack of significant correlation between vitamin B_{12} levels and metformin dose is consistent with findings by de Jager et al., who reported that cumulative duration rather than daily dose was more predictive of vitamin B_{12} reduction among metformin users [10]. In contrast, Liu et al. found a modest association between higher metformin doses and greater decline in serum B_{12} levels; however, their cohort included a wider age range and greater variability in treatment duration, which may explain the discrepancy [11].

The dietary patterns in our population, with a high proportion of vegetarian participants (55.7%), did not show a statistically significant impact on vitamin B_{12} decline. This finding is similar to the results of Bauman et al., who reported that although vegetarian diet can predispose to lower baseline B_{12} stores, metformin-induced reduction occurred irrespective of dietary intake patterns [12]. However, Reinstatler et al. observed a stronger association between vegetarian diet and severity of vitamin B_{12} deficiency, particularly in patients with poor dietary cobalamin intake, suggesting that diet may exert an additive effect in susceptible individuals [13].

In terms of hematological parameters, our study found that complete blood count indices remained within normal limits despite significant biochemical decline in vitamin B_{12} levels. This observation is in concordance with Pouwels et al., who reported that hematological manifestations often lag behind biochemical depletion and may remain subclinical for extended periods [14]. Similarly, Bell et al. observed that early metformin-associated B_{12} deficiency may not present with anemia or macrocytosis initially, emphasizing the need for biochemical screening rather than reliance on hematological changes alone [15].

Overall, the trajectory of vitamin B_{12} depletion in our study is consistent with the broader evidence base indicating that long-term metformin therapy significantly influences cobalamin status. The variation in magnitude and predictors across studies highlights the influence of population characteristics, dietary practices, baseline vitamin B_{12} status, and study methodology.

CONCLUSION

The present study demonstrates that long-term metformin therapy in patients with Type 2 diabetes mellitus is associated with a significant, progressive, and time-dependent reduction in serum vitamin B_{12} levels. Although overt hematological abnormalities were not observed, the increasing prevalence of borderline and deficient vitamin B_{12} status highlights the risk of subclinical deficiency, which may remain clinically silent in the early stages. The decline in vitamin B_{12} levels appeared to be independent of metformin dose, duration of diabetes, dietary pattern, and lifestyle factors, suggesting a direct effect of prolonged metformin exposure. These findings underscore the importance of routine monitoring of serum vitamin B_{12} levels in patients receiving long-term metformin therapy to enable early identification and timely intervention, thereby preventing potential neurological and hematological complications.

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