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Original Research Article

Effect of Metformin Therapy on Serum Vitamin B12 and Folate Levels in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Metformin is the first-line oral hypoglycemic agent for the management of type 2 diabetes mellitus (T2DM). Although it is highly effective and well tolerated, prolonged use has been associated with impaired vitamin B12 absorption, which may result in hematological and neurological complications. The effect of metformin on serum folate levels remains inconclusive.

Aim: To study the effect of metformin on serum vitamin B12 and folate levels in patients with type 2 diabetes mellitus.

Materials and Methods: A hospital-based observational cross-sectional study was conducted among 150 patients with type 2 diabetes mellitus receiving metformin therapy. Adult patients treated with metformin for at least six months were enrolled after obtaining informed consent. Patients receiving vitamin B12 or folic acid supplementation and those with conditions affecting vitamin B12 metabolism were excluded. Demographic and clinical data were recorded, and venous blood samples were collected for estimation of serum vitamin B12 and folate levels using standard laboratory methods. Hemoglobin, mean corpuscular volume (MCV), and peripheral blood film findings were also assessed. Statistical analysis was performed using appropriate statistical tests, and a p-value <0.05 was considered statistically significant.

Results: Among the 150 participants, 82 (54.7%) were males and 68 (45.3%) were females, with the majority belonging to the 41–45 years age group. Mean serum vitamin B12 levels decreased significantly following metformin therapy (872.3 ± 86.5 pg/ml vs. 724.8 ± 188.7 pg/ml; $p<0.001$). Serum folate levels showed a slight decline (10.5 ± 1.9 ng/ml vs. 10.1 ± 1.8 ng/ml), which was not statistically significant ($p>0.05$). Mean corpuscular volume increased significantly, while hemoglobin levels decreased significantly after treatment ($p<0.001$). Peripheral blood film examination demonstrated macrocytic changes in 7 (4.7%) patients following metformin therapy.

Conclusion: Metformin therapy in patients with type 2 diabetes mellitus is associated with a significant reduction in serum vitamin B12 levels, whereas serum folate levels remain largely unaffected. Long-term metformin use may contribute to hematological alterations, emphasizing the need for periodic monitoring of vitamin B12 status and timely supplementation to prevent deficiency-related complications.

Keywords: Type 2 diabetes mellitus; Metformin; Vitamin B12; Folate; Mean corpuscular volume; Hemoglobin.

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common chronic metabolic disorders worldwide and is characterized by persistent hyperglycemia resulting from insulin resistance and impaired insulin secretion. The global prevalence of diabetes continues to increase, making it a major public health concern due to its associated microvascular and macrovascular complications.[1] Metformin is the recommended first-line oral antihyperglycemic agent for the management of T2DM because of its effectiveness, safety profile, cardiovascular benefits, and low risk of hypoglycemia.[1]

Despite its well-established therapeutic benefits, prolonged use of metformin has been associated with reduced absorption of vitamin B12, potentially leading to vitamin B12 deficiency.[2] The proposed mechanism involves interference with calcium-dependent absorption of the vitamin B12–intrinsic factor complex in the terminal ileum.[6] Several studies have demonstrated a significant association between long-term metformin therapy and decreased serum vitamin B12 concentrations, particularly in patients receiving higher doses and longer durations of treatment.[2–5]

Vitamin B12 plays an essential role in DNA synthesis, erythropoiesis, and neurological function. Deficiency may result in megaloblastic anemia, peripheral neuropathy, cognitive impairment, and other neurological manifestations.[8–10] Folate is another important water-soluble vitamin involved in nucleic acid synthesis and one-carbon metabolism. Although the effect of metformin on folate levels is less consistent than its effect on vitamin B12, altered folate status has been reported in some patients receiving long-term metformin therapy.[8,10]

Early identification of vitamin B12 and folate deficiencies is clinically important because the symptoms may overlap with diabetic peripheral neuropathy, leading to delayed diagnosis and inappropriate management.[3,4] Therefore, assessment of serum vitamin B12 and folate levels in patients with T2DM receiving metformin therapy may help identify individuals at risk and improve patient outcomes.[2,5]

The present study was undertaken to evaluate the effect of metformin on serum vitamin B12 and folate levels in patients with type 2 diabetes mellitus.

Material and Methods

A hospital-based observational cross-sectional study was conducted among 150 patients diagnosed with type 2 diabetes mellitus attending the outpatient and inpatient departments of the study hospital. Adult patients receiving metformin therapy for at least six months were included after obtaining written informed consent. Patients receiving vitamin B12 or folic acid supplementation, those with chronic liver disease, renal failure, pregnancy, alcoholism, gastrointestinal disorders affecting vitamin absorption, or other causes of vitamin B12 deficiency were excluded.

Demographic and clinical details, including age, sex, duration of diabetes, duration and dose of metformin therapy, were recorded using a structured proforma. Venous blood samples were collected under aseptic precautions for estimation of serum vitamin B12 and folate levels using standard laboratory methods.

Data were entered into Microsoft Excel and analyzed using appropriate statistical software. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. A p-value <0.05 was considered statistically significant.

Results

Table 1 shows the distribution of study participants according to age and sex. Out of 150 patients, 82 (54.7%) were males and 68 (45.3%) were females. The majority of participants belonged to the 41–45 years age group, comprising 84 (56.0%) patients, followed by 39 (26.0%) patients in the 46–50 years age group and 27 (18.0%) patients in the 36–40 years age group.

Table 2 compares the mean serum vitamin B12, folate, mean corpuscular volume (MCV), and hemoglobin (Hb) levels before and after six months of metformin therapy. A significant reduction in serum vitamin B12 levels was observed after treatment (872.3 ± 86.5 pg/ml vs. 724.8 ± 188.7 pg/ml; $p<0.001$). Serum folate levels showed a slight decline; however, the difference was not statistically significant ($p>0.05$). MCV increased significantly following metformin therapy (82.3 ± 2.5 fl vs. 85.9 ± 5.7 fl; $p<0.001$), while hemoglobin levels showed a significant decrease (14.8 ± 0.7 g/dl vs. 14.2 ± 0.8 g/dl; $p<0.001$).

Table 3 depicts the peripheral blood film findings before and after six months of metformin therapy. Before treatment, all 150 (100%) patients had a normocytic blood picture. After treatment, 143 (95.3%) patients remained normocytic, whereas 7 (4.7%) developed a macrocytic blood picture. No patient exhibited a microcytic blood film during the study period. The observed change in peripheral blood film pattern was statistically significant (Chi-square = 4.61, $p = 0.032$).

Table 1: Distribution of study participants according to age and sex (N = 150)

Age group (years)	Males N (%)	Females N (%)	Total N (%)
36–40	13 (8.7)	14 (9.3)	27 (18.0)
41–45	45 (30.0)	39 (26.0)	84 (56.0)
46–50	24 (16.0)	15 (10.0)	39 (26.0)
Total	82 (54.7)	68 (45.3)	150 (100)

Table 2: Mean ± SD of various parameters before and after 6 months of metformin therapy (N = 150)

Parameters	Before 6 months of metformin	After 6 months of metformin	P value	Significance
Vitamin B12 (pg/ml)	872.3 ± 86.5	724.8 ± 188.7	<0.001	Highly significant
Folate (ng/ml)	10.5 ± 1.9	10.1 ± 1.8	>0.05	Not significant
MCV (fl)	82.3 ± 2.5	85.9 ± 5.7	<0.001	Highly significant
Hb (g/dl)	14.8 ± 0.7	14.2 ± 0.8	<0.001	Highly significant

Table 3: Peripheral blood film changes observed during the study period (N = 150)

Peripheral blood film	Before	After
Normocytic	150	143
Macrocytic	0	7
Microcytic	0	0

Discussion

The present study evaluated the effect of metformin therapy on serum vitamin B12 and folate levels in patients with type 2 diabetes mellitus (T2DM). The findings demonstrated a significant reduction in serum vitamin B12 levels after six months of metformin therapy, while serum folate levels showed a slight but statistically non-significant decline. In addition, a significant increase in mean corpuscular volume (MCV) and a significant reduction in hemoglobin levels were observed, indicating the hematological impact of vitamin B12 deficiency.

Vitamin B12 deficiency has increasingly been recognized as an important adverse effect of prolonged metformin therapy. Metformin is believed to reduce vitamin B12 absorption by interfering with calcium-dependent uptake of the intrinsic factor-vitamin B12 complex in the terminal ileum. Persistent deficiency may contribute to hematological abnormalities and neurological complications, particularly in patients receiving long-term treatment.[11] The present findings are consistent with previous reports demonstrating a significant association between metformin therapy and reduced serum vitamin B12 concentrations.[12]

The observed decline in vitamin B12 levels in the present study is also supported by systematic reviews and meta-analyses, which concluded that both higher doses and longer duration of metformin therapy significantly increase the risk of vitamin B12 deficiency.[13] Since diabetic peripheral neuropathy and vitamin B12 deficiency neuropathy often present with similar clinical manifestations, unrecognized deficiency may delay appropriate treatment and worsen patient outcomes.

Although serum folate levels showed a slight decline after metformin therapy, the difference was not statistically significant. Similar observations have been reported by previous investigators, suggesting that metformin has a more pronounced effect on vitamin B12 than on folate metabolism.[11,13] Nevertheless, simultaneous assessment of folate status remains important because combined deficiencies may aggravate megaloblastic anemia and elevate homocysteine concentrations.

The significant increase in MCV and decrease in hemoglobin observed in this study further support the hematological consequences of declining vitamin B12 levels. Macrocytic changes in peripheral blood film were identified in a small proportion of patients after treatment, indicating early morphological evidence of vitamin B12 deficiency. These findings are in agreement with previous studies reporting macrocytosis and megaloblastic changes among diabetic patients receiving prolonged metformin therapy.[14]

Recent evidence emphasizes the importance of periodic monitoring of vitamin B12 levels in patients receiving long-term metformin, particularly among elderly individuals and those receiving higher daily doses. Early identification and supplementation can prevent irreversible neurological damage while improving hematological parameters.[15] Therefore, routine screening of serum vitamin B12 levels may be considered as part of the long-term management strategy for patients with T2DM treated with metformin.

Overall, the present study reinforces existing evidence that prolonged metformin therapy is associated with a significant decline in serum vitamin B12 levels without causing a significant reduction in serum folate concentrations. Regular biochemical assessment and timely supplementation in deficient patients may reduce the risk of complications and improve the quality of diabetes care.

Conclusion

The present study concludes that metformin therapy in patients with type 2 diabetes mellitus is associated with a significant reduction in serum vitamin B12 levels, accompanied by significant changes in hematological parameters such as increased mean corpuscular volume and reduced hemoglobin levels. In contrast, serum folate levels were not significantly affected by metformin therapy. These findings highlight the importance of periodic monitoring of vitamin B12 status in patients receiving long-term metformin treatment. Early detection and appropriate vitamin B12 supplementation may help prevent hematological abnormalities and neurological complications, thereby improving the overall management and clinical outcomes of patients with type 2 diabetes mellitus.

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