

Vitamin B₁₂ Levels in Patients with Type 2 Diabetes Mellitus on Metformin

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ABSTRACT

Vitamin B₁₂ is required for proper hematopoiesis, cardiovascular and neurocognitive function. Vitamin B₁₂ deficiency is highly prevalent among patients with type 2 diabetes mellitus (T2DM) on metformin therapy. **Aim:** This study was carried out to evaluate the serum levels of vitamin B₁₂ in patients with T2DM on metformin therapy. **Material and methods:** Hundred patients with T2DM within the age group of 45-80 years were recruited into this cross-sectional study. All the patients were on metformin therapy for a minimum of 5 years. **Results:** Vitamin B₁₂ deficiency and borderline deficiency observed were 10.6% and 29% and 60.4% did not have vitamin B₁₂ deficiency. B₁₂ levels were lower in patients with more than 10 years of duration of diabetes and was statistically significant with a p value of 0.004. The average B₁₂ levels in patients on metformin dose of >1,000 mg was 349 pg/dL and in patients with metformin dose above 1,000 mg was 215 pg/dL. The difference between the two groups was statistically significant with a p value of <0.002. **Conclusion:** There is a high prevalence of B₁₂ deficiency among diabetic patients. It is important to screen for vitamin B₁₂ deficiency before initiating metformin and later annually.

Keywords: Vitamin B₁₂ deficiency, diabetes mellitus, peripheral neuropathy, metformin

Vitamin B₁₂ is an essential micronutrient required for proper hematopoiesis, cardiovascular and neurocognitive function. Biochemical and clinical vitamin B₁₂ deficiency is highly prevalent among patients with type 2 diabetes mellitus (T2DM) on metformin therapy. B₁₂ deficiency presents with a variety of clinical manifestations like megaloblastic anemia, pancytopenia, impaired memory, dementia, peripheral neuropathy and subacute combined degeneration of the spinal cord.

Metformin is the most commonly prescribed antidiabetic drug in patients with T2DM and is a cornerstone in the treatment of T2DM. It is well-tolerated in most of the patients.

Due to the numerous benefits of metformin, some side effects are usually ignored and rarely investigated. One such side effect is vitamin B₁₂ deficiency.

Vitamin B₁₂ or cobalamin plays an important role in DNA synthesis, hematopoiesis and neurological function. Vitamin B₁₂ deficiency is associated with hematological and neurocognitive dysfunction.¹ This study was hence carried out to evaluate the serum levels of vitamin B₁₂ in patients with T2DM on metformin therapy.

MATERIAL AND METHODS

A total of 100 patients with T2DM within the age group of 45-80 years were recruited into this cross-sectional study. All the patients recruited were on metformin therapy for a minimum of 5 years.

Exclusion Criteria

Patients with gastrectomy, small bowel resection, recent intake of vitamin B₁₂, liver disease, chronic kidney disease and thyroid disease, patients on histamine 2 receptor blockers and vegetarians were excluded from this study. Participants were enrolled into this study after obtaining a written informed consent from each one of them.

Determination of serum vitamin B₁₂ level was by using high performance liquid chromatography (HPLC). Vitamin B₁₂ deficiency was defined as serum

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concentration of <200 pg/dL and borderline deficiency as 200-300 pg/dL. Concentrations >300 pg/dL were considered as normal. Statistical analysis was done using SPSS (Version 20.0) and the results were analyzed.

RESULTS

Vitamin B₁₂ deficiency and borderline deficiency observed were 10.6%, 29% and 60.4% did not have vitamin B₁₂ deficiency.

Most of the patients were between the ages of 50-59 years - 39%, followed by >60 years - 25%, 40-49 years - 24% and 30-39 years - 12% (Fig. 1). Of them, 62% were females and 48% were males. The patients were divided according to the duration of diabetes into two groups: less than 10 years and more than 10 years of diabetes mellitus. The characteristics of patients with duration of therapy with metformin and B₁₂ levels are shown in Table 1.

The difference between B₁₂ levels in patients below 10 years of duration of diabetes and more than 10 years of duration of diabetes was statistically significant with a p value of 0.004.

B₁₂ Levels and Metformin Dose

Forty-six percent of patients were on a dose of <1,000 mg of metformin per day and 54% were on a dose of >1,000 mg of metformin per day. The average B₁₂ levels in patients on metformin dose of <1,000 mg was 349 pg/dL and the levels in patients with metformin dose above 1,000 mg was 215 pg/dL (Fig. 2). The difference between the two groups was statistically significant with a p value of <0.002.

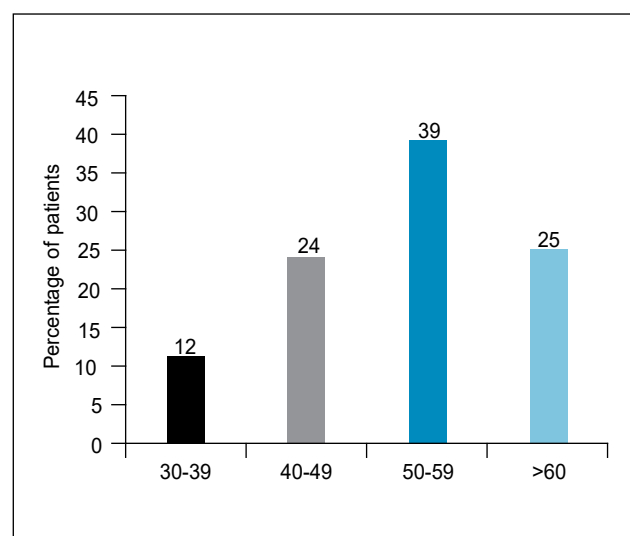


Figure 1. Diabetics on metformin.

Table 1. Characteristics of the Patients Including Levels of B₁₂ According to Duration of Diabetes

Characteristics	<10 years	>10 years
Age	54 ± 34	57 ± 51
BMI	25.62 ± 4	26.64 ± 32
Systolic BP	134 ± 14.24	136 ± 16.52
Diastolic BP	82 ± 11.22	84 ± 11.24
B ₁₂ levels (pg/dL)	422.34	224.21

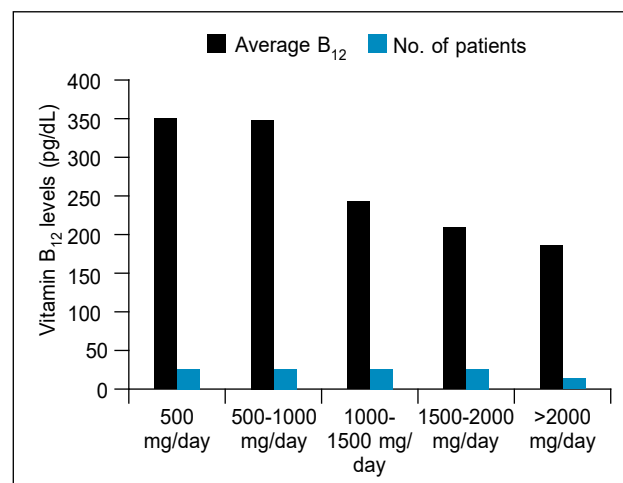


Figure 2. Average B₁₂ levels in patients according to dosage of metformin.

DISCUSSION

Reports have shown that long-term metformin use has a significant impact on the concentration of vitamin B₁₂ in patients with T2DM.

Vitamin B₁₂ or cobalamin plays an important role in DNA synthesis, hematopoiesis and neurological function. Vitamin B₁₂ deficiency is associated with hematological and neurocognitive dysfunction.¹

The source of vitamin B₁₂ is animal proteins. Vitamin B₁₂ deficiency is due to insufficient dietary intake among alcoholics and vegetarians, malabsorption due to chronic atrophic gastritis, pernicious anemia, chronic pancreatitis, celiac disease and drugs like metformin and proton pump inhibitors.

Vitamin B₁₂ deficiency results in disruption of the methylation process and increased accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has toxic effects on neurons and the vascular endothelium. This methylation reaction is essential in the conversion of dietary folate to its active metabolic form, tetrahydrofolate. Vitamin B₁₂ is the co-factor that mediates the conversion of methylmalonyl

coenzyme A (CoA) to succinyl-CoA. Vitamin B₁₂ deficiency leads to increase in serum methylmalonic acid (MMA), causing defective fatty acid synthesis of the neuronal membranes.² Vitamin B₁₂ is also essential in the synthesis of neurotransmitters like serotonin and dopamine.³ Deficiency of dopamine and serotonin occurs with vitamin B₁₂ deficiency, which is the cause of the neurocognitive or psychiatric manifestations. Axonal demyelination, degeneration and later death due to vitamin B₁₂ deficiency manifests as severe peripheral or autonomic neuropathy, subacute combined degeneration of the spinal cord, dementia and delirium.⁴ Hyperhomocysteinemia due to its cellular and vasculotoxic effects, is also associated with an increased risk of cardiovascular events.⁵ Several cross-sectional studies⁶ and case reports⁷ have demonstrated an increased frequency of vitamin B₁₂ deficiency among T2DM patients. Metformin use has been demonstrated as the main factor associated with vitamin B₁₂ deficiency among patients with T2DM.⁸ Studies on type 2 diabetic patients on metformin intake have demonstrated that the prevalence of vitamin B₁₂ deficiency is from 5.8% to 33%.⁹ In a study by Qureshi et al,⁹ a high prevalence of vitamin B₁₂ deficiency of 33% was seen in adult patients with T2DM.

Metformin-induced Vitamin B₁₂ Deficiency Among Patients with T2DM

In a randomized controlled trial by DeFronzo et al, metformin reduced the serum vitamin B₁₂ levels by 22% and 29% when compared to glyburide and placebo, respectively.¹⁰ The risk of developing metformin associated vitamin B₁₂ deficiency is increased by age, metformin dose and duration of treatment.¹¹ In a case-control study done in China among 155 adult Chinese diabetic patients on metformin and 310 controls, for every 1 g/day increase in metformin there was an odds ratio (OR) of 2.9 (95% confidence interval [CI], 2.15-3.87) for developing vitamin B₁₂ deficiency. Among patients treated with metformin for ≥3 years, the adjusted OR was 2.4 (95% CI, 1.46-3.91) when compared with diabetic patients who received metformin for ≤3 years.¹¹

Decrease in vitamin B₁₂ absorption following metformin use starts as early as the 4th month of treatment.¹² Clinical features of vitamin B₁₂ deficiency develop only by 5-10 years, as there are a large amount of B₁₂ stores in the body (liver), which do not get depleted that fast.¹³

The mechanisms to explain metformin-induced vitamin B₁₂ deficiency in patients with T2DM are:

- Alterations in small bowel motility which stimulates bacterial overgrowth and vitamin B₁₂ deficiency
- Competitive inhibition of vitamin B₁₂ absorption
- Alterations in intrinsic factor (IF) levels
- Interaction with the cubulinendocytic receptor¹³
- Inhibition of the calcium-dependent absorption of the vitamin B₁₂-IF complex at the terminal ileum (This inhibitory effect is reversed with calcium supplementation).¹⁴

Vitamin B₁₂ deficiency in patients with type 1 diabetes mellitus (T1DM) are due to:

- Pernicious anemia due to chronic autoimmune gastritis is high among patients with T1DM, more than 3 to 5 folds when compared to general population.¹⁵
- Exhibit autoantibodies to intrinsic factor (AIF) type 1 and 2¹⁵ and parietal cell antibodies (PCA)¹⁶ especially those diabetic patients with glutamate decarboxylase-65 (GAD-65) antibodies and HLA-DQA1*0501-B1*0301 haplotype.¹⁷
- Autoimmune hypothyroidism, celiac diseases are frequent comorbidities in patients with T1DM and they directly affect vitamin B₁₂ metabolism.¹⁸ Dyserythropoiesis due to thyroid hormone deficiency, defective absorption due to reduced bowel motility, bacterial overgrowth and bowel wall edema also contribute to B₁₂ deficiency.¹⁸

Celiac disease is associated with enteropathy, chronic diarrhea and anemia due to malabsorption of folate and vitamin B₁₂.¹⁹

CONCLUSION

Clinical and biochemical vitamin B₁₂ deficiency is highly prevalent among patients with both T1DM and T2DM. As there is a high prevalence of B₁₂ deficiency among diabetic patients, it is clinically important to screen for vitamin B₁₂ deficiency before initiating metformin and later annually. Vitamin B₁₂ levels should be checked regularly among elderly patients with a history of long-term use of metformin (≥3-4 years), use of high doses of metformin (≥2 g/day), worsening diabetic distal polyneuropathy in the absence of other hematological abnormalities.²⁰ Vitamin B₁₂ levels <200 pg/mL is diagnostic of vitamin B₁₂ deficiency. Measurement of serum MMA or homocysteine concentrations is more sensitive and specific for screening type 2 diabetic patients with borderline serum vitamin B₁₂ concentrations of

200-400 pg/mL and mild-hematological manifestations. Normal levels of serum homocysteine and MMA concentrations are 5-15 $\mu\text{mol/L}$ and $<0.28 \mu\text{mol/L}$.²⁰ It is important to screen vitamin B₁₂ levels at diagnosis, then yearly for 3 years, then once in 5 years for life or if there are clinical signs of B₁₂ deficiency.¹⁵

Treatment of B₁₂ deficiency in adult patients with T2DM, intramuscular vitamin B₁₂ in doses of 1,000 μg daily for a week, then weekly once for 4 weeks to correct vitamin B₁₂ deficiency. Associated folate deficiency should be treated with oral folate in doses of 5 mg daily for 1-4 months.

Large, well-designed studies have to be done in future on vitamin B₁₂ deficiency screening in diabetic patients and optimal supplementation dose among type 1 and type 2 diabetic patients to formulate guidelines for care of diabetic patients.

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