CLINICAL STUDY

Vitamin B_{12} Levels in Patients with Type 2 Diabetes Mellitus on Metformin

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ABSTRACT

Vitamin B_{12} is required for proper hematopoeisis, cardiovascular and neurocognitive function. Vitamin B_{12} 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 B_{12} 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 B_{12} deficiency and borderline deficiency observed were 10.6% and 29% and 60.4% did not have vitamin B_{12} deficiency. B_{12} 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 B_{12} 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 B_{12} deficiency among diabetic patients. It is important to screen for vitamin B_{12} deficiency before initiating metformin and later annually.

Keywords: Vitamin B_{12} deficiency, diabetes mellitus, peripheral neuropathy, metformin

Vitamin B_{12} is an essential micronutrient required for proper hematopoeisis, cardiovascular and neurocognitive function. Biochemical and clinical vitamin B_{12} deficiency is highly prevalent among patients with type 2 diabetes mellitus (T2DM) on metformin therapy. B_{12} 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 B_{12} deficiency. Vitamin B_{12} or cobalamin plays an important role in DNA synthesis, hematopoeisis and neurological function. Vitamin B_{12} deficiency is associated with hematological and neurocognitive dysfunction.[1] This study was hence carried out to evaluate the serum levels of vitamin B_{12} 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 B_{12}, 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 B_{12} level was by using high performance liquid chromatography (HPLC). Vitamin B_{12} 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 B_{12} deficiency and borderline deficiency observed were 10.6%, 29% and 60.4% did not have vitamin B_{12} 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 B_{12} levels are shown in Table 1.

The difference between B_{12} 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.

B_{12} 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 B_{12} 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.

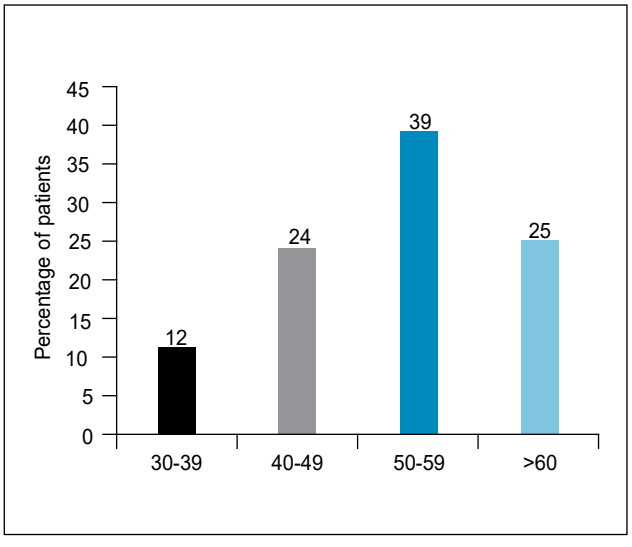

Figure 1. Diabetics on metformin.

Table 1. Characteristics of the Patients Including Levels of B_{12} 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

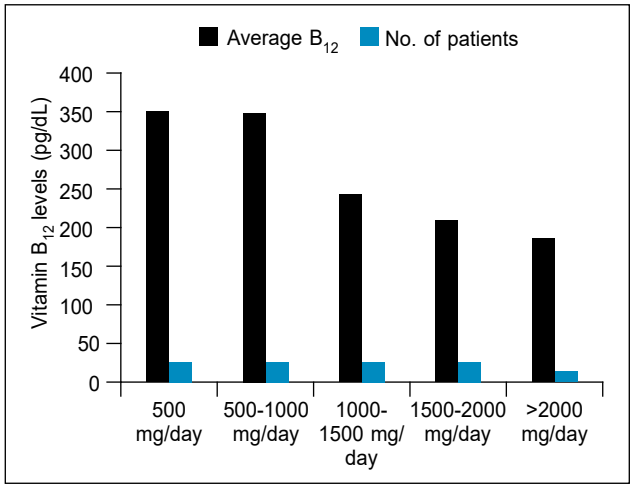

Figure 2. Average B_{12} levels in patients according to dosage of metformin.

DISCUSSION

Reports have shown that long-term metformin use has a significant impact on the concentration of vitamin B_{12} in patients with T2DM.

Vitamin B_{12} or cobalamin plays an important role in DNA synthesis, hematopoeisis and neurological function. Vitamin B_{12} deficiency is associated with hematological and neurocognitive dysfunction.[1]

The source of vitamin B_{12} is animal proteins. Vitamin B_{12} 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 B_{12} 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 B_{12} is the co-factor that mediates the conversion of methylmalonyl

coenzyme A (CoA) to succinyl-CoA. Vitamin B_{12} deficiency leads to increase in serum methylmalonic acid (MMA), causing defective fatty acid synthesis of the neuronal membranes.[2] Vitamin B_{12} is also essential in the synthesis of neurotransmitters like serotonin and dopamine.[3] Deficiency of dopamine and serotonin occurs with vitamin B_{12} deficiency, which is the cause of the neurocognitive or psychiatric manifestations. Axonal demyelination, degeneration and later death due to vitamin B_{12} 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 studies[6] and case reports[7] have demonstrated an increased frequency of vitamin B_{12} deficiency among T2DM patients. Metformin use has been demonstrated as the main factor associated with vitamin B_{12} deficiency among patients with T2DM.[8] Studies on type 2 diabetic patients on metformin intake have demonstrated that the prevalence of vitamin B_{12} deficiency is from 5.8% to 33%.[9] In a study by Qureshi et al,[9] a high prevalence of vitamin B_{12} deficiency of 33% was seen in adult patients with T2DM.

Metformin-induced Vitamin B_{12} Deficiency Among Patients with T2DM

In a randomized controlled trial by DeFronzo et al, metformin reduced the serum vitamin B_{12} levels by 22% and 29% when compared to glyburide and placebo, respectively.[10] The risk of developing metformin associated vitamin B_{12} 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 B_{12} 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 B_{12} absorption following metformin use starts as early as the 4th month of treatment.[12] Clinical features of vitamin B_{12} deficiency develop only by 5-10 years, as there are a large amount of B_{12} stores in the body (liver), which do not get depleted that fast.[13]

The mechanisms to explain metformin-induced vitamin B_{12} deficiency in patients with T2DM are:

- Alterations in small bowel motility which stimulates bacterial overgrowth and vitamin B_{12} deficiency
- Competitive inhibition of vitamin B_{12} absorption
- Alterations in intrinsic factor (IF) levels
- Interaction with the cubulinendocytic receptor[13]
- Inhibition of the calcium-dependent absorption of the vitamin B_{12}-IF complex at the terminal ileum (This inhibitory effect is reversed with calcium supplementation).[14]

Vitamin B_{12} 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 2[15] 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 B_{12} metabolism.[18] Dyserythropoiesis due to thyroid hormone deficiency, defective absorption due to reduced bowel motility, bacterial overgrowth and bowel wall edema also contribute to B_{12} deficiency.[18]

Celiac disease is associated with enteropathy, chronic diarrhea and anemia due to malabsorption of folate and vitamin B_{12}.[19]

CONCLUSION

Clinical and biochemical vitamin B_{12} deficiency is highly prevalent among patients with both T1DM and T2DM. As there is a high prevalence of B_{12} deficiency among diabetic patients, it is clinically important to screen for vitamin B_{12} deficiency before initiating metformin and later annually. Vitamin B_{12} 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 B_{12} levels <200 pg/mL is diagnostic of vitamin B_{12} deficiency. Measurement of serum MMA or homocysteine concentrations is more sensitive and specific for screening type 2 diabetic patients with borderline serum vitamin B_{12} concentrations of

200-400 pg/mL and mild-hematological manifestations. Normal levels of serum homocysteine and MMA concentrations are 5-15 μmol/L and <0.28 μmol/L.[20] It is important to screen vitamin B_{12} levels at diagnosis, then yearly for 3 years, then once in 5 years for life or if there are clinical signs of B_{12} deficiency.[15]

Treatment of B_{12} deficiency in adult patients with T2DM, intramuscular vitamin B_{12} in doses of 1,000 μg daily for a week, then weekly once for 4 weeks to correct vitamin B_{12} 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 B_{12} 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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