

Original Research Article

Serum vitamin B12 and homocysteine levels in type 2 diabetes patients on metformin

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ABSTRACT

Background: Metformin is reported to induce vitamin B12 deficiency, however India data is limited. The present study was conducted to assess the vitamin B12 and homocysteine levels among type 2 diabetes mellitus patients taking metformin.

Methods: The present observational study included 60 patients with diabetes mellitus, 30 patients on metformin therapy (at least 6 months) and 30 patients not on metformin use. Laboratory investigations were sent for vitamin B12, homocysteine and HbA1c.

Results: Mean vitamin B12 level was 159.67 ± 95.73 and 510.73 ± 206.18 pmol/l in the metformin and non-metformin group respectively (p value < 0.001). Serum homocysteine levels were found to be significantly higher in the metformin group as compared to non-metformin group (17.39 ± 4.97 vs 14.43 ± 2.43 $\mu\text{mol/l}$, p value < 0.01). Folate levels were similar in the two study groups (22.71 ± 3.67 vs 27.54 ± 2.83 mmol/L). Haemoglobin A1c levels were significantly higher in the metformin group as compared to non-metformin group (7.85 ± 0.97 vs 7.25 ± 0.80 respectively). Triglycerides, high density lipoprotein and total cholesterol levels were found to be similar between the two study groups. However, low density lipoproteins were significantly higher in the metformin group as compared to the non-metformin group (3.7 ± 0.92 vs 3.4 ± 0.86 mmol/l).

Conclusions: Our results show metformin use in type 2 diabetes mellitus patients was associated with significantly lower vitamin B12 levels and significantly high homocysteine levels.

Keywords: Diabetes mellitus, Homocysteine, Metformin, Vitamin B12

INTRODUCTION

Metformin is the most commonly prescribed oral hypoglycaemia agent as it has been shown to have beneficial effects on carbohydrate metabolism, weight loss, and vascular protection.¹ The European Association for the Study of Diabetes (EASD) and American Diabetes Association (ADA) also recommend metformin as the first line treatment option along with lifestyle intervention for hyperglycaemic management in type 2 diabetes mellitus.² Apart from the routine side effects like

abdominal distress and diarrhoea, scientific evidence suggests metformin reduces vitamin B12 uptake in the terminal ileum and thus long-term use of metformin is associated with low vitamin B12 levels.³ Vitamin B12 plays a vital role in optimal functioning of the nervous and haematological system. The clinical presentation of vitamin B12 deficiency generally includes haematological and neurological manifestations. Neurological signs and symptoms may take many forms, including peripheral neuropathy which generally manifests as numbness and paresthesia. Vitamin B12

deficiency may worsen the peripheral neuropathy which is caused by the pathophysiology of type 2 diabetes mellitus. In addition, vitamin B12 deficiency leads to raised levels of homocysteine, which itself is strongly linked with cardiovascular diseases.⁴ The present study was conducted to assess the vitamin B12 and homocysteine levels among type 2 diabetes mellitus patients taking metformin.

METHODS

Study method and sampling

The present observational study was conducted in the Department of Medicine, Dr. D. Y. Patil Medical College and Hospital, Navi Mumbai, in which a total of 60 patients with diabetes mellitus were studied. Metformin group consisted of patients with type 2 diabetes mellitus with ongoing treatment with metformin with duration of metformin use ≥ 6 months while no metformin group consisted of patients with type 2 diabetes mellitus who had never received metformin.

All patients were diagnosed with diabetes mellitus based on American Diabetic Association criteria.² We included patients who had symptomatic peripheral neuropathy, as assessed using the TCSS.⁵ The metformin group had patients on metformin for at least 6 months. We excluded patients who discontinued metformin within 6 months, received vitamin B12 and calcium supplementation, malabsorption syndrome, Type 1 diabetes mellitus, diagnosed with other forms of neuropathy and alcoholism, pregnancy, pernicious anemia and chronic kidney disease. Patients were explained the purpose of the study and an informed written consent was obtained from them before enrolment. The study was approved by the Institutional Ethics Committee.

Data collection and data analysis

Eligible patients were enrolled and detailed history about their diabetes mellitus and other relevant past medical history was obtained. Blood samples were drawn, and serum was stored at -20°C , which were used to estimate serum total Vitamin B12 levels. Vitamin B12 estimation was done by a solid phase, competitive chemiluminescent enzyme immunoassay on an analyzer using commercial kits. Vitamin B12 deficiency was defined as levels below 150 pmol/l and borderline deficiency levels between 150 and 221 pmol/l.⁶ Serum homocysteine levels were measured using the chemiluminescent analyser. Blood samples were also sent for lipid profile testing. The investigators filled and compiled the data obtained.

The data were analysed using SPSS software (version 23, IBM). The data were presented as means and standard

deviation or frequency distribution. A p value of less than 0.05 was considered statistically significant.

RESULTS

In the present study, a total of 60 patients were included, 30 each in metformin and non-metformin group. The mean age of the patients in metformin and non-metformin group was 57.27 ± 9.38 and 58.63 ± 10.22 years respectively (Table 1).

Gender distribution was observed to be similar in both the study groups (female: male = 13:17 vs 15:15). Mean duration of diabetes mellitus was found to be similar in both the study groups (5.4 ± 1.01 vs 5.2 ± 1.27 years). Of the 30 patients on metformin, 17 patients were taking a glyburide, 10 gliclazide and 5 were on insulin as well. Of the 30 patients not receiving metformin, 9 patients were taking glyburide and gliclazide each and 15 were on insulin. Laboratory investigations revealed that mean vitamin B12 level was 159.67 ± 95.73 and 510.73 ± 206.18 pmol/l in the metformin and non-metformin group respectively (p value < 0.001). Serum homocysteine levels were found to be significantly higher in the metformin group as compared to non-metformin group (17.39 ± 4.97 vs 14.43 ± 2.43 $\mu\text{mol/l}$, p value < 0.01). Folate levels were similar in the two study groups (22.71 ± 3.67 vs 27.54 ± 2.83 mmol/L). Haemoglobin A1c levels were significantly higher in the metformin group as compared to non-metformin group (7.85 ± 0.97 vs 7.25 ± 0.80 respectively). Triglycerides, high density lipoprotein and total cholesterol levels were found to be similar between the two study groups (Table 2). However, low density lipoproteins were significantly higher in the metformin group as compared to the non-metformin group (3.7 ± 0.92 vs 3.4 ± 0.86 mmol/l).

Table 1: Baseline characteristics of the patients included in the study.

Patient variables	Metformin (n=30)	Non-metformin (n=30)
Mean age (years)	57.27 ± 9.38	58.63 ± 10.22
Gender		
Female	13	15
Male	17	15
Duration of diabetes mellitus (years)	5.4 ± 1.01	5.2 ± 1.27
Medication taken		
Metformin	30	0
Glyburide	17	9
Gliclazide	10	9
Insulin	5	15

Table 2: Comparing laboratory investigations among the two study groups.

Laboratory investigations	Metformin (n=30)	Non-metformin (n=30)	P value
Serum vitamin B12 (pmol/l)	159.67±95.73	510.73±206.18	<0.001
Serum homocystein (µmol/l)	17.39±4.97	14.43±2.43	<0.01
Folate (mmol/l)	22.71±3.67	27.54±2.83	0.067
HbA1c (%)	7.85±0.97	7.25±0.80	<0.01
Triglycerides (mmol/l)	1.8±0.74	2.0±0.81	0.058
Low density lipoprotein (mmol/l)	3.7±0.92	3.4±0.86	<0.01
High density lipoprotein (mmol/l)	1.2±0.5	1.2±0.6	0.34
Total cholesterol (mmol/l)	5.8±1.1	5.4±0.9	0.12

DISCUSSION

The present study included type 2 diabetes mellitus patients, with or without metformin therapy. It was found that mean vitamin B12 level were significantly lower in the metformin group as compared to the non-metformin group. Studies done on western populations have shown vitamin B12 deficiency to be prevalent in 5.8% to 8.6% of type 2 diabetes mellitus patients.⁷ However, Raizada et al reported 35% of Indian diabetics to have vitamin B12 deficiency.⁸ This could be due to differences in the dietary habits of the two populations. Metformin prevents the absorption of Vitamin B12 in the ileum and this is caused by inhibition of calcium dependent channels in the ileum. Indian diets have also been reported to be low in calcium, which could be another factor causing higher prevalence of Vitamin B12 deficiency. It is known that prolonged use of metformin cause Vitamin B12 deficiency by this mechanism.⁹ Duration and dose of metformin have been associated with severity of vitamin B12 deficiency, as shown by Ko SH et al. The authors found that patients with Vitamin B12 deficiency had a longer duration of metformin use ($p<0.001$), a larger daily dose of metformin ($p<0.001$) than the patients without Vitamin B12 deficiency.¹⁰ A meta-analysis by Liu Q et al also confirmed that metformin induces a reduction in Vitamin B levels.¹¹ This evidence has been contended by few studies which showed that metformin might improve metabolism of vitamin B12 and only lowered the inactive form of vitamin B12 (holo-haptocorrin, holoHC) rather than the active form of vitamin B12 (Holotranscobalamin, holoTC).¹²

We observed that the serum homocysteine levels were significantly higher in the metformin group as compared to non-metformin group. Metformin users were found to have slightly higher homocysteine levels than non-users.¹³ De Jager et al, a randomized controlled trial of 4.3 years treatment with metformin resulted in a minor statistically significant increase in homocysteine concentrations.¹⁴ However, some studies have reported that decreases in vitamin B12 after metformin treatment did not lead to an increase in homocysteine levels.¹⁵ This could be interpreted as a simple plasma decrease of vitamin B12 levels, rather than a true tissue deficiency. This needs further investigations.

There are a few limitations of this study. First, small sample size of our study may limit the generalizability of our findings. Second, we did not collect information relating to haematological parameters like mean corpuscular haemoglobin. Third, patients were not asked about the medications not related to diabetes, higher intake of proton pump inhibitors could lead to vitamin B12 deficiency.

CONCLUSION

In our study population of type 2 diabetes mellitus, metformin use was associated with significantly lower vitamin B12 levels and significantly high homocysteine levels. Further study is required to understand the impact of duration of diabetes on vitamin B12 levels and the effect it will have on the long term clinical outcome of these patients. Role of supplementing vitamin B12 in type 2 diabetes mellitus patients taking metformin also needs to be investigated in future studies.

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