

EFFECT OF METFORMIN ON THE LEVEL OF VITAMIN B12 AND FOLATE IN PATIENTS OF TYPE 2DM, (DIABETES MELLITUS)

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Abstract

Background and Aim: Due to the clinical benefits of metformin, its associated side effects such as vitamin B12 deficiency are rarely investigate and usually overlooked. In the reports of previous study done; showed that there is association of deficiency of vitamin b12 and use of metformin. The primary aim of the present study is check the assessment of metformin on the folate and vitamin B12 levels. The study population consist of patients diagnosed with type 2 diabetes mellitus. Other objectives of the current study were; to assess and compare the serum level of vitamin B12 before and after metformin therapy, to find out the correlation of serum level of vitamin B12 with haemoglobin and MCV values, to assess and compare the serum levels of folate before and after metformin therapy & to find out the correlation of serum level of folate with haemoglobin level and MCV values.

Material and Methods: This is a hospital based randomized cross-sectional observational study. Each and every eligible case of type 2 diabetes attending medicine OPD, Tertiary care Institute of India for the duration of 1 year were included in the study. The metformin 500 mg was given twice daily for 6 months, the folate and serum vit. B12 levels of all the included patients were recorded.

At the end of the study period of 6 months the foalte levels and serum vit. B12 levels were reevaluated.

Results: On follow up data analysis the significant correlation was found between the change in MCV levels and serum vitamin B12 levels. A non significant correlation was found in the individuals after undergoing the treatment for metformin; between the change in MCV levels and serum folate level.

Conclusion: With the use of high dose and long duration of metformin there is significant low level of serum vitamin B12 level in individuals. Therefore for the patients diagnosed with type 2 diabetes mellitus regular check up for vitamin B12 levels should be done and sometimes its advised to prescribe them with vitamin B12 suppliments.

Key Words: Diabetes Mellitus, Metformin, Serum Folate levels, Vitamin B12 level

Introduction

The group of metabolic disorder which share the phenotype of hyperglcaemia is called as diabetes mellitus. Due to complex interaction of environmental factors and genetic factors there are different types of diabetic mellitus (DM). As per the etiological factors that leads to hypoglycemia includes: decreased glucose utilization, reduced insulin secretion and increase in production of glucose.¹⁻³

The metabolic dysregulation associated with DM causes secondary changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. There are two different categories of diabetes mellitus: Type I & Type II. When there is total or near to total amount of insulin deficiency it leads to type I diabetes mellitus. When there is impaired insulin secretion, different level in resistance of insulin and increase in glucose production it leads to type II diabetes mellitus.¹ The sugar test done with no caloric intake for at least 8 hours is defined as fasting sugar test. When the sugar test is done at any time regardless of the meal intake is defined as random blood sugar test.

The first line of oral treatment for increase in blood sugar levels in diabetic mellitus is metformin. However metformin is regarded for having very few side effects.⁴ In the earlier study done by Bercholdt et al. in 1969 concluded that metformin can leads to decrease in gastrointestinal tract absorption of vitamin B12 which leads to decrease in level of vitamin B12 level in body. After that there have been constant publication regarding the above matter.⁵ Due to increase in use of metformin it is reported that upto 30% of patients showed decrease in vitamin B12 levels in the body.^{6,7} Also some studies have shown that there is inverse relation of duration and dose of metformin and decrease in level of vitamin B12 in body.⁸⁻¹¹ Since large prospective studies have clarified this relationship recently,^{12,13} the 2017 American Diabetes Association treatment guidelines now recommend regular monitoring of vitamin B12 levels in patients with diabetes taking metformin.¹⁴

Due to variability in measurement methods the level of serum vitamin B12 is considered as an unreliable marker for overall vitamin B12 deficiency.¹⁵ The key component in one carbon pathway is the homocysteine that plays

important role in methylation of DNA & is involved in various intracellular vitamin B12 reactions.¹⁶ Due to increase in homocysteine levels there is increase in cardiovascular risk, cancer, chronic kidney diseases and is also known to be associated to vitamin B12 deficiency. Therefore, increased homocysteine levels in patients with serum vitamin B12 deficiency can more accurately predict vitamin B12 deficiency in true tissues.¹⁶ There are many studies that have investigated homocysteine increases in diabetic patients, but there is still controversy.^{17,18}

There are reports that show the association between vitamin B12 and Metformin usage.^{19,20} The mechanism that results in the decrease in vitamin B12 levels and use of metformin in diabetic mellitus is still not clear. However one of the possibilities suggested is change in the bowel motility that leads to increase in bacterial growth and results in vitamin B12 deficiency. Other theory includes inactivation of Vitamin B12 absorption, interaction with cubulin endocytic receptor and change in intrinsic factors levels. Also, inhibition of the calcium dependent absorption of vitamin B12-intrinsic factor (IF) complex at the terminal ileum has been suggested as one of the mechanisms.^{21,22,23} Although decrease in vitamin B12 levels following metformin use typically starts as early as the 4th month²⁴ clinical features of vitamin B12 deficiency become apparent by 5 years owing to the large body stores in the liver that are not quickly depleted. This is however influenced by increasing age and dose of metformin.^{25,26}

In the patients with type 2 diabetes mellitus the assessment of vitamin B12 is of clinical importance. It may result in peripheral neuropathy and can be mistaken for diabetic neuropathy in the patients on metformin treatment.²⁶ This may lead to unnecessary use of anticonvulsants, tricyclic antidepressants and other medications for diabetic neuropathy.^{27,28,29}

The main objective of the present study was to assess the levels of vitamin B12 and usage of metformin in diabetic mellitus patients population in India. Other objectives of the study were to compare & assess the serum level of vitamin B12 and find the correlation with MCV & haemoglobin levels, to compare the vitamin B12 level before and after the metformin therapy, to assess and compare the serum levels of folate before and after metformin therapy and to find out the correlation of serum level of folate with haemoglobin level and MCV values.

Material and Methods

This is a hospital based randomized cross-sectional observational study. Each and every eligible case of type 2 diabetes attending medicine OPD, Tertiary care Institute of India for the duration of 1 year were included in the study. The study was conducted after taking due approval from the institutional ethical committee. Study population Patients of type 2 diabetes, who are not taking metformin,

not having deficiency of vitamin B12 and/or folate and not taking any medication, which can affect the level of vitamin B12 and/or folate and who are not on any diet, which significantly affect the level of vitamin B12 and/or folate. Total 141 patients of type 2 diabetes those were not on metformin therapy and those were not taking vitamin B12 and/or folate and those were not taking any medication, which affect the level of vitamin B12 and/or folate and those were not taking any diet which significantly affects the level of vitamin B12 and/or folate.

The exclusion criteria included with patients of colectomy, inflammatory bowel diseases, gastrectomy and pernicious anemia. Patients with acute illness, presence of cancer, diagnosed with acute coronary syndrome with history of 3 months, presence of serious organ damage characterized by liver cirrhosis, estimated glomerular filtration rate (GFR) < 30 mL/min/1.73 m² or symptoms of heart failure. Patients with hematological disorders other than anemia, pregnant women, and vegetarian patients were also excluded. The 141 patients were between 19 to 86 years of age, with a mean age of 59.1 ± 10.7 years, and 58.5% were male.

Statistical analysis

At the end of the study the data was collected and entered in the excel sheet of computer program. The SPSS version 15 was used for the analysis of the collected data. For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

At the onset of the study 154 patients were enrolled. After 6 months on follow up 13 patients did not turn up and hence their data were not available. Rest of the 141 patients were evaluated and analyzed below: The study population is divided into three age groups with mean age 59 years. We had 13 male patients & 12 female in 36-40 years age group; 44 male patients & 34 female in 41-45 years age group; 22 male patients & 16 female in 46-50 years age group. In total 79 patients were male and 62 were female in study population (Table 1). Mean and standard deviation of various parameters of both the visits of patients i.e. 0 months and then at 6 months after taking metformin are shown in (Table 2).

Peripheral blood film which was done at the start of the study was found normocytic for all of the patients. After 6 months of metformin therapy 5 patients showed Macrocytic changes in peripheral blood film (Table 3). Correlation between change in serum vitamin B12 level and change in Hemoglobin concentration in our study. We found significant Correlation between change in serum vitamin B12 level and change in MCV in our study on follow up. We found a significant negative correlation. Correlation between change in level of serum folate and change in MCV after 6 months of metformin. We found a non-significant correlation between these two parameters.

Table 1: Distribution according to age and sex

Age Group (Years)	Males n	Females n	Total n
36-40	13	12	25
41-45	44	34	78
46-50	22	16	38
Total	79	62	141

Table 2: Mean±SD of various parameters before and after metformin group patients

Variables	Before 6 months of metformin	After 6 months of metformin	P value
Vitamin B12 (pg/ml)	867.2±85.1	735.6±190.7	0.02*
Folate (ng/ml)	10.6±1.70	10.09±1.74	0.14
MCV (fl)	81.90±2.47	84.90±5.41	0.001*
Hb (g/dl)	14.7±0.5	14.26±5.86	0.03*

*indicates statistically significance at $p \leq 0.05$

Table 3: Peripheral blood film changes observed during study period

Peripheral blood film	Before	After
Normocytic	141	136
Macrocytic	0	5
Microcytic	0	0

Discussion

Previous study result have shown that higher dose and longer duration of metformin have cause greater impact on vitamin B12 levels in patients with type 2 Diabetes Mellitus.¹⁹⁻²¹ The percentages of patients with vitamin B12 deficiency and with borderline deficiency observed in this study are in line with the report of Nervo et al.²² This observation could be an indication of nutritional deficiency or a consequence of metformin use. In current study 141 patients were classified in three different age groups like 36-40 years, 41-45 years, 46-50 years. Out of 141 patients 79 were found to be males and 62 were females. In current study we found a significantly positive correlation between change in vitamin B12 and change in hemoglobin concentration before and after metformin treatment. It shows that change in level of vitamin B12 positively correlates with change in concentration on hemoglobin.

The “golden standard” for defining vitamin B12 deficiency is not yet established, since there are various methods for measuring serum vitamin B12, corresponding normal values, and research results. One study reporting lower specificity showed that 60% of patients had symptoms of vitamin B12 deficiency when B12 level was < 200 pg/mL, and 90% at < 100 pg/mL.³⁴ In general however, serum vitamin B12 levels can be interpreted as follows: > 300 pg/mL, B12 deficiency is unlikely (probability of 1%–5%); 200 to 300 pg/mL, B12 deficiency possible (probability of 5%–15%); < 200 pg/mL, consistent with B12 deficiency (specificity of 90%–100%).^{32,35}

In the study we found significant but negative correlation between these two parameters. It states that when there is a change in level of vitamin B12, it inversely affects the level of MCV significantly. In one of the previous study done by Wulffele MG et al 850 mg thrice a day of metformin treatment was administered to patients for 16 weeks and this was found to be associated with a decrease in serum vitamin B12 level by 14%. The observed lower level of vitamin B12 in patients with T2DM who have been on metformin for 10 years or more compared with patients with < 10 years history of metformin use corroborates earlier studies.^{32,33,34} De jager et al.¹² showed that the negative impact of metformin use on vitamin B12 level becomes profound with increasing years of metformin use. This observation has been attributed to either or a combination of alteration in small bowel motility, alteration in intrinsic factor levels, interaction with the cubulin endocytic receptor and inhibition of the calcium dependent absorption of vitamin B12-intrinsic factor complex at the terminal ileum.^{9,10}

In our study we found a non significant correlation between change in serum folate level and change in hemoglobin concentration. It was found that change in level in folate does not significantly affect the concentration of hemoglobin. Wulffele MG et al administered 16 weeks treatment with metformin 850 mg thrice a day and found that the treatment was associated with a decrease in folate concentration of 7% which was non significant.³⁶

We found a non significant correlation between change in serum folate level and change in MCV It states that change in serum folate doesn't significantly affect the level of

MCV. Sahin et al reported that after treatment, metformin use was associated with an increase in levels of Hcy by 2.36 micromol/l and decreases in folate and vitamin B12 concentrations by -1.04 ng/ml and -20.17 pg/ml.³⁷ Metformin also significantly decreased body weight. In controls, there was no change in Hcy, folic acid, vitamin B12, TG, LDL, total-C, HbA1c, insulin, or HOMA levels. Homocysteine change did not correlate with insulin, folate, or vitamin B12 changes in the metformin and rosiglitazone groups.³⁸⁻⁴⁰

An association between obesity and poor glycaemic control has been reported.⁴¹ Nagrebetsky et al⁴² showed that there is a significant association between index of glycaemic control and lower glycated haemoglobin and lower BMI. These reports could explain the observed higher BMI in patients on high dose of metformin compared with patients on low dose. Limitation of study indicates the non-inclusion of age-matched control group and small sample size.

Conclusion

We concluded from end of study that higher dose and longer duration of metformin leads to decrease in serum vitamin B12 level. Hence there should be routine analysis of the vitamin B12 levels in the patients with diabetic mellitus and are under regular dose of metformin. This will lead to early start of vitamin B12 supplements in the patients with metformin. This can result in preventive method. This routine investigations leads to early detection and prevention of decrease in vitamin levels of patients.

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