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Original Article

Serum Vitamin B12 and Folic Acid Status of Patients with Type II DM on Metformin

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Abstract

Background: *Over the last decades, long term treatment with metformin has been recognized as a possible cause of alterations in serum B12 and folic acid value of type 2 diabetic patients. Metformin can affect the absorption of vitamin B12 and folic acid which ultimately results in neurological and hematological complications has been designed to assess serum vitamin B12 and folic acid status of type II diabetic patients on metformin.*

Materials and methods: *This hospital based cross sectional study was conducted in Department of Physiology, Chittagong Medical College in collaboration with Department of Medicine and Department of Endocrinology, Chittagong Medical College Hospital from July 2021 to June 2022. Total 70 diagnosed type 2 diabetic patients were included in the study among which 35 patients aged between 45-55 years and on metformin therapy for more than 2 years were taken as case group and in control group age and sex matched 35 diagnosed diabetic patients not treated with metformin were included. Serum vitamin B12 and folic acid were measured in all patients. Collected data was analyzed by SPSS-26.*

Results: *Values of serum vitamin B12 were significantly lower in metformin exposed diabetic patients compared to metformin unexposed patients ($p<0.001$). Folic acid levels of metformin exposed diabetic patients were also decreased but it was not statistically significant ($p>0.05$). Duration and dose of metformin had significant negative correlation with vitamin B12 level. But serum folic acid levels were not correlated with dose and duration of metformin use.*

Conclusion: *The results of this study concludes that long term use of metformin can alter vitamin B12 and folic acid level in type 2 diabetic patients.*

Key words: Diabetes Mellitus (DM); Folic acid; Metformin; Vitamin B12.

INTRODUCTION

Diabetes Mellitus is recognized as a serious public health concern that considerably influences human life and health expenditures.[1] It is a clinical syndrome characterized by hyperglycemia due to absolute or relative insulin deficiency. Diabetes is one of the world's leading causes of morbidity and mortality.[2]

Type 1 and type 2 diabetes are two general types of diabetes mellitus. Type 2 diabetes, accounting for about 90% to 95% of all cases of diabetes, is initially caused by insulin resistance.[3] Obesity, physical inactivity, ethnicity and genetic factors act as important determinants of insulin resistance.[4] Globally about 422 million individuals are affected by type 2 diabetes mellitus.2 According to the World Health Organization, the number of type 2 diabetic patients is expected to double within the next 25 years.[4]

Metformin is one of the most extensively used antidiabetic agents which is taken by almost 120 million people worldwide.[6] It is being used as monotherapy or in combination with other medications. Metformin, a biguanide, has been established as first line therapy for treatment of type 2 diabetes due to its efficacy, safety, multiple metabolic and cardiovascular benefits.[7]

Common side effects of metformin are gastrointestinal symptoms like nausea, abdominal distress, discount at soft stool, diarrhoea etc.[6] Patients on long term metformin therapy were found to be at risk of vitamin B12 and folic acid deficiency.[8,9,10] Vitamin B12 is essential for DNA synthesis. It is needed for the conversion of homocystein to methionine and methylmalonyl CoA to succinyl CoA for systhesis of myelin sheath.[11] Worldwide 3.9% to 51% diabetic patients on metformin had suffered from borderline to severe vitamin B12 deficiency.[12-17]

Exposer to metformin for more than 2 years was observed by the researchers as potential risk factor for developing B12 deficiency in diabetic individuals.13,16 Vitamin B12 level was significantly lower in patients who had been on metformin for more than 10 years. Similarly patients at a dose of 1000 mg/day had lower vitamin B12 level compared to the patients consuming less than 1000 mg/day.[18]

Metformin associated B12 deficiency leads to the damage of nervous system causing neuropathy. The most common symptoms are numbness, tingling, mood changes, poor energy and brain fog.16 37.8% of diabetic patients on metformin therapy for at least six months had subnormal serum B12 levels including 31.1% B12 deficiency and 6.7% borderline B12 status in Bangladesh.12 Several other observational studies, systematic reviews and meta-analyses have reported about association between long term and higher dose of metformin therapy with borderline or absolute vitamin B12 deficiency.[9,19,20] Insignificant drop in serum B12 level was documented in diabetic patients using metformin for at least one year.[1]

The mechanism by which metformin causes vitamin B12 deficiency has not been clearly established. The calcium dependent membrane action required for intrinsic factor-B12 complex uptake by the ileal receptors is thought to be altered by metformin.[11] Metformin may also affect the absorption of vitamin B12 by slowing of bowel transit time which results in bacterial overgrowth with consequential B12 deficiency.[18] Other theories include alteration of bile acid metabolism, increased accumulation of vitamin B12 in liver or reduction of intrinsic factor secretion by gastric parietal cells.[11,19]

Folic acid is an essential vitamin for human and obtained from diet specially fruits and vegetables.[21] 7% to 34% diabetic patients receiving metformin showed significant decrease in plasma folic acid level in different studies.[21,22] Decrease in serum folate levels of diabetic individuals after treatment with metformin were observed through multiple randomized placebo controlled trials.[22,23] No significant alteration in folate level of diabetic individuals taking metformin was also found by multiple studies.[24,25]

Folate reduces to tetrahydrofolate and acts as one carbon donor for methylation and DNA synthesis.[21] It is not yet established whether metformin competes with folic acid or folate transporter protein expression is downregulated by metformin.[22]

Long term use of metformin associated vitamin B12 and folic acid deficiency can be remained undiagnosed and untreated due to lack of proper monitoring. Routine assessment of serum vitamin B12 and serum folate level needs to be considered during long term metformin treatment for the well being of diabetic patients. Therefore this study has been designed to assess serum vitamin B12 and folic acid status of type II diabetic patients on metformin.

MATERIALS AND METHODS

This hospital based cross sectional study was conducted in Department of Physiology, Chittagong Medical College in collaboration with Department of Medicine and Department of Endocrinology, Chittagong Medical College Hospital from July 2021 to June 2022. Total 70 type 2 diabetic patients were included in the study among which 35 patients aged between 45-55 years and on metformin therapy for more than 2 years were taken as case group. 35 age and sex matched diabetic patients not treated with metformin were taken as control group. Patients having prior gastrectomy, gut resection, inflammatory bowel disease, liver disease, chronic kidney disease or malignancy were excluded. Vegetarians, alcoholics, pregnant and lactating mothers were also excluded.

Ethical clearance of this study was given by Ethical Committee of Chittagong Medical College. Administrative permission from Hospital Authority, Medicine Department and Endocrinology Department of Chittagong Medical College was taken prior to conduct this study. After screening by inclusion and exclusion criteria, 70 patients were recruited into this study. Informed written consent was taken from everyone and all the participants were interviewed by researcher. Then data was recorded in a predesigned case record form. Serum vitamin B12 and folic acid were measured in all patients. Analysis of liver enzyme (SGPT) and serum creatinine was done for detection of liver and kidney pathology to exclude the subjects.

Comparison of continuous and categorical data between two groups was done by Student's unpaired 't' test and Chi-square test respectively as test of significance. Pearson's correlation test was done to observe the correlation of dose and duration of metformin with vitamin B12 and folic acid. In the interpretation of results, p value <0.05 was considered as statistically significant.

RESULTS

Table I Comparison of age, sex, S.Creatinine and SGPT level between metformin exposed (Case) and metformin unexposed (Control) diabetic patients (n=70)

Attributes	Exposed Patients (n=35)	Unexposed Patients (n=35)	p value (test statistics)
Age (Years)	50.9 ±4.1 (45-55)	49.6 ±4.3 (45-55)	0.210^{ns} (t=1.267)
Sex: Male	7 (20%)	7 (20%)	1.0^{ns} (χ^2= 0.0)
Sex: Female	28 (80%)	28 (80%)	
S. Creatinine (mg/dl)	0.96±0.24 (0.48-1.3)	0.87±0.18 (0.52-1.3)	0.09^{ns} (t=1.704)
SGPT (IU/L)	27.42±12.29 (10.0-50.0)	28.71±11.02 (10.0-50.0)	0.647^{ns} (t=–0.461)

Unpaired student's 't'- test was done for age, S.Creatinine and SGPT. Values are expressed as Mean ± SD (Standard Deviation) n= number of subject, ns= statistically not significant (p>0.05), values in parenthesis indicate range.

Chi-square test was done for sex. Values in parenthesis indicate percentage.

Table I shows both groups were similar in age with similar proportion of male and female. There was insignificant difference between S. Creatinine and SGPT level of metformin exposed diabetic subjects and metformin unexposed subjects (p >0.05).

Table II Comparison of serum vitamin B12 and folic acid level between metformin exposed (Case) and metformin unexposed (Control) diabetic patients. (n=70)

Attributes	Exposed Patients Mean±SD (Range) (n=35)	Unexposed Patients Mean±SD (Range) (n=35)	p value (t value)
Serum vitamin B12 (pg/ml)	362.4±153.2 (148.4-796.0)	739.9±299.9 (302.0-1439.0)	<0.001** (–6.630)
Serum folic acid(ng/ml)	7.4±4.5 (1.3–20.0)	9.0±6.2 (1.4–24.0)	0.231ns (–1.207)

Unpaired student's 't'- test was done. Values are expressed as Mean ± SD (Standard Deviation), n= number of subject, ns= statistically not significant, ** = statistically significant.
Table II shows significantly lower serum vitamin B12 in metformin exposed (Case) diabetic subjects compared to metformin unexposed (Control) subjects (p<0.001). The mean serum folic acid level was lower in the metformin exposed cases than the metformin unexposed cases, but the difference was not statistically significant (p >0.05).

Table III Correlation of duration and dosage of metformin use with vitamin B12 and folic acid. (n=35)

Attributes	Vitamin B12 p value (r value)	Folic acid p value (r value)
Duration of metformin use	0.000** (-0.817)	0.749 ns (0.056)
Dosage of metformin	0.000** (-0.750)	0.265 ns (0.194)

r= Pearson Correlation coefficient, ns= statistically not significant (p>0.05), **= statistically
significant (p<0.001).
Table III shows that duration and dosage of metformin have significant negative correlation with vitamin B12 but have no correlation with folic acid level.

DISCUSSION

Age and sex matched (p>0.05) 70 diabetic patients were enrolled in the study among which 35 subjects were exposed to metformin and 35 subjects were not on metformin (Table I). Values of serum creatinine and Serum Glutamate Pyruvate Transaminase (SGPT) were within normal range in all participants (Table I).

In this study significant decrease in B12 value of metformin users compared to non users of metformin was found (p<0.001) (Table II). This study result was consistent with several previous studies.[6,22,27] Serum vitamin B12 value in patients with T2DM of this study had significant negative correlation with duration and dosage of metformin use (Table III)

There are multiple probable mechanisms regarding deficiency of vitamin B12 in metformin users. Metformin might diminish the uptake of cobalamin by cell membrane receptors of terminal ileum.11 Metformin is also thought to reduce the motility of small intestine. Other proposed mechanisms for B12 deficiency in metformin users include reduced secretion of intrinsic factor by gastric parietal cells and impaired enterohepatic circulation of vitamin B12 due to change in bile acid metabolism.[11,18] Opposite finding was revealed by some authors. In their study, diabetic subjects on metformin for minimum one year did not have vitamin B12 deficiency. They supposed that longer use of metformin by diabetic subjects might show different result regarding vitamin B12 status.[1]
There was slight decrease in folic acid level of metformin treated patients in comparison with metformin free patients in this study. But the result was statistically not significant (p>0.05) (Table II). Similar insignificant alterations in folate status of metformin users were found by multiple other investigators.[25] Serum folic acid value in patients with T2DM of this study had no correlation with duration and dosage of metformin use (Table III) Dietary pattern like sufficient intake of vegetables could be an important factor for this result.[22]

Some researchers also observed significant decrease in serum folic acid level of metformin exposed diabetic subjects in their studies.[27] In this study, it is observed that long term use of metformin affects vitamin B12 and folic acid status of type 2 diabetic patients. As this is an observational study, further longitudinal studies are necessary to establish the fact. Thus metformin mediated vitamin deficiencies can be halted at initial stage.

LIMITATIONS

The limitations of this study are short period of study, small sample size and it is conducted in only one tertiary level hospital.

CONCLUSION

The result of this study concludes that vitamin B12 levels are significantly decreased and folic acid levels are insignificantly decreased in metformin exposed diabetic individuals. Duration and dose of metformin is related to the changes in serum B12 level of diabetic people. Serum vitamin B12 and folic acid levels should be checked periodically to diagnose and treat the deficiency at earliest time.

DISCLOSURE

All the authors declared no competing interest.

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