

ORIGINAL ARTICLE

VITAMIN B12 STATUS IN LONG-TERM METFORMIN TREATED TYPE 2 DIABETES MELLITUS PATIENTS

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Abstract:

Background: The association between long-term use of metformin and low vitamin B12 levels in patients with type 2 diabetes mellitus (T2DM) has been proven. However, the prevalence among metformin induced vitamin B12 deficiency in T2DM patients showed considerable variations among the studies. The potential of deficiency to cause new onset or worse preexisting peripheral neuropathy in T2DM patients has been investigated with conflicting results. The objective of this study is to determine the frequency of vitamin B12 deficiency among patients with T2DM patients on metformin therapy compared to those who are not taking metformin. **Methods:** This cross-sectional analytical study was conducted at the Department of Medicine, in Sylhet M. A. G. Osmani Medical College & Hospital, Sylhet. Total 100 patients' with T2DM were selected according to inclusion and exclusion criteria following informed written consent. They were divided into two groups; each group contains 50 patients. Group-I included patients who were taking metformin for ≥ 1 year and Group-II included patients who were not taking metformin for at least 1 year. Detailed history taking, proper clinical examinations and relevant investigations were done. After collection of all the required data, final analysis was carried out by using the SPSS version 22.0. **Results:** Among 100 patients of T2DM, mean age were 55.4 ± 15.62 and 55.24 ± 16.76 years, respectively among Group-I and Group-II. They were similar in terms of demographic profile. About 16.0% patients of group-I had vitamin B 12 deficiency and 6.0% had vitamin B 12 deficiency in Group-II. The mean serum Vitamin B 12 was 481.52 ± 283.37 (pgm/mL) in group-I and 600.85 ± 294.2 (pgm/mL) in group-II. The difference of serum Vitamin B 12 was statistically significant ($p < 0.041$) between two groups. Reduced vitamin B 12 also found to be positively associated with duration of metformin intake ($p < 0.05$) and presence of neuropathy ($p < 0.05$). **Conclusion:** Metformin is significantly associated with decrease the level of vitamin B12.

Keywords: Diabetes mellitus, Metformin, Vitamin B12

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Introduction:

Diabetes mellitus (DM) may be defined as a group of metabolic disorder characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both.¹ Diabetes mellitus affects about 536.6 million people worldwide with prevalence 10.5% and In Bangladesh, a total 13.1 million diabetic people in 2021 with national prevalence of 12.5%.² Metformin is belongs to Biguanide class of anti-diabetic drugs. All previous and recent recommendations propose that metformin therapy has to be initiated, along with lifestyle modification at the time of DM diagnosis, if no contraindications. The exact mechanisms of metformin actions are not clear, but it is effective only in the presence of insulin. It reduces blood glucose primarily by suppressing glucose production by the liver and increased glucose uptake.³ In addition to suppressing hepatic glucose production, metformin also increases insulin sensitivity which enhances glucose uptake in the peripheral tissue, increases fatty acid oxidation, and decreases glucose absorption from the gut.⁴

The most common adverse effect of metformin therapy is GI upset and rarely lactic acidosis. Metformin therapy also an iatrogenic cause for new onset of peripheral neuropathy or exacerbation of preexisting peripheral neuropathy in T2DM patients.⁵ Vitamin B12 is a water-soluble vitamin obtained mainly from foods of animal protein. The main clinical spectrum of B12 deficiency is neurological and haematological changes. Vitamin B12 deficiency induced neuronal damages manifests as severe peripheral or autonomic neuropathy, sub-acute combined degeneration of the spinal cord, delirium and dementia.⁶⁻⁷ Vitamin B12 deficiency is traditionally diagnosed by haematological changes typically in the setting of megaloblastic anemia. Previous studies have shown increased prevalence of vitamin B12 deficiency in patients with T2DM patients on Metformin therapy, ranged between 5.8% and 52.0%.⁸ The deficiency occurs as dose and duration dependent manner. It may occur even within 6 weeks to 3 months after commencing metformin therapy.⁹ However, there are some conflicting reports refuted this association. The exact mechanism by which metformin causes vitamin B12 deficiency is little understood. It is thought to be due to either alteration in small bowel motility, which stimulates small bowel bacterial overgrowth and subsequent vitamin B12 deficiency, or by directly decreasing vitamin B12 absorption process.¹⁰⁻¹¹ Peripheral

neuropathy is caused by both diabetes mellitus and vitamin B12 deficiency which may produce overlapping clinical pictures. However, non-diabetic neuropathies may be present in patients with diabetes and may be treatable with therapeutic vitamin B12 or vitamin B complex mixtures containing B12 supplementation.

Methods:

This was a Cross-sectional analytical study conducted within the period starting from March 2018 to November 2019. Study population were non-pregnant adults with type 2 DM currently on Metformin therapy attending either OPD (Outpatient care) or IPD (Inpatient care) of Sylhet MAG Osmani Medical College Hospital (SOMCH) and equal number of age and sex matched T2DM patients who are not on metformin therapy. About 100 cases (metformin treated 50 and non-metformin treated 50 cases) were included. Cases were selected from IPD or OPD Department of Medicine, Sylhet MAG Osmani Medical College Hospital based on inclusion and exclusion criteria. Peripheral neuropathy elicited by using Toronto Clinical Scoring System, including Symptoms scores, Reflex scores & Sensory test scores. Monofilament 10 grams force for light touch, 128 hertz tuning fork for vibration sense, reflex hammer for knee & ankle jerk were used. Data was analyzed by using SPSS (Statistical Package for the Social Sciences) program (version 22.0). Results expressed in frequencies or percentages (mean \pm SD and median). Comparison of vitamin B₁₂ level in subgroups was done by Student's unpaired t-test. Frequency of reduced vitamin B₁₂ using recommended cut-off values compared in between subgroups by Chi-square test. Pearson's correlation test is used to observe correlation among the different variables (HbA1c, vitamin B₁₂, Random plasma glucose, Age, Metformin use etc.) and P value $>$ 0.05 considered as significant. Ethical clearance was taken from the ethical review committee of Sylhet MAG Osmani Medical College.

Results:

Total study population were divided into two groups, respondents who received metformin were included in Group I and who did not receive Metformin were included in Group II. It was observed that more than one fourth (26.0%) patients belonged to age 41-50 years in group I and 11(22.0%) in group II. The mean age was 55.4 \pm 15.62 years in group I and 55.24 \pm 16.76 years in group II. More than half (56.0%) patients were male in group I and 22(44.0%) in group

II. Almost three fourth (72.0%) patients belonged to duration of DM 1-10 years in group I and 46(92.0%) in group II. More than one third (36.0%) patients were smoker in group I and 17(34.0%) in group II. More than three fourth (78.0%) patients belonged to BMI ≤ 25 (kg/m²) in group I and 40(80.0%) in group II. The mean BMI was 23.63 ± 2.52 (kg/m²) in group I and 24.02 ± 2.53 (kg/m²) in group II. The mean SBP was 125.04 ± 16.93 (mmHg) in group I and

123.9 ± 14.51 (mmHg) in group II. The mean DBP was 77.44 ± 8.44 (mmHg) in group I and 78.68 ± 9.13 (mmHg) in group II. Almost one fourth (22.0%) patients had anaemia in group I and 8(16.0%) in group II. More than one third (36.0%) patients had HTN in group I and 17(34.0%) in group II. The differences of duration of DM was statistically significant ($p < 0.05$) between two groups but other parameter was not statistically significant ($p > 0.05$) between two groups.

Table I

The serum vitamin B₁₂ level of the participants in two groups (n=100)

Level of vitamin B ₁₂	Group I(n=50)	Group II(n=50)	Total (n=100)	P value
	No. (%)	No. (%)		
Normal range	34 (68%)	41(82%)	75	
Borderline deficient	8 (16%)	6 (12%)	14	^a 0.200
Deficient	8 (16%)	3 (6%)	11	
Mean±SD	481.52±283.37	600.85±294.2		^b <.0041
Range (min,max)	102,900	102,1100		
Total	50 (100)	50 (100)	100	

Table II

Distribution of the study patients by peripheral neuropathy (n=100)

Peripheral Neuropathy	Group I(n=50)		Group II(n=50)		p value
	n	%	n	%	
Presence of Neuropathy					
Mild neuropathy	22	44%	12	24%	
Moderate neuropathy	8	16.0	3	6.0	0.153
Severe neuropathy	3	6.0	1	2.0	
No neuropathy	28	56.0	38	76.0	

Table III

The association of serum vitamin B₁₂ level and the duration of metformin use (n=50) in group I

Duration of metformin use	Level of vitamin B ₁₂ of diabetic patients who received metformin		P value
	Within normal range (n=34) No. (%)	Below normal range (n=16) No. (%)	
	Mean ± SD	7.1±3.86	

Table IV

The relation between level of vitamin B₁₂ deficiency and daily dose of metformin (n=50) in group I

Dose of metformin (gm/day)	Level of vitamin B ₁₂ in diabetic patients who received metformin		P value
	Within normal range (n=34) n(%)	Below normal range(n=16) n(%)	
	0.5 - <1	12(35.3%)	
1 - <1.5	14(41.2%)	9(56.3%)	0.196
1.5 - <2	8(23.58%)	4(25.0%)	
≥2	0(0.0%)	1(6.25%)	

Table V*The relative frequency of peripheral neuropathy with respect to the level of vitamin B₁₂ in both group (n = 100)*

Peripheral neuropathy	Level of vitamin B ₁₂ in participants of both group (n=100)		Total	P value
	Within normal range(n=75)	Below normal range(n=25)		
	n (%)	n (%)		
Present	14(18.7%)	20(80.0%)	34	0.001
Absent	61(81.3%)	5(20.0%)	66	

Discussion:

Metformin is a preferred hypoglycemic drug used for treatment of type-2 DM along with lifestyle modifications and has been used for more than 50 years. This cross-sectional, analytical, and observational study aimed to determine the status of vitamin B₁₂ deficiency among metformin-treated and metformin-naïve type 2 DM patients. It is also compared the prevalence rates of peripheral neuropathy among both groups. Total study population were divided into two groups, respondents who received metformin were included in Group I and who did not receive metformin were included in Group II. Both groups of participants were matched for age. The mean age was 55.4±15.62 years in group I and 55.24±16.76 years in group II. In study of Owhim et al¹² they found the mean age of diabetic patients was 55.80 ± 9.3 years with majority belonging to age group of 51- 60 years which was nearly similar to present study.¹² It is important to match both groups for age because the risk of developing metformin treatment induced vitamin B₁₂ deficiency significantly influenced by increasing age, about 20% of elderly people without diabetes in a previous study were reported to have vitamin B₁₂ deficiency.¹³ No statistical difference was found in age, gender, between two groups.

In our study, the overall prevalence of subnormal level of vitamin B₁₂ was 25 % (deficiency and borderline deficiency were 11% and 14%, respectively). Vitamin B₁₂ deficiency differed in the two groups, which were 16 % in the metformin-treated group and 6 % in the non-metformin user group, statistical difference was not found in between two groups (p = 0.200). Statistical different was found in the mean total serum vitamin B₁₂ level of 481.52±283.37pg/ml and 600.85±294.2pg/ml metformin-treated and metformin-naïve groups, respectively, (p =<0.041) was reported in this study, with vitamin B₁₂ deficiency defined as values below 200 pg/mL. Our observation of a correlation of subnormal level of vitamin B₁₂ with metformin use is consistent with the results of previous studies. In one study it was found that in metformin users, B₁₂ deficiency was present in 14.1%

(95% CI 9.2-20.4) and in non-metformin users 4.4% (95% CI 1.6-9.4%).¹⁴ Alharbi et al¹⁵ found in Saudi individuals that the prevalence of B₁₂ deficiency was 7.8% overall, but 9.4% vs 2.2% in metformin users and non-metformin users, respectively.¹⁵ Statistical association had been found between the duration of metformin use and level of serum vitamin B₁₂ (p = 0.029). Low levels of vitamin B₁₂ are observed when metformin was taken for more than 5 years. There have been reports of decreased serum B₁₂ levels occurring as early even three to four months after the beginning of metformin therapy.⁸ For this reason we had included the patients who had taken metformin for at least one year as a standard period comparing to other studies. However, according to most study reports, vitamin B₁₂ deficiency occurs only after five to ten years of metformin therapy.⁵ This delay of starting or onset of B₁₂ deficiency may be due to the significant hepatic storage of vitamin B₁₂.¹⁶ Statistical association had not been found between the daily intake of metformin and serum vitamin B₁₂ level (p = 0.196) that is reduction of serum vitamin B₁₂ level does not correlated with increasing daily dose of metformin in our study. This negative correlation of daily metformin dose might be due to lower amount (the mean of total daily dose of metformin was 1134±418.75 mg) in this study compared to a dose of more than 2000 gm/day in one observation that showed low levels of B₁₂ is directly proportional to higher daily use.¹⁵ Since, we feel that this fact highlights the possibility of this complication in patients on higher dose of metformin over an extended period of time and clinicians should be aware of this association and should consider regular screening for vitamin B₁₂ deficiency because it can be easily treated.

Statistical difference was not found significant between metformin users and non-users type 2 diabetic patients regarding peripheral neuropathy (44% versus 24%, respectively, p = 0.153) as well as in metformin-treated participants with regards to normal and subnormal vitamin B₁₂ (p = 0.136). Alharbi et al.¹⁵ also didn't find significant differences in presence of neuropathy between the metformin users

& non-metformin users ($p > 0.05$).¹⁵ Our results are in-line with those of a recently published study which reported that metformin use was not associated with the presence of diabetic peripheral neuropathy in T2DM patients.¹⁷ But our results are matched with the case - control study of Wile and Toth who reported more severe neuropathy among T2DM patients on metformin compared to non-metformin group.⁵

According to our study there was association between level of vitamin B₁₂ and development of peripheral neuropathy among the patients of diabetes of both groups, where the lower level of vitamin B₁₂ increases development of peripheral neuropathy ($p = 0.001$). Our findings are similar to some other study results. In those with diabetic neuropathy, altered (low and borderline) vitamin B₁₂ level was 64% (95% CI: 47–78%) compared to 17% (95% CI: 10–26%) in patients without diabetic neuropathy.¹⁸ But Ahmed et al¹⁹ didn't find significant difference in presence of neuropathy between those with normal and deficient vitamin levels (36.8% vs 32.3%, $p = 0.209$).¹⁹ Vitamin B₁₂ mal-absorption is a chronic complication of metformin therapy which can present with irreversible neuronal damage ranging from paresthesia and decreased peripheral sensation.²⁰ Unfortunately, the symptoms of diabetic neuropathy overlap with impaired vibration sensation and proprioception, as well as paresthesia, which have also been found to be associated with vitamin B₁₂ deficiency. Therefore, it has been suggested that neuropathy and vitamin B₁₂ deficiency symptoms should be routinely assessed in individuals with diabetes mellitus, via standard neurological examination.¹⁵

Therefore, we regard this as a statistically substantial percentage of vitamin B₁₂ deficiency in metformin use and as a valuable manual for the clinicians to consider it a vital element during the care of the patients with diabetes, predominantly when there has been the use for longer durations and significantly higher dosage of metformin treatment. Though the exact medical importance along with the effect of insufficiency is unidentified, some have proposed that there may very well be an important risk factor for precipitation and/or deterioration of neuropathies along with anemias in a population who are already susceptible to these complications due to the presence of underlying co-morbid diabetes.

Conclusion:

Serum vitamin B12 deficiency occurs more frequently in patients of metformin treated T2DM patients and peripheral neuropathy was more among those having subnormal level of vitamin B12.

Recommendations:

So, our recommendation is that vitamin B12 should be measured prior to initiation of metformin therapy and later annually in patients of T2DM who are on long-term metformin therapy (eⁿ1 year).

Limitations:

Detailed dietary history is not taken in this study. Therefore, Vitamin B12 deficiency due to metformin or poor dietary habit could not be ruled out. Sample size was relatively smaller, comparison with different subgroup in association with low vitamin B12 could not show significant difference.

Conflict of Interest:

The author stated that there is no conflict of interest in this study

Funding:

No specific funding was received for this study.

Ethical consideration:

The study was conducted after approval from the ethical review committee. The confidentiality and anonymity of the study participants were maintained

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