

# An Observational Study to Evaluate the Effect of Different Dosage of Metformin on Serum Vitamin B12 in a Cohort of Eastern Indian Type 2 Diabetes Subjects

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## ABSTRACT

**Aim:** Vitamin B12 deficiency is one of the major and common problem in subject with type 2 diabetes and this situation might get worsened in subjects who are on metformin therapy. The main aim of this study was to evaluate the effect of different dosage of metformin on serum Vitamin B12 in Indian Type 2 Diabetes (T2DM) subjects.

**Methods:** This observational study was carried out in a tertiary care clinic where 365 patients were treated with metformin  $\leq 1000$  mg for at least 3 years ( $5 \pm 0.9$ ) and 360 patients were on metformin  $\geq 1500$  mg at least 3 years ( $5 \pm 0.8$ ). Demographical data was recorded in a predesigned format and blood glucose level was performed in a NABL accelerated pathological laboratory. Serum vitamin B12 was measured by using an immunoassay method.

**Results:** Mean serum vitamin B12 levels in subjects who received higher dose of metformin ( $\geq 1500$  mg) ( $259.19 \pm 123.02$ ) were significantly lower than in subjects who received lower dose of metformin ( $\leq 1000$  mg) ( $307.31 \pm 107.9$ ) (p value 0.037). In addition, mean vitamin B12 in serum levels was found to be in vegetarians ( $321.13 \pm 103.04$ ) were significantly lower than B12 in serum levels was found to be in non-vegetarian ( $389.41 \pm 105.9$ ) (p value 0.01).

**Conclusion:** Among T2DM patients on metformin therapy vitamin B12 deficiency is high. Lowering levels of vitamin B12 is directly related to higher dose of metformin.

**Keywords:** Metformin, Serum Vitamin B12, Type 2 Diabetes (T2DM), Eastern Indian.

## INTRODUCTION

Berchtold *et al.* in 1969 was first described that Vitamin B12 deficiency is a known adverse effect of chronic metformin use [1] and there after several RCT was done internationally to establish the concept and also confirmed this deficiency was having population based variations of 5 to 40%. [2-5]

After lifestyle modification and exercise, American Diabetes Association (ADA) considered metformin as the first drug of choice. Metformin reduced risk of cardiovascular mortality in T2DM [6] improves peripheral insulin sensitivity [7] and also having beneficial effects on vascular protection with weight loss. [8]

The mechanism by which metformin therapy lowers the vitamin b12 deficiency is still unknown. It has been hypothesised that metformin stimulate small bowel bacterial overgrowth by alterations in small bowel motility which leads to vitamin B12 deficiency or it could decrease the absorption of vitamin B12. [9-11]

Association between metformin use and vitamin B12 deficiency in Indian T2DM population who belongs to its eastern parts, is yet to be elucidated despite the growing evidence of metformin-induced vitamin B12 deficiency.

The main aim of this observational comparative study was to evaluate the effect of different dosage of metformin on serum Vitamin B12 in eastern Indian Type 2 Diabetes (T2DM) subjects.

## METHODS

This study was carried out in a tertiary care clinic where 365 type2 DM subjects who were on metformin  $\leq 1000$  mg for at least 3 years and 360 type2 DM subjects treated who were on metformin  $\geq 1500$  mg at least 3 years were selected. Demographical data was recorded in a predesigned format and blood biochemistry was performed in a NBL accelerated pathological laboratory. Serum vitamin B12 was measured by using an immunoassay method.

Subjects of both the gender having age between age  $\geq 18$  years were identified and recruited during their regular clinical visit. Minimum dose of Metformin was selected as 500 mg and maximum dose of metformin was 2000 mg.

Subject with Type 2 Diabetes or any of the following comorbidities like history

of thyroid disease or malnutrition or inflammatory bowel disease or pernicious anaemia or past history of gastrointestinal surgery were excluded from the study.

The data was recorded in an Excel sheet of Microsoft Office and analysed using statistical test comparisons were performed using software like analysis of covariance (ANCOVA).

## RESULT

Mean age of the study group was  $52.12 \pm 4.01$  years and in both the groups subjects were on metformin for more than 3 years duration. 45% patients in metformin  $\leq 1000$  mg study group and 51% patients in metformin  $\geq 1500$  mg study group were male. Following table 1 describe the demographic data in detail.

**Table 1: Demographic profile of the patients**

Variables	Metformin $\leq 1000$ mg (N=365)	Metformin $\geq 1500$ mg (N=360)	P value
Mean age (years)	$52.61 \pm 4.32$	$51.37 \pm 4.49$	0.29
Years with diabetes and Metformin	$5 \pm 0.9$	$5 \pm 0.8$	0.01
Male	163 (45%)	186 (52%)	0.89
Female	202 (55%)	174 (48%)	0.92
Mean BMI	$24.27 \pm 3.73$	$24.61 \pm 2.98$	0.61

Table 2 demonstrated the distribution of mean fasting Blood Glucose (FBG), Post Prandial Blood Glucose (PPBG) and glycated Hb (HbA1c) in both the study groups.

**Table 2: Distribution of mean fasting Blood Glucose (FBG), Post Prandial Blood Glucose (PPBG) and glycated Hb (HbA1c) in both the study groups.**

Variables	Metformin $\leq 1000$ mg (N=365)	Metformin $\geq 1500$ mg (N=360)	P value
FBG (mg/dl)	$123.14 \pm 8.02$	$128.03 \pm 6.43$	0.03
PPBG (mg/dl)	$161.21 \pm 13.89$	$163.29 \pm 14.03$	0.03
HbA1c (mg/dl)	$6.89 \pm 0.5$	$6.87 \pm 0.5$	0.04

Mean serum vitamin B12 levels in subjects who received higher dose of metformin ( $\geq 1000$  mg) ( $259.19 \pm 123.02$ ) were significantly lower than in subjects who received lower dose of metformin ( $\leq 1000$  mg) ( $307.31 \pm 107.9$ ) (p value 0.037). (Table 3)

**Table 3: Distribution of mean vitamin B12 in both the study group.**

Variables	Metformin $\leq 1000$ mg (N=365)	Metformin $\geq 1500$ mg (N=360)	P value
Serum vitamin B12 (pg/ml)	$307.31 \pm 107.9$	$259.19 \pm 123.02$	0.037

Sub analysis of the data reveals that a large number of subjects in both the groups reveals that there was a large number of subjects were on borderline in both the groups. Elaborated data shared in table 4.

**Table 4: Distribution of different range of serum Vitamin B12 level**

Groups	Low <200 pg/mL	Borderline 200–300 pg/mL	Normal Range >300 pg/ml
Metformin $\leq 1000$ mg (N=365)	69 (19%)	171 (47%)	125 (34%)
Metformin $\geq 1500$ mg (N=360)	78 (22%)	197 (55%)	91 (25%)

In addition, mean vitamin B12 in serum levels was found to be in vegetarians ( $321.13 \pm 103.04$ ) were significantly lower than B12 in serum levels was found to be in non-vegetarian ( $389.41 \pm 105.9$ ) (p value 0.01). (Table 5)

**Table 5: Distribution of vitamin B12 between vegetarian and non-vegetarian.**

Variables	Vegetarians (N= 49)	Non-vegetarian (N=76)	P value
Mean Vitamin B12 levels (pg/ml)	$321.13 \pm 103.04$	$389.41 \pm 105.9$	0.01

## DISCUSSION

From this observational study it can be concluded that treatment with metformin as an oral anti diabetic agent to treat type 2 diabetes subjects resulted in statistically significant reduction in serum vitamin B12 concentration. Diabetic peripheral neuropathy (DPN) can cause or exacerbate by Low B12 levels due to prolonged metformin use. [12] Cerebral functions like memory, cognition, alertness etc. also can be altered by low serum B12 levels. 20% of the adult diabetic population suffer from DPN and it is considered as a common disorder. [13]

For the development and function of brain and nerve function Vitamin B12 plays a critical role. The most significant concern is that because vitamin B12 is essential to the formation and maintenance of the myelin sheath thus even a patient who is managing their blood sugars at healthy levels with high dose of metformin could still experience severe and permanent nerve damage. According to the National Institutes of Health, as much as 15% of people without diabetes, B12 deficiency is considered common. It had been observed in several study that vitamin B12 deficiency is associated with dosage and duration of metformin. [14]

Qureshi et al., documented a high prevalence of vitamin B12 deficiency of 33% among adult patients with T2DM and vitamin B12 deficiency was defined as serum vitamin B12 concentrations  $<150$  pg/ml. [15] Even in this trial 19% subjects have reported with low serum Vitamin B12 level ( $<200$  pg/mL) in Metformin  $\leq 1000$  arm and the same was 22% in Metformin  $\geq 1500$  arm. In addition to this there were a large number of subjects in both the groups belongs to borderline which is a major

concern. Like this study De-Jager et al and Ting R et al also confirmed that clinical factors like high dose ( $>1$  g/day) and long-term (4 years) metformin treatment known to be associated with vitamin B12 deficiency. [16,17] Bring R also demonstrated that, the adjusted odds ratio was 2.4 (95% confidence interval, 1.46-3.91) among patients using metformin for  $\geq 3$  years, compared with those who had received metformin for  $\leq 3$  years. Even in this trial, mean years with diabetes and Metformin use was  $5 \pm 0.9$  years in one arm and in another it was  $5 \pm 0.8$  years, which once again confirmed that long duration of metformin uses can be a key factor for developing vitamin B12 deficiency.

In this study it has been found that, mean vitamin B12 in serum levels was found to be in vegetarians ( $551.13 \pm 293.04$ ) were significantly lower than B12 in serum levels was found to be in non-vegetarian ( $679.41 \pm 285.9$ ) (p value 0.01). This once gain confirms that low vitamin B12 levels is associated with vegetarian diet. Due to cultural and religious beliefs in India, a country with a large proportion of vegetarians very high prevalence of vitamin B12 deficiency among the general population has been reported previously like this trial. [18,19]

Very high body fat or BMI also have a strong association with deficiency of vitamin B12. [20,22] Even in this observational study there was an association of high BMI with deficiency of vitamin B12 in serum levels. Though the main reason of association of obesity with vitamin B12 deficiency is still unknown but it can hypothesize that poor dietary content, malabsorption, unhealthy diet frequently seen in obese patients which leads to the comorbidities with vitamin B12 deficiency.

[23-25] Among obese T2DM patient's metformin is frequently prescribed at higher doses with long duration period, there is the need for regular monitoring of vitamin B12 levels. Screening for vitamin B12 deficiency among patients with T2DM is still not advocating by any published guidelines, screening for vitamin B12 deficiency prior to initiation of metformin and later annually among elderly patients with history of long term use of metformin ( $\geq 3$ -4 years), use of high doses of metformin ( $\geq 2$  g/day) is highly recommended.

Considering the regular screening of vitamin-B12 as a cost factor, the peripheral smear can be done in T2DM patients on long-term metformin to detect macrocytic anemia followed by biochemical analysis periodically.

## CONCLUSION

Among T2DM patients on metformin therapy in eastern India, Vitamin B12 deficiency is high. Lowering levels of Vitamin B12 is directly related to higher dose of metformin. Male are having much lower levels of vitamin b12 as compared to female. There is the need for regular monitoring of vitamin B12 levels.

**Conflicts of interest:** The authors state that they have no conflicts of interest.

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