

# Serum Vitamin B12 Levels in Type 2 Diabetes Patients on Metformin Compared to those Never on Metformin: A Cross-sectional Study

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## Abstract

**Context:** There are limited data about the effect of metformin use on serum Vitamin B12 levels in type 2 diabetes patients from India. **Aims:** We studied serum Vitamin B12 levels in patients with type 2 diabetes mellitus who were receiving metformin and compared them to those never treated with metformin. **Subjects and Methods:** A total of 183 patients (“metformin” group 121, “no metformin” group 63) of type 2 diabetes from the endocrinology clinic of a tertiary care center in North India were studied. Serum Vitamin B12 levels were measured in all patients. Diabetic neuropathy symptom score (DNS) and diabetic neuropathy examination score (DNE) were used to assess peripheral neuropathy while hemoglobin and mean corpuscular volume (MCV) were used to assess anemia. **Results:** The serum Vitamin B12 levels were  $267.7 \pm 194.4$  pmol/l in metformin group and  $275.1 \pm 197.2$  pmol/l in the no metformin group ( $P = 0.78$ ). When adjusted for duration of diabetes, metformin use was associated with a  $87.7 \pm 37.7$  pmol/l (95% confidence interval [CI],  $-162.1$ – $3.3$ ,  $P = 0.02$ ) lower serum Vitamin B12 levels. No significant increase in the prevalence of neuropathy (DNS and DNE scores), anemia, or MCV was found in the Vitamin B12 deficient patients (levels  $<150$  pmol/l) as compared to patients with normal Vitamin B12. However, serum Vitamin B12 levels for the entire cohort were higher by  $12.2 \pm 3.0$  pmol/l (95% CI  $6.4$ – $18.0$ ,  $P < 0.001$ ) for every 1 year increase in the duration of diabetes. **Conclusions:** Metformin use was associated with a lower serum Vitamin B12 levels when adjusted for duration of diabetes. Increasing duration of diabetes was associated with higher serum Vitamin B12 levels.

**Keywords:** Diabetic neuropathy score, metformin, type 2 diabetes, Vitamin B12 levels

## INTRODUCTION

Metformin is a first-line drug in the pharmacotherapy of type 2 diabetes. Apart from the low cost, good efficacy, and beneficial effects on body weight, the relatively safe adverse effect profile has justified the widespread use of metformin. Several studies have reported association of Vitamin B12 deficiency in type 2 diabetes patients treated with metformin.<sup>[1-6]</sup> The reported Vitamin B12 deficiency in the general population of India varies from 12% to 67%.<sup>[7,8]</sup> The impact of metformin use on an already Vitamin B12 deficient population is an interesting question. This study was done to further explore this question.

## SUBJECTS AND METHODS

The patients were recruited from the endocrinology outpatient department of a tertiary care hospital from August 2014 to December 2015, after taking informed consent.

Patients were defined as type 2 diabetes if they met the American Diabetic Association criteria with an age at diagnosis above 30 years.<sup>[9]</sup> Pregnancy, pernicious anemia, malabsorption syndrome, gastrointestinal surgery, autoimmune thyroid disease, chronic hepatitis, chronic kidney disease stage 4 and above, chronic alcohol abuse, and the use of oral/parenteral Vitamin B12 or multivitamin supplements were exclusion criteria.

An observational study was conducted from August 2014 to December 2015. The study was approved by the Institute

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Ethical Committee. “Metformin” group consisted of patients with type 2 diabetes mellitus with ongoing treatment with metformin with duration of metformin use  $\geq 3$  months while “no metformin” group consisted of patients with type 2 diabetes mellitus who had never received metformin. The patients who did not consume eggs or any form of meat were considered as vegetarian while those consuming eggs or meat were considered as nonvegetarian. History about diabetes onset, dose, and duration of metformin usage as well as recent (within 3 months) glycosylated hemoglobin, hemoglobin, and mean corpuscular volume (MCV) were obtained from hospital records. The hospital records, patient’s prescriptions, and medicines were also searched for prescription of any Vitamin B12-containing supplements and patients were shown a list of commonly available multivitamins containing Vitamin B12 and were asked about their use at any time in the past.

Assessment for peripheral neuropathy was done using diabetic neuropathy symptom score (DNS) and diabetic neuropathy examination score (DNE). DNS score  $\geq 1$  was considered suggestive of neuropathy while DNE score  $\geq 3$  was considered suggestive of peripheral neuropathy.<sup>[10,11]</sup>

Blood samples were drawn, and serum was stored at  $-20^{\circ}\text{C}$ . Later, these were used to estimate serum total Vitamin B12 levels. Vitamin B12 estimation was done by a solid phase, competitive chemiluminescent enzyme immunoassay on Immulite 1000 analyzer using commercial kits from Siemens Healthcare Diagnostics Inc., (New York, USA). The calibration range of this assay was 111–885 pmol/l. The interassay coefficients of variation (CV) were 7.4% (310 pg/ml), 6.1% (660 pg/ml), and 17% (1192 pg/ml) while the intra-assay CV were 11.3% (159 pg/ml), 10.3% (204 pg/ml), 6.7% (401 pg/ml), 5.3% (736 pg/ml), and 5.9% (1308 pg/ml). Vitamin B12 deficiency was defined as levels below 150 pmol/L and borderline deficiency levels between 150 and 221 pmol/L.<sup>[12]</sup>

Statistical analysis was performed using Statistical Package for Social Sciences SPSS version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were described as mean and standard deviation. The categorical variables were stated as proportions or percentages. The comparison between the “metformin” group and “no metformin” group was done with Student’s *t*-test for continuous variables and Chi-square test for categorical variables. Linear regression analyses were carried out to study the effect of duration since the diagnosis of diabetes, use of metformin, and duration of metformin use on serum Vitamin B12 levels.  $P < 0.05$  was considered statistically significant.

## RESULTS

A total of 183 patients were studied. The mean age of the study population was  $49.8 \pm 10.2$  years. Table 1 shows the baseline characteristics of the “metformin” and “no metformin” groups. The two groups were comparable except for duration of diabetes which was significantly greater in the metformin group. Duration of metformin use was  $27.3 \pm 35.8$  months (range 3–180 months). Maximum daily dose of

metformin was  $834.1 \pm 754.2$  mg (range 500–2550 mg). The cumulative dose of metformin was  $980.6 \pm 1576.1$  g (range 75–10,950 g).

The mean unadjusted Vitamin B12 was not significantly different in the “metformin” and “no metformin” groups:  $267.7 \pm 194.4$  pmol/l (95% confidence interval [CI] 233.6–302.7) versus  $275.1 \pm 197.2$  pmol/l (95% CI 229.3–334.1), respectively,  $P = 0.78$ . However, the mean duration of diabetes was longer in the “metformin” group as compared to the “nonmetformin” group ( $5.6 \pm 4.7$  years vs.  $2.0 \pm 3.9$  years,  $P < 0.001$ ). Vitamin B12 deficiency was present in 35.5% of “metformin” group and in 33.8% of the “no metformin” group ( $P = 0.93$ ) while borderline deficiency was seen in 22.3% of “metformin” group and 21% of “no metformin” group.

On univariate linear regression analysis with Vitamin B12 levels as the dependent variable and metformin use (no and yes) as the predictor variable, metformin use was associated with a  $8.7 \pm 30.9$  pmol/l (95% CI  $-69.7$ – $52.3$ ,  $P = 0.78$ ) lower Vitamin B12 level. On univariate linear regression analysis with Vitamin B12 levels as the dependent variable and duration of diabetes as the predictor variable, Vitamin B12 levels were  $12.2 \pm 3.0$  pmol/l (95% CI 6.4–18.0,  $P < 0.001$ ) higher for every 1 year increase in the duration of diabetes.

To further study the association of Vitamin B12 levels and duration of diabetes, we performed a stratified analysis. Figure 1 shows the box plot of serum Vitamin B12 with duration of diabetes categorized into newly diagnosed ( $< 1$  year), 1–5 years, and  $\geq 5$  years. Serum Vitamin B12 levels were higher by 41.5 pmol/l in patients with diabetes duration of 1–5 years compared to those with recently diagnosed diabetes ( $P = 0.31$ ). Serum Vitamin B12 levels were higher by 119.2 pmol/l in patients with duration of diabetes  $\geq 5$  years compared to those with recently diagnosed diabetes ( $P < 0.01$ ). Similarly, serum Vitamin B12 levels were 77.7 pmol/l higher in  $\geq 5$  years diabetes duration group compared to 1–5 year duration of diabetes group ( $P = 0.01$ ).

On univariate linear regression analysis with Vitamin B12 levels as the dependent variable and duration of metformin use as the predictor variable, duration of metformin use predicted a

**Table 1: Baseline characteristics of “metformin” and “no metformin” groups**

Variable	Metformin (n=121)	No metformin (n=62)	P
Age (years)	50.1±11.5	49.6±9.5	0.78
Sex, n (%)			
Male	66 (54.5)	30 (48.3)	0.43
Female	55 (45.5)	32 (51.7)	
Vegetarian (%)	61 (50.4)	39 (62.9)	0.10
Duration of diabetes (years)	5.64±4.71	1.99±3.86	<0.001
BMI (kg/m <sup>2</sup> )	26.25±4.21	27.02±4.45	0.25
Serum creatinine (mg/dl)	0.86±0.30	0.72±0.29	0.16
HbA1c (%)	8.08±1.6	8.33±1.77	0.40

BMI: Body mass index, HbA1c: Glycosylated hemoglobin

0.8 ± 0.4 pmol/l (95% CI -0.004–1.7 pmol/l,  $P = 0.05$ ) lower Vitamin B12 levels for every 1 month increase in the duration of metformin use. On stratifying duration of metformin use into no metformin use, 0–2 years, 2–5 years, and more than 5 years, it was found that a 20.2 pmol/l ( $P = 0.62$ ) and 37.1 pmol/L lower serum Vitamin B12 concentration was observed in individuals with a 0–2 years and 2–5 year duration of metformin use, respectively, compared with the group which had not received metformin. In contrast, the serum concentration of Vitamin B12 was higher by 45.2 pmol/l ( $P = 0.29$ ) in individuals who had received metformin for more than 5 years compared to those who had never received metformin [Figure 2]. To understand the interplay of duration of diabetes and metformin use on serum Vitamin B12 levels, a stratified analysis was carried out. A multivariate linear regression analysis with serum Vitamin B12 levels as the dependent variable and metformin use (no/yes) and duration of diabetes (stratified as 1–5 years and ≥5 years) as predictor variables were done to adjust for the duration of diabetes. In this analysis, metformin use group was associated with a 87.7 ± 37.7 pmol/l (95% CI, -162.1–3.3,  $P = 0.02$ ) lower serum Vitamin B12 levels. The serum Vitamin B12 levels were 102.7 ± 48.0 pmol/l (95% CI, 8.0–197.4,  $P = 0.03$ ) higher in the 1–5 year duration of diabetes group while they were 192.1 ± 51.9 pmol/l (95% CI, 89.8–294.5,  $P \leq 0.001$ ) higher in ≥5 year duration of diabetes group.

The serum Vitamin B12 levels in the “metformin” and “no metformin” groups stratified according to duration of diabetes (new onset, 1–5 years and ≥5 years) are shown in the box plot given in Figure 3. The serum Vitamin B12 levels were 97.5 pmol/l lower in the “metformin” group as compared to the “no metformin” group in the new onset diabetes (<1-year duration since diagnosis of diabetes) category. In the 1–5 year duration of diabetes category, the serum Vitamin B12 levels were 30.8 ± 45.7 pmol/l ( $P = 0.50$ ) lower in the “metformin” group as compared to the “no metformin” group. On analyzing the ≥5 years duration of diabetes category, the serum Vitamin B12 levels were 203.9 ± 75.8 pmol/l ( $P < 0.001$ ) lower in the “metformin” group as compared to the “no metformin” group.

On comparing patients with Vitamin B12 deficiency to those with normal Vitamin B12 levels, Vitamin B12-deficient patients did not have significantly higher percentage of DNE or DNS positives. Mean hemoglobin and MCV were also not significantly different in the Vitamin B12 deficiency and normal Vitamin B12 groups [Table 2].

## DISCUSSION

The present study involving 183 patients with type 2 diabetes mellitus (121 metformin and 62 without metformin) showed lower Vitamin B12 levels with metformin use, when adjusted for duration since diagnosis of diabetes, which is consistent with other studies published earlier.<sup>[1-6]</sup> Without adjusting for duration of diabetes, there was neither a significant difference in serum Vitamin B12 levels nor in the prevalence of Vitamin

B12 deficiency. However, the prevalence of Vitamin B12 deficiency in patients on metformin in our study is higher than that reported in Western literature. In different studies, Vitamin B12 deficiency was found in 5.8%, 8.6%, 6.3%, of patients

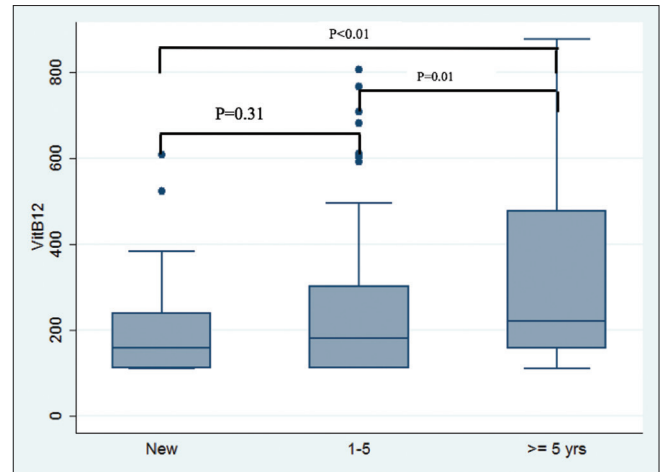


Figure 1: Vitamin B12 levels and duration of diabetes (new onset, 0–5 years and ≥5 years)

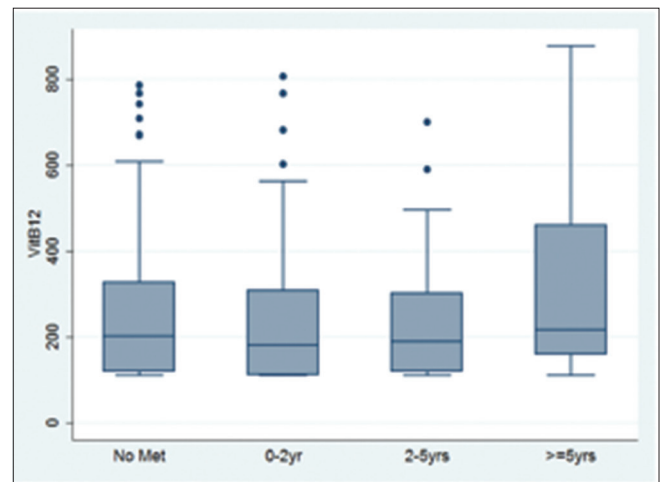


Figure 2: Effect of duration of metformin use on serum Vitamin B12 levels

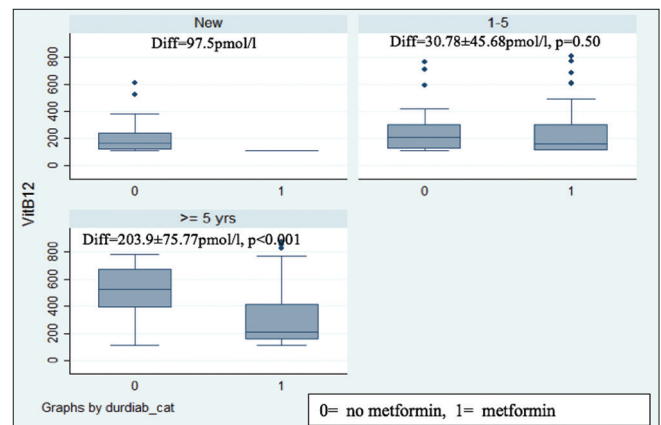


Figure 3: Vitamin B12 levels by metformin use and duration of diabetes

**Table 2: Neuropathy scores and hemoglobin in Vitamin B12 deficient and normal Vitamin B12 patients**

Parameter	Vitamin B12* <150 pmol/L (n=58)	Vitamin B12 >150 pmol/L (n=125)	P
DNE positive <sup>†</sup> , n (%)	19 (32.8)	49 (39.2)	0.40
DNS positive <sup>‡</sup> , n (%)	25 (39)	62 (52.1)	0.14
Hb (g/dl), mean±SD	12.80±1.56	12.23±1.79	0.11
MCV (fl), mean±SD	85.33±5.99	86.34±6.15	0.43

\*Serum Vitamin B12 levels, <sup>†</sup>DNE score  $\geq 3$ , <sup>‡</sup>DNS score  $\geq 1$ .

DNE: Diabetic neuropathy examination, DNS: Diabetic neuropathy symptom, Hb: Hemoglobin, MCV: Mean corpuscular volume, SD: Standard deviation

with type 2 diabetes mellitus on metformin.<sup>[1-3]</sup> The higher prevalence in our study (35.5%) is not surprising considering that the prevalence of Vitamin B12 in the apparently healthy populations in India have been reported to be as high as 33.3%–67%.<sup>[6,7]</sup> A predominantly vegetarian diet could be one of the causes of higher prevalence of Vitamin B12 deficiency in India.<sup>[7]</sup>

Metformin-induced Vitamin B12 deficiency has been ascribed to the binding of the hydrophobic tail of biguanide to the hydrocarbon core of membranes. The biguanide group being positively charged (protonated) gives a positive charge to the membrane and can displace divalent cations such as calcium. The uptake of Vitamin B12 into the ileal cells is calcium dependent and can thus be impaired by metformin.<sup>[13]</sup> Indian diets have also been reported to be low in calcium.<sup>[14]</sup> This could be another factor causing higher prevalence of Vitamin B12 deficiency. However, in a cross-sectional study of two different ethnic groups with type 2 diabetes mellitus in India and the United Kingdom, the prevalence of Vitamin B12 deficiency of 12% was found in the Indian population compared to 27% in a European population.<sup>[15]</sup> The authors cited the use of fermented foods by the Indian population as the probable cause of lower Vitamin B12 deficiency, but it underscores the dependence of Vitamin B12 levels on dietary factors, which vary in different populations.

An interesting finding from our study is the statistically significant rise in Vitamin B12 with increasing duration of diabetes. In a cross-sectional study from the US comprising 1621 patients with type 2 diabetes mellitus (575 on metformin and 1046 not on metformin) and 6867 persons without diabetes, the diabetes without metformin group had the lowest prevalence of Vitamin B12 deficiency (2.4%) as compared to 5.8% in the diabetes on metformin group and the 3.3% in the group without diabetes.<sup>[1]</sup> Interestingly, patients not on metformin group in that study had a statistically significant longer duration of diabetes. However, diabetes not on metformin group included patients who might have earlier received metformin and the authors speculated that

these patients might have been diagnosed with Vitamin B12 deficiency earlier and hence either treated for Vitamin B12 deficiency or given dietary modifications to increase Vitamin B12 levels in the diet. We did make an active effort to exclude patients who had been given Vitamin B12-containing supplements for any indication (review of available medical records was done, and patients were asked about the use of Vitamin B12-containing multivitamin supplements), but these preparations are available over the counter, and we cannot be sure that patients had never taken these medications earlier. In addition, the higher Vitamin B12 levels with greater duration of diabetes were seen in the “no metformin” group also who were not at the risk of metformin-related Vitamin B12 deficiency (as they never received metformin) and hence are less likely to have received Vitamin B12 treatment.

The impact of Vitamin B12 deficiency on hematological parameters such as MCV and hemoglobin as well as on peripheral neuropathy (DNS and DNE scores) was studied. We did not find any increase in MCV or decrease in hemoglobin in the Vitamin B12-deficient patients. The DNS and DNE scores were positive in a greater percentage of patients who were not Vitamin B12 deficient; however, this group also had a longer duration since the diagnosis of diabetes. In other words, Vitamin B12 deficiency as defined by serum Vitamin B12 levels did not impact hematological or neurological parameters assessed in our study. Similar findings have been reported earlier.<sup>[16]</sup> However, serum total Vitamin B12 levels may not accurately reflect Vitamin B12 status of the body and therefore biochemical deficiency often does not result in clinical deficiency.<sup>[17]</sup>

The limitation of our study is that we did not measure functional markers of Vitamin B12 deficiency (serum homocysteine and serum methylmalonic acid levels) which may better reflect the status of Vitamin B12 levels in the body as compared to serum Vitamin B12 levels. In addition, our sample size was small.

The strength of this study is that the “no metformin” group included only those patients who had never taken metformin. The reason for choosing such a group was to exclude a possible long-term effect of metformin use on Vitamin B12 status. Vitamin B12 is stored in the liver and several years may pass before the stores are depleted and detectable Vitamin B12 deficiency manifests.<sup>[17]</sup> Thus, including patients with a history of metformin use in the no metformin group is a potential confounder which we excluded in this study.

## CONCLUSIONS

In our study, metformin use was associated with a significantly lower serum Vitamin B12 levels when adjusted for duration of diabetes. Serum Vitamin B12 deficient patients did not have a higher prevalence of anemia or neuropathy. Further study of the impact of duration of diabetes on serum Vitamin B12 levels and of functional markers of Vitamin B12 deficiency on hematological and neurological parameters will be interesting.



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## Conflicts of interest

There are no conflicts of interest.

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