

# Status of Serum Vitamin B12 in Type 2 Diabetes Mellitus Patients under Metformin Therapy

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## ABSTRACT

**Introduction:** Type 2 diabetes mellitus, a prevalent global health problem, is a major cause of morbidity and mortality. Metformin, the most widely used hypoglycemic agent can lead to a deficiency of vitamin B<sub>12</sub>. Very less information is available regarding the relationship between metformin therapy and vitamin B12 deficiency in the Nepalese population.

**Objectives:** The study aims to evaluate the level of vitamin B<sub>12</sub> in cases of diabetes mellitus under metformin therapy and to access the relationship of serum vitamin B<sub>12</sub> level with the duration of metformin therapy.

**Methods:** A hospital-based cross-sectional study with 300 diagnosed diabetes mellitus cases under metformin therapy were enrolled in the study after the consent was taken. The prior ethical clearance was taken and the duration of the study was from December 2021 to June 2022.

**Results:** The study showed 78% (n=234) of the total diabetic cases under metformin therapy had lower vitamin B<sub>12</sub>. In 50-60 years, age group, 91% of them were vitamin B<sub>12</sub> deficient. Vitamin B<sub>12</sub> deficient cases were more with the increase in duration and dose of metformin therapy. All the diabetics who were under medication for 16-20 years had lower vitamin levels. About 94% (n=200) of the diabetics taking more than 1000 mg/day were vitamin B<sub>12</sub> deficient whereas only 39% (n=34) of diabetics taking less than 1000mg/day were found to be deficient.

**Conclusions:** Vitamin B12 is deficient in diabetics under long-term and higher doses of metformin therapy. Diabetic patients who are undergoing metformin treatment should monitor their vitamin B12 status as they are more prone to vitamin B12 deficiency.

Keywords: Diabetes mellitus; metformin; vitamin B<sub>12</sub>.

## INTRODUCTION

Type 2 Diabetes Mellitus (T2DM), one of the commonest endocrine disorders, is a prevalent global health problem and is on a constant rise.<sup>1</sup> This stands to be a major cause of morbidity and mortality for millions of populations worldwide.<sup>2</sup>

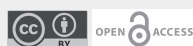
Metformin, the most widely used hypoglycemic agent is considered the first-line drug in DM due to various reasons such as cost-effectiveness, beneficial effect on weight, high potency, least side effects, long-standing evidence of safety, etc. A documented data of approximately 120 million diabetic patients is under metformin treatment worldwide.<sup>3</sup>

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The deficiency of vitamin B<sub>12</sub> in T2DM is induced by prolonged metformin therapy and is a least known and understood side effect. It is often a scenario with prolonged use, higher doses taken, and with the elderly.<sup>4</sup> Despite a high prevalence of T2DM in Nepal, restricted information regarding its side

effects is available to date. This study aims to assess the status of vitamin B<sub>12</sub> levels in diabetics under metformin therapy. The findings of the study will be beneficial to understand the pattern of vitamin B<sub>12</sub> deficiency in T2DM patients and probably help in the better therapeutic management of T2DM.

## METHODS

This is a hospital-based observational cross-sectional study carried out at National Medical College, Birgunj, Nepal for six months duration from 17<sup>th</sup> December 2021 to 16<sup>th</sup> June 2022. The study population consisted of 300 diagnosed cases of diabetic mellitus (type 2), visiting the outpatient departments of internal medicine or wards. All the cases were confirmed diabetics as per ADA criteria and already receiving metformin treatment for DM. A nonprobability purposive sampling technique was used to enroll the subjects for the study. Ethical clearance (Ref. F-NMC/574/078-079) was taken from the Institutional Review Committee (IRC), National Medical College, Birgunj, Nepal. The proper informed consent was taken by the subjects enrolled in this study.

Diagnosed cases of DM under metformin therapy in the age group of 30 to 60 years, those willing to participate in the study were the inclusion criteria. All the participants were Nepalese citizens. The exclusion criteria for this study were those with chronic renal failure, diagnosed cases of benign and malignant tumors, ages below 30 years and above 60 years, and those not willing to participate in the study. The samples collected were analyzed using Beckman Access2, California, and a normal range of Vitamin B<sub>12</sub> was taken as per the standard guideline

and reference given by the producing company.

All the data were entered in Microsoft Excel 2010 and converted to SPSS version 22 accordingly. The Chi square test was applied to compare the categorical variables. The numerical data were expressed in the mean and standard deviation. Independent t-test was used to compare the mean and the standard deviation between low and normal vitamin B<sub>12</sub> groups. Pearson correlation was used to find out the correlation between the serum vitamin B<sub>12</sub> statuses with other variables. The P value of less than 0.05 was considered statistically significant.

## RESULTS

The finding of this study showed that 78% (n=234) of the study participants who were diagnosed cases of diabetes mellitus (type 2) and under metformin therapy, were deficient in serum vitamin B<sub>12</sub>. The remaining 22% of diabetics under metformin therapy, however, had normal serum vitamin B<sub>12</sub>. The maximum number of participants in this study, about 44% were in the 50-60 years age group, followed by 30% from the 41-50 years age group and about 23% from less than 40 years of age group. Among participants in the 50-60 years age group, 91.6 % of them were vitamin B<sub>12</sub> deficient as shown in Table 1.

Vitamin B<sub>12</sub> deficient cases were more with the increase in duration and dose of metformin therapy. All the diabetics (100%), who were under metformin therapy for 16-20 years had lower vitamin B<sub>12</sub> levels. About 94% (n=200) of the diabetics taking more than 1000 mg/day were vitamin B<sub>12</sub> deficient whereas only 39% (n=34) of diabetics taking less than 1000mg/day were found to be deficient.

**Table 1: Distribution of vitamin B<sub>12</sub> status in different age groups.**

S. No.	Age group (years)	Total Participants	Low Vitamin B <sub>12</sub> n (%)	Normal Vitamin B <sub>12</sub> n (%)	p-value*
1	<40	79	40 (50.6%)	39(49.4%)	0.00
2	41-50	90	74 (82.2%)	16(17.8%)	
3	50-60	131	120 (91.6%)	11(8.4%)	

**Table 2: Distribution of vitamin B<sub>12</sub> status based on gender.**

S. No	Gender	Total Participants	Low Vitamin B <sub>12</sub> n (%)	Normal Vitamin B <sub>12</sub> n (%)	p-value*
1	Female	156	124 (79.5%)	32(20.5%)	0.00
2	Male	144	110 (76.4%)	34(23.6%)	

**Table 3: Medication years and vitamin B<sub>12</sub> status.**

S. No.	Medication Years	Low Vitamin B <sub>12</sub> n (%)	Normal Vitamin B <sub>12</sub> n (%)	P value*
1.	<5	38(43.7%)	49(56.3%)	0.00
2.	6-10	142(89.9%)	16(10.1%)	
3.	11-15	45(97.8%)	1(2.2%)	
4.	16-20	9(100%)	0(0%)	

In this study, 52% (n=156) of the total participants who were diagnosed cases of diabetes mellitus (type 2) and under metformin therapy were female, compared to 48% male (n=144).

Among the diabetics under metformin therapy for 6-10 years (about 53% of the total participants), 89.9% (n=142) were found to be deficient in vitamin B<sub>12</sub>. However, the percentage of vitamin B<sub>12</sub> deficient participants increased with a longer duration of metformin therapy, i.e., 97.8% in 11-15 years of metformin therapy and 100% in 16-20 years of metformin therapy as shown in Table 3.

Lower serum vitamin B<sub>12</sub> was seen with the participants taking higher doses of metformin per day as shown in Table 4.

The correlation analysis revealed the correlation of serum vitamin B<sub>12</sub> status with other variables. Significant negative correlations were seen with age (-0.48, 0.00), duration of diabetes mellitus diagnosed (-0.52, 0.00), medication years (-0.52, 0.01), doses of metformin (-0.71, 0.01), fasting blood glucose (-0.33, 0.01), postprandial blood glucose (-0.26, 0.01), HbA1C (-0.19, 0.01) and MCV (-0.34, 0.01), as depicted in table 6.

**Table 4: Dose of metformin per day and vitamin B<sub>12</sub> status.**

S. No.	Dose of metformin/day	Low Vitamin B <sub>12</sub> n (%)	Normal Vitamin B <sub>12</sub> n (%)	P value*
1.	<1000	34(39.1%)	53(60.9%)	0.00
2.	1100-2000	200(93.9%)	13(6.1%)	

**Table 5: Comparison of variables between low and normal vitamin B<sub>12</sub> status**

S. No.	Variables	Vitamin B <sub>12</sub>	Mean ± SD	P value*
1.	Glucose F (mg/dL)	Low	109.93 ± 16.82	0.00
		Normal	99.44 ± 13.84	
2.	Glucose PP (mg/dL)	Low	166.77 ± 35.48	0.00
		Normal	149.26 ± 20.85	
3.	HbA1c (%)	Low	6.34 ± 1.09	0.04
		Normal	6.05 ± 0.7	
4.	Vitamin B <sub>12</sub> (pg/mL)	Low	146.19 ± 28.49	0.01
		Normal	310.94 ± 107.27	
5.	Hb (gm%)	Low	11.28 ± 1.64	0.01
		Normal	13.04 ± 1.30	
6.	MCV (fL)	Low	94.37 ± 9.19	0.01
		Normal	87.58 ± 6.61	

**Table 6: Correlation of serum vitamin B<sub>12</sub> status with variables.**

S. No.	Variables	r value	P value*
1	Age	-0.48	0.00*
2	Gender	0.06	0.2
3	Smoking	0.03	0.5
4	Alcohol	-0.01	0.8
5	Years of Diagnosis of DM	-0.52	0.00*
6	Medication Duration (years)	-0.52	0.01*
7	Dose of Metformin	-0.71	0.01*
8	Glucose fasting	-0.33	0.01*
9	Glucose PP	-0.26	0.01*
10	HbA1c	-0.19	0.01*
11	Vitamin B12	1	-
12	Hemoglobin	0.51	0.01*
13	MCV	-0.34	0.01*

## DISCUSSION

This is a hospital-based observational cross-sectional study comprising 300 participants who were diagnosed cases of diabetes mellitus (type 2) under metformin therapy was carried out for 6 months. The finding of this study showed that 78% (n=234) of the study participants who were diagnosed with cases of diabetes mellitus (type 2) and under metformin therapy, were deficient in serum vitamin B<sub>12</sub>. The remaining 22% of diabetics under metformin therapy, however, had normal serum vitamin B<sub>12</sub>. The maximum number of participants in this study, about 44% were in the 50-60 years age group, followed by 30% from the 41-50 years age group and about 23% from less than 40 years of age group. Among the 50-60 years age group, 91.6 % of them were vitamin B<sub>12</sub> deficient. vitamin B<sub>12</sub> deficient cases were more with the increase in duration and dose of metformin therapy. All the diabetics (100%), who were under metformin therapy for 16-20 years had lower vitamin B<sub>12</sub> levels. About 94% (n=200) of the diabetics taking more than 1000 mg/day were vitamin B<sub>12</sub> deficient whereas only 39% (n=34) of diabetics taking less than 1000mg/day were found to be deficient.

Vitamin B<sub>12</sub>, a water-soluble vitamin is required for hematopoiesis, the functioning of the central nervous

system as well as the peripheral nervous system, and in DNA synthesis of all cells. The common and frequent adverse effects related to metformin are not severe and usually lead to temporary and self-restricting gastrointestinal (GI) disturbance.<sup>5</sup> Apart from GI this issues, other clinically important adverse effects that have been reported include vitamin B<sub>12</sub> malabsorption. A study that was done in 2016 in the Brazilian population receiving extended metformin therapy concluded that the frequency of vitamin B<sub>12</sub> deficiency ranges from 5.8% to 30%.<sup>6</sup> The reported vitamin B<sub>12</sub> deficiency in the general Indian population varies from 12% to 67% which is very much high as compared to that in the western population with only 5-7%.<sup>7,8</sup> The results of this study showed similar findings to the study done in 2012, that showed patients under metformin therapy had statistically significant lower serum vitamin B<sub>12</sub> levels compared to those not receiving metformin (P < .0001; 95 % confidence interval [CI] = -220 to -84 pg/mL).<sup>4</sup>

It is postulated that a predominantly vegetarian diet or strict vegans could be one of the causes of the higher prevalence of vitamin B<sub>12</sub> deficiency in India.<sup>7</sup> This can also apply to the Nepalese population as well. A deficiency of serum vitamin B<sub>12</sub> in our body causes megaloblastic anemia, neuropathy, memory,

and cognitive impairment. Neuropathy, one of the established common complications in DM is similar to the clinical features of serum vitamin B<sub>12</sub> deficient neuropathy (indistinguishable by nerve conduction studies also). There's the probability that vitamin B<sub>12</sub> deficient neuropathy can be misdiagnosed as diabetic neuropathy or it may contribute to worsening of diabetic neuropathy in the long run of disease course.<sup>4</sup>

Serum vitamin B<sub>12</sub> deficiency in the individual induced by metformin has been ascribed to the binding of the hydrophobic tail of biguanide to the hydrocarbon core of membranes. The biguanide group that is found to be positively charged (protonated) gives a positive charge to the membrane and can displace divalent cations such as calcium. The study done in 2006 showed that after adjusting for confounders, the clinically important and statistically significant association of serum vitamin B<sub>12</sub> deficiency with both dose and duration of metformin therapy, which is similar to the findings of this study. The study states that each 1-gram/day metformin dose increment conferred an odds ratio of 2.88 (95% confidence interval, 2.15-3.87) that is developing vitamin B<sub>12</sub> deficiency ( $p < .001$ ). For the participants who used metformin for 3 years or more, the adjusted odds ratio was found to be 2.39 (95% confidence interval, 1.46-3.91) ( $p = .001$ ) compared with those participants receiving metformin tablets for less than 3 years.<sup>9</sup> The uptakes of vitamin B<sub>12</sub> from the diet into the ileal cells are calcium-dependent and so metformin intake can interfere with the uptake of vitamin B<sub>12</sub>.<sup>10</sup>

The first case of serum vitamin B<sub>12</sub> deficiency-induced megaloblastic anemia due to long-term (8 years) metformin therapy was reported in 1980.<sup>11</sup> A study in India done in 2017, reported similar results of this study that metformin intake for diabetes was associated with a low level of serum vitamin B<sub>12</sub> when adjusted for duration of diabetes. The increase in the duration of diabetes was associated with a higher number of deficient serum vitamin B<sub>12</sub> levels. The serum vitamin B<sub>12</sub> levels were  $267.7 \pm 194.4$

pmol/L in the metformin group and  $275.1 \pm 197.2$  pmol/L in the no metformin group ( $p = 0.78$ ). But when adjusted for duration of diabetes, metformin use was associated with  $87.7 \pm 37.7$  pmol/L (95% confidence interval [CI], -162.1--3.3,  $p = 0.02$ ) i.e., lower serum Vitamin B12 levels.<sup>11</sup>

As per the review of literature, it has been stated in a study that reduced absorption of vitamin B<sub>12</sub> manifested as decreased serum vitamin B<sub>12</sub> levels, occurred as early as 3-4 months after the initiation of metformin treatment. It also states that, with the increase in duration i.e.; 5-10 years after metformin therapy, symptomatic deficiency of vitamin B<sub>12</sub> occurred. This finding can be justified by the fact that the body can store of vitamin B<sub>12</sub> is huge amount i.e., 2500 µg, in comparison to per day loss/requirement (1–2 µg), and depletion of the pre-existing body store vitamin B<sub>12</sub> thus takes 3-15 years.<sup>12</sup> It has been mentioned that biochemical deficiency is considered when serum vitamin B<sub>12</sub> levels are between 200pg/ml - 300pg/ml and symptoms suggestive of vitamin B<sub>12</sub> deficiency occur when levels go below 200pg/ml. Vitamin B<sub>12</sub> supplements can reverse hematological manifestations within days to weeks. Reversal of neurological damage is still doubtful.<sup>12</sup>

A study done in 2021 in Nepal, showed similar results of this study. The mean serum vitamin B<sub>12</sub> level is estimated as low as the duration of metformin treatment increases. The gender, and age relation with the development of vitamin B<sub>12</sub> deficiency was not significant. Out of 210 patients, 107 patients were having severe vitamin B<sub>12</sub> deficiency and 63 patients had a borderline vitamin B<sub>12</sub> deficiency level which shows that the deficiency increases per longer use of metformin therapy, which shows a prevalence of 50.95%.<sup>13</sup> Similarly, a study in India during 2019 showed, 164 of 408 cases (on metformin therapy for >3 months) had low vitamin B<sub>12</sub> as against 22 out of 242 controls (never on metformin therapy). But, average serum vitamin B12 was low in cases with less duration of DM when compared to cases with longer duration.<sup>14</sup>



The smaller sample size is the limitation of this study. The study could not incorporate the confounding factors that may also lead to lower vitamin B<sub>12</sub> levels. The study also did not compare serum vitamin B12 levels with the matched non-diabetic population.

## CONCLUSION

Vitamin B<sub>12</sub> is deficient in diabetics under long-term and higher doses of metformin therapy. The dose of metformin taken has a significant effect on lowering the level of vitamin B<sub>12</sub>. Similarly, the duration of metformin therapy in diabetes mellitus is also significantly related to vitamin B<sub>12</sub> deficiency. The results can be undertaken by medical

professionals and evaluation of serum vitamin B12 of the diabetic patients on metformin therapy should be regularly monitored. It is also recommended that the pre-evaluation of serum vitamin B12 will be of great concern before starting metformin therapy in newly diagnosed cases of diabetes mellitus. It can also be advised that prophylactically vitamin B12 supplementation can be instituted in diabetic patients on metformin therapy.

**Conflict of Interest:** None

NJHS

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