

ORIGINAL RESEARCH ARTICLE

EVALUATION OF SERUM VITAMIN B12 LEVELS IN TYPE 2 DIABETES MELLITUS PATIENT ON METFORMIN THERAPY ATTENDING A TERTIARY CARE HOSPITAL

Mahesh Raj Ghimire<sup>1\*</sup>, Sundar Pandey<sup>1</sup>, Deepak Aryal<sup>1</sup>, Pooja Shah<sup>1</sup>, Binita Soti<sup>2</sup>, Sulochana Ghimire Thapa<sup>3</sup>, Shakti Shrestha<sup>4</sup>

<sup>1</sup>Department of Internal Medicine, Devdaha Medical College and Research Institute, Rupandehi, Nepal

<sup>2</sup>Maharajgunj Nursing Campus, Institute of Medicine, Kathmandu, Nepal

<sup>3</sup>Department of Nursing, Universal College of Medical Sciences, Bhairahawa, Nepal

<sup>4</sup>The University of Queensland, 20 Cornwall Street, Australia

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**\*Correspondence to:** Mahesh Raj Ghimire, Department of Internal Medicine, Devdaha Medical College and Research Institute, Bhaluwai, Rupandehi, Nepal.

Email: [ghimiremaheshraj@gmail.com](mailto:ghimiremaheshraj@gmail.com)

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**ABSTRACT**

**Background:** Metformin for type 2 diabetes mellitus has more beneficial effect with few side effects like vitamin B12 deficiency. So, this study aimed to determine the level of vitamin B12 in patients with type 2 diabetes mellitus using metformin and its association with dose and duration of metformin use.

**Methods:** This was descriptive, cross-sectional, hospital-based study. A total of 128 diabetic patients using metformin for more than 6 months were enrolled. After taking informed consent, considering exclusion criteria, level of serum B12 were quantified by chemiluminescent enzyme immunoassay. Vitamin B12 level <300pg/dl as inadequate and ≥300pg/dl as adequate; metformin 1000mg as low dose; more than 1000mg as high dose were considered. Also, metformin used 1000mg /day taken as constant and comparing it with duration of > 10 years with duration of < 10 years. Data were entered in Microsoft Excel 2013 and analyzed on SPSS V25.

**Results:** Our study results 64.1% had adequate (>300pmol/l) and 35.9% had inadequate (≤300pmol/l) vitamin B12 level in patients with type 2 diabetes using metformin. Level of vitamin B12 deficiency was statistically associated with increasing dose of metformin used (p<0.001) and long term used of metformin used (p<0.019).

**Conclusions:** This study concluded that there is prevalence of deficiency of Vit B12 level in type 2 diabetes mellitus patients using metformin. Also, this deficiency level depends on duration and dose of metformin used.

**INTRODUCTION**

Metformin is common and first line drug for type 2 diabetes mellitus with more beneficial effect and few side effects.<sup>1</sup> However, since Berchtold et al first reported in 1969 that metformin could cause vitamin B12 deficiency by reducing vitamin B12 absorption in gastrointestinal tract, such findings are constantly published.<sup>2</sup> It's been reported that an average 6% to 30% of patients could show vitamins B12 deficiency due to Metformin use.<sup>3</sup> In addition, some studies have reported that serum vitamin B12 levels were related inversely to the dose and directly with duration of metformin use respectively.<sup>4</sup> Since, large prospective studies have clarified this relationship recently, the 2017 American Diabetes Association treatment guideline now recommend regular monitoring of vitamin B12 level in patient with diabetes taking metformin.<sup>2,5,6</sup>

As, food habit and food content of our Nepalese society is different from the western nations, so level of vitamin B12 evaluation may turn out to be different in Nepalese population. Anemia and peripheral neuropathies are two important manifestation of decrease vitamin B12 level.<sup>7</sup> Many western nations have guidelines of evaluating vitamin B12 level in their

usual practice.<sup>8</sup> But in Nepal, we prescribe vitamin B12 directly without evaluating its level. So, this study will help and guide clinician for better management of metformin induced vitamin B12 deficiency diseases.

The study aimed to investigate the level and prevalence of vitamin B12 deficiency level among patients with type 2 diabetes mellitus under Metformin treatment and examine how the duration and dose of Metformin affected serum B12 level in our population.

**METHODS**

This was descriptive, cross-sectional hospital-based study conducted from 28<sup>th</sup> April 2022 to 29<sup>th</sup> June 2022 at Internal Medicine department of Devdaha Medical College (DMCRI), Bhaluhui, Rupandehi, Nepal. Ethical approval was taken by Institutional Review Committee (IRC-DMCRI) from DMCRI. After taking informed written consent, participants diagnosed as type 2 diabetes mellitus under metformin therapy for more than 6 months and fulfilling inclusion criteria were enrolled.

Those who were newly diagnosed type 2 diabetes with metformin use duration less than 6 months, with previous and suspected pernicious anemia, pregnant women, diagnosed type 1 diabetes, increased serum creatinine more than 1.7 for male and 1.5 for female, and those who underwent colectomy, gastrectomy, diagnosed inflammatory bowel disease (IBD) were excluded. Also, those who were vegetarian and those priorly taking injectable vitamin B12 as therapy were excluded. Total 128 participants were enrolled by purposive sampling technique.

Sample size was determined from the study conducted by Katsaros T et. Al. and the prevalence of deficiency of serum vitamin B12 was 9% observe in type 2 diabetes patients using biguanides (metformin).<sup>2</sup> Sample size was calculated using formula:

$$n = Z^2pq/e^2$$

Where,

Z= statistic for a level of confidence. (For the level of confidence of 95%, which is conventional, Z value is 1.96).

p= expected proportion in population

q= (1-p)e= absolute error or precision.

(e is considered 0.05)

$$n = (1.95)^2 \times 9\% \times 91\% / (0.05)^2$$

$$= 3.8 \times 0.09 \times 0.91 / 0.0025$$

$$= 124.488$$

So, we enrolled total of 128 patients.

After explaining the effect of metformin use on diabetics and its complications, investigations were sent as a routine follow up management so that extra expenses were minimized. Dose of metformin users were grouped into 3 categories; those taking 500mg twice daily or 1000mg per day, 850mg twice daily or 1700mg per day and 1000mg twice daily or 2000mg per day; and considered low, intermediate, and high dosage respectively. A level of vitamin B12 less than 300pg/dl is taken as inadequate and more or equal to 300pg/dl is taken as adequate. Also, duration of use of metformin by diabetic patients was categorized into 5 groups i.e., 0-5 years; 5.1-10; 10.1-15; 15.1-20 and 25.1 to above. Also, for strength of association between duration of metformin use and level of vitamin B12 deficiency, it was assessed by considering dose of metformin constant as 1000mg per day and compare it with

equal or less than 10 years to more than 10 years.

Statistical analysis was done by SPSS (Statistical package for social science) version 25. Descriptive statistics was represented as Mean  $\pm$  Standard deviation (S.D) with 95% confidence intervals for continuous data (age, duration of metformin use, vitamin B12 level,) and categorical data (gender, religion, occupation, address, income, marital status, alcohol use, smoking, metformin dose and level of vitamin B12) was depicted as frequency number. For measuring the association between variables, we used inferential statistics. Statistical significance was assumed at  $p < 0.05$ .

## RESULTS

In our study, total 128 participants were enrolled and the mean age for male and female was  $57.88 \pm 12.01$  Standard Deviation (SD) years and  $57.68 \pm 9.80$  SD years respectively. Of the total participants 59.4% were female

Serum vitamin B12 level

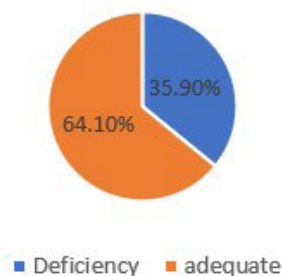


Figure 1: Pie chart of level of serum vitamin B12 in type 2 diabetes mellitus

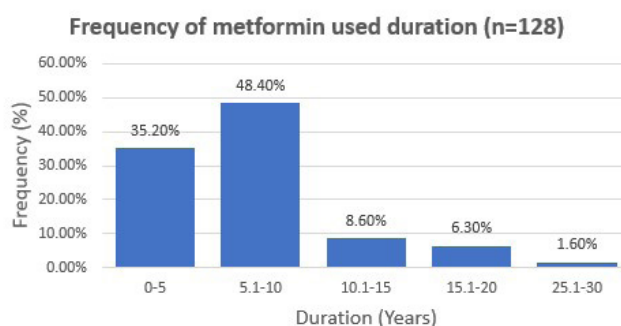


Figure 2: Bar Diagram of frequency of metformin used by type 2 diabetes mellitus

Table 1: Baseline clinic-demographic profile of type 2 diabetes mellitus patients

Parameter	Type 2 diabetes mellitus (mean $\pm$ SD, %) (n=128)		
	Male (N=52)	Female(N=76)	Total (n=128)
Age (years)	57.88 $\pm$ 12.01	57.68 $\pm$ 9.8	57.8 $\pm$ 10.7
Gender	40.6 %	59.4%	
Metformin Used Duration (years)	8.36 $\pm$ 5.06	7.92 $\pm$ 4.82	8.10 $\pm$ 4.91
Serum B12 level (pg./dl)	344.03 $\pm$ 149.49	371.23 $\pm$ 190.36	360 $\pm$ 175

Almost one third (35.9%) of participants were having less than 300pg/dl level of serum B12 level, which was considered as inadequate level as shown in Figure 1. The mean of serum B12 level in type 2 diabetes mellitus with metformin use was 344.03pg/dl  $\pm$  149.49 SD in male, 371.23 pg/dl  $\pm$  90.36 SD in female and 360 pg/dl  $\pm$  175 SD in total, as shown in table 1. Nearly half (48.4%; n=128) of the patients using metformin were belongs to duration of (5.1 to 10) years group as shown in figure 2 along with mean duration of metformin used was 8.36 years  $\pm$  5.06 SD in male and 7.92 years  $\pm$  4.82 SD in female as shown in table 1. More than half i.e., 62.5% were using low dose metformin i.e., 500mg twice daily every day as treatment dose as shown in Table 2.

Table 3 depicts that most of the respondent (78%) using metformin less or equal to 1000mg per day had normal vitamin

**Table 3: Association between Respondents' Metformin use and Vitamin B12**

Variable	Vit B12 normal(n=80) No. (%)	Vitamin B12 deficiency(n=48) No. (%)	$\chi^2$	p-value
Metformin dose/day				
$\leq$ 1000mg/day	63 (78.8%)	17 (21.3%)	24.036	<0.001*
>1000mg/day	17 (35.4%)	31 (64.6%)		
Metformin dose of 1000mg per day				
$\leq$ 10 year	58 (69.9%)	25 (30.1%)	5.486	<0.019*
>10 year	22 (48.9%)	23 (51.1%)		

\*p value <0.05, variable significantly associated with Vit B12

## DISCUSSION

Type 2 Diabetes mellitus is one of the non - communicable diseases, being a major health problem in developing country like ours. Diabetes is one of the risk factors of many leading disease like coronary heart disease, cerebrovascular disease etcetera which need to get control as early as possible. Metformin is first line drug that is being used worldwide. Despite of having many good reasons to become first line of drug, it has few side effects like decrease serum vitamin B12, lactic acidosis etc.<sup>9,10</sup>

The reported prevalence of serum vitamin B12 level varies in different population group. In our study, the mean serum vitamin B12 level is 360  $\pm$  175 pg/dl which is lower comparable to Indian study population which was 410  $\pm$  230.7 pg/dl. Also, mean serum B12 levels among American adults with diabetes and metformin user was 430.2  $\pm$  13 pg/dl and in Korea mean level was with 665.7  $\pm$  246.7 pg/dl which was quite higher than our study population.<sup>5,6,11</sup> Unfortunately, there was not any research study done in Nepalese population till date showing the deficiency level of serum vitamin B12 in type 2 diabetes mellitus using metformin.<sup>12,13</sup>

In our study, vitamin B12 deficiency was present in 35.9% of patients using metformin daily as a treatment. The Studies from Nepal, showing prevalence of vitamin B12 deficiency in diabetes patients with metformin use were 43.7% which was done by Thakur S. et al. and 50.95 % which was done by

B12 level, while nearly two-third (64.6%) using metformin more than 1000mg per day had vitamin B12 deficiency. This was statistically significant as p<0.001. Similarly, most of (70%) of the respondent using 1000mg metformin per day for equal or less than 10 years had normal vitamin B12 level, comparing this with those taking same amount of metformin for more than 10 years had more than half (51.1%) level of deficiency of vitamin B12. This is also statistically significant as p<0.019.

**Table 2: Frequency of Dose of metformin used in type 2 diabetes mellitus**

Dose (mg per day)	Frequency (%)
Low (1000)	80 (62.5)
Intermediate (1700)	15 (11.7)
High (2000)	33 (25.8)

Malla D. et al.<sup>12,13</sup> Both studies were done in National Academy of Medical Sciences, Bir Hospital. A study done in India and published in 2017, by Raizada N. et.al. showed prevalence of vitamin B12 deficiency was 35.5% of diabetics using metformin.<sup>14</sup> But, internationally prevalence of deficiency of vitamin B12 level in diabetics patients using metformin is quite variable.

The result of our study comparing association of dose and duration of metformin used in diabetic patient with serum vitamin B12 level is similar with a study done in Korea by Jiwoon Kim et. al.<sup>15</sup> published in 2019, results in serum vitamin B12 deficiency occurred in 22.2% of patients (n=247). After adjusting for confounders, a 1 mg increase in daily metformin dose was associated with a 0.142 pg/mL decrease in vitamin B12 (P<.001). This result is similar with our study as deficiency of vitamin B12 level is significantly associated with increasing dose of metformin used. Also, considering a study showing association of duration of metformin used and deficiency of vitamin B12 level in type 2 diabetes mellitus patients, published by Jiwoon Kim et. Al: This study results that vitamin B12 deficiency was statistically significant with duration of metformin used.<sup>15</sup> Similarly, when we went through many other studies, considering only duration of metformin used in diabetes patient, there is association between two parameters, like our study.<sup>4,16</sup> Considering daily dose of metformin 1000mg, those using metformin use more than 10 years were having more chance of developing deficiency of vitamin B12 level than those using less or equal to 10 years, like our study.

Complications due to vitamin B12 deficiency like peripheral

neuropathy and anemia can be prevented by doing regular measurement of vitamin B12 concentration in type 2 diabetes mellitus using metformin.

There are few limitations in our study like lack of proper knowledge about disease and its treatment leads to recall bias using different multivitamins, patient taking metformin-based therapy intermittently, single centered, less sample size with limited time.

## CONCLUSION

Though different international diabetes guidelines like American diabetes association have clearly mention about the supplements of vitamin B12 to those who were using metformin based anti- diabetic drugs, we do not practice routinely. Currently, there are no published guidelines advocating routine screening for vitamin B<sub>12</sub> deficiency among patients with type 2 diabetes undergoing metformin treatment.

Although the clinical significance of vitamin B<sub>12</sub> deficiency remains unclear, our data suggest routine vitamin B12 monitoring in patients with type 2 diabetes mellitus, especially in those using metformin. This study concluded that there is prevalence of deficiency of Vit B12 level in type 2 diabetes mellitus patients using metformin. Also, this deficiency level depends on duration and dose of metformin used.

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