

Assessment of Vitamin B12 deficiency in diabetic patients on metformin attending a tertiary care center

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ABSTRACT

Introduction: Diabetes mellitus is one of the most encountered diseases. Vitamin B12 deficiency is highly prevalent among the adult individuals with diabetes mellitus under the Metformin therapy.

Objective: Objective of this study is to assess the prevalence of Vitamin B12 deficiency among the diabetic individuals under Metformin therapy.

Methodology: A descriptive cross-sectional study was conducted at the department of Biochemistry, Nepalese Army Institute of Health Sciences (NAIHS), Sanobharyang from January 2024 to January 2025. A total of 255 diabetic patients under Metformin therapy were included in this study. Data collected included their age, glycated hemoglobin (HbA1c) level and Vitamin B12 level. Chi square test was used to find the association of different variables.

Results: Among the total 255 participants in this study, 150 (58%) are female. Mean age group of participants was 55.14 years and mean value of HbA1c was 6.18. In this study 156 (61%) of participants have Vitamin B12 deficiency. The highest prevalence of Vitamin B12 deficiency was seen among the age group >60 years i.e., 55 (64.7%). Also the deficiency was highest among the female 95 (63.3%) than in male 61 (58.1%). Comparing with HbA1c, Vitamin B12 deficiency is higher i.e., 78.6% among the poor control group and least deficiency were seen among the good control group i.e., 59.9%.

Conclusion: This study concludes that in diabetic patients, Metformin therapy could be a major factor related to Vitamin B12 deficiency. It increases with age and poor control of diabetes as well. Screenings of Vitamin B12 deficiency or supplementation in such individuals were recommended.

Key words: Diabetes mellitus; Metformin; Vitamin B12 deficiency

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INTRODUCTION

The prevalence of Diabetes Mellitus (DM) as well as impaired glucose tolerance is rising steadily in global scenario, with Nepal reporting a pooled prevalence of 8.4 %. This prevalence data was found to be increasing with advancing age.^{1, 2, 3}

Vitamin B12 is an essential micronutrient required for erythropoiesis, DNA synthesis and nerve development. Laboratory diagnosis of vitamin B12 deficiency depends upon low serum Vitamin B12 levels or elevated methylmalonic acid and homocysteine level.^{4, 5, 6} The mechanism by which Vitamin B12 deficiency occurs in patient may be due to inhibition of vitamin B12 intrinsic factor complex absorption. Chronic use of metformin has been reported as one of the cause Vitamin B12 deficiency.^{7, 8, 9} Prior studies have also indicated that the occurrence of Vitamin B12 deficiencies due to metformin differed immensely and ranged between 6% to 30%.¹⁰

Because of very few studies in this regard in our context, no consensus has been reached on whether metformin induces Vitamin B12 reduction in the Nepalese population. We, therefore, aim to assess the level and prevalence of Vitamin B12 deficiency in diabetic patient under metformin therapy and their association especially among different age group and glycemic control group.

METHODOLOGY

This descriptive cross-sectional study was conducted in the Department of Biochemistry (NAIHS), following the approval of the Institutional Review Committee (IRC) of NAIHS (Reg Number 883). Data were collected from January 2024 to January 2025. Irrespective of gender, all type 2 diabetic patients on metformin therapy aged more than 20 years and those coming to the laboratory for sample collection were included in this study. Patients who were taking vitamin B12 supplements and with known GI disorders like Malabsorption Syndrome, Pernicious Anemia and critically ill patients were excluded from this study. Convenience sampling was done and the sample size was calculated using the following formula,

$$\text{Sample size } (n) = Z^2pq/(d)^2$$

Where, Z = confidence level at 95% (standard value 1.96)

P = prevalence of Vitamin B12 deficiency was 81% in type 2 diabetics under metformin¹¹

$$q = 1 - p$$

d = margin of error (5%)

$$\text{Now, the sample size } (n) = Z^2pq/(d)^2 = 234$$

Considering 10% non-response rate, final sample size is 255.

All eligible patients were enrolled during the study period. After taking written consent, questionnaire was used to collect relevant data. Under aseptic condition, 5ml of blood sample was collected by venipuncture from the median cubital vein and it was divided between two collecting tubes. For estimation of Vitamin B12, clot activator and serum separator containing gold capped tube was used and for estimation of HbA1c anticoagulants containing purple capped tube was used. After completion of sample collection, HbA1c and Vitamin B12 were estimated. Vitamin B12 was measured with chemiluminescent immunoassay by utilizing Beckman Coulter. HbA1c was estimated with HPLC method by utilizing Automatic Glycohemoglobin Analyzer ADAMS A1C HA-8180V. Vitamin B12 level

200pg/ml and above was considered normal and below 200pg/ml is considered deficiency.¹² Similarly, HbA1c levels were categorized as good control (<7%), fair control (7.1%-8.9%) and poor control (9%) groups.¹³ The collected data were entered in Microsoft Excel and transferred into statistical package for social sciences, IBM SPSS Statistics for Windows version 16 (IBM Corp., Armonk, N.Y., USA). In this study age groups and gender are independent variables whereas Vitamin B12 is a dependent variable. The continuous data (Age, HbA1c and Vitamin B12) are presented in mean and standard deviation. Categorical data (Vitamin B12, gender) are presented in frequency and percentage. Chi square test was used to find the association of Vitamin B12 with the HbA1c, age groups and gender. The p value of less than 0.05 was considered as statistically significant.

RESULTS

The total numbers of participants included in this study were 255. Participant's age ranged from 20 years to 86 years with the mean age of 55.14 ± 14.17 . In this study more than half of the participants were female 150 (58%, table 1).

Vitamin B12 value ranged from 60pg/ml to 977pg/ml. The mean HbA1c value was (6.2 ± 0.91) % with minimum reported value of 4.5% and maximum of 9.1%

A total of 156 (61%) participants were found to have Vitamin B12 deficiency while 99 (39%) participants had normal Vitamin B12 levels (Table 2).

The comparison of Vitamin B12 deficiency among different age group revealed that among above 60 years group more than two-third 55 (64.7%), of the participants had deficiency of vitamin B12 followed by individuals aged 40-60yrs 82 (60.3%). Though, the distribution of deficiency increased with age, this difference is not statistically significant. Regarding sex, 95 (63.3%) of female and 61 (58.1%) of male had deficiency of Vitamin B12. There is no significance difference between sex and Vit. B12 level since p value is more than 0.05 Comparing HbA1c control with Vitamin B12 deficiency 11 (78.6%) of the poor control group, 18 (62.1%) of the fair control group and 127 (59.9%) of the good control group had deficiency of vitamin B12 level. This shows that, as glycemic control worsens, Vitamin B12 deficiency prevalence increases. There is no significance difference between HbA1C grouping and Vit. B12 level since p value is more than 0.05 (Table 3).

Table 1: Age and sex distribution of participants

Age(years)	Frequency(%)
20 – 40	34 (13.3)
40- 60	136 (53.3)
≥60	85 (33.3)
Mean ± SD	55.14 ±14.17
Sex	
Male	105 (41%)
Female	150 (58%)

Table 2: Prevalence of Vitamin B12 deficiency among the participants

Vitamin B12	Frequency (%)
Normal	99(39)
Deficiency	156 (61)

Table 3: Association of Vitamin B12 status with Age, Sex and HbA1c control

Variables	Level of Vitamin B12		p- value
	Normal n (%)	Deficiency n (%)	
Age group in years			
20 - 40	15(44.1)	19(55.9)	0.64
40- 60	54(39.7)	82(60.3)	
60 and above	30(35.3)	55(64.7)	
Sex			
Male	44(41.9)	61 (58.1)	0.39
Female	55 (36.7)	95(63.3)	
HbA1c control			
Good control	85 (40.1)	127(59.9)	0.38
Fair control	11(37.9)	18 (62.1)	
Poor control	3(21.4)	11 (78.6)	

DISCUSSION

In our study, majority of participants were adults in the 40-60 years age group followed by older age group i. e >60 years. Among the participants, majority of Vitamin B12 deficiency was observed in older age group i. e >60 years and minimum number of Vitamin B12 deficiency was seen in younger age group i.e 20-40 years. Clarke et al. have reported similar findings in 2004 where higher prevalence of Vitamin B12 deficiency was found among older adults i. e >75 years.¹⁴ Also, Vitamin B12 deficiency is found to be more prevalent with advancing age. This increased risk of Vitamin B12 deficiency is attributed to insufficient dietary intake, malabsorption associated with age related changes in gastrointestinal function,

higher incidence of pernicious anemia and chronic use of interfering medication like metformin.¹⁵

Among the participants of this study, 61% were found to have Vitamin B12 deficiency. A meta-analysis by Chapman et al. in 2016 has reported that, 10 out of 17 observational studies showed a statistically lower level of Vitamin B12 in patients on metformin therapy.¹⁶ The primary outcome of the study done by Niafar et al, another meta-analysis, also suggested Vitamin B12 deficiency in Metformin taking group.¹⁷ A study done in 2021 by Thakur et al. also reported Vitamin B12 deficiency in 43.7% of diabetics treated with metformin therapy.¹⁸ Hamdi et al, Jager et al and Farook et al. also show similar results where Vitamin B12 deficiency is associated with higher dose and duration of metformin use.^{12, 19, 20} A significant association of metformin use and Vitamin B12 levels was found in 17 out of 21 studies considered by Atkinson et al. in their systematic review.²¹ The efficacy, low risk and potential for usage with other anti-diabetic drugs has established metformin as the most widely used oral hypoglycemic drug worldwide. On the other hand, several theories describe the interference of metformin with Vitamin B12 metabolism by compromising enterohepatic Vitamin B12 circulation, increasing Vitamin B12 hepatic storage, decreasing IF production and decreasing intestinal motility with bacterial overgrowth leading to Vitamin B12 deficiency. To improve the diagnosis of vitamin B12 deficiency, methyl malonic acid and homocysteine can be used as it increases sensitivity and specificity for detecting Vitamin B12 deficiency.²²

Though age specific and gender specific prevalence of Vitamin B12 was seen in our study, the association was not at a statistically significant level. This finding is similar with the result obtained by another study done by Malla et al. Their study also shows that the deficiency increases as per longer use of metformin and prevalence of Vitamin B12 deficiency is 50.95%.¹¹

The number of participants included in our study were similar with the study done by Akabuwai et al (2016) and Hamdi et al. (2020). They have found vitamin B12 deficiency more prevalent among the female and their finding is similar with our study.^{12, 23} Another study done by Khan et al, among the patients having Vitamin B12 deficiency has reported a significant difference between females and males. This finding is similar with our study where 63.3% of female were found to be deficient. Ageing, lack of physical activities and estrogen deficiency in postmenopausal women are thought to be reasons

behind Vitamin B12 deficiency especially in patients on metformin therapy.²⁴

In this study, Vitamin B12 deficiency increases with worsening glycemic control. But the association of Vitamin B12 deficiency with HbA1c level is not statistically significant. However, Akabwai et al. have reported a statistically significant association between higher HbA1c ($\geq 7\%$) and Vitamin B12 deficiency. This suggests that individuals with higher HbA1c levels can be recommended for screening of Vitamin B12 as well.²⁴

Interestingly, one study done by Phan et al, revealed lower HbA1c levels which were statistically associated with Vitamin B12 deficiency. This outcome may be attributed to the fact that diabetic patients achieving lower HbA1c were intensively treated with higher dose of metformin.²⁵

LIMITATION

The sample size was small and all the data were collected in the single center.

CONCLUSION

Vitamin B12 deficiency is more prevalent in patients under metformin and the focus should shift towards identification of the cause of such deficiency for holistic patient management.

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