



Original Article

Vitamin B12 Status in Metformin Treated Diabetics

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Abstract

Introduction: Metformin is most commonly used drug for patients with type 2 diabetes mellitus (T2DM) patients. Metformin related vitamin B12 deficiency can cause anaemia. According to various reports, Long-term metformin intake can cause malabsorption of vitamin B12 in almost 30% of patients. It may lead to lower levels of serum vitamin B12 concentration in 14% to 30% of cases. Peripheral neuropathy may be a side effect of metformin induced vitamin B12 deficiency in patients with T2DM. Each 1000 mg/day of metformin dose increment increases the risk of vitamin B12 deficiency significantly. The longer is the duration of treatment with Metformin the higher is the risk of Vitamin B12 deficiency.

Methods: Cross sectional Hospital Based observational Study. Patients attending to OPD and medical wards of National Academy of Medical Sciences, Bir Hospital were enrolled in this study. All T2 DM patients of the age group 20-80 years of both sex who were taking Metformin for a minimum of 3 months duration and at a dose of at least 1000mg/day. Determination of serum vitamin B12 level of the patients was determined using high performance liquid chromatography (HPLC) in bir hospital. Vitamin B12 deficiency was categorised as a serum concentration of <200 pg/dl and borderline deficiency as 200–300 pg/dl. Concentrations >300 pg/dl was considered as normal. Pearson's chi-square tests was used to test the differences in the proportion of categorical variables, and independent t-tests was used for evaluating the difference between the means of two continuous variables. Pearson correlation analyses were performed to examine the linear relationship between serum vitamin B12 and metformin use. Receiver operator characteristic (ROC) curve analysis was used to evaluate the relationship between the duration of metformin use and vitamin B12 deficiency and to determine the reflection point (cut-off value). The area under the curve (AUC) with 95% confidence interval (CI) was calculated. P values <0.05 was considered to be statistically significant

Results: A total of 231 patients were enrolled in this study conducted from June 2019 till July 2020 at National Academy Of Medical Sciences, Bir hospital, Kathmandu in Nepal. 112 (48.5%) were males and 119 (51.5%) were females. Of all the participants, 109 (47.2%) were <60 years of age while 122 (52.8%) were >60 years of age. 193 (83.5%) were non-vegetarians and 38 (16.5%) were vegetarians. 177 (76.6%) patients were consuming more than 1.5G of metformin daily while 54 (23.4%) patients were consuming metformin a dose between 1-1.5G daily. Regarding The duration of metformin consumption, 101 (43.7%) patients were taking metformin for 1-5 years duration, while 113 (48.9%) patients were taking it for 5-10 years and 17 (7.4%) patients taking it for less than a year. Among all the patients, vitamin B12 deficiency was found in 108 (43.7%) metformin treated patients. Vitamin B12 deficiency were found more > 60 years of age (P=0.035) and in regular alcohol consumers (P=0.009) more often. Metformin dosage (P=0.102) or its duration (P=0.090) were not found statistically associated with vitamin B12 deficiency. Vitamin B12 deficiency was not significantly associated with Vegetarians (P=0.132) too.

Conclusion: Vitamin B12 deficiency was found in 43.7% of diabetics who were treated with Metformin. The deficiency was significantly found among >60 years of age (P=0.035) who were regular alcohol consumers (P=0.009).

Keywords: T2DM, Metformin, Vitamin B12 status.

Introduction

Metformin is widely used antidiabetic drug for patients with type 2 diabetes mellitus (T2DM)¹. Metformin has beneficial effects on carbohydrate metabolism and weight loss². Metformin related vitamin B12 deficiency can cause anemia³. Long-term metformin intake causes malabsorption of vitamin B12 in almost 30% of patients. It may lead to low levels of serum vitamin B12 concentration of 14% to 30%⁴. Peripheral neuropathy may be a side effect of metformin induced vitamin B12 deficiency in patients with T2DM⁵. The dose of metformin also plays significant role in reducing the serum levels of vitamin B12. Each 1000 mg/day of metformin dose increment increases the risk of vitamin B12 deficiency⁴. Metformin reduces vitamin B12 concentration in both long (>3 years) as well as short (<3 years) treatment with metformin¹. But, the longer is the duration of treatment the higher is risk of Vitamin B12 deficiency. Studies have revealed that vitamin B12 levels of metformin treated T2DM patients for more than 4 years were significantly lower than control (MD, 2152.2 pg/mL; 95%CI, 2220 to 284 pg/mL, P, 0.0001)¹. While few other studies have reported that it may or may not lower vitamin B12 level.

B12 deficiency is a silent epidemic with serious consequences. Long term use of metformin has been associated with malabsorption of vitamin B12 leading to its deficiency. The south Asian due to cultural, religious and food habits who rely on vegetarian diet mostly are at higher risk of developing vitamin B12 deficiency than other part of the world. Thus, we designed this study to assess the status of vitamin B12 in type 2 diabetes mellitus patients on metformin therapy who are attending hospitals in the central (Kathmandu) and peripheral (Saptari) regions of Nepal.

In our context, in Nepal, no consensus has been reached on whether metformin induces vitamin B12 reduction. We therefore wanted to assess the association between metformin and vitamin B12 reduction especially among different subgroups of the patients who are at risk of developing vitamin B12 deficiency in terms of their age, sex, ethnicity,

food habits along with metformin therapy in Type 2 diabetics.

Methods

Type of Study

Cross sectional Hospital Based observational Study.

Place of Study

Patients attending to OPD and medical wards of National Academy of Medical Sciences, Bir Hospital, Kathmandu, Nepal.

Sample Size Calculation

$$N = (Z_{\alpha}^2) (P) (Q) / d^2$$

Where, N=required sample size, Z_{α} =z variates corresponding to desired reliability level (1.96 for 95% reliability)

P=estimated proportion in the population, Q=100-P (if P is in %), d=maximum tolerable error=10% of P P=30%. (Ting RZ, et al have mentioned in their study "Risk factors of vitamin B12 deficiency in patients receiving metformin" published in Arch Intern Med 166: 1975–1979 and have reported that reduction of serum vitamin B12 concentration occurs in 14% to 30%)⁴.

$$\text{Thus, } N = (1.96)^2 \times 0.3 \times 0.7 / 0.03^2$$

$$N = 233$$

Inclusion Criteria

1. T2 DM patients of the age group 20-80 years of both sex and are on Metformin.
2. Duration of treatment with metformin for a minimum of 3 months.
3. Dosage of metformin of at least 1000mg/day.

Exclusion Criteria

1. Newly diagnosed Type 2 Diabetic Patients with any co existing cause for B12 deficiency.
2. Pregnant ladies and Type 2 Diabetic Patients on other drugs.
3. Malabsorption syndromes and Pernicious anemia, prior vitamin B12 injections, gastrectomy, colectomy, inflammatory bowel disease, and strict vegetarianism (Vegan).
4. Patients with any severe medical illness, such as sepsis, severe infection, malignancy, liver cirrhosis, heart failure, or renal failure (serum creatinine levels > 1.7 mg/dL for men and > 1.5 mg/dL for women).

Measurements

Sample collection and storage: After an overnight fast of about 8-10 hours, 5 ml of venous blood was collected from each participant and dispensed into plain bottles. The samples were allowed to retract and then spun at 4000 rpm for 10 minutes to obtain serum samples which were kept at -200°C until analysed for vitamin B12 level.

Determination of serum vitamin B12 level: Serum vitamin B12 levels of the patients were determined using high performance liquid chromatography (HPLC) in bir hospital. Vitamin B12 deficiency was defined as a serum concentration of <200 pg/dl and borderline deficiency as $200-300$ pg/dl. Concentrations >300 pg/dl were considered as normal. The serum vitamin B12 was quantified by chemiluminescent enzyme immunoassay. The blood glucose level was measured using an automated enzymatic method, and the HbA1c level was determined by high-performance liquid chromatography⁵.

Statistical Analysis

SPSS 17 version was used for analysis. Clinical characteristics and parameters were expressed as the mean \pm standard deviation (SD), or numbers (percentage). Pearson's chi-square tests were used to test the differences in the proportion of categorical variables, and independent t-tests was used for evaluating the difference between the means of two continuous variables. Pearson correlation analyses were performed to examine the linear relationship between serum vitamin B12 and metformin use. The variables were analysed using univariate analysis, and the multivariate analysis depending on the variables and their associations to examine. Multiple logistic regression analysis were performed to assess the independent predictive effect of the variables on the risk for vitamin B12 deficiency. Receiver operator characteristic (ROC) curve analysis was used to evaluate the relationship between the duration of metformin use and vitamin B12 deficiency and to determine the reflection point (cut-off value). The area under the curve (AUC) with 95% confidence interval (CI) was calculated. P

values <0.05 were considered to be statistically significant

Results

A total of 231 patients were enrolled in this study conducted between June 2019 and July 2020 at National Academy Of Medical Sciences, Bir hospital, Kathmandu in Nepal. 112 (48.5%) were males and 119 (51.5%) were females. Of all the participants, 109 (47.2%) were <60 years of age while 122 (52.8%) were >60 years of age. 193 (83.5%) were non-vegetarians and 38 (16.5%) were vegetarians. 177 (76.6%) patients were consuming more than 1.5G of metformin daily while 54 (23.4%) patients were consuming metformin a dose between 1-1.5G daily. Regarding The duration of metformin consumption, 101 (43.7%) patients were taking metformin for 1-5 years duration, while 113 (48.9%) patients were taking it for 5-10 years and 17 (7.4%) patients taking it for less than a year. Among all the patients, vitamin B12 deficiency were found in 108 (43.7%) metformin treated patients. Vitamin B12 deficiency were found more >60 years of age ($P=0.035$) and in regular alcohol consumers ($P=0.009$). Metformin dosage ($P=0.102$) or its duration ($P=0.090$) were not found statistically associated with vitamin B12 deficiency. Vitamin B12 deficiency was not significantly associated with Vegetarian ($P=0.132$),

a. Duration of Metformin intake and Vitamin B12 status.

Serum Vit.B12 status	Duration of metformin intake			Total
	1-5 year	5-10 year	<12 months	
Vitamin B12 deficiency	41 38.0%	61 56.5%	6 5.6%	108 100.0%
Normal	60 48.8%	52 42.3%	11 8.9%	123 100.0%
Total	101 43.7%	113 48.9%	17 7.4%	231 100.0%

$P=0.09$

Vit.B12 is not significantly associated with Duration of diabetes

b. Metformin dosage and vitamin B12 status

Serum Vit.B12 status		Metformin Dose		Total
		1G-1.5G	>1.5G	
Vit. B12 deficiency		20	88	108
		18.5%	81.5%	100.0%
Normal		34	89	123
		27.6%	72.4%	100.0%
Total		54	177	231
		23.4%	76.6%	100.0%

P=0.102

Vit.B12 is not significantly associated with Metformin Dose

C. Alcohol consumption and B12 status

Serum Vit.B12 status	Alcohol				Total
	-	+	++	+++	
Vitamin B12 deficiency	0	46	2	60	108
	.0%	42.6%	1.9%	55.6%	100.0%
Normal	6	69	2	46	123
	4.9%	56.1%	1.6%	37.4%	100.0%

P=0.009

Vitamin B12 deficiency is significantly associated among Alcohol consumers

-:no alcohol consumption

+:social alcohol consumption

++:more than 3 times a week alcohol consumption

+++almost daily alcohol consumption

D. Age group and Vitamin B12 status

Serum Vit.B12 status	Age		Total
	<60yr	>60yr	
Vitamin B12 deficiency	43	65	108
	39.8%	60.2%	100.0%
Normal	66	57	123
	53.7%	46.3%	100.0%
Total		109	231
		47.2%	52.8%

P=0.035

Significantly associated with age group and vitamin B12 levels

Discussion

This observational study tried to find out the magnitude of the problem of vitamin B12 deficiency among Metformin treated diabetic patients in Nepal. The study also tried to analyze the risk of deficiency in high risk sub group populations. The study enrolled 231 participants for about 12 months. All of them were T2DM patients taking Metformin since at least 3 months and greater duration at a minimum daily dosage of 1 gram or more. The parameters recorded were age, gender, serum levels of vitamin B12, Metformin dose and duration, vegetarian /non vegetarian status, alcohol consumption habits etc. Metformin related vitamin B₁₂ deficiency was significantly being influenced by increasing age, especially who were regular alcohol consumers. However, apart from this special sub group of population, the risk of vitamin B12 deficiency was not found statistically significant in relation to the dose or duration of metformin usage, age or sex of the study populations. This study showed that vitamin B12 deficiency was evident in 108/231 (43.7%) among metformin treated T2DM patients. Vitamin B12 deficiency was found more often in people of > 60 years of age (P=0.035) and who were involved in regular alcohol consumption (P=0.009). Metformin dosage (P=0.102) or its duration (P=0.090) were not found statistically associated with vitamin B12 deficiency. Vitamin B12 deficiency was not significantly associated with vegetarian (P=0.132) either.

On contrary to these findings, various other studies on literature reviews have shown that the prevalence of metformin induced vitamin B12 deficiency is very high while few other studies have shown that there is no or minimum effect of Vitamin B12 deficiency among chronic metformin users.

Metformin which delays glucose absorption, by reducing small bowel motility⁶. According to some study reviews, GI effects are more frequently observed in patients while they are on metformin treatment. Metformin also induces B12 malabsorption which leads to defect in binding of B12-intrinsic factor (IF) complex and a reduction of

B12 absorption⁶. Also, metformin related alterations in IF levels and/or ileal morphological structure lead to B12 reduction since the B12-IF complex binds to the ileal cell surface receptor^{7, 8}. There has been conflicting reports on the clinical significance of biochemical change in serum vitamin B12 concentrations. Some studies showed that lower serum B12 concentration caused by metformin within the normal range could be clinically meaningful while others show no conclusive clinical side effects. A Greek cohort including 600 diabetic patients showed association between metformin prescription and vitamin B12 dependent megaloblastic anemia³. Cognitive impairment and the progression of diabetic peripheral neuropathy may also be accelerated by metformin in a vitamin B12 dependent manner⁹. So, it was implied that the decrease of serum B12 concentration within the normal range should not be overlooked¹. However, some studies showed that metformin may even improve B12 metabolism¹⁰, or only decreases the inactive form of vitamin B12 (known as holo-haptocorrin, holoHC), rather than the biological active form (known as holo-haptocorrin, holoTC)^{11, 12}. On the other hand, some other studies just provided the opposite results¹³ and the lowered serum level of vitamin B12 caused by metformin was reported to be clinically meaningful¹. Geographic, religious, sociocultural and food habits of the different countries have different impact on metformin induced vitamin B12 deficiency. Prevalence of vitamin B12 deficiency varies with population and B12 cut-off used in the studies. According to a national survey from U.S., consumption of routine vitamin B12 supplement may not be sufficient for the biochemical B12 reduction with metformin use. The recommended amount of vitamin B12 by the Institute of Medicine (IOM) may not be sufficient in metformin treated patients¹⁴. However, several other studies^{15, 16} have found that treatment with vitamin B12 in a higher dose could reverse some of these disadvantages and vitamin B12 deficiency. Therefore, the supplement of vitamin B12 may be reasonable, and the dose

should be individualized according to different ethnicity and habits.

Jayashri, R., Venkatesan, U., Rohan, M. et al. published their study in *Acta Diabetol* (2018). They determined the prevalence of vitamin B12 deficiency in an urban south Indian population in patients of glucose tolerance. The mean levels of vitamin B12 significantly lower in patients with glucose intolerance. The prevalence of absolute vitamin B12 deficiency was 14.9%. The prevalence of vitamin B12 deficiency was higher among T2DM (18.7%). The increased deficiency were seen in increasing age and abdominal obesity. Men and vegetarians had twice the chance of vitamin B12 deficiency than women and non-vegetarians, respectively¹⁷.

Among others, one of the important limitations of this study design is that there is no comparative group or control group among the study population to compare metformin and non metformin group for their vitamin B12 status. It was just an observational study which examined the evidence of the vitamin B12 status among metformin users. So, further comparative or cohort study may be required to prove the fact that metformin use has been statistically linked to vitamin B12 deficiency. However, the concluding remarks from this study could be metformin is associated with vitamin B12 deficiency and this association is strong in elderly who regularly consume alcohol. Thus, in this sub group of diabetic patients metformin should be prescribed with vitamin B12 supplementation to avoid its deficiency and possible complications related to its deficiency.

Conclusion

Vitamin B12 deficiency was found in 43.7% of diabetics who were treated with Metformin. The deficiency was found statistically significant among >60 years of age (P=0.035) specially among those whom were involved in a regular alcohol consumption (P=0.009).

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