

Original Research Article

Correlation of vitamin B12 deficiency with metformin therapy in type 2 diabetes mellitus patients

S. B. Nayyar¹, Manbir Kaur Dhillon^{1*}, Sahiba Kukreja²

¹Department of Medicine, ²Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar, Punjab, India

Received: 17 December 2019

Accepted: 23 December 2019

*Correspondence:

Dr. Manbir Kaur Dhillon,

E-mail: bickyradio@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Diabetes mellitus is an endocrine and metabolic disorder, characterized by hyperglycemia. Metformin is the first line pharmacotherapy recommended by ADA. It has been recognized that metformin use is associated with serum vitamin B12 deficiency. The objective of this study is to determine the prevalence of serum vitamin B12 deficiency among Indian patients with type 2 diabetes mellitus on metformin therapy compared to those who are not on metformin.

Methods: Here, 90 patients of type 2 diabetes mellitus of both sexes were included in this cross-sectional study conducted from January 2018 to August 2019 in SGRDIMS, Sri Amritsar. They were divided into two groups; Group A of 60 subjects of type 2 diabetes mellitus on metformin therapy >1 year and group B of 30 subjects of type 2 diabetes mellitus not on metformin or had stopped taking metformin 6 months back. Serum vitamin B12 levels were measured using a Chemiluminescence method. Data was statistically analysed.

Results: The serum vitamin B12 levels were 190.02 ± 90.75 pmol/l in group A (metformin users) and 586.9 ± 243.69 pmol/l in group B (not metformin users) (p value=0.002). A significant negative correlation existed between the serum vitamin B12 and duration and dose of metformin use ($r = -0.676$) and ($r = -0.855$) by using Pearson's correlation coefficient in group A.

Conclusions: Metformin is associated with decrease in serum vitamin B12 levels. Annual screening of serum vitamin B12 is recommended for patients of type 2 diabetes mellitus who are on metformin therapy for longer duration and /or in higher doses.

Keywords: Metformin, Type 2 diabetes mellitus, Vitamin B12

INTRODUCTION

“Every fifth Indian will be a diabetic and every fifth Diabetic in the world will be an Indian”¹

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “diabetes capital of the world”. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the

elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people.²

Diabetes mellitus is a metabolic disorder that is increasingly becoming a public health concern. The disease is associated with a variety of systemic macrovascular and microvascular complications.³ The metabolic dysregulation associated with diabetes mellitus leads to secondary pathophysiological changes in multiple organ systems which result in various complications, responsible for the morbidity and

mortality associated with the disease. About 80% of the diabetic population lives in developing countries, the largest numbers in the Indian subcontinent and in China. India had 69.2 million people living with diabetes (8.7%) as per the 2015 data. Of these, it remained undiagnosed in more than 36 million people.

Metformin is a first line medication for the treatment 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.⁴ Metformin induces vitamin B 12 malabsorption, which may increase the risk of developing vitamin B 12 deficiency i.e., clinically important and treatable condition. As metformin has been prescribed worldwide and treatment periods increase, the prevalence of metformin-induced vitamin B12 deficiency may have also significantly increased. However, the relationship between metformin use and vitamin B12 deficiency in the Asian population has not been widely investigated.⁵

Vitamin B12 deficiency can lead to hematological abnormalities, cognitive disorders, and neuropathy. This vitamin is obtained from animal origin food, so its deficiency may be related to eating habits, changes in the absorption or metabolic mechanisms.⁶

The present study was undertaken due to paucity of Indian studies evaluating the prevalence of serum vitamin B12 deficiency and its association with duration and dose of metformin use in Type 2 diabetics on metformin therapy.

METHODS

The present hospital based study included 90 subjects of type 2 diabetes mellitus visiting OPD/Indoor of SGRDIMSR, Sri Amritsar from January 2018 to August 2019.

A cross-sectional comparative study was conducted in the Department of Medicine in collaboration with the Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar.

The American Diabetes Association criteria for the diagnosis of type 2 diabetes mellitus:

- A1C \geq 6.5%
- FPG \geq 126 mg/dL (7.0 mmol/L)
- 2-hour PG \geq 200 mg/dL (11.1 mmol/L) during an OGTT
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose \geq 200 mg/dL (11.1 mmol/L).

Relevant history, bio-data and consent of the patient was taken. The subjects were divided into two groups. Group A of 60 subjects of type 2 diabetes mellitus on metformin therapy >1 year and group B of 30 subjects of type 2

diabetes mellitus not on metformin or had stopped taking metformin 6 months back.

Inclusion criteria

- Type 2 Diabetes mellitus patients
- Age >30 years.

Exclusion criteria

- Diabetic patients having Pulmonary Koch's, Bronchial asthma or chronic co-morbid conditions.
- Patients taking multi vitamins, vitamin B12 or calcium supplements or having history of these drugs intake in last 3 months.
- Type 1 diabetes mellitus
- History of Malabsorption Syndrome, Intestinal infections
- Habit of alcohol consumption, smoking

Patients were verified with inclusion and exclusion criteria. All patients and their relatives were informed about the study in their vernacular language and written consent was taken. A detailed history of each patient along with complete clinical examination was done. Routine investigations like complete blood count, urine complete examination, renal function tests, serum electrolytes- sodium, potassium, calcium, HBA1C and serum vitamin B12 were done.

This study was carried out after approval from Institutional Ethical Committee. The data from the present study was systematically collected, compiled and statistically analysed to draw relevant conclusions using SPSS Statistics-20 version. The observations were tabulated in the form of mean \pm Standard Deviation (SD). Continuous variables were analysed using Analysis of Variance (ANOVA). In parametric data, student-t test was used. Quantitative variables were correlated using chi-square test. The data was analysed and level of significance was determined as its 'p' value with $p < 0.05$ as significant, $p < 0.001$ as highly significant and $p > 0.05$ as non-significant.

RESULTS

A total of 90 patients were studied. The youngest patient was 31 years and oldest was 86 years. The mean age of patients in years was 57.53 ± 11.86 in group A and 55.37 ± 13.93 in group B. Maximum patients were in the 40-60 year age group. The mean age of the two groups was not significant (as the p value was 0.443). 35% patients in group A and 33.3% patients in group B were male. The baseline characteristics of the two groups were comparable as shown in Table 1.

The mean BMI of the group A was 29 ± 4.94 and of group B was 28.67 ± 4.71 . The difference in mean BMI of the two groups was statistically not significant (p value=0.760).

Table 1: Baseline characteristics of the subjects.

Variable	Group A (metformin)	Group B (no metformin)	p value
Age (years)	57.53±11.86	55.37±13.93	0.443
Sex, n (%)			
Male	21(35%)	10(33.3%)	
Female	39(65%)	20(66.7%)	
Blood urea nitrogen	21.92±7.67	20.6±9.08	0.545
Serum creatinin	1.4±0.58	2.14±1.69	0.053
Systolic blood pressure	128.5±14.94	125±10.09	0.251
Diastolic blood pressure	85.83±7.87	87±5.96	0.477
Hemoglobin	10.3±2.36	10.31±2.69	0.988

The duration of diabetes in group A was 8.9±4.80 years and of group B was 7.53±3.27 years. The difference in mean duration of diabetes of the two groups was statistically not significant (p value=0.24). The mean fasting plasma glucose in the group A was 144.78±14.74 and in group B was 138.67±17.98. The difference in mean fasting plasma glucose of the two groups was statistically not significant (p value=0.088) (Table 2).

Table 2: Distribution of duration of diabetes, fasting Plasma glucose and HbA1c.

	Group A Mean±SD	Group B Mean±SD	p Value
Duration of diabetes (Years)	8.9±4.80	7.53±3.27	0.24
Fasting plasma glucose	144.78±14.74	138.67±17.98	0.088
HbA1c (%)	9.48±1.95	9.16±2.22	0.002

The mean serum vitamin B12 in group A was 190.02±90.75 and in the group B was 586.9±240.69. The difference in mean serum Vitamin B12 levels of the two groups was statistically significant (p value=0.002). The metformin users had significant reduction in the serum vitamin B12 levels as shown in Table 3.

Table 3: Distribution of serum vitamin B12.

	Group A Mean±SD	Group B Mean±SD	p value
Serum Vitamin B12	190.02±90.75	586.9±240.69	0.002

The mean levels of vitamin B 12 showed a steady decline with increasing duration of metformin use in years. The mean serum vitamin B12 levels were 275.73±118.26, 234.78±100.36, 112.27±55.07 and 63.14±28.08 in <5 years, 5-10 years, 11-15 years and >15 years respectively. The levels were remarkably reduced in those taking metformin for >10 years. The difference of the groups

was statistically significant (p value =0.015) as shown in Table 4.

Table 4: Distribution of mean vitamin B12 levels according to duration of metformin use in years.

Duration of metformin use in years	Study group		
	n	%	Mean±SD
< 5	26	43.3	275.73±118.26
5-10	9	15.0	234.78±100.36
11-15	11	18.3	112.27±55.07
>15	14	23.3	63.14±28.08
p value	0.015		

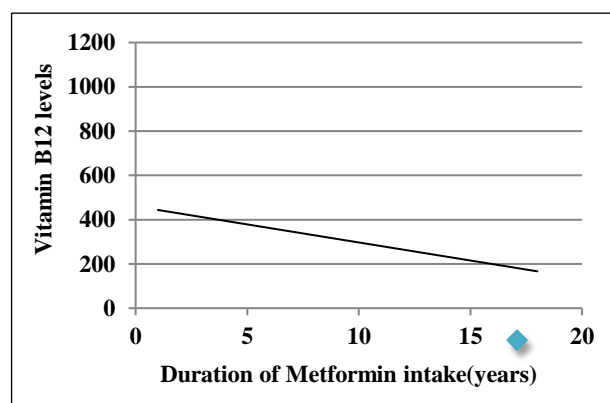


Figure 1: Negative correlation between duration of metformin use in years and serum vitamin B12 levels.

There was a negative correlation between duration of metformin use in years and serum vitamin B12 levels (r=-0.676) by using Pearson’s correlation coefficient in study group as with the increase in duration of metformin use, the levels of serum vitamin B12 declined as shown in Figure 1.

The mean levels of vitamin B12 showed a clear decline as the dose of metformin increased. The mean serum vitamin B12 levels were 371.69±52.66, 208.65±88.45, 119.06±52.37 and 37.2±13.89 with 500 mg, 1000 mg, 1500 mg and 2000 mg respectively. The levels of serum vitamin B12 showed a decline with increasing dose of metformin use. The difference of the groups was statistically significant (p value was 0.006) as shown in Table 5.

Table 5: Distribution of mean serum vitamin B12 according to dose of metformin.

Doses (in mg)	Study group		
	(n=60)	%	Mean ± SD
500	13	21.7	371.69±52.66
1000	20	33.3	208.65±88.45
1500	17	28.3	119.06±52.37
2000	10	16.7	37.2±13.89
p value	0.006		

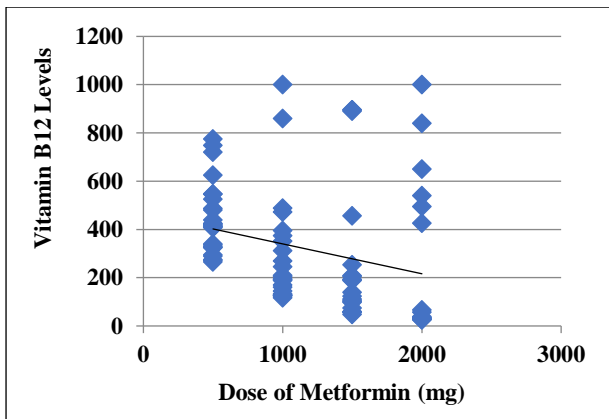


Figure 2: Negative correlation between dose of metformin and serum vitamin B12 level.

There was a negative correlation between dose of metformin use in mg and serum vitamin B 12 levels ($r=0.855$) by using Pearson's correlation coefficient in study group as with the increase in dose of metformin use, the levels of serum vitamin B12 declined as shown figure 2.

DISCUSSION

Metformin therapy with lifestyle modification is recommended as a first line therapy in majority of type 2 diabetes mellitus patients. One of the recently documented side effect of metformin therapy is vitamin B12 deficiency. Limited data is available on this topic from South Asian sub-continent. In this study we found very high (66.67% %) prevalence of vitamin B12 deficiency in Type 2 Diabetes mellitus patients on metformin therapy. In a study conducted by Nishant Raizada et al, the prevalence was found to be 35.5%.⁴ This was not surprising considering that the prevalence of Vitamin B12 in the apparently healthy populations in India had been reported to be as high as 33.3%-67%.^{7,8} A predominantly vegetarian diet could be one of the causes of higher prevalence of Vitamin B12 deficiency in India.⁷

The mean age in the present study was 57.53 ± 11.86 years in group A and 55.37 ± 13.93 years in Group B. The difference between these two values was non-significant. In this study the most common age group was from 40-60 years, constituting 53.3 %, followed by >60 years (35.5%). There were 31 males and 59 females included in the study. It included more females than males and alcoholics and smokers were excluded. This study was done in pure vegetarian population. In a study conducted by Turkai Alharbi et al, the metformin-using group included 319 participants with an average age of 57.8 ± 0.6 years, and the non-metformin users included 93 participants with an average age of 56.6 ± 1.4 years.⁹

The difference in mean Hemoglobin and mean HbA1C of the two groups was not statistically significant (p value >0.005). In a study done by Holay MP et al, parameters

like age, HbA1c, typing of anemia did not show any statistically significant difference between two groups.¹⁰ Sun Hye Ko et al, found that there was no association of serum vitamin B12 levels with sex, age, BMI, and HbA1c value.⁵

In the present study, the mean MCV of study group was 89.77 ± 5.98 and of control group was 90.87 ± 4.88 . There was no significant difference between the mean MCV values of the two groups as the p value was >0.005. Rudra Prasad Roy et al, in 2016 found no patient having any hematological evidence of Vitamin B12 and folic acid deficiency, i.e. macrocytic anemia.¹¹

In the present study, the mean duration of diabetes in the study group was 8.9 ± 4.80 and in the control, group was 7.53 ± 3.27 . This was not statistically significant (p value=0.24). In a study done by Tukai Alharbi et al, the average duration of diabetes was also higher among the metformin users ($p < 0.01$).⁹

There are no consensus on cut-off point to define mild, moderate, severe vitamin B12 deficiency. Authors defined 210 to 150 pg/ml as mild, 100-149 pg/ml as moderate and <99 pg/ml as severe vitamin B12 deficiency.

Metformin influenced not only vitamin B12 but also serum levels of homocysteine. Homocysteine levels were not measured in this study subjects due to financial constraints.

In the present study, mean Vitamin B12 levels in the study group was 190.02 ± 90.75 and in the control group was 586.9 ± 243.69 . The difference in Vitamin B12 of the two groups was statistically significant (p value=0.002). Reinstatler et al, in their study found that the geometric mean serum B12 concentration among those with type 2 diabetes taking metformin was 317.5 pmol/L. This was significantly lower than the geometric mean concentration in those with type 2 diabetes not taking metformin (386.7 pmol/L; $p=0.0116$) and those without diabetes (350.8 pmol/L; $p=0.0011$).¹²

In the present study the mean serum vitamin B12 concentration in males was 172.05 ± 84.81 and in females 199.69 ± 97.87 . Sun Hye Ko et al, in their study observed similar results as the mean serum vitamin B12 concentration was 665.7 ± 246.7 pg/mL (644.1 ± 243.3 pg/mL in men, 664.0 ± 249.3 pg/mL in women), and there was no significant difference according to sex.⁵

Dose of metformin had inverse relation to serum vitamin B12 levels in the present study. Authors had compared vitamin B12 level among subgroups of patients taking metformin 500 mg, 1000 mg, 1500 mg, and >1500 mg and difference was found to be statistically significant p value (<0.005). There was a steady decline in the levels of vitamin B12 as the doses of metformin increased. In a study done by Turkai Alharbi et al, the vast majority of metformin users (301; 94.36%) were on a regimen of 1000–2000 mg/day, while 12(3.76%) and 6(1.88%) were

on regimens of >2,000 mg/day and <1000 mg/day, respectively.⁹

Duration of metformin had inverse relation to serum vitamin B12 levels in the present study the levels of vitamin B12 showed a progressive and significant decline with the increasing duration of metformin use.

The mean levels of vitamin B 12 in patients taking metformin for <5 years, 5-10 years 11-15 years and >15 years were 275.73±118.26, 234.78±100.36, 112.27±55.07 and 63.14±28.08 respectively. This observation was consistent with other previous studies. In a study done by Turkai Alharbi et al, they observed that levels of serum vitamin B12 were lower when metformin was taken in a dose greater than 2,000 mg/day and for a period exceeding 4 years. The majority of metformin users i.e., 196 (61.13%) had been using it for more than 4 years, while 123 (38.56%) had been using it for 1-4 years, and only 1 patient (0.31%) had been using it for less than 1 year.⁹

The main strength of this study was that we had divided metformin use by dosage and length of use like many previous studies focus only on one factor, either dose or duration. This study compared the prevalence of vitamin B12 deficiency in metformin users and non-users, and subdivided metformin use on the basis of two factors: length of use and dosage per day. The presence of a control group made it possible to compare prevalence of vitamin B12 deficiency in a similar population not using metformin.

The limitation of the study was that we used only serum vitamin B12 levels to define deficiency; metabolites such as methylmalonic acid and total homocysteine were not measured. They are considered more sensitive markers of Vitamin B12 deficiency. Authors did not evaluate concomitant folate deficiency. The sample size of this study was small.

Due to lack of follow-up in this study, we were not able to show that Vitamin B12 supplementation or change over to other anti-diabetic drug could improve the condition.

CONCLUSION

This study concluded that metformin which is first line oral hypoglycaemic agent as recommended by ADA is significantly associated with decrease in vitamin B12. It was demonstrated that vitamin B12 deficiency occurs more frequently in patients with type 2 diabetes mellitus in those taking larger amounts of metformin with longer duration of metformin. Currently, there are no published guidelines advocating routine screening for vitamin B12 deficiency among patients with type 2 diabetes mellitus undergoing metformin treatment. Although the clinical significance of vitamin B12 deficiency remains unclear, this study suggests the need for routine vitamin B12 monitoring in patients with type 2 diabetes mellitus, especially those taking metformin dose \geq 1,000 mg per

day and for >10 years, even in the absence of haematological abnormalities.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Joshi SR, Parikh RM. India; the diabetes capital of the world: Now heading towards hypertension. *J Assoc Phys Ind.* 2007;55(Y):323.
2. Huizinga MM, Rothman RL. Addressing the diabetes pandemic: A comprehensive approach. *Ind J Med Res.* 2006;124(5):481.
3. Nareddy VA, Boddikuri IP, Ubedullah SK, Papareddy A, Miriyam MK. Correlation of Vit B12 levels with metformin usage among type 2 diabetic patients in a tertiary care hospital. *Int J Adv Med.* 2018;5(5):1128-32.
4. Raizada N, Jyotsna VP, Sreenivas V, Tandon N. Serum vitamin B12 levels in type 2 diabetes patients on metformin compared to those never on metformin: a cross-sectional study. *Ind J Endocrinol Metabol.* 2017;21(3):424.
5. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, et al. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *J Korean Med Sci.* 2014;29(7):965-72.
6. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. *Revista Da Associacao Medica Brasileira.* 2011;57(1):46-9.
7. Ingole JR, Patel RD, Ingole SJ, Pandave HT. Opportunistic screening of Vitamin B12 deficiency in IT professionals presenting for routine health check-up. *J Clin Diag Res: JCDR.* 2015;9(12):OC01.
8. Yajnik C, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. *Japi.* 2006;54(775):82.
9. Alharbi TJ, Tourkmani AM, Abdelhay O, Alkhashan HI, Al-Asmari AK, Rashed AM, et al. The association of metformin use with vitamin B12 deficiency and peripheral neuropathy in Saudi individuals with type 2 diabetes mellitus. *PloS One.* 2018;13(10):0204420.
10. Holay MP, Vyawahare M, Shekar K. Serum B12 Levels in Type II Diabetics on Metformin Therapy and its association with Clinical Neuropathy. *IJBABN.* 2017;8(8):321-6.
11. Roy RP, Ghosh K, Ghosh M, Acharyya A, Bhattacharya A, Pal M, et al. Study of Vitamin B12 deficiency and peripheral neuropathy in metformin-treated early Type 2 diabetes mellitus. *Ind J Endocrinol Metabol.* 2016;20(5):631.

12. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements: the National Health and Nutrition Examination Survey, 1999-2006. *Diab care.* 2012;35(2):327-33.

Cite this article as: Nayyar SB, Dhillon MK, Kukreja S. Correlation of vitamin B12 deficiency with metformin therapy in type 2 diabetes mellitus patients. *Int J Adv Med* 2020;7:200-5.