



## PREVALENCE OF VITAMIN B-12 DEFICIENCY IN TYPE 2 DIABETES PATIENTS ON LONG-TERM METFORMIN THERAPY

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**ABSTRACT** **Background:** Vitamin B-12 or cobalamin is a water-soluble vitamin that plays a very fundamental role in DNA synthesis, optimal haemopoiesis, and neurological function. The clinical picture of vitamin B12 deficiency, hence, is predominantly of features of haematological and neurocognitive dysfunction. **Objectives of the Study:** To determine the prevalence of Vitamin B12 deficiency in type2 diabetic patients on long-term metformin therapy (for >2years). **Material and Methods:** Cross-sectional, observational, prevalence study conducted from November 2018 to October 2019 in Postgraduate Department of Medicine, Government Medical College, Jammu on 100 diabetic patients who were on long term (>2 years) metformin therapy. Serum Vitamin B12 levels were determined by the ADVIA Centaur vitamin B-12 assay, which is a competitive immunoassay using direct chemiluminescent technology. Vitamin B12 level <21pg/ML was taken as vitamin B-12 deficiency. **Results:** In our study, the total number of patients were 100 among which 51% were males, majority 52% were in the age group of 41-60 years, majority 75% were on non-vegetarian diet and majority 41% were having 2-5 years duration on metformin therapy. Among the studied patients, the prevalence of Vitamin B-12 deficiency was reported among 38% patients. Among those with Diet, Majority among Vegetarians (56%) had Vitamin B-12 deficiency, and the association was found to be statistically significant. While those with gender differentiation, majority males (41.2%) were found to be Vitamin B-12 deficient, but the association was found to be statistically insignificant. The average Vitamin B-12 Levels among males were higher 437.9 pg/ML while in females it was lower 388.2pg/ml, moreover, it was also lower among vegetarians 199.8pg/ml while higher in non-vegetarians 484.87pg/ml. **Conclusion:** Metformin is significantly associated with a decrease in Vitamin B12 levels in the body, and Vitamin B12 deficiency is more common among vegetarian population on prolonged metformin therapy than non-vegetarian population.

**KEYWORDS :** Diabetes, Metformin therapy, Vitamin B-12 Deficiency

### INTRODUCTION:

Diabetes is a known disease for 200 years and is characterized as a group of metabolic disorder that share the phenotype of hyperglycaemia state causing polydipsia, polyphagia and polyuria. As per the data, about 285 million people had diabetes till 2010 with 90% having type-2 Diabetes Melmed S *et al.*(1) and by 2030, the incidence will be doubled as estimated by Wild S *et al.*(2). In India, 7.1% of India's adult population is affected with diabetes, i.e. nearly 62 million people are affected. The high incidence is because of a combination of genetic susceptibility plus adoption of high-calorie diet and sedentary lifestyle by India's growing middle class (3). Regarding management of type-2 Diabetes, multidisciplinary approach is preferred. Life style intervention in high-risk group results in 35-58% reduction in incidence of type-2 Diabetes (4). Metformin is recommended as initial therapy after life style modification for type-2 diabetes (5). In America metformin was approved in 1996 and since then its use is increased dramatically following UKPDS study, when it was found to be effective in decreasing diabetes related chronic complications and mortality in type-2 obese patients (6).

Metformin acts on several tissues via activation of the adenosine monophosphate-activated protein kinase (AMPK) system to reduce serum glucose. The primary effect of metformin is to suppress hepatic gluconeogenesis (7). One of the risky side effects of metformin is lactic acidosis, which can be overcome with judicious use of metformin. Other side effects like abdominal distress and diarrhoea appear within first few days of initiation of metformin but disappear after discontinuation of metformin therapy (5). Another clinically relevant side effect of metformin therapy is vitamin B12 deficiency. Vitamin B-12 or cobalamin is a water-soluble vitamin that plays a very fundamental role in DNA synthesis, optimal haemopoiesis, and neurological function. The clinical picture of vitamin B12 deficiency, hence, is predominantly of features of haematological and neurocognitive dysfunction (8).

The principal source of vitamin B12 includes liver, egg yolk, meat, cheese, etc. About 2/3 to 4/5 of body's content of vitamin B12 is stored in liver(9).

Vitamin B12 is absorbed mainly in terminal ileum with the help of

intrinsic factor secreted from parietal cells of stomach. Vitamin B12 can also be absorbed by the process of passive diffusion in the small intestine which doesn't require intrinsic factor. However, only about 1% of the vitamin B12 dose is absorbed by passive diffusion. In other words, when 100µg of vitamin B12 is administered, approximately 1µg is likely to be absorbed. The various causes of vitamin B12 deficiency includes nutritional deficiency, gastric mucosal damage, pernicious anaemia, drugs like metformin and Proton Pump Inhibitors (PPIs) etc. Vitamin B12 malabsorption was observed in 30% of patients taking long-term metformin therapy and low serum levels of vitamin B12 were observed in about 20% of cases having vitamin B12 malabsorption (10). Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as the 4th month. However, because of storage in liver, clinical symptoms of vitamin B12 deficiency may manifest after 5–10 years (9). The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include:

1. Alterations in small bowel motility which stimulate bacterial overgrowth and consequential vitamin B12 deficiency.
2. Competitive inhibition or inactivation of vitamin B12 absorption.
3. Interactions in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor.

Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum (8). Increase in metformin dose by 1g /day increases risk of vitamin B12 deficiency by greater than two-fold. Subjects consuming metformin for over ten to twelve year and daily dosage  $\geq$  2g showed about a fourfold higher risk of vitamin B12 deficiency compared to those with metformin use of less than four years and daily dosage of  $\leq$ 1g. (11). Vitamin B12 deficiency may have serious consequences such as megaloblastic anaemia, myelopathy and neuropathy, and subnormal cobalamin concentrations have been associated with dementia. Megaloblastic anaemia because of metformin associated vitamin B12 deficiency has been reported, but it can be treated successfully with cyanocobalamin. Symptoms of B12-related neuropathy can be misinterpreted as diabetes neuropathy (12). The progression of neurological damage could be managed by early detection of vitamin B12 deficiency and with appropriate B12 supplementation. However, this vitamin B12 deficiency may lead to permanent neurological damage if it is misdiagnosed as diabetic neuropathy (13).

**OBJECTIVES OF THE STUDY:**

To determine the prevalence of Vitamin B12 deficiency in type2 diabetic patients on long-term metformin therapy (for >2years).

**MATERIAL AND METHODS:**

**Study Design:** Cross-sectional, observational, prevalence study. **Study Period:** November 2018 to October 2019. **Study Area:** Postgraduate Department of Medicine, Government Medical College, Jammu. **Sample Size:** A total of 100 diabetic patients on long term (>2 years) metformin therapy. **Inclusion criteria:** Patients who have given written informed consent, Patients diagnosed as Type 2 Diabetes Mellitus as per ADA guidelines, Type 2 Diabetic patients of age >18yrs, Type 2 Diabetic patients on intake of metformin for >2yrs. **Exclusion criteria:** Patients who have not given written informed consent, Type 1 diabetes mellitus, Diabetic complications, Intake of calcium, Malabsorption Syndrome, Intestinal infection, Partial/total gastrectomy, Patients on vitamin B12 supplements, Hypothyroidism. **Procedure:** Serum Vitamin B12 levels were determined by the ADVIA Centaur vitamin B12 assay which is a competitive immunoassay using direct chemiluminescent technology. Vitamin B12 level <211pg/mL was taken as vitamin B 12 deficiency. **Statistical analysis:** Depending upon the data available appropriate statistical test were applied qualitatively. **Ethical Approval:** The study had no ethical issue pertaining to animal or human experimentation and the proposal for the same was presented to the Institutional Ethical committee before the commencement of the study and the same was cleared by the committee.

**RESULTS:**

Characteristics of the study participants, dietary pattern and duration of their metformin therapy are described in [Table 1]. In our study, the total number of patients were 100 among which 51% were males, majority 52% were in the age group of 41-60 years, majority 75% were on non-vegetarian diet and majority 41% were having 2-5 years duration on metformin therapy.

Among the studied patients, the prevalence of Vitamin B-12 deficiency was reported among 38% patients. [Table 2] Vitamin B12 deficiency in patients on metformin therapy as per their diet and gender is described in [Table 3]. Among those with Diet, Majority among Vegetarians (56%) had Vitamin B-12 deficiency and the association was found to be statistically significant. While those with gender differentiation, majority males (41.2%) were found to be Vitamin B12 deficient but the association was found to be statistically insignificant. Average Vitamin B12 levels in the studied population as per gender and dietary pattern is shown in [Table 4]. The average Vitamin B-12 Levels among males were higher 437.9 pg/mL while in females it was lower 388.2pg/mL, moreover it was also lower among vegetarians 199.8pg/ml while higher in non-vegetarians 484.87pg/ml. [Table 5] shows the dietary pattern in both female and male patients and average vitamin B 12 level according to dietary pattern. It was quite evident from the table that Vitamin B-12 levels were higher among those with non-vegetarian diet and with male gender. Vitamin B-12 deficiency according to the duration of metformin therapy is shown in [Table 6]. Among patients who were taking metformin for 6-10 years and > 10 years; 60.7% and 61.3% patients were vitamin deficient whereas patients who had taken metformin therapy for 2-5 years had deficiency in only 4.9% this showed that duration of therapy has role to play in causing vitamin B12 deficiency.

**DISCUSSION:**

Diabetes is a group of metabolic disorder that show the phenotype of hyperglycaemia. Over the period of time, the metabolic dysregulation associated with diabetes causes pathophysiological changes in multiple organs. However, a common potential interaction of metformin with vitamin B12 is well documented but is poorly studied by the physicians who prescribe metformin to their diabetic patients. This study group comprise 100 patients attending OPD and admitted patients in Government Medical College Jammu. Total of 100 patients were taken out of which 51 patients were male and 49 patients were females. Maximum patients belonged to the age group of 41-60 years (52%). In our study 25 % patients were vegetarian and 75 % were non vegetarian.

Our study showed 38 % of the total patients who were on metformin therapy for >2 years were found to be Vitamin B-12 deficient. Among males 41.2 % and among females 32.7 % were found to be vitamin B-12 deficient. 56 % vegetarian patient were vitamin B12 deficient and 32 % non-vegetarian patients were vitamin B12 deficient and has a p

value of 0.032 (via Pearson chi square method) which is significant. Among patients who were taking metformin for 6-10 years and > 10 years; 60.7% and 61.3% patients were vitamin deficient whereas patients who had taken metformin therapy for 2-5 years had deficiency in only 4.9% this showed that duration of therapy has role to play in causing vitamin B12 deficiency. Our study is supported by hospital based cross sectional studies conducted by Tomkins GH *et al.*, (10) who evaluated the vitamin B12 status of the 71 patients on prolonged metformin therapy. Vitamin-B12 malabsorption has been found in 21 (30%) of 71 diabetic patients taking long-term metformin therapy in addition to dietary management. Kalitsa FK *et al.*, (14) who studied that prevalence of megaloblastic anaemia among 40% patients on long term metformin therapy when compared to non-metformin therapy due to reduced serum vitamin B12 levels & is reversible with cyanocobalamin treatment. Pongchaidecha M *et al.*, (15) who studied that there was a significant depletion in the levels of serum vitamin B12 among patients who had been on long term metformin treatment. Ting RZ-W *et al.*, (16) who studied that 50% of patients have metformin related vitamin B12 deficiency. Clinically important & statistically significant association of vitamin B12 deficiency has been noticed in patients using metformin >3yrs when compared to those receiving for <3yrs. Pflipsen MC *et al.*, (17) who studied that 22% of diabetic patients had metabolically confirmed vitamin B12 deficiency. Patient on metformin had lower serum vitamin B12 levels as defined by serum vitamin B12 levels < 350pg/ml and were at increased risk of developing vitamin B12 deficiency. Mahajan R *et al.*, (18) who showed that deficiency of vitamin B12 is a known sequelae of metformin therapy. Jager JD *et al.*, (19) who studied that there was a mean decrease of vitamin B12 concentration in 19% patients on long term metformin therapy for >4years which is preventable. Wile DJ *et al.*, (20) in a who conducted a prospective case control study with 122 T2DM patients a studied that Metformin treated patients had depressed cobalamin levels (231 pmol/L vs. 436 pmol/L for controls; p <0.001) and elevated fasting methylmalonic acid (MMA) and homocysteine levels. Vitamin B12 deficiency was seen in 31% of patients on Metformin as opposed to 3% in case of controls (p<0.001). Iftikhar R *et al.*, (21) who did a case control study on 112 diabetic patients; they observed that serum vitamin B12 levels were low in 35 patients (31%) on metformin as compared to only 9 patients (8.6%) among controls, (p value 0.002). Mean B12 levels were significantly low in metformin group 311 pg/ml ( $\pm 194.4$ ), p value 0.03. Dose of metformin had inverse correlation with vitamin B12 levels and the difference was statistically significant with p value< 0.001. Kiran MD *et al.*, (22) who compared vitamin B12 levels in diabetic patients on metformin and other antidiabetics and they noticed that there was reduction in vitamin B12 levels with metformin with levels of 272.5pg/ml compared to 714.6pg/ml with other antidiabetics at the end of first period. The levels increased from 272.5pg/ml to 615.9 pg/ml at the end of second period after receiving the combination of metformin and methyl cobalamin. Agarwal P *et al.*, (23) who conducted case control study in total of 100 subjects and they observed that, mean serum vitamin B12 levels in the study group was 431.84 $\pm$ 265.76 and in control was 744.76 $\pm$ 271.927; and the difference was statistically significant. Mean serum vitamin B12 levels in vegetarians (547.27 $\pm$ 303.011) were significantly lower than in non-vegetarian (699.22 $\pm$ 307.992) (p value 0.029). Damião CP *et al.*, (24) who conducted a cross sectional clinical trial with 462 T2DM patients on metformin in Brazil.30 The study demonstrated vitamin B12 deficiency in 22.5% of patients on metformin as compared to 7.4% of controls. The mean serum vitamin B12 was 272 pg/ml and was significantly (p <0.001) lower than controls with 348 pg/ml. Rana R *et al.*, (24) who studied 60 diabetic patients. Serum vitamin B12 level mean was 424 $\pm$ 158 pg/ml in metformin group while it was 619 $\pm$ 177pg/ml in non-metformin. Verma VK *et al.*, (25) who studied Vitamin B12 levels among vegetarian and non-vegetarian diabetic population receiving prolonged Metformin therapy .Statistical analysis of 93 patients included in study showed that vitamin B12 deficiency is common among the vegetarian (56.52%) and non-vegetarian (35.71%) population but the difference is not statistically significant (p value=0.29) which is more in favour of metformin associated vitamin B12 deficiency in non-vegetarian population. Alharbi TJ *et al.*, (26) who studied 412 T2DM patients; 319 patients were taking metformin and 93 were taking other antidiabetic drugs. The prevalence of B12 deficiency was 7.8% overall, but 9.4% and 2.2% in metformin users and non-metformin users, respectively. Alvarez M *et al.*, (27) who studied vitamin B12 levels in 162 patients. Altered vitamin B12 levels (borderline or low) were found in about 29

% of the patients (47 cases 95% CI). The prevalence of borderline levels of Vitamin B12 was 20.9% and the prevalence of low levels was 7.4%. Metformin dose was significantly inversely associated with vitamin B12 levels.

**Table 1: Characteristics of the study participants, dietary pattern and duration of their metformin therapy**

Variables		n (%)
Gender	Male	51 (51)
	Female	49 (49)
Age Group	20-40	28 (28)
	41-60	52 (52)
	>60	20 (20)
Dietary Pattern among patients	Vegetarian	25 (25)
	Non vegetarian	75 (75)
Duration of Metformin therapy among patients	2-5 years	41(41)
	6-10years	28 (28)
	>10years	31(31)

**Table 2: Prevalence of Vitamin B-12 in the studied population**

Vitamin B 12 level	No. of patients.	Percentage
Deficiency	38	38.00%
Normal Levels	62	62.00%
Total	100	100.00

**Table 3: Vitamin B12 deficiency in patients on metformin therapy as per their diet and gender**

Characteristics		No. of patients	Vitamin B12 deficient	% with Group	P value
Diet	Vegetarian	25	14	56.0%	0.032*
	Non vegetarian	75	24	32.0%	
Gender	Male	51	21	41.2%	0.446
	Female	49	17	32.7%	

\*p value < 0.05 significant

**Table 4: Average vitamin B12 levels in the studied population as per gender and dietary pattern**

Gender	Average vitamin B12 levels
Male	437.9 pg/mL
Female	388.2pg/mL
Dietary pattern	Vitamin B12 level
Vegetarian	199.8pg/ml
Non vegetarian	484.87pg/ml
Total population	413.5pg/ml

**Table 5: Dietary pattern in both female and male patients and average vitamin B 12 level according to dietary pattern.**

Dietary pattern	No. of patients (Female)	Vitamin B12 level
Vegetarian	12	233.6pg/ml
Non-vegetarian	37	441.22pg/l
Total	49	388.21pg/l
Dietary pattern	No. of patients (Male)	Vitamin B12
Vegetarian	13	177.46 pg/mL
Non-vegetarian	38	527.02pg/mL
Total	51	437.9pg/mL

**Table 6: Vitamin B-12 deficiency according to the duration of metformin therapy**

Duration	No. of patients.	No. of patients who are deficient	Percentage of deficient patient in each range	p value
2-5 years	41	2	4.9%	0.001*
6-10 years	28	17	60.7%	
> 10 years	31	19	61.3%	
Total	100	38	38.0%	

\*p value < 0.05 significant

## CONCLUSION:

From present study we conclude that metformin which is first line oral hypoglycaemic agent as recommended by ADA is significantly associated with decrease in vitamin B12. Vitamin B12 deficiency is more common among vegetarian population on prolonged metformin therapy than nonvegetarian population and the difference is statistically significant. Vitamin B12 deficiency was also detected

more in patients who had taken metformin for >5years which was also statistically significant. This showed that diet and duration of metformin therapy has also role to play in causing the deficiency. Hence routine screening of vitamin B12 deficiency is recommended in patients on prolonged metformin therapy.

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**Conflict of Interest:** None

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