

Status of Vitamin B12 Level in T2dm Patients on Metformin As Monotherapy For ≥ 6 Months

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Abstract

Introduction- Type 2 diabetes, formerly known as adult-onset diabetes, is a form of diabetes that is characterized by high blood sugar, insulin resistance, and relative lack of insulin.^[4] Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy which can result in blindness, kidney failure, and poor blood flow in the limbs which may lead to amputations.^[5] Metformin is generally recommended as a first line treatment as there is some evidence that it decreases mortality;^[7] It is the most prescribed anti-diabetic drug in patients with type 2 diabetes mellitus (T2DM) and hence, considered a cornerstone in the treatment of T2DM.^[8] the mechanism through which metformin induces vitamin B₁₂ deficiency in patients with T2DM is presently unclear. Some of the suggested mechanisms include alteration in small bowel motility, which stimulates bacterial overgrowth and consequential vitamin B₁₂ deficiency. Others include competitive inhibition or inactivation of vitamin B₁₂ absorption, alteration in intrinsic factor levels and interaction with the cubulin endocytic receptor. Also, inhibition of the calcium dependent absorption of vitamin B₁₂-intrinsic factor (IF) complex at the terminal ileum has been suggested as one of the mechanisms.^[15-17]

Material and method : we carried out this Cross sectional Observational Study from october 2018 till august 2020.

The study group was include 50 consecutive cases of Type 2 Diabetes Mellitus on metformin from the department of Medicine and 50 healthy age and sex matched controls. The duration of metformin therapy and dosage history was determined for each patient by review of their medical record, and these dosages was confirmed verbally by the patients; these data was used to calculate a cumulative lifetime dose of metformin for each patient. Use of other anti-diabetic agents was also being recorded.

Result-

In metformin group, 15 (30%) patients Vitamin B12 Levels were found normal (>220) In 14(28%) cases it was adjudged as possible (151-220), however in remaining 21 (42%) patients vitamin B12 Levels were found deficient (≤ 150). Total number of patients taking vegetarian diet under Metformin group was 22 (44%) & in Healthy control group was 20 (40%) and aggregate total of vegetarian patients in both the groups were 42 (42%). Further patients taking Mix diet under Metformin group were 28 (56%), and those under Healthy control group were 30 (60%) and aggregate total of Mix diet patients in both the groups were 58 (58%).

Conclusion- We showed that in patients with T2DM being treated with Metformin alone had a greater risk of reduction in vitamin B12 levels. The findings of this current study provided a strong case for predictable assessment of vitamin B12 levels during treatment with Metformin.

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I. Introduction

Diabetes mellitus is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when they do occur. hyperglycemia and resulting from the combination of resistance to insulin action, Type 2 diabetes mellitus consists of an array of dysfunctions characterized by inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Poorly controlled type 2 diabetes is associated with an array of microvascular, macrovascular, and neuropathic complications.

Microvascular complications of diabetes include retinal, renal, and possibly neuropathic disease. Macrovascular complications include coronary artery and peripheral vascular disease. Diabetic neuropathy affects autonomic and peripheral nerves.

Type 2 diabetes is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free

fatty acids and proinflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat.

Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B12 deficiency among patients with T2DM^[102-104]. Studies assessing type 2 diabetic patients on metformin have reported the prevalence of vitamin B12 deficiency to range from 5.8% to 33%^[96,105,106]. This wide variation in the reported prevalence could probably be explained by the varied study definitions of vitamin B12 deficiency. American Diabetes Association suggests using metformin as the first-line medical therapy for type-2 diabetes. When used alone, metformin rarely causes hypoglycaemia in older people. It increases insulin sensitivity and improves weight loss and the lipid profile. Its side effects include lactic acidosis in patients who experience heart failure, renal failure, and among alcoholic patients, as well as vitamin B12 deficiency.

Decrease in vitamin B12 absorption and levels following metformin use typically starts as early as the 4th month. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations 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.^[113]

The hydrophobic tail of biguanides such as metformin, extends into the hydrocarbon core of membranes. The protonated biguanide group gives a positive charge to the surface of the membrane, which displaces divalent cations. Thus, the biguanides alter membrane potentials and affect their calcium-dependent functions. Metformin also has an effect on the cubilin, which may affect B12-intrinsic factor complex absorption and result in the deficiency.^[112]

This means that metformin therapy carries a potential risk for development of vitamin B12 deficiency. The study highlights the necessity of checking B12 status during metformin treatment in order to avoid this potential adverse drug reaction and preserve the beneficial effects of metformin.

Material and method – this Cross sectional Observational Study was conducted in the Departments of Medicine subharti Medical College & Subharti Hospital, Meerut Uttar Pradesh from October 2018 to August 2020 which include 50 consecutive cases of Type 2 Diabetes Mellitus on metformin from the department of Medicine and 50 healthy age and sex matched controls.

Inclusion Criteria

- Age more than 30 yrs of both sex.
- Patients of Type 2 diabetes mellitus diagnosed as per American Diabetes Association 2018 guidelines on treatment with metformin for at least 6 months.

Exclusion Criteria

- Patients on or with a prior history of Vitamin B12 supplements (oral or parenteral)
- H/O malabsorption syndromes, gastrectomy, bariatric surgery, ileal resection, jejunal diverticulosis, ileocolic fistula, Crohn's disease etc
- Patients will be excluded if they had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine clearance less than 60 ml/minute.
- Patients who are seropositive for HIV
- Therapy with colchicines, para-aminosalicylates, anticonvulsants like phenytoin, cytotoxic drugs, PPI.
- Alcohol consumption >2 units/ day (1U=8-10g)
- Pregnant patients
- Peripheral neuropathy with principal cause other than diabetes
- On other Anti Diabetic Drugs
- T1DM

Serum Vitamin B12 levels [estimated by chemiluminiscent immunoassay Test done by ELISA Method] Patients to be tested was asked to come to laboratory, sit on a chair, roll up their sleeves above elbow. Venous blood samples were collected using full aseptic measures. kept in closed bottles which will be held in vertical position. And test was performed at the same day, on the basis of results of B12 levels, patients were classified into normal level (>220 pg/ml), possible B12 deficiency (150-220 pg/ml), and definite deficiency (<150pg/ml)^[135].

Statistical Analysis:

Data was analyzed using Statistical Package for Social Sciences, version 23 (SPSS). Results for continuous variables are presented as mean \pm standard deviation, whereas results for categorical variables are presented as number (percentage). The level $P < 0.05$ was considered as the cutoff value or significance.

II. Result

The study group was include **50** consecutive cases of Type 2 Diabetes Mellitus on metformin and **50** healthy age and sex matched controls.

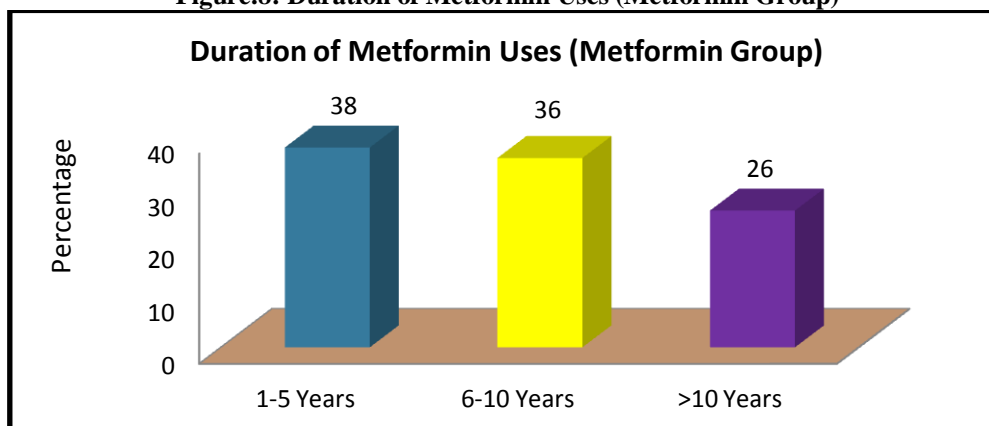
Table: 1. Age distribution among case & control group.

Age Group	Metformin Group (n=50)		Healthy Control Group(n=50)	
	No of cases	Percentage	No of Cases	Percentage
40 – 60	18	36	19	38
61 – 80	25	50	23	46
>80	07	14	08	16
Total	50	100	50	100
Mean & SD	64.320±11.95		63.840±12.83	
p Value	0.0.685 (NS)			

Table.8: Duration of Metformin Uses (Metformin Group)

Duration of metformin use	Frequency (n=50)	Percentage
1-5 Years	19	38
6-10 Years	18	36
>10 Years	13	26

Figure.8: Duration of Metformin Uses (Metformin Group)

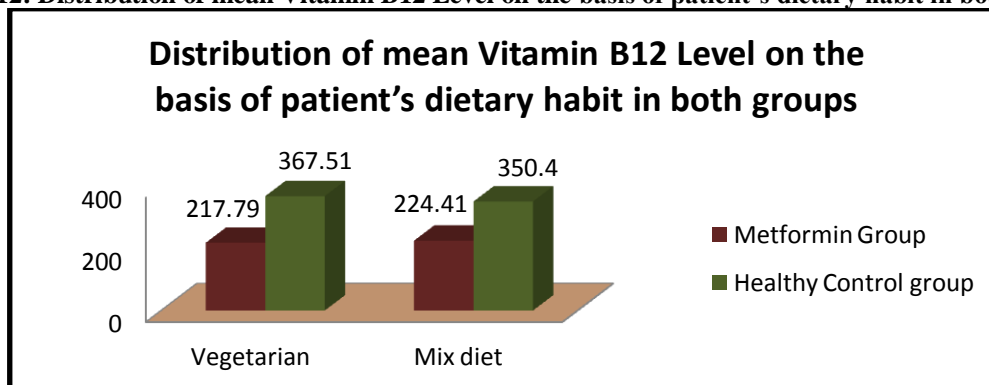


19 (38%) patients were such who were consuming the metformin from 1-5 years only. While 13 (26%) were taking metformin for over >10 years, 18 (36%) were consuming the drug for last 6-10 years.

Table No. 12: Distribution of mean Vitamin B12 Level on the basis of patient’s dietary habit in both groups.

Dietary habit	Vitamin B12 Level				P value
	Metformin Group (n=50)		Healthy Control Group(n=50)		
	Mean	SD	Mean	SD	
Vegetarian	217.79	± 122.58	367.51	±194.56	0.0001
Mix diet	224.41	±120.16	350.4	±193.55	0.0002

Figure.12: Distribution of mean Vitamin B12 Level on the basis of patient’s dietary habit in both groups.

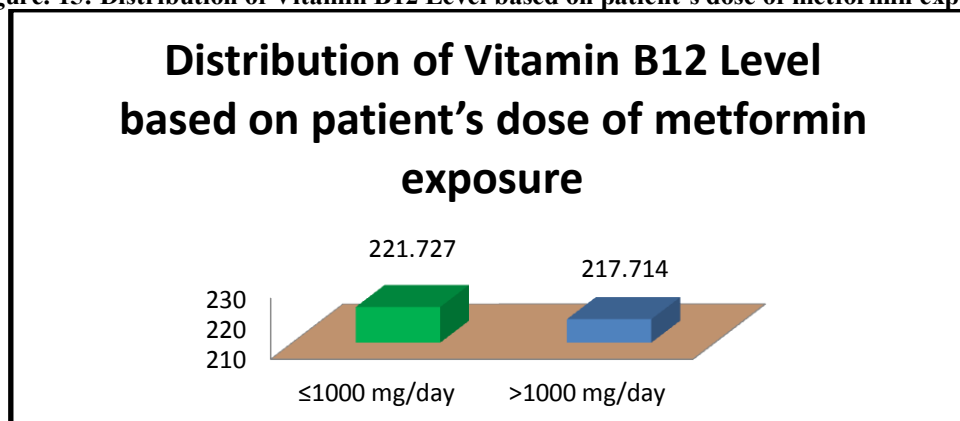


There was found highly significant association between vitamin B12 level in both groups on the basis of dietary habit of patients ($p < 0.01$), it shows diet does not any relation between vitamin b12 level and drugs use in both groups. In metformin group Vitamin b12 deficiency was more prevalent.

Table.15: Distribution of Vitamin B12 Level based on patient’s dose of metformin exposure

Dose of metformin exposure	N=50 (%)		vitamin B12 Level		P value
	No	%	Mean	SD	
≤ 1000 mg/day	22	44	221.727	± 135.97	0.317
> 1000 mg/day	28	56	217.714	± 95.42	

Figure. 15: Distribution of Vitamin B12 Level based on patient’s dose of metformin exposure

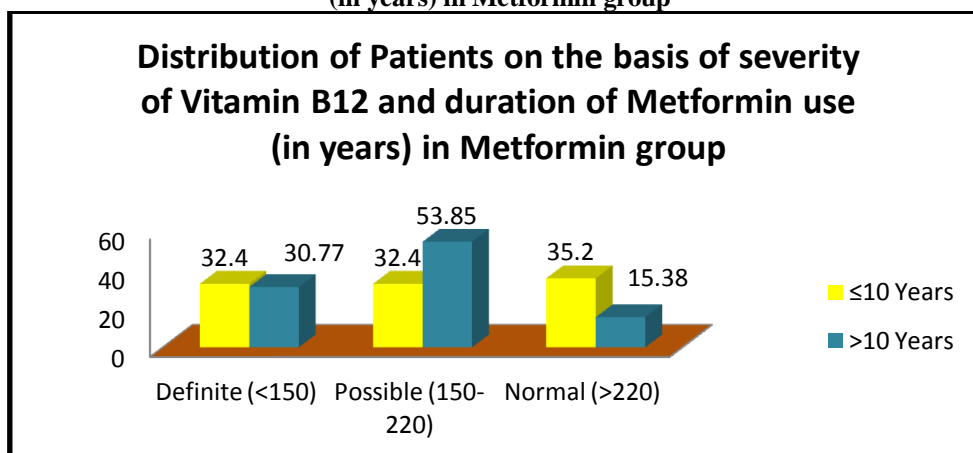


There were 22 (44%) patients who were had metformin more than 1000mg per day whereas 28 (56%) patients who were taking less than 1000mg per day. Who were taking more dose they had more Vitamin B12 deficiency. The association between duration of metformin exposure was found to be non-significant ($p > 0.05$)

Table.20: Distribution of Patients on the basis of severity of Vitamin B12 and duration of Metformin use (in years) in Metformin group

VitaminB12 Levels	Duration of Metformin use (n=50)				P value
	≤ 10 Years (n = 37)		> 10 Years (n=13)		
	No	%	No	%	
Definite (< 150)	12	32.4	04	30.77	0.29
Possible (150-220)	12	32.4	07	53.85	
Normal (> 220)	13	35.2	02	15.38	

Figure.20: Distribution of Patients on the basis of severity of Vitamin B12 and duration of Metformin use (in years) in Metformin group



The following table shows the distribution of patients on the basis of severity of Vitamin B12 and duration of Metformin use (in years) in Metformin group and it was found that the patients with duration of metformin use ≤ 10 years were having the definite severity of Vitamin B12 level in majority 12 (32.4%) followed by the patients with duration of metformin use for > 10 years 4 (30.77%)

III. Discussion

This present study was aimed at estimation of serum vitamin B12 levels in the patients of T2DM who were on treatment with metformin. This fact was compared with Healthy control group by estimating vitamin B12 levels. Study included 100 patients (50 case-Metformin group & 50 Healthy control group) with a mean age of 64.320 years and a standard deviation of 11.95 years, mean duration of diabetic was 9.90 ± 4.63 years, majority 58% were female and rest 42% were males. Mean age of the study group (metformin group) was 64.32 ± 11.95 years, while it was 63.84 ± 6.02 in control group, this is similar to Agarwal P et al^[139] study that also reported mean age of the study group was 51.98 ± 5.17 years, while it was 49.28 ± 5.08 in control group. Study done by Gupta K et al^[140] reported mean age of all studied patients was 57.8 years that similar to our study.

On the basis of dietary habits, total 22 (44%) patients were vegetarian (28, 56% Mix diet from metformin group, 40% vegetarian & 60 % Mix diet from Healthy control group) correlation of BMI (kg/m^2) between the patients of the both groups was found highly significant ($P < 0.001$). The prevalence of Vitamin B12 deficiency in patients on metformin in our study is higher than that reported in Western literature. In different studies, Vitamin B12 deficiency was found in 5.8%, 8.6%, 6.3%, of patients with type 2 diabetes mellitus on metformin as reported by Reinstatler L et al^[142], Calvo Romero JMet al^[143] and NervoMet al^[144]. The higher prevalence in our study (42%) is not surprising considering that the prevalence of Vitamin B12 in the apparently healthy populations in India have been reported to be as high as 33.3%–67%.^[145] A predominantly vegetarian diet could be one of the causes of higher prevalence of Vitamin B12 deficiency in India. In the present study 68% patients were found to be vegetarian^[146-148].

In metformin group, 15 (30%) patients Vitamin B12 Levels were found normal (>220) In 14(28%) cases it was adjudged as possible (151-220), however in remaining 21 (42%) patients vitamin B12 Levels were found deficient (≤ 150). In healthy control group we have found 33 (66%) patients Vitamin B12 Levels were found normal (>220). adjudged as cases were 09(18%), and deficient was only 08(16%) out of 50.

In our study, was found highly significant association between vitamin B12 level in both groups on the basis of d19 (38%) patients were such who were consuming the metformin from 1-5 years only. While 13 (26%) were taking metformin for over >10 years, 18 (36%) were consuming the drug for last 6-10 years. In our study Comparison of Vitamin B12 level between both groups on the basis of patients age was found to be significant ($p < 0.05$)

The association between duration of diabetes exposure was found to be significant (p Value 0.003). There were 13 (26%) patients who were had metformin since more than 10 years whereas 37 (74%) patients were less than from 10 years. Who were taking for more time they had more Vitamin B12 deficiency. The association between duration of metformin exposure was found to be significant ($p < 0.05$) in our study.

In this study, were 22 (44%) patients who were had metformin more than 1000mg per day whereas 28 (56%) patients who were taking less than 1000mg per day. Who were taking more dose they had more Vitamin B12 deficiency. The association between duration of metformin exposure was found to be non-significant ($p > 0.05$) Distribution of Patients on the basis of severity of Vitamin B12 and duration of Metformin use (in years) in Metformin group we have found, The following table no 20 shows the distribution of patients on the basis of severity of Vitamin B12 and duration of Metformin use (in years) in Metformin group and it was found that the patients with duration of metformin use ≤ 10 years were having the definite severity of Vitamin B12 level in majority 12 (32.4%) followed by the patients with duration of metformin use for > 10 years 4 (30.77%)

However, this study has important **limitations**. We have used only the serum Vitamin B12 level to define vitamin deficiency and we have not measured homocysteine levels, which is more sensitive indicators of Vitamin B12 status than serum Vitamin B12 levels. Additionally, we have not recorded the amount of non-supplemental Vitamin B12 ingestion and therefore the daily intake of the Vitamin B12 from food resources has been ignored prior to blood sampling. In addition, our sample size was small.

We thus **recommend** that patients on metformin for more than a year should have their Vitamin B12 levels checked yearly and parenteral supplementation provided if found deficient. Parenteral supplementation is to be preferred because enteral mechanisms are proposed to be compromised by metformin..

IV. Conclusion

We showed that in patients with T2DM being treated with Metformin alone had a greater risk of reduction in vitamin B12 levels. Current guidelines throw light on metformin, a cornerstone in the treatment of T2DM, but make no recommendations on the detection and prevention of vitamin B12 decrease during metformin treatment. The findings of this current study provided a strong case for predictable assessment of vitamin B12 levels during treatment with Metformin. Further study of the impact of duration of diabetes on serum Vitamin B12 levels and of functional markers of Vitamin B12 deficiency on hematological parameters will be interesting.

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