Research

VITAMIN B12 LEVELS IN TYPE 2 DIABETES PATIENTS TAKING METFORMIN ALONE FOR MORE THAN SIX MONTHS

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Abstract
Background: Status of Vitamin B12 level in T2DM patients on Metformin as Monotherapy more than six months. **Materials and Methods:** It was Cross sectional Observational Study. The study was conducted in the Departments of Pharmacology and collaboration with the department of Medicine & Biochemistry M.G.M. Medical College & L.S.K. Hospital, Kishanganj, Bihar. 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 during July 2021 to August 2022. **Result:** The mean age of metformin group was 64.320±11.95 & Control group was 63.840. Majority of patients 22 (44%) were harboring the disease of diabetes for last >10 years a good number of patients 18 (36%) were ailing for over 6-10 Years. Only 10 (20%) patients were ill for last 1-5 Years. 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. 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. **Conclusion:** Metformin alone increases the likelihood of low vitamin B12 levels in patients with type 2 diabetes, as we shown. Metformin is a staple in the treatment of type 2 diabetes, but there are no recommendations for monitoring vitamin B12 levels or taking any action to avoid a drop in B12 levels from occurring while using metformin in the current guidelines.

INTRODUCTION

Approximately 90% of all instances of diabetes in 2019 were thought to be caused by type 2, and this amounted to an estimated 392 million people worldwide in 2019.[1,2] This amounts to roughly 6% of the global population.[2] The prevalence of diabetes is similar in both the industrialised and developing world.[1] However, this is still a rarity among the world's poorest nations.[3]

Interventions in lifestyle, reduction of other cardiovascular risk factors, and maintenance of normal blood glucose levels are central to the management of type 2 diabetes.[4]

Multiple types of diabetes treatments are currently on the market. Metformin is the most commonly given anti-diabetic medicine in patients with type 2 diabetes mellitus (T2DM), and is thus regarded a cornerstone in the treatment of T2DM.[5] There is some evidence that it reduces mortality, hence it is widely recommended as a first-line treatment.

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Excellent improvement in the cardiovascular morbidity and mortality associated with T2DM is characterised by this anti-hyperglycemic medication, which is generally well tolerated by most patients (except for modest gastrointestinal side effects).[6]

Metformin has several possible positive clinical outcomes, thus its potential negative health effects are often overlooked and understudied. Vitamin B12 insufficiency is one such unwanted outcome.[7]

Water-soluble vitamin B12, also known as cobalamin, is essential for healthy blood cells, nerves, and the nervous, cognitive, and circulatory systems. It has a role in cellular processes such as DNA synthesis, fatty acid metabolism, and energy generation. Vitamin B12 is responsible for its physiological effects by enabling homocysteine methylation to methionine, which is then activated into S-adenosyl methionine, which gives its methyl group to methyl acceptors.[8] Like vitamin B12, methylmalonic acid (MMA) accumulates in the blood when the conversion of methyl malonyl coenzyme A (coA) to succinyl coA is impeded, leading to abnormal fatty acid production in the neuronal membranes.[9]

Patients with type 2 diabetes should have their vitamin B12 levels evaluated. Metformin-treated patients may experience a peripheral neuropathy that mimics the symptoms of diabetic neuropathy.[10]

Vitamin B12 deficiency has also been linked to slower nerve conduction velocities and diminished responses to mild touch as measured by monofilament detection.[11] There is a risk that this will result in people taking medicine for diabetic neuropathy when they don't need to.[12]

Despite widespread acceptance of metformin's efficacy in treating type 2 diabetic mellitus (T2DM), its potential side effects are often neglected and understudied, particularly in our region (Kishanganj). Therefore, the purpose of this research was to assess vitamin B12 serum levels in metformin-treated type 2 diabetics.

MATERIALS AND METHODS

It was Cross sectional Observational Study. The study was conducted in the Departments of Pharmacology and collaboration with the department of Medicine & Biochemistry M.G.M. Medical College & L.S.K. Hospital, Kishanganj, Bihar. 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 were used to calculate a cumulative lifetime dose of metformin for each patient. Use of other anti-diabetic agents was also being recorded during July 2021 to August 2022.

Inclusion Criteria
- 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 malabsorptionsyndromes, gastrectomy, bariatricsurgery, ilealresection, jejunaldiverticulosis, ileo-colic fistula, crohn'sdiseaseetc
- 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

Ethical Consideration
All issues including ethical issues of the protocol were evaluated by the Institutional Review Board and have been approved.

Methodology
The patients were evaluated as per the standard protocol specially concentrating on-
- Age
- Gender
- Duration of diabetes
- Duration and dose of metformin prescribed; cumulative metformin dose
- H/O pain, numbness, paraesthesias, numbness, weakness, ataxias along with duration
- H/O hypertension, CAD, nephropathy, retinopathy
- Physical examination(including height, weight and BMI)

Baseline routine investigations:
- Haemoglobin (CMG Method)
- FBS, PPBS, (GOD POD Method)
- HbA1C (HPLC Method)
- Urea (GLDH Method)
- Creatinin(Jaffe Method)
- Lipid Profile (Total Cholesterol, HDL, LDL, VLDL, Triglycerides)
- Urine R/M and 24-hour urinary protein
- Vitamin B-12
- ECG.
- USG abdomen

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.

Special Investigations like

Serum Vitamin B12 levels [estimated by chemiluminiscent immunoassay Test done by ELISA Method] Patients to be tested was be 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 perform 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).[13]

Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences, version 23 (SPSS). Results for continuous variables are presented as mean ± 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.

RESULTS

In Metformin group were mean age (Year),weight (kg), Height (cm), BMI (kg/m2), & mean Duration of the disease (years) was 64.32±11.95, 67.280 ± 6.31, 162.00 ± 5.85, 25.56 ± 1.73, 12.18±7.64 respectively, the corresponding values for healthy control group were Age (Year) 63.84±12.83 , weight (kg) 57.840 ± 5.20, Height (cm)160.02 ± 7.73 , BMI (kg/m2) & 22.758± 1.78 The statistical variation in the BMI (kg/m2) between the patients of the two groups weight was significant P0.01.

Table 1: Anthropometric measurement among case & Control Group

Anthropometric Parameter	MetforminGroup (n=50)		Healthy Control Group(n=50)		P value
	Mean	SD	Mean	SD	
Mean Age (Year)	64.320	±11.95	63.840	12.83	0. 685(NS)
Mean weight (kg)	67.280	±6.31	57.840	±5.201	0.01 (S)
Mean Height (cm)	162.000	±5.85	160.340	±7.47	0.09(NS)
BMI (kg/m^2)	25.566	±1.72	22.758	±1.78	0.737(NS)
Duration of the disease (Months)	9.900	±4.63	-	-	-

Table 2: Distribution of patients under study on the basis of availability of vitaminB12 levels in both groups.

VitaminB12 Levels	MetforminGroup (n=50)		HealthyControl Group(n=50)	
	No of cases	Percentage	No of cases	Percentage
Deficient (≤150)	21	42	08	16
Possible (151-220)	14	28	09	18
Normal (>220)	15	30	33	66
Total	50	100	50	100

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.

Table 3: Vitamin B12 Level distribution based on duration of diabetes in studied patients

Duration of diabetes	Vitamin B12 LevelMetforminGroup (n=50)		p Value
	Mean	SD	
≤10 Years	246.857	±136.36	0.003
>10 Years	193.650	±79.58	

The association between duration of diabetes exposure was found to be significant (p Value 0.003)

Table 4: Distribution of mean Vitamin B12 Level on patient's duration of taking metformin

Duration of metformin exposure	N=50 (%)		vitamin B12 Level		P value
	No	%	Mean	SD	
≤10 Years	37	74.0	237.270	±126.52	0.006
>10 Years	13	26.0	181.461	±68.32	

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).

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Table 5: 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	

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 6: Distribution of patients on the basis of their Vitamin B12 level in both groups with HbA1c severity

Glycosylated Hemoglobin (HbA1c)	Metformin group (n=50)			
	Frequency (%)		Vitamin B12 level (Mean±SD)	
	No	%	Mean	SD
<7%	16	32	325.812	±125.25
7- 8%	16	32	197.937	±98.51
>8%	18	36	153.222	±29.27

In metformin group, 18 (36%) patients had HbA1c more than 8% followed by 16 (32%) patients in HbA1c level 7-8% and rest were in less than HbA1c 7%, more severity of HbA1c shows more deficiency of Vitamin B12.

Table 7: Distribution of all studied patients on the basis of severity of Vitamin B12 and their age group for Metformin group

VitaminB12 Levels	Age wise distribution (Metformin group)						P value
	41-60 Years (n=18)		61-80 Years (n=25)		> 80 Years (n=7)		
	No	%	No	%	No	%	
Definite (<150)	7	38.89	9	36.0	5	71.43	0.383
Possible (150-220)	5	27.78	7	28.0	2	28.57	
Normal (>220)	6	33.33	9	36.0	0	0.0	

The following table shows the distribution of all studied patients on the basis of severity of Vitamin B12 and their age group for Metformin group and it was found that the majority of patients having the definite level of severity of Vitamin B12 were of age group 61-80 years 9 (36.0%) followed by 41-60 years 7 (38.89%) and the least were of >80 years 5 (71.43%) also the association was found to be insignificant (p>0.05)

Table 8: Distribution of Patients on the basis of severity of Vitamin B12 and Duration of diabetes in Metformin group

VitaminB12 Levels	Metformin groupDuration of diabetes(n=50)				P value
	≤10 Years (n=28)		>10 Years (n=22)		
	No	%	No	%	
Definite (<150)	09	32.1	07	31.8	0.190
Possible (150-220)	08	28.6	11	50.0	
Normal (>220)	11	39.3	04	18.2	

The above table shows the distribution of patients on the basis of severity of Vitamin B12 and Duration of diabetes in Metformin group and it was found that the patients with duration of diabetes for >10 years were having the severity of Vitamin B12 level in majority 11 (50.0) followed by patients with duration of diabetes ≤10 years 10 (35.71%) and possibility of severity of VB12 in 7 (25.0%) while in > 10 years diabetes the possibility of severity of Vitamin B12 was 7 (31.82%)

Table 9: Distribution of Patients on the basis of severity of Vitamin b12 and Metformin daily dose (in mg) in Metformin group

VitaminB12 Levels	Metformin daily dose (n=50)				P value
	≤1000mg (n = 22)		>1000mg (n=28)		
	No	%	No	%	
Definite (<150)	6	27.3	10	35.7	0.816
Possible (150-220)	9	40.9	10	35.7	
Normal (>220)	7	31.8	8	28.6	

The above table shows the distribution of Patients on the basis of severity of Vitamin b12 and Metformin daily dose (in mg) in Metformin group and it was found that the majority of patients with metformin daily dose >1000 mg were having definite severity of Vitamin B12 levels 10 (35.7%) followed by those having metformin daily dose ≤1000 mg 6 (27.3%).

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)

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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 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 reported mean age of all studied patients was 57.8 years that similar to our study.

In our study, In metformin group 21 (42%) were male & 29 (58%) female whereas in Healthy control group 23 (46%) were male & 27 (54%) female. 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/m2) between the patients of the both groups was found highly significant (P<0.001).

Similarly, in study done by Agarwal P et al [14] 22 (44%) were male & 28 (58%) female in study group (metformin group) and 24 (48%) were male & 26 (52%) were females in control group; also found similar percentage of patients in vegetarian diet in both groups and also correlation of BMI of both groups was found significant (p<0.05).

In our study, out of 50 cases majority of patients 22 (44%) were harboring the disease of diabetes for last >10 years, 18 (36%) were ailing for over 6-10 Years & only 10 (20%) patients were ill for last 1-5 Years and mean duration of diabetic of all patients was 9.90±4.63 years. Study done by Gupta K et al,[13] mean age of diabetic was reported 10.2 years which was similar to our findings.

In our study, in metformin group & Healthy control group the level of Fasting blood sugar was 152.800 ± 10.50 & 86.400 ± 7.86 which shows statistically significant p value (p<0.01) and levels of HbA1c was 7.768 ± 5.26 & 5.260 ± 0.33 respectively which also shows that level of HbA1C is better controlled with Healthy control group with highly significant p value(p<0.01) and; Level of Vitamin B12 level, in metformin group & Healthy contral group was 222.760±760 & 362.380±192.43 respectively, this shows that vitamin B12 levels were low in metformin group when compared to Healthy control group and had highly significant p value (p<0.01).

Similarly, in study done by Agarwal P et al,[14] the mean glycosylated hemoglobin in study group was 7.84±1.07 as compare to 8.32±1.22 in control groups, it was significant difference between them (p<0.05) and also significantly lower mean S. vitamin B12 was observed in cases as compared to controls (p=0.000) i.e similar to our findings.

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 Comparison of Vitamin B12 level between both groups on the basis of patients age was found to be significant (p<0.05)

In this study, 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.

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)

In metformin group, 18 (36%) patients had HbA1c more than 8% followed by 16 (32%) patients in HbA1c level 7-8% and rest were in less than HbA1c 7%, more severity of HbA1c shows more deficiency of Vitamin B12.

In this study shows the distribution of all studied patients on the basis of severity of Vitamin B12 and their age group for Metformin group and it was found that the majority of patients having the definite level of severity of Vitamin B12 were of age group 61-80 years 9 (36.0%) followed by 41-60 years 7 (38.89%) and the least were of >80 years 5 (71.43%) also the association was found to be insignificant (p>0.05)

The distribution of patients on the basis of severity of Vitamin B12 and Duration of diabetes in Metformin group and it was found that the patients with duration of diabetes for >10 years were having the severity of Vitamin B12 level in majority 11 (50.0) followed by patients with duration of diabetes ≤10 years 10 (35.71%) and possibility of severity of VB12 in 7 (25.0%) while in > 10 years diabetes the possibility of severity of Vitamin B12 was 7 (31.82%)

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 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 > 10years 4 (30.77%)

Distribution of Patients on the basis of severity of Vitamin b12 and Metformin daily dose (in mg) in Metformin group, the distribution of Patients on the basis of severity of Vitamin b12 and Metformin daily dose (in mg) in Metformin group and it was found that the majority of patients with metformin daily dose >1000 mg were having definite severity of Vitamin B12 levels 10 (35.7%) followed by those having metformin daily dose ≤1000 mg 6 (27.3%).

Distribution of Patients on the basis of severity of Vitamin B12 and HbA1c levels in Metformin group, we have found the the distribution of Patients on the basis of severity of Vitamin B12 and HbA1c levels in Metformin group and it was found that the majority of patients with definite severity of Vitamin B12 were having HbA1c level >8 9(50.0%) followed by HbA1c level between 7-8 5(29.4%) and the least were the patients with HbA1c level <7 2 (13.3%).

Agarwal P et al also reported similar significant association among both groups for mean S. vitamin B12 in patients with duration of diabetes more than 5 years (p<0.001).Additionally, metformin alters bacterial flora through an effect on gastrointestinal motility resulting in bacterial overgrowth similar to blind loop syndrome, which will further lead to VB12 malabsorption. In our study we also found association vitamin B12 deficiency in age more than 65 years. In the metformin group, duration of metformin exposure on the basis of duration of diabetes was found to be highly significant among the patients of both groups (p<0.001). In the same group, 21 (42%) patients were used more than 1000mg/day and they were more vitamin B12 deficient than patients who were used more than 1000 mg/day. The association between dose of metformin exposure was found to be non-significant (p>0.05) in the present study.

The strengths of this study include its population based sampling. Moreover, the presence of a control group makes it possible to compare prevalence of VB12 deficiency in a similar diabetic patients not taking metformin. The reason for choosing such a group was to exclude a possible long-term effect of metformin use on Vitamin B12 status. Vitamin B12 is stored in the liver and several years may pass before the stores are depleted and detectable Vitamin B12 deficiency manifests. Thus, including patients with a history of metformin use in the no metformin group is a potential confounder which we excluded in this study.[14]

CONCLUSION

Metformin alone increases the likelihood of low vitamin B12 levels in patients with type 2 diabetes, as we shown. Metformin is a staple in the treatment of type 2 diabetes, but there are no recommendations for monitoring vitamin B12 levels or taking any action to avoid a drop in B12 levels from occurring while using metformin in the current guidelines. This study's results bolster the case for routinely measuring vitamin B12 levels in patients using Metformin. It would be fascinating to learn more about the correlation between the length of time someone has had diabetes and their serum Vitamin B12 levels, as well as the effects of functional markers of Vitamin B12 insufficiency on haematological parameters.

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