



Assessment of Serum Vitamin B12 Levels in Metformin-Treated Type 2 Diabetes Patients

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ABSTRACT

BACKGROUND: Diabetes is a problem with your body that causes blood glucose (sugar) levels to rise higher than normal. This is also called hyperglycemia.¹ Type 2 diabetes is the most common form of diabetes. **AIM AND OBJECTIVE:** To study Vitamin B12 levels in patients of Type 2 Diabetes treated or on treatment with metformin (at least 1 year). **METHOD AND MATERIAL:** This hospital-based study was conducted in Department of Physiology and Department of Endocrinology, and Biochemistry, Index Medical College, Hospital and Research Centre Indore MP, India. **RESULT:** Majority were females (56%) than males (44%) in our study. Mean age of all patients was 64.08±12.30 years & mean duration of diabetic was 12.25±7.69years. 38% patients were smokers. History of CAD was found in 63 (21%) patients, mean Weight (kg), Height (cm), mean BMI (kg/m²), mean SBP (mmHg) & mean DBP (mmHg) was 68.20±6.18, 161.01±6.87, 26.29±1.98, 134.71±17.19, was 82.63±10.52 respectively. **CONCLUSION:** Patients were divided into equally 2 groups (150 were in metformin exposure & 150 were in OHA/insulin exposure); Metformin group consisted of 63 (42%) Male & 87 (58%) female whereas OHA /insulin group was comprised of 69 (46%) male & 81 (54%) female.

INTRODUCTION

Diabetes is a problem with your body that causes blood glucose (sugar) levels to rise higher than normal. This is also called hyperglycemia.¹ Type 2 diabetes is the most common form of diabetes. If one has type 2 diabetes it's body does not use insulin properly. This is called insulin resistance. At first, your pancreas makes extra insulin to make up for it. But, over time it isn't able to keep up and can't make enough insulin to keep your blood glucose at normal levels.**Error! Bookmark not defined.**

Metformin 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.¹ It is an anti-hyperglycemic agent that is usually well tolerated in most of the patients (except for mild gastrointestinal side effects) and it is characterized by excellent improvement in the cardiovascular morbidity and mortality associated with T2DM.² Metformin is one of the few oral hypoglycemic agents that have been shown to improve cardiovascular morbidity and mortality among diabetic patient.^{4,5} The unique effect of metformin on restoring sensitivity to insulin has greatly improved the prognosis of diabetic patients and provided added protection against vascular complications.⁶ Previous studies suggest a higher prevalence of vitamin B12 (VB12) deficiency in diabetic patients treated with metformin.^{7,8} Although the association between metformin and VB12 deficiency has been widely described, little is known about its underlying pathophysiology. Vitamin B12 (VB12), also known as cobalamin, is a water-soluble vitamin that is essential for the normal function of the nervous system and erythropoiesis, through its required role in DNA synthesis. VB12 is essential for three enzymatic processes including the conversion of: a) homocysteine to methionine, b) methylmalonic acid to succinyl coenzyme A, and c) 5-methyltetrahydrofolate to tetrahydrofolate.^{9,10}

Vit. B12 deficiency manifests itself with hematologic findings in the form of, macrocytic (megaloblastic) anaemia and, in advanced cases, pancytopenia. Clinical signs and symptoms are divergent and go beyond the hematologic system. Nervous system is commonly affected in VB12 deficiency and causes varying degrees of sensory neuropathy progressing to combined sclerosis of the spinal cord in severe cases.^{11,12}

In this study our primary aim was to define the prevalence of Vitamin B12 deficiency in Diabetes mellitus-2 patients treated with metformin, and compare them to those patients receiving other hypoglycemic agents or insulin. Our primary endpoint was the occurrence of serum Vitamin B12 concentrations below 148 pmol/L. We hypothesized that the prevalence of cobalamin deficiency is higher in patients receiving metformin as a part of their oral hypoglycemic regimen.

MATERIAL & METHOD

All issues including ethical issues of the protocol have been evaluated by the Institutional Review Board and have been approved. The study was conducted in the Departments of Physiology, Endocrinology, and Biochemistry at Index Medical College, Hospital and Research Centre Indore MP, India.

STUDY DURATION: January 2021 - December 2022

STUDY DESIGN: Cross sectional Observational Study

STUDY LOCATION: This hospital-based study was conducted in Department of Physiology and Department of Endocrinology, and Biochemistry, Index Medical College, Hospital and Research Centre Indore MP, India.

SAMPLE SIZE: 300 patients

INCLUSION CRITERIA:

- Patients of Type 2 diabetes mellitus diagnosed as per American Diabetes Association 2016 guidelines on treatment with metformin for at least 1 year, with or without other OHA.

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, ileo-colic fistula, crohn's disease etc
- Patients were excluded if they had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine clearance less than 60 ml/minute.
- Therapy with colchicines, para-aminosalicylates, anticonvulsants like phenytoin, cytotoxic drugs, PPI.
- Pregnant patients
- Peripheral neuropathy with principal cause other than diabetes
- Not consenting to participate in the study

The patients were evaluated as per the standard protocol specially concentrating on-

1. Age
 2. Gender
 3. Duration of diabetes
 4. Duration and dose of metformin prescribed; cumulative metformin dose.
 5. History of pain, numbness, paraesthesias, numbness, weakness, ataxias along with duration.
 6. History of hypertension, CAD, nephropathy, retinopathy.
1. Physical examination (including height, weight and BMI)

Baseline routine investigations like:

- ❖ Hemogram (ESR, Hb%, TLC, DLC, Platelet count, TRBC count, MCV, MCH, MCHC, PCV and Peripheral smear for type of anemia)
- ❖ HEMOGLOBIN INVESTIGATION: -

was done by autohematology analyzer (lab life nobel, RFCL limited new delhi). Analyzer reagents (Diluent, rinse, lyse, probe, cleaner, E-Z cleaner) controls and calibrators are components of analyzer system.

- ❖ HEMOGLOBIN ESTIMATION

Using calorimetric method this analyzer calculates hemoglobin concentration (gm/l)

$Hb(gm/dl) = \text{constant} * \log_{10} (\text{blank photocurrent}/\text{sample photocurrent})$

- ❖ RBC

RBC is number of erythrocytes measured directly by counting the erythrocytes passing the aperture.

❖ PLT

PLT is also measured directly by counting the platelets passing through aperture.

❖ MCV

Based on RBC histogram, this analyzer calculates the mean cell volume and expresses the result in fl. This analyzer calculates the HCT (%), MCH and MCHC.

- ❖ $MCV \text{ (in fl)} = PCV \% / RBC \text{ Count (in million / cubic mm)} * 10$
- ❖ $MCH \text{ (in pg)} = HB / RBC \text{ Count (in million / cubic mm)}$
- ❖ $MCHC \text{ (in g/dl)} = HB / HCT \% * 10$
- ❖ RDW-CV

Based on RBC histogram, this analyzer calculates the CV of erythrocyte distribution width.

- ❖ **FBS, PPBS:** - methodology: - (glucose oxidase peroxidase method)
- ❖ **Special Investigations like**
- ❖ Serum Vitamin B12 levels [estimated by chemiluminiscent immunoassay technique by VITROS model of Johnson and Johnson]. This device works on the principle of competitive binding. This depends on competition between B12 present in the sample with a horseradish peroxidase (HRP)- labeled B12 for a limited number of binding sites on a biotinylated binding protein conjugate. B12 present in patient sample is released from endogenous binding proteins by alkaline denaturation and stabilized to prevent oxidation. An aliquot of the treated sample is transferred into a streptavidin coated well and B12-HRP and biotinylated binding protein conjugates added. Following a competitive binding reaction, the binding protein complexes are captured by streptavidin on the wells. Unbound conjugate is removed by washing. The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent is added to the wells. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of vitamin B12 percent.
- ❖ Venous blood samples were collected using full aseptic measures. Blood samples were at -30 degree Celsius, kept in closed bottles which was held in vertical position. 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).
- ❖ Complete standardized neurological examinations were performed in all patients with DPN, including tone, power, deep tendon reflexes, sensory function, Romberg testing, gait, and tandem gait. Tandem gait was recorded as the number of heel to toe steps along a straight line.
- ❖ On the basis of data collected from the history of neurological symptoms and clinical examination of the nervous system, neuropathy was graded using Toronto clinical scoring system (TCSS).¹³ Nerve conduction studies were done in patients having TCSS more than 6 on clinical neurological examination.

STATISTICAL ANALYSIS

The statistical package for social sciences, version 20 (SPSS Inc., Chicago, IL), was used to analyze the data. The results for categorical variables were provided as frequency/number (%), whereas the results for continuous variables were displayed as mean \pm standard deviation. The parametric independent Student's t test was used to compare continuous two independent groups. The chi-square (χ^2) test was used to compare discrete (categorical) groupings. The significance level, or cutoff value, was set at $P < 0.05$.

RESULT AND DISCUSSION

DESCRIPTIVE STATISTICS

Majority were females (56%) than males (44%) in our study. Mean age of all patients was 64.08 ± 12.30 years & mean duration of diabetic was 12.25 ± 7.69 years. 38% patients were smokers. History of CAD was found in 63 (21%) patients, mean Weight (kg), Height (cm), mean BMI (kg/m^2), mean SBP (mmHg) & mean DBP (mmHg) was 68.20 ± 6.18 , 161.01 ± 6.87 , 26.29 ± 1.98 , 134.71 ± 17.19 , was 82.63 ± 10.52 respectively.

Table No. 1: Demographic details of studied patients

Characteristics	Studied Subjects (n=100)	
Sex	Male	132 (44%)
	Female	168 (56%)
Age (Year)	64.08 ± 12.30	
Duration of diabetes (Year)	12.25 ± 7.69	

Currently smoking (%)	114 (38%)
History of CAD (%)	63 (21%)
Weight (kg)	68.20±6.18
Height (cm)	161.01±6.87
BMI (kg/m ²)	26.29±1.98
SBP (mmHg)	134.71±17.19
DBP (mmHg)	82.63±10.52
Patients H/O Hypertension	111 (37%)
Family H/O Diabetes mellitus	141 (47%)
Family H/O Hypertension	99 (33%)

Detailed Biochemical Parameters of patients under study were analyzed and mean values were recorded in the table as under.

Table No.2: Distribution of biochemical parameters of studied patients

Biochemical Parameters	Studied Subjects (n=100)
Fasting Blood Sugar (mg/dL)	156.01±9.68
Post Prandial Blood Sugar (mg/dL)	209.40±13.71
Glycosylated Hemoglobin (%)	7.45±1.19
Triglycerides (mg/dL)	160.45±20.19
Cholesterol (mg/dL)	177.04±35.08
High Density Lipoproteins (mg/dL)	44.58±5.69
Low Density Lipoproteins (mg/dL)	99.88±23.16
Hemoglobin (g/dL)	14.08±1.44
Serum Creatinine (mg/dL)	1.10±0.39
Serum Urea (mg/dL)	29.51±4.95
Vitamin B12	291.21±174.46
U. Albumin	1.13±1.0
U. Sugar	1.22±1.02

Patients were divided into 2 groups; Metformin group (n=150) and OHA /insulin group (n=150), the difference in the hematological parameters of the two groups were recorded in respect of fasting blood sugar (mg/dL), post prandial blood sugar (mg/dl), glycosylated hemoglobin (%), urea, Vitamin B12 & Triglycerides (mg/dL) were statistically highly significant (P<0.05). In case of all other parameters like, cholesterol (mg/dl), low density lipoproteins (mg/dl), serum creatinine (mg/dl), urine for albumin & urine for sugar the variation between the patients of the two groups were not significant (P>0.05).

Table No. 3: Distribution of studied patients on the basis of age

Age Group	Metformin group (n=150)	OHA /insulin group (n=150)	Total
41-60	54 (36%)	57 (38%)	111 (37%)
61-80	75 (50%)	69 (46%)	144 (48%)
>80	21 (14%)	24 (16%)	45 (15%)

Metformin group consisted of 63 (42%) Male & 87 (58%) female whereas OHA /Insulin group was comprised of 46 (46%) male & 81 (54%) female. Total male patients in both groups were 132 (44%) and female patients were 168 (56%).

Table No.4: Gender wise distribution of studied patients

Gender	Metformin group (n=150)	OHA /insulin group (n=150)	Total
Male	63 (42%)	69 (46%)	132 (44%)
Female	87 (58%)	81 (54%)	168 (56%)

Total number of patients taking vegetarian diet under Metformin group was 99 (66%) & in OHA /Insulin group was 105 (70%) and aggregate total of vegetarian patients in both the groups were 204 (68%). Further patients taking Mix diet under Metformin group were 51 (34%), and those under OHA /Insulin group were 45 (30%) and aggregate total of Mix diet patients in both the groups were 96 (32%).

DISCUSSION

Assessment of vitamin B12 deficiency in patients with T2DM is of clinical importance. It can present as peripheral neuropathy and may be mistaken for diabetic neuropathy in patients on metformin treatment. **Error! Bookmark not defined.** Also, low vitamin B12 levels have been reported to be associated

with poor nerve conduction velocities and poorer responses to light touch by monofilament detection. **Error! Bookmark not defined.** So, primary aim of the study was to define the prevalence of Vitamin B12 deficiency in Diabetes mellitus-2 patients treated with metformin, and compare them to those patients receiving other hypoglycemic agents. Our primary endpoint was the occurrence of serum Vitamin B12 concentrations below 148 pmol/L. Alvarez M et al **Error! Bookmark not defined.** conducted a cross-sectional study of the Vitamin B12 deficiency and diabetic neuropathy in patients taking metformin and concluded that the Vitamin B12 deficiency is highly prevalent, especially in patients with diabetic neuropathy. In their study an inverse correlation was found between diabetic neuropathy and the plasma level of vitamin B12. Higher doses of metformin and male sex were factors related to lower levels of vitamin B12. Yashaswini L **Error! Bookmark not defined.** performed a hospital based observational study on serum vitamin B12 levels in type 2 diabetic women and its correlation with metformin therapy and concluded that the metformin treatment in cases is associated with higher prevalence of Vitamin B12 deficiency which increases with duration of DM and dose of metformin. Raizada N et al **Error! Bookmark not defined.** performed a cross-sectional study on serum Vitamin B12 Levels in Type 2 Diabetes Patients on Metformin Compared to those Never on Metformin and concluded that Metformin use was associated with a lower serum Vitamin B12 levels when adjusted for duration of diabetes. Increasing duration of diabetes was associated with higher serum Vitamin B12 levels.

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 patient of T2DM who were not on metformin but rather taking some other OHA/Insulin by estimating vitamin B12 levels. Study included 300 patients (150 case-Metformin group & 150 control-OHA/Insulin group) with a mean age of 64.08 years and a standard deviation of 12.30 years, mean duration of diabetic was 12.25±7.69 years, majority 56% were female and rest 44% were males. Mean age of the study group (metformin group) was 64.32±11.87 years, while it was 63.84±12.75 in control group (OHA/Insulin group), this is similar to Agarwal P et al¹ 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 al¹⁰¹ reported mean age of all studied patients was 57.8 years that similar to our study.

In our study, total male patients in both groups were 132 (44%) and female patients were 168 (56%). In metformin group 63 (42%) were male & 87 (58%) female whereas in OHA /insulin group 69 (46%) were male & 81 (54%) female. On the basis of dietary habits, total 204 (68%) patients were vegetarian (99, 66% from metformin group, 105, 70% from OHA/insulin group) whereas total 96 (32%) patients were on mix diet (51, 34% from metformin group, 45, 30% from OHA/insulin 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, 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 300 cases majority of patients 147 (49%) were harboring the disease of diabetes for last >10 years, 96 (36%) were ailing for over 6-10 Years & only 45 (15%) patients were ill for last 1-5 Years and mean duration of diabetic of all patients was 12.25 years. In Metformin group duration of the disease of diabetic was 12.18±7.59 years where as in OHA/insulin group it was 12.92±6.80 years and correlation between them was found to be non-significant (p=0.375). Study done by Gupta K et al **Error! Bookmark not defined.** mean age of diabetic was reported 10.2 years which was similar to our findings.

CONCLUSION

1. Out of 300 studied patients, 56% were female, mean vitamin B12 level was 291.21±174.46.
2. Patients were divided into equally 2 groups (150 were in metformin exposure & 150 were in OHA/insulin exposure); Metformin group consisted of 63 (42%) Male & 87 (58%) female whereas OHA /insulin group was comprised of 69 (46%) male & 81 (54%) female.
3. Neuropathy was present in 54 (18%) patients only, out of which 36 were from metformin group and 18 from OHA/Insulin group. NCV done of all 54 patients and 24 out of 36 from metformin group had significant changes in NCV and 12 out of 18 from OHA/Insulin group respectively.

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