VITAMIN B$_{12}$ STATUS IN PROLONGED USE OF METFORMIN IN TYPE 2 DIABETES PATIENTS IN BHILAI, CHHATTISGARH, INDIA

Original Research Article

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Abstract

Background: Due to the immense advantage of the use of Metformin in controlling blood sugar levels in type II DM patients, an imperative side effect of the drug usage like deficiency of Vit B12 is often disregarded and hardly ever being scrutinized. With the above background information, our study focused on estimating the level of Vit B12 among Type 2 DM patients with prolonged use of metformin drugs. **Materials and Methods:** A cross-sectional study in the Institution was conducted in Type II Diabetes Mellitus subjects who were taking Metformin therapy for a minimum of 5 years or more attending OPD within 2 months study period. Serum Vitamin B12 levels of the type II DM participants were assessed by using Mindray's Chemiluminescent Immunoassay (CLIA), in serum with a setting of a lower limit to 125pg/ml. After ethical permission data were collected. With Consent blood sample was collected along with the review of the medical record. After collection, data was spread in an Excel sheet. Calculation of data was done by use of Excel sheet and SPSS version 22 free. Statistical data were expressed in proportion and mean $\pm$ SD and an appropriate test was applied to show the link between the variables with a statistically significant p-value of < 0.05. **Result:** With Vitamin B12, the difference was more in, < 10 years duration of metformin use than with >10 years duration and the difference was statistically significant (0.04). **Conclusion:** Vitamin B12 deficiency occurs in type 2 DM subjects taking Metformin drug for a long time.

INTRODUCTION

Diabetes Mellitus has emerged as a frequent metabolic malfunction disease in the modern world. It is a highly taxing health issue in the 21st century. It is affecting millions of people, near about 6-7% of the world's populace.

Type II Diabetes also known as Insulin Independent Diabetes Mellitus (NIDDM), is the commonest form of the disease comprising about 90% of the Diabetic population of many nations. If preventive measures are not taken, it is approximated that 438 million people will have diabetes by 2030.

In people with Type-II diabetes, blood sugar must be controlled either through diet, with oral hypoglycaemic drugs, or in severe cases with exogenous insulin. During the past decade, an increase of 30% in the occurrence of DM has been noted in our country among younger individuals. Food habits, sedentary life, and overall poor lifestyles are the main causative aspects for the climb in the prevalence of Type II DM.

WHO study reveals that Metformin is the secure and most effective generic medication required in a health system. It is hence the most prescribed drug for patients of T2DM and has emerged as a milestone in diabetic control.[1] It was the 4th most prescribed medication in western countries with more than 81 million prescriptions.

Metformin is regarded as a "wonder drug" and is very well tolerated by almost all patients of T2DM with colossal improvement in coronary complications.[2] However there are numerous side effects of using metformin for glycemic control which is usually uncared for, one of such is the insufficiency of Vitamin B12.[3,4]

The vitamin is of primal importance information of blood, genome synthesis, brain functioning, and various metabolic functioning of fat and energy.[5]

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Due to the excellent glycemic control by metformin, various reports and guidelines suggest its preference with parallel modifications in overall lifestyle along with the initiation of therapy for Type II DM. It participates in methylation reactions and acts by decreasing glucose production by the liver, increasing utilization of glucose by cells, and increasing the insulin sensitivity of body tissues.[6]

One risky effect of lactic acidosis in such glycemic control can also be conquered by the utilization of the drug Metformin. It is also reported to derail fat metabolism in the brain.[7] Previously researchers have made efforts to demonstrate the relationship between metformin use and Serum deficiency of Vitamin B12. Although is still foggy about the precise machinery of, Vitamin B12 deficiency by metformin use in T2DM subjects. Ideas to answer it although was seen in literature like decrease of Vitamin B12 absorption, inactivation of the vitamin by metformin, modulation of intrinsic factors (IF's), and alterations of receptor mechanism for Vitamin B12 absorption.[8-10] The drug itself has adversities like nausea, distress of the abdomen; soft stools, and diarrhea which again disappears slowly or after cessation of the drug therapy. Nonetheless, malabsorption of vitamin B12 might not be simply detected until a few months due to large stores in the liver which do not deplete easily.[11] The clinical features of Vitamin B12 deficiency, however, are usually demonstrated after 5-7 years of long metformin use and also depend on dosage and advancing age of the individual.[12,13]

Vitamin B12 also known as Cobalamine refers to a group of cobalt-containing vitamer compounds containing Methylcobalamine, Cyanobalamine, 5-deoxy-adenosine Cobalamine. It is a water-soluble vitamin and is a very stable form that is used as a reference compound for measuring serum Cobalamine concentrations. It is precisely involved in DNA production, fat metabolism optimal functioning of body vasculature, blood formation, and neurocognitive functions.[5]

The incidences of Vitamin B12 deficiency fluctuate from 5.8% to 31% in patients undergoing prolonged use of Metformin. Assessment of Deficiency of Vitamin B12 in Type II DM patients is of medical importance as lower levels of Vitamin B12 have been described and also allied with peripheral neuropathy and diabetic neuropathy in patients of metformin treatment.[14] It is also been associated with poor nerve conduction.[15] There had been reports of needless use of drugs due to lack of clinical aye for the condition and treatment of neuropathy seen in diabetes.[14,16-18]

Although at hand a relationship had been established between vitamin B12 deficiency and the use of metformin drug, the factual difficulty has not yet been truthfully computed.

No specific literature and no systematic study have been carried out in the Chhattisgarh population; therefore we planned to carry out this study at our hospital.

MATERIALS AND METHODS

This Medical College-based cross-sectional study was taken up in Type II Diabetes Mellitus patients who were on prolonged Metformin therapy with the age group between 30-70 years. The sample size of the study was 84, with 10% precision and 95% C. I by the prevalence of 3 % of TypeII DM patients in Chhattisgarh with the previous study.

All the participants were taken from the OPD of the Department of Medicine, Sankaracharya Institute of Medical Sciences, Bhilai, Chhattisgarh within 2 months duration. Participants were confirmed for the usage of the metformin drug for at least 5 years or more. Adult Diabetic Type II Patients with the following conditions:

1. Mentally Uncooperative, bothered psychological state, expectant and lactating females, and Diabetes Insipidus patients.
2. Patients with diabetes taking drugs known to cause neuropathy like Ioniazide, Linezolid, Metronidazole, and Phenytoin, and 3. Patients with other chronic disorders were disqualified from the study.

Determination of Serum Vitamin B 12 level was based on the principle of the ELISA. Serum Vitamin B12 levels of the type II DM participants were assessed by Mindray's CL-series Chemiluminescent Immunoassay analyzer (CLIA), in serum or plasma collected in EDTA, sodium heparin, and lithium heparin as recommended for this assay. The assay required 50μl of sample for a single test. Conversion factors: pmol/lx 1.36=pg/ml, pg/mlx0.738= pmol/l was used. The Vitamin B12 (CLIA) reagent kit had a limit of blank of $\leq$ 50 pg/ml, a limit of detection of $\leq$ 125 pg/ml, and a limit of quantitation of $\leq$ 150 pg/ml. The lower boundary of normal serum of Vitamin B12 level depends upon the procedure used for the testing and it is set at about 125pg/ml. The upper limit of this assay is 2000 pg/ml. Serum vitamins less than 200 pg/ml are diagnostic of vitamin B12 deficiency while values from >2000 pg/ml are considered as Upper Reference values of vitamin B12.

After ethical permission data were collected. A written Consent form was taken from all the participants, and the blood sample was collected along with the review of the medical record.

Statistical Analysis: After collection, data was spread in an Excel sheet. Calculation of data was done by use of Excel sheet and SPSS version 22. Statistical data were expressed in proportion and mean $\pm$ SD, also expressed in suitable tables and diagrams. An appropriate test was applied to show the association between Metformin drug and other variables in study subjects. p-value less than 0.05 will be considered statistically significant.

RESULTS

A total of 70 patients were selected and enrolled for our study, with an age group between 30 to 70 years who were diagnosed as having Diabetes Mellitus Type 2 and were on Metformin drug as an anti-diabetic treatment for a prolonged time. The subjects were selected within a Study period of July to August 2021. Among the study participants, 75.7% were male and the rest 24.3% were female adult diabetic patients. Sex distribution of study subjects according to age group showed in Figure 1. 40.0% of study subjects were female with 60.0% were male in the age group of lesser than 40 years, followed by 16.6% of female and 83.4% male in the age group of 40 -60 years and 41.18% female and 58.82% male in more than 60 years age group respectively showing a majority of the male population in each age group. 68.57% of study participants belong to the age group between 40-60 years, followed by 24.29% age group above 60 years and only 7.14% were between the age group of 30-40 years. This shows a majority of study participants belong to the age group of 40-60 years, followed by least among 30-40 years while considering both these age groups with a history of Diabetes Mellitus and oral intake of Metformin anti-diabetic drug of prolonged duration it contributes 75.71% of total study population showing the incidence of Diabetes Mellitus in early age that is before 40 years is increasing slowly.

The mean ± SD weight of study subjects was 54.31 ± 11.83 in kg, with a median of 54.00 kg and a range between 32.50- 88.00 kg. The mean ± SD Height of study subjects was 1.58 ± 0.76 meters, with a median of 1.58 meters and a range between 1.28-1.75 meters. The Body Mass Index of adult diabetic patients showed 21.55± 4.30 as Mean ± SD, with a 21.24 Median and a range between 13.44 - 33.33.

Vitamin B12 Mean ± SD among adult diabetics were 159.10±57.67 pg/ml, with a median value of 161.29 pg/ml and a range between 74.56 to 351.31pg/ml respectively. Adult diabetics with severe deficiency of vitamin B12 account for 42.8% followed by 42.9% of moderate deficient vitamin B12 and mild shortage contributes 10.0% of the study participants respectively. Sex distribution according to deficiency of Vitamin B12 grading was presented in figure 2. Only 4.3% of study participants had the normal status of vitamin B12 in the body and they were males contributing 5.7%. 73.3% of males and 26.7% of females were diagnosed with severe deficiency of vitamin B12. The deficient vitamin B12 group had 73.35 males with 26.7% of female participants. 85.7% of males and 14.3 % of female participants showed a Mild shortage of vitamin B12.

A single tablet of Metformin was 500mg, and 65.7% of study subjects were on 500mg of metformin. 21.4% were taking 1000mg followed by 12.9% on 1500mg of Metformin drug. 82.6% of males and 17.4% of females were on OD Dose. 66.7% of males with 33.3% of females were taking BD Dose followed by 55.6% of males and 44.4% of females on TDS Dose.

According to the Indian Asian Classification of Body Mass Index (BMI), 27.1% of participants were underweight (<18.5), followed by 30.0% with normal BMI (18.5-2.9), 25.7% of adult diabetics were Obese- At risk (23.0-24.9), 14.3% were classified as Obese Class I and only 2.9% were in Obese Class II respectively. 52.6% were on OD Dose of metformin with 47.4% on BD/TDS Dose among underweight, 61.9% were on OD Dose with 38.1% on BD/TDS Dose in an adult diabetic with normal BMI, 83.3% Obese Risk participants were on OD Dose and rest 16.7% taking BD/TDS of Metformin drug. 70.0% of Obese Class I were on OD Dose with 30.0% on BD/TDS Dose of anti-diabetic drug. Half of the participants were taking OD and the other half BD/TDS Dose in Obese Class II.

The frequency distribution of Vitamin B12 among study subjects shows the normal distribution in Fig. 2. The mean of vitamin B12 was 159.10 ± 57.67pg/ml in adult diabetics participants. The one-sample T-test shows a significant difference in the study population with a p-value of 0.00 (23.081 test value, df=69) statistically. On testing, correlation with the Pearson test revealed a non-significant positive correlation (0.223 (0.064)) between Vitamin B12 and the age of study subjects.

The mean for males was 163.00 ± 61.68 and for females was 146.95 ± 42.10, the Students-test shows no disparity between male and female vitamin B12 levels with the test value of 0.998. The difference between male and female vitamin B12 levels was statistically non-significant (0.322 (df=68)) in study subjects shown in Table 2. The study participants were divided into 2 groups based on the duration of use of metformin ie<10 years and >10 years. There was a significant deficiency of Vitamin B12 among patients of T2DM subjects receiving prolonged metformin observed both in <10 and >10 years of drug use. As depicted in [Table 1] there was a strong relationship between Vitamin B12 levels and long-term use of metformin among T2DM subjects. Likewise, when all study participants were divided into another 2 groups based on the dosage of metformin use [Table 2], there was hardly any significant relationship seen between Vitamin B12 levels and dosage of metformin among all participants of T2DM.

In [Table 3], the relation of study variables of participants with the duration of Metformin intake for years had been shown. With age the difference was more in, less than 10 years duration of metformin use than with more than 10 years duration and the difference was statistically non – significant (0.49). With Weight and height, the difference was more in, less than 10 years duration of metformin use than with more than 10 years

duration, and the difference was statistically non–significant (0.78) with weight, and the difference was statistically significant (0.05) with height. With BMI the difference was less in less than 10 years duration of metformin use than with more than 10 years duration and the difference was statistically non –significant (0.50). With Vitamin B12, the difference was more in, less than 10 years duration of metformin use than with more than 10 years duration and the difference was statistically significant (0.04).The relation of study variables to a dose of metformin intake was presented in [Table 4]. With age, Weight, and BMI the difference was more in study subjects having less than 1000 mg/day dose of metformin use than in those with more than 1000mg/day dose use and the difference was statistically non –significant for age (0.45), for Weight (0.48), and BMI (0.30). With Height and Vitamin B12, the difference was more in study subjects having less than 1000 mg/day dose of metformin use than in those with more than 1000mg/day dose use and the difference was statistically non –significant for Height (0.84) and Vitamin B12 (0.84).
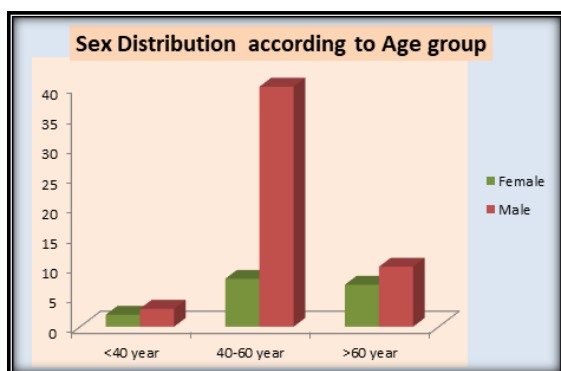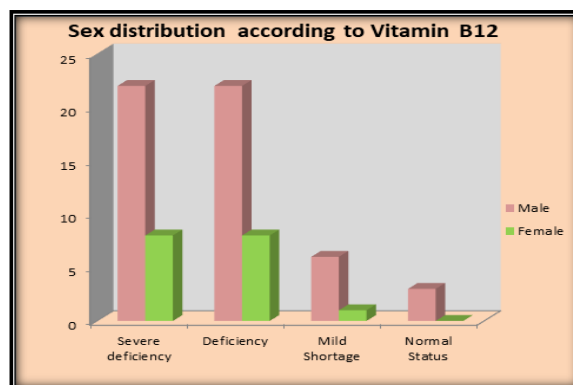

Figure2: Sex distribution of study participants according to Vitamin B12 grading (n=70)
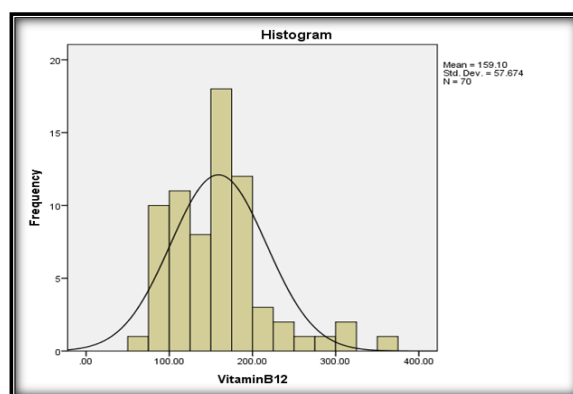
Figure 3: Frequency distribution of Vitamin B12 among study subjects (n=70)

Figure 1: Sex distribution of study participants according to age group (n=70)

Table 1: Grading of Vit B12 deficiency 19, 20.

Grading of Vitamin B12 deficiency	Values (in pg/ml)
Severe Deficiency	Value < 150 pg/ml
Deficiency	Value < 200 pg/ml
Mild shortage	Value 200-300 pg/ml
Normal status	Value 300-900 pg/ml
Hypervitaminosis	Value > 1000 pg/ml

Table 2: Distribution of Vitamin B12 according to gender (n=70)

	Gender	N	Mean± SD	T test	p-value
Vitamin B12	Male	53	163.00 ± 61.68	0.998	0.322 (df=68)
	Female	17	146.95± 42.10		

Table 3: Distribution of study subjects according to characteristic with duration of Metformin taken in years.(n=70)

Variables	<10 years (n=57)Mean± SD	>10 years (n=13)Mean± SD	p-value
Age	53.8772 ± 9.81	51.7692± 11.28	0.49
Weight (in kg)	54.5053 ±12.11	53.4846 ± 10.91	0.78
Height (in meter)	1.5942 ± 0.07	1.5500 ± 0.04	0.05*
BMI	21.3879± 4.31	22.2723 ± 4.34	0.50
Vitamin B12 (pg/ml)	165.8304 ± 55.41	129.6146 ± 60.31	0.04*

Table 4: Distribution of characteristics of study subjects according to dose of Metformin drug (n=70)

Variables	<1000mg/day(n=46) Mean± SD	>1000mg/day (n=24) Mean± SD	p-value
Age	54.2174 ± 8.59	52.0833 ± 12.45	0.45
Weight (in kg)	55.0391 ± 11.78	52.9292 ±12.05	0.48
Height (in meter)	1.5802 ± 0.08	1.5971 ± 0.06	0.84
BMI	21.9389 ± 4.22	20.8108 ± 4.44	0.30
Vitamin B12 (pg/ml)	158.857 ± 49.93	161.0575 ± 71.37	0.84

DISCUSSION

From our recent learning, it was observed that T2DM had affected males more than females across any age or sex group category. It was apparent that the majority of the subjects had developed the T2DM, early in their life before the age limit of 40 years with most advised and were users of metformin drug a hypoglycaemic drug for casual management of the disease complication. Although the dosage of the Metformin drug did not greatly influence the status of Vit B12 levels in either age or sex parameters, however, the duration of usage of the hypoglycaemic drug did. It was also understood that most subjects with Vit B12 deficiency were either underweight or Obese. It was observed in our present study, that use of metformin had a noteworthy influence on Vitamin B12 levels in T2DM patients.[21-23] This observation of Vitamin B12 deficiency among T2DM patients was concordant with previous studies by K.S. Akimlade et al &Nervo et al.[24] The close watch could be recognized probably due to paucity of nutrition in prolonged metformin use. This low level of Vit B12 observed among T2DM patients with prolonged (<10 years) usage of the drug metformin, was concordant with other previous research theories.[12,23,25,26] An isolated study by De jayer et al however had shown depressing levels of Vitamin B12 with prolonged usage of the drug metformin in T2DM patients.[13] His outcome was concordant with the findings of our study. The scenario may be answered by the lower GI motility or in combination with overproduction of bacterial growth in the GI tract leading to Vitamin B12 deficiency. There may also be derangement in the production of intrinsic factors (IF's) or deceased Ca++dependent Vitamin B12 absorption.[8,10,11] Few research concepts had also been seen in the literature regarding Vitamin B12 depletion from the liver due to higher doses of metformin use,[13,27,28] which was again not as per our findings. Although the exact mechanism of the effect of metformin use affecting Vitamin B12 level is not clearly understood, as per our study lower Vitamin B12 absorption may attribute to liver depletion of Vitamin B12 stores with prolonged use of metformin among T2 DM patients. Although Ting RZ,[12] had previously shown that 1gm of daily usage of metformin in T2DM subjects increases Vitamin B12 deficiency by a ratio of 2.88, our observation of Vitamin B12 levels in T2DM patients who have been using the drug metformin with higher doses had hardly any relationship.

A small number of researchers like Viikari. J et al and Nagrebetsky et al had previously correlated obesity with poor diabetic control.[29,30] They confidently reported the strong relationship between low BMI &glycemic Index i.e. HbAlc. But as per our findings, there was hardly any mentionable relationship between BMI and poor diabetic control neither with prolonged use of metformin nor with higher doses of the drug use among T2DM subjects.

CONCLUSION

As per our present study, it could be concluded that deficiency of Vitamin B12 is very common among Diabetes patients using metformin as a hypoglycaemic drug. Prolonged use of Metformin (< 10 years of usage) has a strong grip on modulating Vitamin B12 levels in T2DM subjects. There is a lower level of serum Vitamin B12 observed in our present study among T2DM patients taking metformin for more than 10 years. A deficiency was also seen among underweight subjects, Obese participants, but with an insignificant association, hence regular monitoring of Vitamin B12 levels is advised for all T2DM patients having prolonged use of the medication for maintaining blood sugar levels so that there could be some identification for patients who would need and benefit from Vitamin B12 supplement for a healthier approach to glycemic control. Due to fewer data in the literature and great demographic variations, extended research with a larger sample size is needed to validate the hypothesis.

Summary

The link and mechanism of Vitamin B12 deficiency with prolonged use of Metformin is presently not well studied and unclear. Due to the copious medical profit of Metformin, certain potential adverse effects by and large are uncared for, and rarely investigated; one of such side effects is Vitamin B12 deficiency. Previous studies have pointed out that the occurrence of Vitamin B12 deficiency is taxing to Metformin use. There is need of larger sample size and organized setup to better understand the status of higher doses and prolonged use of the drug in T2DM.

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