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Original Research Article

Assessment of Vitamin B12 Deficiency in Chronic Metformin Treated type 2 Diabetic Patients

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Abstract

Abstract

Background: Metformin is one of the most widely used oral hypoglycemic agents. Vitamin B12 deficiency due to metformin therapy may not be easily detected without close attention. Monitoring for vitamin B12 has been recommended for patients with type 2 diabetes, especially those on long-term metformin treatment. Peripheral neuropathy due to vitamin B12 deficiency may be confused with diabetic peripheral neuropathy or may contribute to the aggravation of diabetic peripheral neuropathy.

Objectives: This study was conducted with the objective to assess the relation between metformin therapy duration, dose and vitamin B12 levels in chronic metformin treated type 2 diabetic patients.

Materials and Methods: The study was conducted at tertiary care hospital between January 2017- August 2018. Patients of Type 2 diabetes with metformin therapy for one or more than one year duration were taken as cases and those type 2 diabetes patients without metformin therapy were taken as controls. Laboratory investigations including FBS, PPBS, Hb1AC and Vitamin B12 levels were performed and comparison of these parameters between cases and control was done.

Results: There was no statistically significant difference in mean FBS, PPBS and Hb1Ac% between cases and control groups. Among cases, 47% patients were taking metformin dose of ≤1000 mg/day while 28% patients were on higher dose of metformin i.e. >1500 mg/day. Mean year of metformin usage by case group was 5.6 years. Duration of metformin use 500mg or more varied from lowest 1 year to highest 12 years. Significantly lower mean S. vitamin B12 was observed in cases (333.15pg/ml) as compared to controls (467.78pg/ml).

Conclusion: Metformin therapy is associated with vitamin B12 deficiency among diabetic patients.

Keywords: Diabetes, Metformin, Vitamin B12 deficiency.

Introduction

Metformin is one of the most widely used oral hypoglycemic agents. Most of the current global clinical practice recommendations, including those of the American Diabetes Association, the European Association for the Study of Diabetes, and the Korean Diabetes Association, propose that metformin, if there are no contraindications, should be initiated with concurrent lifestyle modifications at initial diabetes diagnosis[1,2].

Most of the side effects due to metformin are mild and usually include gastrointestinal symptoms, such as abdominal distress, soft stools, and diarrhea[3]. Generally, these side effects appear shortly after the initiation of metformin and promptly disappear after discontinuation. However, insidious or asymptomatic side effects resulting from long-term treatment, such as vitamin B12 deficiency, may not be easily detected without close attention. Serum vitamin B12 levels have been reported to be inversely associated with the dose and duration of metformin use[3,4]. These studies reported that an average of 10 to 30% of patients exhibited mal absorptive vitamin B12 deficiency[3,4].

Although the clinical significance of vitamin B12 deficiency related to metformin treatment is debatable, monitoring for vitamin B12 has been recommended for patients with type 2 diabetes, especially those on long-term metformin treatment. Clinically, vitamin B12 deficiency could lead to altered mental status, megaloblastic anemia, and neurological damage[5]. Unfortunately, diabetic neuropathy symptoms can overlap with paresthesias, impaired vibration sensation and proprioception. Therefore, peripheral neuropathy due to vitamin B12 deficiency may be confused with diabetic peripheral neuropathy or may contribute to the aggravation of diabetic peripheral neuropathy.[6] The progression of neurologic damage due to vitamin B12 deficiency can be stopped by early detection and treatment with cobalamin supplementation[5]. However, if this occurrence is misdiagnosed as diabetic neuropathy, permanent neurological damage may occur[6].

As metformin has been prescribed worldwide and treatment periods increase, the prevalence of metformin-induced vitamin B12 deficiency may have also significantly increased. However, the relationship between metformin use and vitamin B12 deficiency has not been widely investigated. This study focused on the correlation of vitamin B12 deficiency and the factors associated with metformin.

Methodology:

Study Design: Analytical Cross sectional study

Study Period: January 2017- August 2018

Study Population: Type 2 Diabetic patients, who were on Metformin therapy for 1 or more years were enrolled from tertiary care hospital.

Inclusion Criteria: 100 cases of Type 2 diabetes mellitus patients who were on metformin therapy for 1 or more years.

Exclusion Criteria:

- Patients with type 1 diabetes mellitus, pregnant women, prior vitamin B 12 injections, gastrectomy, colectomy, IBD & vegetarianism.
- Patients with known chronic kidney disease, heart failure, liver cirrhosis, and known malignancy.
- Patients with over-the-counter medications, calcium supplements, histamine-2 blocker, and proton pump inhibitor.

Study Method: The work up included history, physical examination and Laboratory Evaluation of all patients,

- CBC
- Peripheral smear study,
- Renal function tests,

- Liver function tests,
- FBS, PPBS.
- HbA1C
- Serum Vitamin B12 assay.
- Urine- Routine and Microscopy.

In present study, there are 2 groups divided as: Cases: Type 2 DM with metformin therapy for 1 or more than 1 year. Control: Type 2 DM not on Metformin.

Statistical Analysis:

The data obtained was coded and entered into Microsoft Excel Worksheet. The categorical data was expressed percentages and chi-square test was used to compare the data. The continuous data was expressed as mean ± standard deviation (SD) and the comparison was done using unpaired 't' test. A probability value ('p' value) of less than 0.05 was considered as statistically significant.

Results:

In our study, highest number of patients were within age of 51-60 years (35% and 33% in cases and control group respectively) followed by age of 41-50 years (22% and 25% in in cases and control group respectively) Mean age of cases was found to be 51.90±10.47 and that of control group was 52.33±10.53; which was statistically non-significant (p=0.8023). Sex wise distribution shows that male (68% and 60% in cases and control group respectively) were more predominant than female (32% and 40% in cases and control group respectively). Among cases 58% patients and among controls 54% patients had duration of diabetes between 5 to 10 years, followed by 29% and 35% patients with less than 5 years of duration of diabetes in cases and controls respectively. Patients with duration of diabetes more than 10 years were 13% and 11% among cases and controls respectively. There was statistically significant difference in mean duration of diabetes between both the groups (P<0.05). Mean duration of diabetes is higher i.e. 8.21 years among cases than controls i.e.6.99 years [Table 1]

Table 1: Demographic variables of patients

Variables		Cases Group (n=100)	Control Group (n=100)	P value
Age groups (years)	31-40	21 (21%)	19 (19%)	
	41-50	22 (22%)	25 (25%)	
	51-60	35 (35%)	33 (33%)	
	61-70	17 (17%)	18 (18%)	
	71-80	05 (05%)	05 (05%)	
Mean age± SD		51.90±10.47	52.33±10.53	p=0.80
Sex	Male	68 (68%)	60 (60%)	p=0.30
	Female	32 (32%)	40 (40%)	
Duration of DM (years)	< 5 Yrs	29 (29%)	35 (35%)	
	5 – 10 Yrs	58 (58%)	54 (54%)	
	>10 Yrs	13(13%)	11 (11%)	
Mean duration time		8.21 ± 4.1	6.99 ± 3.09	P<0.05

In the present study, mean fasting blood sugar was 128.24 ± 16.63mg/dl among cases and 132.50 ± 15.20 mg/dl among control group patients. Mean post prandial blood sugar was 228.46±40.71mg/dl in cases and 225±41.09 mg/dl among control group patients. Mean Hb1Ac% was 7.96 ± 1.07 in cases and 8.22 ± 1.22 among control group patients. There was no statistically significant difference in mean FBS, PPBS

and Hb1Ac% between cases and control groups (P>0.05). [Table 2] Mean Haemoglobin was 13.82 ± 1.4 gm/dL among cases group and 14.1 ± 1.53 gm/dl in control group patients. Mean MCV was 91.62 ± 4.67 among cases group and 91.23 ± 3.74 gm/dl in control group patients, the difference was not statistically significant. [Table 2]

Table 2: Comparison of investigation between cases and control group

Investigations	Cases Group (n=100)	Control Group (n=100)	P value
	Mean ± SD	Mean ± SD	
FBS	128.24 ± 16.63	132.50 ± 15.20	p=0.06
PPBS	228.46±40.71	225±41.09	p=0.55
HBA1C (%)	7.96±1.07	8.22±1.22	p=0.11
Hb (gm/dl)	13.82 ± 1.4	14.1 ± 1.53	p=0.12
MCV (fl)	91.62 ± 4.67	91.23 ± 3.74	p=0.51
Vitamin B12	333.15 ± 233.97	467.78 ± 387.32	p<0.01

Among cases, 47% patients were taking metformin dose of ≤1000 mg/day while 28% patients were on higher dose of metformin i.e. >1500 mg/day. Mean year of metformin usage by case group was 5.6 years. Duration of metformin use of 500mg or more varied from lowest 1 year to highest 12 years. [Figure 1]

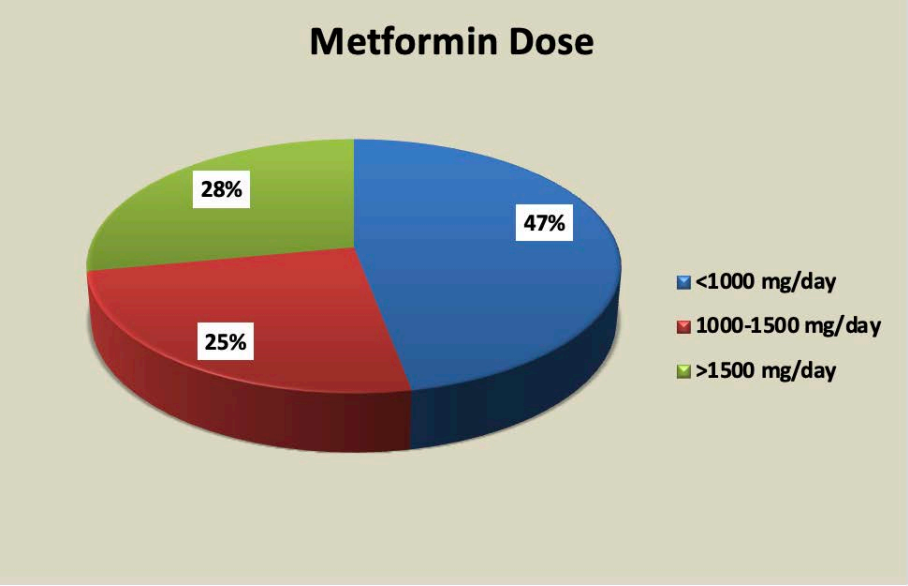

Figure 1: Distribution of diabetic patients according to metformin dose (N=100)

In the cases group S. vitamin B12 ranged from 52 to 1480pg/ml with a mean of 333.15 ± 233.97. In the control group S. vitamin B12 ranged from 342 to 743pg/ml with a mean of 467.78 ± 387.32. Significantly lower mean S. vitamin B12 was observed in cases as compared to controls (p<0.01) [Figure 2]

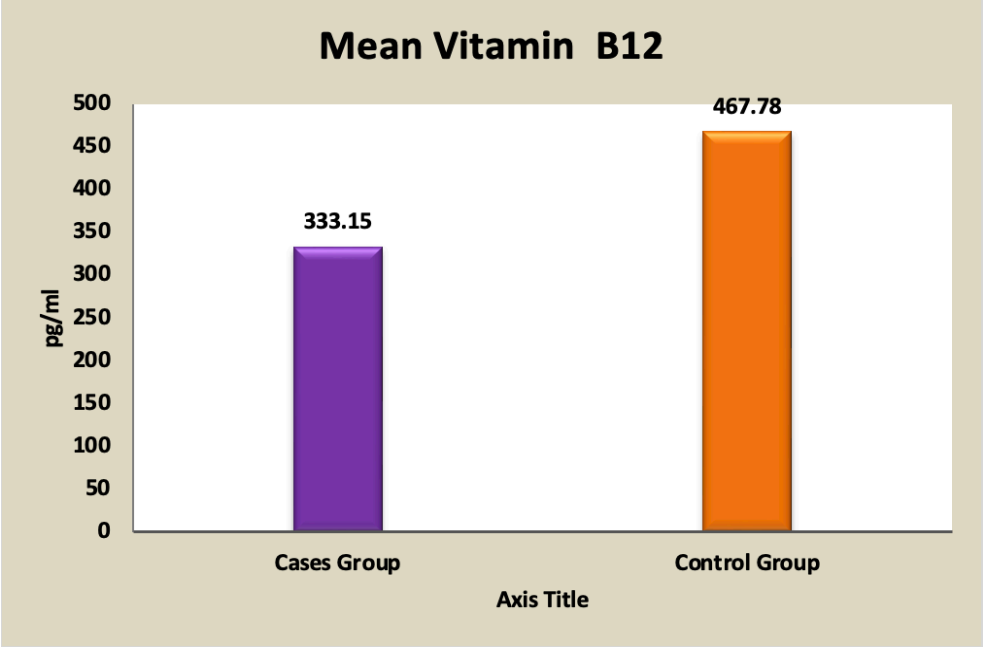

Figure 2: comparison of serum vitamin B12 levels between cases (n=100) and controls (n=100)

The dose of metformin was found to have statically significant effect on B12 levels (p value < 0.05). Mean daily dose of metformin with vitamin B12 deficiency was 1985.8 grams while it was 1507 ± 589.2 grams among patients with normal vitamin B12 levels. [Table 3]

Table 3: Comparison of Vitamin B12 deficiency and normal vitamin B12 groups among metformin therapy cases (N=100)

	Vitamin B12 Deficiency group (n=31)	**Vitamin B12 Normal group (n=69)**	**P value**
Age in years	52.1±9.52	52.31±11.62	0.0839
Male	20 (64.5%)	48 (69.6%)	0.8993
Female	11(35.5%)	21 (30.4%)	0.8993
Hb	14.01 ± 1.23	13.54 ± 1.32	0.0959
MCV	91.53 ± 3.66	90.47 ± 4.05	0.2158
HbA1C	8.02 ± 1.03	7.86 ± 1.14	0.5056
Duration of DM(years)	8.4 ± 3.5	7.7 ± 4.2	0.4201
Duration of metformin (years)	5.7 ± 2.8	5.4 ± 2.7	0.6126
Dose of metformin (mg)	1985.8 ±560.61	1507 ± 589.2	**<0.0001**
Vitamin B12 levels	124.4 ± 87.9	436.9 ±314.2	**<0.0001**

Discussion:

Our study indicated metformin therapy is associated with vitamin B12 deficiency among diabetic patients. Vitamin B12 levels are significantly lower in diabetic patients taking metformin as compared to those who are not on metformin treatment.

P Agarwal et al[7] study have found similarly in the cases group mean S. vitamin B12 as 431.84±265.76pg/ml comparable with in the control group 744.76±271.927pg/ml which was statistically significant. Similarly, kim et al[8] in their study also found that prevalence of vitamin B12 deficiency of 22.2% in patients with type 2

diabetes taking long-term metformin, with larger daily doses significantly associated with vitamin B12 deficiency.

The "golden standard" for defining vitamin B12 deficiency is not yet established, since there are various methods for measuring serum vitamin B12, corresponding normal values, and research results. One study reporting lower specificity showed that 60% of patients had symptoms of vitamin B12 deficiency when B12 level was <200 pg/mL, and 90% at <100 pg/mL[9]. In general; however, serum vitamin B12 levels can be interpreted as follows: >300 pg/mL, B12 deficiency is unlikely (probability of 1%–5%); 200 to 300 pg/mL, B12 deficiency possible (probability of 5%–15%); <200 pg/mL, consistent with B12 deficiency (specificity of 90%–100%)[10,11]. On this basis, biochemical vitamin B12 deficiency was defined as serum B12 <300 pg/mL, not accompanied by serum folic acid deficiency, in this study[10].

The prevalence of vitamin B12 deficiency after metformin use has been reported to be 6% to 30%, varying with race, standard values of vitamin B12, and metformin usage[12-17]. A large prospective study reported a 20.3% prevalence of vitamin B12 deficiency after 9.5 years of metformin use[16] and a large dataset provided by the National Health and Nutrition Examination Survey showed a 22% prevalence when vitamin B12 deficiency was defined as levels below 300 pg/mL[12]. A recent study of 799 patients by the College of Medicine of the Catholic University of Korea showed a lower prevalence of vitamin B12 deficiency (9.5%) and higher mean B12 levels (662.5±246.7 pg/mL) than in the present study.

Some studies have focused on the impact of the duration and doses of metformin on vitamin B12 deficiency. In the previously mentioned study, both the duration and daily dose of metformin were significantly associated with vitamin B12 deficiency, with cut-off values of 4 years and 1100 mg/d, respectively[14]. In another study, metformin dose had a negative relationship with vitamin B12 levels, and an increase of 1 mg in the daily metformin dose was associated with a 0.042 pg/mL decrease in vitamin B12 levels, but the duration of metformin use did not show significant effects.[5] Similarly, several further studies have reported that daily dose is a more strongly associated with vitamin B12 deficiency than duration[18,19]. Our findings indicate that a 1 mg/d increase in metformin dose decreased vitamin B12 levels by 0.142 pg/mL, but the duration of use did not have significant effects. Altogether, the results of these studies and our study indicate that it is important to account for metformin dose in recommendations for screening for vitamin B12 deficiency, and not just the duration of metformin use.

The clinical significance of vitamin B12 deficiency lies in megaloblastic anemia and neuropathy[20,21]. In this study, anaemia was a significant factor related to B12 deficiency, even after adjusting for other confounders; but in other studies, there were few patients with typical megaloblastic anemia (MCV >100 fL)[14]. In all, 13 patients showed MCV >100 fL, and only 1 was vitamin B12-deficient. This may be because various factors, such as age and nutrition, play parts in vitamin B12 deficiency, along with other factors, such as iron deficiency anemia and anemia due to chronic disease. This result is supported by other studies which found that 30% of patients, even those with anemia due to vitamin B12 deficiency, had normal MCVs[22-24]. Since this study followed a cross-sectional design, we cannot judge the causal relationship, but our results indicate vitamin B12 deficiency affected anemia.

The mechanism by which metformin reduces serum vitamin B12 levels has not been elucidated, but the most likely hypothesis is that metformin interferes with

calcium-dependent membrane action responsible for vitamin B12 intrinsic factor absorption in the terminal ileum[25]. Few studies have investigated whether such absorption difficulties caused by metformin could be improved by supplementation of multivitamins, which usually contain small amounts of vitamin B12 at 2 to 30 ug/d. A recent review paper recommended the use of vitamin supplements for patients on long-term metformin treatment to prevent decreases in vitamin B12, albeit without direct evidence[26]. Moreover, a retrospective study showed that among metformin users, multivitamin use was associated with lower prevalence of vitamin B12 deficiency (adjusted OR=0.14; 95% CI=0.04, 0.54) compared to those not using multivitamins[27]. However, in most other studies that investigated the supplementation with multivitamins, no significant influence on vitamin B12 deficiency was observed[12,14,19]. Kim et al[8] found that the mean vitamin B12 level in patients taking multivitamin was 715.1±340.4 pg/mL, and that in patients who did not take multivitamin was 410.8±173.4 pg/mL (P<.001). After adjusting for various confounders, our findings indicate that multivitamin supplementation was significantly associated with lower prevalence of vitamin B12 deficiency and a reduced OR of developing a deficiency. They further suggested that results with such a meaningful difference were derived, as the study investigated and compared patients whose records showed that they had regularly consumed vitamin supplements for more than 6 months.[8] However, the lack of a detailed grasp of the vitamin B12 dose contained in multivitamin was one of the major limitations of their study. Further well-designed studies should be conducted to confirm the validity of multivitamin use for preventing B12 deficiency.

Conclusion:

Our findings demonstrates that long term use of Metformin predisposes to Vitamin B12 deficiency among Type 2 DM patients. With increasing dose of daily Metformin consumption there is increased risk of getting comparatively lower blood levels of Vitamin B12. A well-defined guideline regarding the initiation and frequency of Vitamin B12 blood level monitoring among Diabetic patients on prolonged Metformin therapy is an urgent need of the day. The necessity of prophylactic Vitamin B12 supplementation also needs to be investigated and scrutinized.

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