

Serum B12 Levels in Type II Diabetics on Metformin Therapy and its association with Clinical Neuropathy

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Abstract

Background: Metformin use in type II DM has also been known to cause B12 deficiency in as reported many studies. Iatrogenic neuropathy caused by Metformin induced B12 deficiency can add to burden of peripheral neuropathy that already exists in diabetic patients.

Aims and Objectives: 1) To determine the serum vitamin B12 levels in patients of type II DM on metformin therapy and compare it with those not on metformin therapy; 2) To correlate serum vitamin B12 levels with dose and duration of metformin therapy; 3) To investigate association between peripheral neuropathy and serum vitamin B12 levels.

Method: The present two year hospital based cross sectional study enrolled total 132 cases of type 2 diabetes mellitus and divided into two groups, ("metformin" group 70 and "non-metformin" group 62). Serum vitamin B12 levels were measured in all patients. Toronto clinical scoring system (TCSS) and nerve conduction velocity (NCV) tests were used to assess peripheral neuropathy. The correlations of vitamin B12 levels with Toronto clinical score, nerve conduction studies, cumulative dose and duration of metformin therapy was done.

Results: The serum vitamin B12 levels were significantly lower in patients who consumed metformin (292.64 ± 152.82 pg/ml) as compared to non-metformin, (406.91 ± 126.59 pg/ml) group. There was a significant negative correlation of serum vitamin B12 levels with cumulative dose and duration of metformin therapy. The incidence of neuropathy by TCSS and NCV test was significantly higher in metformin group with a positive correlation with cumulative dose and duration of metformin and a negative correlation with serum vitamin B12 levels.

Conclusion: Metformin use is significantly associated with decrease in vitamin B12 levels and increased incidence of neuropathy, this is dependent on dose and duration of metformin therapy.

Keywords: Metformin, Vitamin B12, Diabetes mellitus (DM), Peripheral neuropathy, Toronto clinical scoring system (TCSS), Nerve conduction velocity (NCV).

1. Introduction

Vitamin B12 deficiency is common in vegans and can give rise to hematological and neurological manifestations. It can coexist with Type 2 Diabetes mellitus and is an independent risk factor for cardiovascular disease [1-3]. Metformin is a first line drug in the pharmacotherapy of type 2 diabetes. Apart from the low cost, good efficacy and beneficial effects on body weight, the relatively safe adverse effect profile has justified the widespread use of metformin.

However metformin use in Type II Diabetics has also been known to cause B12 deficiency in many studies [4-6]. Iatrogenic neuropathy caused by metformin induced B12 deficiency can add to burden of peripheral neuropathy that already exists in diabetic patients. In various surveys and cross-sectional studies across the world, it has been associated with 10 - 50% reduction in B12 levels [7-9]. This association between the use of metformin and vitamin B12 deficiency was conclusively proved by two randomized

placebo controlled trials which showed a decrease in 22% [10] over a period of 29 weeks and 19% [2] decreases over a period of 4yrs respectively. Some authors have also questioned the clinical relevance of these findings since B12 deficiency is asymptomatic at most times. However in the past decade many case reports have emerged showing that metformin induced B12 deficiency can cause insidious neuropathy which may be attributed to poor glucose control in Type II DM [3,11].

Due to paucity of Indian studies evaluating the prevalence of B12 deficiency and its association with neuropathy in Type II Diabetics on Metformin therapy, the present study was undertaken.

2. Materials and Methods

This hospital based cross sectional observational study was done among the indoor and outdoor cases of type 2 diabetes mellitus in medicine wards, medicine OPD and diabetes specialty clinic at tertiary care hospital, during the period of 2 years (from Jan 2015 to Dec 2016). Total 132 cases of type II DM on metformin (for a period of more than 18 months) and on other oral hypoglycemic drugs were included in the study. Patients with history of type 1 DM, chronic alcoholism, pregnancy, chronic diarrhea, patient already receiving B12 in therapeutic doses, documented evidence of hypothyroidism, sickle cell anaemia, CKD with severe anaemia and history of GI surgery (example: bariatric surgery) were excluded from the study. The study was approved by the Institutional Ethical Committee and written informed consent was obtained from all the patients.

A detailed history of duration of diabetes and type of oral hypoglycemic drug intake was taken. Patients were divided into two groups: those taking metformin and those who were not taking metformin. History of other associated co-morbid conditions like ischemic heart disease, hypertension, dyslipidemia and personal habits of smoking and alcoholism were enquired through an interview with the patient and care giver. Patients who had history of chronic alcoholism were excluded from the study. Further, these patients underwent a thorough general examination for vitals (pulse, blood pressure), weight was measured and BMI was also calculated. This was followed by a thorough systemic examination. All patients were subjected to standardized neurological examination including power, tone, deep tendon reflexes, and sensory function. Romberg's test was performed in case of any gait abnormality. On the basis of data gathered from history of neurological symptoms and standardized examination, neuropathy in the patient was graded using Toronto clinical scoring system (TCSS) by an investigator blinded to the laboratory results.

Each patient was questioned as to presence or absence of pain (characteristic of neuropathic pain such as

burning, stabbing, or shock-like), numbness, tingling, and weakness in the feet; the presence or absence of similar upper-limb symptoms; and the presence or absence of unsteadiness on ambulation. Sensory testing was performed at the first toe and rated as normal or abnormal. The outcome, the clinical neuropathy score, is a continuous variable ranging from a minimum of 0 (no neuropathy) to a maximum of 19 points. Six points are derived from symptoms, eight from lower-limb reflexes, and five from sensory examination distally at the toes.

On the basis of the score, patients were graded into classes of: 1) no neuropathy: 0-5; 2) mild neuropathy: 6-9; 3) moderate neuropathy: 10-12; 4) severe neuropathy >12.

All patients were subjected to serum vitamin B12 assay which was done using fully automated bidirectional interfaced chemiluminescent immune assay method. Serum samples were stored at room temperature (15-30 degrees centigrade) for not longer than 2 hours. The normal laboratory reference range for serum vitamin B12 was 211 pg/ml – 911 pg/ml. Complete blood count was done through fully automated cell counter. The normal range of mean corpuscular volume was 80fl to 96fl. So considering this, the cases were divided into 3 categories. Cases having mcv below 80fl, between 80-96fl and above 96fl. Nerve conduction velocity (NCV) tests were performed using RMS SALUS EMG Machine kit in all patients and results were assessed according to the ADA diabetic neuropathy protocol. Median, ulnar, tibial nerve conduction studies, F-wave, and median, ulnar, and sural NCVs were recorded at room temperature maintained at 22–24°C. Standard NCVs were used. Polyneuropathy types were described as either demyelinating or axonal.

2.1 Statistical Analysis

The data was analyzed using SPSS version 16. Baseline characteristics like age, weight, HbA1c, fasting and postmeal blood sugar levels, haemoglobin levels were compared by relevant independent sample t tests. The correlations of vitamin B12 levels, Toronto clinical score and nerve conduction studies were done with cumulative metformin dose and duration using rank correlation test. The significance level was taken as 5%.

3. Observations and Results

Total 132 patients were selected for the study and divided into metformin group and non metformin group. The mean age of patients in metformin and non-metformin group was 63.65 ± 11.37 years and 62.29 ± 12.80 years respectively. Maximum number of patients in the present study belonged to age group of 60.1 - 70 years they being 38.57% in metformin and 41.93% non-metformin group. Parameters like age, HbA1c, typing of anemia did not show any statistically significant difference between two groups

but baseline parameters like weight, fasting and post prandial blood sugar, mean corpuscular volume and Toronto clinical score for peripheral neuropathy showed a highly significant difference in metformin group as compared to non-metformin group. The serum vitamin B12 levels were

significantly lower in patients who consumed metformin (292.64 ± 152.82 pg/ml) as compared to non-metformin, (406.91 ± 126.59 pg/ml) group which was statistically significant (Table 1).

Table 1: Comparison of baseline characteristics between two groups

Factor	Metformin Group	Non-Metformin Group	P Value
Age(years)	63.65± 11.37	62.29 ±12.80	0.517
Weight(kg)	67.4 ± 12.16	76.96 ± 10.89	<0.0001
HBA1C levels	7.91 ± 2.01	7.99 ± 2.12	0.8270
Fasting blood sugar (mg/dl)	155.15 ± 52.33	203.46 ± 63.08	<0.0001
Post meal blood sugar (mg/dl)	229.58 ± 69.86	266.48 ± 81.86	0.0060
Mean corpuscular volume (fl)	88.92 ± 7.06	83.07 ± 9.15	0.0001
Vitamin B12 level (pg/ml)	292.64 ± 152.82	406.91 ± 126.59	<0.001
Toronto Clinical Score	6.98± 3.87	5.16± 3.70	0.0067
Haemoglobin (g/dl)	10.67 ± 1.80	10.44 ± 2.01	0.4893

There was a significant negative correlation (pearson correlation -0.256) between the cumulative dose of metformin and serum vitamin B12 levels (Figure 1). The p value was statistically significant (P value =0.032). In addition to this there was a significant negative correlation between the duration of metformin therapy and serum vitamin B12 levels (P value 0.027) (Figure 2).

Figure 1: Scatter diagram showing negative correlation between dose of metformin and serum vitamin B12 level

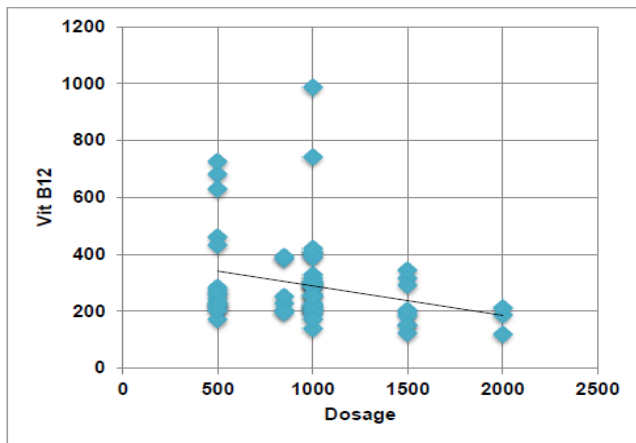
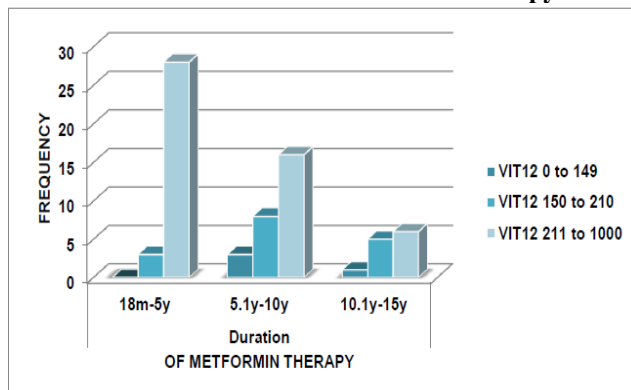
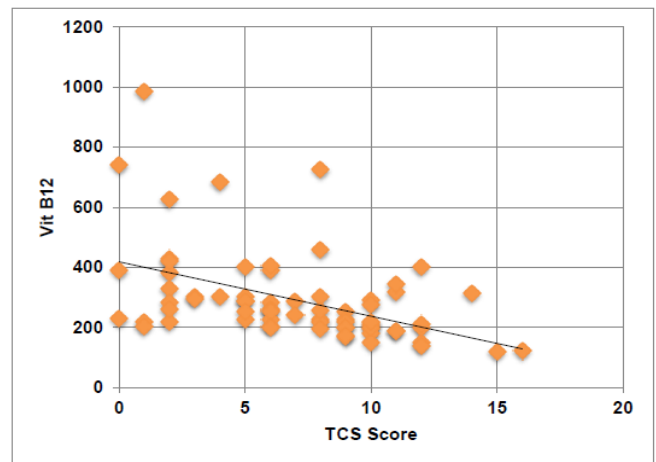


Figure 2: Negative correlation between vitamin B12 levels and duration of metformin therapy



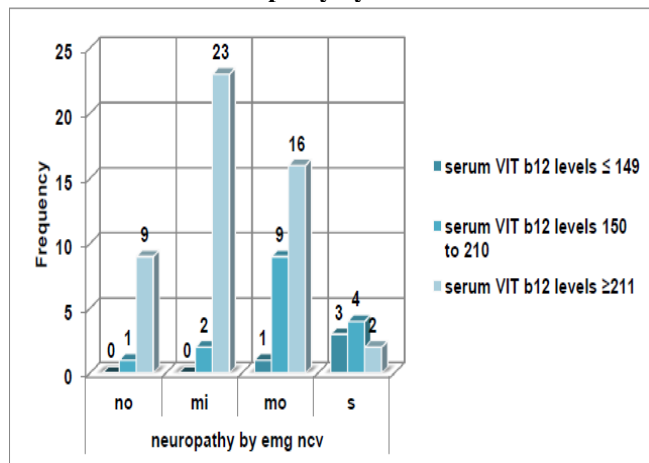
The present study also revealed that there was a significant difference in the mean Toronto clinical score between the metformin exposed (6.98 ± 3.87) and non-metformin exposed group (5.16 ± 3.70) along with a significant positive correlation between Toronto clinical score and cumulative dose of metformin. Also we found significant negative correlation between serum vitamin B12 levels and Toronto clinical score (P value<0.0001) (Figure 3).

Figure 3: Scatter diagram showing negative correlation between Toronto clinical score and serum vitamin B12 level



The incidence of neuropathy by Toronto clinical score as well as nerve conduction studies was significantly higher in metformin exposed group with a positive correlation with cumulative dose and duration of metformin and a negative correlation with serum vitamin B12 levels (Figure 4).

Figure 4: Correlation between serum vitamin B12 levels and neuropathy by EMG NCV



4. Discussion

Current and most likely explanation for Metformin induced vit B12 malabsorption and deficiency is that metformin has an effect on calcium dependent membrane action on terminal ileum. Intrinsic factor complex is calcium dependent and metformin interferes with this absorption.

Iatrogenic neuropathy caused by metformin induced B12 deficiency can add to burden of peripheral neuropathy that already exists in Diabetic patients. This hypothesis has been studied extensively in previous studies.

The result of present study, comprising of 132 cases of Diabetes studied in two groups (Those with on metformin and other not on metformin therapy) revealed low serum B12 level i.e. mean serum vitamin B12 level in metformin group was 292.64±152.82 pg/ml and 406.91±126.59 pg/ml in the non-metformin group, this difference was statistically significant. But mean age of the cases did not influence on outcome of the study in both the groups. These observations were comparable with results of the other studies reported by various authors [4,12,15]

All these studies showed low serum vitamin B12 levels in patients of type 2 DM on metformin therapy. The mean serum vitamin B12 level in our study was low as compared to other studies for which no explanation could be found. In current study due to financial restraints we could not carry out serum homocysteine and folate levels which are more efficient markers of vitamin B12 deficiency.

We found significant negative correlation (pearson correlation - 0.256) between cumulative dose of metformin and serum vitamin B12 levels, (P value = 0.032). This shows that as the cumulative dose of metformin goes on increasing there is significant decrease in the serum vitamin B12 levels. Vitamin B12 forms a complex with cubulin

(endocytic) receptor at ileum for absorption. This B-12 endocytic receptor complex is normally taken by ileal cell surface by calcium dependent process. Metformin with its protonated biguanide group binds to the B12-cubulin complex and imparts positive charge to it, alters membrane potential and competitively repels the divalent calcium ions thus preventing calcium dependent uptake, leading to malabsorption of B12. It has also been proposed to act by increasing bacterial overgrowth, altering bowel motility, and by direct inhibition of B12 absorption.

The present study observed negative correlation between duration of metformin therapy & serum B12 levels This negative correlation between the duration of metformin therapy and serum vitamin B12 levels was statistically significant (p value 0.027). More the duration of metformin therapy lower the serum B12 level.

There is plethora of articles showing significant negative correlation between serum B12 levels and cumulative doses & long duration metformin therapy [5,15-17].

Though serum B12 levels in metformin users have been studied across the world, only few studies have explored the additional burden of neuropathy it might impose on them. The total number of patients who had neuropathy by TCS (i.e score of more than 5) in metformin group were 46 (65.71%) and 29 (46.77%) in non-metformin group. There was a significant difference in the mean TCS between metformin (6.98± 3.87) and non-metformin group (5.16± 3.70). This shows that the incidence of neuropathy was more in metformin group as compared to non-metformin group. Also there was a significant positive correlation between the Toronto clinical score and the cumulative dose of metformin, (p value<0.0001). Moreover there was a significant negative correlation between the serum vitamin B12 levels and Toronto clinical score. Our results were similar to the study carried out by Singh *et al* [12] and Wile *et al* [18].

There was smaller but significant difference in neuropathy scores in our study than observed by Wile *et al*. [18]. Since the present study included patients with both asymptomatic and symptomatic neuropathies to make it more representatives, this might have led to a smaller difference in our study. Our study differs from study carried out by Dunstan *et al* [19] in which no difference in neuropathy scores were found using NTSS score. However, we hypothesize this might have to do with the nature of NTSS score, which is based on history of sensory symptoms alone, rather than reliable and well validated TCSS used in our study, which is easy to calculate, highly reproducible, and includes elements of both history and examination.

The present study also performed nerve conduction studies in addition to TCS to have accuracy for detection of

peripheral neuropathy. The total number of patients who had neuropathy by nerve conduction in metformin group was 60 (85.71%) as compared to 37 (39.45%) in non-metformin group, this difference was highly significant.

The study further tried to correlate doses of Metformin and neuropathy by NCV & significant positive correlation was observed between cumulative dose of metformin & neuropathy reported on NCV. Also 90% (9 cases out of 10) of patients who do not have neuropathy by EMG NCV have serum vitamin B12 levels more than 211 pg/ml while 77.7% (7 of 9) of patient who have severe neuropathy have serum vitamin B12 levels less than 211pg/ml. This shows that there was significant negative correlation (p value <0.001) between serum vitamin B12 levels and neuropathy by EMG NCV.

Similar kind of results was also observed previously in one of the cross-sectional study carried out by Roy *et al* (2016) [20]. Hence, the present study shows that the incidence of neuropathy by nerve conduction studies was significantly higher in metformin group than non-metformin exposed group and also there was a significant positive correlation between the cumulative dose of metformin and neuropathy by EMG NCV.

5. Conclusion

From the observations of present study, we concluded that the patients with type 2 DM on metformin therapy have lower serum vitamin B12 levels and a greater incidence of neuropathy by both Toronto clinical scoring system and nerve conduction studies as compared to non-metformin group. This decrease in the serum vitamin B12 levels and the worsening of clinical neuropathy is dependent on the dose and duration of metformin therapy. Toronto clinical score is simple, reproducible tool for clinical detection of peripheral neuropathy.

6. Strengths and Limitations

The study has many strong points which include- 1. Patients of all age groups ranging from 30 to 90 years with both short term and long term exposure to metformin (from 18 months to 15 years) were included in the study. 2. We did prior power calculations. 3. We performed neurological screening and examination of both symptomatic as well as asymptomatic patients in both the groups. 4. We not only screened for neuropathy by valid scale like Toronto clinical scoring system but also by nerve conduction studies and thus we could address the limitation of previous studies.

There are some limitations of the study which include- 1. It is a hospital based cross sectional study and could not be applied to the general population, 2. The study

period was limited we could not see the effects of vitamin B12 supplementation on the reversal on neuropathy in the metformin exposed group, 3. Larger sample size with therapeutic effect of vitamin B12 on the reversal of neuropathy in type 2 DM patients on metformin needs to be studied.

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