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Original Article

Effect of Vitamin B_{12} Supplement in Metformin Treated Diabetic Patients and it's Correlation to Peripheral Neuropathy

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ABSTRACT

Diabetes is an increasingly prevalent disorder with a range of systemic complications including diabetic peripheral neuropathy (DPN), which occurs in up to 50% of diabetic patients and causes sensory, motor, and/or autonomic dysfunction. Metformin is considered a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with type 2 diabetes. In addition, it is one of a few antihyperglycaemic agents associated with improvements in cardiovascular morbidity and mortality, which is a major cause of death in patients with type 2 diabetes. Metformin does, however, induce vitamin B_{12} malabsorption, which may increase the risk of developing vitamin B_{12} deficiency. Hence it is of interest to investigate interrelation between metformin consumption and peripheral neuropathy in the context of vitamin B_{12} administration.

The objectives of this research project are: 1. To assess the correlation between vitamin B_{12} level with nutritional status and metformin use (dose relation). 2. To find out the correlation between vitamin B_{12} deficiency and development of neuropathy in diabetic patients who are taking metformin. 3. To do an intervention in patients who's having vitamin B_{12} deficiency and analyze improvement of neuropathy status by B_{12} replacement oral versus parenteral (i.m).

The results of this research show that there is a significant correlation between vitamin B_{12} deficiency and dose of metformin. Also there is a significant association between vitamin B_{12} deficiency and diet of the patient. It also shows an important connection between vitamin B_{12} deficiency and status of neuropathy. It was observed that when there is severe vitamin B_{12} deficiency, the symptoms of peripheral neuropathy worsen. The supplementation of vitamin B_{12}, i.e. oral tablet and parenteral (i.m injection), results in a significant improvement of peripheral neuropathy symptoms.

Keywords: Diabetes, Vitamin B_{12} deficiency, Metformin and Peripheral Neuropathy.

1. INTRODUCTION

Diabetes mellitus (DM) is a disease associated with risk of cardiovascular diseases, which cannot be fully justified by important risk factors such as hyperglycemia, hypertension, and dyslipidemia [1].The incidence of diabetes in the world is showing an increasing trend with each passing day [2].

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is increasingly becoming a pandemic in developed and developing world [3].

T2DM is nowadays becoming a public health concern. The disease is associated with a variety of systemic macrovascular and microvascular complications. Diabetic peripheral neuropathy (DPN) is the most common complication, and it may eventually develop in up to 50% of patients [4], causing sensory, motor and/or autonomic dysfunction [5].

Metformin is the first-line treatment for patients with type 2 diabetes due to its low cost and low incidence of hypoglycemia. It has low rate of drug-drug interactions because of renal excretion and improvement of cardiovascular morbidity and mortality [6].

The major mechanisms of metformin action, as an insulin sensitizer, are the following: inhibiting hepatic glucose production, increasing peripheral tissues sensitivity (i.e., muscle and fat) to insulin, and thus, decreasing insulin secretion and also reducing the absorption of blood glucose in the intestine [7]. However, metformin causes reduction in vitamin B_{12} absorption leading to vitamin B_{12} deficiency which is a clinically important condition.

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin involved in the optimal functioning of the hemopoetic, neuro-cognitive and vascular systems. It is involved in DNA synthesis, fatty acid metabolism and energy production [8].

The B_{12}-intrinsic factor complex uptake by ileal cell membrane receptors is known to be calcium-dependent, and metformin affects calcium-dependent membrane action(9). The resulting B_{12} deficiency can be reversed by administering calcium, and this seems to be the clearest mechanism of action of Metformin [9].

Metformin has a good safety profile and limited side effects although early discontinuations due to gastro-intestinal intolerance occur in up to 20% of cases. Malabsorption of vitamin B_{12} under metformin treatment has been known for decades [10].

Vitamin B_{12} deficiency causes an increase in homocysteine level. This may negatively impact patient's health, as elevated homocysteine levels are associated with an increased risk of cardiovascular disease [11].

Also, Vitamin B_{12} plays a crucial role in the nervous system. It is a coenzyme for methyl malonyl-CoA mutase, the action of which is required for myelin synthesis [12]. Impaired myelin formation can lead to neuropathy, neuropsychiatric abnormalities, myelopathy, and optic nerve atrophy [12].

Clinical evidence of vitamin B_{12} deficiency-related neuropathy includes loss of vibratory sensation, diminished proprioception, and loss of cutaneous sensation in the lower limbs [13].

The National Health and Nutrition Examination Survey (NHANES) data reported that oral B_{12} supplementation reduced the rate of B_{12} deficiency by two-thirds in those without diabetes, but there was no association seen in those taking metformin [14].

The aim of this study is first to find out the relation between metformin dose, vitamin B_{12} deficiency and severity of peripheral neuropathy. It has also an objective to find out whether oral and injectable vitamin B_{12} supplementation are a possible methods to assess the efficacy of these supplements in reducing the symptoms of peripheral neuropathy.

2. MATERIALS AND METHODS

Subjects:

Total (200) type 2 diabetic patients with metformin therapy and symptoms of neuropathy of different sex and age had been enrolled in the study which was conducted at Poona Diabetes centre, East Street, Pune, India. Adults aged more than 30 years on treatment of oral metformin for at least 6 months and suffering from pain / subjective symptoms (subjective symptoms including pain, numbness, hyperesthesia, coldness in the extremities, muscular weakness, dizziness, and orthostatic fainting) were included in the study.

Exclusion criteria:

Exclusion criteria included following categories of patients:

1. Non diabetic peripheral neuropathy patients (alcoholic neuropathy, carpal tunnel syndrome, sequelae of cerebrovascular disease, etc).
2. Unstable glycemic control (HbA1C ≥ 10).
3. If the patients had severe hepatic or renal disorder, history of alcoholism, ongoing pregnancy and history of malabsorption.
4. If the patients were receiving other experimental medications for diabetic neuropathy or any other medication that affects symptoms of diabetic neuropathy like tetracycline antidepressant, SSRI etc.
5. If the patients were participating in other interventional studies.

Informed consent and ethical approval:

Ethical approval (ECR/354/INST/MH/2013/100/2013-2014) was obtained from Institutional Ethics Committee (IEC), Inamdar Multispeciality Hospital, Pune, India. The participants in the study were informed about the details of the study and written consents were obtained from each of them.

Observational phase:

After taking the written consent of each patient, the patients had undergone observational phase of the study. They were screened and divided into different groups according to different parameters.

Screening involved case history: demographic, health, diabetic and treatment history were recorded. Serum vitamin B_{12} level test for each patient was recorded so as to check whether the patient can be included in the study and also to keep those parameters as initial reading.

The study was designed according to vitamin B_{12} level of the patients. Patients were divided in to three groups: sufficiency or normal vitamin B_{12} level (where B_{12} level was ≥ 400 pg/ml), insufficiency vitamin B_{12} level (where B_{12} level was between 251-400 pg/ml) and deficiency or low vitamin B_{12} level (where B_{12} level was < 250 pg/ml).

After checking of serum vitamin B_{12} level of the patients, they were compared with different parameters:

- The patients were arranged according to their duration of diabetes. Patients were divided in to: patients who were diabetic for (≤ 60 months), patients who were diabetic for (61 – 120) months and patients who were diabetic for (>120) months.
- The patients were arranged according to their metformin treatment. Patients were divided as per their metformin dose in to: patients who were taking metformin (≤1000 mg/day), patients who were taking metformin between (1000 – 1500 mg/day) and patients who were taking metformin (>1500 mg/day).
- Duration of metformin was also included where patients should be on metformin treatment for not less than six months. Patients were divided according to their metformin duration in to: patients who were on metformin treatment for (≤12 months), patients who were on metformin treatment for (13 – 24) months and patients who were on metformin treatment for (25 – 36) months.
- The patients were categorized according to their diet status. Indian individuals were divided into vegetarians and non-vegetarians.
- Mean corpuscular volume and hemoglobin level were also included. Patients were divided into normal level and abnormal level.
- Toronto Clinical Scoring System (TCSS) and Neuropathy Total Symptoms Score-6 questionnaire (NTSS-6) for evaluation of the severity of peripheral neuropathy of each patient were recorded and kept as initial reading. These scoring systems gave information about the patient and decision made whether he/she neuropathic or non-neuropathic. Patients were classified according to their neuropathy status in to neuropathic patients and non-neuropathic patients.

Interventional phase:

After completion of the observational phase, second phase was started (i.e. the interventional phase). The interventional phase was including selection of patients who fit into the inclusion criteria i.e. these patients were checked for their serum vitamin B_{12} level. The patients who were B_{12} deficient (serum vitamin B_{12} is ≤250 pg/ml) followed supplement of vitamin B_{12} (methylcobalamin).

The treatment of vitamin B_{12} deficient patients was divided in two types of treatment:

1. Oral treatment where the patients took oral methylcobalamin tablet (1500 mg/day) for 180 days and
2. Parenteral treatment in that methylcobalamin injection (1000 umg/week I.M) for six months as follows: one injection per week for five weeks, one injection on alternative week for five weeks and then one injection per month for three months.

After completion of treatment duration, the patients were subjected for evaluation of neuropathy status through Toronto Clinical Scoring System (TCSS) and Neuropathy Total Symptoms Score-6 questionnaire (NTSS-6) so that the effect of this treatment on improvement of neuropathy was correlated.

Statistical analysis:

Data analysis was done by using SPSS (Statistical Package for Social Sciences) version 20:0. Qualitative data variable was expressed by using frequency and percentage (%). Chi-sequare year/ Fisher's exact test used to find the association of vitamin B_{12} level with various risk factors. P-value <0.05 was considered as significant.

3. RESULTS AND DISSCUSION

The study results give us an idea about the correlation between vitamin B_{12} level and various parameters in diabetic patients. Observations in table 1 indicate that there is a correlation between vitamin B_{12} level and duration of diabetes mellitus. It was found that there is no significant difference between vitamin B_{12} level and duration of the disease (p-value = 0.154) i.e. diabetes itself doesn't cause vitamin B_{12} deficiency, (see table1). However other studies have shown that vitamin B_{12} deficiency is more common among patients with type 2 diabetes mellitus [15-17]. One recent study confirmed that higher vitamin B_{12} deficiency with greater duration of diabetes was seen [18], which is not in conformity with our findings.

Table 2 presents the correlation between vitamin B_{12} level and metformin dose. Among 200 patients, 99 patients were taking metformin dose of ≤1000 mg/day where 55 patients were having normal vitamin B_{12} level and 24 patients were suffering from deficiency in vitamin B_{12} level. At higher dose of metformin (>1500 mg/day), out of 61 patients 19 patients only were having normal vitamin B_{12} level and 24 patients were suffering from deficiency in vitamin B_{12} level. It confirmed that there is a significant correlation between vitamin B_{12} level and dose of metformin (p-value = 0.014). Several studies confirmed that vitamin B_{12} deficiency in type 2 diabetes mellitus is related to long exposure to metformin [19-21].

Table 3 shows the correlation between vitamin B_{12} level and duration of metformin treatment. It was found that there is no significant correlation between vitamin B_{12} level and duration of metformin treatment (p-value = 0.768). However, it is found that: long-term follow-up data support the evidence that metformin is associated with vitamin B_{12}

deficiency, and routine measurement of vitamin B_{12} for metformin-treated individuals should be considered [22].

The study results show that most of the patients were taking metformin for less than one year i.e. 127 patients out of 200 were taking metformin for less than one year. The maximam duration for metformin treatment presented in our study is three years and it shows insignificant difference to cause vitamin B_{12} deficiency. It means that metformin treatment may require more than three years to cause deficiency in vitamin B_{12} level. (See table 3)

Correlation between vitamin B_{12} level and diet of the patients is presented in table 4. It was found that there is a significant difference between vegetarian and non-vegetarian patients. (p-value = 0.001). Among 56 vegetarian patients, there were 13 patients having deficiency in vitamin B_{12} level and out of 144 non-vegetarian patients there were 46 patients having deficiency in vitamin B_{12} level. Thus our observation indicates that non-vegetarian diabetic patients are more prone for vitamin B_{12} deficiency. Probably processing of non-vegetarian food might be destroying vitamin B_{12} content.

Some recent studies indicate that deficiency of vitamin B_{12} was more common among vegetarian population on prolonged metformin therapy than non-vegetarian population [23, 24], which is not in conformity with our findings.

Table 5 presents the correlation between vitamin B_{12} level and MCV (Mean corpuscular volume). It was found that there is no significant correlation between vitamin B_{12} level and MCV (p-value = 0.823).

The earlier studies show that there was no correlation between vitamin B_{12} levels and MCV [25, 26], which is in conformity with our findings.

Correlation between vitamin B_{12} level and hemoglobin level showed that there is no significant difference between vitamin B_{12} level and hemoglobin level (p-value = 0.288). These results are probably raised because 128 patients out of 200 patients were having normal hemoglobin level. (See table 6).

A recent review presented that increased incidence of anaemia among patients treated using metformin was noticed. However, vitamin B_{12} deficiency was also observed with lack of anemia [27], which is in conformity with our findings.

Table 7 shows the correlation between the scores of Toronto Clinical Scoring System (TCSS) at baseline i.e. initially before starting supplementation of vitamin B_{12} and after following up of patients for evaluation of the severity of neuropathy. Our results indicate that there is a significant difference (p-value <0.05) between the severity symptoms of neuropathy. Scoring showed that the result at baseline was (mean ± SD) 8.64+1.13 while after follow up and completion of six months of vitamin B_{12} treatment was 2.53+1.50. It indicates that vitamin B_{12} supplement reduces the severity of peripheral neuropathy in diabetic patients.

An earlier research paper presented that the patients with type 2 DM on metformin therapy had lower serum vitamin B_{12} levels and a greater incidence of neuropathy by Toronto clinical scoring system as compared to non-metformin group [28].

Table 8 shows results about the correlation between the scores of Neuropathy Total Symptoms Score-6 questionnaire (NTSS-6) at baseline i.e. initially before starting supplementation of vitamin B_{12} and after following up of patients for evaluation of the severity of neuropathy. Our results indicate that there is a significant difference (p-value <0.05) between the severity symptoms of neuropathy. Scoring showed that the result at baseline was (mean ± SD) 8.88+1.75 while after follow up and completion of six months of vitamin B_{12} treatment was 3.08+2.39. It indicates that vitamin B_{12} supplement reduces the severity of peripheral neuropathy in diabetic patients. (See figure 2).

One study confirmed that a statistically significant difference in clinical neuropathy scoring systems between the groups, with the metformin-exposed group having higher scores, indicating clinically more severe peripheral neuropathy [29].

NTSS-6 scores are also compared between capsule and injection for supplementation of vitamin B_{12}. The results presented that there is no significant difference (p-value = 0.988) between the two scores. It indicates that both capsule and injection of vitamin B_{12} supplement are responsible for effective reducing of symptoms of peripheral neuropathy. (See figure 3).

There are sublingual tablets and oral spray of vitamin B_{12} supplement which need to be investigated and may have better efficacy than oral tablet and injection as their destination are away from metformin pathway.

Table 1: Vitamin B_{12} count in correlation to duration of diabetes mellitus

Duration of DM	Vitamin B_{12} count			Total	p-value
	≤ 250	251 – 400	> 400		
≤ 60 month	23	12	38	73	0.154
61- 120 month	22	13	23	58	
> 120 month	14	18	37	69	
Total	59	43	98	200	

Table 2: Vitamin B_{12} count in correlation to metformin dose

Metformin dose	Vitamin B_{12} count			Total	p-value
	≤ 250	251 – 400	> 400		
≤ 1000	24	20	55	99	0.014
1000 – 1500	11	5	24	40	
> 1500	24	18	19	61	
Total	59	43	98	200	

Table 3: Vitamin B_{12} count in correlation to the duration of metformin

Duration of Metformin	Vit B_{12} group			Total	p-value
	≤ 250	251 – 400	> 400		
≤ 12 month	35	32	60	127	0.768
13 - 24 month	12	5	16	33	
25 - 36 month	5	2	7	14	
> 36 month	7	4	15	26	
Total	59	43	98	200	

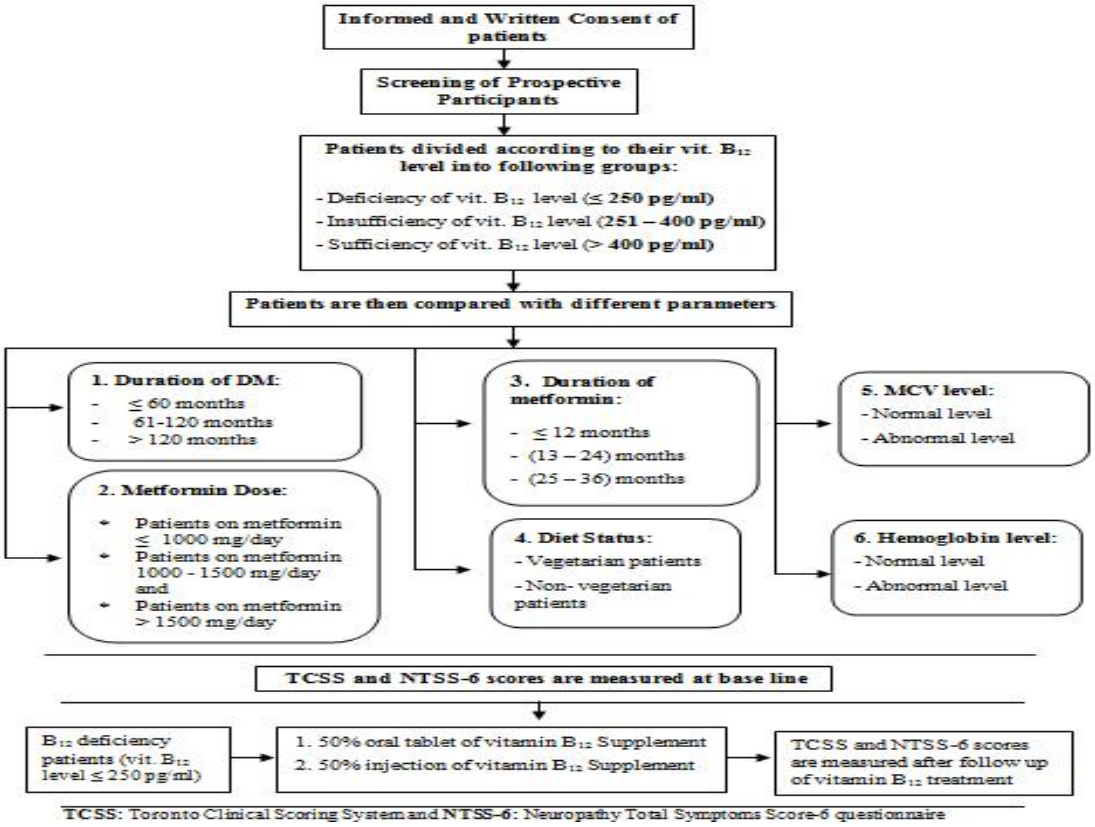

Fig 1: Flow chart for the proposal work

Table 4: Vitamin B_{12} count in correlation to diet of patient

Diet	Vitamin B_{12} count			Total	p-value
	≤ 250	251 – 400	> 400		
Vegetarian	13	4	39	56	< 0.001
Non Vegetarian	46	39	59	144	
Total	59	43	98	200	

Table 5: Vitamin B_{12} count in correlation to MCV (Mean corpuscular volume)

MCV group	Vit B_{12} group			Total	p-value
	≤ 250	251 - 400	> 400		
Normal	39	29	61	129	0.823
Abnormal	20	14	37	71	
Total	59	43	98	200	

Table 6: Vitamin B_{12} count in correlation to hemoglobin level

Hemoglobin group	Vit B_{12} group			Total	p-value
	≤ 250	251 - 400	> 400		
Normal	36	32	60	128	0.288
Abnormal	23	11	38	72	
Total	59	43	98	200	

Table 7: Correlation between TCSS scores at baseline and after follow up

Variables	TCSS Baseline (n=58)	TCSS At follow up (n=58)
Min	6	1
Max	11	6
Mean	8.64	2.53
SD	1.13	1.50
Median	9	2

Table 8: Correlation between NTSS-6 scores at baseline and after follow up

NTSS 6 score	At Baseline (n=29)	At Follow up (n=29)	p-value
Mean	8.88	3.08	< 0.001
SD	1.75	2.39	
Median	8.99	3.33	

Table 9: Correlation of NTSS-6 scores in capsule and injection treatment

NTSS Score	Capsule (n=16)	Injection (n=13)	p-value
Mean	3.06	3.10	0.966
SD	2.24	2.66	

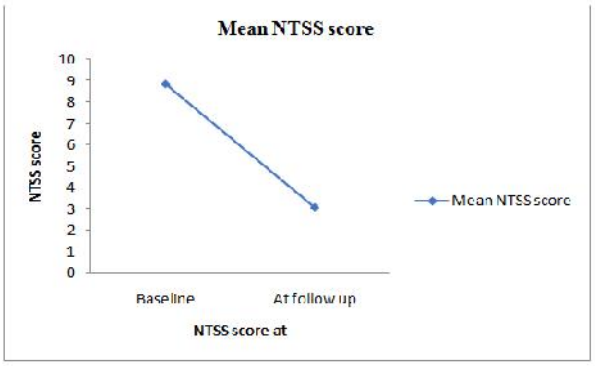

Fig 2: Mean of NTSS-6 score at baseline and after follow up

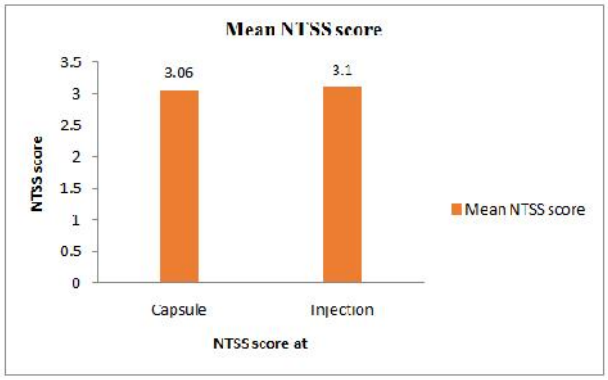

Fig 3: Mean of NTSS-6 score in capsule and injection treatment

4. CONCLUSION

This study indicates that diabetic patients on metformin therapy are prone for vitamin B_{12} deficiency. Probably vitamin B_{12} deficiency is related to metformin and not to the disease itself.

Vitamin B_{12} deficiency can cause elevation of symptoms of peripheral neuropathy, this study shows that supplementation of either tablet or injection of cobalamin is an effective method to reduce peripheral neuropathy symptoms in diabetic patients on metformin therapy.

Thus our study indicates that metformin induced vitamin B_{12} deficiency and peripheral neuropathy can be corrected by oral / parenteral of cobalamin (vitamin B_{12}).

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