International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Is vitamin B_{12} deficiency responsible for neuropathy in people with type 2 diabetes on metformin?

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Abstract

Aims: Metformin is the first line monotherapy for achieving glycemic control in type 2 diabetes mellitus. However, metformin induces vitamin B12 malabsorption, which may increase the risk of developing vitamin B12 deficiency. The present study, therefore, aims at identifying the decrease in vitamin B12 levels in diabetics on metformin therapy and its effect on nerve conduction.
Methods: Sixty subjects, including 30 type 2 diabetics and 30 without diabetes were enrolled into the study and their nerve conduction parameters were assessed along with the evaluation of their serum vitamin B12 levels.
Results: Vitamin B12 levels were found to be deficient in diabetics on metformin as compared to age-matched, healthy controls ($p<0.05$). Nerve conduction latency was increased and conduction velocity decreased in diabetics in contrast to the controls ($p<0.05$). The decrease in vitamin B12 levels showed a significant negative correlation with the dose ($r = -0.7$, $p<0.05$) and duration ($r = -0.5$, $p<0.05$) of metformin exposure, whereas, the dose and duration of metformin therapy had no association with the nerve conduction parameters ($p>0.1$).
Conclusions: Although the vitamin B12 levels were deficient and nerve conduction parameters worsened in people with diabetes on metformin, but the worsened neuropathy cannot be attributed to the deficient vitamin B12 levels.
Keywords: Metformin, Vitamin B12 levels, Nerve conduction latency, Nerve conduction velocity, Neuropathy.

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***Article History:**
Received: 19/07/2019
Revised: 18/08/2019
Accepted: 18/08/2019
DOI: https://doi.org/10.7439/ijbr.v10i8.5233

QR Code

How to cite: Khattar D, Khaliq F. and Neelam Vaney. Is vitamin B12 deficiency responsible for neuropathy in people with type 2 diabetes on metformin? *International Journal of Biomedical Research* 2019; 10(08): e5233. DOI: 10.7439/ijbr.v10i8.5233
Available from: https://ssjournals.com/index.php/ijbr/article/view/5233

1. Introduction

The incidence of diabetes in India is showing an increasing trend.[1] As diabetes has become an increasingly prevalent disorder, morbidity due to its wide range of systemic complications is also on a rise. Diabetic peripheral neuropathy occurs in about 50% of people with diabetes and causes sensory, motor or sensory-motor dysfunction.[2] Several pathogenic mechanisms attribute to the severity of this neuropathy and these include – microangiopathy, oxidative stress, mitochondrial dysfunction, insulin deficiency and advanced glycation end products.[3-6] However, Metformin induced vitamin B12 deficiency is a recently talked about factor that adds on to the burden of neuropathy that may already exist in people with diabetes.[7]

Metformin, which is a biguanide is the first line monotherapy, for achieving glycemic control in type 2 diabetes, because of its efficacy, known side-effect profile, and relatively low cost.[8] Metformin activates adenosine monophosphate dependent protein kinase (AMPK) and enters cells through organic cation transporters, thereby acting to reduce plasma glucose mainly by suppressing hepatic gluconeogenesis.[9] However, the present study discusses one of its disadvantages i.e. the vitamin B12 deficiency induced by it.[10]

Whether this vitamin B12 deficiency induced by Metformin is responsible for neuropathy in people with diabetes is yet not clear. According to Bell [11], vitamin B12 deficiency in people with diabetes on Metformin usually presents with peripheral neuropathy which may be misdiagnosed for diabetic neuropathy, thereby resulting in progression of the central and/or peripheral neuronal damage which can otherwise be controlled with vitamin B12 replacement. On the other hand, Marar *et al* [12], observed that although the vitamin B12 levels, of people with diabetes taking Metformin, were significantly reduced

as compared to the controls, but this vitamin B12 deficiency did not correlate with degree of neuropathy in these people, which may be otherwise present in them. Therefore, the neuropathy occurring in people with diabetes on Metformin may not be solely due to vitamin B12 deficiency. Literature holds ambiguity that Metformin use is associated with Vitamin B12 deficiency and also talks about the increasing incidence of neuropathy in Type II diabetics.

Stimulus related potentials (SRPs) are records of the changes in electrical potentials in the nervous system in response to an adequate external stimulus. They reflect the functional integrity of the anatomical sensory/motor pathways in the brain or spinal cord or at periphery.[13] In most neurological studies, SRP's are considered to be a useful adjunct. However these have not been studied adequately in vitamin B-12 deficiency induced neuropathy. We had earlier evaluated cognition and functional integrity of the auditory pathway by evoked potential studies in patients with diabetes taking metformin.[14.15] It was concluded that cognitive impairment and deficient auditory conduction observed in these patients, cannot be attributed to the vitamin B12 deficiency. As an extension to our earlier work we now propose to objectively evaluate the neural involvement in this group by means of electrophysiological investigations. The results of the present study would guide the management of neuropathy in Type II diabetics thereby contributing a new outlook to the current management of diabetic neuropathy.

The present study therefore aims at finding whether vitamin B12 deficiency results from Metformin use in people with diabetes and if yes, whether this vitamin B12 deficiency is responsible for neuropathy indicated by electrodiagnostic parameters in people with diabetes taking Metformin.

2. Material and methods:

2.1 Setting:

The study was carried out in the Electrophysiological Lab, Department of Physiology, UCMS & GTB Hospital, Delhi. The patients were recruited from the Diabetic Clinic, Department of Endocrinology & Metabolism, UCMS & GTB Hospital, Delhi. The control group subjects were randomly chosen from the hospital staff and general community. Both, the people with diabetes and the control group subjects were age matched. A written informed consent was obtained from all the participants, for nerve conduction recordings and sample collection, prior to enrolment into the study. Ethical clearance was obtained from the institutional ethical committee.

2.2 Type of study: Case - control (Pilot) study.

2.3 Subjects:

- Study group: 30 type 2 diabetes mellitus patients taking a minimum dose of 1g/day of Metformin for a period of at least 6 months.
- Control group: 30 normal healthy subjects of the same age group.

Subjects with history of head injury, epilepsy, migraine, drug abuse, malabsorption, type 1 diabetes, any other metabolic disorders or neurological abnormality were excluded from the study. Subjects on vitamin B12 supplementation, subjects taking Metformin for diseases other than diabetes, subjects taking oral hypoglycemics other than Metformin or in combination with Metformin were also excluded from the study.

Relevant history and examination to rule out exclusion criteria and to look for the presence of abnormality or disease if any was done. Height and weight of all subjects was measured and BMI calculated.

2.4 Nerve conduction evaluation:

The recordings were done using Octopus 4 M/C NCV/EMG/EP system by Biostar healthcare, India. Recordings were done in the Median nerve in the upper limb and Tibial & Sural nerves in the lower limb as follows:

Median nerve: Sensory conduction velocity of median nerve was measured by orthodromic stimulation. The recording electrode for orthodromic conduction was placed 3cm proximal to the distal wrist crease and reference was placed 3cm proximally, both between the flexor carpi radialis and palmaris longus tendons. Stimulus was applied at the base of the second digit. For median motor nerve conduction study (NCS), the recording electrode was placed close to the motor point of abductor pollicis brevis (APB) and reference electrode 3cm distal at first metacarpophalangeal joint. A supramaximal stimulation was given at wrist (3cm proximal to the distal wrist crease) and at elbow (near the volar crease of the brachial pulse).

Tibial nerve: For tibial nerve conduction, the active surface recording electrode was placed on abductor hallucis longus (AHL). Surface stimulation was used behind and proximal to the medial malleolus and in the popliteal fossa along the flexor crease of the knee, slightly lateral to midline in popliteal fossa.

Sural nerve: The surface electrode was placed between the lateral malleolus and Tendo Achilles and the nerve was stimulated antidromically 10-16cm proximal to the recording electrode, distal to the lower border of gastrocnemius at the junction of middle and lower third of leg. During the recording, person lied prone with the feet hanging from the table and the legs relaxed.

For all the recordings, stimulus was always applied by keeping the cathode towards the recording electrode. While evaluating the nerve conduction, conduction velocity was calculated by measuring the distance between the point of stimulus and the recording electrode or between the two points of stimulus, if two stimuli had been applied, using a measuring tape and dividing it by the latency of the curve obtained in response to the stimulus.

2.5 Serum Vitamin B12 levels:

Blood (5ml) was collected in plain vials. The samples were allowed to clot for two hours at room temperature. The samples were then centrifuged at approximately 3000 rpm for 30 minutes. Serum was then used for Vitamin B12 assay which was based on the principle of ELISA.

2.6 Statistical Analysis:

Analysis was done using SPSS – 20 statistical package. The two groups were compared by independent 't' test. Data has been presented as mean ± SD. P value <0.05 has been considered as significant. The vitamin B12 levels were correlated with different parameters of nerve conduction using Spearman's rho correlation coefficient. Also vitamin B12 levels were correlated with the dose and duration of Metformin use.

3. Results

The anthropometric parameters were comparable in the two groups. The mean glycosylated hemoglobin (HbA1c) value in the diabetics was 7.14±1.3% and the mean duration of diabetes was 4.15±2.5 years. The mean dose of Metformin being taken by the diabetics in the study was 1.4 g/day and the mean duration for which it had been taken was 4 years.

Median nerve motor & sensory latency was increased and sensory conduction velocity was decreased significantly in the study group as compared to controls. Tibial motor nerve conduction latency was significantly increased and conduction velocity decreased in the study group as compared to controls. Similarly, sural sensory nerve conduction latency was significantly increased and conduction velocity decreased in the study group as compared to controls ($p<0.05$).

Table 1: Nerve conduction parameters and Vitamin B12 levels in subjects and controls

Nerves			Controls	Study Group	p- value
Median nerve	Motor	Latency	3.5 ± 0.96	4.4 ± 1.6	.015*
		Conduction velocity	49.5 ± 12.7	46.2 ± 10.9	0.279
	Sensory	Latency	1.7 ± 0.3	2 ± 0.5	.007*
		Conduction velocity	48.7 ± 8.3	41.7 ± 10.5	.005*
Tibial Nerve	Motor	Latency	5.7 ± 1.4	7.1 ± 2.2	.004*
		Conduction velocity	47.3 ± 20.8	26.4 ± 13.9	.00*
Sural Nerve	Sensory	Latency	2.9 ± 1.3	5.5 ± 2.7	.00*
		Conduction velocity	48.6 ± 12.6	27.1 ± 11.1	.00*
Serum Vit. B12 Levels			274.5 ± 64	143 ± 42.8	.00*

***P < 0.05**

- Median motor nerve latency significantly delayed in study group as compared to controls
- Median sensory nerve latency significantly delayed and conduction velocity reduced in study group subjects as compared to controls.
- Tibial nerve latency significantly delayed and conduction velocity reduced in study group subjects as compared to controls.
- Sural nerve latency delayed and conduction velocity reduced in study group subjects as compared to controls.
- Serum Vitamin B12 levels significantly reduced in study group subjects as compared to controls.

The study group subjects were divided into two groups based on the duration of Metformin intake. It was observed that serum Vitamin B12 levels decreased in subjects taking Metformin for 5 or more years. Whereas, latencies & conduction velocities of median, tibial and sural nerve showed no significant difference between the two groups.

Table 2: Nerve conduction parameters and Vitamin B12 levels amongst people with diabetes taking metformin for < 5 years and those taking it for ≥ 5 years.

Nerve			Metformin intake for < 5 years	Metformin intake for ≥ 5 years	p - value
Median nerve	Motor	Latency	4.5 ± 1.9	4.4 ± 0.9	0.885
		Conduction velocity	47 ± 11.2	44.7 ± 10.6	0.593
	Sensory	Latency	2 ± 0.5	2 ± 0.5	0.656
		Conduction velocity	41.5 ± 9.8	42.1 ± 12.2	0.882
Tibial Nerve	Motor	Latency	7.6 ± 2.3	7 ± 1.3	0.46
		Conduction velocity	27.9 ± 15.4	23.3 ± 9.2	0.41
Sural Nerve	Sensory	Latency	5.5 ± 2.8	5.5 ± 2.7	0.996
		Conduction velocity	26.9 ± 12.1	27.5 ± 9.3	0.891
Serum Vit. B12 Levels			163.5 ± 36.9	101 ± 13.7	.00*

***P < 0.05**

- Serum Vitamin B12 levels present a significant decline as the duration of Metformin intake increased.
- Nerve conduction parameters did not vary significantly with increasing duration of Metformin.

The cases were divided into two groups based on the dose of Metformin that they were taking. On comparing these two groups, the mean difference in serum Vitamin B12 levels decreased in subjects taking ≥ 1500 mg of Metformin per day, while no significant results were obtained when median motor, median sensory, tibial and sural nerves were compared amongst the two groups.

Table 3: Nerve conduction parameters and Vitamin B12 levels amongst people with diabetes based on the dose of metformin

Nerve			Dose of metformin < 1500 mg	Dose of metformin ≥ 1500 mg	p - value
Median Nerve	Motor	Latency	5.1 ± 2.6	4.1 ± 1	0.127
		Conduction velocity	50 ± 8	44.6 ± 11.7	0.213
	Sensory	Latency	1.9 ± 0.3	2 ± 0.5	0.448
		Conduction velocity	41.9 ± 8.4	41.6 ± 11.4	0.949
Tibial Nerve	Motor	Latency	8.2 ± 2.2	6.6 ± 2	0.074
		Conduction velocity	20.5 ± 11.9	28.9 ± 14.1	0.131
Sural Nerve	Sensory	Latency	4.8 ± 2	5.8 ± 3	0.327
		Conduction velocity	27.6 ± 8.4	27 ± 12.3	0.899
Serum Vit.B12 levels			183.3 ± 38.4	118 ± 20.4	.00*

***P < 0.05**

- Serum Vitamin B12 levels present a significant decline as the dose of Metformin intake increased.
- Nerve conduction parameters did not vary significantly with increasing dose of Metformin.

Vitamin B12 levels were found to have a significant negative correlation with the dose of Metformin ($r = -0.7$, $p<0.05$) and also a significant negative correlation with the duration of Metformin intake ($r = -0.5$, $p<0.05$). However, no significant correlation was found between the vitamin B12 levels and the nerve conduction parameters ($p>0.1$).

Figure 1: Showing representative sensory nerve conduction waves in controls (a) and study group (b).

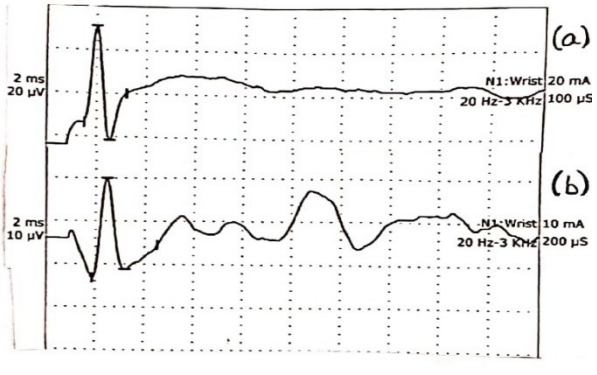

Figure shows increased latency and decreased conduction velocity in the study group (b) as compared to the controls (a).

4. Discussion:

The present study observed that the serum vitamin B12 levels were found to be significantly lesser in cases as compared to the controls. Vitamin B12 forms a complex with cubulin (endocytic) receptor which is taken by the ileum for absorption and this absorption is a 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 vitamin B12.[16] This mechanism has been made evident by the fact that Metformin-induced vitamin B12 deficiency can be treated with calcium supplementation.[16,17,18] It has also been proposed to act by increasing bacterial overgrowth, altering bowel motility, and by direct inhibition of vitamin B12 absorption.[17]

Bell (2010),[11] in his case report strongly presents his view, that Metformin results in malabsorption of vitamin B12 at the terminal ileum and thereby, results in vitamin B12 deficiency and vitamin B12 deficiency is responsible for neuropathy in his patient. Some other studies also observed similar results.[7,19] They demonstrated that patients with type 2 diabetes who were treated with Metformin for > 6 months had lower vitamin B12 levels and that vitamin B12 levels had a significant correlation with the dose of Metformin. They also found that Metformin treated group had a more severe neuropathy as compared to controls as indicated by the higher NIS (Neuropathy Impairment Score) and TCSS (Toronto Clinical Scoring System). However, they were unable to demonstrate significant group differences on electrophysiological parameters.[7]

The present study also showed that vitamin B12 levels were lower in people with diabetes treated with Metformin as compared to the controls and deficiency gets worsened as the dose and duration of Metformin treatment increases while both the dose and duration of Metformin treatment did not have any impact on nerve conduction

parameters. Meaning thereby, that although the neuropathy was worse in Metformin treated group, but this worsening cannot be attributed to the deficient vitamin B12 levels.

Likewise, Marar *et al* (2011)[12] and some other works also demonstrated similar results.[20,21] The presence of vitamin B12 deficiency was more in the Metformin exposed group as compared to the non-Metformin exposed group and a significant inverse relationship existed between the vitamin B12 levels and the dose and duration of Metformin.[14,15] Despite the significantly increased prevalence of vitamin B12 deficiency among the Metformin group in comparison to controls, it was not associated with neuropathy. Therefore, this study does not support that vitamin B12 levels are responsible for neuropathy in people with diabetes treated with Metformin.[12]

Our study also showed a significant increase in latency and a significant reduction in conduction velocity of median sensory, tibial and sural nerve conduction in cases when they were compared to the controls. While, considering the median motor nerve conduction, the latency increased significantly, whereas, the conduction velocity decreased, although not significantly, in cases, when compared with controls.

Prolonged motor latencies and slowed conduction velocities are indicative of demyelination. Precisely, in demyelination, with loss of myelin thickness, nerve conduction is slowed, and, if severe enough, saltatory conduction fails (conduction block).[22] Since, the present study shows lengthened motor latencies and decreased tibial motor conduction velocity, therefore, we can say that metformin is producing demyelinating type of neuropathy. However, with increasing motor axon loss some of the largest fastest conducting fibers will be lost. Therefore, distal motor latency will be slightly prolonged (<120% of normal limit) and conduction velocity slightly slowed (>80% of normal limit).[22] If the results of the present study are considered, prolongation of latency and decreased conduction velocity can be attributed to demyelinating type of neuropathy. However, there is no clear evidence to differentiate between the two.

In symptomatic diabetic neuropathy, there is slowing of nerve conduction velocity owing to demyelination and loss of large myelinated fibers, and a decrease in nerve action potentials owing to loss of axons.[23] Purely demyelinating neuropathy is rare in patients with diabetes,[24] so it is a combination of demyelination and axonal loss. It may be so that the increased incidence of neuropathy that we find in Metformin exposed group is the neuropathy which commonly exists in type 2 diabetes.[12]

Chronic hyperglycemia in diabetes mellitus is responsible for tissue injury including injury to peripheral nerves, thereby resulting in peripheral neuropathy which is abnormal function of peripheral nerves. The mechanisms that cause tissue injury in diabetes probably involve multiple effects of high glucose concentration and other metabolic abnormalities on proteins of endothelial and vascular smooth muscle cells. The resultant impairment in blood supply to tissues results in damage. To worsen the condition, hypertension, secondary to renal injury and atherosclerosis, secondary to abnormal lipid metabolism develops in patients with diabetes and amplify the tissue damage caused by elevated glucose levels. Peripheral neuropathy is a microvascular complication of diabetes and it is a result of chronic hyperglycemia. It correlates with fasting and postprandial glucose levels as well as with haemoglobin A_{1C} (HbA_{1C}). [25]

5. Limitations

Ideally, if the diabetics on Metformin were compared with diabetics not taking Metformin rather than normal subjects, it would have answered the research question better. However, since in our clinical set-up, every person diagnosed with diabetes is started with Metformin as the first line treatment and even those taking combination therapy have Metformin included in their treatment, so, taking this group was not possible for us.

6. Conclusion

Vitamin B12 levels were lower in people with diabetes treated with Metformin as compared to the controls and this deficiency gets worsened as the dose and duration of Metformin treatment increases. Neuropathy as indicated by nerve conduction parameters was worse in Metformin treated group, but this worsening cannot be attributed to the deficient vitamin B12 levels.

Declaration of interest: none.

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