Nerve Conduction Studies in Type-II Diabetic Mellitus with and without Metformin Therapy & its Association with Vitamin B12

Suman Pokhrel[1], Dilip Thakur[2], Nirmala Limbu[2], Robin Maskey[3], Binod Kumar Lal Das[4]

[1]*Department of Nutrition and Dietetics, Central Campus of Technology, Hattisar, Dharan, Nepal*

[2]*Department of Basic and Clinical Physiology, BP Koirala Institute of Health Sciences, Dharan, Nepal*

[3]*Department of Internal Medicine, BP Koirala Institute of Health Sciences, Dharan, Nepal*

[4]*Department of Biochemistry, BP Koirala Institute of Health Sciences, Dharan, Nepal*

CORRESPONDENCE

Mr. Suman Pokhrel
Teaching Assistant, Department of Nutrition and Dietetics
Central Campus of Technology, Hattisar, Dharan
Email: pokhrelisuman@gmail.com
Orchid ID: https://orcid.org/0000-0003-9812

ARTICLE INFO
Article History
Submitted: 10 December, 2023
Accepted: 10 January, 2024
Published: 8 February, 2024

Source of support: None
Conflict of Interest: None

ABSTRACT

Introduction: Type II Diabetes Mellitus (T2DM) is a heterogeneous group of metabolic disorders characterized by insulin resistance & and impaired insulin secretion. Metformin is a first line treatment oral hypoglycemic agent for patients with T2DM. Conversely, it has been found that the use of metformin is associated with malabsorption of vitamin B12, which may lead to more detrimental effects on peripheral nerves.

Methods: A comparative cross-sectional study was conducted enrolling type II diabetic patients (Group A) with metformin therapy for more than 6 months (n=30), type II diabetics (Group B) without metformin exposure (n=11) and healthy controls (n=30). Nerve conduction study parameters of median, tibial, common peroneal & and sural nerves, serum glucose and serum vitamin B12 levels were measured. A way ANOVA (post hoc: Tukey) test was used to compare the variables using SPSS. 22.0.

Results: T2DM with metformin therapy showed significantly longer latencies and lower amplitudes of both sensory and motor nerves when compared to healthy controls and T2DM without metformin therapy. NCS parameters showed more deleterious effects on the median, tibial and sural nerves of diabetic patients with metformin therapy. Diabetics undergoing metformin treatment had reduced vitamin B12 levels as compared to those without metformin therapy [194.03 (164.86-223.53) vs. 297.82 (258.99-363.00), p=0.001] and healthy controls [194.03 (164.86-223.53) vs. 287.50 (204.25-351.50), p=0.001]. Serum vitamin B12 level showed a strong negative correlation (significant at p<0.01 level) with duration of metformin exposure/ treatment in metformin-exposed diabetics.

Conclusion: Long-term metformin therapy in diabetic patients is associated with significant vitamin B12 depletion, leading to alteration in motor and sensory NCS parameters. Thus, we recommend regular vitamin B12 screening and oral/ parenteral vitamin B12 supplementation to diabetic patients on metformin therapy.

Keywords: Diabetes Milletus; Metformin; Vitamin B12.

INTRODUCTION

Diabetes mellitus is a syndrome caused by a relative or an absolute lack of insulin, the leading outcome of which is peripheral neuropathy presented as a chronic and diffuse distal symmetric polyneuropathy (DSPN) with or without autonomic dysfunction.[1] Type 2 diabetes mellitus results from insulin resistance, usually with relative insulin deficiency or insulin secretory defect with insulin resistance. Usually, type II diabetes mellitus is treated initially by lifestyle modifications (dietary pattern, physical exercise) and medication (oral anti-diabetic agents). Metformin is the most widely used oral first-line treatment drug due to its low cost, low incidence of hypoglycemia, and low rate of drug-drug interactions. Conversely, it has been found that continuous use of metformin is associated with the deficiency of Vitamin B12 aka. Cobalamin. Many studies had estimated the rate of prevalence of metformin-induced vitamin B12 deficiency to be around 10-20% of treated patients[2], while others have reported level as low as 5% and even higher than 30%.[3,4] As metformin interferes the absorption of cobalamin, the latter can lead to progressive deterioration of nerve tissue and is frequently misdiagnosed as diabetic

peripheral neuropathy.[5] Long-term (more than 6 months) metformin therapy has been hypothesized to cause vitamin B12 deficiency and aggravate peripheral neuropathy in type 2 diabetes patients. Therefore, we aimed to investigate the sensory and motor nerve conduction variables of peripheral nerves in type 2 diabetes patients with and without metformin exposure.

METHODS

Hospital based comparative cross-sectional study was carried out in department of basic and clinical physiology in collaboration with department of biochemistry at B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. Type 2 diabetes mellitus patients, of either sex and aged more than 15 years, diagnosed as per American Diabetes Association (ADA) 2015 guidelines were included in the study. The study consisted of 60 subjects out of which, Group A consisted of 30 asymptomatic type 2 diabetics undergoing metformin treatment for more than 6 months, Group B consisted of 30 asymptomatic type 2 diabetics who never took metformin but other medications. Exclusion criteria included patients with clinically diagnosed systemic diseases like hypertension, type I diabetes, neuropathic diseases, history suggestive of malabsorption disorders, gastrectomy, chronic smoking habits, alcoholism and under any medication beside metformin that alters the nerve conduction study and vitamin B12 level.

A detailed history (duration of diabetes, types & duration of oral hypoglycemic drug intake) and clinical examination were performed in all the recruited patients. Anthropometric and cardio-respiratory variables were measured using standard techniques. Nerve conduction study (NCS) variables were recorded using digital Nihon Kohden machine (Japan) at a room temperature of 24±2ºC. Variables of compound muscle action potential (CMAP) of bilateral median, tibial and common peroneal nerves were recorded. Similarly, sensory nerve action potential (SNAP) of median and sural nerves were measured. The serum blood glucose level was estimated using an automated enzymatic method (Cobas, Roche Diagnostics) and serum vitamin B12 levels were measured by using chemiluminescent enzyme immunoassay (MAGLUMI 1000 CLIA system). The serum vitamin B12 level <200 pg/ml was considered as vitamin B12 deficiency.

The statistical analysis was done using statistical package for social sciences (SPSS) 22.0 version. One-way ANOVA was applied to compare the parametric variables. Mann-Whitney U test was used to compare the non-parametric variables between groups. Spearman's correlation test was used to correlate the NCS variables and serum vitamin B12 levels. The data were expressed as mean ± standard deviation (SD) for parametric variables and as median (Interquartile range) for non-parametric variables. P value less than 0.05 ($p<0.05$) was considered statistically significant

RESULT

Baseline anthropometric (age, weight, height, BMI, upper/lower limb length) and cardiorespiratory variables (systolic & diastolic blood pressure, respiratory rate, pulse rate) were comparable between the groups. The duration of diabetes mellitus after its diagnosis was also found insignificant between metformin exposed diabetics [3.50 (2.75 – 7.00)] and metformin unexposed diabetics [3.00 (3.00 - 7.00)]

1. Nerve conduction study variables:

1.1 Median motor nerve conduction study variables

Nerve conduction velocity of right median nerve was significantly low in metformin exposed diabetics. Likewise, proximal onset latency of both right and left median nerves were significantly prolonged in Group A. F-waves minimum latency of left median nerve was significantly longer in T2DM with metformin therapy than those without metformin while distal latencies, distal & proximal amplitudes, F-waves maximum and F-waves minimum of bilateral median nerves were found insignificant.

1.2 Tibial motor nerve conduction study variables

The right tibial motor nerve distal latency, proximal latency and F-waves minimum latency were significantly longer in diabetics with metformin therapy. Similarly, right tibial nerve conduction velocity was significantly low in diabetics with metformin therapy than those without metformin therapy. Regarding left tibial nerve, proximal amplitude and nerve conduction velocity were found significantly lower in diabetics with metformin therapy. Remaining NCS variables were comparable between both groups. Nerve conduction study variables of both right and left tibial motor nerves are presented in Table 1.

1.3 Common peroneal motor nerve conduction study variables

All the NCS variables for common peroneal motor nerves were found to be insignificant between Group A and Group B.

Table 1: Comparision of tibial motor nerve conduction study variables

	Right Tibial Nerve Mean ± SD			Left Tibial Nerve Mean ± SD		
Motor NCS Variables	**Group A**	**Group B**	**p value**	**Group A**	**Group B**	**p value**
Distal latency	3.77±0.88	3.08 ±0.34	0.020*	3.33±0.63	3.70±0.95	NS
Proximal latency	12.67±1.61	10.47±2.00	0.001*	12.35±1.58	11.30±1.62	NS
Proximal amplitude	6.74±2.74	8.22±2.48	NS	6.86±3.20	9.18±2.00	0.046*
Proximal amplitude	38.02±6.49	48.08±15.74	0.024*	37.27±5.38	44.64±6.81	0.012*
Conduction velocity (m/s)	38.02±6.49	48.08±15.74	0.024*	37.27±5.38	44.64±6.81	0.012*

1.4 Sensory nerve conduction study variables

The onset latency was significantly longer in bilateral median sensory nerves in type 2 diabetics with metformin therapy as compared to diabetics without metformin therapy. Similarly, amplitude and nerve conduction velocity of right sensory median nerve were significantly low in metformin exposed diabetics. Regarding the sural nerves, amplitudes of both right and left sural nerves were significantly lower in type 2 diabetics with metformin therapy as shown in table 2.

Serum Glucose Level and Serum vitamin B12 level

Fasting and postprandial serum glucose level were comparable between the diabetics with metformin therapy and diabetics without metformin therapy. Serum vitamin B12 level was significantly lower in type 2 diabetics with metformin therapy in comparison to diabetics without metformin therapy (table 3).

Table 2 : Comparision of median sensory nerve conduction study variables

	Right Median Nerve Mean ± SD			Left Median Nerve Mean ± SD		
Sensory NCS Variables	**Group A**	**Group B**	**p value**	**Group A**	**Group B**	**p value**
Onset latency	2.23 ±0.34	1.94±0.14	0.019*	2.27 ±0.36	1.99±0.22	0.034*
Amplitude	17.01±8.16	27.11±10.46	0.018*	20.01 ±12.00	27.78±10.33	NS
Conduction velocity	45.80±7.03	51.61±3.90	0.030*	45.10 ±6.90	50.85±6.06	NS
	Right Sural Nerve			**Left Sural Nerve**		
Onset Latency	2.62 ±0.33	2.47±0.37	NS	2.71 ±0.61	2.53 ±0.40	NS
Amplitude	10.83 ±5.71	16.72±5.26	0.001*	9.40 ±4.00	21.59 ±9.45	0.001*
Conduction velocity	38.64±4.61	41.28±6.19	NS	38.73 ±8.70	40.32 ±5.95	NS

Table 3: Comparison of serum vitamin B12 levels among type 2 diabetics with metformin therapy & type 2 diabetics without metformin therapy.

Variables	Median (Interquartile range)		p value
	Group A	Group B	
Serum Vitamin B12 Level	194.03 (164.86- 223.53)	297.82 (258.99 – 363.00)	0.001*

*** Mann- Whitney test was applied.**

DISCUSSION

No significant differences in any anthropometric and cardio-respiratory findings was encountered between the two groups which reduced the possibility of acting it as a confounder. Fasting & postprandial serum glucose level and also duration of diabetes after diagnosis were insignificant between the groups, these assets strengthen our objectives allowing relevant comparison to be made between outcome variables of both targeted groups.

Our study showed significant decrease in the serum vitamin B12 level in metformin exposed diabetics. Biochemical vitamin B12 deficiency was present in 18 (60%) of subjects with diabetes under metformin therapy. The result was in accordance with the similar studies.[3,4,7,8] Metformin interferes and impairs calcium-dependent membrane activity in the ileum leading to vitamin B12 malabsorption. Metformin along with its protonated biguanide group binds to the B12-cubulin complex and imparts positive charge which alters membrane potential and competitively repels the divalent calcium ions thus preventing calcium dependent uptake, leading to vitamin B12 malabsorption.[9,10] This mechanism supports the underlying cause behind the deficiency of serum vitamin B12 level in diabetics with metformin therapy. Reduced serum B12 concentration (<200pg/ml) within 6-8 months of metformin exposure in diabetic subjects was found in our study as was shown by study done by Bauman et al.[9] Our study showed high negative correlation between duration of metformin treatment in diabetics and serum vitamin B12 level which was in accordance with the result of many studies.[7,8,11,12]

In our study, motor NCS showed significant longer proximal latency and lower conduction velocity of bilateral median nerve in diabetics with metformin therapy. The significant reduction in vitamin B12 level in diabetic subjects with metformin therapy can be attributed to changes in normal myelin function which finally leads to differences in latencies and conduction velocities altering the propagation of action potential.[9,13,14] We did not come across any published study involving electrophysiological evaluation of motor nerves in asymptomatic individuals with and without metformin exposure. It was evident from other studies involving symptomatic subjects that the features of both axonal as well as demyelinating sensory-motor neuropathy were seen in nerve conduction studies of vitamin B12 deficient subjects.[15,16,17,18] It is thereby clear that ongoing alteration in myelin due to long term use of metformin, associated with depletion of vitamin B12 leads to neuropathy. In the early period, despite of vitamin B12 depletion and alteration in NCS variables, subjects are asymptomatic; however, it takes time for neuropathy symptoms to appear.

Regarding the tibial nerves, the longer latencies and lower conduction velocity may be due to either decreased myelination or beginning of axonal impairment (metabolic effect) as a result of vitamin B12 depletion, potentially leading to clinical neuropathy. Our results did not show any significant differences on onset latency, amplitude and nerve conduction velocity of bilateral common peroneal nerves between diabetic groups. Similar to our results, Leishear et al. showed no any significant association between vitamin B12 deficiency and common peroneal nerve CMAP amplitude however in their study, common peroneal nerve conduction velocity was worse in vitamin B12 deficient subjects.[19] This dissimilarity with ours can be explained firstly as differential sensitivities of nerves towards ongoing alteration in myelin due to long term use of metformin. It may be well predicted that neither all the nerves get affected simultaneously, nor all the parameters of single nerve. Secondly, the study population in their study were quite older as compared to ours.[19] However, the subjects were symptomatic, some study found significantly reduced CMAP amplitude and conduction velocity of peroneal nerve.[18]

A study suggested that some nerve fibres might be more susceptible to damage than others, in particular, the small caliber or non-myelinated fibres, while others with diameter large enough to sustain the normal conduction velocities may be spared.[20,21] This might explain the lower SNAP amplitudes of sural nerves in our study. Other NCS variables of bilateral sural nerves were comparable as in accordance to the study by Wile & Toth.[15] Similar result regarding sural nerve amplitude was found by Kalita et al. but their study showed decreased sural nerve conduction velocity, however, the subjects were symptomatic in their study.[18] The adverse effect of metformin on serum vitamin B12 level have been studied extensively across the world but its consequences on peripheral nerve function was rarely assessed by NCS. Clinical scoring

system like Toronto Clinical Scoring System (TCSS) and Neuropathy Impairment Score (NIS) were used by some of the studies but the reported findings regarding the alteration of peripheral nerve functions in diabetics with metformin therapy were in concordance to our study.[15,22] Overall, our study showed potential risk of serum vitamin B12 deficiency and significant electrophysiological NCS changes in diabetics with metformin therapy. The results of many studies were in accordance to ours and some showed no significant association between duration of metformin therapy and peripheral neuropathy. [6,19,23] indistinguishable from vitamin B12 related neuropathy.

Despite having comparable serum glucose level (fasting and PP) and duration of diabetes (after diagnosis) between two diabetic groups, only T2DM with metformin therapy showed significant longer latencies and lower amplitudes of both sensory and motor nerves.

CONCLUSION

Since the intake of drug metformin was the only unravelling factor between these 2 groups of diabetes, we can conclude that metformin causes potential risk of vitamin B12 deficiency and ongoing alteration of peripheral nerve function in T2DM with metformin therapy. However, it takes time for the symptoms of neuropathy to appear clinically. Long term metformin therapy in diabetic patients is associated with significant vitamin B12 depletion, leading to alteration in motor and sensory NCS parameters. Thus, we recommend regular vitamin B12 screening and oral/ parenteral vitamin B12 supplementation to the diabetic patients on metformin therapy.

REFERENCES

1. Boulton A, Vinik A, Arezzo J, Bril V, Feldman E, Freeman R et al. Diabetic Neuropathies: A statement by the American Diabetes Association. Diabetes Care. 2005;28(4):956-962. [DOI: 10.2337/diacare.28.4.956] [PubMed|FullText | DOI]

2. Calvo Romero J, Ramiro Lozano J. Vitamin B12 in type 2 diabetic patients treated with metformin. Endocrinología y Nutrición (English Edition). 2012;59(8):487-490. [FullText | DOI]

3. Reinstatler L, Qi Y, Williamson R, Garn J, Oakley G. Association of Biochemical B12 Deficiency with Metformin Therapy and Vitamin B12 Supplements: The National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care. 2011;35(2):327-333. [PubMed|FullText |DOI]

4. Nervo M, Lubini A, Raimundo F, Moreira Faulhaber G, Leite C, Fischer L et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Revista da Associação Médica Brasileira (English Edition). 2011;57(1):46-49. [FullText| DOI]

5. Boylu E, Şenol M, Saraçoğlu M. P125 The peripheral neuropathy in asymptomatic patients with vitamin B12 deficiency. Clinical Neurophysiology. 2008;119:S103. [FullText | DOI]

6. Chen S, Lansdown A, Moat S, Ellis R, Goringe A, Dunstan F et al. An observational study of the effect of metformin on B12 status and peripheral neuropathy. The British Journal of Diabetes & Vascular Disease. 2012;12(4):189-193. [FullText DOI]

7. Pongchaidecha M, Srikusalanukul V, Chattananon A, Tanjariyaporn S. Effect of metformin on plasma homocysteine, vitamin B12 and folic acid: a cross-sectional study in patients with type 2 diabetes mellitus. Journal of the Medical Association of Thailand.2004;87(7):780-787. [PubMed]

8. Iftikhar R, kamran S, Qadir A, Iqbal Z, Usman H. Prevalence of Vitamin B12 deficinecy in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. Pan African Medical Journal. 2013; 16:67. [PubMed|FullText | DOI]

9. Bauman W, Shaw S, Jayatilleke E, Spungen A, Herbert V. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227-1231. [PubMed|FullText | DOI]

10. Gilligan M. Metformin and Vitamin B12 Deficiency. Archives of Internal Medicine. 2002;162(4):484-485. [PMID: 11863489] [PubMed|FullText|DOI]

11. De Jager J, Kooy A, Lehert P, Wulffele M, van der Kolk J, Bets D et al. Long-term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo-controlled trial. BMJ. 2010;340(4):2181-2181. [PubMed|FullText | DOI]

12. Ting R. Risk Factors of Vitamin B12 Deficiency in Patients Receiving Metformin. Archives of Internal Medicine. 2006;166(18):1975-79. [PubMed|FullText|DOI]

13. Stabler S. Vitamin B12Deficiency. New England Journal of Medicine. 2013;368(2):149-160. [PubMed|FullText | DOI]

14. Misulis K, Head T. Essentials of clinical neurophysiology. Amsterdam: Butterworth-Heinemann; 2003. [FullText]

15. Wile D, Toth C. Association of Metformin, Elevated Homocysteine, and Methylmalonic Acid Levels and Clinically Worsened Diabetic Peripheral Neuropathy. Diabetes Care. 2009;33(1):156-161. [PubMed|FullText | DOI]

16. Kumar S, Vijayan J, Jacob J, Alexander M, Gnanamuthu C, Aaron S. Clinical and laboratory features and response

to treatment in patients presenting with vitamin B12 deficiency-related neurological syndromes. Neurology India. 2005;53(1):55. [PubMed|FullText | DOI]

17. Puri V, Chaudhry N, Gulati P, Tatke M, Singh D. Recurrent tumefactive demyelination in a child. Journal of Clinical Neuroscience. 2005;12(4):495-500. [FullText| DOI]

18. Kalita J, Chandra S, Bhoi S, Agarwal R, Misra U, Shankar S et al. Clinical, nerve conduction and nerve biopsy study in vitamin B12 deficiency neurological syndrome with a short-term follow-up. Nutritional Neuroscience. 2013;17(4):156-163. [PubMed|FullText | DOI]

19. Leishear K, Boudreau R, Studenski S, Ferrucci L, Rosano C, Rekeneire N et al. Relationship Between Vitamin B12 and Sensory and Motor Peripheral Nerve Function in Older Adults. Journal of the American Geriatrics Society. 2012;60(6):1057-1063. [PubMed|FullText | DOI]

20. Brismar T, Sima A, Greene D. Reversible and irreversible nodal dysfunction in diabetic neuropathy. Annals of Neurology. 1987;21(5):504-507. [PubMed|DOI]

21. Brismar T. Abnormal Na-Currents in Diabetic Rat Nerve Nodal Membrane. Diabetic Medicine. 1993;10(S2):110S-112S. [PMID: 8392921] [PubMed| DOI]

22. Singh A, Kumar A, Karmakar D, Jha R. Association of B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes patients. Journal of Postgraduate Medicine. 2013;59(4):253. [PubMed|FullText | DOI]

23. Moore E, Mander A, Watters D. More Adverse Neurological Consequences of Metformin-Induced Vitamin B12 Deficiency. Southern Medical Journal. 2011;104(7):541-542. [PubMed|FullText | DOI]