Delayed auditory conduction in diabetes: is metformin-induced vitamin B12 deficiency responsible?

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Summary

The present study aims to evaluate the functional integrity of the auditory pathway in patients with diabetes taking metformin. A further aim is to assess its association with vitamin B12 deficiency induced by metformin. Thirty diabetics taking metformin and 30 age-matched non-diabetic controls were enrolled. Stimulus-related potentials and vitamin B12 levels were evaluated in all the subjects. The diabetics showed deficient vitamin B12 levels and delayed wave III latency and III–V interpeak latency in the right ear and delayed Na and Pa wave latencies in the left ear compared with the controls. The dose and duration of metformin showed no association with the stimulus-related potentials. Therefore, although vitamin B12 levels were deficient and auditory conduction impairment was present in the diabetics on metformin, this impairment cannot be attributed to the vitamin B12 deficiency.

KEY WORDS: auditory conduction, metformin, stimulus-related potentials, vitamin B12 levels.

Introduction

Metformin, a biguanide, is the first-line oral hypoglycemic agent used to achieve glycemic control in type 2 diabetes mellitus (Marar et al., 2011). It acts via activation of the adenosine monophosphate-activated pro-

tein kinase system to reduce serum glucose, primarily by suppressing hepatic gluconeogenesis (Diamanti-Kandarakis et al., 2010). However, there are several side effects of metformin, such as gastrointestinal upset and, rarely, lactic acidosis (Marar et al., 2011). The side effect of metformin discussed in the present study is vitamin B12 deficiency (Marar et al., 2011; Pflipsen et al., 2009; Ting et al., 2006). Vitamin B12 or cobalamin is a water-soluble vitamin that plays a fundamental role in DNA synthesis, optimal hematopoiesis and neurological function. Therefore, vitamin B12 deficiency clinically manifests with features of hematological and neurocognitive dysfunction (Oh and Brown, 2003).

Diabetic neuropathy is a frequent complication of type 2 diabetes occurring in about 50% of people with this condition; it causes sensory, motor or sensory-motor dysfunction (Boulton et al., 2005; Gupta et al., 2010). However, the above rate actually refers only to the peripheral neuropathy observed in diabetes, whereas the term diabetic neuropathy is usually taken to mean both peripheral and autonomic neuropathy in patients with diabetes.

With the recent advances in non-invasive electrophysiological techniques, detailed exploration of sensory pathways in the central nervous system has become possible (Gupta et al., 2010; Bastianello et al., 2008; Anjana et al., 2010). The non-invasive methods currently employed for evaluating neurophysiological functions in various disease states include the measurement of brainstem auditory evoked potentials (BAEPs). These are obligate neuronal responses to an auditory stimulus, and therefore stimulus-related potentials, and, on the basis of their timing, they have been divided into three types:

- Early latency or auditory brainstem response (ABR) – (0 – 8 msec),
- Middle latency or mid-latency response (MLR) – (8 – 50 msec),
- Long latency or slow vertex response (SVR) – (50 – 300 msec) (Picton et al., 1974).

The ABR is as a non-invasive clinical tool used in characterizing the electrophysiological phenomenon of neural excitation, conduction and transmission along the auditory pathway in the brainstem, while the MLR and SVR represent conduction in the central auditory cortex; this means that the integrity of the thalamocortical projections, the primary auditory cortex and association cortex can be assessed by

recording the MLR and SVR (Vaney et al., 2011). There is scarce literature on the cause of auditory involvement in type 2 diabetes being treated with metformin. Metformin is associated with worsened neuropathy (Bell, 2010; Wile and Toth, 2010). This is an observation common to various studies; however, whether this worsening is due to B12 deficiency induced by metformin, or is, instead, part and parcel of diabetic neuropathy itself, is debated. Therefore, the aim of the present study was to evaluate the presence of auditory conduction abnormalities in people with diabetes being treated with metformin, and the vitamin B12 status of these subjects, and to establish whether there is any correlation between the degree of auditory impairment and vitamin B12 levels in this group.

Materials and methods

Setting and subjects

This case-control pilot study was carried out in the Electrophysiology Laboratory, Department of Physiology, UCMS & GTB Hospital, Delhi. The study group patients were recruited from the Diabetes Clinic, Department of Endocrinology & Metabolism, UCMS & GTB Hospital, Delhi. The control group subjects were randomly chosen from the hospital staff. Written informed consent to stimulus-related potential recordings and sample collection was obtained from all the participants prior to their enrollment in the study. Ethical clearance was obtained from the institutional ethics committee.

The study group comprised 30 type 2 diabetes mellitus patients who had been taking a minimum dose of 1g/day of metformin for a period of at least six months, while the control group was made up of 30 age-matched, normal healthy subjects.

Subjects with a 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, and subjects taking oral hypoglycemics other than metformin, or in combination with metformin, were also excluded from the study.

The subjects underwent a relevant history and examination to rule out exclusion criteria and to look for the presence of any abnormalities or disease. We measured the height and weight of all subjects and calculated their body mass index (BMI).

Auditory brainstem response

The ABR recording was done from the scalp using an Octopus 4 M/C NCV/EMG/EP system (Biostar Healthcare, India), with silver chloride disk electrodes placed at standard scalp locations according to the 10-20 International system. The electrodes were placed at the vertex of the head (reference electrode),

forehead (ground electrode) and ear lobes (active electrodes) after cleaning the scalp and skin site with alcohol followed by application of Skinpure™ skin preparation gel and Elefix™ EEG paste (Nihon Kohden, Tokyo, Japan). The skin electrode contact impedance was kept below 10KΩ.

The ABR was recorded using a click stimulus with a click duration of 0.1 msec delivered at an intensity of 90 dB; the contralateral ear was masked with a noise level of 60 dB. About 1000 responses were averaged. Absolute peak latencies and amplitudes of waves I, II, III, IV and V, as well as interpeak latencies (IPLs) of I–III, III–V and I–V were determined for each ear separately.

Mid-latency response

The MLR was recorded using a click stimulus with a click duration of 0.1 msec which was delivered at an intensity of 90 dB, while the contralateral ear was masked with 60 dB. Five hundred responses were averaged. Latencies of negative and positive waves, i.e. Na and Pa, were recorded.

Slow vertex response

The SVR was recorded using a click stimulus with a click duration of 0.1 msec, which was delivered at an intensity of 90 dB, while the contralateral ear was masked with 60 dB. A hundred responses were averaged. Absolute peak latencies of N1 and P2 were recorded.

Serum vitamin B12 levels

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

Statistical analysis

The statistical analysis was done using the SPSS 20 statistical package. The two groups were compared by unpaired t test. Data are presented as mean values and standard deviation. A p-value <0.05 was taken as significant. All the parameters in the study were correlated with baseline data using Spearman's rho correlation coefficient. Also, vitamin B12 levels were correlated with the different parameters of the stimulus-related potentials. Analysis of covariance was applied to rule out confounding factors.

Results

In all the subjects stimulus-related potentials were successfully recorded and no complications or poor results were observed.

Although the cases and controls in the present study

were selected from the same age group, their mean age differed significantly, the mean age of the study group being 51.53 ± 9.9 years and that of the control group 45.35 ± 9.5 years. The study group comprised 13 males and 18 females while the control group included 11 males and 19 females. No significant difference was found between the study group and the control group in other baseline characteristics, such as height, weight and BMI. The mean glycosylated hemoglobin (HbA1c) value in the diabetics was $7.14 \pm 1.3\%$ and the mean duration of diabetes was 4.15 ± 2.5 years. All the subjects in the present study were on metformin therapy alone, with no other medication. No subject was suffering from any diabetic complication or any other systemic condition.

Measurement of ABRs showed (Table I) that the

absolute latency of wave III and the III–V IPL were significantly delayed in the study group as compared to the control group in the right ear. Instead, the absolute latencies of waves I, II, IV and V, as well as the I–III and I–V IPLs, did not show any significant difference. Similarly, the amplitudes of these waves did not differ significantly between the controls and the study group (and neither did the MLR or SVR amplitudes).

We also observed (Table I) a significant decrease in the amplitude of wave V in the study group in the left ear. The absolute latencies and IPLs in the left ear did not differ significantly between the groups.

Evaluation of MLRs (Table II) showed delayed latencies of waves Na and Pa in the left ear in the study group as compared to the controls, while the SVRs did not differ significantly.

Table I - Auditory brainstem responses: comparison of the controls and the study group.

		Waves	Controls	Study Group	p-value
ABR right ear	Latencies	I	1.6 ± 0.3	1.7 ± 0.2	0.115
		II	2.6 ± 0.2	2.7 ± 0.2	0.245
		III	3.5 ± 0.2	3.7 ± 0.3	0.005*
		IV	4.6 ± 0.3	4.6 ± 0.5	0.685
		V	5.4 ± 0.3	5.4 ± 0.3	0.86
	Interpeak latencies	I – III	2 ± 0.4	2.1 ± 0.2	0.279
		I – V	3.8 ± 0.3	3.9 ± 0.4	0.226
		III – V	1.7 ± 0.3	1.9 ± 0.4	0.034*
	Amplitudes	I – Ia	0.2 ± 0.2	0.2 ± 0.2	0.82
		V – Va	0.4 ± 0.2	0.4 ± 0.2	0.142
ABR left ear	Latencies	I	1.5 ± 0.4	1.5 ± 0.4	0.542
		II	2.5 ± 0.2	2.7 ± 0.2	0.16
		III	3.5 ± 0.3	3.5 ± 0.4	0.728
		IV	4.6 ± 0.4	4.6 ± 0.4	0.829
		V	5.4 ± 0.3	5.5 ± 0.3	0.353
	Interpeak latencies	I – III	2.1 ± 0.5	2 ± 0.6	0.865
		I – V	3.9 ± 0.5	4 ± 0.5	0.234
		III – V	1.9 ± 0.5	2 ± 0.5	0.311
	Amplitudes	I – Ia	0.8 ± 0.9	0.8 ± 0.9	0.882
		V – Va	0.6 ± 0.4	0.4 ± 0.2	0.004*

Abbreviation: ABR=auditory brainstem response. * $p < 0.05$.

Table II - Middle latency responses and slow vertex responses: comparison of the controls and the study group.

		Waves	Controls	Study Group	p-value
MLR right ear	Latencies	Na	15.2 ± 1.9	15.3 ± 1.8	0.903
		Pa	22.6 ± 2.9	23.2 ± 3.1	0.445
SVR right ear	Latencies	N1	77.2 ± 17.9	83.4 ± 13.6	0.131
		P2	145.9 ± 27.4	149.8 ± 16.5	0.5
MLR left ear	Latencies	Na	14.3 ± 1.9	15.2 ± 1.7	0.004*
		Pa	23.1 ± 2.9	24 ± 3.4	0.04*
SVR left ear	Latencies	N1	78 ± 10.9	79 ± 15.9	0.76
		P2	142.6 ± 15.2	150.5 ± 19.4	0.08

Abbreviations: MLR=middle latency response; SVR=slow vertex response. * $p < 0.05$.

The study group, i.e. people with diabetes taking metformin, had significantly deficient serum vitamin B12 levels (143 ± 42.8 pg/ml) as compared to the control group (274.5 ± 64 pg/ml) ($p = .00$).

The above results were observed even after applying analysis of covariance for confounding factors such as age and gender. When the stimulus-related potentials and serum vitamin B12 levels in diabetic subjects were correlated with duration of diabetes no significant correlation was obtained. However, when correlations were calculated between each of the stimulus-related potential parameters and HbA1c, the latencies of wave III after both right ($p = 0.04$) and left ear ($p = 0.036$) stimulation were positively correlated and the I–III IPL ($p = 0.016$) in the right ear and III–V IPL ($p = 0.017$) in the left ear were also positively correlated.

The study group was divided into two groups according to the duration of metformin intake: <5 years and ≥ 5 years. Serum vitamin B12 levels were significantly lower (101 ± 13.7 pg/ml) in the patients taking metformin for ≥ 5 years than in those taking metformin for <5 years (163.5 ± 36.9 pg/ml; $p = 0.00$). No significant differences were found in the ABRs, MLRs or SVRs.

The study group was also divided into two groups according to the dose of metformin taken: <1500 mg and ≥ 1500 mg of metformin per day. Serum vitamin B12 levels were significantly lower in the patients taking ≥ 1500 mg metformin per day (118 ± 20.4 pg/ml) than in those taking <1500 mg (183.3 ± 38.4 pg/ml; $p = 0.00$). However, the ABRs, MLRs and SVRs did not show significant differences.

Vitamin B12 levels were found to show a significant negative correlation with the dose of metformin ($r = -0.7$) and also a significant negative correlation with the duration of metformin intake ($r = -0.5$). However, no significant correlation was found between vitamin B12 levels and the stimulus-related potentials.

Figure 1 shows representative brainstem-evoked response audiometry (BERA) waves in the right ear in controls (a) and in the study group (b).

Discussion

In the present study, recording of ABRs revealed, in the members of the study group, a significant delay in the absolute latency of wave III and in the III–V IPL in the right ear along with a decrement in the amplitude of wave V in the left ear.

The ABR is a series of potentials (BAEPs) arising from the acoustic nerve and brainstem that are volume conducted to surface recording electrodes at the scalp (Stockard et al., 1992). The ABR waveforms are labeled from I to V. Wave I is believed to reflect activity in the auditory nerve; waves II and III, activity in the cochlea and superior olivary nuclei, and waves IV and V, activity in the lateral lemniscus and inferior colliculus (Stockard et al., 1992). The latency of the waveforms denotes the conduction time along the auditory pathway. The amplitude of the ABR waveforms depends on the number of neural elements activated by the sound stimulus and the degree of synchronized

Figure 1- Representative BERA waves in the right ear in controls (a) and study group (b).

activity of these neural elements (Don and Kwong, 2002). The IPLs reflect neural conduction in the corresponding segments of the central auditory pathway: IPL I–V is a measure of total conduction time, IPL I–III is a measure of conduction from the acoustic nerve to the pontomedullary portion, and IPL III–V is a measure of pontomesencephalic conduction time. Abnormally prolonged IPLs reflect dysfunction of central auditory conduction (Stockard et al., 1992).

The delay in IPL III–V and the reduction in the amplitude of wave V are evidence of a central conduction delay at the brainstem-to-midbrain level. We found no significant difference in waves I and II in the two groups, which implies that the eighth nerve transmission time was normal in people with diabetes taking metformin. However, the delay in wave III latency must implicate higher structures, probably at the level of the superior olivary nucleus. In an earlier work, too, it was observed that type 2 diabetes mellitus patients showed significant prolongation of the absolute latencies of I and III ($p = 0.001$) and of the I–III, III–V and I–V IPLs in the left ear ($p = 0.001$), while absolute latencies of I and V and IPL III–V were significantly prolonged in the right ear (Mahallik et al., 2014). Another study observed that the same five measures were significantly altered in diabetics with both right and left ear stimulation: the latencies of waves III and V, the IPLs I–III and I–V, and the amplitude of wave V (Donald et

al., 1981). With right ear stimulation, wave IV was also altered in amplitude. The latencies of waves I and II and the amplitudes of waves I, II and III were not significantly different from those of the normal group, upon stimulation of either ear. Similar results were observed by others as well (Gupta et al., 2010; Fidele et al., 1984).

The present study also observed delayed latencies of MLR waves Na and Pa in the left ear of the people in the study group. The MLR and SVR represent conduction in the central auditory cortex. Thus, integrity of the thalamocortical projections, the primary auditory cortex and association cortex can be assessed by using the MLR and SVR (Stockard et al., 1992). Another study in diabetics did not find any difference in the MLR between these patients and normal controls (Mukhopadhyay et al., 1992). Likewise, one study also reported that neither MLR nor SVR differed significantly between diabetics and normal controls (Di Leo et al., 1997). Therefore, it is possible that there is patchy distribution of nervous system anatomofunctional anomalies in diabetes (Pozzessere et al., 1991). The variable involvement of waves in both ears, observed in the present study, might also be due to the shorter duration of diabetes in the members of our study group, as a longer duration of diabetes is a definite risk factor for the development of central conduction delay (Gupta et al., 2010).

The present study also found lower serum vitamin B12 levels in the people with diabetes taking metformin compared with the normal age-matched controls. This serum vitamin B12 deficiency worsened as the dose or duration of exposure to metformin increased. Vitamin B12 forms a complex with cubulin, an endocytic receptor, which is taken up 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 the membrane potential and competitively repels the divalent calcium ions, thus preventing calcium-dependent uptake; this leads to malabsorption of B12 (Gilligan, 2002). The fact that metformin-induced B12 deficiency can be treated with calcium supplementation provides confirmation of this mechanism (Gilligan, 2002; Andres et al., 2000; Bauman et al., 2000). Metformin has also been suggested to act by increasing bacterial overgrowth, altering bowel motility, and by direct inhibition of B12 absorption (Andrs et al., 2000).

Bell (2010), in his case report, strongly presents his view that metformin results in malabsorption of vitamin B12 at the terminal ileum, thus leading to vitamin B12 deficiency, and that this B12 deficiency is responsible for neuropathy in his patient. There is a risk that this neuropathy may be misdiagnosed as diabetic neuropathy, with the result that the central and/or peripheral neuronal damage, which can otherwise be controlled with vitamin B12 replacement, is allowed to progress (Bell, 2010).

However, the B12 deficiency observed in the present study showed no association with the degree of dete-

rioration of the stimulus-related potentials, which means that the auditory conduction impairment observed in our subjects with diabetes taking metformin was not due to the B12 deficiency induced by the drug. This impairment is probably linked to poor glycemic control or duration of diabetes. Neural involvement in diabetes could pathogenetically be due to the adverse effects, on the brain, of chronic hyperglycemia or recurrent episodes of hypoglycemia (Biessels et al., 1994). Chronic hyperglycemia can lead to both metabolic and vascular disturbances in the brain. Studies in diabetic rats have shown regional reductions in cerebral blood flow within weeks (Harik and LaManna, 1988; Duckrow et al., 1987) to months (Jakobsen et al., 1990) after diabetes induction. Studies of diabetic patients report regional decreases in cerebral blood flow and impaired cerebrovascular reactivity as well as structural alterations in the cerebral vasculature, including thickening of capillary basement membranes (Mankovsky et al., 1996). These functional and structural changes in the vasculature could impede the delivery of nutrients and oxygen to the brain, thus affecting cerebral energy metabolism.

In conclusion, vitamin B12 levels were decreased in people with diabetes taking metformin and this deficiency worsened with dose and duration of metformin exposure. Diabetes patients taking metformin were also found to show auditory conduction abnormalities, however these abnormalities cannot be attributed to deficient vitamin B12 levels.

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