Is Metformin-Induced Vitamin B12 Deficiency Responsible for Cognitive Decline in Type 2 Diabetes?

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ABSTRACT

Introduction: Diabetes mellitus has its deleterious effects on various aspects of cognition such as memory function, executive function, and information-processing speed. The present study aims to assess cognition in diabetes patients and also tries to find its association with Vitamin B12 deficiency induced by metformin. Materials and Methods: Thirty diabetics taking metformin and thirty nondiabetic controls were enrolled. Event-related potentials (ERPs) and serum Vitamin B12 levels were evaluated in them. Results: Vitamin B12 levels were found to be deficient, and latencies of waves P200 and P300 were prolonged in the diabetics as compared to the controls. The dose and duration of metformin had no association with the ERPs. Conclusions: Although the Vitamin B12 levels were deficient in diabetics on metformin, this is not the reason behind the cognitive impairment found in them.

Key words: Cognition, event-related potentials, metformin, Vitamin B12 levels

INTRODUCTION

The incidence of diabetes in the world is showing an increasing trend with each passing day. The therapeutic approach to type 2 diabetes mellitus usually commences with a single hypoglycemic agent, which most of the times, is metformin, a biguanide. It reduces hepatic glucose production and improves peripheral glucose utilization slightly. Metformin activates adenosine monophosphate (AMP)-dependent protein kinase and enters cells through organic cation transporters. Metformin is the drug of choice because it not only reduces serum glucose but also improves lipid profile and brings about modest weight loss. However, there are several disadvantages to the use of metformin, the one which the present study discusses is Vitamin B12 deficiency induced by it. The mechanism behind this remains unclear; however, it is thought to be due to either alteration in small bowel motility, resulting in bacterial overgrowth and subsequent B12 deficiency, or by directly decreasing Vitamin B12 absorption.

The deleterious effects of diabetes mellitus on the retinal, cardiovascular, and peripheral nervous systems...
Several tools and tests have been devised and standardized by physiologists and cognitive scientists to assess cognitive functions, which include Bender-Gestalt test,[7] Wechsler Memory test,[8] Halstead-Reitan Categories Test,[9] Trail Making Tests,[10] and the Mini-Mental State Examination.[11] However, electrophysiological tests have proven to be a reliable tool to document cognitive impairment in diabetes, even at an early stage of the disease.[12] Cognitive functions of the brain are evaluated by using event-related potentials (ERPs) which are those potentials of the electroencephalogram (EEG) that are evoked by the perception of or the preparation for events, and they include an early sensory-evoked potential and a late cognitive response (p300 component). In other words, it is evoked by unexpected stimuli and indicates the amount of processing required by a given stimulus.[13,14]

The mechanism underlying cognitive impairment in type 2 diabetes mellitus is not completely understood. Vitamin B12 deficiency induced by metformin[13,14] might be responsible for the cognitive impairment in these patients. Therefore, the present study aims at identifying whether metformin therapy induces Vitamin B12 deficiency in type 2 diabetics and whether this B12 deficiency correlates with the degree of cognitive impairment in them by employing ERPs as a tool to study cognitive functions.

MATERIALS AND METHODS

Setting
The study was carried out in the electrophysiological laboratory, Department of Physiology, UCMS and GTB Hospital, Delhi. The patients were recruited from the Diabetic Clinic, Department of Endocrinology and Metabolism, UCMS and GTB Hospital, Delhi. The control group subjects were randomly chosen from the hospital staff. Both, the people with diabetes and the control group subjects were age-matched. A written informed consent was obtained from all the participants, for ERPs’ recordings and sample collection, prior to enrollment in the study. Ethical clearance was obtained from the Institutional Ethical Committee.

Type of study: Case–control study.

Subjects
- Study group: Thirty type 2 diabetes mellitus patients taking a minimum dose of 1 g/day of metformin for at least 6 months
- Control group: Thirty 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, and subjects taking oral hypoglycemic 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 were measured and body mass index (BMI) calculated.

Event-related potentials
The recording was done from the scalp of the subjects using Octopus 4 M/C NCV/EMG/EP system by Biostar healthcare, India. The recording was done in a sound-proof room, using the silver chloride disk electrodes placed at standard scalp locations of the 10–20 international system. The electrodes were placed at vertex of head (reference electrode), forehead (grounding electrode), and ear lobes (active electrodes) after cleaning the scalp and skin site with alcohol followed by Skinpure™ skin preparation gel and EEG paste Elefix™. The skin electrode contact impedance was kept at <10 kΩ. During the recording session, subject was instructed to fix his eyes on a particular spot on the wall in front to avoid electro-oculographic artifacts due to eye movement.

The auditory ERPs were recorded using an “oddball paradigm” wherein two stimuli (target and nontarget) were presented in a random order by headphones. The target stimulus was a 2 KHz beep sound with 20% occurrence and the nontarget was a 1 KHz click with 80% occurrence. The auditory stimuli had 10 ms rise/fall time, 100 ms duration, and intensity of 90 dB. The evoked potentials were filtered with a band pass of 0.1–50 Hz and averaged for 100 responses. The response time ranged from 0 to 500 ms.
ERPs were recorded from the scalp of the subjects while the subjects performed psychomotor task on the computer. The subjects had to press a button on the response pad with the thumb of their dominant hand on the hearing of target stimulus (beep) delivered by the headphones. The peak latencies of the ERPs were evaluated from stimulus onset (stimulus artifact) to the peak point of the particular wave, i.e., the point of greatest amplitude. Amplitude was measured as the distance of the corresponding peak from the baseline. Peak latencies of N100, P200, N200, and P300 and amplitude of N200–P300 wave were recorded and evaluated.

**Serum Vitamin B12 levels**

Blood (5 ml) was collected in plain vials. The samples were allowed to clot for 2 h at room temperature. The samples were then centrifuged at approximately 3000 rpm for 30 min. Serum was then used for Vitamin B12 assay which was based on the principle of ELISA. Serum Vitamin B12 levels <200 pg/ml were defined as deficient levels.

**Statistical analysis**

Analysis was done using SPSS 20 Statistical Package (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). The two groups were compared by unpaired t-test. Data have been presented as mean ± standard deviation. *P* < 0.05 has been considered statistically significant. Vitamin B12 levels were correlated with the dose and duration of metformin use using the Spearman’s rho correlation coefficient. Further, the Vitamin B12 levels were correlated with latencies of the ERPs.

**RESULTS**

The cases and controls in the present study were selected from the same age group, but their mean age differed significantly, the mean age of the study group was 51.53 ± 9.9 years and that of the control group 45.35 ± 9.5 years. Analysis of covariance has been applied to eliminate confounders such as age and gender. The age- and gender-adjusted values obtained for all parameters are reported in the result, which have not been found to be significantly different from that obtained earlier. The study group had 13 males and 18 females and the control group included 11 males and 19 females. There was no significant difference in other baseline characteristics such as height, weight, and BMI among the study group and the control group. The mean glycosylated hemoglobin (HbA1c) in the diabetics was 7.14 ± 1.3%, and the mean duration of diabetes was 4.15 ± 2.5 years. All the subjects in the present study were only on metformin therapy with no other medication. None of them were suffering from any diabetic complication or any other systemic condition.

The ERPs showed [Table 1] delayed latencies of waves P200 and P300 among the study group in comparison to the control group (*P* = 0.034 and 0.001, respectively). The latencies of waves N100 and N200 and the amplitude of wave P300, i.e., N200–P300, did not show significant difference between the two groups.

The serum Vitamin B12 levels were significantly deficient (*P* = 0.00) in the study group, i.e., in people with diabetes taking metformin (143 ± 42.8 pg/ml) as compared to the control group (274.5 ± 64 pg/ml).

The study group was divided into two groups according to the duration of metformin intake [Table 2]. One group included people with diabetes taking metformin for <5 years and the other included those taking metformin for ≥5 years. Serum Vitamin B12 levels were significantly lower (*P* = 0.00) in those taking metformin for ≥5 years as compared to those taking metformin for <5 years. However, on comparing the ERPs among these two groups, no significant difference was found in either the latencies or the amplitudes.

The study group was also divided into two groups according to the dose of metformin [Table 3]. One group included people with diabetes taking <1500 mg of metformin per day and those taking metformin ≥1500 mg/day. Serum Vitamin B12 levels were significantly lower (*P* = 0.00) in those...
taking ≥1500 mg metformin per day as compared to those taking <1500 mg. However, on comparing the ERPs among these two groups, no significant difference was found in either the latencies or the amplitudes. Vitamin B12 levels were found to have 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 the Vitamin B12 levels and the ERPs.

**DISCUSSION**

The present study showed significantly delayed latencies of waves P200 and P300 in the people with diabetes taking metformin when compared to normal age-matched controls. The N100 and P200 components of the ERPs reflect the activity occurring in neural areas activated by sensory stimuli and are independent of the subject’s attention.\[15\] The N200 component is related to the degree of unexpectedness of the stimulus.\[16\] The P300 component of the ERPs is associated with psychological processing. It is generated from various sites of the brain including the cortical and subcortical areas, particularly the auditory cortex, hippocampus, amygdala, brainstem, and thalamic structures.\[17\] The P300 wave is believed to reflect cognitive processes underlying attention allocation and memory updating\[18\] and its amplitude indicates the amount of difficulty encountered in differentiating target from nontarget stimuli in the “oddball” paradigm of the ERP.\[19\] The present study therefore observed cognitive impairment in the diabetics taking metformin.

Through the present study, we also observed that serum Vitamin B12 levels were deficient in diabetics taking metformin while they were not deficient in the control group. In addition, this Vitamin B12 deficiency was worse as the duration and dose of metformin exposure progressed. Various mechanisms have been proposed that may underlie this B12 deficiency induced by metformin, including:

- Alterations in small bowel motility which stimulates bacterial overgrowth and subsequent Vitamin B12 deficiency
- Competitive inhibition or inactivation of Vitamin B12 absorption
- Alterations in intrinsic factor (IF) levels
- Interaction with the cubilin endocytic receptor\[20\]
- Inhibition of the calcium-dependent absorption of Vitamin B12-IF complex at the terminal ileum. This inhibitory effect can be reversed with calcium supplementation.\[21\]

The present study demonstrated that the Vitamin B12 deficiency worsened with the increase in dose and duration of metformin. Likewise, Marar et al. also demonstrated similar results. The presence of Vitamin B12 deficiency was more in the metformin exposed group as compared to the nonmetformin exposed group and a significant inverse relationship existed between the B12 levels and the dose and duration of metformin.\[11\] Another study demonstrated that patients with type 2 diabetes who were exposed to metformin for >6 months had lower Vitamin B12 levels and that Vitamin B12 levels had a significant correlation with the dose of metformin.\[22\]

Patients with type 2 diabetes mellitus have been found to have cognitive impairment.\[23\] The present study also abides by this fact. Type 2 diabetes has been associated with decreases in psychomotor speed, executive function,\[23\] verbal memory, processing speed,\[24\] complex motor functioning, and working memory.\[23\] However, since the present study observed that the ERPs did not have any correlation with the Vitamin B12 levels, Vitamin B12 deficiency does not underlie the cognitive impairment seen in diabetics. Various studies have proposed that several mechanisms pertaining to diabetes are responsible for this cognitive impairment. Biessels et al.\[25\] mentioned that atherosclerosis, microvascular disease as a result of insidious ischemia, advanced protein glycation and oxidative stress as a result of glucose toxicity, and insufficient insulin action are major factors responsible for dementia in type 2 diabetics. However, for cognitive dysfunction, either blood glucose disorders such as hyperglycemia or insulin disorders such as insulin resistance or microvascular damage may be held responsible.\[4\]

Cognitive impairment due to hyperglycemia can be attributed to neuronal changes as a result of advanced glycosylated end-product production and oxidative stress.\[25,26\] Cognitive dysfunction is also associated with the action of insulin. There is a large number of insulin receptors in the hippocampus and cerebral cortex, which play a central role in memory.\[23\] Insulin induces the release of β-amyloid peptide (Aβ) in cells to the cell exterior and promotes the expression of insulin-degrading enzyme (IDE). In case of insulin resistance, hyperinsulinemia causes downregulation of insulin receptors and also less insulin comes into the brain.\[27\] Moreover, as insulin is degraded by IDE, in the high insulin state, IDE gets consumed resulting in an increase in Aβ causing cognitive impairment. Another mechanism that may underlie cognitive disruption in type 2 diabetes is diabetic microvascular disease.\[28\] It has been proposed that damage to cerebral small vessels as a result of ischemia or damage to nerve sheaths is
probably responsible. Abnormal polyol pathway and myoinositol metabolism could alter glucose metabolism in the frontal lobe, resulting in cognitive impairment.

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 setup, 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.

CONCLUSIONS

- It is known that there is Vitamin B12 deficiency in diabetes; however, this cannot be demonstrated by the findings of this particular study
- Treatment with metformin also seems to contribute to B12 deficiency as there is a dose- and duration-related correlation
- People with type 2 diabetes have impaired cognitive functions, but Vitamin B12 deficiency does not seem to be responsible for it as no significant correlation was found between the Vitamin B12 levels and the ERPs.

Hence, treatment of diabetes adequate to check the progression of the disease process must be paid importance. This can keep a check on the cognitive dysfunction and thus provide a better quality of life to people suffering from type 2 diabetes mellitus.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Marar O, Senturk S, Agha A, Thompson C, Smith D. The prevalence of Vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin. R Coll Surg Irel Stud Med J 2011;4:16-20.
2. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: An old medication of new fashion: Evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. Eur J Endocrinol 2010;162:193-212.
3. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of Vitamin B12 deficiency in patients receiving metformin. Arch Intern Med 2006;166:1975-9.
4. Kawamura T, Umemura T, Hotta N. Cognitive impairment in diabetic patients: Can diabetic control prevent cognitive decline? J Diabetes Investig 2012;3:413-23.
5. van den Berg E, Kessels RF, Kappelle LJ, de Haan EH, Biessels GJ; Utrecht Diabetic Encephalopathy Study Group. Type 2 diabetes, cognitive function and dementia: Vascular and metabolic determinants. Drugs Today (Barc) 2006;42:741-54.
6. Khattar D, Sodhi C, Parmod J, Dutta A. Correlating estrogen levels and cognitive functions in regularly menstruating females of reproductive age group and post-menopausal women of North India. J Family Reprod Health 2010;5:92-6.
7. Sadock BJ, Sadock VA. Kaplan and Sadock’s Synopsis of Psychiatry: Behavioral Sciences/Clinical Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2003.
8. Wechsler D. WMS-R Manual. New York: Psychological Corporation; 1987.
9. Reitan R, Davidson L, editors. Clinical Neuropsychology, Current Status and Applications. New York: John Wiley; 1974.
10. Reitan RM. Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills 1958;8:271-6.
11. Bird TD, Miller BL. Alzheimer’s disease and other dementias. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL, editors. Harrison’s Principles of Internal Medicine. 16th ed., Vol. II. USA: McGraw Hill; 2005. p. 2393-406.
12. Pozzessere G, Valle E, de Crignis S, Cordischi VM, Fattapposta F, Rizzo PA, et al. Abnormalities of cognitive functions in IDDM revealed by P300 event-related potential analysis. Comparison with short-latency evoked potentials and psychometric tests. Diabetes 1991;40:952-8.
13. Anjana Y, Khaliq F, Vaney N. Event-related potentials study in attention deficit hyperactivity disorder. Funct Neurol 2010;25:87-92.
14. Khaliq F, Alam KK, Vaney N, Singh TB. Sensory, cognitive and motor assessment of children with poor academic performance: An auditory evoked potential study. Indian J Physiol Pharmacol 2010;54:255-64.
15. Picton TW, Hillyard SA. Human auditory evoked potentials. II. Effects of attention. Electroencephalogr Clin Neurophysiol 1974;36:191-9.
16. Ritter W, Ford JM, Gaillard AW, Harter MR, Kutus M, Näätänen R, et al. Cognition and event-related potentials. I. The relation of negative potentials and cognitive processes. Ann N Y Acad Sci 1984;425:24-38.
17. Smith ME, Halgren E, Sokolik M, Baudena P, Musolino A, Liegeois-Chauvel C, et al. The intracranial topography of the P3 event-related potential elicited during auditory oddball. Electroencephalogr Clin Neurophysiol 1990;76:225-48.
18. Picton TW. The P300 wave of the human event-related potential. J Clin Neurophysiol 1992;9:456-79.
19. Donchin E, Coles MG. Is the P300 component a manifestation of context updating? Behav Brain Sci 1988;11:357-74.
20. Andrès E, Noel E, Goicot B. Metformin-associated Vitamin B12 deficiency. Arch Intern Med 2002;162:2251-2.
21. Bauman WA, Shaw S, Jayatilleke E, Spungen AM, Herbert V. Increased intake of calcium reverses Vitamin B12 malabsorption induced by metformin. Diabetes Care 2000;23:1227-31.
22. Wile DJ, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. Diabetes Care 2010;33:156-61.
23. Munshi M, Grande L, Hayes M, Ayres D, Suhl E, Capelson R, et al. Cognitive dysfunction is associated with poor diabetes control in older adults. Diabetes Care 2006;29:1794-9.
24. Messier C. Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. Neurobiol Aging 2005;26 Suppl 1:26-30.
25. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. Risk of dementia in diabetes mellitus: A systematic review. Lancet Neurol 2006;5:64-74.
26. Strachan MW. The brain as a target organ in Type 2 diabetes: Exploring the links with cognitive impairment and dementia. Diabet Med 2011;28:141-7.
27. Young SE, Mainous AG 3rd, Carnemolla M. Hyperinsulinemia and cognitive decline in a middle-aged cohort. Diabetes Care 2006;29:2688-93.
28. Ding J, Strachan MW, Reynolds RM, Frier BM, Deary IJ, Fowkes FG, et al. Diabetic retinopathy and cognitive decline in older people with type 2 diabetes: The Edinburgh Type 2 Diabetes Study. Diabetes 2010;59:2883-9.
29. Ito S, Nagaawa T, Abe M, Mori T. Strain vessel hypothesis: A viewpoint for linkage of albuminuria and cerebro-cardiovascular risk. Hypertens Res 2009;32:115-21.
30. Ryan CM. Diabetes, aging, and cognitive decline. Neurobiol Aging 2005;26 Suppl 1:21-5.