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RESEARCH ARTICLE

A comparative study of event-related potential P300 between normal individuals and individuals with prediabetes

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ABSTRACT

Background: Prediabetes is the early stage of Type 2 diabetes mellitus. Altered glucose metabolism, atherosclerosis, and inflammation of blood vessels are seen in prediabetes. This may lead to neurodegeneration and cognitive decline. Event-related potential P300 can easily detect cognitive decline before the appearance of any neurological manifestations. **Aims and Objective:** The aim of this study is to assess and compare the P300 latencies in normal subjects and individuals with prediabetes. **Materials and Methods:** The study included 25 individuals with prediabetes and 25 healthy controls between the age group of 20 and 50 years. After obtaining informed consent, the study subjects were evaluated by history, general physical, and systemic examination. Serum HbA1c level was estimated. P300 was recorded using the standard auditory oddball paradigm from the vertex (Fz, Cz, and Pz) in response to stimuli presented monaurally through head phones. The peak latencies of P300 of target stimuli were calculated. **Results:** Mean P300 latency in normal individuals was 305.15 ms ± 27.80 and in individuals with prediabetes 383.98 ms ± 22.02 which is statistically significant ($P < 0.05$). **Conclusion:** Increase in P300 latencies indicates the existence of a cognitive decline in individuals with prediabetes compared to healthy individuals.

KEY WORDS: Event-related Potential P300; Prediabetes; Cognitive Function; Serum HbA1c

INTRODUCTION

Prediabetes is defined as a state in which blood glucose levels are elevated above the normal range but not high enough to be classified as diabetes mellitus (DM) according to the criteria of American Diabetes Association (ADA).[1,2] It is frequently associated with obesity, dyslipidemia, and hypertension.[2] Prediabetes has also been found to be associated with early carotid atherosclerosis, coronary artery calcification as well as other vascular abnormalities.[3]

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In the British Whitehall II study done by Tabak et al., it has been found that people with diabetes had increased blood glucose level 13 years before the diagnosis of Type 2 diabetes.[4] This pattern of glycemic changes was also observed in other studies.[5,6] Insulin sensitivity was also found to be reduced with a higher insulin level throughout the 13-year observation period of Whitehall II study. They have also observed that insulin secretion was reduced just before the diagnosis of diabetes. These findings indicate that insulin resistance and beta cell dysfunction start years before the development of diabetes, which could be a period of prediabetes.[4,7,8] The gradual increase in insulin resistance and subsequent beta cell dysfunction in prediabetes eventually lead to the development of Type 2 diabetes. According to ADA expert panel, up to 70% of individuals with prediabetes develop Type 2 diabetes in the subsequent years.[9]

In prediabetes hyperglycemia, increased free fatty acids and insulin resistance provoke molecular mechanisms that alter

the function and structure of blood vessels.[3] Atherosclerosis, altered vascular flow, insulin resistance, hyperglycemia, accumulation of advanced glycosylation end products, and formation of reactive oxygen species are hypothesized to be the probable cause of neurological complications even in prediabetes.

The neurological deficits may have an early onset in prediabetes but remain unnoticed due to their subtlety. Neurological defects may occur in the form of slowness of intellectual functions, lethargy, memory deficits, depression, and cognitive dysfunction.

Prediabetes, DM, and Alzheimer's disease have been found to share multiple common pathogenic factors such as hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, and acute hypoglycemic episodes.[10-13] In prediabetes, the blood glucose level is higher than the normal, and also there is some degree of insulin resistance[14] which may contribute to the cognitive decline.

Cognition refers to all the mental activities involved in receiving information, comprehending it, sorting, retrieving, and using it. It is associated with goal directed behavior and helps the individual in adjusting with the changing environmental needs.

The cognition can be assessed by several methods such as Addenbrooke's Cognitive Examination test, Montreal cognitive examination, and critical flicker frequency. Measurement of event-related potential (ERP) P300 latencies is a standardized method of assessing cognition. ERPs are the electrical potentials recorded in the scalp which is associated with specific sensory, perceptual, cognitive, or motor events. P300 is a frequently investigated ERP that occurs at about 300 ms following task-related stimuli.[15] It is a large, broad, vertex positive wave usually elicited following an infrequent stimulus (oddball paradigm). It is generated by multiple, relatively independent foci, or by a central integrated system with widespread connection and impact throughout the brain. P300 has been used extensively for the objective evaluation of cognitive functions.

Very few studies have been done to assess the cognitive function in prediabetes. Hypothesis of this study was that there may be a cognitive impairment in prediabetic condition. Early diagnosis and management of this state may prevent the progress of cognitive decline. Therefore, the objective of this study was to assess and compare the P300 latencies in normal subjects and individuals with prediabetes.

MATERIALS AND METHODS

Ethical clearance was obtained from Institutional Ethical Committee. Twenty-five prediabetic patients from the Department of Endocrinology, Ramaiah hospital, were included as cases and 25 age-matched controls were taken from the neighborhood. The study was undertaken between October 2014 and July 2016. The study group comprised of both male and female patients with prediabetes in the age group of 20-50 years. Control group included age-, sex-, and education-matched normal subjects. Patients with the past or present history of psychiatric and neurological disorders, history of ear and eye diseases, chronic alcoholics and chronic smokers, history of diabetes, thyroid, renal, and liver disorders, and patients on drugs which alter the psychomotor function were excluded from the study.

Methods of Recording

Testing procedures were explained, and consent was obtained from the cases and controls. A detailed history was taken. A thorough general physical examination and systemic examination were done. The subjects were divided into the two groups based on their serum HbA1C level. Serum HbA1C level was estimated by high-performance liquid chromatography. Cognitive function was assessed using the auditory oddball paradigm for ERP P300 latencies.

Electrode Placement

The subjects were seated comfortably in a semi-darkened, acoustically shielded, air-conditioned room. The electrode placement sites were cleaned with spirit. Ag/AgCl disc electrodes were placed with conductive paste at Fz, Cz, Pz, C3, P3, A1, and A2 of the 10-20 International system (Figure 1).

Fz was taken as the grounding electrode, and A1 and A2 were used as reference electrodes. All the electrodes were connected to a junction box, and skin to electrode impedance was kept at <5KΩ (Figure 2).

ERP P300

ERP P300 was recorded using the Nihon Kohden Neuropack MEB 2200 version 03.02 (Figure 3). P300 recording consisted of stimulus presentation through an oddball paradigm in which an unexpected infrequent stimulus was interspersed among frequent stimuli. The procedure was explained to the subjects. They were familiarized with the stimuli and requested to remain alert and awake during the process. The subject was presented with 300 stimuli as a sequence of two distinguishable sound stimuli, one of which occurred frequently (frequent stimulus/non-target) for 240 times and the other infrequently (rare stimulus/target) for 60 times. The frequency of the frequent stimulus was 750 Hz and that of the rare stimulus was 2000 Hz. The subjects were asked to press a button whenever they heard the target sound. The evoked responses to the rare stimuli were filtered with a band pass

1-30 Hz and averaged. Samples contaminated with artifacts were auto discarded. A high-pass setting of 0.01 Hz and a low-pass setting of 100 Hz were employed. The positive wave appearing about 300 ms after the stimulus was marked as P300 (Figure 4), and the latency was measured at the point of maximum amplitude. Peak latencies of P300 were also compared among the groups.

Statistical Analysis

Statistical analysis was done using SPSS 17.0. All parameters were summarized using mean and standard deviation (SD). Students *t*-test was used to compare mean differences in all the parameters between the two groups.

RESULTS

The study was conducted on 25 normal subjects (Group 1) and 25 individuals with prediabetes (Group 2). The participants were between the age group of 20 and 50 years. In Group 1, 15 (60%) were females, and 10 (40%) were males. In Group 2, 13 (52%) were females, and 12 (48%) were males. The gender distribution in the study groups was comparable ($P = 0.687$). Gender distribution is shown in Figure 5.

The mean body mass index (BMI) of the normal subjects and individuals with prediabetes was 23.07 ± 2.19 and 27.84 ± 1.88 kg/m^2, respectively, and the difference was statistically significant. BMI in study groups is shown in Figure 6.

The study groups had significantly different levels of HbA1C (Figure 7) reflecting their blood glucose status. The mean HbA1C levels in Groups 1 and 2 were 5.14 ± 0.21 and 6.02 ± 0.21%, respectively.

P300 Latencies

The P300 latencies were normally distributed among the study groups. These results were described in terms of mean and SD.

P300 latencies were significantly prolonged ($P < 0.05$) in case of individuals with prediabetes compared to normal individuals (Table 1 and Figure 8).

The latencies of P300 in Fz position for Groups 1 and 2 were 324.80 ± 39.67 and 385.44 ± 29.43 ms, respectively. The difference in latencies was statistically significant ($P < 0.005$).

Mean P300 latencies in Group 2 in Cz and Pz position were 385.85 and 389.48.85 ms, respectively, which were significantly higher than normal subjects.

The mean P300 latencies in C3 position for Groups 1 and 2 were 302.69 and 385.74 ms, respectively. The difference in latencies was statistically significant ($P < 0.005$).

The latency of P300 in P3 position was also significantly higher in Group 2 compared to Group 1.

DISCUSSION

In our study, there was a statistically significant difference in HbA1C levels between Groups 1 and 2. This difference reflects their different blood glucose status supporting the diagnosis of prediabetes in Group 2 according to ADA criteria.[1] BMI was higher in individuals with prediabetes compared to normal subjects. The findings of this study showed the presence of cognitive decline in prediabetes stage which was noted by significantly increased P300 latencies in Group 2 compared to Group 1.

Saczynski et al. found in their study that compared with normoglycemics, persons with impaired fasting glucose and diagnosed Type 2 diabetes had a higher average BMI[16] which supports the finding of our study. Higher BMI in individuals with prediabetes compared to normal subjects indicates the relationship between obesity and abnormal glucose regulation. Prolonged P300 latencies in prediabetes compared to normal subjects suggest the presence of cognitive decline in prediabetes state even before developing Type 2 DM. In the study conducted by Luchsinger et al., executive function was found to be worsened in prediabetic stage.[17] Xu et al. concluded in their study that diabetes and prediabetes accelerate the progression from mild cognitive impairment to dementia and the risk effect was found to be more on prediabetes with a hazard ratio of 4.96.[18] Result of the study conducted by Nazaribadie et al. showed significant differences in cognitive function in patients with Type 2 DM and prediabetes when compared with normal subjects.[19] These results support the findings of the present study. Hyperglycemia leads to formation of advanced glycation end-product (AGEs) which can cause degeneration of neurons, glial cells, and myelin sheath.[10,11] AGE-mediated brain injury may be a cause of cognitive decline in prediabetes and Type 2 DM. Increased blood glucose level causes to activation of protein kinase C (PKC). Activated PKC can lead to atherosclerotic changes in the blood vessels which can retard the blood flow to the brain.[3] Hyperglycemia is also proposed to cause end-organ damage by increasing the generation of reactive oxygen species.[10] Combined effect of

Table 1: P300 latencies of Group 1 (Controls) and Group 2 (Cases)

Parameters	Group 1 Mean±SD	Group 2 Mean±SD	P-value
FZ-AR P300	324.80±39.67	385.44±29.43	<0.001*
CZ-AR P300	307.82±33.87	385.85±27.57	<0.001*
PZ-AR P300	308.07±30.08	389.98±48.34	<0.001*
C3-AR P300	302.69±28.55	385.74±22.85	<0.001*
P3-AR P300	306.04±30.07	383.90±22.75	<0.001*

* Significant-*P* value: P<0.05

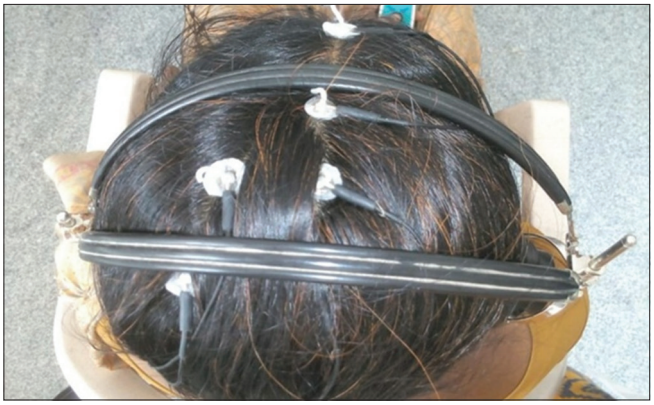

Figure 1: Scalp electrodes in P300 recording

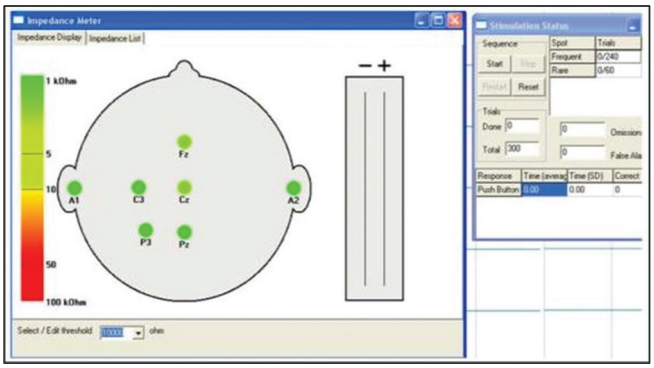

Figure 2: Impedance at scalp-electrode interface for P300

Figure 3: Galileo NT instrument

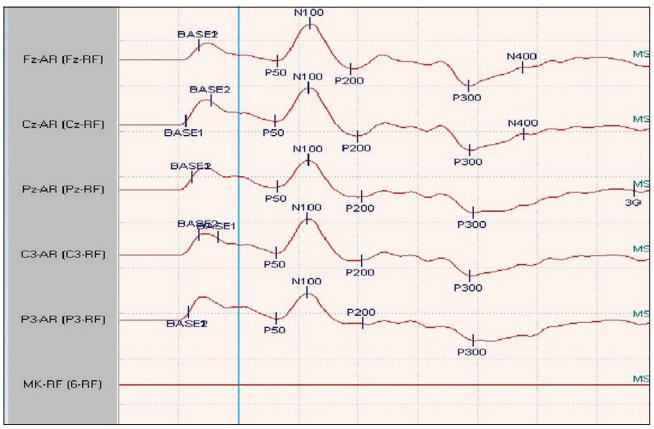

Figure 4: P300 waveforms

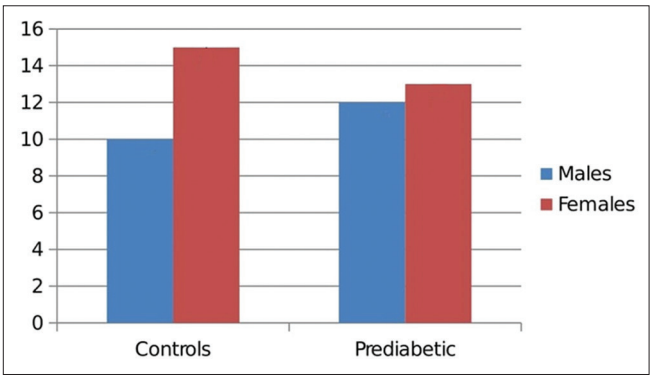

Figure 5: Distribution of the patients according to gender

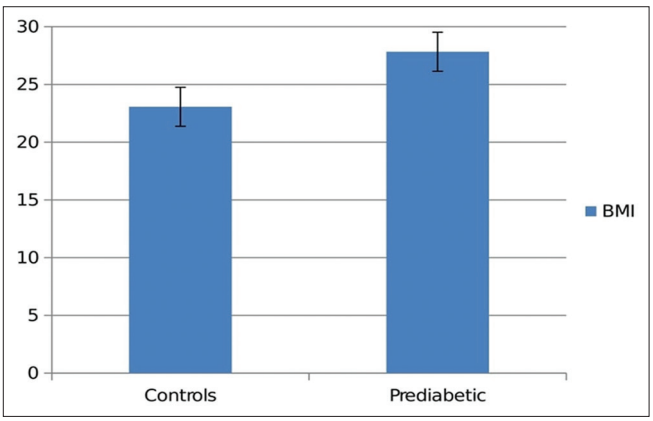

Figure 6: Body mass index in study groups

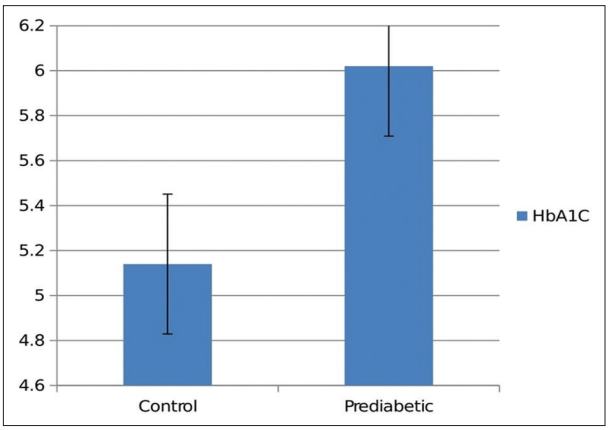

Figure 7: HbA1C level in study groups

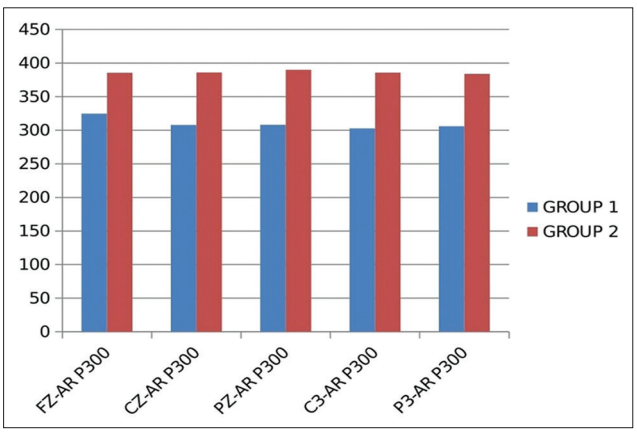

Figure 8: P300 latencies of study groups

all these factors has been postulated to cause neuronal damage in the brain. In the presence of neuronal damage, processing of information may be delayed, leading to impaired cognitive function. Other likely mechanisms of cognitive dysfunction in Type 2 DM are atrophy in the region of hippocampus and amygdala.[20]

Very little is known about the cognitive function of people with prediabetes because of a limited number of studies available in the literature. In the present study, the cognitive function was compared between individuals with prediabetes and normal subjects. Cognition was assessed by electrophysiological test using ERP P300 which is a well-established method for the assessment of cognition. It is important to know the cognitive status in individuals with prediabetes to avoid further deterioration of cognition to maintain a better quality of life. An early onset of cognitive decline in prediabetes stage may have a negative impact on the personal and professional life of an individual. Self-care ability of individuals with prediabetes will be affected due to cognitive impairment which can also hasten the development of DM. All prediabetic patients should be screened for cognitive decline along with other neurological examination at the time of diagnosis and regularly thereafter to improve the quality of life. Limitation of our study was that the severity of cognitive dysfunction could not be assessed. Future studies may be directed toward follow-up of prediabetic people. Effective management of blood glucose level on the cognitive function in prediabetes can be investigated.

CONCLUSION

P300 latencies are increased in individuals with prediabetes compared to normal subjects. Increase in latencies of P300 suggests the existence of cognitive decline in prediabetes stage itself before the development of Type 2 DM.

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