



ORIGINAL ARTICLE

A Comparative Study of the Detection of cAMP response element binding protein (CREB) in the Peripheral Blood of Alzheimer's Patients and the Healthy subjects as a Biomarker for the diagnosis of AlzheimerMasih Falahatian^{1*}, Ahmad Chitsaz¹¹ School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran* Corresponding Author: Masih Falahatian, Email: masih.falahatian@gmail.com

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ABSTRACT

Introduction: Alzheimer's disease is a neurodegenerative disease, which usually helps some biomarkers, such as amyloid proteins, to diagnose the disease. Therefore, the purpose of this study was to compare the expression of a protein binding protein to the adjuvant responder to circular adenosine monophosphate (CREB) in peripheral blood of patients to Alzheimer's and healthy elderly people as a biomarker for diagnosing Alzheimer. **Materials and Methods:** In this case-control study, 32 patients with Alzheimer's disease and 32 normal blood samples were taken. Using real time PCR, CREB expression was evaluated. **Results:** The mean CREB level in the case group was 0.89 ± 0.30 and in the control group was 1.01 ± 0.03 . The mean of BDNF level in the case group was significantly higher than the control group ($P < 0.001$). There was no significant relationship between the level of CREB with age, sex, MMSE score and Cornell scale for depression in dementia ($P > 0.05$). **Conclusion:** Reducing CREB levels in people with Alzheimer's disease can be a factor in diagnosis in comparison to healthy people.

INTRODUCTION

Dementia or cognitive disorder is one of the most important symptoms of Alzheimer's disease (AD) which occurs due to neurodegeneration in the central nervous system. Alzheimer's disease is most often observed in elderly people and as reported, the risk of developing Alzheimer at the age of 65 to 100 in males is 33% and in females is 45% (1). Consequently, the Alzheimer's disease causes many economic loss and health issues for the society.

Various research have suggested that the period of the Alzheimer's disease is a chronic period and neurodegeneration has begun many years before the appearance of the clinical symptoms (2). Moreover, the fact that the process of the illness is prolonged provides the time needed to study the causes and symptoms of the disease. Having known the fact that hyperphosphorylation of the tau proteins, the formation of intracellular neurofibrillary coils and formation of beta-amyloid plaques in extracellular space are the main reasons of the long period of the development of Alzheimer's disease (3,4). Considering the long period of the disease, the early diagnosis of the Alzheimer is of utmost importance

and nowadays the researchers try to study and discover novel methods and specifically biomarkers by implementing them we are able to diagnose the disease in its preliminary stages (5).

The binding protein to the responding component to the cyclic adenosine monophosphate (cAMP)-response element binding (CREB) cyclic proteins are a member of proteins the function of them is the growth, differentiation, and survival of vital neurons and are widely spread in the nervous system (6). Various research have shown that CREB has a substantial role in neuronal axonal growth and conduction and also releasing neurotransmitters (7 – 10) which has made this factor an important factor in neurodegeneration disorder pathophysiology and in Alzheimer's disease in specific. In this field, there have been research, which suggest the decrease of this protein in the brain of the individuals diagnosed with Alzheimer (11, 12). In addition, there also have been research with contrary results (13). In spite of the existing strong pieces of evidence on the connection between the alterations in CREB and the pathophysiologic characteristics of the AD, some points have remained unknown. For instance, knowing if the levels of CREB in

the blood flow precisely reflect the same idea about the brain or not and also how are the CREB levels in various peripheral blood cells like endothelial cells and peripheral blood mononuclear cells which synthesize it. Minding the aforementioned matter, the considerable point is that the blood cells and the brain cells have similar genes in 80% of the cases of expression, therefore measuring the intracellular peripheral blood RNA can turn to an important and valid apparatus in detecting and expressing genes which are also expressed in central nervous system (14). According to the limited research about the role of the serum level of CREB in the early stages of Alzheimer's disease and also to the fact that studying the expression of the gene of this protein in peripheral blood helps discover the expression of the CERB gene in the central nervous system, thus in this research project we have tried to achieve benefit from CERB as a biological marker in diagnosing the Alzheimer's disease by measuring the expression of the gene of the CERB protein in peripheral blood of the individuals diagnosed with Alzheimer and comparing them to the healthy people and eliciting its connection with the severance of the symptoms and the duration of being infected to the disease.

MATERIALS AND METHODS

In this case-control study, the population studied are the patients diagnosed with Alzheimer who are selected among the visiting patients to the internal department of Neurosurgery and clinic, and the healthy working personnel of Al Zahra Hospital. The factors to exclude the individuals from the study population for the healthy people include having a record of any psychiatric or neurological disease, or cancer or record of taking any drugs that suppress the immune system. The criteria for including individuals in the study were having the age above 50, being diagnosed by an experienced neurologist with Alzheimer and receiving a lower mark than 24 in mini-mental state examination (MMSE). Moreover, in case of having cancer, being addicted to drugs, taking anti-depression or antipsychotic medicine in the past two months and addiction to alcohol or drugs and having stroke-induced aphasia the individual would be excluded from the study. The data collection method was through a clinical interview with the patient, taking blood sample and studying the gene and investigating gene expression of brain-derived neurotrophic factor (BDNF) using real-time PCR device. Cognitive decline severance was assessed through the MMSE questionnaire which included 11 questions each question of which had a score between 1 to 5 out of the total score of 30. Cognitive decline severance is divided to three severances using this method the score of 0 to 17 of which represents the severe cognitive decline, 18 to 23 represents the mild cognitive decline and 24 to 30 represents no cognitive decline.

The peripheral blood samples (5 ml) in the tubes containing ethylenediaminetetracetic acid (EDTA) are gathered and centrifuged in the room temperature for 35 minutes with the

speed of 1200. Afterwards, the layer containing lymphocyte is collected and is washed twice in magnesium phosphate-calcium buffer saline (PH=7.4). The total RNA is extracted from the lymphocytes. Using the spectrophotometry and electrophoresis gel, quantity and the purity of the extracted RNA is determined. One microgram of a total RNA is reverse transcribed in the form of cDNA using reverse transcription kit. The beta-actin gene is used as a housekeeping gene for normalizing the target gene expression. Oligonucleotide primers are ordered for beta-actin propagation and BDNF gene are also ordered for performing real-time PCR, design and synthesis. The synthesized cDNA is used for all real-time PCR reactions along with SYBR Green I Master Mix (the Genetbio kit manufacturer company from South Korea). Annealing heat is optimized for all primer pairs.

After the collection of data, they were entered in the statistical software SPSS ver. 22, thereafter, the qualitative data were displayed in the form of frequency or percentage and the quantitative data were displayed as mean and standard deviation. In order to determine the normality of the data, Kolmogorov-Smirnov test was used and to compare the qualitative data between groups, Chi Square test and to compare the quantitative data between groups, the independent t test were used. The P-value beneath 0.05 was considered as significance level.

RESULTS

In this research, 32 patients participated in the case group (13 males and 19 females) and 32 individuals took part in the control group (15 males and 17 females). Among the two groups there was no significant difference based on age and sex ($P > 0.05$). The average CREB blood level in the case group was 0.89 ± 0.30 and in the control group, it was 1.01 ± 0.03 , based on the independent t test the average level of BDNF in the case group was significantly more than the control group ($P < 0.001$). In addition, the average score achieved in the MMSE questionnaire was 11.68 ± 6.99 , and the average score achieved in the Cornell scale for depression in dementia questionnaire was 12.21 ± 6.63 (Table 1).

There was no significant difference between the two sexes according to the average CREB blood level ($P = 0.65$). Furthermore, the Pearson correlation indicated that there is no significant correlation between the CREB blood level, age ($P = 0.61$, $r = -0.06$), MMSE questionnaire score ($P = 0.44$, $r = -0.10$) and the score of the Cornell scale for depression in dementia questionnaire ($P = 0.58$, $r = 0.10$).

DISCUSSION

According to what was discussed, finding a marker that can be used for early diagnosis of Alzheimer's disease is of utmost importance. Consequently, in this study, we showed that the amount of the CREB gene expression in the individuals with Alzheimer is significantly less compared to the

Table 1. The variables studied in the study in both case and control groups

Variables	Case	Control	P-value
Age	71.34±5.86	67.50±4.81	0.18
Gender	Male	13 (40.6%)	15 (46.9%)
	Female	(59.4%) 19 19 (59.4%)	(53.1%) 17 17 (53.1%)
Blood Levels of CREB (pg/ml)	0.89±0.30	1.01±0.03	0.001>
Mean of MMSE	11.68±6.99	-	-
Mean of Cornell scale for depression in dementia	12.21±6.63	-	-

normal people. Also, the results achieved through our research coincided with the results of a research published by Pugagenti et al. (11). They proved that the amount of the CREB gene expression in the brain of the rats with Alzheimer decreases. Another research showed that the level of the CREB in the hippocampus and cortical neurons in the brain of the rats with Alzheimer decreases (15). Another research conducted on rats also has reported similar results to the previously mentioned research and coincided with the results achieved through our research (16). It is worth mentioning that the level of the CREB in the brain and in peripheral blood in individuals with Alzheimer can depend on various factors including race and the amount of the progression the disease (17). Although meanwhile, there have been research conducted which represented the increase in the level of CREB in patients with Alzheimer. In a recent research conducted by Platnic et al. (13) it is shown that the activity of CREB in peripheral blood lymphocyte in individuals with Alzheimer has increased. This result has not been in favor of the results achieved throughout the research.

CONCLUSION

Therefore, referring to the studies performed on animal samples cannot be a good comparison criterion against the human samples. The current study is among the few studies which have measured the level of CREB peripheral protein gene in individuals diagnosed with Alzheimer. Due to this fact we can refer in this way that considering the matter in which the blood cells and also the brain cells have similar genes expression in 80 percent of the cases and the received results of this research based on the decrease in CREB peripheral protein gene expression in peripheral blood of individuals diagnosed with Alzheimer, these results can place doubt on the results of its prior made research results. Moreover, it is worth mentioning that based on this idea we can consider the CREB protein as a substantial marker in Alzheimer's disease the decrease in the level of which can be used for early diagnosis of the Alzheimer's disease.

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AUTHOR CONTRIBUTIONS

None

CONFLICT OF INTERESTS

None

ETHICAL STANDARDS

None

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