Is Anti-Cyclic Citrullinated Peptide Antibody a Good Value Biomarker for Alzheimer Disease?

Rokhsareh Meamar[1,2], Gholamreza Askari[3], Majid Ghasemi[1,4], Mohammad Reza Aghaye Ghazvini[5], Sahar Vesal[1], Mostafa Sharifkhah[1], Nazanin Alaei Faradonbeh[6], Leila Dehghani[2]

[1]Isfahan Neurosciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Medical Sciences, Najafabad Branch, Islamic Azad University, Isfahan, Iran, [3]Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Department of Neurology, Medical School, Isfahan University of Medical Sciences, Isfahan Iran, [5]Isfahan Center of Health Research, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran, [6]Young Researchers Club, Najafabad Branch, Islamic Azad University, Isfahan, Iran

Correspondence to:
Ms. Leila Dehghani,
Department of Medical Sciences,
Najafabad Branch, Islamic
Azad University, Isfahan, Iran.
E-mail: L_Dehghani2002@yahoo.com

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ABSTRACT

Background: Alzheimer's disease (AD) is one of the most important neurodegenerative disorder. Anti-cyclic citrullinated peptide (anti-CCP) may all be involved in the development of vascular disease such as AD. The aim of this study is detection of seropositivity for anti-CCP antibody in AD patients.

Methods: In our study, 30 patients with AD and 29 healthy controls (age and-sex matched) were recruited. Homocysteine and anti-CCP was measured by spectrophotometrically and immunoassay.

Results: Mean ± SE anti-CCP was higher significantly between AD (13.6 ± 3) and healthy subjects (4.8 ± 0.2) (P = 0.006). In the patients, anti-CCP serum level was in high range (32.1%) of abnormal levels, but there was no significant difference in serum homocysteine in AD patients compared with controls. There is no correlation between anti-CCP and homocysteine levels in AD patients (P = 0.75), but between age and anti-CCP level observed a significantly correlation (P = 0.04).

Conclusions: It needs more studies to clarify confirmation the role of anti-CCP antibody production in AD patients.

Keywords: Alzheimer`s disease, anti-cyclic citrullinated peptide, homocysteine

Original Article

INTRODUCTION

Alzheimer's disease (AD) is one of the most important neurodegenerative disorder and fifth major cause of death for people above 65 years in the United States.[1] Growing evidence for progressive dementia in this disease, suggests that oxidative stress and micro vascular injury play a critical role in development of AD.[2] Nevertheless, specific serum markers to determine the presence of disease have not been recognized. Recent studies presented that immunological and metabolic laboratory markers, homocysteine, C-reactive protein, tumor necrosis factor alpha and Anti-cyclic citrullinated peptide (anti-CCP) may all be involved in the development of vascular disease such as AD patients.[3,4] Anti-CCP antibody is

can also be detected in different diseases especially, in early rheumatoid arthritis (RA) patients.[5] Satoh *et al*. reported that anti-CCP antibody seems to be a simple and early serologic biomarker for AD among dementia patients.[6] In this study, detection of anti-CCP antibody in eight of the 42 AD in Asian patients was confirmed.[7] Peptidylarginine deiminases (PADs) catalyze arginine deimination into citrulline leading production to citrullinated in a post-translational manner.[7] Expression of citrullinated proteins and PAD2 has been shown in astrocytes, and the hippocampi of AD patients. Considering the acceptable hypothesis, that an accumulation of citrullinated proteins leading to the presence of anti-CCP antibody serum,[1,2] the aim of our study was detection of seropositivity for anti-CCP antibody in AD patients.

METHODS

In this study, 30 patients with AD and 29 healthy controls (age- and sex-matched healthy subjects) were recruited. Diagnosis of AD was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke, and AD and Related Disorders Association.[8]

Patients with a history drug abuse or intoxication, metabolic abnormalities severe head injury, or seizure disorders, and who were treated with electroconvulsive therapy, major depression, cerebrovascular disease, and dementia caused by diseases other than AD were not included in the study.

Measurement of homocysteine, anti-CCP antibody and blood samples were drawn, after more than 12 h of fasting, by standard methods. The serum fraction were separated by centrifugation, as soon as possible, and stored at −20°C for no more than 10 days.

Homocysteine was spectrophotometrically measured by a commercial kit from Axis-Shield Diagnostics Ltd., (Dundee, Scotland). In this method, briefly, homocysteine react with serine to form cystathionine which then is broken down to form homocysteine and pyruvate. Pyruvate is enzymatically converted to lactate and the rate of Nicotinamide adenine dinucleotide (NAD)+ produced during this reaction is directly proportional to the concentration of homocysteine and level over than 20 μmol/L was regarded as abnormal level.

Anti-CCP antibodies were quantitatively measured by a solid phase enzyme immunoassay kit from Aesku diagnostics (Wendelsheim, Germany) and level over than 12 U/ml < was considered as abnormal level.

Statistical analysis

Standard statistical methods (mean, SE) were utilized to summarize parametric values and *t*-test and K-square were employed to compare the control group versus group of patients affected by AD. The difference between variables was assessed by Pearson correlation. The data were analyzed using SPSS 16.0 statistical package (SPSS Inc.). $P \leq 0.05$ was regarded as significant.

RESULT

The study included 29 patients with AD, and 30 healthy controls. As shown in Table 1, mean ± SE anti-CCP was higher significantly between AD (13.6 ± 3) and healthy subjects (4.8 ± 0.2) ($P = 0.006$). In the patients, anti-CCP serum level was in high range (32.1%) of abnormal levels, but there was no significant difference in Serum homocysteine in AD patients compared with controls. Our data identified that homocysteine was detected in 17.9% of AD patients compared with 3.4% healthy subjects. There is no correlation between anti-CCP and homocysteine levels in AD patients ($P = 0.75$) but between age and anti-CCP level observed a significantly correlation ($P = 0.04$).

DISCUSSION

In our study, we observed significantly difference in detection of anti-CCP between AD and control group in small our population. Anti-CCP antibody was found in nine out of 28 AD patients (32.1%), but not detected in none of the normal group. In accordance

Table 1: Summary of the clinical profiles of the Alzheimer disease patients

Variables	AD (*N*=29)	Control (*N*=30)	*P* value
Age (years)	74.5±0.4	71±0.9	0.85
Homocystein (mean±SE)	13.2±1.5	12.7±0.5	0.76
Anticyclic (mean±SE)	13.6±3	4.8±0.2	0.006

with our study, detection of anti-CCP antibody in eight of the 42 AD patients was observed (19.1%) but not detected in any of the 30 patients with other neurological disorders in Japan.[6]

In the other hand, Bodil Roth *et al*. presented 2-3% of AD or multiple sclerosis (MS) with seropositivity of anti-CCP antibody.[9] Anti-CCP antibodies have been identified in patients with different autoimmune disease.[10,11]

The most important pathological event in AD is accumulation of two main abnormal protein aggregates, senile plaques and neurofibrillary tangles in hippocampus and cerebral cortex.[12]

In the other hand, levels of PAD2 are more than three-fold higher in the hippocampus than in the cortex of rat brains.[13] Under hypoxic conditions, PAD2 activates and citrullinates various cerebral proteins.[14]

In addition, abnormal expression of citrullinated proteins and PAD2 especially in the hippocampus of AD has been presented[6]; All of the collected data suggest the involvement of citrullinated protein in human neurological disorder.[7] Five different types of PADs have been recognized in mammals.[15]

The importance of PAD2 in AD[6] and PAD4 in RA patients in production of anti-CCP has been suggested.[16]

In meta-analysis, a strongly positive association was observed between PADI4 and RA not only in the Japanese, but also in European population.[17] CCP antibody positive represents more severe damage than who seronegative for CCP antibody.[18] However, in our study only a correlation between CCP antibody titer and age was seen. Recently, the role of PAD4 in MS brain was illustrated, leading to destabilisation of myelin protein.[19] Nevertheless, in some studies anti-CCP antibody was not detected in sera from MS patients.[6,3] Another biomarker such as elevation of plasma total homocysteine is considered a potential risk-factor for AD.[20]

However, recently Nilsson *et al*. suggested that that plasma homocysteine is not primarily concerned in the pathogenesis of AD rather other main determinant influence plasma homocysteine in AD patients. [21] Furthermore, in our study didn't observe significantly difference in homocysteine level between patients and control. Further laboratory examination with large number of patients is necessary to clarify confirmation role of anti-CCP antibody production in AD patients.

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