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Original Article

Study on the changes in serum C-reactive protein and amyloid protein levels in elderly patients with suspected cognitive impairment

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Abstract: Objective: To study the changes in serum C-reactive protein and plasma amyloid protein levels in suspected Alzheimer's disease (AD) patients. Patients and methods: Sixty patients that likely had AD according to preliminary diagnosis were selected as the observation group, while 60 healthy patients who did not have AD were selected as the control group. General patient characteristics, serum C-reactive protein (CRP), plasma amyloid beta Aβ1-40 and Aβ1-42 levels were observed in both groups. The 60 patients in the observation group were divided into a progressive group (n=13) and a non-progressive group (n=47) according to disease progression. General patient characteristics, CRP, plasma Aβ1-40 and Aβ1-42 levels were compared between the two groups, and risk factors of disease progression were analyzed. Results: There were significant differences in serum CRP and Aβ1-42/Aβ1-40 between the two groups (all P<0.001). In terms of education level, mini-mental state examination (MMSE) score, Aβ1-42, Aβ1-40, and Aβ1-42/Aβ1-40, the non-progressive group had higher levels than the progressive group, while the non-progressive group had significantly lower CRP than the progressive group (P<0.05). Logistic regression analysis of the involved factors showed that two indicators, Aβ1-42/Aβ1-40 and CRP, could be considered risk factors for disease progression. Conclusion: Serum CRP levels were high and Aβ1-42/Aβ1-40 ratio was low in suspected AD patients and both could be used as risk factors for disease progression in suspected AD patients.

Keywords: Plasma amyloid beta 1-40, plasma amyloid beta 1-42, Alzheimer's disease, serum C-reactive protein, amyloid protein levels, risk factors

Introduction

Alzheimer's disease (AD) is one of the most common types of senile dementia, accounting for 60-80% of all senile dementia, and the incidence of AD increases with the age of the patients. The morbidity is 5-10% in patients over 65 years old, while in patients over 85 years old, the morbidity increases to 20-50%; also, female patients have higher morbidity than male patients [1, 2]. Due to the high disease morbidity rates, AD has caused great harm to human health, and the prevention and treatment of the disease has also brought heavy economic and social burdens [3]. The pathogenesis of AD is still not clear. Many studies on the pathogenesis of AD have found that it is related to gene mutations, plasma amyloid beta (Aβ) deposition, tau protein phosphorylation, cell oxidation, cell apoptosis, cell injury and other factors [4, 5], and the Aβ toxicity hypothesis remains dominant in all pathogenesis studies [6]. Moreover, the onset of AD is relatively insidious and early stages are often clinically neglected. Studies have shown that in the preclinical and early clinical stages of AD, although pathophysiological changes have begun to occur, no significant changes are observed in biochemical markers detected in blood or cerebrospinal fluid [7]. Therefore, it is

a new research direction to seek biochemical markers with high sensitivity and specificity for early diagnosis of AD.

In recent years, the role of inflammatory cytokines in the development and progression of AD has also been studied in depth. Microglia make up 10% of all cells in the nervous system. After nervous system injury caused by disease or trauma, a large number of microglial cells are activated. The role of activated microglia is similar to that of macrophages, and in healthy patients is to aid in the clearance and phagocytosis of damaged or dead cells, while in AD patients, their main role is to clear Aβ deposition [8, 9]. In addition, studies on AD patients and AD model rats have found that microglia release a large number of inflammatory cytokines around Aβ plaques, including interleukin-1, interleukin-6, interleukin-10, and tumor necrosis factor [10, 11], and may have damaging effects on peripheral neurons [12].

In the present study of Aβ-induced AD, Aβ1-40 and Aβ1-42 were found to play major roles. However, serum C-reactive protein (CRP) is the most frequently detected inflammatory cytokine in the clinic [13]. This protein is synthesized in the liver in response to interleukin-6 and other inflammatory factors [14]. Both stable expression of Aβ in cerebrospinal fluid, and significantly decreased expression in the cerebrospinal fluid due to deposition in brain tissue in AD patients can help guide clinicians in the diagnosis of AD [5]. However, cerebrospinal fluid testing cannot be easily performed in clinical diagnosis of early stage AD patients. In order to seek better clinical indicators and provide a simpler test method for early clinical diagnosis, this study will detect plasma Aβ1-40, Aβ1-42, Aβ1-40/Aβ1-42 and serum CRP to provide a more extensive clinical basis for the diagnosis of early suspected AD in patients [15].

Materials and methods

Clinical data

This study was approved by the Sichuan Provincial People's Hospital ethics committee. Sixty patients admitted to the neurology department of Sichuan Provincial People's Hospital from January 2016 to December 2017 with preliminary diagnosis of AD were selected as the observation group, and 60 healthy patients excluded from AD diagnosis during the same period were selected as the control group. All patients were between 50 and 80 years old and signed and an informed consent form.

Inclusion criteria

(1) The diagnostic criteria for suspected AD patients refers to the 2011 National Institute on Aging and Alzheimer's Association diagnostic guidelines [16]. (2) All the included patients were assessed with mini-mental state examination (MMSE) to evaluate their mental state [17]. The scoring system has a total score of 30 out of 19 items. If the subject's MMSE score is less than 24 points, the patient can be considered to have dementia or other diseases that impair cognitive function. (3) The patients were between 50 and 80 years old.

Exclusion criteria

(1) Patients with cardiopulmonary insufficiency; (2) Patients with malignant tumors; (3) Patients with incomplete clinical data; (4) Patients with a history of brain trauma or surgery.

Methods

Blood test evaluation: For each patient, two 5 mL tubes of venous blood were collected. The collected blood samples were stored in a sterile ethylenediamine tetra acetic acid (EDTA) tubes. After 15 minutes of storage at 4°C in the refrigerator, serum and plasma were separated using a centrifuge at a speed of 3300 RPM. The isolated plasma was added to a phosphate buffer solution with 40 μL of protease inhibitor and stored in the freezer at -80°C, while the serum was used for the determination of serum CRP by immunoturbidimetric assay. Enzyme linked immunosorbent assay (ELISA) was used to determine Aβ1-40 and Aβ1-42 in the plasma. The above two kits were purchased from Ke Lei Biological Technology Co., Ltd., Shanghai, China. The instrument used to complete the testing was the Beckman automatic biochemical analyzer (Beckman company, USA).

Follow-up: Sixty patients in the observation group were followed up by telephone or outpatient follow-up for one year, and changes in the patients' conditions and the number of cases that progressed to AD were recorded. AD diagnosis was based on the ICD-10 diagnostic criteria [18]. Patients were divided into a progressive group and a non-progressive group ac-

Table 1. Comparison of general data and baseline data between the two groups

Item	Observation group (n=60)	Control group (n=60)	X^2/t	P
Gender (male/female)	33/27	28/32	0.834	0.361
Age (years old)	68.6±9.1	68.3±8.3	0.177	0.860
Education level (years)	8.17±3.05	8.97±2.65	0.534	0.128
MMSE score	23.07±2.20	27.42±1.79	11.884	<0.001
Combined disease				
Diabetes	35	31	0.539	0.463
Hypertension	39	33	1.250	0.264
Psychiatric history	3	1	0.259	0.611

Note: MMSE, mini-mental state examination.

Table 2. Comparison of serum CRP and plasma Aβ1-40 and Aβ1-42 between the two groups

Item	Observation group (n=60)	Control group (n=60)	X^2/t	P
CRP (mg/L)	6.68±1.83	4.72±1.40	6.612	<0.001
Aβ1-42 (ng/L)	18.05±4.83	19.55±3.37	1.971	0.051
Aβ1-40 (ng/L)	45.15±10.60	41.78±9.30	1.850	0.067
Aβ1-42/Aβ1-40	0.40±0.029	0.47±0.042	11.668	<0.001

Note: CRP, serum C-reactive protein; Aβ1-40, plasma amyloid beta 1-40; Aβ1-42, plasma amyloid beta 1-42.

cording to whether the disease progressed to AD.

Statistical methods

SPSS 22.0 statistical software was used in this study. Continuous variables were represented as mean ± standard deviation ($\bar{x}$ ± sd). Data with a normal distribution and homogeneity of variance were tested using the t-test, and those that did not have a normal distribution and homogeneity of variance were tested using the rank sum test. Enumeration data were expressed in % and subjected to the Pearson chi-square test. Taking whether the disease progressed to AD as the dependent variable; age, gender, education level, MMSE score, Aβ1-42, Aβ1-40, Aβ1-42/Aβ1-40, CRP, and coexisting diseases such as hypertension, diabetes, and mental illness as independent variables; the risk factors for disease progression were analyzed using Logistic regression analysis. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value. P<0.05 was considered statistically significant.

Results

Comparison of general data

There was no significant difference in gender, age or education level between the two groups (all P>0.05). The MMSE scores of patients in the observation group were significantly lower than those in the control group (P<0.001, **Table 1**).

Comparison of serum CRP and plasma Aβ1-40 and Aβ1-42 between the two groups

Serum CRP in the observation group was significantly higher than that in the control group (P<0.001). The Aβ1-42/Aβ1-40 ratio in the observation group was significantly lower than in the control group (P<0.001). No statistical difference was seen between the two groups in Aβ1-42 and Aβ1-40 levels (P>0.05, **Table 2**; **Figures 1** and **2**).

Comparison of general data between patients in the progressive group and those in the non-progressive group

By the 1-year follow-up, 13 of the 60 patients in the observation group had progressed to AD. After comparing the non-progressive group with the progressive group, we found that there were no statistically significant differences between the two groups in terms of gender, age, and coexisting disease. The non-progressive group was higher than the progressive group in terms of education level, MMSE score, Aβ1-42, Aβ1-40 and Aβ1-42/Aβ1-40, while the non-progressive group was lower than the progressive group in terms of CRP, with statistically significant differences (all P<0.05, **Table 3**).

Analysis of risk factors for disease progression in patients

Taking whether the disease progressed to AD as the dependent variable, and age, gender, education level, MMSE score, Aβ1-42, Aβ1-40, Aβ1-42/Aβ1-40, CRP, and coexisting diseases such as hypertension, diabetes, and mental ill-

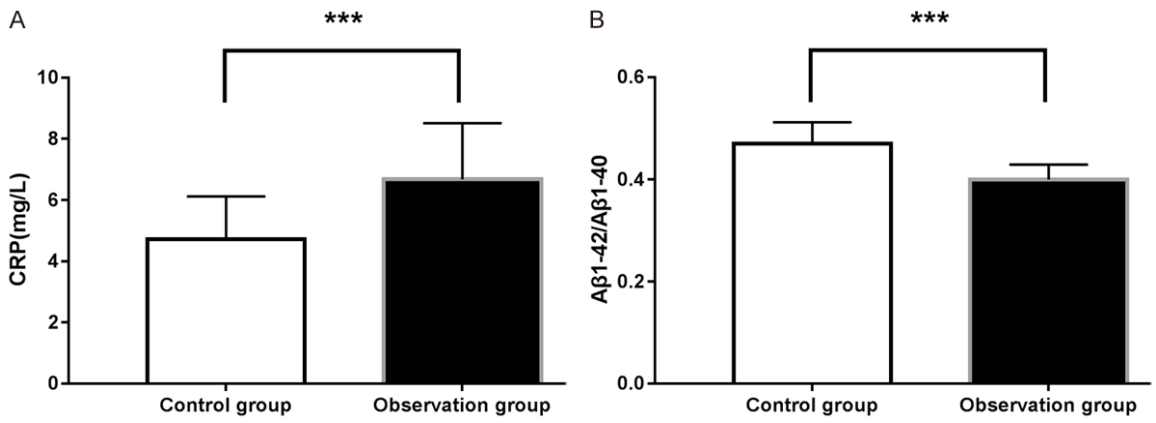

Figure 1. Comparison of CRP and Aβ1-42/Aβ1-40 between the two groups. A. Comparison of CRP between the two groups; B. Comparison of Aβ1-42/Aβ1-40 between the two groups ***P<0.001. CRP, serum C-reactive protein; Aβ1-40, plasma amyloid beta 1-40; Aβ1-42, plasma amyloid beta 1-42.

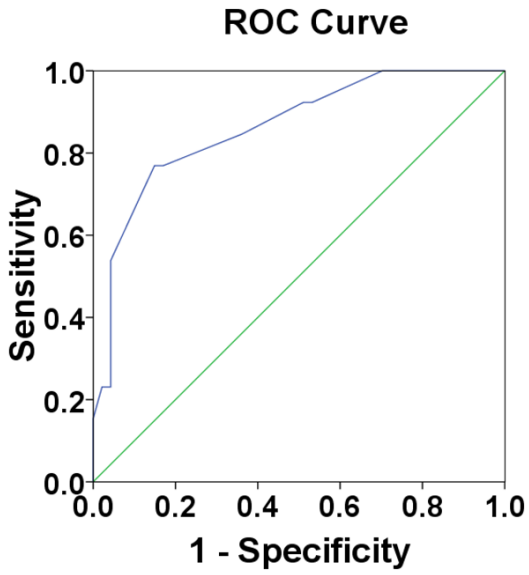

Figure 2. The ROC diagnostic curve of Aβ1-42/Aβ1-40. The green line is 50% reference line and the blue line is ROC curve. ROC, receiver operating characteristic; Aβ1-40, plasma amyloid beta 1-40; Aβ1-42, plasma amyloid beta 1-42.

ness as independent variables, logistic regression analysis was performed on the factors involved. The results showed that the OR values of Aβ1-42/Aβ1-40 and CRP were greater than 1 and P was less than 0.05, so these were considered risk factors for disease progression (**Table 4**).

ROC curve of Aβ1-42/Aβ1-40 for disease progression

The ROC curve was further used to evaluate the predictive value of Aβ1-42/Aβ1-40 for AD diagnosis. The area under the ROC curve of Aβ1-42/Aβ1-40 for patients with AD was 0.863 and the sensitivity and specificity were 0.769 and 0.830, respectively (**Figure 2**).

ROC curve of CRP for disease progression

The ROC curve was used to evaluate the predictive value of CRP for AD diagnosis, and it was found that the area under the ROC curve of CRP for patients that had progressed to AD was 0.812, with a sensitivity of 0.979 and a specificity of 0.692 (**Figure 3**).

Discussion

With an increase in age, the morbidity of AD also increases, thus the prevention and treatment of this disease has been a heavy burden on society [3]. At present, treatment of this disease mainly aims to delay disease progression and improve the clinical symptoms of the patients [19]. Studies on the pathogenesis of AD have found that the main pathological changes are as follows: (1) Aβ is deposited on nerve tissue and gradually forms senile plaques [20, 21]. (2) Tau protein is phosphorylated and aggregates in the cell to form neurofibrillary tangles [22, 23]. At present, the Aβ theory is still dominant. In previous studies, Aβ has been shown to play a critical role in the development of AD [24, 25]. In the course of research on Aβ, drugs targeting Aβ are widely used in the clinical treatment of AD.

Previous studies have shown that the expression of Aβ is decreased in the cerebrospinal fluid of AD patients [5]. However, cerebrospinal

Table 3. Comparison of general data between patients in the progressive group and those in the non-progressive group

Item	Non-progressive group (n=47)	Progressive group (n=13)	X^2/t	P
Gender (male/female)	26/21	7/6	0.009	0.925
Age (years old)	68.2±10.0	68.1±7.1	0.043	0.966
Education level (years)	8.57±2.92	6.69±3.15	2.021	0.048
MMSE score (points)	24.62±1.97	22.08±1.85	4.162	<0.001
Aβ1-42 (ng/L)	19.26±4.39	13.69±3.86	4.154	<0.001
Aβ1-40 (ng/L)	47.17±9.87	37.85±10.25	2.991	0.004
Aβ1-42/Aβ1-40	0.41±0.013	0.36±0.043	12.622	0.002
CRP (mg/L)	6.23±1.49	8.31±2.06	4.072	<0.001
Combined disease				
Diabetes	28	7	0.137	0.711
Hypertension	31	8	0.087	0.767
Psychiatric history	2	1	0.000	1.000

Note: MMSE, mini-mental state examination; CRP, serum C-reactive protein; Aβ1-40, plasma amyloid beta 1-40; Aβ1-42, plasma amyloid beta 1-42.

Table 4. Analysis of risk factors for disease progression in patients

Factors	OR	95% CI	P
Gender	1.115	0.631-1.597	0.927
Age	1.031	0.879-1.724	0.421
Education level	0.203	0.541-1.294	0.387
MMSE score	1.541	0.500-438.201	0.506
Aβ1-42	5.282	0.000-1.177	0.057
Aβ1-40	1.762	0.804-42.242	0.081
Aβ1-42/Aβ1-40	2.484	2.158-2.874	0.002
CRP	1.085	1.201-7.288	0.018
Diabetes	0.276	0.103-0.579	0.127
Hypertension	0.502	0.212-0.847	0.598
Psychiatric history	1.098	0.856-1.935	0.725

Note: MMSE, mini-mental state examination; CRP, serum C-reactive protein; Aβ1-40, plasma amyloid beta 1-40; Aβ1-42, plasma amyloid beta 1-42.

fluid examination cannot be performed well in the early stage diagnosis and exclusion of suspected patients, so we are looking for more convenient biomarkers. Previous studies on plasma Aβ1-40 and Aβ1-42 have found that plasma Aβ1-40 and Aβ1-42 are present at high levels in patients who are developing AD and these factors are associated with increased disease risk [26]. On the other hand, recent studies have found that Aβ1-40 (46.76±10.10) and Aβ1-42 (16.92±5.70) are decreased in plasma from patients with AD, and their ratio (0.37±0.11) was also decreased [27]. Combining the results of several studies, it was found that plasma levels of Aβ1-40 and Aβ1-42 in patients with mild cognitive impairment could be increased, unchanged or decreased [26, 28, 29], but studies on the ratio of Aβ1-42/Aβ1-40 showed that the ratio (0.42±0.13) decreased in patients with mild cognitive impairment, and the ratio (0.38±0.12) decreased more significantly in patients with AD [29]. The role of inflammatory cytokines in the development and progression of AD has been a research hotspot in recent years. Studies have found that a variety of inflammatory cytokines are increased in patients with AD, and neuroinflammatory reactions occur in patients with AD. During the inflammatory response, microglia and astrocytes are over-activated and can produce toxic substances, such as the inflammatory cytokine interleukin-6, that damage neurons, thus leading to neuron degeneration and apoptosis [30]. In early stage AD patients, plasma interleukin-6 expression is increased [31]. However, in another study, there was no difference in serum interleukin-6 expression between patients with mild cognitive impairment and normal subjects [32]. CRP is a protein synthesized by the liver in response to interleukin-6 and other inflammatory factors, and is a commonly used clinical indicator [14]. This study found that there was no significant difference in plasma Aβ1-40 and Aβ1-42 between suspected AD patients and the normal control group, that serum CRP in the observation group was higher than that in the control group, and that the Aβ1-42/Aβ1-40 ratio in the observation group was lower than that in the control group, which is consistent with the prior studies discussed above.

MMSE evaluation scale used in this study is used for intelligence status assessment in monitoring disease progression and is the most influential clinical tool used to evaluate dementia at present. However, this scale has some degree of error due to the influence of patient education level [33]. In this study, patients with and without disease progression to AD were further analyzed, and it was found that there

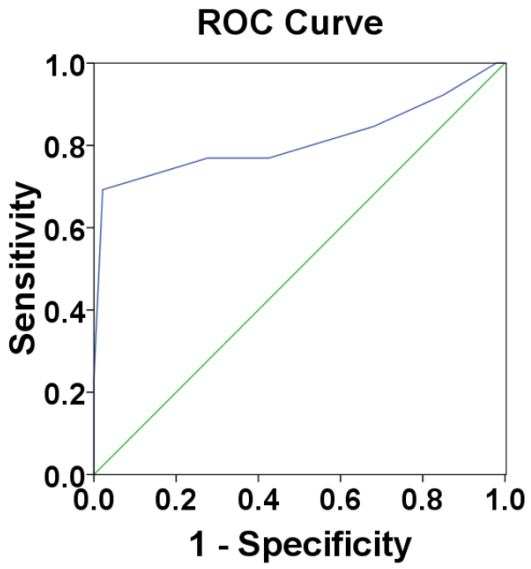

Figure 3. The ROC diagnostic curve of CRP. The green line is 50% reference line and the blue line is ROC curve. ROC, receiver operating characteristic; CRP, serum C-reactive protein.

were differences in education level and MMSE between these two patient groups. However, the education level and MMSE could not be used as risk factors for disease progression after multivariate analysis because the influence of education level introduces error in the scale. However, previous studies have found that Aβ is deposited in the brain and is closely related to the progression of cognitive impairment [34]. Another study found that the ratio of Aβ1-42/Aβ1-40 was 0.40±0.13 in patients with mild cognitive impairment, while that of AD patients was 0.36±0.14, indicating the degree of cognitive decline was proportional to the degree of Aβ1-42/Aβ1-40 ratio decline [35]. In this study, we found that although the plasma levels of Aβ1-42 and Aβ1-40 in the progressive group were significantly lower than those in the non-progressive group, multivariate analysis showed that these two factors could not be used as risk factors for disease progression. However, analysis of Aβ1-42/Aβ1-40 showed that the ratio in the progressive group was significantly lower than that in the non-progressive group, and multivariate analysis suggested that the ratio of Aβ1-42/Aβ1-40 could be used as a risk factor for disease progression. Previous studies examining CRP have found that serum CRP is increased in AD patients and correlated with cognitive function decline [36]. Our study found that serum CRP level in suspected AD patients was significantly higher than that in the normal control group. In addition, serum CRP of patients that didn't progress to AD was lower than that of patients that did progress to AD, and multivariate analysis suggested that CRP could be used as a risk factor to predict disease progression. The ROC curve was further used to evaluate the predictive value of Aβ1-42/Aβ1-40 and CRP indicators, and it was found that both of them had good diagnostic value, which was consistent with the above studies.

Deficiency and prospect: the sample size of this study was small and will need to be further expanded in future research. In this study, the follow-up time was short and there were many external influencing factors, so it will also be necessary to increase the follow-up time in prospective studies.

In conclusion, serum CRP levels are higher in suspected AD patients and the Aβ1-42/Aβ1-40 ratio is lower than in normal controls. Serum CRP and Aβ1-42/Aβ1-40 can be used as risk factors for disease progression in suspected AD patients.

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Disclosure of conflict of interest

None.

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