

Complement factor H levels are decreased and correlated with serum C-reactive protein in late-onset Alzheimer's disease

Os níveis de fator H do complemento estão diminuídos e correlacionados com a proteína C-reativa sérica na doença de Alzheimer de início tardio

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia. Despite numerous studies on the subject, the pathologies for AD are still unclear and there is still no ideal biomarker for diagnosis. The present study aimed to investigate clinical significance of human complement factor H (CFH) in patients with late-onset AD. **Methods:** The present prospective study included 187 late-onset AD patients who went to our hospital from January 2015 to December 2017. One hundred patients with mild cognitive impairment (MCI) and 80 healthy individuals who were age and gender matched to AD patients were enrolled as controls. Demographic data such as age, gender, and education duration were recorded. Blood samples were collected and serum levels of C-reactive protein (CRP), CFH, and brain-derived neurotrophic factor (BDNF) were determined by Enzyme-linked immunosorbent assay (ELISA). The mini-mental state examination (MMSE) score was measured for all patients. **Results:** No significant difference was found in age, gender, and education duration for all participants. The MMSE scores showed AD patients had lower MMSE scores than the other two groups. All factors of CFH, CRP, and BDNF were dramatically decreased in AD patients compared with the MCI and the healthy control. Levels of CFH were found to be positively correlated with levels of CRP; however, no significant correlation was found between CFH and BDNF, nor CFH and MMSE. **Conclusion:** CFH was decreased in late-onset AD patients, and serum levels of CFH was correlated with serum levels of CRP, but not MMSE and BDNF. These results may provide more clinical evidences for the role of CFH in AD patients.

Keywords: complement factor H; late-onset Alzheimer's diseases; C-reactive protein.

RESUMO

A doença de Alzheimer (DA) é a causa mais comum de demência. Apesar de inúmeros estudos sobre DA, suas patologias ainda não são claras e ainda não existe um biomarcador ideal para o diagnóstico da condição. O presente estudo teve como objetivo investigar a significância clínica do fator H do complemento humano (CFH) em pacientes com DA de início tardio. **Métodos:** O presente estudo prospectivo incluiu um total de 187 pacientes com DA de início tardio que foram ao nosso hospital entre janeiro de 2015 e dezembro de 2017. Cem pacientes com comprometimento cognitivo leve (CCL) e 80 indivíduos saudáveis com idade e sexo pareados com pacientes com DA foram incluídos como controle. Dados demográficos como idade, sexo e duração da educação foram registrados. As amostras de sangue foram coletadas e os níveis séricos de proteína C-reativa (PCR), CFH e fator neurotrófico derivado do cérebro (BDNF) foram determinados pelo ensaio imunoabsorvente ligado à enzima (ELISA). O escore do miniexame do estado mental (MEEM) foi medido para todos os pacientes. **Resultados:** Não foram encontradas diferenças significativas em idade, sexo e duração da educação para todos os participantes. Pacientes com DA tinham os menores escores de MEEM em relação aos outros dois grupos. Todos os fatores de CFH, PCR e BDNF diminuíram drasticamente em pacientes com DA em comparação com o CCL e o controle saudável. Os níveis de CFH mostraram correlação positiva com os níveis de PCR; no entanto, não foi encontrada correlação significativa entre CFH e BDNF, nem CFH e MEEM. **Conclusão:** A CFH diminuiu nos pacientes com DA de início tardio e os níveis séricos de CFH foram correlacionados com os níveis séricos de PCR, mas não o MEEM e o BDNF. Esses resultados podem fornecer mais evidências clínicas do papel da CFH em pacientes com DA.

Palavras-chave: fator H do complemento; doença de Alzheimer de início tardio; proteína C-reativa.

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Alzheimer's disease (AD), the most common cause of dementia, is an age-related neurodegenerative disorder which influences millions of people globally, especially for the elderly¹⁻³. Despite numerous studies on AD within more than a century time, the pathologies for AD are still unclear^{4,5}. Moreover, there is still no ideal biomarker for AD diagnosis^{6,7}.

Many factors are found to express abnormally in AD patients or animals, such as tumor necrosis factor (TNF)- α ⁸, brain-derived neurotrophic factor (BDNF)⁹, homocysteine¹⁰, and C-reactive protein (CRP)¹¹. BDNF and CRP are both widely studied factors in AD development. Lower BDNF levels were found in AD patients, higher BDNF serum levels were correlated with slower cognitive decline in AD patients¹². For CRP, lower levels of CRP were observed in AD patients than the healthy¹³. Besides, among the factors, the human complement factor H (CFH), which is considered to play an important role in the stimulation of pro-inflammatory responses, has been noticed in AD development^{14,15}. Animal researches showed CFH was down-regulated in AD models¹⁶; however in clinical studies, the results are still controversial¹⁷. Some researches indicate CFH is abnormally expressed in AD, however others showed no significant difference was found^{15,18}. Similar dispute is also found in levels of CRP. Despite the findings that CRP was decreased in AD patients, a recent study provided opposite results^{19,20}.

In the present study, we aimed to investigate levels of CFH in AD patients, as well as its correlation with CRP, BDNF, and MMSE. These results might give more clinical evidences for the role of CFH in AD patients, as well as provide some new research directions for mechanisms of AD development.

METHODS

Patients

This prospective study included 187 late-onset AD patients who went to the Outpatient Department of Neurology in Dezhou People's Hospital from January 2015 to December 2017. All patients who met the inclusion criteria below were consecutively enrolled during the study period. The inclusion criteria were: patients ≥ 65 years and patients diagnosed with AD according to criteria of National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)²¹. Additionally, 100 patients with mild cognitive impairment (MCI) and 80 healthy individuals who were age and gender matched to AD patients were enrolled as controls according to NINCDS-ADRDA criteria. Patients with other severe system diseases such as severe liver, renal, heart diseases, severe inflammation, and other brain diseases like trauma, psychiatric diseases, were excluded. Written informed consent was obtained from all patients. The present study was approved by ethic committee of Dezhou People's Hospital.

Data collection and measurement

Demographic data such as age, gender, and education duration were recorded. Briefly, fasting venous blood samples (5 mL) were collected in tubes without EDTA to obtain serum within 24 hours of admission. The supernatant was obtained after centrifugation with $1500 \times g$ for 10 min, at 4°C, and then the supernatants were harvested and stored at -70°C. Serum levels of CRP (ab99995, sensitivity < 2 pg/mL, Abcam, USA), CFH (LS-F21748, sensitivity 23.438 ng/mL, LifeSpan Bioscience, USA), and BDNF (ab99978, sensitivity < 80 pg/mL, Abcam, USA) were determined by Enzyme-linked immunosorbent assay (ELISA) using commercial ELISA kits according to manufacturer's instructions. The mini-mental state examination (MMSE) score was measured for all patients.

Statistical analysis

The measurement data was expressed by mean \pm SD. The normality of data was confirmed by both Kolmogorov-Smirnov and Shapiro-Wilk tests. Chi-square test was used to compare the rates. Comparisons for continuous data among three groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Pearson's analysis was performed for correlation between different factors. A $p < 0.05$ was considered as statistically significant. All calculations were made using SPSS 20.0.

RESULTS

Basic clinical characteristics for all patients

In the present study, 187 late-onset AD patients were included with mean age 75.2 ± 6.1 , male: female 101:86, and mean education duration 6.6 ± 2.7 . One hundred MCI patients and 80 healthy individuals were regarded as controls. No significant difference was found in age, gender, and education duration for all participants. The MMSE scores showed AD patients had the lowest MMSE scores than the other two groups ($p < 0.05$, Table 1).

CFH was decreased in late-onset AD patients

To investigate alteration of CFH in AD patients, serum levels of CFH, as well as CRP and BDNF, were determined using ELISA. Results showed the CFH levels were significantly decreased in late-onset AD patients compared with the MCI and the healthy control ($p < 0.05$, Table 2 and Figure 1). Similarly, both CRP and BDNF levels were dramatically decreased in AD patients compared with the other two groups ($p < 0.05$). These results indicated serum levels of all CFH, BDNF, and CRP were decreased in late-onset AD patients.

Serum levels of CFH was correlate with levels of CRP but not MMSE and BDNF

To further study relationship among CFH, CRP, BDNF, and MMSE, Pearson's analysis was performed. As shown in

Table 1. Basic clinical characteristics for all patients.

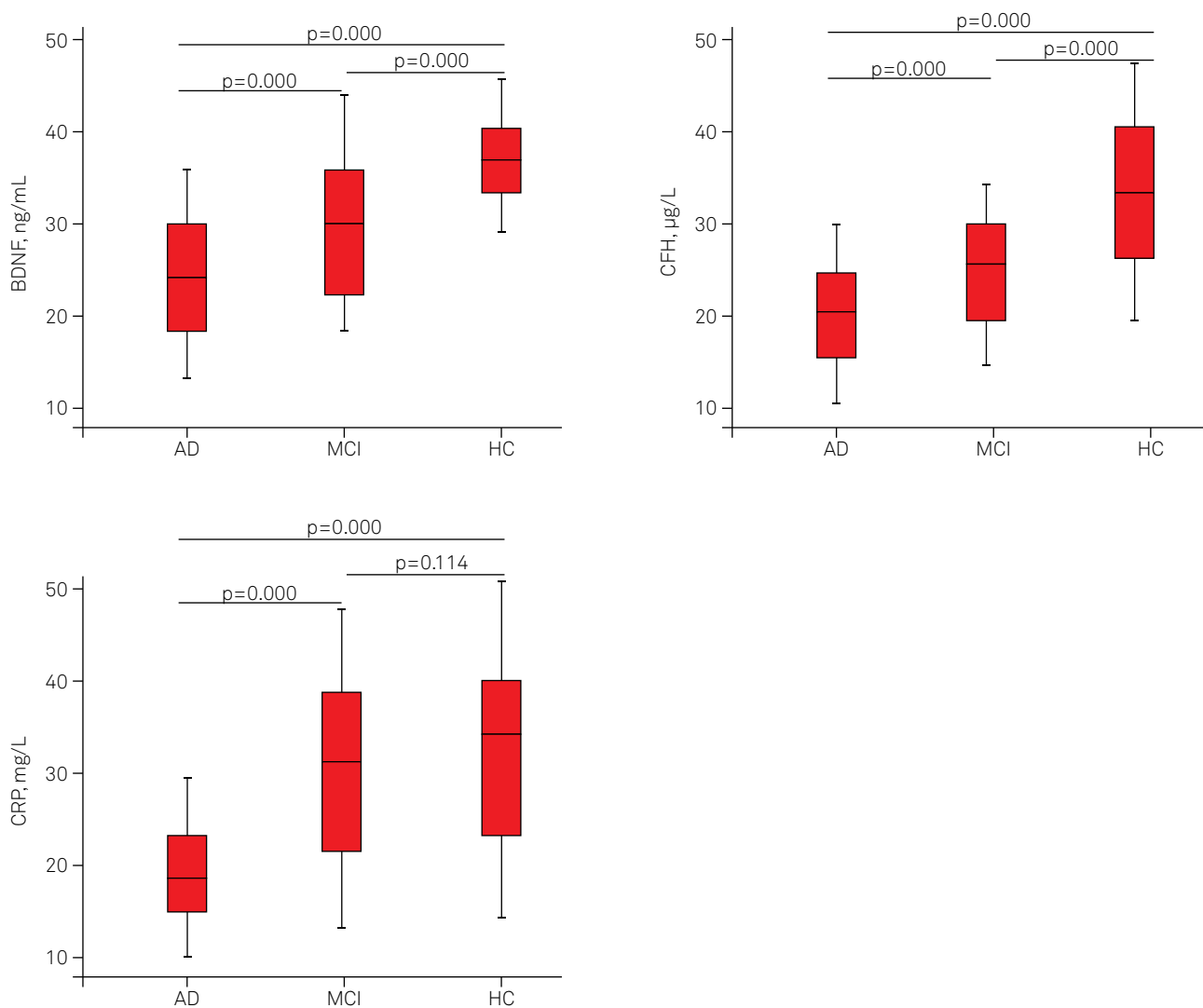
Variables	AD, n=187	MCI, n=100	Healthy, n=80	p-value#
Age, year	75.2±6.1	75.3±5.7	74.9±5.4	0.920
Gender, male: female	101:86	60:40	48:32	0.611
Education duration	6.6±2.7	6.3±3.0	6.2±2.8	0.426
MMSE	19 (11~27)*	27 (25~30)*	29 (28~30)	0.000

AD: Alzheimer's disease; MCI: mild cognitive impairment.*p<0.05, compared with the healthy control; #chi-square test was used to compare the gender, and comparisons for continuous data among three groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test.

Table 2. Serum levels of CFH, BDNF, and CRP in different groups.

Variables	AD, n=187	MCI, n=100	Healthy, n=80	p-value
CFH, µg/L	481.0±97.4	568.9±101.9	712.8±146.6	0.000
BDNF, ng/mL	24.4±6.9	29.6±7.7	36.9±4.6	0.000
CRP, mg/L	1.8±1.0	4.0±2.0	4.5±2.0	0.000

CFH: complement factor H; CRP: C-reactive protein; BDNF: brain-derived neurotrophic factor. Comparisons for continuous data among three groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test.



CFH: complement factor H; CRP: C-reactive protein; BDNF: brain-derived neurotrophic factor; AD: Alzheimer's disease; MCI: mild cognitive impairment.

Figure 1. Serum levels of CRP, CFH, and BDNF in AD, MCI patients and the control.

Figure 2, levels of CFH were found to be positively correlated with levels of CRP ($p < 0.05$). However, no significant correlation was found between CFH and BDNF, nor CFH and MMSE, suggesting CFH and CRP might have deeper interaction, which needs more studies to confirm.

DISCUSSION

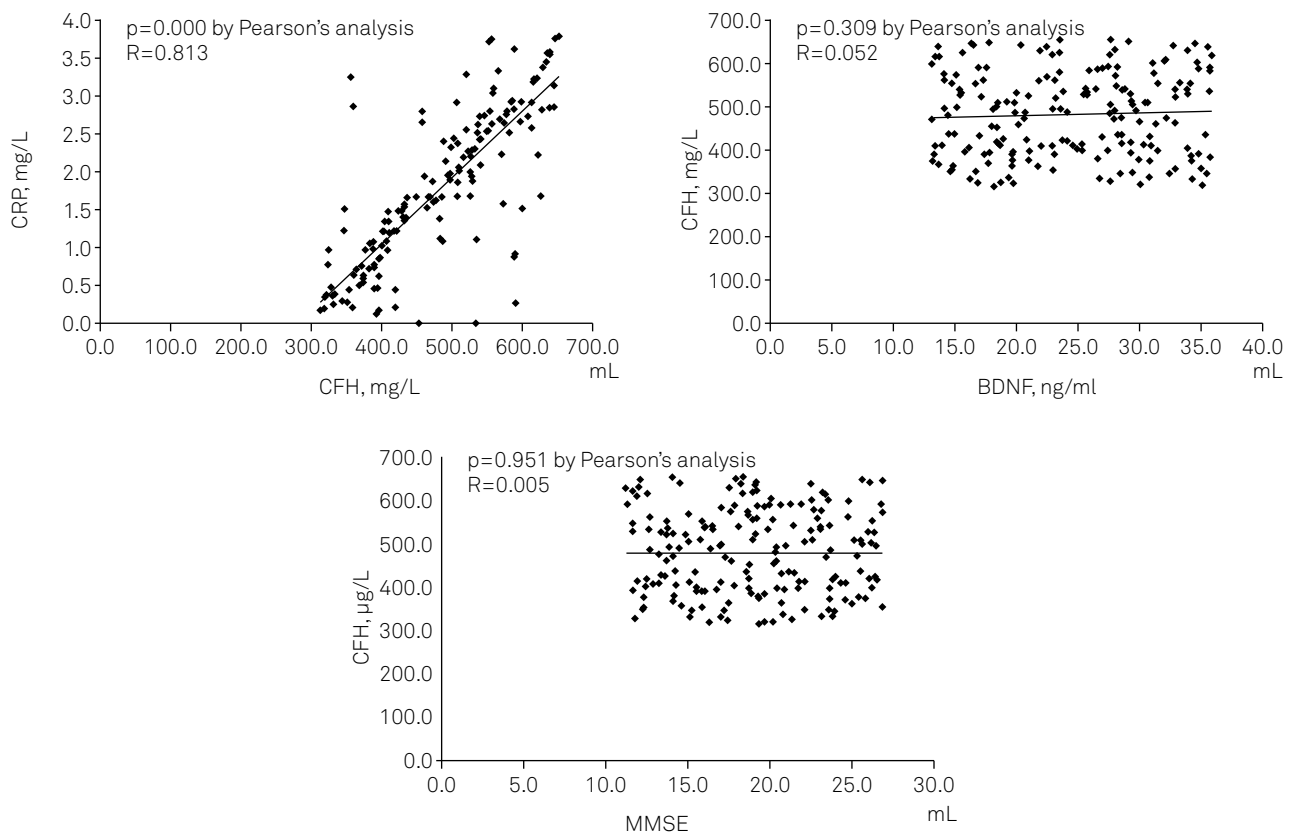
Despite numerous studies on pathology and mechanisms for AD, the molecular mechanisms of AD are still unclear, and the biomedical for early diagnosis of AD is still insufficient. In the present study, we further confirmed that CFH was decreased in late-onset AD patients, and we for the first time demonstrated serum levels of CFH was correlated with serum levels of CRP, but not MMSE and BDNF.

In the present study, we found CFH was decreased in late-onset AD patients. The role of CFH in development of AD was reported in both animal models and clinic. In animal models, most studies demonstrated CFH was decreased in AD. Lukiw et al. investigated miRNAs in AD brain and their correlation with CFH, finding that miR-125b, miR-146a, and miR-155 were all upregulated in AD and could further be downregulated the CFH levels in AD brain²². Alexandrov et al. also reported CFH levels showed dramatically decrease in

different AD mice models¹⁶. Similar results were also found by Li's team, which showed miR-155 was upregulated and CFH was decreased in Down syndrome (DS) and AD²³.

However, clinical findings for CFH in AD patients demonstrated different results. In 2011, Wang et al. showed complement 3 and CFH were upregulated in cerebrospinal fluid of AD patients and were correlated with the severity of impairment in AD using MMSE²⁴. However, in 2013, Gezen et al. demonstrated CFH was downregulated in late-onset AD patients, despite not showing significant difference in early-onset patients²⁵. Interestingly, Williams et al. recently reported that no significant difference of plasma CFH levels was found in AD patients and the healthy control¹⁸. In our research, the results supported the downregulation levels of CFH. We speculate the different results might be due to different study populations, which still needs more clinical evidences and mechanism researches to confirm.

The abnormal expression of CRP has also been reported in AD patients. Nilsson et al. found CRP levels were decreased in AD patients and were related to cognitive function and survival time¹³. It was also considered that individuals with AD had significantly lower levels of plasma CRP than individuals with MCI and normal aging²⁶. In our study, we also found CRP was decreased in AD patients. In addition, we showed serum levels of CRP were positive correlated with CFH. The present



CFH: complement factor H; CRP: C-reactive protein; BDNF: brain-derived neurotrophic factor; MMSE: mini-mental state examination.

Figure 2. Correlation between CFH vs. CRP, CFH vs. BDNF, and CFH vs. MMSE.

study also has some limitations. First, we only included a limit of cases; secondly, the underlying mechanisms for role of CFH in development of AD and its possible relationship with CRP are still unknown. All these need further studies to reveal.

In conclusion, we conducted a prospective observational study to investigate clinical significance of CFH in late-onset

AD patients. Results showed CFH was decreased in late-onset AD patients, and serum levels of CFH was correlated with serum levels of CRP, but not MMSE and BDNF. These results might give more clinical evidences for the role of CFH in AD patients, as well as provide some new research directions for mechanisms of AD development.

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