Int J Clin Exp Med 2016;9(9):18569-18573
www.ijcem.com /ISSN:1940-5901/IJCEM0026977

Original Article

The correlative analysis of IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin 1 receptor antagonist in different types of dementia in patients with cognitive dysfunction

Ping Sun, Shaobo Shi, Ligang Wang, Zhongguo Sun, Shenghai Wang, Yongdong Zhang, Chunhui Qu, Chunxia Wang

Qingdao Mental Health Center, Qingdao 266034, Shandong, China

Received November 16, 2016; Accepted August 15, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Cognitive dysfunction (cognitive impairment, CI) refers to the clinical syndrome with cognitive function impairment. Studies have shown that IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin 1 receptor antagonist exhibited obvious differences in different types of dementia patients with cognitive dysfunction. 80 dementia patients were divided into two groups: alzheimer's disease (AD) group and vascular dementia (VD) group. 40 healthy individuals acted as the control group (HC). Enzyme-linked immunosorbent method was used to detect the serum level of IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin 1 receptor antagonist. The polymorphism of IL-1RA was genotyped by PCR. In AD group, the MMSE score was significantly lower than that in HC group ($P < 0.05$). TNF-α and hs-CRP concentration in AD and VD group were significantly increased comparing with HC group ($P < 0.05$). Comparing to VD group, hs-CRP in AD group was lower ($P < 0.05$), however, TNF-α level was significantly higher ($P < 0.05$). Genotype frequency A2/A2 homozygous and A1/A2 heterozygous in the AD group were significantly higher than that in the VD group ($P < 0.05$). Level of TNF-α and Hcy were negatively correlated with MMSE score ($P < 0.05$). In conclusion, TNF-α and Hcy were involved in the pathogenesis of dementia in patients with cognitive dysfunction and IL-1RA polymorphism may also participate in onset process of dementia.

Keywords: IL-1β, IL-6, TNF-α, Hcy, hs CRP, Interleukin 1, dementia, cognitive function

Introduction

As the aging process is accelerated, the prevalence of dementia and cognitive dysfunction increase year by year. Alzheimer's disease is considered to be the main cause of dementia unless the patient with vascular risk factors, typical systemic vascular events and cerebrovascular diseases [1-3]. Several studies indicated that alzheimer's disease is a major cause of dementia in the elder people in European and American countries. Simple mental state scale (MMSE) is a kind of judgment and screening tool for assessment of cognitive impairment in patients, and it can reflect the patient's nervous psychological state to a certain extent [4-7]. The aim of the present study was to explore the correlation of IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin 1 receptor antagonist with cognitive dysfunction in patients with different types of dementia.

Materials and methods

General information

80 cases of different types of dementia patients from January 2013 to January 2015 in Qingdao Mental Health Center were enrolled in this study. According to the types of dementia, it can be divided into two groups: alzheimer's disease (AD) and vascular dementia (VD). In the AD group, 17 patients were the male and other 23 cases were female with the average age being 79.96±8.45 and mean of the education period being 5.82±4.10 years; In VD group, 18 patients were male and 22 patients were female with the average age being 80.11±8.29 and mean period of the education being

Table 1. Demographic information of included participants

	AD group	VD group	HC group	F/χ^2	P
Gender (M/F)	17/23	18/22	20/20	0.470	0.791
Age (Years)	79.96±8.45	80.11±8.29	77.89±6.93	0.982	0.414
Years of education	5.82±4.10	5.73±4.23	5.02±4.54	0.417	0.660

5.73±4.23 years. The other selected 40 cases of healthy individuals were served as normal control group (HC), including 20 female and 20 male with a median age of 77.89±6.93 years. All patients were in line with the diagnosis standard in the statistical manual of the United States ruling out of serious illness such as heart, liver and kidney; the initial cases; late-life depression and anxiety disorders and other mental illness; brain atrophy or cerebral infarction head according to CT and MRI.

Two groups of patients showed no significant differences regarding the sex ratio, average age, the fixed year of the education ($P > 0.05$), and the data was comparable.

The protocol of this study has been pre-approved by the ethical committee in Qingdao Mental Health Center. Informed consents have been obtained from all participants.

Method

We detected the serum level of IL-1β, IL-6, TNF-α, Hcy, hs-CRP by enzyme-linked immunosorbent method as follows.

Blood was coagulated about 15 min at room temperature after extraction of venous blood in patients with 5~10 ml, and then centrifuged 20 min at 2000 r/min. Take supernatant after the serum was isolated, and save it at -20°C to avoid repeated freezing and thawing. Serum levels of IL-1β, IL-6, TNF-α, Hcy, hs-CRP were detected by assay kit which was purchased from R&D companies in the United States. First of all, the standard of the sample was diluted and added, then incubated, liquored, washed followed by addition of enzyme standard reagent 50 μl (except the blank well), incubated and washed, colored (15 min at 37°C avoiding light), terminated (added terminated liquid 50 μl) and standard curve drawing. Absorbance (OD value at 450 nm) of each well was measured. The results were calculated according to the standard curve [5, 8].

In terms of genetic test, firstly, PCR product was detected by polyacrylamide gel electrophoresis of deformation, and genotype of migration was detected by silver staining; Secondly, polymorphism of IL-1RA was detected in order to test variable number tandem repeats in no. 2 introns (VNTR) [9-11].

Observational index

Firstly, comparing MMSE scale component score in the three groups. The scores of patients were performed using simple intelligent mental state examination (MMSE), including: directional force (1~10), attention and calculation (14~18), memory (19~21), named (22~23), language fluency (24~29), depending on the space (30), executive function. The total score is 30 and the higher score indicated that better function. A range of different cultural levels: 27 points or more was considered as normal; no culture with 17 points or less, primary cultural with 20 points or less, secondary cultural with 22 points or less, and university culture with 23 points or less were considered as dementia [12].

Secondly, the indicators were compared in the three groups, including: the concentration of IL-1β, IL-6, TNF-α, Hcy, hs-CRP.

Thirdly, IL-1RA genotype and gene frequency were compared. Genotype included A1/A1, A2/A2, A1/A2 [13].

Fourthly, correlation of the indexes (IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin receptor antagonist) with MMSE score was performed.

Statistical analysis

SPSS17.0 statistical software was used for data analysis. Average age, education years, MMSE scale component score, levels of IL-1β, IL-6, TNF-α, Hcy, hs-CRP and interleukin 1 receptor antagonist were expressed as the mean ± SD. Statistically significant differences in groups were analyzed using t test or a chi-square test. Multivariate regression analysis was performed to evaluate the relationship between each indicator and MMSE score. $P < 0.05$ was considered statistically significant.

Table 2. MMSE score in three groups of patients

Groups	N	Orientation	Immediate memory	Computing forces	Delayed memory	Name	Language fluency	Visual space function	Total score
AD group	40	4.13±0.68	1.47±0.32	2.03±0.73	0.63±0.43	1.00±0.00	0.77±0.30	2.03±0.21	12.19±3.78
VD group	40	5.34±0.55*	1.58±0.21*	2.67±0.20*	1.43±0.30*	1.00±0.00*	0.92±0.21*	2.44±0.27*	13.63±3.60*
HC group	40	10.03±0.00Δ	3.01±0.02Δ	5.00±0.38Δ	2.73±0.48Δ	2.00±0.00Δ	1.76±0.46Δ	4.77±0.47Δ	28.69±1.34Δ

AD group vs. HC group, ΔP > 0.05; AD group vs. VD group, *P < 0.05; VD group vs. HC group, *ΔP < 0.05.

Table 3. The indicators in the three groups of patients

Groups	N	IL-1β	IL-6	TNF-α	Hcy	hs-CRP
AD group	40	23.28±13.00	26.38±9.48	48.66±8.15	28.51±11.72	6.60±1.28
VD group	40	24.40±14.42*	29.71±16.26*	40.81±7.56*	28.78±10.85*	8.95±10.02*
HC group	40	24.53±0.48Δ	28.90±13.80Δ	38.10±8.72Δ	14.30±3.19Δ	5.75±1.40Δ

AD group vs. HC group, ΔP > 0.05; AD group vs. VD group, *P < 0.05; VD group vs. HC group, *ΔP < 0.05.

Table 4. The IL-1RA genotype and gene frequency in the three groups

Groups	n	Genotype			Gene frequency	
		A1/A1	A1/A2	A2/A2	A1	A2
AD group	40	22	13	5	58	22
VD group	40	23*	7*	2*	66*	14*
HC group	40	29Δ	8Δ	2Δ	66Δ	14Δ

AD group vs. HC group, ΔP > 0.05; AD group vs. VD group, *P < 0.05; VD group vs. HC group, *ΔP < 0.05.

Results

Demographic information of included participants

No significant differences were observed among AD group, VD group and HC group (P > 0.05) regarding gender, age or years of education (**Table 1**).

MMSE score in three groups of patients

Compared with HC group, there were significant differences of MMSE score in AD, VD group (P < 0.05). Comparison between AD and VD group, orientation, immediate memory and computing forces and delayed memory, visual space function, the total score in AD groups were lower than that in HC group (P < 0.05) (**Table 2**).

The indicators in the three groups of patients

Compared with TNF-α level in the HC group, hs-CRP concentration in AD and VD group were obviously higher (P < 0.05) with lower level of hs-CRP in AD group than that in the VD group (P < 0.05) (**Table 3**). These data suggested that serum levels of hs-CRP and Hcy may be involved in the development of cognitive impairment in patients.

The IL-1RA genotype and gene frequency in the three groups

Frequency of A2 allele of IL-1RA gene in AD group was obviously higher than that in VD group (P < 0.05) (**Table 4**), and genotype frequency of A2/A2 homozygous and A1/A2 heterozygous in the AD group were significantly higher than that in the VD group (P < 0.05) (**Table 4**).

The relationship between each indicator and MMSE score

In order to evaluate which indicator might be associated with MMSE score, multivariate regression analysis was performed. The results showed that levels of TNF-α and Hcy were found to be associated with MMSE score (P < 0.05) (**Table 5**).

Discussion

In recent years, it is popular to explore the pathogenesis of AD and VD. Levels of IL-6, TNF-α, IL-1β seem to have a direct relationship with AD and VD patients, because these parameters were higher than those in healthy people [14, 15]. In addition, animal experiments showed that TNF, CRP and other inflammatory cytokines played a vital role in AD disease, because these inflammatory factors indirectly stimulated neurons, and inhibited cholinergic neurons relay, leading to the central cholinergic system damage [16]. To some extent, these studies indi-

Table 5. Multivariate regression analysis of each indicators with MMSE score

Indicator	Bi	SBi	T	P	Beta
Gender	0.825	0.647	0.834	0.387	0.092
Age	1.246	0.863	1.416	0.073	0.105
Years of education	5.724	4.853	0.973	0.425	0.116
TNF-α	-18.273	14.925	-9.348	< 0.001	-0.452
Hcy	-7.461	5.884	-6.573	< 0.001	-0.316
IL-1β	10.382	7.046	1.538	0.069	0.141
IL-6	0.865	0.711	1.027	0.126	0.088
hs-CRP	-4.283	3.076	-0.825	0.501	-0.124
IL-1RA genotype	1.907	0.825	-1.494	0.062	-0.136

cated that there is a direct relationship between the inflammatory factors and cognitive impairment in patients with dementia.

Previous studies has shown focal inflammatory reaction in the brain in patients with AD, and a large number of inflammatory factors such as TNF-α and IL-1β were activated. It was hypothesized that immune and inflammatory responses and the onset of dementia has certain relevance, and are likely to have close relationship with cytokines [17]. A large amount of precipitation was found in brain tissue of familial and sporadic AD. This is because the amyloid protein (APP) is the precursor of the β-amyloid, and IL-6, IL-1β, which were produced in the brain and to the local, can promote the synthesis of nerve cells in the APP. β-AP produced by abnormal metabolism of APP precipitated in brain tissue, leading to AD [18].

Studies have found that Hcy levels are influenced by many factors, such as heredity, nutrition, age, gender, race and other factors. Related reports suggest that folic acid is an important factor affecting Hcy levels, and supplements of vitamin can effectively elevate Hcy levels. Other research suggested that Hcy was associated with cognitive dysfunction. Data in these studies showed that plasma level of Hcy in AD and VD patients is higher than that in control group, indicating that high level of Hcy is an independent risk factor for VD. Hofman reported that Hcy was involved in almost the entire process of cognitive impairment and was associated with dementia and cognitive decline [18]. Further research showed that high serum levels of Hcy may lead to cognitive damage by cognitive cerebrovascular or cortical and hippocampal atrophy [19].

CRP, which was induced by cytokines IL-6 and TNF, is a kind of acute inflammatory protein markers, and has anti-inflammatory effect. The mechanism of its anti-inflammatory effect is activated by the immune response. CRP level can increase in serious infections, physical damage, autoimmune disease, tumor, vascular injury, ischemia or necrosis cases and was generally considered as a unique inflammatory markers. CAI etc. found that high serum levels of HCY and hs-CRP were involved in the pathogenesis of VD. High levels of HCY can damage endothelial function, and CRP is considered as the indicator of the degree of nerve function damage, condition monitoring and prognosis evaluation. As a result, the hs-CRP and HCY concentration monitoring in cognitive function in patients with has a certain guiding significance for the diagnosis and prognosis judgment. Interleukin 1 is a kind of inflammatory cytokine and plays an important role in the pathogenesis of disease. In the present study, frequency of A2 allele IL-1RA gene in AD group was obviously higher than that in VD group ($P < 0.05$), and genotype frequency of A2/A2 homozygous and A1/A2 heterozygous in the AD group were significantly higher than that in the VD group ($P < 0.05$), consistent with a previous study showing IL-1 gene may be involved in the onset of dementia in Parkinson's disease [19].

To sum up, the levels of TNF-α and Hcy were involved in different types of dementia in patients with cognitive dysfunction and the polymorphism of IL-1RA gene may also participate in the pathogenesis of different types of dementia.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chunxia Wang, Qingdao Mental Health Center, No. 299, Nanjing Road, Shibei District, Qingdao 266034, Shandong, China. Tel: +86-0532-86669022; Fax: +86-0532-86669022; E-mail: chunxiawang123@yeah.net

References

[1] Wisniewski T and Goni F. Immunotherapy for Alzheimer's disease. Biochem Pharmacol 2014; 88: 499-507.

[2] Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D and Jones E. Alzheimer's disease. Lancet 2011; 377: 1019-1031.
[3] Castelo-Branco C and Soveral I. The immune system and aging: a review. Gynecol Endocrinol 2014; 30: 16-22.
[4] Vital TM, Hernandez SS, Stein AM, Garuffi M, Corazza DI, de Andrade LP, Costa JL and Stella F. Depressive symptoms and level of physical activity in patients with Alzheimer's disease. Geriatr Gerontol Int 2012; 12: 637-642.
[5] Lambracht-Washington D and Rosenberg RN. Active DNA Abeta42 vaccination as immunotherapy for Alzheimer disease. Transl Neurosci 2012; 3: 307-313.
[6] Koppel J, Goldberg TE, Gordon ML, Huey E, Davies P, Keehlisen L, Huet S, Christen E and Greenwald BS. Relationships between behavioral syndromes and cognitive domains in Alzheimer disease: the impact of mood and psychosis. Am J Geriatr Psychiatry 2012; 20: 994-1000.
[7] Wunder A, Schoknecht K, Stanimirovic DB, Prager O and Chassidim Y. Imaging blood-brain barrier dysfunction in animal disease models. Epilepsia 2012; 53 Suppl 6: 14-21.
[8] Tan ZS, Beiser AS, Vasan RS, Roubenoff R, Dinarello CA, Harris TB, Benjamin EJ, Au R, Kiel DP, Wolf PA and Seshadri S. Inflammatory markers and the risk of Alzheimer disease: the Framingham Study. Neurology 2007; 68: 1902-1908.
[9] Zhao J, Tang H, Sun J, Wang B, Chen S and Fu Y. Analysis of cognitive dysfunction with silent cerebral infarction: a prospective study in Chinese patients. Metab Brain Dis 2012; 27: 17-22.
[10] Carrero I, Gonzalo MR, Martin B, Sanz-Anquela JM, Arevalo-Serrano J and Gonzalo-Ruiz A. Oligomers of beta-amyloid protein (Abeta1-42) induce the activation of cyclooxygenase-2 in astrocytes via an interaction with interleukin-1beta, tumour necrosis factor-alpha, and a nuclear factor kappa-B mechanism in the rat brain. Exp Neurol 2012; 236: 215-227.
[11] Winblad B, Andreasen N, Minthon L, Floesser A, Imbert G, Dumortier T, Maguire RP, Blennow K, Lundmark J, Staufenbiel M, Orgogozo JM and Graf A. Safety, tolerability, and antibody response of active Abeta immunotherapy with CAD106 in patients with Alzheimer's disease: randomised, double-blind, placebo-controlled, first-in-human study. Lancet Neurol 2012; 11: 597-604.
[12] Kirshner HS. Mild cognitive impairment: to treat or not to treat. Curr Neurol Neurosci Rep 2005; 5: 455-457.
[13] Jeremias J, Ledger WJ and Witkin SS. Interleukin 1 receptor antagonist gene polymorphism in women with vulvar vestibulitis. Am J Obstet Gynecol 2000; 182: 283-285.
[14] Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M and Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. Med Princ Pract 2015; 24: 1-10.
[15] Sperling R, Salloway S, Brooks DJ, Tampieri D, Barakos J, Fox NC, Raskind M, Sabbagh M, Honig LS, Porsteinsson AP, Lieberburg I, Arrighi HM, Morris KA, Lu Y, Liu E, Gregg KM, Brashear HR, Kinney GG, Black R and Grundman M. Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. Lancet Neurol 2012; 11: 241-249.
[16] Leblhuber F, Walli J, Artner-Dworzak E, Vrecko K, Widner B, Reibnegger G and Fuchs D. Hyperhomocysteinemia in dementia. J Neural Transm (Vienna) 2000; 107: 1469-1474.
[17] Terrando N, Eriksson LI, Ryu JK, Yang T, Monaco C, Feldmann M, Jonsson Fagerlund M, Charo IF, Akassoglou K and Maze M. Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 2011; 70: 986-995.
[18] Matousek SB, Hein AM, Shaftel SS, Olschowka JA, Kyrkanides S and O'Banion MK. Cyclooxygenase-1 mediates prostaglandin E(2) elevation and contextual memory impairment in a model of sustained hippocampal interleukin-1beta expression. J Neurochem 2010; 114: 247-258.
[19] Pflanzner T, Janko MC, Andre-Dohmen B, Reuss S, Weggen S, Roebroek AJ, Kuhlmann CR and Pietrzik CU. LRP1 mediates bidirectional transcytosis of amyloid-beta across the blood-brain barrier. Neurobiol Aging 2011; 32: 2323 e2321-2311.