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

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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...
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].

**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 1. Demographic information of included participants**

<table>
<thead>
<tr>
<th></th>
<th>AD group</th>
<th>VD group</th>
<th>HC group</th>
<th>F/χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>17/23</td>
<td>18/22</td>
<td>20/20</td>
<td>0.470</td>
<td>0.791</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>79.96±8.45</td>
<td>80.11±8.29</td>
<td>77.89±6.93</td>
<td>0.982</td>
<td>0.414</td>
</tr>
<tr>
<td>Years of education</td>
<td>5.82±4.10</td>
<td>5.73±4.23</td>
<td>5.02±4.54</td>
<td>0.417</td>
<td>0.660</td>
</tr>
</tbody>
</table>

In terms of genetic test, firstly, PCR product was detected by polyacrylamide gel electrophoresis of deformation, and genotype of migration 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.
Analysis of dementia patients with cognitive dysfunction

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-

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Table 2. MMSE score in three groups of patients

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

Table 3. The indicators in the three groups of patients

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Genotype</th>
<th>Gene frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1</td>
<td>A1/A2</td>
<td>A2/A2</td>
</tr>
<tr>
<td>AD group</td>
<td>40</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>VD group</td>
<td>40</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>HC group</td>
<td>40</td>
<td>29</td>
<td>8</td>
</tr>
</tbody>
</table>

AD group vs. HC group, *P > 0.05; AD group vs. VD group, *P < 0.05; VD group vs. HC group, **P < 0.05.
cated that there is a direct relationship between the inflammatory factors and cognitive impairment in patients with dementia.

Previous studies have 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].

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.

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