A comparative study of innate immunity markers in Alzheimer's disease, Mixed dementia and Vascular dementia

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Background: Innate immunity is currently considered to be an important component of pathological cascade in old age dementia. The aim of this study was to evaluate activity of inflammatory markers such as leukocyte elastase and alpha-1 proteinase inhibitor, as well as concentration of C-reactive protein and interleukin-6 in blood plasma of patients with pure AD, mixed dementia and VaD of varying severity.

Methods: Several parameters of innate immunity such as leukocyte elastase activity (LE), the functional activity of alpha-1-proteinase inhibitor, the concentration of C-reactive protein (CRP) and interleukin-6 (IL-6) were measured in blood plasma of elderly patients with main forms of dementia including: Alzheimer’s disease (AD), mixed dementia (MD) and vascular dementia (VaD).

Results: A total of 161 in-patients and 39 control subjects were included in the study. The levels of innate immunity markers were found to depend on the severity of dementia. The decreased activity of LE in AD and MD is suggested to be due to the alteration of neutrophils degranulation activity or to changes in enzyme properties. A negative correlation between the level of IL-6 and degree of cognitive impairment in AD (Spearman ρ = -0.43; p<0.05) indicates significantly that levels of proinflammatory cytokines may characterize the severity of the process having the great impact on (lower) total MMSE score. Elevated level of CRP is likely to be a biological marker of an early stage of disease in VaD.

Conclusions: Our results confirm the presence of an inflammatory component in the pathological cascade of AD, MD and VaD.

Keywords: Alzheimer’s disease, Mixed dementia, Vascular dementia, Innate immunity, Leukocyte elastase activity, Functional activity of alpha-1-proteinase inhibitor; C-reactive protein, Interleukin-6
Introduction

Inflammation is an important feature of brain pathology in old age dementia, including Alzheimer’s disease (AD), mixed dementia (MD) and vascular dementia (VaD) [1-3]. The pathway of these diseases includes the neuroinflammation reactions accompanied with the increasing of proinflammatory cytokines level in brain. Proinflammatory cytokines are synthesized by activated microglia which is believed to be activated by accumulated extracellular beta-amyloid. Microglial activation plays a protective role and prevents the formation of amyloid plaques at the initial stages of dementia. However, the disease is accompanied by dysfunction of microglia, that includes a 2.5-fold increase in synthesis of proinflammatory cytokines and inhibition of expression of genes involved in the process of elimination of beta-amyloid [4-6].

A possible relationship between neuroinflammation (in the brain) and systemic innate immune responses (in the bloodstream) is widely discussed in literature. Many studies revealed a high level of proinflammatory cytokines in the blood of patients with AD [7-9]. Other authors reported a decrease [10] or no change of these indices level compared to control [11]. The discrepancies in these results may be explained by differences in subjects included in those studies (such as the size of subject pool, the inclusion criteria, stage of the disease, comorbidity, etc.).

The relationship between the level of peripheral inflammatory markers and clinical characteristics of patients, including the form and severity of dementia, was studied insufficiently. However, the establishing of such relationship (if it exists) is extremely important as it could help to improve the reliability of diagnostics and laboratory monitoring of disease progression. In addition, the detection of systemic inflammation markers in blood can help provide an early (preclinical) diagnostics of various forms of dementia and to draw attention to persons with high risk for a dementia.

The aim

The aim of this study was to evaluate activity of inflammatory markers such as leukocyte elastase and alpha-1 proteinase inhibitor, as well as concentration of C-reactive protein and interleukin-6 in blood plasma of patients with pure AD, mixed dementia and VaD of varying severity.

Leukocyte elastase (LE) is the member of the serine proteases family and one of the markers of neutrophil degranulation activity. It is localized in the azurophilic granules of neutrophil leukocytes [12, 13]. Neutrophil activation and degranulation results in the release of this protease into the extracellular space as proteolytically active enzymes and is itself the result of the adhesion of neutrophils to the vascular wall or death of cells [14, 15]. LE may increase vascular permeability, as well as produce cytotoxic effects on endothelial cells due to its proteolytic activity [16, 17].

Alpha-1 proteinase inhibitor (α1-PI), or alpha-1 antitrypsin is synthesized primarily by hepatocytes. It is involved in the inhibition of LE, in particular, and in the number of other proteases [17].

C-reactive protein (CRP) is synthesized primarily in the liver and manifests an acute phase of inflammation. Neurons as well as monocytes and lymphocytes could be a source of CRP [18, 19]. This protein is controlled by pro-inflammatory cytokines (mainly IL-1β and IL-6) [20]. It was shown that CRP is a reliable marker of vascular damage and inflammation in the walls of arteries [18]. The association between the level of CRP in the serum and the risk and severity of vascular disease was the basis for its use as an independent predictor of myocardial infarction, stroke and vascular death [21, 22].

IL-6 – glycoprotein (21-28 kDa), a pleiotropic cytokine with a wide range of biological activity, is produced by lymphoid and non-lymphoid cells [23, 24]. T-and B-lymphocytes, eosinophils, mast cells, astrocytes and microglia can secrete IL-6 [25-27]. Proinflammatory mediator IL-6 regulates the synthesis of other mediators of inflammation and itself is regulated by other cytokines (TNF-α, IL-1β). A major function of IL-6 is regulation of maturation of antibody-producing B-lymphocytes and production of immunoglobulins. IL-6 is involved in activation of T cells, in induction of the synthesis of many acute-phase proteins, fibrinogen, alpha 1-antithrombin, haptoglobin, serum amyloid A, CRP, etc. The inhibition of the synthesis of pro-inflammatory cytokines such as IL-1β and TNF-α, may have hormone-like effects on the liver, maintaining glucose homeostasis. IL-6 plays a key role in the development of inflammatory and immune response to infection and tissue injury.

Methods

A total of 161 in-patients (54–94 years old) of psychogeriatric unit of Moscow psychiatric hospital were included in the study. A control group consisted of 39 elderly persons (55–79 years old) without any mental or severe physical disorders. The control group was recruited from healthy aged persons who were examined by general practitioner and did not reveal a cognitive impairment. Patients and control group were matched for the men/women ratio and age distribution. All participants signed an informed consent to take part in the study. The study protocol was approved by local ethics committee of Mental health research center.

According to comparative design of the study all patients were divided into 3 groups. The first group included 84 patients (57 men and 27 women) with ‘pure’ AD. The second group comprised 54 patients (37 men and 17 women) diagnosed as MD. The third group made up of 23 patients (20 men and 3 women) with a diagnosis of VaD.

The diagnosis of probable Alzheimer’s disease was established according to the ICD-10 and NINCDS-ADRDA criteria.
criteria [28]. Mixed dementia was diagnosed in cases of possible Alzheimer’s disease with medical history of cerebrovascular disease (e.g. hypertension, TIA/stroke), clinical signs and focal MRI findings of infarcts, lacunes and white matter periventricular hyperintensities. Hippocampal atrophy was most consistent in AD-cases, but it might occur in patients with MD as well. AD associated with cerebrovascular disease is now considered to be the most frequent type of dementia [29-32]. Diagnostic criteria of ICD-10 and NINDS-AIREN [33] were used to diagnose probable vascular dementia.

Clinical assessment of cognitive impairment severity (mild, moderate or severe dementia) was based on the results of Clinical Dementia Rating (CDR) [34] and psychometric assessment (Mini–Mental State Examination – MMSE) [35] and on clinical picture of disorders (Table 1).

Male subjects were overrepresented in the study because most of the patients were recruited from the men’s unit of the hospital. Such gender distribution is a side effect differed from the prevalence of dementia in population with well-known bias of aged females with dementia. A male/female ratio was closed in AD and MD groups, while there was a predominance of males in the group of patients with VaD. The compared groups did not differ significantly on the other main characteristics of disease such as the index age, age of onset, degree of dementia severity.

Blood samples were collected between 8:30–9:30 am by standard venepuncture technique using Greiner Vacuette K3E K3EDTA tubes after twelve hours of fasting. Plasma was collected after sample centrifugation (750x g, 22 °C) for 15 min and was used for analysis either immediately after preparation, or stored at +2-8°C is not more than a day or stored at -20-37°C during the month until being analyzed.

LE activity was determined by enzymatic method using a specific substrate N-tert-butoxy-carbonyl-alanine-β-nitrophenyl ester (BOC-Ala-ONp), and its activity was presented in nmol/min·ml units [36]. Functional activity of the α1-PI was determined by the spectrophotometric method and evaluated in IU/ml (inhibitory units/ml) [37]. The concentration of CRP was measured by ELISA (IMTEK, Russia) in mg/l. The level of IL-6 was measured by ELISA (VECTOR-BEST, Russia) in pg/ml. The analytical sensitivities of LE activity, the functional activity of α1-PI, the concentration of CRP and IL-6 assays were 40 nmol/min·ml, 5 IU/ml, 1 mg/l and 0.5 pg/ml respectively.

Statistical data processing was performed using non-parametric statistical software Statistica-7 (for Windows, StatSoft., Inc, USA). Mann-Whitney U-test was used to compare two independent groups, Spearman correlation coefficient was used and ANOVA test were used to analyze three or more independent groups with each other. Data are presented as median [Q1; Q3] (Median [25th; 75th Percentile). A confidence level p<0.05 was used.

Results

Main study results are summarized in Tables 2, 3 and 4.

Alzheimer’s disease

There was a significant decrease in activity of LE (p<0.0001) and increase in activity of α1-PI, IL-6 and CRP levels (p<0.0001, p<0.01, p=0.06 respectively) in the group of patients with AD compared to control (Table 2).

There was a significant positive correlation between

<table>
<thead>
<tr>
<th>Table 1. Common characteristics of clinical materials (median [25%; 75th percentile]).</th>
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<tr>
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<tr>
<td>Alzheimer’s disease (AD)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Total number (n)</td>
</tr>
<tr>
<td>Male/female ratio</td>
</tr>
<tr>
<td>Age of plasma sampling (yrs)</td>
</tr>
<tr>
<td>Early onset of dementia (≤65)</td>
</tr>
<tr>
<td>Late onset of dementia (&gt;65)</td>
</tr>
<tr>
<td>Duration of illness (yrs)</td>
</tr>
<tr>
<td>Severity of dementia</td>
</tr>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
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<tr>
<td>Severe</td>
</tr>
<tr>
<td>MMSE</td>
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<td>CDR</td>
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</table>

CDR = Clinical Dementia Rating; MMSE = Mini–Mental State Examination.
CRP and IL-6 (Spearman r=0.53, p<0.05) in all patients with AD, i.e. these two measures are directly related to each other and could probably reflect the effect of IL-6 on induction of CRP synthesis in the liver. In contrast, a negative correlation between activity LE and IL-6, CRP levels was found in patients with Alzheimer’s disease (r=-0.36, r=-0.25, p<0.05 respectively), i.e. reduction of LE activity is accompanied by increased levels of IL-6 and CRP (Table 4).

A positive correlation between total score of cognitive scale MMSE and LE activity (r=0.34, p<0.05) and negative correlation between the concentrations of IL-6, CRP plasma levels and total score of cognitive scale MMSE (r=-0.43, r=-0.24, p<0.05 respectively) were demonstrated in patients with Alzheimer’s disease, i.e. decrease LE activity and elevat-

Table 2. Parameters of blood plasma in different forms and stages of dementia in aged (Median [25th; 75th Percentile]).

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Total number</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39</td>
<td>LE (nmol/min·ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>210.7 [187.9; 231.0]</td>
</tr>
<tr>
<td>AD total</td>
<td>84</td>
<td>185.5 [158.0;208.4]***</td>
</tr>
<tr>
<td>Mild dementia</td>
<td>21</td>
<td>198.7 [181.4;220.9]</td>
</tr>
<tr>
<td>Moderate dementia</td>
<td>36</td>
<td>185.2 [155.5;214.9]***</td>
</tr>
<tr>
<td>Severe dementia</td>
<td>27</td>
<td>166.3 [146.4;192.2]****##</td>
</tr>
<tr>
<td>MD total</td>
<td>54</td>
<td>182.0 [149.0;205.0]***</td>
</tr>
<tr>
<td>Mild dementia</td>
<td>14</td>
<td>186.6 [171.7;214.5]*</td>
</tr>
<tr>
<td>Moderate dementia</td>
<td>26</td>
<td>183.9 [147.4;207.4]***</td>
</tr>
<tr>
<td>Severe dementia</td>
<td>16</td>
<td>167.0 [135.0;200.9]***</td>
</tr>
<tr>
<td>VaD total</td>
<td>23</td>
<td>205.0 [164.6;235.4]</td>
</tr>
<tr>
<td>Mild dementia</td>
<td>9</td>
<td>209.5 [190.0;218.0]</td>
</tr>
<tr>
<td>Moderate dementia</td>
<td>9</td>
<td>203.0 [162.0;244.0]</td>
</tr>
</tbody>
</table>

* = p<0.05, **= p<0.01, ***= p<0.001, **** = p<0.0001 - compared to control
## = p<0.05 - compared to mild dementia
α1-PI = Alpha-1 proteinase inhibitor; AD = Alzheimer’s disease; LE = Leukocyte elastase; MD = Mixed dementia; VaD = vascular dementia

Table 3. Parameters of blood plasma in AD, MD and VaD depending on the disease onset (Median [25th; 75th Percentile]).

<table>
<thead>
<tr>
<th>Examined groups</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LE (nmol/min·ml)</td>
</tr>
<tr>
<td>Control</td>
<td>211.4 [190.2;231.0]</td>
</tr>
<tr>
<td>AD</td>
<td>190.0 [173.0;220.3]</td>
</tr>
<tr>
<td>Late onset of dementia (n=51)</td>
<td>181.0 [147.0;198.7]</td>
</tr>
<tr>
<td>MD</td>
<td>159.4 [140.0;233.3]</td>
</tr>
<tr>
<td>Late onset of dementia (n=44)</td>
<td>182.8 [158.6;201.8]</td>
</tr>
<tr>
<td>VaD</td>
<td>213.8 [205.2;248.4]</td>
</tr>
<tr>
<td>Late onset of dementia (n=15)</td>
<td>193.2 [164.0;218.0]</td>
</tr>
</tbody>
</table>

* - p<0.05, **- p<0.01, ***- p<0.001, ****- p<0.0001 - compared to control
# - p<0.05 - compared to early onset of dementia
α1-PI = Alpha-1 proteinase inhibitor; AD = Alzheimer’s disease; LE = Leukocyte elastase; MD = Mixed dementia; VaD = vascular dementia
ed levels of IL-6 and CRP are related to more marked dementia severity in AD. It was found a positive correlation between age of patient and the level of IL-6 (r = 0.33) (Table 4).

Next, investigated immunological parameters (activity/level LE, α1-PI CRP, IL-6) were determined in patients with AD of varying severity of dementia.

A group of patients with mild AD (Table 2) was characterized by a significant increase in activity of α1-PI compared to control (p<0.0001). The activity of LE, the level of CRP and IL-6 in this group of patients were not statistically different from control.

Patients with moderate AD were characterized by a significant increase in activity of α1-PI, level of IL-6 (p<0.0001 and p<0.05 respectively). LE activity was significantly lower compared to control (p<0.01).

There was a significant increase in activity of α1-PI as well as CRP level and IL-6 (p<0.0001, p<0.01, p<0.001 respectively) and a significant decrease of the LE activity in patients with severe AD compared to control (p<0.0001).

Comparison between all three subgroups of patients with AD depending on dementia severity showed that the LE activity was significantly reduced and CRP level was increased in severe dementia compared to mild dementia (p<0.001, p<0.05 respectively).

Patients with early onset of dementia were characterized by a significant increase in the functional activity of α1-PI compared to control (p<0.0001) (Table 3). It was found a negative correlation between IL-6 and MMSE (r=-0.62). Besides of it there was a positive correlation between IL-6 and degree of dementia severity (r=0.44). So an increasing of IL-6 level was associated with decreased MMSE score and deterioration of dementia (Table 4).

Patients with late onset of dementia were characterized by a significant decrease of LE activity, increase in the functional activity of α1-PI, CRP and IL-6 levels compared to control (p<0.0001, p<0.0001, p<0.05 and p<0.001 respectively) (Table 3). The LE-activity was significantly lower in late onset of dementia while IL-6 level was significantly higher than in dementia with early onset (Table 3).

It was revealed a positive association of LE and MMSE score (r=0.36), but there was a negative correlation between LE and degree of dementia severity at the late stage of disease. Thus a decrease of LE-activity is associated with a progression of dementia. It was also shown that IL-6 level increases with the aging (r=0.32) (Table 4). Thus, LE-activity depends on age of onset and degree of dementia severity. In severe cases of dementia with late onset LE-activity is significantly lower than control indices, while CRP and IL-6 levels are increased (Table 3).

**Mixed dementia**

The group of patients with MD (total) was characterized by the significant decrease in the activity LE and the significant increase of functional activity of α1-PI, concentration of CRP and IL-6 compared to control (p<0.0001, p<0.0001, p<0.01 respectively) (Table 2). The level of IL-6 in this group of patients was increased, but it was not statistically different from control.

Patients with early onset of dementia were characterized by the significant decrease in the activity LE and the significant increase of functional activity of α1-PI, concentration of CRP and IL-6 compared to control (p<0.0001, p<0.0001, p<0.01 respectively) (Table 3). The level of IL-6 in patients with late onset of dementia was significantly higher than in dementia with early onset (Table 3).

Mixed dementia

The group of patients with MD (total) was characterized by the significant decrease in the activity LE and the significant increase of functional activity of α1-PI, concentration of CRP and IL-6 compared to control (p<0.0001, p<0.0001, p<0.01 respectively) (Table 2). The level of IL-6 in this group of patients was increased, but it was not statistically different from control. However, the concentration of CRP was positive correlated with the level of IL-6 (r=0.56, p<0.05) (Table 4).

The CRP and the IL-6 levels were positively correlat-
ed with disease duration in patients with MD (r=0.33 and r=0.37, p<0.05, respectively) (Table 4).

The group of patients with mild MD was characterized by the significant increase of α1-PI activity and decrease of LE activity compared to control (p<0.0001, p<0.05, respectively) (Table 2). The CRP and IL-6 levels in this group of patients were not statistically different from control.

The group of patients with moderate MD was characterized by significant decrease of the LE activity and the significant increase of α1-PI activity and CRP level compared to control (p<0.01, p<0.0001, p<0.01 respectively) The IL-6 level in this group of patients was not statistically different from control (Table 2).

The group of patients with severe MD was characterized by significant decrease of the activity LE and the significant increase of α1-PI activity, the CRP level compared to control (p<0.01, p<0.0001, p<0.05 respectively) The IL-6 level in this group of patients was not statistically different from control (Table 2).

No significant differences in the studied parameters were found depending on dementia severity in patients with MD.

Patients with both early and late onset of MD were characterized by a decrease LE activity and significant increase of α1-PI functional activity and concentration of CRP compared to age-control (Table 3).

No significant differences in the parameters studied between early and late onset of disease in patients with MD have been identified. It was found a negative correlation between EL activity (r=-0.33) at the late stage of mixed dementia. In other words, a decrease of LE activity is associated with progression of dementia severity (Table 4).

**Vascular dementia**

Table 2 shows that the total group of patients with VaD was characterized by a significant increase in activity of α1-PI and level CRP compared to control (p<0.01 and p<0.01 respectively). LE activity and the level of IL-6 in patients of this group did not differ significantly from control. However, the CRP was positively correlated with the level of IL-6 (r=0.52, p<0.05), i.e. these two measures were directly related to each other (Table 3).

The group of patients with mild VaD was characterized by a significant increase in activity of α1-PI and CRP level compared to control (p<0.01, p<0.001 respectively). The activity of LE and IL-6 level in this group of patients were not statistically different from control.

The group of patients with moderate VaD was characterized by increase of α1-PI-activity (p<0.05) and CRP-concentration, but the last parameter did not significantly differ from control. No significant difference of studied parameters was found between mild and moderate VaD.

Patients with both early and late onsets of VaD were characterized by a significant increase in the functional activity of α1-PI and concentration of CRP compared to age-control (Table 3).

No significant differences in the parameters studied between early and late onset of disease in patients with VaD have been identified. However, the correlation between CRP with the disease duration detected only in patients with early onset (r=0.87, p<0.05, Table 4). Thus confirming that CRP concentration could be marker of VaD already at early stage of disease.

**Discussion**

Our results confirm the presence of an inflammatory component in the pathological cascade of AD, MD and VaD [2]. The increased level of CRP at the early stages of the disease may be the most specific diagnostic marker of VaD. An increased α1-PI activity is observed only in patients with AD at the stage of mild dementia, which is consistent with the report by Licastro F. and colleagues [38]. In addition, the level of α1-PI may serve as a peripheral marker of "acute inflammatory response" in the brain at this stage of the disease.

The progression of dementia to moderate and severe levels is followed by an increase in the α1-PI activity and by increase in the level of IL-6 and CRP in patients with AD, however, a significant decrease of LE activity compared to control (p<0.001) is observed. As mentioned above, the LE is released from neutrophils in response to various stimuli during the deployment of inflammatory reactions and is involved in the changes of permeability of the vascular wall. It is possible that the decrease in activity of LE in blood plasma may be due to both change in neutrophil degranulation activity, and changes in properties of the enzyme.

It was shown that the level of IL-6 in patients with AD negatively correlates with MMSE (r = -0.43, p <0.05). In this regard, the level of IL-6 in plasma can serve as a biological markers of the severity of the pathological process. Notably, direct relationship between CRP and IL-6 levels could probably reflect the effect of IL-6 on induction of CRP synthesis in the liver. Interestingly, such relationship persists in AD [39, 40].

Our study has some limitations. The most important is the fact that these markers are unspecific, but these immunological marker indices are considered as evidence of neuroinflammation in the pathway of dementia in aged.

One of the most important strengths of this present study is the comparative design. The study included the main forms of dementia in aged, including AD, mixed dementia and VaD compared to healthy control of the same age. There was some difference of investigated markers of inflammation in neurodegenerative forms of dementia compared to vascular dementia. We found a correlation of its activity/level with severity of cognitive impairment at the different stages of disease.
Abbreviations

a1-PI: Alpha-1 proteinase inhibitor; AD: Alzheimer’s disease; CDR: Clinical Dementia Rating; CRP: C-reactive protein; LE: Leukocyte elastase; MD: Mixed dementia; MMSE: Mini–Mental State Examination; VaD: Vascular dementia

Competing interests

The authors declare no conflict of interest.

References


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  http://dx.doi.org/10.1192/bjp.140.6.566
  http://dx.doi.org/10.1016/0022-3956(75)90026-6
  http://dx.doi.org/10.1016/0165-5728(94)00163-1
  http://dx.doi.org/10.1212/WNL.0b013e318225ae07