Serum Inflammatory Markers in Dementia Disorders

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Abstract

Background: Systemic and local inflammatory responses have been proposed as mechanisms in the initiation and progression of dementia. Several studies described increased levels of proinflammatory cytokines in Alzheimer’s disease patients. Acute phase reactants such as CRP have been detected within the neuritic plaques and neurofibrillary tangles in the brains of patients with Alzheimer’s disease and their elevated levels may predict vascular disease and dementia. Recently, inflammatory markers; α 1-antichymotrypsin, interleukin 6 and to a lesser extent CRP were associated with an increased risk of dementia. Even though several lines of evidence in the literature have shown that inflammation is involved in the pathogenesis of dementia, the results from the evaluation of inflammatory markers in Alzheimer’s disease and vascular dementia patients have been controversial. More over, many of these studies were conducted on CSF. As plasma and CSF levels of many inflammatory markers are significantly inter-related; assessment of such markers in the easily accessible periphery may be as effective as in the CSF and may provide a simple way for the prediction, diagnosis and follow-up of dementia.

Aim: The aim of this work was to study inflammatory markers in peripheral blood of patients with dementia in a trial to clarify their role in disease pathogenesis and progression as well as their potential value as diagnostic biological markers and targets of anti-inflammatory therapy for dementia.

Subjects and Methods: The study included 20 patients with probable Alzheimer’s disease, 20 patients with probable vascular dementia and 20 healthy normal age and sex matched subjects as a control group. Inflammatory markers including C-reactive protein (CRP), interleukin-6 (IL-6) and α 1-antichymotrypsin (ACT) were measured in the serum of both patient groups and control group.

Results: CRP was significantly higher in both Alzheimer disease patients (44.2±6.1) and vascular dementia patients (52.3±5.8) compared to controls (10.6±0.32) (p<0.01). IL-6 was significantly higher in both Alzheimer disease patients (26.4±2.5) and vascular dementia patients (32.4±5.3) compared to controls (9.2±0.5). There was no significant difference between the two patient groups as regards CRP and IL-6 (p>0.05). Serum ACT was significantly higher in Alzheimer’s disease patients (780±19.3) compared to controls (490±16) and vascular dementia patients (512±16.7) (p<0.01). There was no significant difference between vascular dementia patients and control group as regards ACT (p>0.01). There was no significant difference between vascular dementia patients and control group as regards ACT (p>0.01). There was no significant difference between vascular dementia patients and control group.

Conclusion: In this study, alterations of the inflammatory markers were detected in peripheral blood of dementia patients denoting that assay of plasma inflammatory markers might provide an insight in the pathogenesis of dementia and might provide an easier and accessible way in the diagnosis and follow up of dementia compared to their assay in CSF. Serum CRP, IL-6 levels were significantly higher in both patients groups compared to the control group. The fact that these changes were found in both Alzheimer’s disease patients and vascular dementia patients emphasize the common pathophysiological mechanisms in the two dementia subtypes. Serum α 1-antichymotrypsin was significantly higher in Alzheimer’s disease patients than in vascular dementia patients denoting its possible value in the differential diagnosis between the two types. The negative correlation between MMSE score and levels of IL-6 and ACT in both patient groups suggest that IL-6 and α 1-ACT may play a role in defining the aggressiveness of dementia and might be used in the follow-up of disease course and effect of therapy. However, continued further research involving larger study population is needed to further establish the significance of these inflammatory markers in the pathogenesis, diagnosis, follow-up and possible therapeutic options for dementia.

Key Words: Dementia – Alzheimer ’s disease – CRP – IL 6 – Alpha-1 antichymotrypsin.

Introduction

DEMENTIA is a neurological syndrome characterized by impaired cognition that is severe enough to interfere with social or occupational functioning. The two most common forms of dementia are Alzheimer’s disease accounting for 50%-70% of all cases and vascular dementia accounting for 10%-15% of all cases. Although these two forms of dementia represent different clinical presentations and pathology, there is growing evidence that they might share common pathogenesis [1].

Increasing amounts of evidence indicate that inflammatory processes are involved in the neurotoxicity of Alzheimer’s disease [2]. Amyloid deposition in the Alzheimer’s disease brain elicits a range of reactive inflammatory responses including
astrocytosis, microgliosis, and upregulation of proinflammatory cytokines, complement activation, and acute phase reactions [3]. A central event in these inflammatory processes appears to be the activation of microglia by a variety of factors, including beta amyloid and proinflammatory cytokines. Activated microglia in turn release proinflammatory cytokines, such as interleukin (IL)-1 beta and IL-6 that may lead to neuronal death and dysfunction by a variety of mechanisms [4].

Vascular dementia is characterized by a loss of cognitive function and social adaptive functions in individuals with cerebrovascular disease [5]. The clinical presentation of this illness is variable, depending on the site and extent of the lesion or infarct [6]. The pathogenesis of vascular dementia has not been well defined. Chronic inflammation and cytokine dysregulation may play a role similar to that seen in Alzheimer’s disease [7].

Elevated levels of C-reactive protein, a marker of low-grade chronic inflammation is associated with atherosclerosis and an increased cerebrovascular risk [8]. CRP deposits have been immunohistochemically detected in the affected areas of Alzheimer’s disease brain [9]. As an activator of complement system, CRP may be a significant initiator of autodestructive inflammatory processes in the brain and might represent important target for pharmacological interventions in dementia. Studies on CRP concentration in patients with Alzheimer’s disease and Vascular dementia have given discordant results [10-11].

Cytokines are mediators of the inflammatory response. Some cytokines have been involved in the pathogenesis of dementia [12]. Interleukin-6 is a cytokine implicated in inflammation, acute phase responses and cell proliferation whose effects are mediated by a receptor complex including the IL-6 receptor α-subunit and glycoprotein 130. A possible increase in plasma IL-6 levels has been reported in Alzheimer’s disease and vascular dementia [13].

Recently, elevated levels of plasma α₁-antichymotrypsin were associated with an increased risk of dementia [14]. Alpha 1-antichymotrypsin is an acute phase protein and a protease inhibitor produced by the liver and brain. Its involvement in the pathogenesis of dementia has been reported. Elevated concentration was found in cerebrospinal fluid and brain from Alzheimer’s disease patients. Also, α₁-antichymotrypsin has been shown to influence amyloid deposition in vitro and in animal models of Alzheimer’s disease [15].

Most of the reported studies concerning inflammatory markers in dementia are related to their levels in cerebrospinal fluid (CSF). CSF closely reflects the composition of the brain extracellular space, and is likely to have the highest yield in biomarker evaluation. Nonetheless, CSF is not routinely collected in the evaluation and follow-up of dementia and lumbar puncture is not a widespread technique [16].

According to recent research, plasma and CSF levels of many inflammatory markers are significantly interrelated [17]; therefore inflammatory markers assessment in the easily accessible periphery may be as effective as in the CSF, in clear contrast to several other potential biomarkers. The identification of biomarker molecules in blood would be more widely applicable and reduce the need for invasive, expensive or time consuming testing. Alternatively, such measurements might be of use for the identification of subjects at risk for dementia, thus allowing early therapeutic or even prophylactic intervention with anti-inflammatory drugs or novel disease-modifying compounds [18].

A growing body of literature indicates that inflammatory processes are present in the development of dementia. However, studies of plasma and serum biomarkers have not yielded a consistent, easily reproducible, sensitive, or specific marker for dementia diagnosis, differential diagnosis, risk, progression or treatment effects [19].

The aim of this work was to study inflammatory markers in the peripheral blood of dementia patients in a trial to clarify their role in disease pathogenesis and progression and if they could be of value as a biochemical marker for dementia and the development of novel anti-inflammatory therapy targeting these markers.

**Subjects and Methods**

A total of 60 subjects were included in this study, divided into 3 groups:

1. The Alzheimer’s disease group comprised 20 patients with probable Alzheimer’s disease (mean age 72.3 ± 5.2 years, male/female 9/11), diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association (NINCDS-ADRDA) criteria [20] and all had Hachinsky ischaemic score [21] < 4.

2. The vascular dementia group comprised 20 patients with probable vascular dementia (mean
age 70.6±4.3 years, male/female 11/9) diagnosed according to the National Institute of Neurological Disorders and Stroke and the Association Internazionale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) and Hachinski Ischemic Scale (HIS) [21,22]. All had Hachinsky ischemic score >7.

3- The control group comprised 20 age, sex and educational level matched healthy subjects (mean age 69.3±6.4 years, male/female 10/10), they all had MMSE score >28.

Patients were selected from the neurology outpatient clinic and from the neurology department of Cairo University Hospitals. They all had a mini mental state examination (MMSE) score [23] <23. None of the patients were suffering from concomitant medical or metabolic illness known to affect cognition such as hypothyroidism, liver or kidney failure, previous history of other significant neurologic or psychiatric disorders known to cause cognitive impairment such as major depression, history of taking psychoactive drugs, history of alcohol or other substances abuse, recent or past disease known to affect the parameters to be investigated such as autoimmune disease, inflammatory bowel disorders, chronic liver diseases, asthma, allergies, intercurrent infection or other concomitant diseases. Patients with use of non-steroidal anti-inflammatory drugs (NSAIDs), acetylsalicylic acid or steroids during the last 2 months before enrollment were excluded from the study.

All studied groups were subjected to the following:
1- Thorough history taking from a near relative or care giver.
2- Thorough general clinical examination.
3- Thorough neurological and neurovascular examination.
4- Diagnosis of dementia using the Mini-Mental State Examination (M.M.S.E) [23]. The total score is 30. Score of 23 is used to distinguish the normal from the abnormal subjects.
5- Diagnosis of type of dementia using:
• The criteria of the National Institute of Neurological and Communicative Disorders and Stroke, the Alzheimer Disease and Related Disorder Association [20] (NINCDS-ADRDA).
• The criteria of National Institute of Neurological Disorders and Stroke-Association pour La Recherche et L’Enseignement en Neurosciences (NINDS-AIREN) [22].
• Hachinski Ischemic Scale (HIS) [21]: A score of 7 or more is taken as indication of vascular dementia. A score of 0-4 suggested Alzheimer’s dementia. A score 4-7 suggests mixed dementia.

6- Routine laboratory investigations including complete blood picture, ESR, glucose, renal and liver functions tests, lipid profile and thyroid function tests.

7- Assay of IL-6 in serum using the Biosource human Interleukin-6 (hIL-6) EASIA kit (Biosource Europe S.A., Nivelles, Belgium, catalogue number KAC 1261):
• It is based on a solid phase enzyme amplified sensitivity immunoassay technique which uses a blend of monoclonal antibodies directed against distinct epitopes of IL-6. The use of a number of distinct monoclonal antibodies (MAbs) avoids hyperspecificity and allows high sensitive assays with extended standard range and short incubation time. Standards and samples containing IL-6 react with captured monoclonal antibodies (MAbs 1) coated on the microtiter well. After incubation, the excess antigen is removed by washing. MAb 2 (the horseradish peroxidase (HRP)-labelled antibody) is then added. After an incubation period allowing the formation of a sandwich: coated MAb 1- IL-6-MAb 2-HRP, the microtiter plate is washed to remove unbound enzyme labeled antibodies. Bound enzyme-labelled antibodies are measured through chromogenic reaction. Chromogenic solution (TMB +H2O2) is added and incubated. The reaction is then stopped with the addition of stop solution (H2 SO4) and the microtiter plate is then read at 450 nm. Standard curve is plotted and IL-6 concentration in the samples is determined by interpolation from the standard curve. The assay has a detection sensitivity of 2 pg/ml.
• All blood samples were drawn in pyrogen free tubes; between 8 and 11 a.m. to control for diurnal variation. Serum was quickly separated and stored at -70ºC until further analysis.
• IL-6 assay was performed according to manufacturer instructions, in brief, 50 ul of incubation buffer were added to all wells of the microtiter plate, then 100 ul of each standard, control and sample were added to the appropriate wells and incubated for 1 hour at room temperature on a horizontal shaker set at 700 rpm. After washing, 100 ul of anti-IL-6-HRP conjugate and 50 ul diluent were added to all wells. After 1 hour incubation at room temperature and washing, 200 ul of the chromogenic solution was added to each well and incubated for 15 minutes at room temperature after which 100 ul of stop
solution was added into each well. The absorbance was then read at 450 nm within 3 hours.

8- Assay of C-reactive protein (CRP) using the Calbiotech, Inc. (CBI) C-Reactive Protein Ultra Sensitive ELISA Kit Catalog number: CR120C:

- It is based on a solid phase direct sandwich method. The samples and anti-CRP-HRP conjugate are added to the wells coated with MAb to CRP. CRP in the patient’s serum binds to anti-CRP MAb on the well and the anti-CRP second antibody then binds to CRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of CRP in the samples. A standard curve is prepared relating color intensity to the concentration of the CRP.

- Serum CRP assay was performed according to manufacturer instructions, in brief, patient samples and controls were diluted 1:100 by adding 5 ul of samples to 495 ul of sample Diluent. 10 µL of standard, diluted samples and controls were dispensed into the appropriate wells. Then, 100 µl of enzyme conjugate was added to all wells. After Incubation for 60 minutes at room temperature, the liquid was removed from all wells. Wells were washed three times with 300 µl of 1X wash buffer. 100 µl of TMB substrate was added to all wells. After 15 minutes incubation at room temperature, 50 µl of stop solution was added to all wells. Absorbances were read on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

- Expected normal values: 1-10 mg/l.

9- Assay of α_1-antichymotrypsine in serum using the Immunology Consultants Laboratory human α_1-antichymotrypsin ELISA Kit (Immunology Consultants Laboratory, Newberg, USA, catalogue number E-80CYT):

- The Alpha 1-Antichymotrypsin (CYT) test kit is a highly sensitive two-site enzyme linked immunosassay (ELISA) for measuring α_1-antichymotrypsin in biological fluid of humans.

- It is based on double antibody sandwich ELISA. In this assay the Alpha 1-Antichymotrypsin present in samples reacts with the anti-Alpha 1-Antichymotrypsin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-CYT antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound CYT. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, (TMB). The quantity of bound enzyme varies directly with the concentration of CYT in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of CYT in the test sample. The quantity of CYT in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Results

A total of 60 subjects were included in this study: 20 patients with probable Alzheimer’s disease (AD), 20 patients with probable vascular dementia (VaD) and 20 healthy sex and age matched subjects as a control group.

C-reactive protein (CRP), interleukin-6 (IL-6) and α_1-antichymotrypsin (ACT) were measured in serum of both patient groups and the control group. Results of inflammatory markers in the different studied groups are shown in Tables (1, 2,3).

As regards CRP, serum levels mean value was 44.2±6.1 in Alzheimer’s disease patients and 52.3±5.8 in vascular dementia patients, they were both significantly higher than that of controls 10.67±0.32 (p<0.01). However, there was no statistically significant difference between the two patient groups as regards CRP (p>0.05).

IL-6 mean value was 26.4±4.5 in Alzheimer’s disease patients and 32.4±5.3 in Vascular dementia patients, they were both significantly higher than that of controls 9.2±0.5 (p<0.01). However, there was no statistically significant difference between the two patient groups as regards IL-6 (p>0.05).

As regards α_1-antichymotrypsin, the mean value was 780±21 in Alzheimer disease significantly higher than that of controls 490±16 and that of vascular dementia patients 512±19 (p<0.01). There was no significant difference between vascular dementia patient group and control group as regards ACT (p>0.05).

Correlation between serum inflammatory markers levels and dementia severity in the two patient groups is shown in Table (4). There was significant negative correlation between MMES scores and IL-6 and ACT in the two patient groups. Whereas CRP did not correlate with disease severity judged by MMSE score.
Table (1): Comparison of inflammatory markers between AD patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>AD patients</th>
<th>Control group</th>
<th>p value</th>
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<tbody>
<tr>
<td>CRP mg/l (mean±SD)</td>
<td>44.2±6.1</td>
<td>10.67±0.32</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>IL-6 pg/ml (mean±SD)</td>
<td>26.4±4.5</td>
<td>9.2±0.5</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>α1-antichymotrypsin ng/ml (mean±SD)</td>
<td>780±21</td>
<td>490±16</td>
<td>&lt;0.01*</td>
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Table (2): Comparison of inflammatory markers between VaD patients and controls.

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<thead>
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<th></th>
<th>VaD patients</th>
<th>Control group</th>
<th>p value</th>
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<tbody>
<tr>
<td>CRP mg/l (mean±SD)</td>
<td>52.3±5.8</td>
<td>10.67±0.32</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>IL-6 pg/ml (mean±SD)</td>
<td>32.4±5.3</td>
<td>9.2±0.5</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>α1-antichymotrypsin ng/ml (mean±SD)</td>
<td>512±19</td>
<td>490±16</td>
<td>&gt;0.05</td>
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Table (3): Comparison of inflammatory markers between AD patients and VaD patients.

<table>
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<th>VaD patients</th>
<th>p value</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>α1-antichymotrypsin ng/ml (mean±SD)</td>
<td>780±19.3</td>
<td>512±16.7</td>
<td>&lt;0.01*</td>
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Table (4): Correlation between serum inflammatory markers levels and dementia severity in the two patient groups.

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<tbody>
<tr>
<td>CRP</td>
<td>-0.25</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.58</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>α1-antichymotrypsin</td>
<td>-0.62</td>
<td>&lt;0.01*</td>
</tr>
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</table>

Discussion

Dementia is associated with aging and its incidence and prevalence as a major health problem is increasing with the population increasing life expectancy. Epidemiologic studies consistently confirm that Alzheimer’s disease is the most prevalent cause of dementia and vascular dementia is the second [24].

Systemic and local inflammatory responses have been proposed as mechanisms in the initiation and progression of dementia disorders [28]. Several studies described increased levels of pro-inflammatory cytokines in Alzheimer’s disease patients [26]. Acute-phase reactants such as C-reactive protein have been detected in neuritic plaques and neurofibrillary tangles in the brains of patients with Alzheimer’s disease [27]. Elevated levels of serum C-reactive protein, a marker of inflammation, may predict vascular disease [28] and dementia [29].
and have been associated with stroke patients [30]. Recently, inflammatory markers α_1-antichymotrypsin, interleukin-6 and to a lesser extent CRP were associated with an increased risk of dementia.

Even though several lines of evidence in the literature have shown that inflammation is involved in the pathogenesis of dementia, the results from the evaluation of inflammatory markers in Alzheimer patients and vascular dementia patients in these studies have been controversial.

Moreover, most of these studies were conducted on CSF. Although there is clear rationale for pursuing dementia biomarkers in CSF, there is no question that a biomarker derived from the peripheral blood would be more convenient and would probably aid the study, diagnosis and monitoring of clinical progress and therapeutic interventions.

The aim of this work was to study inflammatory markers in peripheral blood of patients with dementia in a trial to clarify their role in disease pathogenesis and progression as well as their potential value as biological markers and targets of anti-inflammatory therapy for dementia.

In this study, serum concentration of C-reactive protein (CRP), interleukin-6 (IL-6) and α_1-antichymotrypsin (ACT) were measured in two groups of dementia patients (Alzheimer’s disease and vascular dementia) and compared with corresponding values in a control group of healthy sex and age-matched subjects.

CRP levels were significantly higher in both Alzheimer’s disease patients and vascular dementia patients compared to normal controls. There was no significant difference between Alzheimer’s patients and vascular dementia patients as regards CRP.

Although CRP was originally thought to be produced almost exclusively by hepatocytes, CRP is now known to be synthesized in brain cells and up regulated in Alzheimer tissue [34,35]. Elevations in serum CRP could be attributed to increased release from the brain which is reflected in serum.

In both Alzheimer disease and vascular dementia, an increase in CRP has been reported. Gupta et al. [11] found a significantly elevated CRP concentration in serum of both Alzheimer’s and vascular dementia patients as compared to controls. Schmidt et al. [29] found strong association between high C-reactive protein plasma levels and late onset Alzheimer disease and vascular dementia. They reported significantly higher CRP levels in cases of VaD and AD than in controls. In 2006, increased serum level of CRP, a marker traditionally associated with vascular risk, was reported to precede by several decades the onset of both VD and AD by Ravaglia et al. [36]. Similarly, Schimdt et al. [29], found a strong positive relation between high CRP concentration at midlife and the risk 25 years later of dementia.

However, Jordanova et al. [37] in 2007, found no association between CRP levels and cognitive decline. They explained this by the fact that the immunoassays were only performed at a single time point. Licastro et al. [10], reported normal CRP levels in Alzheimer’s disease patients. Also, Carusone et al. [38] did not find a significant difference in CRP levels between the vascular dementia cases and controls, they concluded that these results were most likely due to limitations of measuring serum CRP and the possibility that localized increases in CRP may be associated with vascular dementia but not detected with serum measurements.

There was no significant difference between Alzheimer’s patients and vascular dementia patients as regards CRP. This finding was in accordance with Gupta et al. [11], who reported that there was no significant difference between vascular dementia patients and Alzheimer’s patients as regards CRP.

On the other hand, Nilsson et al. [39] in 2008 reported that patients with Alzheimer’s disease showed lower CRP levels than patients with vascular dementia. Schmidt et al. [30] reported higher CRP levels in cases of VaD compared with AD.

As regards IL-6, the mean serum IL-6 value in Alzheimer’s patients was significantly higher than that of controls. It was also significantly higher in vascular dementia patients than in controls but not significantly different from the mean of Alzheimer’s disease patient group. Thus AD and VaD patients, similarly, had higher IL-6 levels compared to the control group.

Elevated IL-6 found in plasma or serum may be produced by blood cells, endothelium or may originate from the brain. Activated by Aβ amyloid accumulation, blood cells may cross the blood-brain barrier and contribute to Alzheimer’s disease degeneration [40,41]. In contrast, inflammatory molecules produced in the brains of demented patients may result in an inflammatory response in the periphery by humoral, neuroendocrine and sympathetic connections that have been clearly
demonstrated [42-43]. Alternatively, dementia may be associated with a more widespread systemic immune dysregulation, detectable in plasma [14].

Serum IL-6 was shown to be elevated in different types of dementia compared to controls by several authors. In 2008, Angelopoulos et al. [44] reported that the levels of interleukin-6 were significantly elevated in the serum of patients with dementia including AD patients and VD patients than in controls. A study by Ozturk et al. [48], 2007, showed high levels of the pro-inflammatory cytokine IL-6 in all patient groups (AD and VD) compared to controls.

Gupta et al. [11] found statistically significant elevations of serum IL-6 in the two dementia subtypes. Maes et al. [46] stated that serum IL-6 levels were significantly higher in AD patients than in controls. Similarly, Bonaccorso et al. [47] reported that AD patients had significantly higher serum IL-6 than age-matched normal volunteers. Kalman et al. [48] also stated that significantly increased levels of IL-6 were found in the severe stage of AD. A strong positive relation between high IL-6 levels at midlife and the risk 25 years later of dementia was reported by Weaver et al. who concluded that increased IL-6 is not an effect but cause of dementia [49].

Contrarily, Bonotis et al. [50], reported that no significant differences were observed on IL-6 cytokine levels between Alzheimer’s disease patients and controls. Angelis et al. [51] reported that proportions of individuals with elevated serum IL-6 concentrations did not differ significantly between dementia patients and controls and Blum-Degen et al. [52] found no significant difference of serum IL-6 between AD patients and control group.

In this study, there was no significant difference between Alzheimer’s patients and vascular dementia patients as regards CRP and IL-6 levels. It was previously believed that most cases of dementia were the outcome of one of the two AD and VaD distinct diseases. However, the clear division between them has recently been challenged. It is now widely believed that vascular risk factors are also associated with Alzheimer's disease and Alzheimer’s disease and vascular dementia may share many common clinical and pathological characteristics as well as same pathogenesis [53,54].

Contrarily, Zuliani et al. [55] reported that serum IL-6 was higher in VD patients compared to AD patients, however, they stated that the higher IL-6 levels found in VD might be not a specific finding, as it might come from several conditions including atherosclerosis and related vascular risk factors, co-morbidity, and frailty. Gupta et al. [11], also reported that the rise in serum IL-6 was higher in vascular dementia patients compared to Alzheimer's patients.

The confounding results regarding serum CRP and IL-6 in different studies could be attributed to differences in sample collection protocols, assay methodology, assay sensitivity, small sample sizes, heterogeneous patient populations, effects of disease severity age and co-morbid inflammatory illness [56]. Circulating cytokines have a short half-life, may reach high concentrations at sites of release but much lower concentrations after dilution in blood or they may bind to molecules that do not permit their detection by immunological methods. [57]. All of these possibilities may factor into the contradictory results of several studies.

Alpha_1-Antichymotrypsin (ACT) is commonly associated with amyloid plaque deposits in Alzheimer’s disease brains. Elevated levels in brains, cerebrospinal fluid and sera from AD patients have been reported. Still, the value of ACT as a biomarker of dementia is unclear [58]. Previous studies of blood and CSF levels of ACT in dementia have been conflicting.

In this study serum α_1-antichymotrypsin in Alzheimer’s patients was significantly higher than that of controls and of vascular dementia patients. There was no significant difference between vascular dementia patients and controls as regard α_1-antichymotrypsin.

The source of the elevated ACT in blood in Alzheimer disease is unknown. Although it is not known whether ACT leaks from CNS into the peripheral vascular system (or vice versa), the elevated levels in CSF and blood suggest a systemic response. Concentrations of ACT in the CSF are 100 times lower than blood concentrations; therefore, the source of any increase in the plasma ACT is not likely to be the CNS [59].

Previous studies of blood and CSF levels of ACT in dementia have been conflicting. Matsubara et al. [60] measured serum alpha_1-antichymotrypsin levels in 38 patients with Alzheimer-type dementia and 20 patients with vascular dementia. Cerebrospinal fluid (CSF) levels of alpha_1-antichymotrypsin were also measured in 15 patients
with Alzheimer-type dementia and 6 with vascular dementia. They reported that serum and CSF levels were significantly higher in patients with Alzheimer-type dementia than in vascular dementia. Serum levels of alpha-1-antichymotrypsin were also significantly elevated in the early stage of Alzheimer-type dementia. They concluded that the measurement of serum levels of alpha-1-antichymotrypsin could be useful as a screening marker for Alzheimer-type dementia.

Similarly, Teunissen et al. [61] stated that alpha-1-antichymotrypsin was significantly higher in Alzheimer’s patients compared to controls. Sun et al. [17] and Licastro et al. [10] also reported elevated ACT in plasma and CSF of AD patients.

Contrarily, Pirttilä et al. [62], reported that alpha 1-antichymotrypsin and were not increased in CSF or serum in Alzheimer's disease. A study by Ozturk et al. [44] in 2007 showed high levels of alpha-1-antichymotrypsin in VD patients only, compared to controls. Engelhart et al. [16] reported that elevated plasma levels of ACT were associated with an increased risk of AD and vascular dementia.

In this study, there was significant negative correlation between MMSE scores and IL-6 and alpha-1-antichymotrypsin in the two patient groups. In 2007, Wright et al. [63] reported that IL-6 was negatively associated with MMSE score and conclude that IL-6 levels were negatively associated with performance. Adjusting for vascular disease and subclinical atherosclerosis did not attenuate the association, suggesting a direct effect on the brain.

Licastro et al. [10] reported that plasma levels of alpha 1-antichymotrypsin negatively correlated with cognitive performances, as assessed by the mini mental state examination. Dekosky and colleagues also reported a negative correlation (increasing ACT as cognition declines); they concluded that the contradiction between different studies may reflect differences in numbers of cases examined and the stage of disease progression [58].

Conclusion:

In this study, alterations of the inflammatory markers were detected in peripheral blood of dementia patients denoting that assay of plasma inflammatory markers might provide an insight in the pathogenesis of dementia and might provide an easier and accessible way in the diagnosis and follow up of dementia compared to their assay in CSF. Serum CRP, IL-6 levels were significantly higher in both patients groups compared to the control group. The fact that these changes were found in both Alzheimer’s disease patients and vascular dementia patients emphasize the common pathophysiological mechanisms in the two dementia subtypes. Serum alpha-1-antichymotrypsin was significantly higher in Alzheimer’s disease patients than in vascular dementia patients denoting its possible value in the differential diagnosis between the two types. The negative correlation between MMSE score and levels of IL-6 and ACT in both patient groups suggest that IL-6 and alpha-1-ACT may play a role in defining the aggressiveness of dementia and might be used in the follow-up of disease course and effect of therapy. However, continued further research involving larger study population is needed to further establish the significance of these inflammatory markers in the pathogenesis, diagnosis, follow-up and possible therapeutic options for dementia.

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