Determinition of Adiponectin, Tumor Necrosis Factor-Alpha, and Adhesion Molecules in Alzheimer’s Disease

Sumru Savaş¹, Burcu Güler², Fulden Saraç³

Objective: Though the data regarding the mechanisms behind neurodegeneration in addition to amyloid plaques and neurofibrillary tangles are not clear, there are emerging data for inflammation and microvascular changes to have a contributory role in the pathology of Alzheimer’s disease (AD). The relationships between numerous biomarkers also need to be investigated. This study aimed to assess inflammatory marker tumor necrosis factor-alpha (TNF-α), adiponectin (a modulator of anti-inflammation), and potential microvascular markers for AD including both intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in Turkish patients with AD and healthy elderly subjects, and the relationships among the variables in patients with AD.

Materials and Methods: In this study, 46 patients with AD and 30 cognitively healthy controls over 60 years of age from the outpatient clinics of Ege University were included. Adiponectin, ICAM-1, VCAM-1, and TNF-α were evaluated by enzyme-linked immunosorbent assays.

Results: The median adiponectin level in the AD group was higher than in the controls (p=0.002). Median VCAM-1, ICAM-1, and TNF-α values for patients with AD and the controls were similar. There were positive correlations between VCAM-1 and both TNF-α and adiponectin in the patients with AD (r=0.540, p<0.001, and r=0.301, p=0.044, respectively).

Conclusion: Though a dramatic rise of adiponectin, and associations of VCAM-1 and both TNF-α and adiponectin in subjects with AD were shown, the clinical significances of these peripheral measurements need to be further investigated.

Keywords: Adiponectin, Alzheimer’s disease, intercellular adhesion molecule-1, tumor necrosis factor-alpha, vascular cell adhesion molecule-1

INTRODUCTION

Alzheimer’s disease (AD) is one of the most important causes of dementia in the aging population. Because of the increased life expectancy, an enhanced AD burden is expected in the future. Though defined by two core pathologies such as plaques and tangles (1), underlying neurodegenerative mechanisms still need to be explored for AD. Amyloid-beta and tau biomarkers are significantly associated with the presence of neuropathological hallmarks of AD. However, they do not assess other biochemical aspects of AD. For the patients with AD with a later onset, a large amount of blood biomarkers have been investigated and published reporting associations of biomarkers with AD in recent years both for exploration of the pathogenesis and for the diagnosis (2–6). Those blood biomarkers have the advantage of the practicality, yet they are not sufficiently accurate (2, 3).

Neuroinflammation and continuing immune responses raised as substantial mechanisms for the pathogenesis of Alzheimer’s type dementia in recent years (6). One hypothesis is that microglia are activated after the accumulation of damage signals caused by numerous factors, and interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), and some trophic factors are consequently released (7). Adiponectin, which is an adipocytokine, has anti-inflammatory properties; and it sensitizes the body to insulin. However, it may also play a role in the etiopathology of AD (8).

Vascular dementia and AD were traditionally considered separate disorders. However, increasing evidence suggests that they may be related, and there is growing evidence suggesting significant roles for potential microvascular biomarkers such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) for AD (4, 5).

Since chronic inflammation and microvascular changes may play pivotal roles for the pathogenesis and the progression of the disease, we hypothesized associations among adiponectin, TNF-α, ICAM-1, and VCAM-1 in patients with AD. To the best of our knowledge, there is no study evaluating those biomarkers and their relations in Turkish patients with AD and cognitively healthy controls except for TNF-α. Therefore, this study was performed to examine the aforementioned factors and the relationships between them.

INTRODUCTION
MATERIALS and METHODS

This case-control study was carried out with adherence to international ethical standards. Izmir First Clinical Investigations Ethics Committee (approval reference number: 09-10/11) approved the work and the protocols. The controls and the patients with AD or the guardians gave informed consent to participate in the study.

Study Population

Individuals over 60 years of age and with an education of more than five years were taken into the study as controls and patients within six months from the outpatient clinic of the Department of Neurology and Geriatrics. Baseline assessments of biochemical analyses and a survey form were carried out for the subjects. We recruited 64 consecutive patients with known AD diagnosis. Mini-Mental State Examination (MMSE) was used to assess cognitive function. Patients with stroke or acute coronary events, malignancy, diabetes, and inflammatory comorbidities were excluded from the study. Ultimately, 46 patients with mild-to-moderate AD with MMSE scores 10–24 were enrolled.

The control group comprised of individuals from the outpatient clinic of Internal Medicine Department of the same institution. Fifty consecutive subjects with minor complaints that were explored and proven not to have major consequences were assessed. Thirty non-demented healthy elderly subjects with MMSE scores ≥25 and to whom the exclusion criteria did not apply were included as controls.

Biochemical Analyses

Having an overnight fasting period, venous blood samples were collected from the subjects in the same laboratory, and within 30 min of sampling, they were centrifuged at 3,000×g for 10 min. Fasting serum samples were stored at –80°C for the analyses of TNF-α, adiponectin, ICAM-1, and VCAM-1.

The serum values for TNF-α, adiponectin, ICAM-1 (soluble), and VCAM-1 were processed by kits which are enzyme-linked immunosorbent assays (Thermo Fisher Scientific, Invitrogen, No.: KHC3011, Camarillo, CA; Life Technologies, Invitrogen, No.: KHP0041, CA, USA; Invitrogen, No.: KHS5411, Camarillo, CA; Invitrogen, No.: KHT0602/KHT0601, CA, USA, respectively) according to the manufacturers’ instructions. The limits of lower determination were as follows: TNF-α=1.7 pg/mL, adiponectin=100 pg/mL, ICAM-1 <0.5 ng/mL, VCAM-1 <0.5 ng/mL.

Statistical Analyses

Before performing the analyses, normality was assessed by the Shapiro–Wilks test. The patients with AD and the controls were evaluated by the Mann–Whitney U test, the chi-square test, and the Spearman’s correlation analyses, where available. The findings were shown as medians (interquartile ranges) for continuous values, and percentages for categorical variables. The significance of statistics was driven with a p < 0.05 value. The version of Windows (18.0, SPSS Inc., USA) of Statistical Package for Social Sciences was used for the statistical tests.

RESULTS

Demographic data and biochemical profile of the subjects are presented in Table 1. The patients with AD and controls were similar regarding mean age and gender. The age range was 65–86 years in the AD group and 61–84 years in the controls. The number of patients with the history of hypertension and dyslipidemia was also similar in patients with AD and the controls. The median levels of serum adiponectin, TNF-α, VCAM-1, and ICAM-1 were not different between males and females in both groups (data not shown). The median (IQR) MMSE scores in the control group and patients with AD were 28 (2) and 17.5 (3), respectively (p<0.001).

The median adiponectin level in subjects with AD was significantly higher than the controls where median levels of TNF-α, VCAM-1, and ICAM-1 were similar in both groups as shown in Table 1.

As presented in Figure 1, adiponectin represented a weak positive correlation with VCAM-1 in patients with AD (r=0.301, p=0.044). Likewise, there was a medium positive correlation with TNF-α and VCAM-1 in subjects with AD (r=0.540, p<0.001) (Fig. 2). Age showed a medium positive correlation with VCAM-1 in controls (r=0.548, p=0.004).

No other significant correlation was observed.

![Figure 1. Correlation between vascular cell adhesion molecule-1 and adiponectin in patients with Alzheimer’s disease](image-url)

<table>
<thead>
<tr>
<th>Table 1. Demographic data and biochemical profile of the patients with Alzheimer's disease and the control group</th>
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<tr>
<td><strong>Characteristics</strong></td>
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<tr>
<td>Age* (year)</td>
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<tr>
<td>Gender (M/F), n</td>
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<tr>
<td>Presence of hypertension, n (%)</td>
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<tr>
<td>Presence of dyslipidemia, n (%)</td>
</tr>
<tr>
<td>Adiponectin* (µg/mL)</td>
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<td>VCAM-1* (µg/mL)</td>
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<td>ICAM-1* (µg/mL)</td>
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<td>TNF-α* (pg/mL)</td>
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*Values are expressed as medians (interquartile ranges). AD: Alzheimer's disease; M: Male; F: Female; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular adhesion molecule-1; TNF-α: Tumor necrosis factor-alpha
Chronic inflammation and microvascular changes may contribute to the development of dementia (5, 6). Multifactorial mechanisms behind neurodegeneration require clarification in AD. In this study, our results show a significant rise in the level of adiponectin for subjects with AD with respect to controls. Besides, we showed positive correlations between VCAM-1 and both adiponectin and TNF-α in patients with AD. This is the first study evaluating aforementioned relationships and biomarkers (except for TNF-α) in Turkish patients with AD.

Adiponectin is a hormone secreted by visceral fat. Despite the fact that adiponectin has protective effects for diabetes and atherosclerosis and anti-inflammatory properties, some authors suggest that patients with AD lose weight that may ultimately lead to lower leptin, higher adiponectin levels, and adiponectin resistance (9–11). Though adiponectin has receptors in neurological tissue and a role in insulin signaling, the relation among adiponectin and dementia is nevertheless in dispute (8, 11). In three cross-sectional studies, authors failed to show significant differences regarding adiponectin levels between patients with AD and controls (9, 12, 13). On the other hand, a study showed lower adiponectin levels in patients with AD across control group (14). In contrast with these findings, it was shown in a prospective study that higher plasma adiponectin level was a risk factor for future dementia and AD for older females (8). Furthermore, in two other cross-sectional studies, patients with AD were found to have increased plasma levels of adiponectin compared to normal controls (15, 16). Besides, in a recent study analyzing biomarkers, adiponectin levels were higher in the patients with AD across healthy controls from the beginning and 36 months later (2). This result is consistent with our study in which we showed that patients with AD had significantly higher adiponectin levels than the control group.

There is growing evidence that late-onset AD is a multifactorial disorder in which patients commonly present complex combinations and manifestations where the most common mixed pathology is AD with vascular pathologies. How, when, and in which conditions vascular lesions get involved with the process and which risk factors predispose patients in developing cognitive impairment are questions to be answered. Though vascular pathologies such as macroinfarcts and microinfarcts are common in older persons with aging, it is suggested that vascular dysfunction may impair the Aβ clearance of the brain, or vascular pathology may be an additional burden on the brains of patients with AD lowering the threshold for cognitive impairment (4). Additionally, several data suggest that other vascular contributions might also be involved in AD such as vascular anatomical defects, dysfunction of the brain blood barrier (BBB), a vascular remodeling defect, and subsequent insufficient cerebral blood flow. A compromised BBB may change the transport across the BBB, increase the entrance of molecules, also having an effect on Aβ accumulation, and may trigger an inflammatory cascade (17). The BBB is a specialized endothelial cell membrane in a high degree, presenting the interface between neural cells and circulating cells of immunity that operates within the neurovascular unit. The neurovascular unit may become dysfunctional in AD and contribute to neuronal injury and cognitive deficit (6). In AD models, targeting adhesion mechanisms that control leukocyte-endothelial interactions reduces memory loss by inhibiting Aβ deposition and tau hyperphosphorylation. Therefore, controlling vascular inflammation and related mechanisms could help to understand the fundamentals of BBB dysfunction and could enable developing new therapeutic approaches (6). Cognitive impairments and vascular diseases may be linked through the development of cerebral endothelial dysfunction that may be due to several diseases such as hypertension and type 2 diabetes. Cerebral endothelial dysfunction may also impair cerebral blood flow and vasoreactivity. Elevated plasma VCAM-1 is presumed as a marker of endothelial dysfunction, both in the brain and systemic circulation. In a study of older patients, the authors have observed an association between increased VCAM-1 levels and both cognitive impairment and increased cerebrovascular resistance suggesting that VCAM-1 is associated with abnormalities in cerebral blood flow regulation (18). The upregulation of VCAM-1 and ICAM-1 by cytokines is also suggested to precede the reduction of the permeability of the microvasculature reducing vasodilation (4–6, 17, 18). In the aforementioned study, increased ICAM-1 levels have been shown in cognitive impairment; however, they are not statistically significant (18). Zuliani et al. found that plasma VCAM-1 levels were increased in patients with AD and vascular dementia (19). In a recent systematic review and meta-analysis evaluating peripheral inflammatory markers in AD, increased plasma VCAM-1 and TNF-α were reported in patients with AD (3). In contrast with these findings, we did not observe significant elevations in serum levels of ICAM-1 and VCAM-1 among patients with AD in our study. Consistent with our data, ICAM-1 and VCAM-1 did not show an association with an elevated risk of AD in a population-based setting (20). On the other hand, in the study by Gupta et al., where VCAM-1 levels changed substantially after 36 months, they suggested that this points out that elevated VCAM-1 might be a repairing reaction to injuries and neuroinflammation when cytokines induce endothelial cells (2). Recently, amyloid-beta oligomer-triggered elevations in TNF-α levels were reported (21). Inflammatory responses accompany AD, as shown in a meta-analysis of cytokines by Swardfager et al. where they reported significant elevations of peripheral serum TNF-α, IL-6, IL-1 beta, transforming growth factor-beta, IL-12 and IL-18 levels in AD (3, 22). However, the evidence was weaker for TNF-α and IL-18. In our study, though we did not observe statistically significant differences in median TNF-α levels of subjects with AD.
and control group, TNF-α levels were lower in patients with AD in comparison with the control group. This discrepancy could be because of the relatively limited sampling of groups. Besides, our patients could possibly be on cholinesterase inhibitors that may reduce peripheral TNF-α concentrations through the role of brain cholinesterase activity. To date, there have been studies investigating the potential of several biomarkers in Turkish patients with AD such as proinflammatory cytokines (23–25), neopterin (23), resistin (24) as well as brain-derived neurotrophic factor, complement factor H (25), and leptin (26). TNF-α levels were increased in patients with AD in a recent study (24). Consistent with this study, TNF-α levels were similar for the patients with AD and controls in our prior study (23).

Once activated, proinflammatory cytokine TNF-α increases expression of adhesion molecules (27). In a recent cell culture study investigating memantine, the authors reported that treating with memantine repressed TNF-α induced elevations of ICAM-1, VCAM-1, and E-selectin (28). Our data support the data of association between VCAM-1 and TNF-α in patients with AD. Though in-vitro studies demonstrate that adiponectin suppresses TNF-α-induced adhesion molecule expression, a positive association between VCAM-1 and adiponectin was previously described in patients with diabetic nephropathy and hyperlipidemia (29, 30). Likewise, we found a positive correlation between adiponectin and VCAM-1 in patients with AD. It may be hypothesized that adiponectin levels might be increased as a compensating reaction (29, 30).

Additionally, age positively correlated with VCAM-1 in a sample of elderly patients with AD or vascular dementia in a study by Zuliani et al. (19). In contrast with this association, age correlated with VCAM-1 in our control group, but not in the AD group. It may be speculated that this result is because of the higher proportion of hypertensive individuals in the control group; however, this higher proportion in the controls was not statistically significant. Besides, though we did not include individuals with stroke or acute coronary events, the availability of vascular diseases may not be definitely eliminated in elderly patients.

The limitations of our study include the limited number of control subjects because of the difficulty in locating healthy elderly subjects and the relatively small sample size of the patients with AD. Besides, the cross-sectional design of the study may preclude showing the temporal relation among the variables.

**CONCLUSION**

The role of circulating immune system cells in AD-related brain damage is still insufficiently understood. Recently, it has been clear that vascular and AD pathologies commonly occur together. Targeting the vascular component would enable the interventions to either reduce the risk of cognitive impairment or maybe decelerate the pathological process in the future. Therefore, the related biological markers of endothelial dysfunction such as VCAM-1 would be of central importance. Overall, understanding and revealing the associations between microvascular biomarkers and other peripheral measurements will help to fill in the gaps for the vicious pathophysiological processes in AD and enable targeting those mechanisms as well and to evaluate therapeutic efficacy. Besides, this is the first study measuring ICAM-1, VCAM-1, adiponectin, and TNF-α together in the Turkish population and would contribute to researches for Turkish patients with AD. Further prospective studies of overlapping factors, especially of circulating adhesion molecules that may be responsible for the onset or/and progression of AD, are required in larger samples that comprise the medications, anthropometric measurements, the severity of the disease, and several other factors including the genetics.

**Acknowledgements:** The authors acknowledge Hatice Uluer for her assistance with the statistical analysis.

**Ethics Committee Approval:** Izmir First Clinical Investigations Ethics Committee (approval reference number: 09-10/11) approved the work and the protocols.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**REFERENCES**

11. Ishii M, Iadecola C. Adipocytokine-derived factors in age-related dementia...
and their contribution to vascular and Alzheimer pathology. Biochim Biophys Acta 2016; 1862(5): 966–74. [CrossRef]


