Alzheimer disease, inflammation, and novel inflammatory marker: resistin

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Background/aim: Inflammation may play an important role in Alzheimer disease (AD) pathogenesis. A growing amount of evidence indicates that resistin has hallmark regulatory functions such as inflammatory states. The aim of this study was to determine whether plasma resistin levels would be useful in the diagnosis of patients with AD and to investigate the relationships between resistin and other inflammatory markers such as hs-CRP and TNF-α.

Materials and methods: In this cross-sectional study, 38 AD patients and 32 control subjects with normal cognitive function aged 65 years and over were included. The diagnosis of AD was made according to DSM-IV and NINCDS-ADRDA criteria. Serum levels of resistin were measured with an enzyme-linked immunosorbent assay method using the human resistin E50 kit.

Results: The median resistin level of AD patients was significantly higher than in the control group (86.3 vs. 70.8 pg/mL, P = 0.002). Overall accuracy of resistin in determining AD was 70.66%, with sensitivity, specificity, PPV, and NPV of 75.0%, 65.5%, 73.0%, and 67.9%, respectively. There was no statistically significant difference between AD patients and control subjects with respect to hs-CRP and TNF-α levels.

Conclusion: Resistin levels may be considered as a predictor of AD and it may predict activation of the immune system in AD pathophysiology.

Key words: Alzheimer disease, inflammation, resistin

1. Introduction

Alzheimer disease (AD), the most common type of dementia, is a major world health problem, influencing approximately one in eight people over 65 years old. Several epidemiological and clinical studies have suggested that inflammation may have an important role in dementia, including AD pathogenesis (1–5). Acute phase response to damaged tissue, expressing amyloid precursor protein, and gene polymorphisms of several inflammatory mediators are some hypotheses suggesting a role of inflammation in AD (6). Several longitudinal studies have also shown that inflammatory markers are related to dementia (2–4,7). Resistin is a 12.5-kDa cysteine-rich peptide produced by adipocytes and is an important factor linking obesity with diabetes (8). Besides its contribution to insulin resistance, it has been shown that resistin can trigger a proinflammatory state by regulating various biological processes, including several inflammatory diseases (9). Moreover, proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1β can increase resistin expression in peripheral blood mononuclear cells, suggesting a potential role of resistin in the process of inflammation (9). Resistin upregulates proinflammatory cytokines and vascular adhesion molecule expression in human endothelial cells, indicating that resistin may play a crucial role in the host response to acute inflammation via a nuclear kappa B-dependent pathway (10,11). Resistin has also been reported to be expressed in chronic disease states, such as rheumatoid arthritis, atherosclerosis, obesity, diabetes, and inflammatory bowel disease (10,12).

Our study was undertaken to investigate whether the levels of resistin were altered in clinically diagnosed

AD patients and its correlation with other inflammatory markers. A clarification of the complex network of immune-inflammatory mediators involved in the development of AD could lead to the identification of new therapeutic targets for the prevention and treatment of AD.

2. Materials and methods

2.1. Patients
In this cross-sectional study, 38 AD patients and 32 control subjects with normal cognitive function aged 65 years and over who were admitted to our outpatient clinic were enrolled.

All the patients underwent a comprehensive geriatric assessment, including mini-mental state examination (MMSE) (13) and clock-drawing tests (14). The diagnosis of AD was made according to DSM-IV (15) and NINCDS-ADRDA (16) criteria after cognitive assessment and neuroimaging was performed using magnetic resonance. In addition, information from a knowledgeable informant was obtained to ensure there was no change in cognitive function for the normal cognitive status group.

Those patients on dialysis; with malignant disease; having severe liver failure, deep vein thrombosis, coronary artery disease, congestive heart failure, cerebrovascular accident, diabetes mellitus, thyroid disorders, or morbid obesity; using antiinflammatory drugs; or with active infection or inflammatory diseases were excluded from the study. The study was conducted in accordance with the guidelines of the Helsinki Declaration.

2.2. Serum samples
Blood samples were obtained from a peripheral vein after an overnight fast without using any anticoagulant and were subjected to centrifugation at a speed of 3000 rpm for 10 min at 4 °C to obtain serum. All serum samples were stored at –80 °C immediately after separation from peripheral blood prior to analysis.

2.3. Determination of plasma resistin concentration, TNF-α, and hs-CRP levels
Resistin values were measured with an enzyme-linked immunosorbent assay (ELISA) method using the human resistin E50 kit (Laboratory Medicine Inc., Reutlingen, Germany). Serum hs-CRP was measured on fresh blood using the N-high sensitivity CRP assay with a CRP HS ELISA kit (DRG Instruments GmbH, Marburg, Germany). TNF-α values were measured by ELISA using the Human TNF-α Total ELISA Kit (Bender MedSystems GmbH, Vienna, Austria).

2.4. Statistical analyses
SPSS 15.0 for Windows was used for analysis. The variables were evaluated by using histograms, probability plots, and analytical methods to identify the normally distributed and skewed variables. Data were presented as mean ± SD for normally distributed variables and as median (minimum-maximum) for skewed continuous variables. Numbers and frequencies were used to show categorical variables. The Mann–Whitney U test was used for skewed data in univariate analysis. Independent sample t-test was performed for comparing normally distributed variables. P < 0.05 was considered statistically significant.

Correlation analysis between resistin and MMSE was performed with the Spearman correlation test. Resistin, hs-CRP, TNF-α, leukocytes, and ESR values for predicting AD were analyzed using receiver operating characteristic (ROC) curve analysis. When a significant cut-off value was observed, the sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and overall accuracy were presented. While evaluating the area under the curve, a 5% type-1 error level was used to accept a statistically significant predictive value of the test variables.

3. Results
Thirty-eight patients with AD [mean ± SD age: 79.86 ± 5.55 years, 19 males (50%)] and 32 patients with normal cognitive function [74.22 ± 7.21 years, 11 males (34.4%)] as a control group were enrolled in the present study. The demographic properties and laboratory parameters of the study population are presented in Table 1. With respect to routine biochemical analysis, only albumin and creatinine levels were found to be decreased in AD patients (P = 0.021 and P = 0.044, respectively). There was no significant difference between groups regarding other laboratory parameters.

The median resistin level of patients with AD [86.3 pg/mL (33.3–455.3 pg/mL)] was significantly higher than in the control group [70.8 pg/mL (20–204.4 pg/mL)] (P = 0.002) (Figure 1). No significant difference was observed between the two groups with respect to h-CRP and TNF-α levels (Table 2).

ROC curve analysis suggested that the optimum resistin cut-off point for AD was 73.33 pg/mL, with sensitivity, specificity, PPV, and NPV of 75.00%, 65.52%, 73.00%, and 67.90%, respectively (AUC: 0.724) (Figure 2). Overall accuracy of resistin in determination of AD was 70.66%. The same analysis for other inflammatory markers is summarized in Table 3.

Spearman correlation analysis revealed a moderate negative relationship between resistin and MMSE (r = –0.348, P = 0.038) (Figure 3).

4. Discussion
In the present study, we demonstrated that patients with AD have elevated resistin levels in comparison with healthy controls. Although hs-CRP and TNF-α levels

Table 1. Demographics properties and laboratory parameters of the study population with AD and normal control subjects.

Parameters	Controls (n = 32)	AD (n = 38)	P
Age (years)	74.2 ± 7.2	79.8 ± 5.5	0.001
BMI (kg/m²)	26.7 ± 2.9	26.7 ± 3.4	0.972
Sex (F/M)	21 (65.6%)/11 (34.4%)	19 (50%)/19 (50%)	0.156
Hemoglobin (g/dL)	13.99 ± 1.26	13.45 ± 1.41	0.116
Leukocytes (mm³ × 10³)	8.8 ± 1.9	7.3 ± 1.8	0.444
Platelets (mm³ × 10³)	233 ± 560	239 ± 861	0.759
Sedimentation (mm/h)	17.07 ± 10.04	20.28 ± 15.31	0.349
Glucose (mg/dL)	88.48 ± 6.02	89.29 ± 12.11	0.072
ALT (U/L)	19.24 ± 4.99	14.70 ± 6.00	0.062
AST (U/L)	22.28 ± 4.06	20.91 ± 6.50	0.314
Albumin (g/dL)	4.39 ± 0.26	4.23 ± 0.25	0.021
Creatinine (mg/dL)	0.87 ± 0.23	1.01 ± 0.30	0.044
GFR (mg/dL)	58.68 ± 4.14	56.15 ± 6.72	0.076
TSH (mU/mL)	2.31 ± 1.55	1.87 ± 1.02	0.093

BMI: Body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GFR: glomerular filtration rate, TSH: thyroid-stimulating hormone.

were also found to be higher in AD patients, this was not statistically significant. The level of plasma resistin is shown to have high sensitivity, specificity, and predictive values in patients with AD. Elevated resistin levels in the plasma of AD patients support the hypothesis that resistin may play a role in the ongoing inflammatory process in AD.

In humans, resistin is mainly expressed in bone marrow, monocytes, macrophages, and the spleen, and proinflammatory mediators such as TNF-α, IL-1α, IL-6, or lipopolysaccharide (LPS) can strongly increase the expression of resistin in peripheral blood mononuclear cells (Figure 4) (11,17–19). In vitro and in vivo resistin is produced with a potent inflammatory character itself and also promotes the activation of mononuclear cells in a nuclear kappa α-dependent manner. Resistin also causes vascular dysfunction through endothelial cell activation, angiogenesis, and accumulation of cholesterol and triglycerides in macrophages (9). Studies have demonstrated the substantial role of resistin in metabolic syndrome, rheumatic diseases, malignant tumors, inflammatory diseases, atherosclerosis, and nonalcoholic fatty liver disease (20–22). Although the current literature data have demonstrated that resistin has various effects on distinct disease states, the relationship between resistin and AD is still obscure. In this respect, high plasma resistin levels that are found in AD patients suggest action through cytokine release during monocyte-macrophage differentiation as playing a key role in the inflammation process.

Figure 1. Resistin levels of patients with AD and controls.

It is strongly believed that inflammation is crucial in the pathogenesis of AD. Inflammatory molecules and processes occur in brain of AD patients (1,23). Systemic inflammatory response results in the production of cytokines such as IL-1, IL-6, and TNF-α, as well as acute phase reactants CRP and α1-antichymotrypsin (ACT) that circulate in the blood and can communicate with the brain (1,24,25). Cytokines and acute phase proteins synthesized
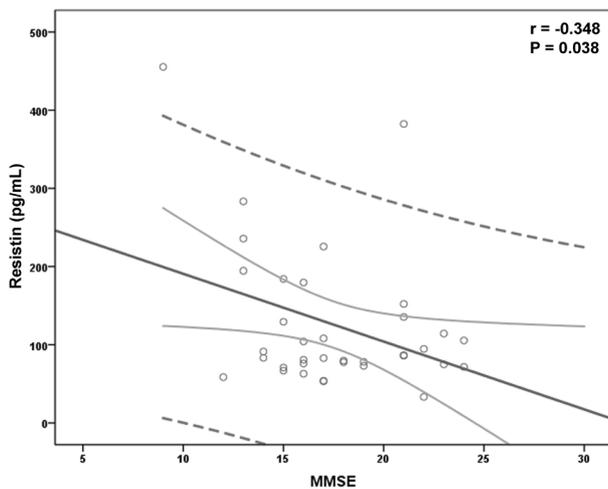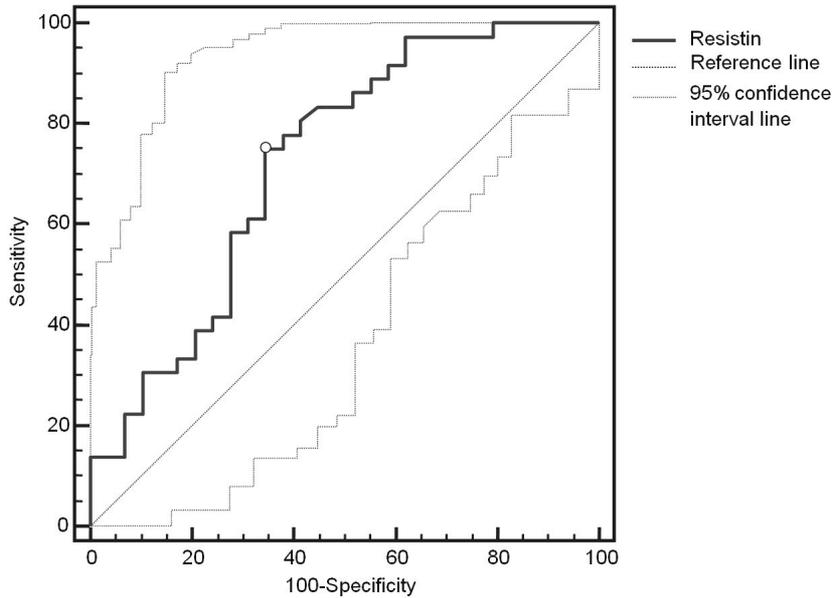
Table 2. Resistin, hs-CRP, and TNF-α levels of AD patients and control subjects.

Parameters	Controls (n = 32)	AD (n = 38)	P
Resistin (pg/mL)	70.80 (20–204.4)	86.37 (33.3–455.3)	0.002
hs-CRP (mg/L)	2.26 (0.20–6.6)	3.27 (0.5–16.9)	0.237
TNF-α (pg/mL)	5.28 (0.10–19.9)	7.82 (0.05–29.46)	0.228

hs-CRP: High-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α.

Figure 2. ROC curves of resistin in patients with AD.

in the periphery activate microglia and astrocytes and lead to the production of proinflammatory cytokines in the central nervous system. The released cytokines lead to neuronal damage through various mechanisms, including neurotransmission alteration, apoptosis, and the production of free radicals, glutamate and nitric oxide (26). In addition, cytokines increase the expression of amyloid precursor protein (APP) and alter the processing of APP for generating larger amounts of amyloidogenic α-amyloid

(27,28). The chronic infusion of IL-1β and TNF-α can also harm the cholinergic neurons that originate within the nucleus basalis (29). Data obtained from postmortem AD brains showed high concentrations of acute phase inflammatory reactants, such as CRP and proinflammatory cytokines, in senile plaques and neurofibrillary tangles (30,31). There is also evidence supporting the hypothesis that inflammatory processes may take place in the early stages of AD (4,32,33).

Table 3. Overall accuracy and ROC analyses of resistin and other markers of inflammation to differentiate AD patients from controls.

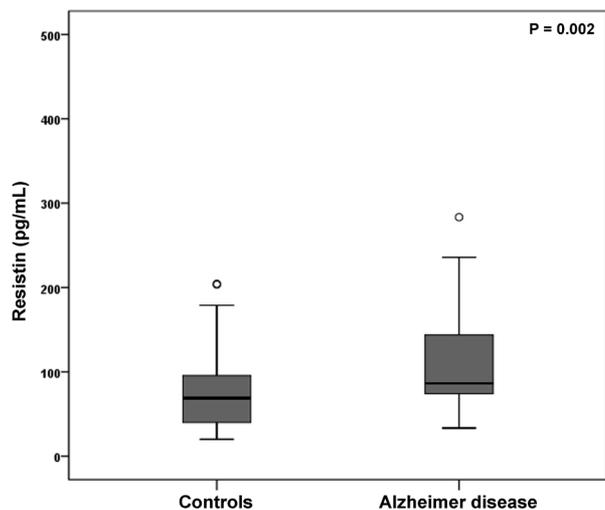|	AUC	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Overall accuracy
Resistin (cut-off: 73.3)	0.724	75.00	65.52	67.9	73.0	70.66
hs-CRP (cut-off: 3.7)	0.590	43.33	75.00	55.3	65.0	57.81
TNF-α (cut-off: 0.2)	0.587	88.89	34.48	71.4	62.7	64.01
Leukocytes (cut-off: 8000)	0.545	38.24	82.76	53.3	72.2	58.59
Sedimentation (cut-off: 21)	0.535	37.5	78.57	52.4	66.7	56.27

Figure 3. Correlation between resistin and MMSE in patients with AD. Lines representing the 95% confidence interval and the 95% prediction interval of the regression line are shown.

Schmidt et al. reported from the Honolulu Asia Aging Study that increased CRP levels at midlife were associated with increased risk of AD development (2). In the Health, Aging, and Body Composition Study, CRP, IL-6, and TNF-α association with cognitive decline were investigated and serum IL-6 and CRP were related to cognitive decline while TNF-α was not (34). In contrast to these findings, Bruunsgaard et al. showed that there was an association between high concentrations of TNF-α and AD (35). Moreover, the Longitudinal Aging Study from Amsterdam revealed that high serum ACT was associated with an increased risk of cognitive decline (3).

In the present study, plasma TNF-α levels were found to be higher in patients with AD, but this was not statistically significant. The results found in this study may be due to differences in assays for detecting TNF-α, including high detection limits, and in the small number of subjects that were enrolled in the study. TNF-α acts at a low concentration and is quickly broken down. Consequently,
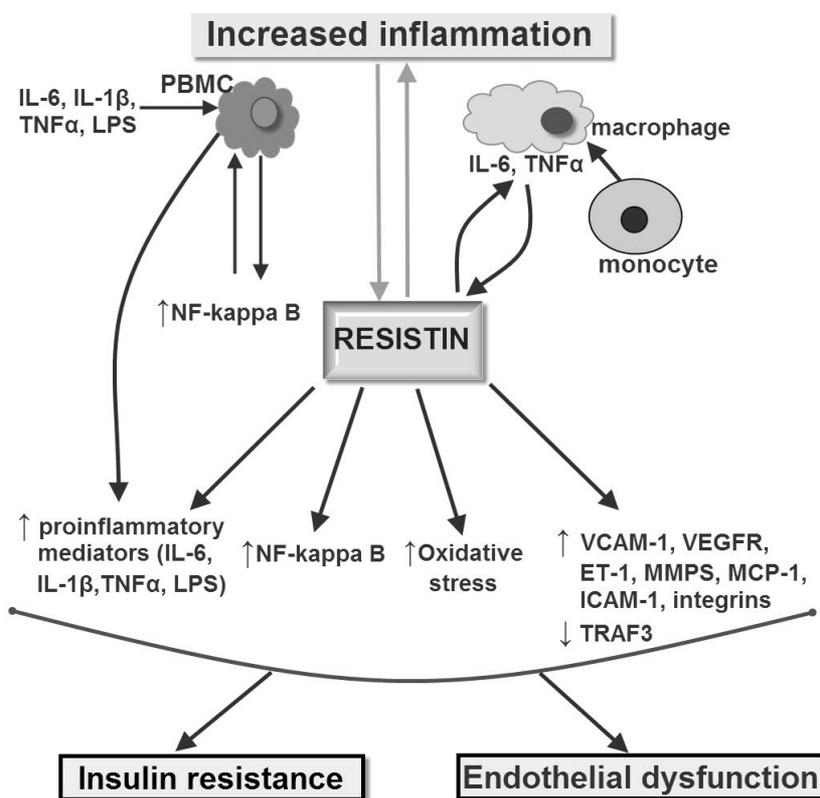

Figure 4. Schematic representation of resistin as a potential regulatory molecule of inflammatory processes. Based on the cellular milieu, release of resistin acts as a signaling pathway in immune cells. Moreover, resistin has many unique features of the proinflammatory cytokines, which activates the transcription factor NF-κB. The maturation/differentiation stage of monocytes/macrophages also upregulates resistin expression that increases after TNF-α, IL-6, IL-1β, and LPS stimulation. VCAM-1, vascular cell adhesion molecule; TRAF3, TNF receptor-associated factor-3; PBMCs, peripheral blood mononuclear cells; ICAM-1, intercellular adhesion molecule 1; ET-1, endothelium-derived vasoactive factor; TNF, tumor necrosis factor; IL, interleukin; VEGFR, vascular endothelial growth factor receptor; MCP-1, monocyte chemoattractant protein; MMPs, matrix metalloproteinases; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa B.

even small differences may be of physiological importance and more sensitive assays must be used (35).

We recognize some limitations inherent to our study design. First of all, our study had a cross-sectional structure and therefore cause and effect could not be evaluated. Secondly, our study had a relatively small sample size. Another limitation was that the patients' characteristics, such as sex and age, were not matched between patients with AD and the control group.

In conclusion, the plasma concentration of resistin was higher in AD patients compared to the control group. Increased plasma resistin levels may be related to ongoing inflammatory processes during AD development. Further studies are essential to better understand the exact association between inflammatory markers and the risk of cognitive decline.

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