Vascular Contributions to Alzheimer’s Disease

Serum IL-8 is a marker of white-matter hyperintensities in patients with Alzheimer’s disease

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Introduction: Neuroinflammation and cerebrovascular disease (CeVD) have been implicated in cognitive impairment and Alzheimer’s disease (AD). The present study aimed to examine serum inflammatory markers in preclinical stages of dementia and in AD, as well as to investigate their associations with concomitant CeVD.

Methods: We performed a cross-sectional case–control study including 96 AD, 140 cognitively impaired no dementia (CIND), and 79 noncognitively impaired participants. All subjects underwent neuropsychological and neuroimaging assessments, as well as collection of blood samples for measurements of serum samples interleukin (IL)-6, IL-8, and tumor necrosis factor α levels. Subjects were classified as CIND or dementia based on clinical criteria. Significant CeVD, including white-matter hyperintensities (WMHs), lacunes, and cortical infarcts, was assessed by magnetic resonance imaging.

Results: After controlling for covariates, higher concentrations of IL-8, but not the other measured cytokines, were associated with both CIND and AD only in the presence of significant CeVD (CIND with CeVD: odds ratios [ORs] 4.53; 95% confidence interval [CI] 1.5–13.4 and AD with CeVD: OR 7.26; 95% CI 1.2–43.3). Subsequent multivariate analyses showed that among the types of CeVD assessed, only WMH was associated with higher IL-8 levels in CIND and AD (WMH: OR 2.81; 95% CI 1.4–5.6).

Discussion: Serum IL-8 may have clinical utility as a biomarker for WMH in AD. Longitudinal follow-up studies would help validate these findings.

Keywords: Alzheimer’s disease; Cognitive impairment; Dementia; Inflammation; Cerebrovascular disease; White-matter hyperintensities; IL-8

1. Introduction

Alzheimer’s disease (AD), characterized by progressive loss of memory and cognitive function, is the most common form of dementia in the elderly [1], and a major...
source of health care burden worldwide. By 2050, the
worldwide prevalence of AD will be quadruple that in
2006, which translates to 1 in every 85 people worldwide
having AD [2].

Originally regarded as an “immune privileged” organ,
the brain is now well known to exhibit key features of
inflammation. Accumulating evidence suggests that neuro-
inflammation plays an essential role in AD pathogenesis
[3], with alterations of brain cytokine levels reported
[4,5]. Furthermore, activated microglia and astrocytes as
well as increased concentrations of inflammatory
mediators have been detected in the vicinity of amyloid
plaques and neurofibrillary tangles in AD brain [3].
Indeed, meta-analyses of observational studies indicate a
significant association between the long-term use of
nonsteroidal anti-inflammatory drugs and lowered risk of
AD [6].

Neuroinflammation, when occurring in the acute phase
and resolved in a timely manner, is beneficial in combating
pathogens and in tissue repair. But a chronic neuroinflam-
matory response may contribute to neurodegeneration [7].
Cytokines released from such neuroinflammatory processes
are known to be mirrored in the periphery, thus allowing
measurements of potential biomarkers [8,9], which at
present include cerebrospinal fluid (CSF) markers
(combined phosphotau and β-amyloid 38/42) and
neuroimaging (hippocampal atrophy, amyloid positron
emission tomography) [10–12]. However, the invasiveness
and high costs associated with neuroimaging and CSF
investigations have hindered their wide clinical
application. In contrast, the minimal invasiveness and
relative low cost of blood-based investigations have stimu-
lated research into assessments of their feasibility as diag-
nostic and prognostic biomarkers. Indeed, several studies
of circulating cytokines have been reported (albeit with
inconsistent findings), with the majority of these studies
focused on staging after a diagnosis of dementia has
been made [4,5,13–16]. As neuroinflammation is thought
to be involved in disease pathogenesis in early stages, it
is important to also investigate inflammatory markers in
predementia stages such as mild cognitive impairment or
cognitive impairment no dementia (CIND), which are
associated with increased risk of AD but may also
represent better prospects for treatment and/or prevention
[17,18].

Besides β-amyloid mismetabolism and tau hyperphos-
phorylation, vascular disease has been suggested to play a
role in AD [19]. Several neuroimaging markers of cerebro-
vascular disease (CeVD), such as white-matter hyperinten-
sities (WMHs), cortical infarct, and lacunes, have been
shown to be associated with AD [20], and may exacerbate
the severity of dementia [21,22]. However, in previous
inflammatory marker studies, the impact of CeVD was
generally neglected. In this study, we aimed to measure
serum proinflammatory cytokines, including interleukin 6
(IL-6), IL-8, and tumor necrosis factor α (TNFα), in a mem-
ory clinic cohort of CIND and AD who underwent neuroi-
maging assessments for CeVD.

2. Methods

2.1. Study cohort

A case–control design was used. Cases (CIND and demen-
tia) with subjective complaints of memory loss and cognitive
impairment on neuropsychological assessment were recruited
from memory clinics from National University Hospital and
Saint Luke’s Hospital in Singapore. Controls were recruited
from both memory clinics and the community and were
defined as those with subjective memory impairment com-
plaints but who were cognitively normal on objective neuro-
psychological assessment. All subjects underwent clinical,
physical, and neuropsychological assessments and neuroi-
maging at the National University of Singapore from August
2010 till May 2014. Ethics approval for this study was ob-
tained from the National Healthcare Group Domain-
Specific Review Board (DSRB reference: 2010/00017; study
protocol number: DEM4233), and written informed consent
had been obtained from participants or their next-of-kin
before study recruitment and procedures. The study was con-
ducted in accordance with the Declaration of Helsinki.

2.2. Questionnaires

Relevant demographic and medical information was
collected by administration of a detailed questionnaire. Data
collected included age, gender, education, marital status,
occupation, smoking, alcohol intake, ability to live indepen-
dently, handedness, previous head trauma, and family history
of dementia. Inquiries on medical history included stroke, car-
diovascular diseases, hypertension, hyperlipidemia, diabetes
mellitus, vitamin B12 deficiency, thyroid disease, urinary,
and bowel incontinence, Parkinson disease, depressive symp-
toms, and psychiatric illnesses and were subsequently verified
by review of the medical records. Barthel activities of daily
living indices were assessed for functional status.

2.3. Neuroimaging

As previously described [23,24], a 3T Siemens Magnetom
Trio Tim scanner with a 32-channel head coil was used for
magnetic resonance imaging (MRI) at the Clinical Imaging
Research Center of the National University of Singapore.
CeVD was assessed using MRI markers for lacunes, cortical
infarcts, and WMHs. Briefly, the presence and quantification
of lacunes and cortical infarcts were defined on FLAIR and
T2 sequences using the STRIVE criteria [25], whereas
WMH grading was based on the Age-Related White Matter
Changes (ARWMC) scale [26]. Significant CeVD was
defined as the presence of cortical infarct and/or presence
of two or more lacunes, and/or confluent WMH (ARWMC
score ≥8) in two regions of the brain [23,24].
2.4. Assessments for cognitive impairment and AD

Diagnosis of cognitive impairment and dementia were made at weekly consensus meetings attended by clinicians and neuropsychologists, where clinical histories, psychometrics, and neuroimages were reviewed. CIND cases were diagnosed by clinical judgment and further confirmation by neuropsychological tests, namely, impairment in at least one domain of the neuropsychological test battery consisting of executive function, attention, language, visuomotor speed, visuoconstruction, verbal memory, and visual memory, in patients who did not meet the criteria for dementia [23,24]. AD cases were diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association criteria. Noncognitively impaired (NCI) subjects were those assessed to be normal by neuropsychological tests.

2.5. Peripheral inflammatory markers

Nonfasting blood samples collected in serum-separating tubes were centrifuged at 2000 rcf for 10 minutes at 4°C, after which aliquots of serum were mixed well and stored at −80°C until use. All samples were subject to only one freeze-thaw cycle. Concentrations of interleukin 6 (IL-6), IL-8, and TNFα were measured in duplicate by multiplex xMAP®-based Luminex assays (Millipore Corp., Billerica, MA, USA) per manufacturer’s instructions. The detectable concentration range of inflammatory markers is from 0.2 to 15,000 pg/mL (IL-6), 0.3 to 10,000 pg/mL (IL-8), and 0.3 to 10,000 pg/mL (TNFα).

2.6. Covariates

Demographic information and information about risk factors such as hypertension, hyperlipidemia, diabetes, smoking, and cardiovascular diseases were collected from physical and clinical interview and medical records and classified as absent or present. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or use of antihypertensive medications. Cardiovascular diseases were classified as a previous history of atrial fibrillation, congestive heart failure, and/or myocardial infarction. Apolipoprotein E (APOE) genotyping using DNA extracted from the buffy coat of blood samples were as previously described [27] for the determination of APOE ε4 carrier status (presence of at least one APOE ε4 allele).

2.7. Statistical analyses

Statistical analysis was performed using SPSS software (version 21, IBM Inc., Armonk, NY, USA). For group comparisons, one-way analyses of variance (ANOVA) were used for normally distributed continuous variables (age); chi-square tests for categorical variables (gender, education, APOE ε4, hypertension, hyperlipidemia, smoking, diabetes, cardiovascular disease); nonparametric Kruskal–Wallis ANOVA with Dunn’s post hoc were used for skewed distributed continuous variables (inflammatory markers). Binary logistic regression was used to assess the association between inflammatory markers and primary diagnosis (NCI, CIND, or AD). The levels of inflammatory markers were included as determinants in the logistic models and were categorized into tertiles, whereas CIND and AD were listed as outcomes. Odds ratios and 95% confidence intervals were reported for both CIND and AD. All the models were adjusted for age, gender, education, APOE ε4 carrier status, diabetes mellitus, hypertension, and cardiovascular diseases. A P-value of <.05 was considered statistically significant for all analyses.

3. Results

A total of 383 elderly subjects (95 NCI, 164 CIND, 124 AD) were recruited, of whom 315 (79 NCI, 140 CIND, and 96 AD) had available blood samples and MRI data. Of
these, 17 NCI (21.5%), 68 CIND (48.6%), and 53 AD (55.2%) subjects had significant CeVD on MRI. Table 1 shows the demographic variables of the study cohort. In comparison to NCI, CIND and AD were older, had lower education levels, higher prevalence of diabetes, hypertension, and cardiovascular disease. The concentration values of inflammatory markers in the samples ranged from 0.3 to 60.0 pg/mL for IL-6, 1.3 to 47.0 pg/mL for IL-8, and 0.9 to 17.5 pg/mL for TNFα. The lowest detectable values were used in statistical analyses for cases whose concentrations fell below detectable range (0.2 pg/mL was used for IL-6 for 28 cases in NCI group, 50 cases in CIND group, and 20 cases in AD group; 0.3 pg/mL was used for TNFα for two cases in NCI group and 1 case in CIND group). As shown in Table 1, levels of IL-6, IL-8, and TNFα were highest in AD and lowest in NCI, with intermediate levels in CIND. The differences were statistically significant for IL-8 in both CIND and AD, whereas IL-6 and TNFα were significantly raised only in AD.

Table 2 shows the multivariate analyses of associations between serum inflammatory markers and clinical diagnoses. There was no association between IL-6 and CIND or AD after adjustment for covariates of age, gender, education, APOE ε4 carrier, diabetes mellitus, hypertension, and cardiovascular diseases. Furthermore, the associations with IL-8 were significant only in the presence of CeVD, and more specifically, with the presence of WMH when adjusted for covariates.

IL-8 (also known as CXCL8) is a chemokine which induces chemotaxis in target cells, migrating neutrophils, basophils, and T cells to the site of infection [28]. In this study, we found that high-serum IL-8 was associated with both CIND and AD only in the presence of CeVD, specifically with significant WMH, independent of age, gender, education, APOE ε4 carrier status, diabetes mellitus, hypertension, cardiovascular diseases, as well as cortical infarct and lacunes. This finding corroborates previous work showing high IL-8 in cognitively impaired patients associated with vascular cognitive impairment [29]. IL-8 has been known to initiate acute inflammation, and our data support the involvement of elevated IL-8 in the chronic neuroinflammation of AD which may be related to cerebrovascular damage [30]. Actually, significant elevations in plasma IL-8 levels in cognitive impairment have also been reported by others [5, 13], although there are also conflicting data showing lower IL-8 level in mild cognitive impairment and AD [31]. The mechanism underlying associations of IL-8 with WMH is at present unclear. The disease processes may be related to microglial activation-associated upregulation of cytokines besides inducible nitric oxide synthase [32], which in turn trigger increases in proinflammatory and pro-oxidant nitric oxide, affect brain microvessel endothelia, and result in white-matter lesions detectable as WMH by MRI [33, 34]. Although these lesions have been shown to be associated with, and predict for, cognitive impairment [35–37], whether such processes actually link IL-8 with white-matter lesions will require follow-up studies.

IL-6 can be produced by a variety of immune cells as well as endothelial cells and fibroblasts and is a primary inducer of acute proteins and hormones which mediate fever and immune cell expansion in response to infection or injury [28]. Interestingly, IL-6 also has anti-inflammatory

4. Discussion

Although all three inflammatory markers (IL-6, IL-8, and TNFα) were found to be significantly different among NCI, CIND, and AD patients, only elevated IL-8 was associated with CIND and AD after adjustment for age, gender, education, APOE ε4 carrier, diabetes mellitus, hypertension, and cardiovascular diseases. Furthermore, the associations with IL-8 were significant only in the presence of CeVD, and more specifically, with the presence of WMH when adjusted for covariates.
effects [38]. This functional dichotomy may underlie conflicting results where a few studies have shown increased levels of serum or plasma IL-6 levels in AD [4,15], whereas other studies reported unchanged peripheral levels of IL-6 between control, mild cognitive impairment, and AD [14,39–41]. In the present study, although higher IL-6 was found in AD, subsequent multivariate analyses did not support associations of serum IL-6 with AD or concomitant CeVD.

TNFα is a proinflammatory cytokine, produced primarily by activated macrophages, T cells, and NK cells. It is a mediator of both acute and chronic inflammation and can activate vascular endothelium and increase vascular permeability [28]. Similar to the other markers, there are inconsistent results on the status of peripheral TNFα in AD with reports of significantly lower [14,42], unchanged [43,44], or increased [45,46] TNFα in mild cognitive impairment and/or AD. The present study showed that, like the other two markers, serum TNFα was increased in AD, although the association was not statistically significant when adjusted for covariates. This suggests confounding by concomitant risk factors. Indeed, there is evidence of TNFα involvement in cardiovascular disease [47–49] which was included as a covariate in our study.

The strengths of this study include the following: (1) a relatively large cohort; (2) inclusion of covariates such as age, gender, education, APOE ε4 carrier, diabetes mellitus, hypertension, and cardiovascular diseases.

### Table 3

<table>
<thead>
<tr>
<th>Inflammatory markers</th>
<th>With CeVD*</th>
<th>Without CeVD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CIND odds ratio (95% CI)</td>
<td>AD odds ratio (95% CI)</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>0.73 (0.3–1.8)</td>
<td>0.84 (0.2–3.4)</td>
</tr>
<tr>
<td>3rd tertile</td>
<td>0.89 (0.4–2.1)</td>
<td>0.96 (0.3–3.6)</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>1.00 (0.4–2.4)</td>
<td>0.31 (0.1–1.5)</td>
</tr>
<tr>
<td>3rd tertile</td>
<td><strong>4.53 (1.5–13.4)</strong></td>
<td><strong>7.26 (1.2–43.3)</strong></td>
</tr>
<tr>
<td>TNFα</td>
<td></td>
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<tr>
<td>1st tertile</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>1.17 (0.5–2.9)</td>
<td>0.86 (0.2–3.6)</td>
</tr>
<tr>
<td>3rd tertile</td>
<td>1.42 (0.6–3.5)</td>
<td>1.32 (0.3–5.6)</td>
</tr>
</tbody>
</table>

Abbreviations: CIND, cognitive impairment no dementia; AD, Alzheimer’s disease; CeVD, cerebrovascular disease; CI, confidence interval; IL, interleukin; TNFα, tumor necrosis factor α.

NOTE. N values for CIND with CeVD = 68; AD with CeVD = 53. Bold text indicates P values <.05.

*Adjusted for age, gender, education, APOE ε4 carrier, diabetes mellitus, hypertension, and cardiovascular diseases.

### Table 4

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>WMH (ARWMC ≥ 8), odds ratio (95% CI)*</th>
<th>Cortical infarct, odds ratio (95% CI)†</th>
<th>≥2 lacunes, odds ratio (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st tertile</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2nd tertile</td>
<td>1.28 (0.7–2.5)</td>
<td>2.10 (0.6–7.6)</td>
<td>0.94 (0.3–3.2)</td>
</tr>
<tr>
<td>3rd tertile</td>
<td><strong>2.81 (1.4–5.6)</strong></td>
<td>3.50 (0.9–13.1)</td>
<td>2.70 (0.9–8.4)</td>
</tr>
</tbody>
</table>

Abbreviations: IL, interleukin; MRI, magnetic resonance imaging; CeVD, cerebrovascular disease; WMH, white-matter hyperintensity; ARWMC, age-related white-matter changes; CI, confidence interval; CIND, cognitive impairment no dementia.

NOTE. N values for CIND with CeVD = 68; AD with CeVD = 53. N values for significant WMH (ARWMC ≥ 8) = 118, cortical infarcts = 28, lacunes (≥2) = 37. Bold text indicates P values <.05.

*Adjusted for age, gender, education, APOE ε4 carrier, hypertension, diabetes mellitus, cardiovascular diseases, presence of cortical infarct, and lacunes.
†Adjusted for age, gender, education, APOE ε4 carrier, hypertension, diabetes mellitus, cardiovascular diseases, WMH, and presence of lacunes.
‡Adjusted for age, gender, education, APOE ε4 carrier, hypertension, diabetes mellitus, cardiovascular diseases, WMH, and presence of cortical infarct.
such as WMH in cognitive impairment and AD. However, follow-up longitudinal studies are needed for validation.

Acknowledgments

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RESEARCH IN CONTEXT

1. Systematic review: Although neuroinflammation is widely considered to play a pathogenic role in Alzheimer’s disease (AD), the extent to which changes in peripheral inflammatory markers may reflect concomitant cerebrovascular disease (CeVD) has not been studied in either cognitive impairments no dementia (CIND), a predementia stage, or in AD, despite evidence in the literature indicating the exacerbating effects of concomitant CeVD on dementia severity.

2. Interpretation: Of the three acute inflammatory markers investigated, only elevated IL-8 was associated with white-matter hyperintensities in CIND and AD, suggesting that interleukin-8 is a potential biomarker for small vessel CeVD in cognitive impairment and AD.

3. Future directions: A case–control design was used for our study. Follow-up longitudinal studies are needed for validation.

References


