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Brief Report

Association of biomarkers for inflammation, endothelial dysfunction and oxidative stress with cognitive impairment: the Epidemiology of Hearing Loss Study

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Key Words
Biomarkers; Cognitive impairment; Inflammation; Oxidative stress

Abstract
Individual biomarkers of inflammation, endothelial dysfunction and oxidative stress have been associated with cognitive impairment. This study explored whether a combination of biomarkers could prospectively identify those who developed cognitive decline. Biomarkers were obtained during the baseline examination of the Beaver Dam Eye Study (1988-90), and cognitive status was assessed during the 5-year follow-up examination of the Epidemiology of Hearing Loss Study (1998-2000). Cognitive impairment was defined as a score of < 24 points on the Mini-Mental State Examination or self- or proxy report of Alzheimer Disease or dementia. Among those with cognitive data, interleukin-6, isoprostanes, protein carbonyl, soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were available for 950 participants and 2,336 had high sensitivity C-reactive protein. Biomarkers of inflammation and endothelial dysfunction were not associated with cognitive impairment. There was a weak inverse association between higher levels of protein carbonyl content and cognitive impairment (OR, 0.8 per quartile of protein carbonyl content, P = 0.045 unadjusted for multiple comparisons). This was not significant on multiple testing and may have been a chance finding. We found that many markers of inflammation and endothelial dysfunction were not associated with cognitive impairment. An inverse association with carbonyl protein, a marker of oxidative stress needs further confirmation.

INTRODUCTION

Increased prevalence of age-related cognitive decline, has led to a need of understanding associated risk factors for Alzheimer's disease (AD) and dementia [1]. Inflammation, endothelial dysfunction and oxidative stress may contribute to the development of cognitive impairment [2, 3]. Combining several markers from these mechanisms, could help identify those at higher risk of developing cognitive decline [4].

Oxidative plasma biomarkers such as protein carbonyl content and isoprostanes may be involved in the pathogenesis of cognitive impairment [5, 6]. However reports on the association of these markers with cognitive decline have been inconsistent [7]. According to a review by Song *et al* [7], some studies have shown higher levels of plasma, cerebrospinal fluid (CSF) and urine isoprostanes to be present in patients with cognitive impairment compared to controls while others have not [7, 8]. These conflicting results may be due to utilization of anti-oxidant vitamins in AD patients [8].

Plasma levels of inflammatory markers such as Interleukin-6 (IL-6) and high sensitivity C-reactive protein (hsCRP), and endothelial dysfunction markers such as soluble intercellular adhesion molecule (ICAM) and soluble vascular cell adhesion molecule (VCAM) may signify early stages in cognitive decline leading to AD [2-4, 9, 10]. However, other studies have reported no association [11, 12].

Research on multiple biomarkers and their relationships with each other may reduce limitations which surround understanding of these associations [13]. The purpose of this paper was to determine if higher levels of these biomarkers were associated with subsequent cognitive impairment in a large population-based cohort study of older adults allowing for control of potential confounders.

MATERIALS AND METHOD

Beaver Dam (WI, USA) residents (n = 5,924) identified in a private census aged 43-84 years in 1987-88, were enrolled in the Beaver Dam Eye Study (BDES) and 4,926 were examined from 1988-90 [14]. Of 4,541 BDES participants alive in 1993, 3,753 enrolled in the Epidemiology of Hearing Loss Study (EHLS), a study of aging [15]. Methods used to identify and recruit participants have been described in previous articles [15, 16]. Cognitive impairment was measured in 1998-2000 as part of the five-year follow-up examination for the EHLS (n = 2,800). As part of the Beaver Dam Chronic Kidney Disease Study, an ancillary study in the same BDES cohort, biomarkers were measured on samples which had been collected in 1988-90 and stored at –80ºC. Baseline samples (n = 4,880) were assayed for hsCRP and a random sample (n = 1,793) of participants was selected for the determination of IL-6, ICAM-1, VCAM-1, isoprostanes and protein carbonyl content. Our study included only participants with both measures of cognitive impairment and biomarker data (n = 2,336 for hsCRP, n = 950 for all other markers).

The Mini-Mental State Examination (MMSE) [17] was used in the 1998-2000 EHLS examination to measure cognitive function. Cognitive impairment was defined as a score of < 24 points on the MMSE or reported diagnosis of AD or dementia in the absence of MMSE score [18].

High sensitivity C-reactive protein was measured in ethylene-diamine-tetraacetic acid (EDTA) plasma using a latex particle enhanced immunoturbidimetric assay kit (Kamiya Biomedical Co, Seattle, WA, USA) on the Hitachi 911 Automatic Analyzer (Boehringer, Mannheim, Germany). IL-6 was measured using the quantitative sandwich enzyme technique of the ELISA QuantiKine High Sensitivity kit from R&D Systems (Minneapolis, MN, USA). The intensity of the color was measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). ICAM-1 and VCAM-1 were measured using the quantitative sandwich enzyme technique of the ELISA ICAM-1 and VCAM-1 parameter kits respectively, from R&D Systems. Protein carbonyls were determined by derivatizing the sample with dinitrophenylhydrazine (DNP) and measuring bound DNP using the Zentech Protein Carbonyl Enzyme Immunoassay method (Zenith Technology Corp. LTD, Dunedin, New Zealand). To measure 8-isoprostane, samples were purified by use of 8-isoprostane affinity sorbent (Cayman Chemical, Ann Arbor, MI, USA) and the 8-isoprostane was detected by EIA (8-Isoprotane EIA Kit, Caymen Chemical).

Participant's age at the EHLS 5-year examination was used for the analysis. Education (categorized as lesser, greater or equal to high school) and smoking (classified as past-, current-, or non-smoker) were included as covariates. Other covariates such as; use of vitamins or non-steroidal anti-inflammatory drugs (NSAIDS), history of stroke or arthritis were assessed from questionnaires. Diabetes mellitus was defined as a total glycosylated hemoglobin > 8% (which corresponds to hemoglobin A1c (HbA1c) > 6.9%), or a history of diagnosed diabetes and current treatment with diabetes medication. All covariates other than age were measured at BDES baseline.

Statistical Methods

To test differences in characteristics between randomly selected participants and other baseline participants, a 2-tailed, unpaired *t*-test was used for continuous variables and the χ^2 test for association was used with contingency tables for dichotomous variables. Multiple logistic regression models were used to estimate odds ratios (ORs) for the associations of biomarker levels (in quartiles) and cognitive impairment. Each marker was evaluated in separate models adjusting only for age, sex, and education (model 1). Additional models (model 2) controlled for other potential confounders, including use of NSAIDS, use of vitamin supplements, history of smoking, diabetes mellitus, history of stroke and arthritis. SAS® (SAS-Institute, NC, USA) was used for analysis.

RESULTS

Participants with cognitive impairment data were 3.7 years younger, had 1.3 more years of education and were more likely to be female than BDES participants in 1988-90 without cognitive data in the EHLS 1998-2000 examination, reflecting the patterns of mortality during the ten year period.

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Of the 2,636 participants with baseline hsCRP and cognitive impairment data, 41.5% (n = 1095) were men. The mean age for all participants with baseline hsCRP was 68.7 years and the mean years of education was 12.5 years. Among study participants with baseline hsCRP data, 8.6% were cognitively impaired at the 1998-2000 follow up examination.

The remaining biomarkers were available in 950 participants of whom 41.9% were men. Their mean age and education were 68.3 years and 12.6 years respectively. In this subset, the subsample and the baseline participants were similar in age and gender distribution. The participants' characteristics are given in Table 1.

Adjusting for age, sex and number of years of education, there was no association of hsCRP with cognitive impairment (OR, 1.02 per quartile: 95% confidence interval [CI], 0.89-1.17). This result did not change with additional adjustment for use of NSAIDS, vitamin supplements, history of smoking, diabetes mellitus, history of stroke and arthritis (OR, 1.05 per quartile, 95% CI, 0.92-1.2). In the subset with additional inflammatory markers, IL-6, (OR, 1.06 per quartile: 95% CI, 0.85-1.33), ICAM-1 (OR 0.95 per quartile: 95% CI, 0.76-1.17) and VCAM-1 (OR, 0.98 per quartile: 95%CI, 0.78-1.23) were not associated with cognitive impairment. Higher protein carbonyl content levels were associated with lower odds of cognitive impairment (OR, 0.8 per quartile, 95% CI, 0.64-1; P = 0.045). Adjusting for use of non-steroidal anti-inflammatory drugs and vitamin supplements, history of stroke, smoking, diabetes and arthritis did not attenuate this association (OR, 0.79 per quartile, 95% CI, 0.64-0.99; Table 2). Isoprostane level was not associated with cognitive impairment (OR, 1.01 per quartile, 95%CI, 0.82- 1.25).

DISCUSSION

Increased inflammation, endothelial dysfunction and oxidative stress at the cellular level may be observed in multiple body tissues, and appear to be associated with dementia in the elderly population [9, 19-22]. Previous studies have reported inconsistent associations between plasma inflammatory and endothelial dysfunction markers, and risk of developing cognitive impairment [12, 23]. Some reports may have been based on older study population with inflammatory assessments closer to cognitive decline [9]. In this study, markers of inflammation and endothelial dysfunction were not associated with cognitive impairment ten years later and only a single marker of oxidative stress, protein carbonyl, was inversely associated with impairment.

Table 1. Participants' characteristics at the 1998-2000 examination

Characteristics	hsCRP group (n = 2636)	Other biomarkers (n = 950)
Male, n (%)	1095 (41.5%)	398(41.9%)
Mean age, year (STD)	68.7(STD = 9.7)	68.3 (STD = 9.8)
Mean education, year (STD)	12.5 (STD = 2.72)	12.6 (STD = 2.74)
Cognitively impaired, n (%)	226 (8.6%)	91 (9.6%)

Table 2. Association between biomarker quartiles and cognitive impairment (estimated ORs and 95% CIs)

Variable	Model 1[a]			Model 2[b]		
	Sample size	OR	95% CI	Sample size	OR	95% CI
hsCRP*	2634	1.02	[0.89, 1.17]	2624	1.05	[0.92, 1.2]
Inflammatory markers						
IL-6*	937	1.06	[0.85, 1.33]	932	1.13	[0.90, 1.42]
ICAM-1*	945	0.95	[0.76, 1.17]	940	0.94	[0.76, 1.17]
VCAM-1*	948	0.98	[0.78, 1.23]	943	0.97	[0.77, 1.23]
Oxidative stress markers						
Carbonyl protein*	948	0.8	[0.64, 1]	943	0.79	[0.64, 0.99]
Isoprostanes*	934	1.01	[0.82, 1.25]	929	1.04	[0.84, 1.29]

*Lowest quartile served as reference for corresponding biomarker; quartile distributions (25^{th}, 50^{th} and 75^{th} percentiles) were hsCRP (0.9, 1.79, 3.66), IL-6 (1.38, 2.06, 3.11), ICAM-1 (234.55, 276.20, 324.58), VCAM-1 (644.72, 761.62, 890.27), carbonyl (0.1, 0.14, 0.21), isoprostanes (95.63, 121.75, 159.98); the quartiles were included as ordered factors (one step quartile) in the analysis

[a]Model 1 = logistic regression adjusting for education, age at cognitive impairment measurement and sex;

[b]Model 2 = additional adjustment for arthritis, non-steroidal anti-inflammatory drugs, history of stroke, diabetes mellitus, smoking status (currently smoking or not) and use of vitamin supplements

OR = odds ratio; CI =confidence interval; hsCRP = highly sensitivity C-reactive protein; IL-6 = interleukin 6; ICAM-1 = intercellular adhesion molecule-1; VCAM-1 = vascular endothelial cell adhesion molecule-1

Changes in biomarker levels over a 10-year period may be better predictors of cognitive impairment compared to one time initial biomarker measures. In their recent study utilizing an elderly population, Jenny *et al* [24] evaluated the relationship between cognitive impairment and changes in inflammatory makers over a 9-year period. They indicated a 2-fold increase in either CRP or IL-6 was a better predictor of cognitive impairment compared to single initial biomarker levels assessed 9-years prior. It is possible that one-time biomarker measurement before assessment of cognitive impairment may explain our finding of no associations between remote estimates of inflammatory process and subsequent cognitive impairment.

Unlike previous studies, our results revealed cognitive impairment had no association with isoprostanes but was inversely associated with protein carbonyl (OR, 0.8 per quartile, P = 0.045). These unexpected findings were contrary to our hypothesis that oxidative stress would be positively associated with cognitive impairment, and may result from chance due to multiple comparisons. However, the association remained significant adjusting for additional confounders. It is also possible higher levels of oxidized protein may be associated with subclinical atherosclerosis [20] which may precede cognitive impairment, and result in uncontrolled confounding.

While MMSE has been used as an indicator of cognitive impairment in population-based studies, it is not as sensitive or specific as a clinical evaluation with a battery of neurocognitive tests. It is possible that misclassification may have limited our findings. The overall young age and expected low prevalence of cognitive impairment in our study population may have contributed to the lack of an association. However, this study has several strengths including its population-based design and ten year lag time between the blood sample and the assessment of cognitive function.

Our results show no relation of inflammatory and endothelial dysfunction biomarkers with cognitive impairment measured 10 years later. However, prospective studies using new diagnostic criteria for mild cognitive impairment and dementia with repeated assessment of protein carbonyl are needed.

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COMPETING INTERESTS

Chidi Obasi was the lead author for this study. All co-authors contributed substantially and approved the final version of this report.

The authors have no conflicts of interest pertaining to this work.

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