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Research article

Plasma gelsolin and matrix metalloproteinase 3 as potential biomarkers for Alzheimer disease

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HIGHLIGHTS

- GSN levels were decreased in plasma of AD.
- MMP3 activity were increased in plasma of AD.
- Both the GSN level and MMP3 activity correlated with the MMSE scores.
- Combination of GSN level, MMP3 activity and clinical data may help in screening AD.

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ABSTRACT

Gelsolin (GSN) levels and matrix metalloproteinase 3 (MMP3) activity have been found to be altered in the plasma in patients with Alzheimer disease (AD). The aim of this study was to determine whether a combination of these proteins with clinical data is specific and sensitive enough for AD diagnosis. In 113 non-demented controls and 113 patients with probable AD, the plasma GSN levels were determined using the enzyme-linked immunosorbent assay (ELISA), and the plasma MMP3 activity was determined using casein zymography. Logistic regression and receiver operating characteristic (ROC) curve analysis were used to determine the diagnostic accuracy of these proteins combined with clinical data. Compared with the controls, the AD patients had significantly lower GSN levels and significantly higher MMP3 activity. Moreover, both the GSN level and MMP3 activity were significantly correlated with the MMSE scores. In AD patients, the GSN level was negatively correlated with MMP3 activity. ROC curve analysis showed that the specificity and sensitivity were 77% and 75.2%, respectively, for the combination of the following candidate biomarkers: GSN level/the total amount of $A\beta_{42}$ and $A\beta_{40}$, plasma MMP3 activity and clinical data. With its relatively high sensitivity and specificity, this combined biomarker panel may have potential for the screening of AD patients.

1. Introduction

Alzheimer disease (AD) affects millions of people around the world. Although there are no treatments to prevent or slow down the progression of this disease currently, early diagnosis may allow early management. Therefore, there is clearly a need for reliable and valid diagnostic biomarkers for early diagnosis of AD. As the ideal biomarker would be one that is present in peripheral blood [1], massive efforts have been directed at identifying such markers. Several studies have reported blood markers of inflammation, oxidative stress, neuronal and microvascular injury, and some of these studies focus on biomarkers that are specific to pathologic hallmarks of AD [2].

Abbreviations: GSN, gelsolin; MMP3, matrix metalloproteinase 3; AD, Alzheimer disease; ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating characteristic; Aß, amyloid beta protein; Tg, transgenic; MMSE, mini-mental status examination; CSF, cerebrospinal fluid; MoCA, Montreal Cognitive Assessment; ADL, activity of daily living scale; CDR, clinical dementia rating scale; HIS, Hahinski's ischemic score; CES-D, Center for Epidemiological Studies Depression scale; NINCDS-ADRDA, the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; EDTA, ethylenediamine tetra-acetate; NC, non-demented controls; IOD, integrated optical density; HT, hypertension; DM, diabetes mellitus.

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Plasma gelsolin (GSN) is a 93 kDa protein [3] that may inhibit the fibrillization of the amyloid beta protein (Aβ) and also defibrillize its preformed fibrils [4]. As a matter of fact, the association between the plasma GSN and pathologic hallmarks of AD has been indicated by a few studies in AD transgenic (Tg) mouse models. In these studies, peripheral elevation of plasma GSN was found to reduce the brain amyloid levels [5,6], as well as increase the Aβ levels in plasma [7]. GSN is fairly unlikely to penetrate the blood–brain barrier when its concentration in blood increases due to its high molecular weight (93 kDa) [6]; therefore, it would be difficult for plasma GSN to defibrillize or bind Aβ peptides directly in the brain. These findings seem to suggest that plasma GSN probably acts, to some extent, as a "peripheral sink protein" to bind Aβ peptides, mimicking the action of antibodies injected into the blood that function as a peripheral sink for Aβ by altering the periphery/brain dynamics [8]. In AD patients, it was found that the plasma GSN level was significantly decreased compared to the controls [9]. Moreover, its level correlated with disease progression rate estimated by mini-mental status examination (MMSE) decline per year [9]. However, the study could not clearly determine whether plasma GSN alone can be used as a biomarker for AD [9].

Matrix metalloproteinase 3 (MMP3) is the main GSN-degrading enzyme [10], and it is found in two active forms that can be distinguished by their molecular weight (45 kDa and 28 kDa) [11]. There is growing evidence showing the correlation of MMP3 with the early pathogenic mechanisms in AD: (i) In a study exploring cerebrospinal fluid (CSF) biomarkers associated with AD-like brain atrophy in 90 healthy individuals, the baseline MMP3 level that interacted with $A\beta_{42}$ present in the CSF was significantly associated with atrophy in the inferior parietal cortex and inferior temporal cortex 4 years later [12]. (ii) CSF MMP3 levels were found to be significantly increased in AD patients compared to the non-demented controls (NC) [13]. Moreover, the CSF MMP3 level was reported to correlate with the CSF *T*-tau and *P*-tau levels in the elderly controls [14]. It has also been revealed that the CSF MMP3 levels negatively correlate with MMSE scores [15]. With regard to the blood MMP3 level, a small-scale study has reported that plasma MMP3 activity is significantly elevated in AD patients compared to the controls [16], which indicates that the change in plasma MMP3 activity is similar to that in CSF MMP3. However, no research has investigated the potential of MMP3 in blood as a diagnostic biomarker for AD [17].

Till date, no reliable and validated peripheral markers have been established for the early detection of AD in the clinic. As plasma GSN and its main degrading enzyme MMP3 are both linked to the pathological mechanisms of AD and the progression or severity of the disease to some extent, they may be attractive candidate biomarkers for AD. In this study, we investigated whether the GSN level combined with MMP3 activity can be used as a diagnostic biomarker for AD.

2. Method

2.1. Subjects

The study randomly recruited 114 NC and 115 patients with sporadic AD from Xuan Wu Hospital in Beijing between July 2011 and December 2012. As three blood samples (1 NC and 2 patients) were unqualified due to hematolysis, there were 113 participants in each group in the end. The subjects underwent a medical interview; physical examination; blood tests; brain MRI; and neuropsychological assessment with the MMSE, Montreal Cognitive Assessment (MoCA), activity of daily living scale (ADL), clinical dementia rating scale (CDR), Hachinski's ischemic score (HIS), and Center for Epidemiological Studies Depression scale (CES-D). The AD patients met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) for the clinical diagnosis of probable AD [18]. Subjects with cancer, subdural hematoma, current alcohol abuse, central nervous system infection, severe congestive heart failure, and severe hepatic or renal insufficiency were excluded from the study because of the possibility of secondary forms of dementia. Subjects with inflammatory diseases (such as rheumatoid arthritis, acute lung disease, chronic obstructive pulmonary disease and sepsis) or subjects who were taking anti-inflammatory medications (such as nonsteroidal anti-inflammatory drugs, antibiotics and steroids) were also excluded. Informed consent was obtained from each subject, either directly or from his or her guardian, and the protocol of this study was approved by the Ethical Committee of the Xuan Wu Hospital of Capital Medical University.

2.2. Blood sample preparation

Non-fasting plasma samples were collected in tubes that contained ethylenediamine tetra-acetate (EDTA), which was used as an anticoagulant. After centrifugation (10 min at 2000 rpm), the plasma samples were aliquoted into polypropylene tubes and stored at −80 °C for further analysis. The APOE genotypes were determined by restriction enzyme digestion as previously described [19]. Subjects were classified as APOEϵ4-positive if they carried at least one copy of the ϵ4 allele.

2.3. Plasma Aβ assay

The levels of $A\beta_{42}$ and $A\beta_{40}$ were determined using commercially available sandwich ELISA kits (Invitrogen, USA). The mean coefficients of within and between assay variations were 3.6% and 4.3%, respectively, for $A\beta_{42}$, and 2.5% and 3.8%, respectively, for $A\beta_{40}$. The progression of Aβ measures were in strict accordance with the kit instruction.

2.4. Plasma GSN and MMP3 assay

The plasma GSN concentration was determined using a human GSN ELISA Kit (Uscn Life Science & Technology Company, Wuhan, China) according to the manufacturer's instructions. Casein zymography was used to determine the activity of plasma MMP3, as previously described [16]. The plasma MMP3 activity determined in a normal person served as the positive control.

2.5. Statistical analysis

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, USA). The Kolmogorov–Smirnov test was performed to ascertain the normality of the distribution of continuous variables. Differences in the plasma GSN level and MMP3 activity between the AD patients and the NC were assessed using a *t*-test and the Mann–Whitney *U*-test, respectively. Spearman tests were used to analyze correlations. A logistic regression analysis was performed to identify the markers that can be used to discriminate between AD patients and the NC. Receiver operating characteristic (ROC) curves were then created using the predicted variables to demonstrate the specificity and sensitivity. $P < 0.05$ was considered to indicate statistical significance.