■ ORIGINAL ARTICLES

Measurement of plasma amyloid-β precursor protein APP770 concentration in healthy young volunteers: Preliminary study of APP770 as a potential biomarker for Alzheimer's disease diagnosis.

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ABSTRACT:

Purpose : APP770, expressed in brain endothelial cells, is a different isoform from neuronal APP695. Soluble APP770 (sAPP770) is produced by APP770 cleavage by α-secretase. sAPP770 has been proposed as a candidate biomarker of Alzheimer disease. To investigate sAPP770 levels in human plasma, a new commercially available sandwich ELIZA system has been developed. This study was undertaken to test whether this system would be useful in determining plasma sAPP770 levels.

Subjects and Methods : Subjects were 20 healthy young volunteers. Plasma sAPP770 concentration was assayed by sandwich ELIZA using the Human APP770 assay kit.

Results: Plasma concentration of sAPP770 in all subjects was 56.80ng/mL. The concentration in male subjects was 44.31ng/mL and in the female subjects was 60.99ng/mL. No significant difference in male and female concentrations was observed. The detection limit of this kit was 0.03ng/mL, and the working range of the assay was 0–6.2 ng/mL.

Conclusion : The sandwich ELIZA method tested in this study is a useful protocol to analyze plasma sAPP770 concentrations.

Key words: Alzheimer disease, APP770, ELIZA

INTRODUCTION

Alzheimer disease (AD) is a very important neurodegenerative disorder that particularly impacts countries like Japan, with an unprecedented aging society. AD is the most common cause of dementia. It is estimated that between 60 to 80% of dementia is AD-related. AD is a progressive and irreversible disease of the brain that slowly impairs memory and cognitive function. Like other types of dementia, the highest risk factor for AD is aging.

AD is characterized by the deposition in the brain of extracellular amyloid-ß peptide (amyloid-ß) into amyloid plaques [1, 2]. Amyloid-ß is generated from amyloid precursor protein (APP) which is a single-pass transmembrane protein with large extracellular and small intracellular domains. APP has eight types of alternatively spliced isoforms involving exons 7 and 8, of which three are most common: APP695, APP751, and APP770 [3, 4]. Of these isoforms, APP695 is predominantly expressed in neurons[5]. Kitazume et al. [6] recently reported that APP770 is expressed in brain vascular endothelial cells where it generates amyloid-ß40/42. Deposition of amyloid-ß derived from APP770 within the walls of small arteries in the brain is observed in approximately 90% of AD patients [7, 8, 9, 10]. APP770 can be cleaved by α-secretase within the amyloid-ß domain to release soluble APP770 (sAPP770)[11]. A sandwich ELISA system to specifically detect sAPP770 in human plasma samples was developed by Kitazume et al.[12] when it was successfully applied to establish sAPP770 as a marker of acute coronary syndrome[12]. Amyloid-ß is a highly sensitive and specific AD biomarker detected in cerebrospinal fluid (CSF)[13]. However, CSF collection cause severe patient pain and involves certain risks such as bleeding into the spinal canal, brainstem herniation, damage to the nerves in the spinal cord and others[14]. As a result, its routine use in screening or as a diagnostic test is limited by the requirement for a lumbar puncture as has been suggested by Stern et al[15]. The aim of this study was to investigate the use of an alternative analytical method to assay plasma sAPP770 as a potential biomarker to estimate the progress stage of AD.

MATERIALS AND METHODS

All subjects gave signed informed consent to take part in this study, which was approved by the Ethical Committees of Kansai University of Welfare Sciences.

1. Participants

This study was based on plasma samples obtained from 20 healthy students (10 males and 10 females) from Kansai University of Welfare Sciences. All participants were between 20 and 21 years of age. The blood sample (5mL) was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma was separated by centrifugation at 3,000 rpm for 10 minutes. The 1 mL of supernatants were collected and stored at -80°C until analyzed.

2. Measurement of sAPP770

sAPP770 concentration was assayed by sandwich ELIZA with the Human APP770 assay kit (IRL27736, Immuno-Biological Laboratories Co., Ltd. Japan).

1) Antigen-antibody reaction

Dilutions (0.10-, 0.19-, 0.39-, 0.78-, 1.55-, 3.1-, 6.2-ng/mL) of standard substrate in the assay kit (Recombinant Human APP770) was prepared with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.05% Tween 20. PBS containing 1% BSA and 0.05% Tween 20 without the standard substrate was used as the blank sample. Each plasma sample was diluted serially（1×, 2×, 4×, 8×, 16×, 32×, 84×, 168×）with the PBS containing 1% BSA and 0.05% Tween 20. 100μL of standard sample solutions and diluted plasma samples were added into each well of a 96-well microplate pre-coated with anti-human APP

OX2(351) rabbit IgG A.P. and incubated at 4°C for overnight.

2) Detection

The plates were washed 7 times with PBS containing 0.05% Tween 20, followed by the addition of 100µL per well of secondary antibody (HRP-labelled anti-Human APP(R101A4) Mouse IgG MoAb Fab' A.P.). The plate was incubated at 4°C for 30minutes. After 9 washes with PBS containing 0.05% Tween 20, 100µL per well of TMB substrate solution was added and incubated in the dark at room temperature. After 30 minutes, the reaction was stopped by adding 100µL of 1N- H_2SO_4. Plates were scanned at 450nm using a microplate reader (SH-1000, Corona Electric Co. Ltd, Japan) within 30 minutes of adding H_2SO_4.

3) Characterization of the ELIZA assay

A standard curve was drawn based on the colorimetric intensity of diluted standard substrate solutions. The sensitivity of this kit was 0.03ng/mL as determined by the National Committee for Clinical Laboratory Standards Evaluation Protocols. The working range of the assay was 0–6.2 ng/mL. The percentage of the recovery test ranged from 71.9% to 76.9%. The antibody using for the detection of the standard substrate in this study specifically recognizes the OX2 domain of the sAPP770ß subunit, generated upon cleavage of APP770 by ß-secretase in human brain microvascular endothelial cells. Antibody specificity for human APP770 is near 100%, with minimal cross-reactivity with APP695 and APP751.

3. Statistical analysis

Data were analyzed using SPSS (IBM SPSS Statistics 24 for Windows). The Mann–Whitney test was used for comparison of plasma sAPP770 concentrations between male and female subjects. P-values <0.05 were considered statistically significant.

RESULTS

The concentrations of sAPP770 in plasma samples are shown in Fig. 1. The median concentration in plasma sAPP770 were 44.31ng/mL in male subjects and 60.99ng/mL in females, respectively (Fig. 1-A). The largest value in male subjects was 123.16 ng/mL and the smallest value was 27.63ng/mL. The largest value in female subjects was 113.40ng/mL and the smallest value was 39.23ng/mL. The median plasma sAPP770 concentration in all subjects (male + female) was 56.80ng/mL (Fig. 1-B). No significant difference in concentrations between male and female was observed (P=0.28).

DISCUSSION

Detection of amyloid-ß from CSF is an established highly sensitive biomarker for AD[13]. However, CSF collection can involve significant discomfort to patients and lead to more serious consequences as mentioned in the Introduction section[14]. In addition to CSF, structural and functional brain imaging are alternative diagnostic tools for AD. To this aim, volumetric magnetic resonance imaging (MRI), functional MRI and positron-emission tomography (PET) with the use of fluoro-deoxy-glucose (FDG) or amyloid tracers have been previously employed[16, 17]. However, there are limitations in these imaging techniques[17]. For example, structural MRI cannot detect the histopathological hallmarks of AD such as amyloid plaques or neurofibrillary tangles, cerebral atrophy is not unique to neuronal damage caused by AD and FDG PET is an expensive technique that involves exposure to radioactivity. Therefore, it is important to develop alternative and less invasive methods to predict AD prior to the onset of symptoms. Plasma levels of amyloid-ß have been a principal focus on blood-based biomarkers. The levels of amyloid-ß alone were not significantly associated with progress of AD[18]. Van Oijen et al.[19] reported that plasma concentrations of amyloid-ß40 and amyloid-ß42 rise with age and are increased in people with mutations that

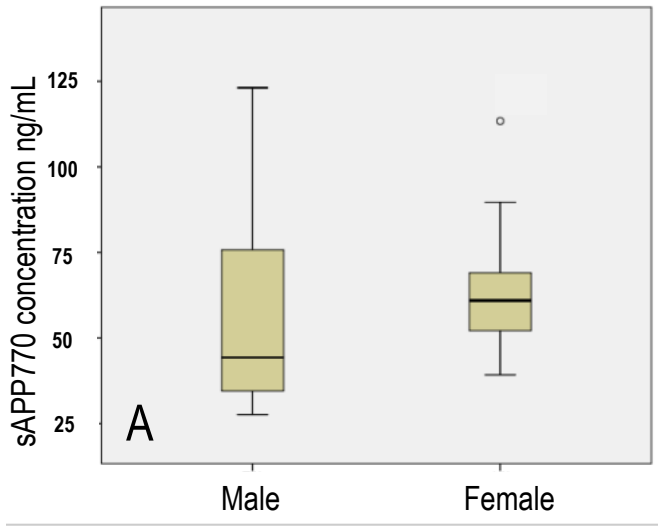

Fig. 1-A

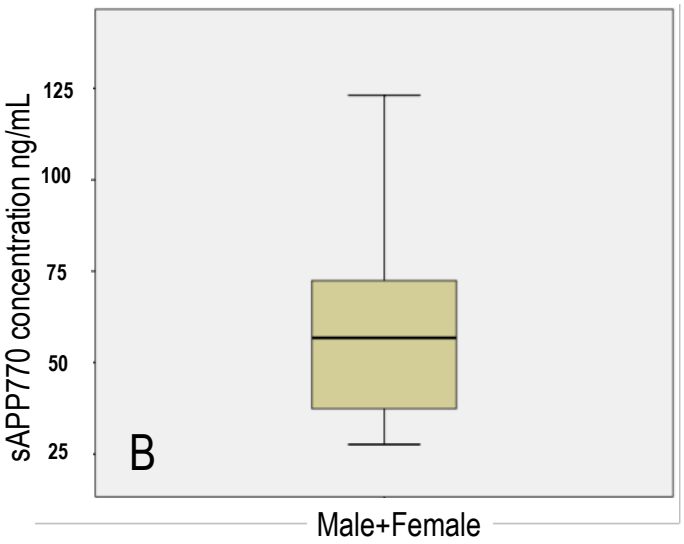

Fig. 1-B

Fig. 1. Box plot of plasma sAPP770 concentrations in young healthy male and female subjects (A). B shows the concentration in total (male + female) subjects.

The lines inside the boxes denote the medians. The boxes mark the interval between the 25th and 75th percentiles. A data point indicated by a small open circle in female box plot is outlier.

cause early-onset AD. However, amyloid-ß42 concentrations may decrease early in the dementia process. Both amyloid-ß 40 and 42 are processed from their precursor protein, APP770. Therefore, plasma levels of APP770 may be informative as of the potential range of plasma amyloid-ß concentrations.

In the present study, we measured plasma sAPP770 concentration in young healthy subjects. The sAPP770 levels obtained in this study (56.80ng/mL) indicate the endogenous level secreted from human brain vascular endothelial cells. Kitazume et al.[12] reported that the plasma level of sAPP770 in patients about 72.4 years old with AD-related diseases but, cognitively normal functions was approximately 100ng/mL. The level of plasma sAPP770 in elders suffering from AD-related disease appears to be consistently higher than those of young healthy subjects. The development of AD with clinically recognized memory loss and cognitive decline progress over a period of 20-years, during which time mild cognitive impairments (MCI), and brain damage may remain undetected. Further work will be required to characterize the age-related changes in plasma sAPP770 concentrations to establish normative values throughout adulthood. Such studies will contribute to our understanding of age-dependent component in the development of AD.

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