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Clinical Study

The level of Alzheimer-associated neuronal thread protein in urine may be an important biomarker of mild cognitive impairment

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ABSTRACT

Increased levels of Alzheimer-associated neuronal thread protein (AD7c-NTP) are often detected in urine in the early course of Alzheimer's disease (AD), which makes it a promising biomarker for AD. However, whether the concentration of urinary AD7c-NTP is increased in patients with mild cognitive impairment (MCI) remains unclear. The aim of this study was to explore the value of urinary AD7c-NTP to assist in the diagnosis of cognitive impairment by comparing differences in urinary AD7c-NTP among normal controls, MCI patients and AD patients. One hundred and seventy patients from the Xuan wu Hospital, Capital Medical University were divided into three groups according to their clinical diagnosis: an AD group (n = 45), an MCI group (n = 60) and a normal group (n = 65). The Mini Mental State Examination and the Montreal Cognitive Assessment scale were used to screen for the diagnosis of AD and MCI, and patients met the diagnostic criteria of the National Institute of Neurological Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. The level of urinary AD7c-NTP was determined using the enzyme-linked immunosorbent assay method. The urinary levels of AD7c-NTP in the AD group (median 2.14 [range 0.49–6.39] ng/ml) and the MCI group (median 1.57 [range 0.4–4.15] ng/ml) were significantly higher than those of the normal group (median 0.53 [range 0.04–2.07] ng/ml). To our knowledge our study is the first to show that the level of urinary AD7c-NTP in MCI patients is higher than in healthy people, which suggests that the level of urinary AD7c-NTP may be an important biomarker for early diagnosis of MCI.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease with cognitive dysfunction its main clinical manifestation. In the USA, 13% of people over the age of 65 and 33% of those over the age of 85 suffer from AD [1], and the prevalence of AD in people aged 55 and older in some cities in China is 2.1% [2–3]. Patients with AD have difficulty with activities of daily life, abnormal behavior, and accompanying diseases of old age, which cause a tremendous burden on the economy, public health services, families and society. At present, there is no effective method to reverse the disease, so treatment is aimed at slowing the course of the disease. Therefore, early diagnosis and intervention to postpone its development has become the major treatment for AD. Mild cognitive impairment (MCI) is a clinical concept that categorizes individuals who are in an intermediate cognitive state between normal cognitive function and dementia [4]. It represents a transitional period between preclinical AD and AD itself [5]. There are some clinical assessment measures for MCI, but early detection of MCI is very difficult for neuropsychologists working in clinical settings, so an effective biomarker that can assist in the diagnosis of MCI would be of great value.

De la Monte and Wands first discovered the neurofilament protein (neural thread protein [NTP]) and neurofibrillary tangles in the brains of a large number of patients with AD in 1992. They also found that NTP may play a role in the process of pathological changes in AD. In 1996, they successfully cloned a 41 KDa protein associated with the AD NTP, which was named AD7c-NTP [6–8].

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AD7c-NTP has now been identified as biochemical marker for the diagnosis of AD, because elevated levels are found in select brain tissue, cerebrospinal fluid (CSF), and urine of patients with AD [9–12]. Urine examination for AD7c-NTP is non-invasive and easier to undertake than other methods such as brain tissue and CSF examinations. However, it remains unclear whether the level of AD7c-NTP in urine is increased in patients with MCI. The aim of the present study was to examine the level of AD7c-NTP in urine to determine if it could assist in the diagnosis of cognitive impairment by comparing differences among normal controls, MCI patients and AD patients.

2. Patients and methods

2.1. Participants

Sixty MCI patients and 45 AD patients were selected from the Clinic of Neurology, and 65 normal participants were selected from the Department of Health Medicine, Xuan wu Hospital between October 2011 and April 2014. All participants underwent extensive examinations, including a series of tests of cognitive function, and a head CT scan or MRI to rule out local brain lesions. The inclusion criteria for the MCI group were: a score of $\geqslant$0.5 in one or more domains of the Clinical Dementia Rating (CDR) scale; a total CDR score $\leqslant$0.5; daily capacity reservations; not meeting the diagnostic criteria for dementia (*Diagnostic and Statistical Manual of Mental Disorders IV* standard); and a Montreal Cognitive Assessment (MoCA) score of >26, indicating normal cognition (25 if <12 years of schooling). The inclusion criteria for the AD group were: meeting the AD diagnostic criteria of the United States National Institute of Neurology Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [5]; and no language barriers (fluent in Chinese). Mini Mental State Examination (MMSE) score thresholds were $\leqslant$17 for the illiterate group, $\leqslant$20 for the primary school educated group and $\leqslant$24 for the secondary school or higher educated group; with a CDR of 1–2 for all patients. The inclusion criteria for the normal participants were: healthy individuals with normal cognitive function (MoCA >26, or >25 in those with <12 years schooling) and MMSE score >24. Exclusion criteria for all groups were head MRI showing local brain lesions, cognitive impairment due to metabolic syndrome, hypertension, diabetes, cancer, recent acute infection, severe cardiac, liver or kidney dysfunction, cerebrovascular disease, severe Parkinson's disease, depression or anxiety, hypothyroidism, intracranial space-occupying lesion, alcohol dependence, cognitive dysfunction after traumatic brain injury, alcohol or drug abuse, renal disease and urine abnormalities. Informed consent was obtained from all participants. This clinical investigation was approved by the Ethics Committee of Xuan Wu Hospital, Capital Medical University, Beijing, China.

2.2. Urine specimen collection

Specimens were taken of the patient's first morning urination after waking in the morning, having emptied the bladder before going to sleep. Ten milliliters of urine were collected from each participant and immediately refrigerated.

2.3. Determination of urine AD7c-NTP

An enzyme-linked immunosorbent assay kit (Anqun Biological Technology, Shenzhen, China) was used to detect the protein level of AD7c-NTP in the urine specimens. The absorbance value of the reaction product was measured at 450 nm with a microplate reader (Multiskan Spectrum, Thermo Scientific, Waltham, MA, USA). The AD7c-NTP concentration in the urine specimen, which is positively correlated with the absorbance value, was calculated according to the standard curve of recombinant human AD7c-NTP peptides measured at the same time.

2.4. Statistical analysis

Data which fit the normal distribution are expressed as mean ± standard error of the mean, or are otherwise expressed as median (range). The age, sex, and neuropsychology demographics of the groups were compared by one-way analysis of variance, followed by Tukey's *post hoc* test, and the urine levels of AD7c-NTP were compared by Kruskal–Wallis test using the Statistical Package for the Social Sciences version 11.5 (SPSS, Chicago, IL, USA). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Participant demographics

Table 1 shows the mean age and sex composition of the three groups (n = 170). The average ages of the MCI group, AD group and normal control group were 67.8, 68.4, and 68.9 years, respectively. There were no significant differences in age or sex among the three groups ($p > 0.05$).

3.2. Neuropsychology differences

As shown in Table 2, the CDR, MMSE and MoCA scores of the AD group were much higher than normal control group ($p < 0.01$).

3.3. Urinary levels of AD7c-NTP

As shown in Figure 1, the mean levels of urinary AD7c-NTP in the MCI group (1.57 ng/ml) and the AD group (2.14 ng/ml) were much higher than the levels seen in the normal control group (0.53 ng/ml, $p < 0.05$).

4. Discussion

Neuropsychological tests such as the MMSE are widely used in clinical studies to evaluate cognitive impairments in order to make a diagnosis. Although diagnostic sensitivity and specificity in the early disease stages are improved by CSF markers [13], more comprehensive test arrays are necessary to improve the reliability of results, particularly in the earliest stage of the disease. Thus, the early diagnosis of cognitive impairment is particularly important. Although the pathogenesis of AD is still not clear, the NTP may help to improve the accuracy of diagnosis of AD [8,11,14].

AD7c-NTP is a transmembrane phosphate protein whose molecular weight is approximately 41 kDa, and it abounds in neurofibrillary tangles, which are related primarily to nerve inflammation and cell death [15]. AD7c-NTP has been suggested as a candidate biomarker molecule because its gene is selectively overexpressed in brains with AD compared with age-matched

Table 1
Clinical characteristics of the three groups

Group	Patients, n	Age, years[a]	Males	Females
MCI	60	67.8 ± 12.7	37	23
AD	45	68.4 ± 12.1	27	18
NC	65	68.9 ± 11.9	40	25

AD = Alzheimer's disease, MCI = mild cognitive impairment, NC = normal controls.
[a] Data are expressed as mean ± standard error of the mean.