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Determination of Alzheimer's disease stage based on plasma cis phosphorylated tau biomarker

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Abstract

Background and Objective: Alzheimer's disease (AD) is the most prevalent cause of dementia globally, with its incidence continuing to increase. Cis phosphorylated tau (Cis p-tau) is postulated as the earliest detectable pathogenic marker in AD and as a novel diagnostic and therapeutic factor. This study was conducted to evaluate stage of AD patients based on plasma level of Cis p-tau.

Materials and Methods: Target population (65-80 years) was chosen from people with AD who visited the neurology clinic of Firouzgar hospital. Selection of cases was according to their medical history and they were divided into control and AD groups at two early and late stages. Individual's cognitive performance was evaluated using Mini-Mental State Exam (MMSE) and Clinical Dementia Rating (CDR) tests besides measurement of plasma level of Cis p-tau.

Results: The results obtained for this study showed that besides significant difference in cognitive indices between control group and two groups of AD patients at early and late stages of the disease, plasma level of Cis p-tau is significantly higher in AD groups (especially in late stage AD group) as compared to the control and healthy group ($p<0.05$).

Conclusion: It seems that routine measurement of plasma Cis p-tau in AD patients may be of clinical diagnostic value to differentiate AD stages and to evaluate efficacy of used therapies. However, further large-scale research studies are still required to affirm this issue.

Keywords: Alzheimer's disease, Cis p-tau, Dementia, Clinical diagnosis

1. Introduction

Alzheimer's disease (AD) is highlighted by the presence of amyloid β (Aβ)-composed plaques and tau neurofibrillary tangles (NFT). AD even at its early stage is associated with cognitive impairment. AD is a common causative factor of cognitive deterioration in elder individuals (1, 2). People afflicted with AD have usually debilitating difficulties in performing daily activities and this complication increases as the disease progresses and some patients commonly experience varying degrees of neuropsychiatric symptoms (3). AD is a foremost healthcare challenging issue with no curative and effective treatment at present (4). Early detection of AD in its first stages is important for testing and applying routine treatments to prevent its progression and to reduce its mortality and healthcare costs (5, 6).

Tau is a microtubule-associated protein which contributes to stabilizing microtubules in neurons.

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Abnormal phosphorylation of tau proteins is a pivotal and determinant attributes of tau pathology in AD patients. Hyperphosphorylated tau (P-tau) proteins are aggregated in the form of helical filaments which finally form intracellular NFTs in neuronal dendritic spines. By gradual formation and deposition of NFTs, normal function of neurons is lost with eventual cell loss (6-8). P-tau protein is present in two arrangements, i.e., trans and cis, of which the latter one has been demonstrated to be highly neurotoxic and more susceptible to accumulate (6, 9). Clinical evidence indicate that in early-stage AD, an increase in the amount of cis and not trans form of P-tau appears in brains of patients with mild cognitive impairment (6, 10, 11).

Cis-tauosis appears long before other tauopathy agents and may be regarded as a precursor of tauopathy and as a specific factor to forecast AD and its progression at earlier phases (6, 11). However, no biosensor has been invented to detect AD on the basis of cis tau pathology (6). Thus, we herein employed a monoclonal antibody against the pathogenic P-tau species to detect and even predict neurodegeneration through a non-invasive procedure using blood samples.

Abnormal accumulation of phosphorylated tau (known as p-tau) protein in the neuronal cell bodies is considered as an important pathogenic factor in AD development. Accumulated p-tau protein is pathologically observed as NFTs in cytoplasmic inclusions of neurons and glial cells (astrocytes and oligodendrocytes) (12).

None of presented markers are considered the exclusive and early biomarker of AD which can be consistently detected at its early phases (6). Hence, there exist an unmet requirement for valid biomarker for diagnosis of AD. This research study was thus undertaken to evaluate stage of AD patients based on plasma level of Cis p-tau.

2. Materials and Methods

This study was a clinical trial research study linked to Iranian Alzheimer's disease Registry of data on AD patients from outpatient neurology clinic in Tehran city (Iran) that was certified by Medical Research Ethics Committee (R.IUMS.REC.1396.32623) and informed consent was also obtained from the patients whom participated in the study. Data were collected from these centers between September 2018 and December 2021. Subjects were entered into this investigation if they were diagnosed with AD by a neurologist and/or psychiatrist based on cognitive tests and clinical examination. The target population of this study was selected from people with AD and with a minimum age of 65 and a maximum age of 80 who visited the neurology clinic of Firouzgar hospital. The selection of these patients was based on medical record information. The groups of this study included control group and Alzheimer's patients groups at early and late stages. In the control group, there were people who did not have any signs of neurodegenerative diseases and were in the age range of 65 to 80 years.

Level of Cis-p tau, trans-p tau, and total tau protein in the plasma of the studied groups was determined using ELISA method and the individual's cognitive and mental disorders were also evaluated using Mini-Mental State Exam (MMSE), Clinical Dementia Rating (CDR), and Geriatric Depression Scale (GDS) tests.

MMSE questionnaire was used to assess cognitive status. This questionnaire is usually used as a practical test to assess cognitive functions. This questionnaire, prepared by Folstein et al in 1975, consists of categories of orientation to time, orientation to place, repetition and registration, attention and calculation, recall, repetition, naming, comprehension, reading, writing, and so like (13). It is a practical and useful method to grade cognitive ability of affected patients by the clinician. The maximum total score is 30; a score of 27-30, 20-26, 10-19, and less than 10 is regarded as normal, mild, moderate, and severe AD, respectively. This questionnaire used in our study has been previously standardized (14).

CDR test is a validated instrument in clinical studies for assessment of AD spectrum, which evaluates three domains of cognition (memory, orientation, and judgment/problem solving) and three domains of function (community affairs, home/hobbies, and personal care) using structured interviews. The scores for the six domains (ranging from 0 to 3) tested can be summed (CDR Sum of Boxes or CDR-SB) (15).

To evaluate severity of depressive symptoms, we employed Geriatric Depression Scale (GDS) test. Tailored for elder people, the GDS test consists of 30 items and adopts a yes-no/agree-disagree response format, simplifying the symptom reporting process. A score between 11 and 20 suggests mild depression, whereas a score of 21 to 30 implies moderate to severe depression (16, 17).

Inclusion criteria for this study were male or female cases, age between 65 and 80 years, and normal or corrected normal ability to see and hear. Exclusion criteria for this study included depression, any other cause of dementia for AD patients (vascular dementia), Any symptoms of disease or abnormalities sufficient to cause memory impairment other than AD (e.g. normal pressure hydrocephalus, progressive supranuclear palsy), stroke history, major structural abnormalities on MRI (eg. infarction, intracerebral malformation), history of seizures, epilepsy, a serious infectious disease affecting the brain in the past, and serious head injury. For control group, the criteria were normal MRI/CT and cognitively normal based on history and psychometric tests and no history of neurodegeneration. In addition, case and control group was matched regarding age and gender.

For statistical analysis, GraphPad Prism software version 7 and one-way ANOVA and t tests were used

with p<0.05 as significant.

3. Results

Table 1 shows demographic data of studied cases in different groups. Obtained data showed significant differences in cognitive indices using MMSE and CDR tests between Alzheimer's patients irrespective of disease stage (p<0.05) as compared to the control group, clearing indicating establishment of AD in affected cases. Regarding GDS, no significant differences were found out between the groups (p>0.05).

Table 1 also indicates data for plasma levels of different forms of p-tau in different groups. Regarding plasma cis p-tau, there was a significant difference between the Alzheimer's groups and the control group (p<0.05) and such significant difference was also obtained between Alzheimer's groups at early and late stages (p<0.05). In addition, no significant differences were obtained between the studied groups regarding plasma trans p-tau and total p-tau (p>0.05).

Table 1: Demographic data of used cases, cognitive and depressive scores, and plasma level of different forms of p-tau in different groups

	Early stage and Late stage (n=78)		Early stage	Late stage	Control	P value
Age	65-80		-	-	65-80	-
Sex	Women	53 %	-	-	49%	-
	Men	47%	-	-	44%	-
Disease history	First degree relatives	65.75%	-	-	-	-
	Second degree relatives	21.91%	-	-	-	-
	Other relatives	5.47%	-	-	-	-
	Unknown	6.84%	-	-	-	-
Mean MMSE score			20.32±5.3* (n=54)	15±5.1*# (n=24)	29.3±2.3 (n=64)	*P<0.05 #P<0.05
Clinical dementia rating (CDR)			0.86±0.81* (n=54)	2.33±1.1*# (n=24)	0.23±0.1 (n=64)	*P<0.05 #P<0.05
Geriatric Depression Scale (GDS)			4.8±2.3 (n=54)	5.1±2.4 (n=24)	1.9±0.6 (n=64)	ns
Plasma cis p-tau (pg/mL)			18.33±3.3* (n=20)	30.13±4.8*# (n=20)	11.12±1.8 (n=20)	*P<0.05 #P<0.05
Plasma trans p-tau (pg/mL)			2.45±1.3 (n=20)	1.99±0.9 (n=20)	2.11±1.1 (n=20)	ns
Plasma total p-tau (pg/mL)			19.23±3.4	30.88±3.2	12.56±1.6	ns

* p<0.05 (versus control)
# p<0.05 (versus early stage)

4. Discussion

Alzheimer's disease (AD) is a prevalent and debilitating neurodegenerative disease and as the most frequent contributing factor for dementia in the elder society in the world. According to World Health Organization (WHO), incidence of AD is continually increasing at a rather fast pace. It has been estimated that over 50 million people in the world are suffering from AD and dementia (18, 19). AD is initially associated with memory disturbances, but with disease advancement, AD patients usually show cognitive impairment, impulsive or unpredictable behavioral difficulties and unusual personality changes (20, 21). Pathologically, AD is typified by neuroinflammation, amyloid-beta (Aβ) plaque, and deposition of neurofibrillary tangle (NFT) which are associated with progressive cognitive deficit and neurodegeneration (22). AD is known as Aβ and tau pathology which finally shows itself with neurodegeneration and cognitive disturbances. The initiation of AD and its progression are determined by an amalgamation of environmental and genetic factors which result in dysfunctions in some brain areas which are responsible for disease clinical symptoms (22, 23). Over the past years, amyloid beta cascade and tau hypotheses have been the two most known hypotheses for explaining AD pathogenesis process (24-26). Aside from these past hypotheses, researchers are also working on novel theories to explain AD etiology and its pathogenesis (22, 27). Some of these new theories include gamma oscillations, lysosomal deficit, and calcium dysregulation which may be leading to presentation of safe and effective therapy for AD (20, 28).

Tau fibrils accumulation and spreading closely

correlates with neurodegeneration and memory decline which is observed during the development of AD. Which factors affect tau spreading are not well-known. In this respect, new research studies on human brains using positron emission tomography have shown widespread tau hyperphosphorylation (29). Plasma level of p tau may have good potential for AD diagnosis and its prognosis. In the primary care clinics, plasma p tau may be used to initially screen for AD pathophysiological process besides regular clinical assessments for possible dementia and cognitive deterioration. It would be an ideal maneuver to monitor for longitudinal alterations in plasma p tau and cognitive ability (30).

Since clinical diagnosis of AD at its early stages is practically difficult, biomarkers have significant role in this field. Biomarkers can serve as diagnostic tools in the initial phases of AD. Currently presented diagnostic tools are frequently expensive, time-consuming, invasive, low access, and inadequately sensitive to detect AD at its initial stages (23, 31). Blood-based markers are considered less invasive and as cheaper approach to detect AD (32).

Findings of this study showed that measurement of plasma Cis p-tau in AD patients may be of clinical diagnostic value to differentiate AD stages and to evaluate efficacy of used therapies. However, further large-scale research studies are still required to affirm this issue.

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Ethical considerations

This research study was approved by Ethics Committee of Iran University of Medical Sciences (Tehran, Iran) (IR.IUMS.FMD.REC.1399.349).

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