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Title: Cerebrospinal Fluid Tau and Phosphotau As Biomarkers in Alzheimer's Disease Diagnosis

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ABSTRACT

Dementia is a progressive disorder that leads to memory loss and cognition impairment and affects daily function. Alzheimer disease (AD) is the main cause of dementia that characterized by loss of memory and cognition. AD pathologically is demonstrated by neuronal atrophy, synapse loss and the unusual reposition of amyloid- β protein ($A\beta$) as senile plaques and hyperphosphorylated tau protein as neurofibrillary tangles (NFT). Tau is a microtubule associated protein mostly expressed in neurons. Site-specific phosphorylation regulates Tau function. In AD, the six adult tau isoforms are unusually phosphorylated that cause to form the paired helical filament. The different conditions of tau phosphorylation eventuate from the function of specific kinases and phosphatases. In recent years some biomarkers such as phospho tau 181, 199 and 231 had been assessed in cerebrospinal fluid (CSF) and blood and had been showed their elevation in AD. This article provides an overview of tau structure, functions, and its involvement in AD and its role as a CSF biomarker.

Key words : Alzheimer, Tau, Phospho tau, Dementia, CSF biomarker, NFT

INTRODUCTION

Dementia also known senility is a progressive disturbance which causes memory loss and cognition impairment and affects daily functioning of patients[1]. Observations show a significant increasing in the number of demented individuals from 25 million in the year 2000 to 63 million in 2030[2]. Alzheimer disease (AD) is the main cause of dementia and illustrates significant social problems that mostly is treated symptomatically [3]. Patients are demonstrated with memory loss then consequently present, confusion, agitation, and behavioral disorders [4]. The diagnosis of AD depends on clinical and neuropathological evaluation [5]. Rapidly progression of this disorder levitates from the molecular pathology in the limbic and related cortexes[6]. In the cognitively normal brain, there is an age-related reduction in brain volume and weight, enlargement of ventricles, and loss of synapses and dendrites in selected areas[7]. But AD pathologically is demonstrated by neuronal atrophy, synapse loss and the unusual reposition of amyloid- β protein ($A\beta$) as senile plaques and hyperphosphorylated tau protein as neurofibrillary tangles (NFT)[8]. Senile plaques are made of $A\beta$ -protein and the gene encoding $A\beta$ is related to AD [9] while, microtubule associated protein tau (MAPT) the gene encoding tau protein is not genetically associated to AD[10]. Illustrations show NFT implicate medial temporal lobe structures (hippocampus and entorhinal cortex) and then progress to temporal, parietal and frontal lobe[11].

Tau

Tau is a microtubule associated protein mostly expressed in neurons [12]. The human tau gene is located over 100 kb on the long arm of chromosome 17 at band position 17q21 and contains 16 exons [13]. Tau protein stabilizes microtubules and authorizes neurite extension and stabilization [14]. (FIG1). The protein expresses in the axons of the central nervous system and was seen mainly in six isoforms with different functions which array from 352 to 441 amino acid and produced by alternative splicing. Abnormal deposition misprocessed Tau is an important pathway Alzheimer's disease. Tau has two ways of controlling microtubule stability: isoforms and phosphorylation. [15, 16]. These isoforms vary from each other by the attendance of either three- (3R) or four-repeat regions (4R) in the carboxy-terminal and the absence or presence of one or two inserts (29 or 58 amino acids) in the amino-terminal [17].

Tau kinases and phosphatases

Protein phosphorylation is one of the major molecular mechanism which regulates protein function in reaction to extracellular stimulation of the nervous system[18]. Phosphorylation disport indispensable roles in signaling pathways and cellular processes like transcription, apoptosis, intercellular communication, and neuronal and immunological functions[19]. (FIG2). Observation

show site-specific phosphorylation of Tau regulates its function [20]. In accordance with these results phosphorylation of a few specific sites of tau is essential in tau-microtubule interactions regulations [21]. In the other hand hyperphosphorylation of tau reduces the biological activity of tau [22]. Illustrations show tau is transiently phosphorylated during development and during anesthesia and hypothermia [23]. Normal adult human brain tau contains 2–3 moles phosphate/mole of tau protein [23]. While in AD, brain tau is three to four-fold more hyperphosphorylated than the normal adult brain tau and in this hyperphosphorylated state is polymerized into paired helical filaments (PHF) [24, 25]. In AD, the six adult tau isoforms are unusually phosphorylated that cause to form the PHF, the main fibrous component of the neurofibrillary lesions [26]. The longest type of adult tau in the human brain (441 amino-acids) has 80 Serine or Threonine residues and 5 Tyrosine residues; therefore, approximately 20% of the molecule has the potential of phosphorylation [27]. Tau is phosphorylated at almost 45 sites in AD brains. Tau aggregations differ in phosphorylation and content of tau isoforms, which enable a molecular classification of tauopathies [28]. Many of the phosphorylation sites are on Serine–Proline and Threonine–Proline sites. But few of them are on non-Serine / Threonine–Proline motives [13]. The different conditions of tau phosphorylation eventuate from the function of specific kinases and phosphatases [29].

The main tau kinases are glycogen-synthase kinase-3 β (GSK-3 β), cyclin-dependent protein kinase 5 (cdk5), cAMP-dependent protein kinase (PKA), and stress-activated protein kinases [30]. Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase that involves in phosphorylation of serine and threonine amino acids [31]. GSK-3 is encoded by two genes, GSK-3 alpha (GSK3A) and GSK-3 beta (GSK3B) [32]. Phosphorylation of Thr231 have the main role in regulating tau-microtubule interactions and Thr231 is the brief site for GSK3 β [33, 34]. Phosphorylation of Thr231 remarkably reduces tau and microtubules binding [34]. Cdk5 is a member of the cyclin-dependent kinases (Cdks) that activated in post-mitotic neurons through the neuron-specific activator p35 [35, 36]. Cdk5-p35 has the main role in brain development and physiological synaptic functions [37]. In pathologic states Cdk5 is hyperactivated by p25 [38], that is the N-terminal shortened form of p35 [39]. There is a reposition of p25 in AD brains with high phosphorylation capability of Cdk5-p25 for tau in comparison with Cdk5-p35 [40]. Between the 16 Ser/Thr-Pro sites in tau, Cdk5 phosphorylates 9–13 residue [41]. The in vitro investigations show Ser202, Thr205, Ser235, and Ser404 are the major residues and Thr153 and Thr212 are the

minor sites of phosphorylation[41]. phosphorylation at serine 422 in tau occurs early and related to cognitive decline in AD[42]. Elevated levels of t-tau and p-tau181 in CSF show degenerative processes in the cortical areas specially affected in Alzheimer disease. p-tau181 can be more significantly associated with neurodegenerative alternations in early AD[43] .The CSF/phospho-tau199 levels in the AD are remarkedably high with 85.2 in sensitivity and specificity.So Csf phospho-tau199 can be a new biomarker in diagnosis of AD [44].

Protein phosphatases(PP2A) is the major tau phosphatase in the brain[45]. All protein phosphatases, involving PP2A, have more broad substrate specificity than protein kinases[46]. PP2A activity is regulated by two endogenous protein inhibitors, I_1^{PP2A} and I_2^{PP2A} [47] .These two inhibitors may be disregulated in AD brain cause to PP2A inhibition [48]

Tau as peripheral biomarkers

Tau first was described in cerebrospinal fluid (CSF) in 1993 [49]. Total Tau in CSF was first quantified by ELISA method that assesses all Tau isoforms [50].The results illustrated that CSF total Tau(CSF-tTau) increases with an age-related pattern. Elevation in CSF-tau is observed in Alzheimer's .the same high CSF-tau concentrations may be seen before the onset of clinical dementia.. [51].Illustrations demonstrated that elevations of CSF-tTau in AD are 2-3 times higher as compared to control group[50].CSF-tTau increase due to Tau exudation from neurons into CSF and is a sign of neuronal damage severity [52]. In fact, There is a growing body of evidence implicating that in acute states like stroke or Creutzfeld Jacob disease (CJD), a marked elevation in CSF-tTau was seen[53, 54]. Bearing this in mind phosphorylated tau (p-Tau) is one of the important biomarkers in early diagnosis of AD. [55].In the other hand elevation of CSF-pTau as compared to controls has found in all ELISA assays [56] .Observations show pTau181, pTau199, and pTau231 assays differ in AD from normal aging[57]. Evidences implicate the specificity of pTau is more than total Tau in differentiating various conditions[58]. Diversity in phospho-epitopes is the main factor in their potential to forejudge AD from other neurodegenerative [59]. In agreement with this conclusion, The best difference between AD and Frontotemporal Dementia (FTD) is pTau231 and between AD and Dementia with Lewy Bodies (DLB) is pTau181[60, 61]. Several studies show that high CSF-tTau and CSF-pTau levels are seen in early AD and the performance of these biomarkers in AD with MMSE (Mini-Mental State Examination) scores above 23-25 is similar to advanced AD[62]. As well as, in MCI progressing to AD during clinical

follow-up, the biomarkers are changed with sensitivities equivalent to AD dementia [63]. PTau at various epitopes including Thr181, Thr231 and Ser 199 is an authentic predictor of the conversion from MCI to AD, with a sensitivity of 66- 100% and a specificity of 66-78%[52] .In addition elevation of tTau/pTau ratio is a valuable marker in differential early diagnosis of dementia[52]. P-tau₂₃₁ and t-tau levels are higher in patients with MCI than in control. Observations show CSF p-tau₂₃₁ levels increase in MCI [64]. In addition High p-tau₂₃₁ levels are related to the point loss in MMSE scores MCI [65]. CSF p-tau₂₃₁ elevation has a prediction role in cognitive decline in patients with MCI[66]{Randall, 2013 #3058}. Other studies point to p-tau 181 concentrations as a introducer of the clinical symptoms of AD [66]. Several studies have provided evidence that CSF t-tau and p-tau_{181p} are related to neuronal and axonal damage[67].In progressive MCI CSF p-tau₁₈₁ and t-tau elevation are related to hippocampal atrophy[68]. P-tau₁₈₁, and t-tau are related to specific model in brain structure changes in AD [69].In table 1 there are some measures of various p-tau and t-tau in different studies in AD or MCI.

DISCUSSION

Alzheimer disease (AD) is the main cause of dementia and illustrates significant social problems that mostly is treated symptomatically [3]. In the cognitively normal brain, there is an age-related reduction in brain volume and weight, enlargement of ventricles, and loss of synapses and dendrites in selected areas[7]. AD pathologically is demonstrated by neuronal atrophy, synapse loss and the unusual reposition of amyloid- β protein ($A\beta$) as senile plaques and hyperphosphorylated tau protein as neurofibrillary tangles (NFT).As a matter of fact Tau is a microtubule associated protein mostly expressed in neurons.These proteins are found mostly in neurons compared to non-neuronal cells. One of tau's main functions is to modulate the stability of axonal microtubules.[8].CSF-tTau increases due to Tau exudation from neurons into CSF and is a sign of neuronal damage severity [52]. There is evidence implicating that the specificity of pTau is more than total Tau in differentiating various conditions[58]. In addition elevation of tTau/pTau ratio is a valuable marker in differential early diagnosis of dementia[52].In recent years serum or CSF tau protein as a most important biomarker have been used in diagnosis of AD. CSF

total tau and phosphotau was the aim of some studies. But because of LP invasiveness researches is low number. However the results of these studies are different, that can be due to the selection of patients in different stages of the disease or using different method. So, more studies with large number of patients in different stages and accurate methods are necessary.

Abrevations

AD: Alzheimer disease

A β : Amyloid- β protein

NFT: Neurofibrillary tangles

MPT: Microtubule associated protein tau

GSK3: Glycogen synthase kinase 3

CDKS: Cyclin-dependent kinases

PP2A: Protein phosphatases

P-TAU: Phospho TAU

MCI: Mild cognitive impairment

CSF: Cerebro spinal fluid

FTD: Frontotemporal Dementia

DLB: Dementia with Lewy Bodies

CJD: Creutzfeld Jacob disease

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Table1: csf tau and phosphotau levels in MCI and AD

Reference	P-tau 181	p-tau231	p-tau199	t-tau
[57, 70]	103 pg/ml CSF	667.5 pg/ml csf	1.7 fmol/ml csf	631 pg/ml CSF
[71, 72]	86.71 pg/ml CSF-AD		20 pg/ml CSF-AD	572.73 pg/ml CSF
[73, 74]	339 pg/ml (CSF-AD) 200.5 pg/ml (CSF-MCI)		58.5 pg/ml CSF-AD	70pg/ml (CSF-AD) 57.5pg/ml(CSF-MCI)
[75]	94.8 pg/ml CSF-AD	64.4 pg/ml CSF-AD		

Abbreviations: AD=Alzheimer disease; MCI= mild cognitive impairment; CSF = cerebrospinal fluid

Fig 1: tau and microtubule interaction

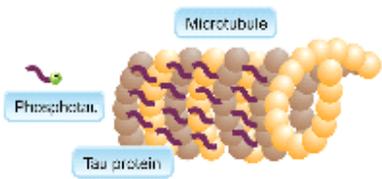
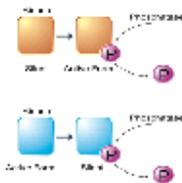


Fig 2: protein phosphorylation with kinases and phosphatases



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