

Biomarkers of Alzheimer's Disease: A Review

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ABSTRACT

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease. Neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles. The biomarkers used in the diagnosis of AD are mostly invasive. Established methods are available to identify biomarkers in cerebrospinal fluid (CSF) and plasma. β -amyloid (1-42) [$A\beta$ (1-42)], total tau (T-tau) and phosphorylated tau (P-tau) are the three biomarkers present in CSF as well as plasma. Increased level of these three biomarkers is associated with AD. These biomarkers in CSF can be measured by enzyme linked immunosorbent assay (ELISA), but measuring these biomarkers in blood need more sensitive and specific assays. Dendritic proteins like neurogranin, synaptosomal associated protein 25 (SNAP-25) and synaptotagmin-1 (SYT1) in CSF can also be used as biomarkers as their level is decreased in AD. Neurofilament light (NFL) level

(increased) in both CSF and plasma can serve as a biomarker for AD. Collection of CSF by lumbar puncture is a measure drawback which limits its use as a diagnostic tool. Biomarkers in non-invasive samples such as urine, saliva, hair and nail are under development and can be used in near future. Level of 8-hydroxy-2-deoxyguanosine (8-OHdG) and isoprostane in urine, cortisol level in saliva, concentration of trace elements in hair and nail can be used as biomarkers in AD.

Keywords: CSF, blood, non-invasive, urine, saliva

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INTRODUCTION

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease. In 1906, a German Psychiatrist Dr. Aloisius Alzheimer firstly observed the symptoms of AD. He found changes in the brain of a woman who died with symptoms like memory loss and unpredictable behavior [1]. The disease is called Alzheimer after his findings. Neuropathological hallmarks of AD are protein aggregation in the brain consisting of plaques and neurofibrillary tangles [2]. Plaques are composed of "amyloid", i.e. proteinaceous deposits. This protein was previously known as amyloid A4 protein but later it is known as β -amyloid or $A\beta$ [3]. The neurofibrillary tangles are composed of hyperphosphorylated tau proteins [4]. AD was initially considered as "presenile" dementia, affecting people between 50-65 years of age, while "senile dementia" affect people after 65years of age [5]. Studies reveal that people with "senile dementia" and "presenile dementia" have same pathological changes composed of amyloid plaques and tangles [6]. Now all dementia cases are termed as AD irrespective of age [7]. So senile dementia was called senile dementia of the Alzheimer type (SDAT) or late onset AD (LOAD) in older patients. The patients before 65 years of age were diagnosed with early onset AD (EOAD). In several studies, EOAD patients have shown more severe AD pathology (i.e., plaque and tangle) than LOAD [8-9]. The pathology in LOAD is not confined to the plaque and tangles formation but also a combination of α -synuclein and deposition of TAR DNA binding protein 43 (TDP-43) with additional microvascular changes along with hippocampal sclerosis [10-11]. The pathophysiological heterogeneity of LOAD leads to unspecific clinical symptomatology and difficulty in diagnosis [8]. For a successful treatment of AD, initially the symptoms need to be properly diagnosed but due to less accuracy and low sensitivity of our current diagnostic operations, it is difficult to treat AD [12-13]. Diagnosis of dementia mostly depends on the history of illness, blood tests and structural imaging of the brain to find out exact cause of the symptoms [14].

Generally, the treatment of AD is done with symptomatic drugs like acetylcholine esterase (AChE) inhibitors, NMDA-receptor antagonists, and with future promising newer

disease-modifying drugs such as secretase inhibitors or $A\beta$ immunotherapies [15]. Invasively obtained fluids, such as blood samples and CSF serve for testing sample in the diagnosis of AD. However, the brain proteins secreted from the brain extracellular space to the CSF are easy to identified as compared to the blood [16]. Future cognitive impairment and the progression of the disease, related to neurofibrillary and amyloid pathologies can be predicted from the CSF. However lumbar punctures are expensive and require highly trained medical personnel. So, it might be of interest to study other tissues that are easier to collect [17]. The use of non-invasive samples (NIS) like urine, saliva, hair, nail etc. can benefit millions of people worldwide, because of painless procedure and increased patient acceptability. Again, samples do not require special storage conditions therefore easily collected and shipped to laboratories for clinical analysis. NIS can be readily used in small, affordable and disposable kits or sensing devices for testing of AD and then can be disposed on daily basis [18]. This review paper describes about the CSF biomarkers and blood biomarkers used for the diagnosis of AD with their diagnostic procedures. It also describes the non-invasive biomarkers under development for diagnosis of AD.

BIOMARKER

Biological markers are known as biomarkers. Their occurrence at a certain quantitative level serves as an indication of a normal or pathogenic biological process. They play a valuable role in the prognosis, diagnosis and monitoring of any disease. The most important factors affecting are the sensitivity, specificity and ease-of-use to make the biomarker a popular method in diagnostic protocols. An ideal molecular biomarker should indicate with 100% confidence whether a patient suffers from a disease or not. There are many biomarkers for AD but no ideal molecular biomarker is available for diagnosis of AD.

CEREBROSPINAL FLUID (CSF) BIOMARKERS FOR AD

There are three key biomarkers which are well-established and validated internationally to diagnose AD in CSF. They

are β -amyloid (1-42) [$A\beta$ (1-42)], total tau (t-tau) and phosphorylated tau (p-tau). These biomarkers have significantly increased the diagnostic validity for AD in recent times, having more than 95% sensitivity and more than 85% specificity [19].

CSF β -amyloid (1-42)

In 1985, $A\beta$ (1-42) peptide was found as the main component of extracellular plaque depositions [20]. $A\beta$ is cleaved from the large amyloid precursor protein (APP) by β - and γ -secretases in synaptic vesicles. It was observed that $A\beta$ is released into the CSF which makes an easiest diagnostic protocol to measure its level [21]. From various studies it was revealed the marked reduction of $A\beta$ 42 in CSF samples with high plaque count in AD patients [22]. The reason behind low CSF $A\beta$ 42 is the aggregation of hydrophobic peptide leads to formation of plaque [23]. Decreased concentration of CSF $A\beta$ 42 was also observed with Lewy bodies in case of dementia [24]. This biomarker can be measured by Enzyme linked immunosorbent assay (ELISA) and mass spectrometry method [25]. Low CSF $A\beta$ 42 is in high concordance with positive amyloid positron emission tomography (PET) status. Hence, CSF $A\beta$ 42 and amyloid PET can be used as a biomarker interchangeably in AD, based on the availability, costs, risk measurement (radiation exposure vs. post lumbar puncture headache) in addition to both physician and patient preferences.

Beta site-amyloid precursor protein (APP)- cleaving enzyme 1 (BACE1)

It is a major β -secretase involved in plaque formation in the brain. BACE is not only found in the brain but also in most tissues in the body [26]. There is overproduction of BACE 1 in CSF of AD patients [27]. It can be measured by western blotting as well as ELISA [28].

CSF tau proteins as Alzheimer biomarkers

In 1987, tau protein was identified in CSF of AD patients [29]. Normally tau proteins bind and stabilize microtubules in neuronal axons [30], and this process is inhibited when tau is phosphorylated.

Total Tau (t-tau)

CSF t-tau is a "state marker", which show the intensity of neurodegeneration [31]. The increased level of CSF t-tau was marked in AD patients. Apart from that the level of CSF t-tau found higher in AD than other neurodegenerative diseases like Creutzfeldt Jakob disease. In healthy controls, total CSF t-tau levels increases with age [32]. But in AD patients the level is higher than age matched control subjects which support the CSF t-tau as a biomarker of AD [33]. CSF tau can be measured by ELISA which was reported first time in 1993 [34]. Then another ELISA method i.e. Innogenetics or INNOTEST assay, had been developed [35]. Studies suggest that there was increase in CSF- tau levels in Mild Cognitive Impairment (MCI) patients. Clinical studies also report that there is high incidence of conversion of MCI into AD. Hence, in AD, CSF t-tau can act as a biomarker [36].

P-tau

Tangles are composed of abnormally hyper-phosphorylated tau protein [37] with three times more phosphorylated sites than normal tau. The P-tau and truncated proteins are the key components of neurofibrillary tangles which is a CSF biomarker for AD [38]. High level of CSF P-tau predicts rapid disease progression in AD. P-tau in CSF can be measured by specific ELISA method (antibody combinations) which recognize the mid-domain phospho-tau epitopes [39]. Increased level of CSF P-tau is seen in AD patients [40]. CSF P-tau levels are not changed in case of acute ischemic stroke [41] but increased in neurodegenerative disorders like AD [42].

PET of CSF T-tau and P-tau were correlated and highly found in preclinical AD [43]. These findings favor use of P-tau and CSF T-tau as biomarkers in disease state. PET scans suggest that increased neurodegeneration in earlier disease stages can be identified before tau aggregates [44]. So, tau PET can be used as a biomarker of AD. An Axonal transport dysfunction mainly occurs due to hyper-phosphorylation of tau in AD [45]. The detection of phosphorylated tau at 181 position found to be increased in Alzheimer's patients compared to controls [46]. The analysis of other phosphorylated forms of tau (phospho-tau-199, 231, 235, 396 and 404) might offer significant improvements during early diagnosis of AD [47]. Phospho-tau-181, 231 can be used as a marker to distinguish AD from controls and other types of dementias like Lewy body dementia (LBD), frontotemporal dementia (FTLD), vascular dementia (VaD) and other neuronal disorders [48]. However, highly sensitive and specific ELISAs are needed for site-specific phosphorylated tau isoforms to improve the diagnostic tool for AD.

Synaptic biomarkers for AD

Synapses are the communication units of the neuronal networks in the brain [49]. Neurotransmitters are stored in the synaptic vesicles of presynaptic domain and released to the synaptic cleft and binds to the post-synaptic receptors to activate a cascade of molecular events thereby transmitting the neuronal signal [50]. A synaptic dysfunction and degeneration is the direct cause of the cognitive impairment in AD [51]. Several literatures showed marked degeneration and loss of synapses in grey matter of the brain in the early stages of AD patients [52]. Severity of synaptic loss is correlated with the degree of cognitive impairment than plaque and tangle [53-55]. Further, animal studies also suggested that both $A\beta$ fibrils and diffusible $A\beta$ oligomers [56] may disturb dendritic spines by different mechanisms [57-58]. Thus, synaptic biomarkers in CSF may serve as diagnostic tool in the pathophysiology of AD.

Few synaptic proteins were also identified in CSF in the 1990s. Neurogranin is a dendritic protein which play an important role in long term potentiation of synapses mainly in the hippocampus and the basal forebrain regions [59-60]. Several studies have shown the increased concentration of CSF neurogranin in AD [61-62]. This synaptic protein neurogranin is specific for AD and predicts future rate of cognitive deterioration. Thus, measurement of neurogranin in CSF can be used as a biomarker for AD. Presynaptic

SNARE complex proteins like synaptosomal-associated protein 25 (SNAP-25), syntaxin-1, and vesicle-associated membrane protein (VAMP) causes fusion of membranes in neurotransmitter exocytosis [63]. The SNAP-25 and synaptotagmin-1 (SYT1) essential for exocytosis of synaptic vesicle leads to release of neurotransmitter [64]. Levels of both SNAP-25 and synaptotagmin-1 decrease in AD brain (SYT1) but increased concentration is seen in CSF [65-67]. It may be used as a biomarker for evaluation of AD. However, this needs validation in future studies.

BLOOD BIOMARKERS FOR AD

Collection of CSF by lumbar puncture is the drawback of using CSF for diagnosis of AD. Additionally, screening of same patients for several years is also difficult. So, there is need to discover the other biomarkers in different body fluids to properly diagnose the AD [68]. Blood is a more challenging matrix than CSF for brain biomarkers [69]. However, measuring biomarkers in blood for AD need more sensitive and specific assays. It can be measured by ultrasensitive immunoassays and mass spectrometry methods [70]. Moreover, it is difficult to establish blood biomarkers for AD. A mutual exchange system has been existing between the molecules from brain and CSF. But, only a minute amount of brain proteins enters to the bloodstream and degraded in blood by proteases, metabolized in the liver and cleared by the kidney [71].

A β in plasma

It has been difficult to establish strong blood biomarkers for A β pathology. Currently, A β can be measured by ELISA methods or other immunoassay techniques [72-73] but there is lack of correlation between the concentration of A β in CSF and plasma [74]. The lack of association with disease pathology may be due to contribution from peripheral tissues to plasma A β [75]. In 2011, Single molecule array (Simoa) technique mainly based on immunocapture of protein biomarker was established which can measure plasma levels of A β 42 [76]. From various studies, it has been reported that in brain various plasma proteins such as pancreatic polypeptide Y, immunoglobulin M, interleukin 17, α 2-macroglobulin, apolipoprotein A1 and other proteins are associated with A β 42 [77-79]. But there is lack of mechanistic understanding of these associations. It has been estimated that up to 90% of blood A β is produced by platelets [80]. Platelet β -secretase activity is increased in both MCI and AD patients. So, platelet β -secretase may become a biomarker of choice for AD [81].

Tau protein in plasma

Tau is a brain-specific protein which can alter the function of other proteins like kinases and phosphatases which are implicated in tau pathology [82]. Several groups of kinases contribute to hyper-phosphorylation of tau, including cyclin-dependent kinase 5 and glycogen synthase kinase-3 (GSK-3). GSK-3 is significantly increased in white blood cells in AD as compared to healthy subjects [83]. A study confirmed that concentration of plasma tau is increased in the dementia stage of AD as compared to healthy subjects [84]. Ultrasensitive immunoassay techniques like Simoa and

immunomagnetic reduction (IMR) can measure tau protein in blood samples [85-87]. Alternatively, T-tau or P-tau can be used as a blood biomarker in AD [88].

Neurofilament light (NFL) in plasma

NFL is an intra-axonal structural protein. It leaks into body fluids upon axonal injury without any specific cause and can be measured by Simoa method [89-90]. Serum or plasma levels of NFL are increased in several non-AD neurodegenerative diseases and are increased in both familial and sporadic AD [91]. In familial AD, increased level of NFL concentration in CSF may serve as a biomarker for AD [92]. It can be a well replicated biomarker for neurodegeneration in AD.

BIOMARKERS IN NON- INVASIVE SAMPLES

Conventionally, biomarkers in AD have mainly focused on invasively obtained fluids such as blood samples and CSF. Biomarkers detected from these fluids help in diagnosis of disease progression related to neurofibrillary tangles and amyloid pathologies. Moreover, current developments of diagnostic devices based on small sensors and ion selective electrodes, to be used at the point-of-care or at point of discharge of non-invasive samples have the potential to disseminate the diagnostic tools to a large fraction of the population in the near future [93].

Urine

The urinary system eliminates waste from the body and regulates different physiological parameters like volume, pressure, pH, electrolytes and metabolites of the blood. Metabolites and hormones usually vary based on body physiological conditions [94]. Aging process and neurological diseases, such as AD are associated with increase in free-radical reactions and the enhancement of protein and DNA oxidative damages, confirmed by postmortem analysis of brain tissues. This knowledge has led to search for markers of the oxidative processes that occur during the development of the disease, considering different hypotheses for the pathology. A well-known biomarker for DNA oxidative damage is the 8-hydroxy-2-deoxyguanosine (8-OHdG), making it a promising biomarker for AD. Various study confirmed increased level of 8-OHdG in AD patients when compared with controls [95-96]. The AD group showed a mean urinary level of 115.7 ± 50 nmol/ μ mol creatinine for 8-OHdG, while the control group had a mean level of 9.28 ± 2.23 nmol/ μ mol creatinine, with $p < 0.001$. Free-radical-induced injury to the phospholipid membrane could induce the fragmentation of lipids, and some fragments could undergo an internal cyclization process producing isoprostanes and neuroprostanes. Two independent studies indicated an increased level of F2-isoprostanes in CSF samples of patients with AD [97-98]. Using GC-MS analysis, it was found that level of isoprostanes in urine significantly increased in MCI and AD patients. The mean level of isoprostanes in controls, MCI subjects and AD patients was 1.5 ± 0.1 ng/mg of creatinine ($p < 0.001$), 3.6 ± 0.3 ng/mg of creatinine ($p < 0.01$) and 4.6 ± 0.2 ng/mg of creatinine ($p < 0.001$) respectively. These findings suggest that isoprostanes can be

a reliable marker in the early stages of the disease. The mean level of the protein in the urine of AD and MCI subjects was significantly higher than healthy controls. It is believed that free amino acids (FAAs) can play a role in the early detection of AD, as they are involved in neurotransmission and neurotoxicity mechanisms [99]. Concentration of amino acids like glycine, histidine, 3-methyl histidine and carnosine are elevated in urine of AD patients whereas concentration of 1-methyl histidine is decreased.

Saliva

Saliva is an aqueous bio-fluid made up of roughly 99% water and 1% of organic and inorganic compounds. Due to its non-invasiveness, easy collection and storage, it can be preferred as biomarker for research and clinical applications as compared to other fluids such as CSF and blood [100]. The fluids presented in saliva are very sensitive to metabolic changes. So many studies have tried to describe the connection between saliva's components and AD [101]. Metabolites like sphinganine-1-phosphate, ornithine and phenyl lactic acid showed increased levels whereas inosine, 3-dehydrocarnitine and hypoxanthine showed decreased levels in AD patients [102]. It was found that the cortisol level increased in AD patients as compared to control subjects. The study also showed that evening cortisol levels were significantly lower than morning levels for both AD patients and controls [103]. Using fast ultra-HPLC coupled with TOF-MS in a high-throughput manner saliva marker of the disease can be identified [104].

Hair

Hair consists of a protein called keratin surrounded by an amorphous matrix [105]. Hair is an inert tissue which has the advantage that trace elements are fixed into its structure. Concentration of trace elements do not vary over a short time scale in hair unlike blood, saliva and urine [106]. So hair can be used on a long-term diagnostic scale. Additionally, hair can be easily collected and stored. So, it has the ability to be widely and cheaply used in clinical diagnostic centers around the world [107]. Trace elements are present in very low concentrations and a small variation may cause a disease. The relationship between trace elements and neurological diseases has been studied since the 1970s [108]. Trace element levels of Br, Zn, Na, K are significantly increased and levels of Co, Ca, Cu, Mn, Fe, Al, Pb, Hg, Cd are significantly decreased in hairs of AD patients [109].

Nail

Nails are made up of keratin and contains traces of many minerals and some metabolites. It may grow at an average rate of 3 mm (0.12 in) a month [110]. As nails are very suitable for storing trace elements over a long period, they are of great importance in the study of diseases that involve those elements. Zinc is the most abundant trace element found in the brain, which plays a vital function in enzymatic processes involving the degradation of A β peptide, and the processing of the amyloid precursor protein. However, in nail sodium showed an increased concentration in AD patients [111]. Whereas trace elements like manganese, iron,

copper, cadmium, mercury and zinc showed lower levels in AD patients [112].

CONCLUSION

Preliminary diagnostic procedures are the essential tool for the successful treatment of any disease. In case of AD, different diagnostic biomarkers have been developed to detect tangle and plaque pathology in the CSF and blood of AD patients. Biomarkers in CSF like T-tau, P-tau and A β 42 have been evaluated in various neurochemical studies with high diagnostic accuracy. Several others promising biomarkers are also there to be explored to detect synaptic loss and dysfunction. Additional biomarkers need to be identified with proper validation to detect pathological changes common to AD and other neurodegenerative proteopathies using noninvasive samples such as urine, saliva, hair and nails. The collection, transportation and storage of such samples are much easier if compared with those required for blood or CSF. Moreover, current developments of diagnostic devices based on small sensors and ion selective electrodes, to be used at the point-of-care or at point of discharge of noninvasive samples, have the potential to disseminate the diagnostic tools to a large fraction of the population in the near future.

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