

# Research Biomarkers For The Diagnosis Of Alzheimer's Disease

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**ABSTRACT---** *Alzheimer's disease (AD) is a neuronal disease that is characterized by the loss of neurons and cognitive impairment. There is no definitive cure and current treatment options are focused more on reducing the damage caused by the disease and providing care for the patients. Early diagnosis of the disease will help in minimizing the damage and give better treatment options. In this review we try to analyze a few important risk factors and identify some biomarkers in blood and cerebrospinal fluid (CSF) which could be used for the diagnosis of AD*

## I. INTRODUCTION

Alzheimer's disease (AD) is a neurological disease with distinct pathological features which includes the appearance of amyloid plaques and neurofibrillary tangles. There are variations in the brain, which scientists assume to have happened decades before any symptoms of AD begins to show. During these pre-clinical stages, the abnormal protein depositions result in the formation of amyloid plaque and tau tangles throughout the brain [1]. The damage primarily affects the hippocampus resulting in neuronal dysfunction which advances to cell death of healthy neurons [2]. The mild form of AD ascends with an increasing damage of the section of the brain governing language, reasons, stimuli and response leading to cognitive impairment [3]. Critical AD stage involves the distribution of the plaques all over the brain and the symptoms include the incapability to communicate and is rendered completely dependent on others [4].

The cause of AD is still unknown. Several factors have been attributed for the progression of the disease, but none has been singled out as the causative factor. Genetic changes, vascular factors,

lifestyle and acquired factors have been attributed for the progression of AD [5]. It has gene related aspects and early-onset AD disease arises between the age of 30s. Few cases are because of changes which are inherited, forming a type called early-onset familial AD disease (FAD) [6].

There is no known treatment for total curing of the disease and treatment is aimed at providing palliative care to the patients. Scientists are working on to find about addressing the underlying disease processes rather than treating symptoms. Various treatment options currently available include drug therapy, cognitive training and palliative care [7].

## II. BIOMARKERS FOR AD DIAGNOSIS

Currently AD is diagnosed only after the symptoms begin to show. Recent diagnostic techniques include analysing the brain scan images to find out the levels of neuronal damage [8]. There are no reliable techniques for the early diagnosis of AD. Research is focussed on establishing quick and reliable techniques for AD diagnosis. Early diagnosis will help in better treatment as well as reduce the burden on families [9]. Biomarkers are quantitatively calculated and assessed for indicating normal body functions and pharmacological response to a therapy [10]. Biomarkers are the primary indicators of all pathological disorder. They can back up diagnosis and progressive treatment of AD [11]. Through incorporation of information obtained by biomarkers linked amyloid accumulation and nervous disintegration, a probable AD progression map could be developed. Lastly, tests for blood biomarkers may be exploited as a diagnostic option [12].

### 2.1. Biomarkers for AD in CSF:

The brain's extracellular space directly contacts CSF and can be extracted by a simple lumbar puncture method [13]. Because CSF is more invasive to the brain than blood, many researchers believe biochemical signs about changes for AD are more possible to be shown in the CSF [14]. Two fundamental CSF biomarkers namely  $A\beta$  and tau protein have been evaluated and suggested for the diagnosis of AD [15]. Directly inserting a micro dialysis catheter into the brain will enable us to use the CSF to study the biochemical changes happening in the brain [16].

#### 2.1.1. $\beta$ -amyloid ( $A\beta$ ):

The plaques formed during AD are composed of  $\beta$ -amyloid, formed via cleaving of the Amyloid Precursor Protein (APP) by proteolytic cleavage carried out by  $\gamma$ -secretase complex found in the brain.

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It is a sequential cleavage initiated by a primary generation of a C-terminal fragment, and further cleavage by gamma-secretase leads to the generation of A $\beta$  40 and A $\beta$  42 [17]. The role of A $\beta$  peptides in initiating the amyloid cascade hypothesis includes glial cell activation and neuroinflammation, synapse and neurotic dysfunction, tau hyperphosphorylation and neuronal cell death with other consequent cognitive deficits [18]. From which, A $\beta$ 40 species is the most abundant, but A $\beta$ 42 is found to be very important to initiate the segregation of A $\beta$  and is considered to be a check point that triggers the amyloid cascade hypothesis of this disease [19]. Owing to its role in AD pathogenesis, it is a very potent biomarker in detecting AD than its shorter counterpart. Though A $\beta$  42 is a high pronounced biomarker of AD diagnosis, but the decreased A $\beta$  42/ A $\beta$  40 ratio is a much more advantageous indicator than reduced A $\beta$  in AD diagnosis [20]. Alternatively the relevance of A $\beta$  38 present in CSF towards AD pathology has also been talked about. The initial existence of A $\beta$  38 in CSF was found by immunoblotting based method and further studies of the same domain assure that there is a chance of increased A $\beta$  38 level in affected subjects than samples collected from a non-dementia individual [21]. Therefore, assessing the levels of A $\beta$  peptides individually and/or A $\beta$  42/40 ratio will be a reliable biomarker.

### 2.1.2. p-Tau:

Research in the recent past suggest that p-tau not only simply marks the axonal disintegration and neuronal breakdown but also is highly linked to the pathology of AD and contributes to the development of NFTs [22]. Recognition of the phosphorylated tau at 181 position is gradually enhanced in affected individuals in comparison to the controls, providing a specific method to discriminate healthy individuals from those of affected population [23]. In recent studies, elevation of p-tau in the CSF have been shown to correlate with the neurofibrillary tangle (NFT) load, therefore it seems to be related with the presence of NFT [24]. For AD, the concentration of p-tau present in CSF has been tested using ELISA based monoclonal antibodies which spots various abnormal phosphorylated sites of p-tau (namely Ser-199, Thr-181 and Ser-231/Thr-235) [25]. Moreover the combination of CSF markers (A $\beta$  and tau) can more prominently discriminate the patients from the controls or any other form of dementia than as individual markers [26]. Wiltfang et al have demonstrated that the ratio of A $\beta$  40 generally remains unchanged and rather the ratio of A $\beta$  42/ A $\beta$  40 has been used to successfully differentiate affected patients from normal individuals [27]. The examining of varying other possible combinations of CSF biomarkers have suggested that increased CSF p-tau / A $\beta$  42 ratio has a higher sensitivity and specificity for finding out that affected individuals [28].

### 2.2. Blood plasma biomarkers:

The use of CSF as a biomarker has a few disadvantages. One such thing is that the collection of CSFs for regular check-up process is both sensitive and has several side effects as well as discomfort to the patients. On the other hand plasma can be collected from blood, in presence of anticoagulants and checked for the factors corresponding to

the progression of AD [29]. A major advantage of searching AD biomarkers in blood is that patients can be easily screened or can perform easy follow ups for diagnosis over for several years. Similarly the levels of both tau and A $\beta$  levels in plasma seems to be upregulated in AD patients in comparison to normal controls [30].

On the basis of their assumed roles in AD treatment a huge number of peptides, amino acids and proteins, different from A $\beta$  in plasma have been examined. Ray et al used protein array technology to identify and predict plasma proteins that could be used for the clinical diagnosis of AD [31]. Common markers of inflammation like TNF- $\alpha$  were associated with the risk of vascular dementia [32]. The changes in the levels of A $\beta$  in AD patients versus normal in plasma are relatively unreliable unlike the changes in the CSF. However, measurement from plasma, serum or blood could still provide an index of AD and could be used as a diagnostic tool [33].

### 2.3. Cholesterol metabolism and vascular disease related markers

There is a direct relationship that exists between serum cholesterol levels, apolipoprotein E and the progression of AD [34]. Excess cholesterol in the brain is being converted to 24 S hydroxycholesterol and the levels of 24 S hydroxycholesterol in both CSF and plasma are elevated in AD affected brains when compared to normal controls [35]. Moreover, Kivipelto et al have stated in a population study the correlation between cognitive impairment and vascular risk factors [36]. Even though, S hydroxycholesterol is not a promising biomarker still its high level should not be ignored. Similarly, the  $\epsilon$ 4 allele of the apoE protein (Apolipoprotein E) throws a chief risk for early and late onset of AD. The presence of  $\epsilon$ 4 allele of apoE increases the risk of plaque formation and raised A $\beta$ 40 levels in the affected brain [37]. Therefore, the presence of the  $\epsilon$ 4 allele of the apoE protein could be determined and as a biomarker for AD diagnosis.

### 2.4. Markers of oxidation & inflammatory response

High levels of protein oxidation, lipid peroxidation and lesser functioning of the antioxidant enzymes in affected AD brains play an important role in neurodegeneration [38]. Both AD as well as the vascular dementia are linked with lower plasma levels of Vitamin A, Vitamin E and carotene when compared to normal controls [39]. Free radicals generated in the plasma resulted in higher production of malondialdehyde in AD patients than controls which could be measured in the fluid as a marker of the oxidation state [40]. Amyloid deposition in the affected brain brings out a diverse type of inflammatory responses which includes microgliosis, up-regulation of inflammatory cytokines [41]. Also, AD may be linked to widespread immune dysregulation that can be detected and the inflammatory molecules that relatively increases in the disease includes IL6, TNF $\alpha$ , IL1 $\beta$  and TGF $\beta$  [42]. The measuring of immune mediators in the serum and plasma of the patient is limited as for the variability of results.

### III. OTHER DISEASE MARKERS & RESULTS

It has been reported that there is an up-raised GSK 3 in WBC of AD patients as compared to non-affected subjects [43]. Also, in the fibroblasts, lymphocytes and RBC's of AD patients the Protein kinase C (PKC) seems to be changed which is an evidence for the point that confirmation of PKC could be an indicative biomarker in the AD pathology [44].

Similarly, failure of the main protein catabolism pathway, known as the Ubiquitin Proteasome Pathway (UPP) leads to A $\beta$  toxicity in AD. Evidence suggested that the ubiquitin levels in the CSF and cortex region of the brain are increased to a multiple fold. This rise in ubiquitin level strongly links with the neurofibrillary alterations in AD brains [45].

A stress response phenomenon, called cellular senescence, resulting in the shortening of the telomere during each cell division process, is a consequence of AD pathology. Shorter telomeres have been found in blood cells of the patient with AD than healthy individuals [46]. In a similar manner, high chance of apoptosis is seen in the CD4+ T-cells and NK cells of AD patients, with greater expression of Bcl 2 in comparison to normal subjects [47]. The damage to the cerebrovasculature, mainly the blood brain barrier and neurovascular unit of the brain outcomes in the less supply of oxygen to the brain. This is the primary cause of ischemic stroke and various neurological dysfunction [48]. The analysis of vasoconstrictor, vasodilator or several cell adhesion molecules [VCAM1, ICAM3, ICAM 1 and E-selectins] may give an enhanced analysis of AD. A progressive decrease in the plasma levels of these cell adhesion molecules have been seen in the AD patients [49]. Therefore, study of biomarkers associated with the damage of cerebrovascular system provides a strong grip in the diagnosis of AD. Micro RNAs that belong to the class of small non-coding RNA molecules are known to play a crucial role in brain disorders. Identifying the regulatory miRNAs in the peripheral blood can help in being a potent source of marker for the diagnosis of dementia and Alzheimer's disease [50].

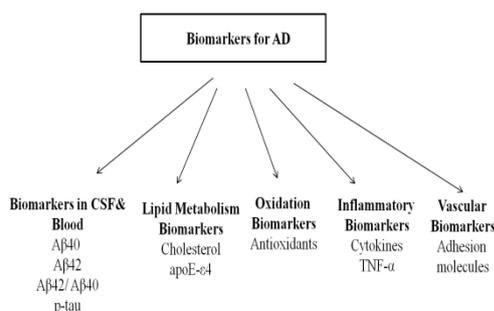


Fig.1. Various Biomarkers used for the early diagnosis of Alzheimer's disease.

### IV. CONCLUSION

AD is a complex disorder causing severe burden to individuals and families. Currently the cure is aimed at relieving distress and providing better life to patients. Moreover, if diagnosed early better treatment options can be

given for the patients. Recent techniques allow us to diagnose AD only after the symptoms become prominent. We hereby propose to give a detailed outline about the various biomarkers which includes biomarkers in blood, CSF and other metabolic biomarkers and their applications in the early detection of AD. Moreover, it gives us a lead to test these biomarkers in future for diagnostic and therapeutic approaches.

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