Review Article

International Journal of Research in Pharmaceutical Sciences

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

# Alzheimer's Disease: Biomarkers And Future Targets For Drug Intervention

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*Article History:*

Received on: 12 Jan 2020
Revised on: 10 Feb 2020
Accepted on: 18 May 2020

*Keywords:*

Alzheimer's disease,
Amyloid beta-peptide,
Biomarkers,
Tau protein

Abstract

Alzheimer's disease is a progressive disorder and the most common types of dementia that develops in the elderly, characterised by gradual memory loss and cognitive skills of an individual. The neurodegenerative disease is characterised by intracellular settling of hyperphosphorylated tau protein like neurofibrillary tangles (NFT) in the neuronal cytoplasm and extracellular settling of beta-amyloid peptide(A$\beta$). These changes lead to cognitive decline due to the loss of synapses and neurons involved in learning, memory and cognitive function. Amyloid precursor protein, presenilin 1, presenilin 2 and TREM2 are some of the genes involved in the development of the disease. The current treatment for AD is the cholinesterase inhibitors such as donepezil, galantamine, rivastigmine, and N-methyl D- aspartate antagonist memantine. Other recommended adjuvant therapy was vitamin D, omega-3 fatty acid, Mediterranean diet, and aerobic exercise. Diagnosis is based upon clinical presentation and imaging biomarkers. This review summarises AD biomarkers such as cerebrospinal fluid biomarkers, circulatory biomarkers, inflammatory markers and oxidative biomarkers which are under study and the future targets of drug intervention. The amyloid-beta peptide, tau protein, and $\beta$-secretase may be the possible sites of drug action in the future.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2563

Production and Hosted by

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## INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder and the most common form of dementia in the elderly. According to the current reports, it is estimated that almost one new case of AD develops every 4 seconds, and the number of people with loss of memory is expected to be about 65—million by 2030 and 115.4 million in 2050 making the disease a global health crisis. The condition is clinically characterised by progressive memory loss and cognitive impairment associated with an impaired performance of normal daily activities, with later deficiency of language related to behavioural disorders like aggressiveness, apathy, and depression (Dillon *et al.*, 2013).

The pathological features are extracellular deposition of beta-amyloid (A$\beta$) plaques and neurofibrillary tangles intracellularly. Both of these are derived from the proteolysis of amyloid precursor protein and hyperphosphorylation of the protein tau, which is associated with microtubules, respectively. Additionally, a massive neuronal loss, mainly in the hippocampus and associated regions of the cortex, is also noted (Zhang *et al.*, 2012). The protein amyloid can also be deposited in the capillary walls, arteries, and arterioles leading to the development of cerebral amyloid angiopathy (Cortes-Canteli *et al.*, 2010). This may lead to degeneration of walls of

blood vessels, worsening of blood flow and also the same predisposes to intraparenchymal haemorrhages.

**Pathophysiology**

The hallmark of AD is the presence of intracellular settling of hyperphosphorylated tau protein like neurofibrillary tangles(NFT) in the neuronal cytoplasm and extracellular settling of beta-amyloid peptide(A$\beta$). The mechanism by which such changes lead to cognitive impairment is still under discussion. This accumulation induces neuronal atrophy and death, an imbalance in calcium homeostasis, inflammation, and exhaustion of neuronal factors. This process ends up in cognitive decline due to damage of synapses and neurons, which includes learning, thinking and other brain functions. The accumulation of A$\beta$ in cerebrum results from an imbalance between its production and clearance.

The A$\beta$ is composed of 36 to 43 amino acids formed by enzymatic proteolysis from the amyloid precursor protein (APP). The APP gene that is found on chromosome 21 in humans also explains why individuals with Down syndrome have a higher rate of early-onset AD. APP gene locus doubling is responsible for an unusual type of advance beginning of AD, which is hereditary. The increased production of cerebral A$\beta$ and hence its deposition is due to overexpression of amyloid precursor protein (APP).

There are two main pathways for the processing of amyloid precursor proteins:

1. Non-amyloidogenic $\alpha$-secretase mediated pathway.
2. Amyloidogenic $\beta$ and $\gamma$-secretase mediated pathway.

**Non-amyloidogenic $\alpha$-secretase mediated pathway**

In this pathway, soluble amyloid precursor protein alpha(sAPP$\alpha$) is formed by the cleaving of amyloid precursor protein by the enzyme alpha-secretase. It has a neuroprotective function and plays an integral part in neuroplasticity and also protects against excitotoxicity.

**Amyloidogenic $\beta$ and $\gamma$-secretase mediated pathway**

In this pathway, soluble amyloid precursor protein beta (sAPP$\beta$) is a mediator. It is responsible for neuronal death and the cleaving of amyloid precursor protein forms a carboxy-terminal complex by $\beta$-secretase, which is later converted to form A$\beta$40 (amyloid-beta 40 amino acid) or A$\beta$42 (amyloid-beta42 amino acid), predominantly A$\beta$40 due to the cleavage by the enzyme $\gamma$-secretase. These pathways co-exist in equilibrium, but the non-amyloidogenic path is being favoured preferentially. The $\gamma$ –secretase is composed of 4 proteins such as presenilin 1 or 2, nicastrin, anterior-pharynx defective- 1, and presenilin enhancer-2. The A$\beta$42 (amyloid-beta peptide containing 42 amino acid) is more likely to aggregate than A$\beta$40 (amyloid-beta peptide containing 40 amino acids). In AD a different form of $\gamma$ –secretase cleave amyloid precursor protein at an incorrect place yielding A$\beta$42. Immunohistochemical analysis revealed that A$\beta$42 is initially accumulated in the amyloid plaques of AD patients at high amounts. Many other reports also support this fact. But the relationship between serum A$\beta$42 levels and cerebral amyloidosis is still not elucidated, and there is a drop in A$\beta$42 levels in the cerebrospinal fluid of AD patients, which may be due to its higher amyloid plaque deposition.

The relative rise in A$\beta$42 peptide levels in early-onset family AD variants is due to mutations of the genes APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2). Under physiological conditions, A$\beta$ peptides are formed in the form of monomeric forms that have a protective function. But an accumulation of this protein creates oligomeric products (dimers, trimers, tetramers) which then form fibrils and then accumulate in senile plaques. Also, these oligomeric products interact with the cell membrane, their receptors and interfere with the intracellular process of a neuron, resulting in degeneration and neuronal toxicity. They interfere with the normal functioning of cholinergic, serotonergic and dopaminergic neurons. Thereby a reduction in control over the amyloidogenic pathway is achieved.

The exact mechanism by which the formation of the neurofibrillary tangles of hyperphosphorylated tau proteins associated with AD is not known. But Blurton-Jones et al. (Blurton-Jones *et al.*, 2009) put forward specific mechanisms. They are

1. The A$\beta$ peptide activates specific kinases The A$\beta$ peptide activates specific kinases glycogen synthase kinase three betas (GSK3$\beta$) that catalyse the hyperphosphorylation of tau proteins resulting in the formation of NFT.
2. Accumulation of A$\beta$ peptide The collection of A$\beta$ peptide initiates neuroinflammation. As a result, pro-inflammatory mediators are released, which triggers tau protein phosphorylation.

**Table 1: Investigational Anti Alzheimer's Drugs with Their Results Under Study**

| Target | Drug | Study Phase | Expected completion date |
|---|---|---|---|
| $B$-Amyloid | CAD106 | 2 | May 2024 |
| | CNP520 | 2 | May 2024 |
| | LY3002813 | 2 | December 2020 |
| | Crenezumab | 3 | October 2022 |
| | Aducanumab | 3 | April 2022 |
| | Gantenerumab | 3 | November 2019 |
| | CT1812 | 2 | Completed October 2016 |
| | Thiethylperazine | 2 | July 2021 |
| | ABvac40 | 2 | February 2021 |
| | ACC-001 | 2 | completed February 2014 |
| | KHK6640 | 1 | Completed December 2017 |
| | UB-311 | 1 | Completed |
| | ABvac40 | 1 | Completed July 2015 |
| BACE 1 | JNJ-54861911 | 2 | October 2022 |
| | Elenbecestat | 3 | December 2020 |
| | LY3202626 | 2 | December 2020 |
| | Verebecestat | 3 | March2021 |
| P-tau | IONIS-MAPTRx | 1,2 | February 2020 |
| | RO7105705 | 2 | September2022 |
| | BIIB-092 | 2 | September 2020 |
| | BIIB-080 | 1 | February 2020 |
| | TPI-287 | 1 | Completed May 2017 |
| Retinoid receptor | Acitretin | 2 | Completed February 2018 |

3. Reduced clearance of tau proteins by the proteasome
4. DepositionofA$\beta$ A$\beta$ deposition interferes with axonal transport leading to inadequate tau protein distribution and its mRNA results in hyperphosphorylation and NFT formation.

Tau protein is a microtubule-associated protein that is involved in stabilising microtubule tubulin polymerisation and the mechanism of intracellular transport. Once hyperphosphorylated, the protein loses its function resulting in neuronal damage and hence cytotoxicity. Tau protein is formed by alternate splicing of the MAPT. The histopathological analysis shows that the cognitive dysfunction in AD patients is mainly due to the distribution of NFT.

**Genetic Factors**

Early-onset AD below 65 years of age accounts for 4-6% of AD cases and Late-onset AD seen in individuals above 65 years of age. They also differ in neuroimaging, neuropathological and neuropsychological parameters.

According to a researcher, (Ballard *et al.*, 2011), the early onset of AD is due to the mutations in the gene APP, PSEN1 and PSEN2 and the late form AD-related with a polymorphism in gene apolipoprotein E- a lipid transport protein (ApoE) especially with E4 allele (occurs in three alleles E2, E3, E4). This genetic mutation accounts for 70% of AD. Over 30 significant mutations have already been identified in the APP gene (located in chromosome 21q21), which is responsible for 15 per cent premature-onset AD events. Genetic variations in the PSEN1 gene (14q24.3) and the PSEN2 gene (1q31-q42) account for 80% and 5% of early AD events.

These gene mutations increase A$\beta$42: A$\beta$40 ratio due to increased expression of A$\beta$42 or a decrease in A$\beta$40 or both. This dysregulation supports early A$\beta$ deposition and promotes amyloidogenic cascade. Other researchers (Campion *et al.*, 1999) illustrate there are many other genes responsible for early-onset AD. ApoE is a lipid transport protein that mediates the movement of cholesterol between cells, which is encoded by the ApoE gene. There are 3 ApoE alleles denoted as E2, E3, E4 which give rise to ApolipoprotienE2(ApoE2), ApolipoprotienE3(ApoE3), Apolipoprotein E4 (ApoE4) iso-

forms respectively. They are present in population in the following proportion E2 5-10 %, E3 65-70 %, E 4 15-20% respectively.

The mechanism by which the ApoE promotes amyloidogenesis is not clearly understood. Still, it has been found that the ApoE2 and ApoE3 improve the clearance of A$\beta$ peptide and hence they have a neuroprotective effect while ApoE4 bind to A$\beta$ peptide promotes its polymerisation and form fibrils. Therefore the deposition causes a neurotoxic effect. Moreover, the protease induced ApoE fragments favour the deposition and produce neuronal injury.

Currently, it was observed that an alteration in the triggering receptors expressed on myeloid cells 2 (TREM2) gene, which is located on the chromosome 6p21 enhances the chance of developing AD by 2.9%. The mechanism underlying this is yet to be clarified.

TREM2 gene responsible for the expression of TREM2 protein is a receptor on the surface of the microglia. Microglia are present in the central nervous system as phagocytic cells. They are activated through the TREM2, and DNAX-activating protein of 12kDa (DAP12) receptors cause the release of chemokines such as C-C motif chemokine ligand 19 (CCL19), C-C- motif chemokine ligand 21 (CCL21) and initiate phagocytosis. The phagocytic capacity of microglia was impaired in knockout models of TREM2 receptors (Mecca *et al.*, 2018). Thus the timely clearance of A$\beta$ peptide deposit is reduced in microglial cells which are deficient in TREM2 receptors favour amyloid plaque deposition (Xiang *et al.*, 2016).

**Biomarkers**

The preliminary diagnosis of AD is made by neurological examination, mental status tests, and brain images. But these tests are difficult to perform in an AD patient who is in an early stage of AD. This is the reason behind the evolution of biomarkers. With an efficient biomarker, effective therapy can be started in the early stage itself and can delay cognitive impairment.

Biomarkers are substances present either inside or outside of the human body, which can influence the occurrence of a disease in the human body. The different established biomarkers for AD are as follows.

**Cerebrospinal fluid Biomarkers**

Since the cerebrospinal fluid is in close contact with the brain and spinal cord, it can have various biochemical and metabolic brain profiles. The different biomarkers determined from the cerebrospinal fluid are A$\beta$ and tau proteins, and phospho-tau expression levels. But this technique is invasive and painful to the patient because the fluid is obtained by lumbar puncture. The sensitivity and the specificity of these tests are more than 95% and 85% respectively.

**Amyloid beta-peptide (A$\beta$42 )**

The concentration of A$\beta$ in the cerebrospinal fluid of AD patients is found to be less than 500 pg/ml (pictograms per millilitre) when compared to healthy patients with 794 $\pm$20 pg/ml of A$\beta$. This may be due to the aggregation of A$\beta$.

**Tau protein**

It is a useful prognostic biomarker for AD. Its concentration gradually increases with age about less than 300 pg/ml (21 to 50 years) and almost less than 500 pg/ml (greater than 71 years). Still, an exponentially high concentration is observed on AD patients of age 51 to 70 years as between 450 and 600 pg/ml respectively.

**Phosphorylated Tau protein**

The Tau protein a specific biomarker of AD which is phosphorylated in around 39 possible sites at position 181. The other distinguished sites phosphorylated are 199, 231, 235, 396 and 400.

**Circulatory biomarkers**

The main advantage of using blood for the diagnosis of AD is that it is readily available, and the follow-up of the patient is possible.

**Circulatory micro ribonucleic acids (miRNA)**

A miRNA is a small non-coding RNA molecule with about 22 nucleotides that help in RNA silencing (RNAi or RNA interference) and gene expression transcription regulation. The dysfunction of miRNA expression in the peripheral blood is a crucial factor in the treatment of AD and brain-related diseases. Schipper et al. (Schipper *et al.*, 2007) studied and reported the expression of down-regulated miRNAs in various samples like peripheral blood, plasma, serum, cerebrospinal fluids, temporal cortex, hippocampus, and extracellular fluid.

This fact was further confirmed by Geekiyanageet.al (Geekiyanage *et al.*, 2012). Another study revealed that downregulation of miRNA 296 and 15a regulate transcription factor Squamosa promoter binding protein-like (Spl) which in turn controls the expression of APP and tau (Bekris *et al.*, 2013; Koyama *et al.*, 2012).

Researchers also suggest that miRNAs are involved in redox reactions and DNA repair mechanisms of cellular functions (Villa *et al.*, 2013). All these conclusions recommend their potential as future therapeutic biomarkers of AD.

**Amyloid markers**

Perez et al. (Pérez *et al.*, 2012) found that significant variation in the ratio of free and cell-bound A$\beta$42 levels in the blood of mild cognitive impairment patients versus age-matched control groups. This indicates the plasma level of A$\beta$ is a precise and accurate biomarker for the diagnosis of AD.

**Inflammatory markers**

Studies showed that neuroinflammation plays a crucial role in the neurodegeneration associated with AD. Several studies were conducted to find out the relation between AD and inflammatory mediators. According to another study, it was observed that the Tumor necrosis factor receptor 1 could be a dominant inflammatory biomarker for a better understanding of AD patients (Laske *et al.*, 2013).

Since vascular injury is also observed in AD and some of the biomarkers of microvascular injury determined are vascular cell adhesion molecule -1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and selectins, higher levels of these substances were observed in the plasma of late-onset of AD patients suggestive of endothelial dysfunction. In another study of AD patients versus controls showed an increase in B-cell lymphoma 2 (Bcl-2), caspases, antioxidant enzyme superoxide dismutase level and enhanced apoptosis of cluster of differentiation 4 types of cell (CD4+ T) and natural killer (NK) cells.

Ceramides, sphingomyelins, and sulfatides play a pivotal role in the functioning of neurons and the synthesis of bioactive metabolites involved in AD. The serum levels of ceramides were altered in another study conducted using mild cognitive impairment patients, AD patients, and their respective controls. Increased levels of ceramides have found to reduce hippocampal volume. This may lead to cognitive impairment in the normal brain.

**Biomarkers of oxidative stress**

Oxidative stress is also a significant cause of AD. Reactive oxygen species level is high in the degenerative parts of the brain of AD patients. This highly reactive oxygen species causes nitration of tyrosine residues, post-translation of proteins and lipid peroxidation. Significant biomarkers of oxidative stress also include DNA oxidation, free fatty acids, iso, and neuro prostane formation, 4 - hydroxy 2 trans nonenal (HNF), lipid peroxidation, protein glutathionylation, and advanced glycation end products (Sharma and Singh).

**Current Treatment**

The currently recommended therapy for AD are

**Cholinesterase inhibitors**

Donepezil, rivastigmine, and galantamine were the commonly used cholinesterase inhibitors for mild, moderate and severe cases of dementia.

**N-methyl D-aspartate antagonist**

Memantine used in moderate to severe cases of AD.

**Vitamin D**

Vitamin D therapy is recommended in patients with deficiency of this Vitamin, as it was found to be a risk factor to dementia.

**Omega - 3 fatty acid**

Omega - 3 fatty acid supplements have shown improvement in thinking and memory in mild cognitively impaired patients in a randomized, controlled, double-blind study involving a small sample size.

**Mediterranean diets**

Mediterranean diets such as whole grain, olive oil, legumes and seafood with restriction of red meat, sweets, dairy and poultry products, and processed foods have decreased dementia in AD patients.

**Aerobic exercise**

Regular aerobic exercise is found to preserve the cognitive function of AD patients with genetic risk factors when compared with the control group. This reflects the protective effect of regular exercise against neurodegeneration. But further research is required to identify the long term effects of physical work.

**Future Treatment**

Future treatment for AD now focused on the phosphorylated tau proteins of NFT and A$\beta$ of senile plaques. But still, controversy exists regarding the best target to slow cognitive decline and how fast the treatment should be initiated.

Another approach is to enhance cognitive function to strengthen the transcortical networks and build up interneuronal connections. According to several studies the best approach is to slow or interrupt the progression of AD is early detection followed by successive therapy in the preclinical phase itself.

**$\beta$- secretase inhibitors**

Another target site is $\beta$ secretase ($\beta$ site amyloid precursor protein cleaving enzyme 1 or BACE 1) which can cleave amyloid precursor protein at the $\beta$ site leading to the formation of $\beta$ amyloid peptide. Verubecestat found to be $\beta$–secretase inhibitor, in a study with rodents and primates, showed a 40-fold reduction in the amyloid-beta level and proved good safety profile in the early stages of clinical trials.

In another study with transgenic mice in 2014, the combination of a monoclonal antibody with $\beta$–secretase inhibitor significantly reduced the amyloid $\beta$ level. Many scientists suggest that this combination therapy may be a grand success in the future treatment of AD patients (Jacobsen *et al.*, 2014).

**Anti- tau**

A different target of concern is phosphorylated tau because tau also plays a major role in the development of AD. There are many tau vaccines understudy that showed safety and efficacy in animal studies. An anti-tau drug in a recent small study involving human subjects imparted positive immune response and given a good safety profile. Several other drugs target tau proteins are under clinical trial but their results are yet to be published. Table 1 shows some of the investigational anti-Alzheimer's drugs with their results under study.

**Neural Circuitry**

In a large scale clinical study, it was concluded that the overall neuronal network dysfunction is the main cause of the progression of clinical symptoms of AD. Another advance in the treatment of AD is with the application of gamma frequency oscillation. Gamma waves, a high-frequency brain wave rhythm concerned with interneuronal communication in the brain and may help to distinguish true and false memories. A study conducted at Massachusetts Institute of technology using a mouse model of AD, in which the desired frequency of gamma radiation was applied to the mouse cortex by using a non - invasive 40-hertz photic stimulator. The treatment decreased A$\beta$ deposition and improved cognitive function which may be due to improvement in interneuronal communication (Iaccarino *et al.*, 2016). Currently, this is also in the early phases of clinical trials employing visual and auditory stimulation.

## CONCLUSIONS

Alzheimer's disease is a great challenge for medicine and the country in the upcoming years. Neuropathological and physiological reasons for the development of the disease are currently being investigated. Rigorous studies have been conducted in this area but still, there is much to be learned. To date, the biomarkers for AD have been amyloid-beta, tau protein, and phosphor tau. The advancement of knowledge in genomics, proteomics and system biology nowadays, several novel blood-based biomarkers especially circulatory miRNA and inflammatory biomarkers which are being developed for better diagnosis, However, they should be validated for proper diagnosis, detection, monitoring of AD progression and estimation of therapeutic relevance.

To improve memory and alertness without altering the progression of the disease medications like memantine and cholinesterase inhibitors can be given. Hence, the treatment option remains supportive and symptomatic without attenuation of the ultimate prognosis. The pathological features associated with AD, A$\beta$, $\beta$-secretase, and phosphorylated tau are the current targets for potential treatment. But the early success in a comparative study in small scale clinical trials are not reproducible in large scale studies. The rising prevalence and mortality of AD along with the growing total healthcare cost makes it a matter of urgency to develop effective means for the diagnosis and successful treatment of this progressive neurodegenerative disease.

## ACKNOWLEDGEMENT

The authors are acknowledged to Vinayaka Mission's Research Foundation (Deemed to be University) for providing the facilities for this research.

**Funding Support:**None

**Conflict Of Interest**

All the authors declare that there is no conflict of interest.

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