1. INTRODUCTION

1.1. Alzheimer's disease

Dementia is a general term used for diseases and conditions characterized by decline in memory or other related skills that affect the ability of a person to carry out day-to-day activities.[100] Alzheimer's disease (AD) is the most common type of dementia. It accounts for 60% to 80% cases of dementia. In 1907, Dr. Alois Alzheimer, a German scientist, reported a neurodegenerative disease characterized by decreased cognitive ability and severe behavioural abnormalities like disorientation, depression, restlessness, irritability and anxiety. AD is an irresistible neurodegenerative disorder characterized by progressive and permanent decline in cognitive functions. It affects people in different ways. Common symptoms include memory loss, difficulty in planning and solving problems, confusion with time and place, difficulty in understanding of visual images or speaking new words etc.[101]

The most common symptoms of AD begin with slowly worsening condition wherein there occurs difficulty in memorizing new things. This is because of disruption of brain cell functions mainly in the regions that are involved in forming new memories. As the neuronal damage spreads, individuals experience other difficulties. Following are the significant signs of AD[102]:

- Memory loss that disturbs daily life.
- Confusion with time or place.
- Trouble in completing familiar tasks at home, at work or during relaxation.
- Challenges in forecasting or resolving problems.
- Difficulty in understanding visual images and spatial connections.
- Different problems with words in talking or writing.
- Misplacing belongings and losing the capability to review steps.
- Poor judgment.
- Withdrawing oneself from work or social events.
- Fluctuations in mood and personality.

A person suffering with AD can survive for an average of four to eight years, while a few may survive as long as 20 years with the AD. According to WHO, worldwide 35.6 million people have dementia, with just over half (58%) of them living in low- and middle-income countries.

1.2. Pathophysiology

Alzheimer's disease is completely related to neuronal death or damage and the proposed causes/hypotheses for the same are as follows,

Cholinergic hypothesis

The emerging role of acetylcholine (ACh) in memory and learning led to the "cholinergic hypothesis of Alzheimer's disease". Consequently it was suggested that degeneration of cholinergic neurons inside basal forebrain and consequent cholinergic neurotransmission loss in the cerebral cortex and different zones contributed essentially to the decay in psychological capacity seen in patients with AD (**Figure 1**).[103]

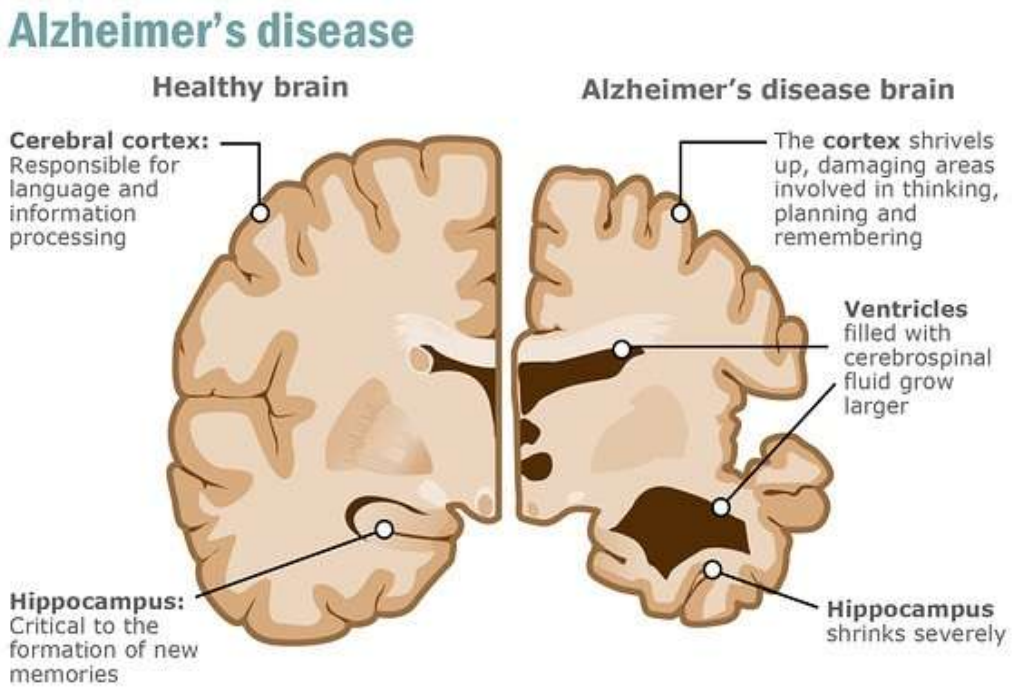

Figure 1: Effect of AD on various regions of brain

Presently, majority of the therapeutic treatments for AD are aimed to inhibit acetylcholinesterase (AChE) to enhance acetylcholine (ACh) levels in brain. AChE inhibitors like tacrine, donepezil, rivastigmine and NMDA receptor antagonist like memantine are currently used for AD treatment. Literature supports the evidence of gradual fall in the levels of AChE in the brain of AD patients, while there occurs a slight increase in the activity of butyrylcholinesterase (BuChE). Further, postmortem tissue analysis of AD patients showed a high level of BuChE in the hallmark lesions of AD. In rats, selective BuChE inhibitor cymserine was found to elevate ACh levels and enhanced long-term CNS potentiation and learning. Therefore both these enzymes emerge as appropriate targets for the development of cholinesterase inhibitors for the treatment of AD. Although AChE and BuChE are produced by different genes they are highly homologous with more than 65% similarity. AChE has two major binding sub-sites, a peripheral anionic site (PAS) and the other a catalytic anionic site (CAS) which is located in the deep gorge of the enzyme structure and is assigned to Ser-His-Glu catalytic triad.[104,105]

Protein misfolding and aggregation

Incorrect protein folding with abnormal conformation can lead to large insoluble protein aggregates. For majority of the proteins, the important structural pattern of the functional part of the protein is its native conformation which is the alpha helix. A protein becomes toxic when major conformational change occurs and it acquires a pattern recognized as the beta sheet. This transition from alpha helix to beta sheet is typical for amyloid deposits. This abnormal conformational change from alpha helix to beta sheet exposes the hydrophobic amino acids and this leads to protein aggregation. As per the amyloid hypothesis and the Tau protein hypothesis protein aggregation is the most acceptable cause of AD.

These two microscopic features responsible for the AD, cause extracellular deposits of amyloid plaques which are amorphous deposits of β-amyloid (Aβ) protein and intraneuronal neurofibrillary tangles which are the filaments of phosphorylated microtubule-associated protein i.e. Tau.

The amyloid deposits are aggregates of Aβ, which is a 40 or 42 unit segment of amyloid precursor protein (APP), produced by the action of specific enzymes called secretases. APP is a 720 amino acid membrane protein. This exists in many cells including CNS neurons. The

cleavage due to α-secretase releases the large extracellular APP domain which is the soluble form and serves various physiological functions. For the formation of Aβ plaques the responsible enzymes are β- and γ-secretases. β-Secretase initiates the APP breaking into intramembrane domain, whereas γ-secretase lacks precision and cuts the APP at different points in the transmembrane domain generating Aβ fragments of various lengths (**Figure 2**). The 40 and 42 unit deposits of Aβ are observed wherein the 42 unit shows a stronger tendency, and observed to be the main culprit in the amyloid formation than 40 unit Aβ deposits. The pathological accumulation of Aβ in the brain leads to decrease in neurotransmission, oxidative stress, neuronal destruction and at the end of clinical symptoms of AD.[106]

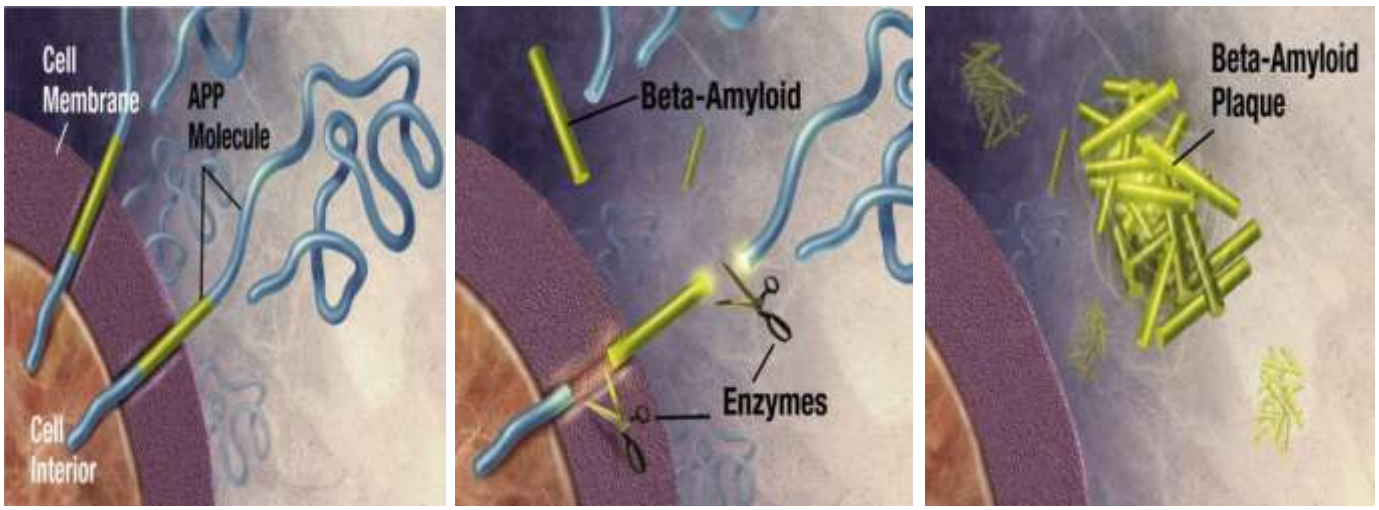

Figure 2: Amyloid hypothesis explaining the origin of AD.

The second main player of protein misfolding is the Tau protein which is the structural part of microtubules (**Figure 3**). Normally it is associated with intracellular microtubules and serves as transportation track for materials along the axons of the neurons. In AD this Tau protein gets phosphorylated by various kinases and dissociates from microtubules to form paired helical filaments intracellularly. When cell death occurs these filaments aggregate externally as extracellular neurofibrillary tangles. The phosphorylation of Tau is also enhanced by Aβ, may be because of over activation of kinases, and the hyper-phosphorylated Tau favours the formation of amyloid deposits.[107]

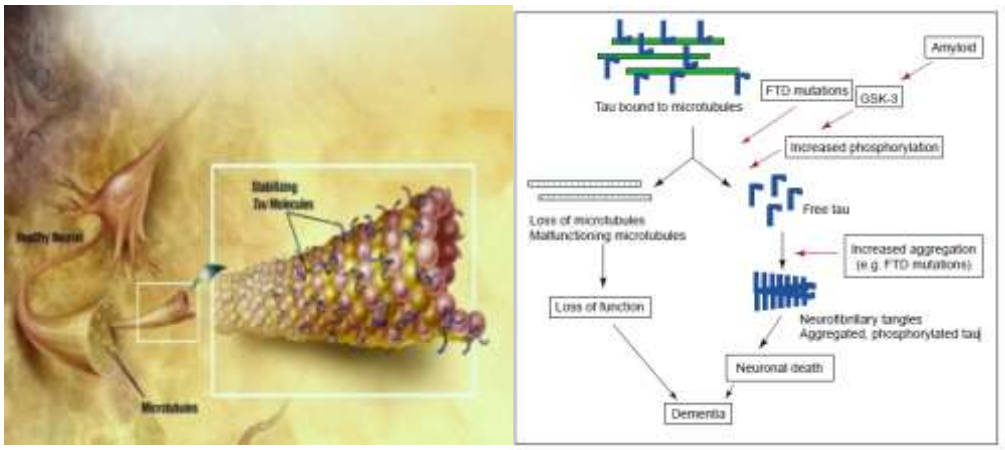

Figure 3: Tau hypothesis for AD

Apart from the cholinergic, Aβ and Tau hypotheses, the other factors responsible for AD include excitotoxicity, apoptosis and oxidative stress.

Excitotoxicity is nothing but the ability of glutamate or related excitatory amino acids to cause the neuronal cell death. This occurs because of over activation of the receptors for glutamate such as, NMDA, AMPA etc which get overactivated by the glutamatergic storm. Excitotoxins like NMDA binds to these receptors as well as the excessive levels of the glutamate pathologically can cause excitotoxicity by permitting the entry of high levels of calcium ions (Ca^{++}) into the cell. This Ca^{++} influx into the cells activates a number of enzymes, including endonucleases, phospholipases and proteases. These enzymes damage the cell components of the cytoskeleton, membrane and DNA.

Apoptosis is genetically planned cell suicide with systemic destruction and no inflammatory response. The recognition of precise genetic and environmental factors accountable for this disease has boosted evidence for a common pathway of neuronal death involving perturbed calcium homeostasis, oxidative stress, mitochondrial dysfunction and activation of cysteine proteases known as caspases. Increase in the levels of oxyradicals and Ca^{++}, generation of Par-4 and translocation of pro-apoptotic Bcl-2 family members (Bax and Bad) to the mitochondrial membrane are observed in the initial stage of apoptosis mainly due to the activation of intracellular cascade of events by the death signals. Certain caspases (caspase-3, -8, -9 for example) also act near the beginning in the cell death process before, or independently of, mitochondrial changes. At the end, the nuclear chromatin becomes condensed and fragmented.[108]

Recent research has highlighted the important role of oxidative stress in the fundamental molecular mechanism of AD. Oxidative damage mainly involves lipid peroxidation from reactive oxygen species derived from metabolism. Oxidative stress occurs when there is an inequity between the formation and quenching of free radicals from oxygen species. These reactive oxygen species (ROS) play an important role in numerous chronic diseases including neurodegenerative diseases. Through pathological reduction-oxidation steps, ROS can denature biomolecules like proteins, lipids and nucleic acids. This can commence tissue damage through necrosis and apoptosis. Thus, oxidative stress plays a central role in the pathogenesis of AD leading to neuronal dysfunction and cell death.[108]

Considering the fact that inhibition of AChE provides symptomatic treatment of AD, and increased levels of BuChE are observed in the brains of AD patients, it was planned to design and evaluate some novel heterocyclic derivatives against animal models of AD.