Chapter 1

Introduction

1.1 Neurodegeneration

Neurodegeneration is an umbrella term for the continuous loss of structure or capacity of the neurons, leading to neuronal death. There are several analogies among the various neurodegenerative disorders including atypical protein aggregations in addition to induction of cell death. Neurodegeneration can be found at several levels of the neuronal networks going from molecular to systemic[1].

The cognitive deficits are observed in several of the other neurodegenerative conditions such as frontotemporal dementia (FTD), vascular dementia, mixed dementia, and dementia with Lewy bodies (LBD) in addition to Alzheimer's disease (AD). Similarly, amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and spinocerebellar ataxias (SCAs) all have an impact on the motor system. Since, aging is a shared risk factor for all of these diseases, the incidence of these dreadful diseases is expected to increase as life expectancy is increasing globally, placing a heavy socioeconomic cost on individuals, families, and communities[2].

Despite their diverse clinical manifestations, reflecting loss of specific neurons and synapses in distinct brain regions, neurodegenerative diseases share common features and mechanisms. Regional aggregation of cytosolic or nuclear proteins is an important characteristic of these disorders. Inclusions of TAR DNA-binding protein (TDP)-43 in ALS and FTD, beta-amyloid (Aβ) plaques and hyperphosphorylated microtubule-binding tau in AD and other tauopathies, aggregates of α-synuclein in PD and other synucleinopathies, and polyglutamine protein aggregates in HD and other CAG-polyglutamine repeat diseases. Additionally, certain aggregates seed and disperse from one area to another, supporting the progression of neurodegenerative diseases[3].

1.2 Parkinson's disease

Parkinson Disease (PD) is the most common neurodegenerative movement disorder. Approximately 1% of the population older than 65 years suffers from this slowly progressive neurodegenerative disease; 95% of PD cases are sporadic. Roughly 1% of the populace past 65 years in age experiences this gradually debilitating neurodegenerative disorder. The symptoms of PD are caused by selective and progressive degeneration of pigmented dopaminergic (DA) neurons in the substantia nigra pars compacta. Existing treatments, such as carbidopa/levodopa, dopamine agonists, monoamine oxidase inhibitors and anticholinergics generally help reduce muscle rigidity, improve speed and coordination of movement and lessen tremor. PD is the most widely recognized neurodegenerative development issue [4].

1.3 Amyotrophic lateral sclerosis

Jean-Martin Charcot first described Amyotrophic lateral sclerosis (ALS) in the 1870s as an age-related disorder that prompts degeneration of motor neurons. The ailment starts in the central nervous system and thereafter spreads gradually throughout the body. ALS, also known as Lou Gehrig's disease, is a complex genetic disorder. It tends to shorten the life expectancy to around 3 years subsequent to the onset of side effects, yet the scope for survival stretches out from a couple of months for some, to decades for just about 5% of patients.

ALS starts with weakness of the limbs in around 65% of the patients. Early symptoms include foot drop, troubled walking, loss of hand dexterity or shoulder weakness. With time, a few patients progress toward becoming anarthric. Gulping issues can prompt drooling, dehydration, hunger with weight reduction and aspiration. Sphincter and sensory function are often spared. Cognition is impaired in 25–50% of patients with around 15% of the patients developing evident dementia.[5].

Elevated levels of glutamate, an excitatory neurotransmitter, is often associated with neurodegeneration. Riluzole, designed to be an antiepileptic drug (AED), showed an inhibition

of presynaptic glutamate release. Although, it's precise mechanism in ALS is unclear. Riluzole is at present the main medication endorsed to moderate the course of ALS [6]. The U.S. Food and Drug Administration recently approved Radicava (edaravone) to treat patients with ALS.

1.4 Huntington's disease

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder brought about by a CAG expansion in the HTT gene described by a strikingly selective cell loss and atrophy in the caudate and putamen manifesting as motor symptoms, behavioral and cognitive impairments. Striatal medium spiny neurons are the most susceptible in HD.

The disease is pathologically characterized by the presence of nuclear and cytoplasmic inclusions, that contain mutant huntingtin and polyglutamine in addition to the neuropathological altered nerve fibres, neurofilaments, tubulin, and microtubule-associated protein 2 and diminished complexin 2 concentrations. [7] Indications of Huntington's infection for the most part begin between ages 30 and 50. However, the symptoms could start by age 2 or as late as 80. The characteristic manifestation of HD is uncontrolled movement of the arms, legs, head, face and upper body. HD likewise causes a decrease in thinking and reasoning skills, including memory, focus, judgment and the capacity to arrange and plan. It also causes changes in the brain which lead to variations in mood, leading to depression, anxiety, and uncharacteristic anger and irritability. Another common symptom is obsessive-compulsive behavior, leading a person to repeat the same question or activity over and over. Symptomatic treatment with Antichoreic drugs such as tetrabenezine or neuroleptics offer some respite to patients with severe chorea which comprises persistent involuntary movements.

1.5 Spinocerebellar ataxia

Spinocerebellar ataxia (SCA) is a cluster (SCA 1,2,3,6,7,10,11) of genetic disorders characterized by progressive movement related issues and lack of co-ordination. It is also associated with complications in speech and swallowing, spasticity and muscle weakness,

ophthalmoplegia. SCA causes quick involuntary eye movements called nystagmus due to ophthalmic muscle weakness. People with SCA1 also experience cognitive impairment. Although, there is no cure or treatment available for SCA, the use of adaptive devices allows the ataxic individuals to attain some independence.

1.6 Spinal muscular atrophy

Spinal muscular atrophy (SMA), an autosomal recessive disorder, is one of the foremost genetic causes of infant deaths. SMA manifests itself through motor neuron degeneration in the spinal cord as a result of the homozygous disruption (by mutation or deletion) of the disease-causing gene—survival motor neuron 1 (*SMN1*) [8].

1.7 Dementia

Dementia is defined by chronic, acquired loss of two or more cognitive abilities caused by brain disease or injury. Memory requires the recording, storage, and retrieval of information. Dementia is typically manifested as a gradually developing loss of memory worsening over time, frequently accompanied by a lack of capacity to acquire new knowledge, especially autobiographical information such as recent events in one's life. .

It is a widespread public health concern and by 2050, there will be 131 million dementia sufferers worldwide, up from the current estimate of 47 million.

1.7.1 Frontotemporal dementia

Frontotemporal dementia (FTD) is a neurocognitive syndrome which is a collection of progressive behavioural dysfunctions, with diminished language and executive functioning. It is the second most frequent cause of dementia after AD. There is currently no approved disease modifying medication for the condition; nonetheless, asymptomatic medications such selective serotonin reuptake inhibitors and atypical antipsychotic medications are often utilised to manage the syndrome.

1.7.2 Dementia with Lewy bodies and Parkinson's Dementia

Dementia with Lewy bodies is a widespread dementia, whereas it develops in up to 80% of the people afflicted with Parkinson's disease. In community-based research, the average prevalence probability of dementia with Lewy bodies was 4.2%, but the prevalence rate in clinic-based studies was 7.5 %.

Lewy bodies and Lewy neurites, followed by neuronal loss, are the hallmarks of Lewy body dementias. Although, some people exhibited significant α-synuclein pathology at autopsy they were devoid of clinical symptoms of Lewy body dementia, making it unclear whether Lewy bodies and Lewy neurites have a neuroprotective or neurotoxic role and to what degree they contribute to the clinical picture.

Braak et. al., proposed that the Parkinson's disease presented pathological similarity with Lewy body pathology, with Lewy bodies beginning in the dorsal IX/X motor nucleus or neighbouring intermediate reticular zone, spreading rostrally into the brainstem (substantia nigra, basal ganglia), then to the limbic system, and finally reaching the neocortex.

The strongest possible identifier for Parkinson's disease dementia is the presence of cortical α-synuclein, whereas amyloid β plays a bigger part in dementia with Lewy bodies. However, the presence of amyloid β pathology and cortical tau neurofibrillary tangles, results in an advanced form of dementia within in the Parkinson's disease dementia, suggesting that the two illnesses collaborate. Rare autosomal dominant inherited mutations in the SNCA and LRRK2 genes has been observed in some cases, although majority of Lewy body dementia cases tend to be sporadic.

Lewy body dementias include visual-spatial and executive impairments along with changes in cognition and arousal. Memory loss does not disqualify the diagnosis of dementia with Lewy bodies. Individuals with pure Lewy body disease had identical free recall abilities but better

delayed recognition memory compared to those with pure AD or mixed pathology, indicating defective retrieval processes.

Rivastigmine, an acetylcholinesterase inhibitor, had a modestly positive impact on cognition, neuropsychiatric symptoms, and daily activities in PD dementia patients, however, donepezil showed inconsistent effects in randomised controlled study.

1.7.3 Alzheimer's disease

AD is acknowledged as a progressive multi-factorial neurodegenerative disorder leading to progressive decline in functional, mental and behavioral abilities. The disease progresses symptomatically from mild to severe and is placed among the top contributors of death worldwide.

1.7.3.1 Diagnosis

The diagnosis of AD went from being a purely pathological one, during the days of Alois Alzheimer (1864–1915), to a clinical and more exclusionary approach in 1984.

The diagnosis of AD was initially limited to the stage of dementia, a clinical illness marked by significant progressive cognitive impairment involving numerous domains, or neurobehavioral symptoms severe enough to impede everyday functioning. The major characteristic that distinguishes dementia from moderate cognitive impairment is the need for dependency in a person suffering from dementia.

The Amyloid/Tau/Neurodegeneration (ATN) framework clearly pave the way for a diagnosis before the stage of Alzheimer's disease-associated dementia, and it makes individualized risk-profiling for patients with mild cognitive impairment feasible.

1.7.3.2 The Pathological hypotheses of AD

1.7.3.2.1 (i) Aβ hypothesis

According to the Aβ hypothesis, Aβ accumulation in the cerebral region occurs mainly through increased Aβ production, $A\beta_{42}/A\beta_{40}$ ratio, Aβ deposition, and decreased Aβ clearance leading to the senile plaque formation and deposition followed by a complex cascade of events such as inflammatory responses, microglia activation, cytokine release, and astrocytosis. Aβ oligomers alter neuronal activity by impairing synaptic functioning and associated signalling pathways [9].

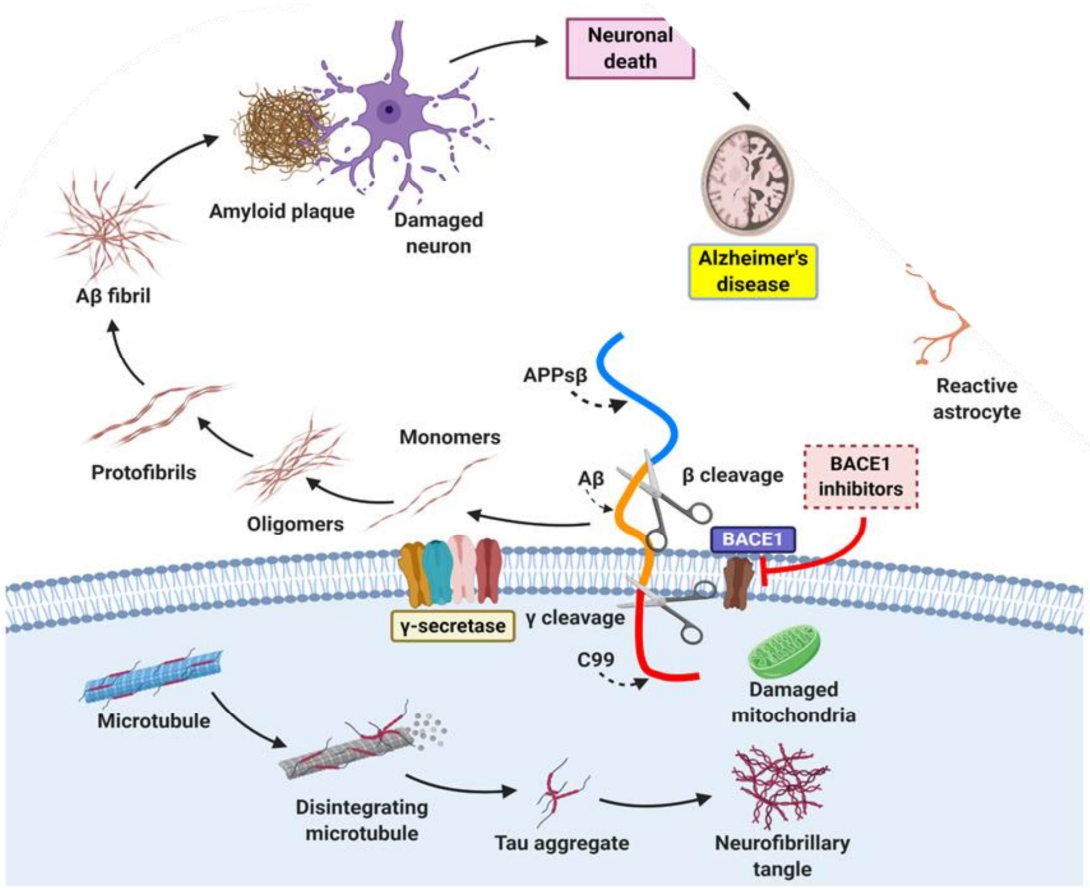

Figure 1.1 Various pathological markers of AD

The lipid transport protein ApoE4 promotes Aβ accumulation and hinders Aβ clearance. metabolic byproducts, cause microinfarcts, and promote the activation of glial cells. Amyloid precursor protein (APP), a transmembrane protein, is cleaved into smaller fragments by the

action of proteolytic enzymes. The β-secretase enzyme breaks down APP to create APP-β along with the C99 β-subunit; also, in the presence of γ-secretase, the β-subunit produces $A\beta_{42}$, which accumulates extracellularly and aids in the formation of harmful Aβ plaques. As opposed to $A\beta_{42}$, which has a very high propensity to aggregate and produce amyloid plaques and deposits, other forms of Aβ, including monomers, dimers, higher oligomers, and fibrillar polymer ($A\beta_{40}$), are less accumulative [10].

Altogether, $A\beta_{42}$ and its various formations are responsible for progressive neuritic injury, neuronal deficits, and cognitive dysfunctions. Hence, preventing the deposition and clearance of Aβ is the most prominent therapeutic approach for managing AD. Furthermore, preventing oligomerization of Aß monomer also prevents aggregation of Aß, which further protects neurons from damage and works as a potential target of AD (**Figure 1.1**)[11].

1.7.3.2.2 (ii) Tau hypothesis

The tau hypothesis involves tau protein, a highly soluble microtubule-associated protein (MAP) that remains phosphorylated in the axonal membrane and stabilizes microtubule assembly by interacting with tubulin in normal conditions [12]. However, activation of the MAP kinase (MAPK) due to the overproduction of inflammatory mediators activates the cyclin-dependent kinase-5 (CDK-5), which leads to tau phosphorylation. Tau protein hyperphosphorylation leads to the formation of insoluble intracellular NFTs (neurofibrillary tangles), which disrupt the plasticity of neurons and cause neurodegeneration. Furthermore, hyperphosphorylation of tau leads to dissociation of microtubule and aggregates as paired helical filaments (PHFs) and NFTs, resulting in neurodegeneration. Thus, the Tau hypothesis also postulates that tau phosphorylation and aggregation primarily cause neurodegeneration. The secondary causing agent is Aβ plaque formation which precedes the tau phosphorylation and aggregation. Hence, inhibition of the CDK-5 enzyme and activation of dephosphorylating enzyme phosphatase 2A provide symptomatic relief in AD. However, preventing tau protein

degradation by inhibiting Heat shock protein 90 (Hsp 90), which is involved in folding denatured proteins, could be used as a treatment option for AD patients. Moreover, increasing the breakdown of polymerized tau, responsible for neurodegeneration, is a rational approach for treating AD.

1.7.3.2.3 (iv) Cholinergic hypothesis

According to the cholinergic hypothesis, cholinergic neurons are impaired in the early stages of AD, involved in Acetylcholine (ACh) production in the basal forebrain being lost, resulting in cognitive dysfunctions[13, 14]. The primary cause behind the cholinergic neuron dysfunctions is the neurofibrillary degeneration in the basal forebrain region of the brain, leading to cholinergic denervation in presynaptic neurons [14]. ACh synthesis gets regulated by the choline acetyltransferase (CAT), a neurotransmitter found in the brain that plays a crucial role in neuromodulation of learning, memory, and cognitive processes [15]. Acetylcholine (ACh) synthesis occurs in neurons from choline and acetyl-coenzyme A by the action of the enzyme acetyltransferase [16]. ACh gets released into the synaptic cleft and acts on presynaptic and postsynaptic muscarinic as well as nicotinic receptors before being destroyed by the enzyme acetylcholinesterase into choline and acetate. Choline is recycled back into neurons. Physostigmine and Donepezil are few of the inhibitors of AChE that prevent the breakdown of ACh into choline and acetate. Acetyl-coenzyme A (AcCoA), acetylcholine receptor (AChR) and Acetylcholinesterase inhibitors (AchEIs) inhibits the breakdown of acetylcholine post synaptically to enhance cholinergic neurotransmission [13, 17]. An increase in acetylcholine is required to get symptomatic relief from AD. So, AchEI drugs are one of the therapeutic approaches of AD.

1.7.3.2.4 (v) Excitotoxic hypothesis

The excitotoxic hypothesis states that the excessive activation of excitatory neurotransmitters such as glutamate leads to neuronal cells' excitotoxicity, leading to neurodegeneration and cell death [18]. The excessive activation of the NMDA type glutamate receptor causes excessive calcium ions into the neuronal cells, which hinders neuronal transmission, damages the nerve cells, and is responsible for neurodegeneration and cell death [19]. In normal condition, NMDA receptors mediate synaptic plasticity, an essential component of memory and learning functions. Studies suggest that excessive activation of the NMDA type glutamate receptor causes excessive entry of calcium ions into the neuronal cells, which affects neuronal transmission, suppresses synaptic functions, and damages the nerve cells. The antagonists of NMDA receptors prevent calcium influx which is an approach to AD treatment.

1.7.3.2.5 (vi) Oxidative stress hypothesis

The oxidative stress hypothesis states that free radicals are responsible for neurodegeneration as neuronal cells are vulnerable to high oxygen consumption and lack of antioxidant enzymes. The common free radicals like hydrogen peroxide radicals (H_2O_2), hydroxyl radicals ($OH\cdot$), and the superoxide radical ($O_2^{-\cdot}$) get generated through mitochondrial oxidative phosphorylation [20]. In normal physiological conditions, 95-98% of ROS comes from the ETS system and mediates oxidative injury [12, 20]. CNS is extremely susceptible to oxidation because it is rich in polyunsaturated fatty acids (PUFAs) with a high metabolic oxidative rate and high transition metals concentration [21]. Aβ itself is oxidizing and generates positive feedback on the APP levels and its proteolytic pathway by generating additional oxidative stress [21]. Aβ also increases the generation of free radicals by entering the mitochondria and thus induces oxidative stress, leading to neurodegeneration [22]. Free radicals damage the neurons as they are vulnerable to it due to a lack of an antioxidant enzyme. Therefore, targeting oxidative stress by using antioxidants might provide benefits to AD patients.

1.7.3.2.6 (vi) Apolipoprotein E hypothesis

The Apolipoprotein E (ApoE) is the glycoprotein mainly found in the liver, followed by the brain[23]. In the CNS region, it gets produced by astrocytes and microglia to play a significant role in the transportation of cholesterol through ApoE receptors[23]. ApoE gene is having three types of allele i.e. ApoE2 (ε2), ApoE3 (ε3) and ApoE4 (ε4) [24]. The ε4 allele increases the aggregation Aß and reduces the clearance of Aß. Hence, found to be associated with three to four-fold higher risks for developing late-onset or sporadic AD [25, 26].

1.7.3.2.7 (vii) GSK-3 hypothesis

GSK-3ß is an isoform of GSK-3, a proline-directed serine/threonine kinase enzyme known as microtubule-binding protein and is involved in glycogen metabolism along with several other cellular processes [27]. In AD patients, over-activity of GSK3 is observed, leading to memory impairment and tau hyperphosphorylation [27, 28]. In addition, this kinase-dependent enzyme is also responsible for Aβ aggregation and deposition and also local plaque-associated microglial-mediated inflammatory responses, leading to neurodegeneration and cell death.