CHAPTER I

INTRODUCTION

1.1. Neurodegenerative diseases- a concern for millions of people worldwide

Neurons play a central role in the proper functioning of the human brain [1,2]. Neural stem cells generate the majority of neurons in childhood, but with an increase in age, their numbers are progressively reduced [3]. Neurons are not immortal, but the progressive loss of neuronal structure and function, or sometimes the neuronal failure to transmit the signal, is known as neurodegeneration, which is the most common cause in the pathophysiology of different brain diseases [4,5]. Therefore, a group of diseases characterized by the degeneration of neurons are collectively known as neurodegenerative diseases (NDs) [5]. NDs principally affect neurons in the central nervous system (CNS), presented by the progressive loss of CNS neurons, resulting in defects in specific brain functions such as memory, cognition, and movement [6].

Acute neurodegeneration defines a pathological feature in which neurons are promptly damaged and generally die in return for a rapid traumatic incident like strokes, head injury, cerebral hemorrhage, ischemic brain damage, and traumatic brain injury [7]. Chronic neurodegeneration is a condition in which the process of neuron degeneration generally initiates slowly but gradually degrades over time resulting in the irreversible loss of a particular neuron population [6]. The example of chronic NDs are Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) [8-10].

In 2019, approximately 349 million disability-adjusted life years (DALYs) and 10 million deaths were reported globally and became the second foremost cause of death after cardiovascular disease worldwide due to NDs [11]. From 1990 to 2019, there was a 1.91% surge in the global burden of NDs, such as stroke, AD, PD, migraine, motor neuron disease, etc [11,12]. As shown in Fig 1.1, significant DALY reductions from 1990 to 2019 were reported globally in most regions of Asia, South America, the Archipelago, Malaya, and Central Africa [11,12].

NDs are categorized by their principal clinical parameter, viz. neurotoxic protein accumulation in the brain, movement disorders, anatomic vulnerability, and cognitive disorders [5,9]. The neuronal dysfunction and death in NDs involve various fundamental mechanisms, viz. proteotoxic stress and its associated aberrations in the ubiquitin-autophagosomal and proteasomal system, neuroinflammation, oxidative stress, and programmed cell death (Fig 1.2) [9,10,13]. Decades of research presented evidence for

hallmarks of NDs, including aberrant proteostasis, pathological protein aggregation, synaptic and neuronal network dysfunction, DNA and RNA defects, altered energy inflammation, homeostasis, and neuronal cell death (Fig 1.3) [14].

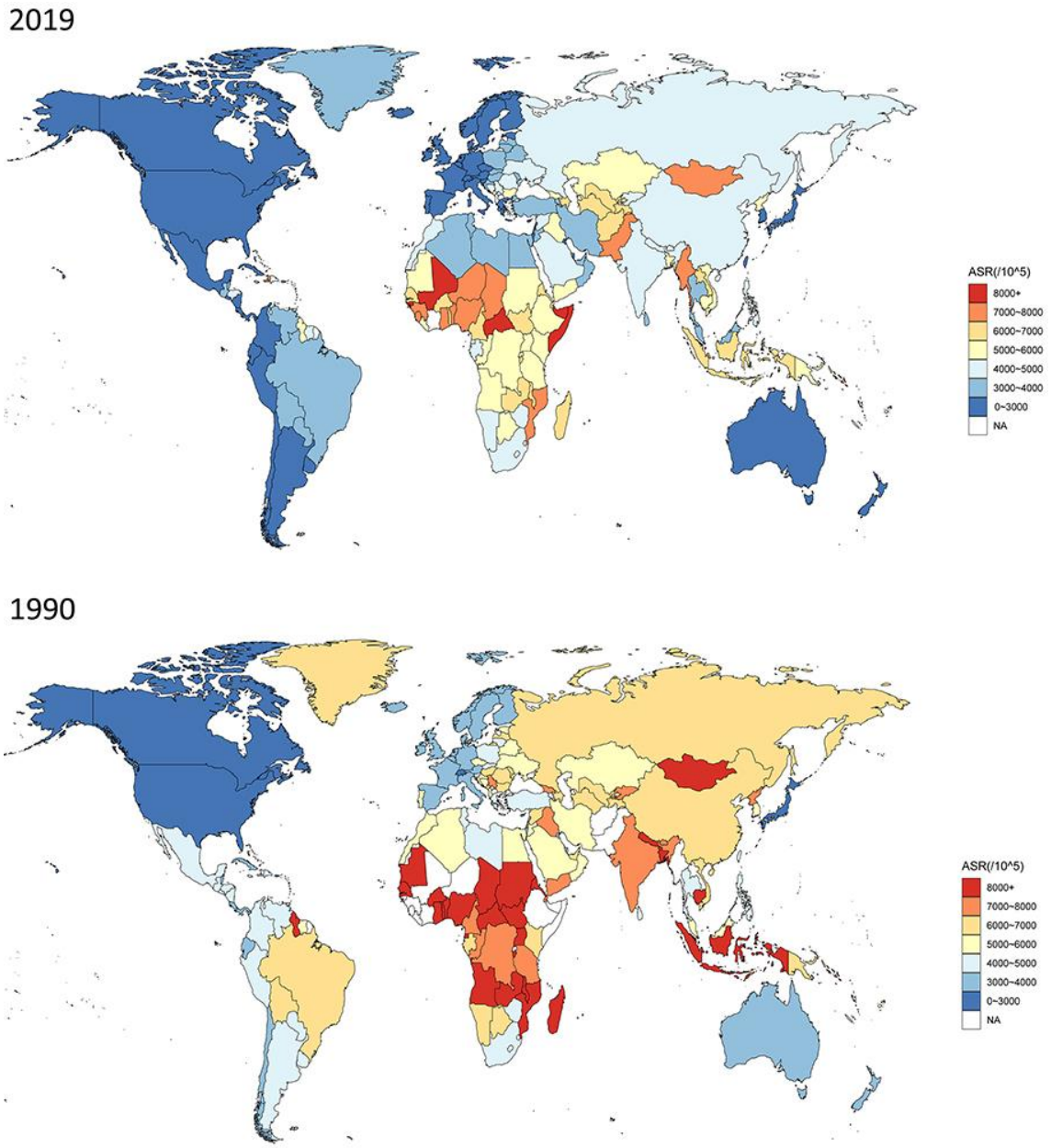

Fig 1.1 Age-standardized DALY rates of neurological disorders for both sexes and all ages among 204 countries and territories. (A) in 2019; (B) in 1990. This figure is adapted from [11].

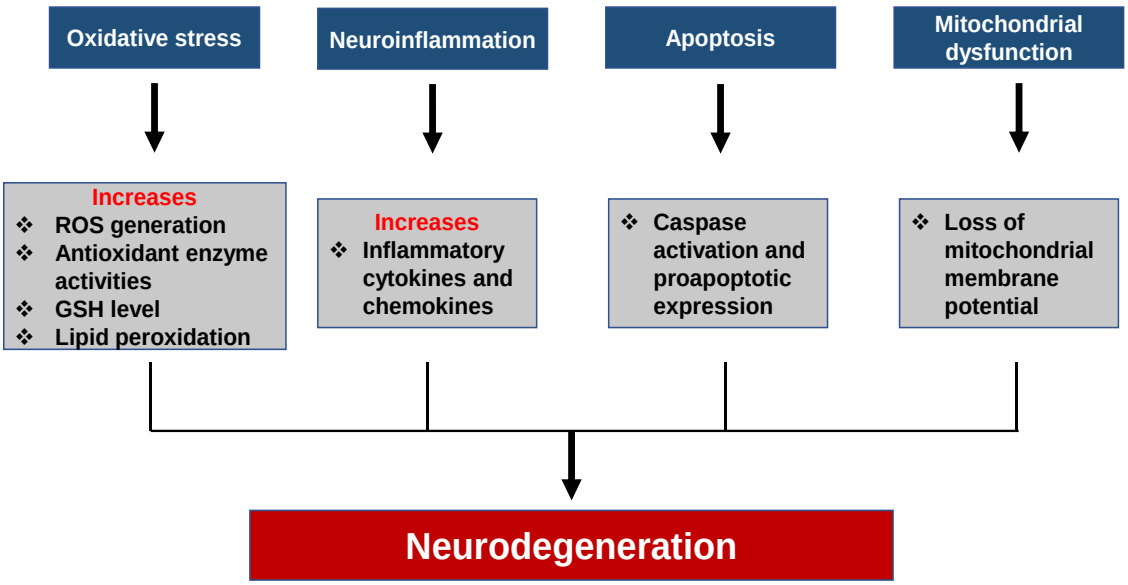

Fig 1.2 Several mechanisms allied with neurodegeneration that is concerned with the progression and pathogenesis of neurodegenerative diseases. This figure is adapted from [13].

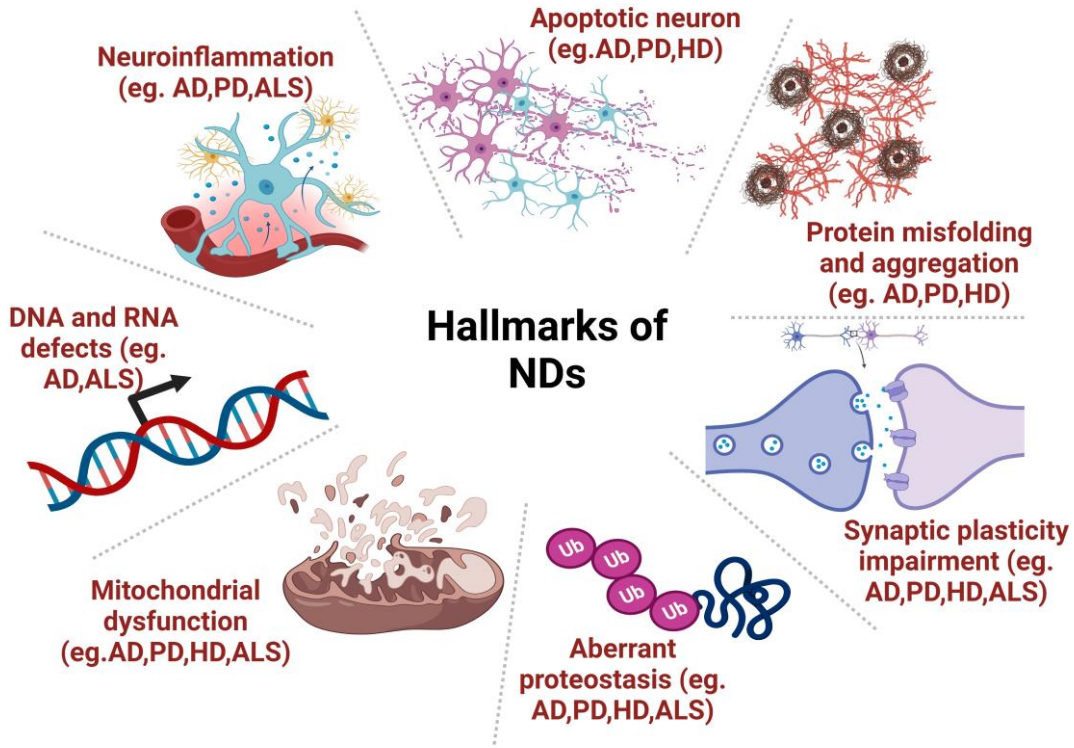

Fig 1.3 Schematic presentation of the neurodegenerative disorder hallmarks and their subcellular location. Created with BioRender.com

1.1.1. Types of neurodegenerative diseases and current treatments

In the subsequent subsection, the basic pathology and available current treatments of some of the important chronic NDs have been discussed. The pathophysiology of the above-mentioned NDs is summarized in Fig 1.4.

1.1.1.1. Alzheimer's disease: AD is described as a loss of synapse and synaptic proteins that correlates with the decline in cognitive function and the presence of neuritic plaques due to deposition of amyloid-β (Aβ) in the medial temporal lobes and neocortical structures [10]. Currently, there are approximately 50 million people suffer from AD worldwide, and this number is expected to double every five years to reach 152 million by 2050 [15]. The burden of Alzheimer's disease affects individuals, their families, and socio-economic conditions and is estimated to cost 1 trillion USD annually worldwide for treatment.

At present, there is no permanent cure for Alzheimer's disease, but there are treatments that only improve symptoms of the disease [15]. Glutamate regulators and cholinesterase inhibitors are two major classes of drugs available for the treatment of AD. Memantine [N-methyl-D-aspartate (NMDA) receptor antagonist] is the only approved glutamate regulator class of medication used to treat AD that is allied to memory and learning. In ordinary people, glutamate binds to NMDA receptors and allows excitatory glutamatergic neurotransmission, which exhibits neuronal plasticity and survival. But the accelerated activity of the NMDA receptor promotes neuronal cell death and contributes to the AD pathogenesis [16]. Cholinesterase inhibitors manage AD function by delaying the failure of acetylcholine (neurotransmitter); however, their side effects are nausea, loss of appetite, increased frequency of bowel movements, and vomiting [17]. Memantine is the only effective drug with cholinesterase inhibitors; however, the drug's efficacy is not satisfactory and is effective only in 50% of patients for a very short period. Constipation, headache, dizziness, and confusion are the side-effects of Memantine, which is a significant concern for AD treatment and management.

1.1.1.2. Parkinson's disease: PD is the second most common neurodegenerative disorder after AD that affects 2-3% of the population [18,19]. PD is characterized by α-synuclein aggregation in the brain and progressive dopaminergic (DAergic) neuronal degeneration in the substantia nigra leading to impaired motor control, rigidity, tremors, postural instability, and slow movement of the patient [20-22]. Studies have reported that in the

nigrostriatal system, axons become damaged before the degeneration of dopaminergic neurons, which results in the loss of synaptic communications [23]. The principal molecular pathogenesis of PD includes various pathways and mechanisms, viz. α-synuclein proteostasis, oxidative stress, mitochondrial dysfunction, axonal transport, and neuroinflammation [24].

Levodopa, apomorphine, monoamine oxidase type B inhibitors, and amantadine are approved by the FDA (USA) for symptomatic therapy of PD. Regrettably, since 1970, no further advanced drug has been approved for PD therapy. The primary target of these drugs is to increase the dopamine (neurotransmitter) level to improve motor symptoms of the disease [25,26]. However, long-term PD medication of these drugs reduces efficacy and leads to other adverse effects, such as motor complications [22,26].

1.1.1.3. Amyotrophic lateral sclerosis: In 1869, Jean-Martin Charcot initially explained ALS as a pure motor neuron disease, which is now known as a multisystem neurodegenerative disease with heterogeneity [27,28]. The clinical manifestation of ALS (weak focal muscle and wasting) spreads with the progression of the disease. The initial symptoms of ALS can differ between patients; most commonly, the onset of weakness in the limb muscles (also known as spinal onset), whereas there are approximately 20-30% of patients have bulbar-onset disease presenting with dysphagia (difficulty in swallowing), dysarthria (difficulty in speech), dysphonia (abnormal voice), and rare masseter weakness [29].

The incidence of ALS prevalent in Europe ranges from 2-3 cases per 1000,000 individuals [28,29]. The mechanism underlying ALS is poorly understood, although various factors such as genetic factors, oxidative stress, excitotoxicity, autoimmune response, neurofilament aggregation, impaired axonal transport, mitochondrial dysfunction, and environmental factors may be involved [13]. ALS is linked with a mutation in a gene encoding the zinc/copper dismutase-1 enzyme [13].

On account of its pathophysiology, other drugs such as ibudalist (cyclic nucleotide phosphodiesterase inhibitors), TRIUMEQ (antiretrovirals used as anti-HIV therapy), tamoxifen (antiestrogen; NCT00214110 under www.clinicaltrials.gov), retigabine (antiepileptic drugs), and mastinib (tyrosine kinase inhibitor; NCT02588677, www.clinicaltrials.gov) are currently being explored for ALS treatment. Although only two drugs, edaravone (free-radical scavenger; NCT01492686, www.clinicaltrials.gov)

and riluzole (antagonist of glutamate receptor), are prescribed to the patients; nevertheless, these drugs only slow down the disease progression but cannot cure the disease and inept at reverting associated symptoms of ALS [30].

1.1.1.4. Huntington's disease: HD is an autosomal dominant and progressive NDS. In basal ganglia, it is presented pathologically by diminished gamma-aminobutyric acid (GABA) and undue dopaminergic activity. Clinical manifestations of HD include movement dysfunction, cognitive impairment, and psychiatric abnormalities [31]. HD occurred by repeat expansion of CAG trinucleotide in the huntingtin (*htt*) gene at the chromosome 4 short arm [32]. Currently, there is no treatment for HD; the only possibility is to manage the symptoms [33,34]. The often-prescribed medication for HD treatment is tetrabenazine (depletes dopamine), aripiprazole, and olanzapine. However, they develop the risk of adverse side effects such as depression, akathisia, dizziness, parkinsonism, or fatigue [34].

The pathophysiology of the above-mentioned NDs is summarized in Fig 1.4.

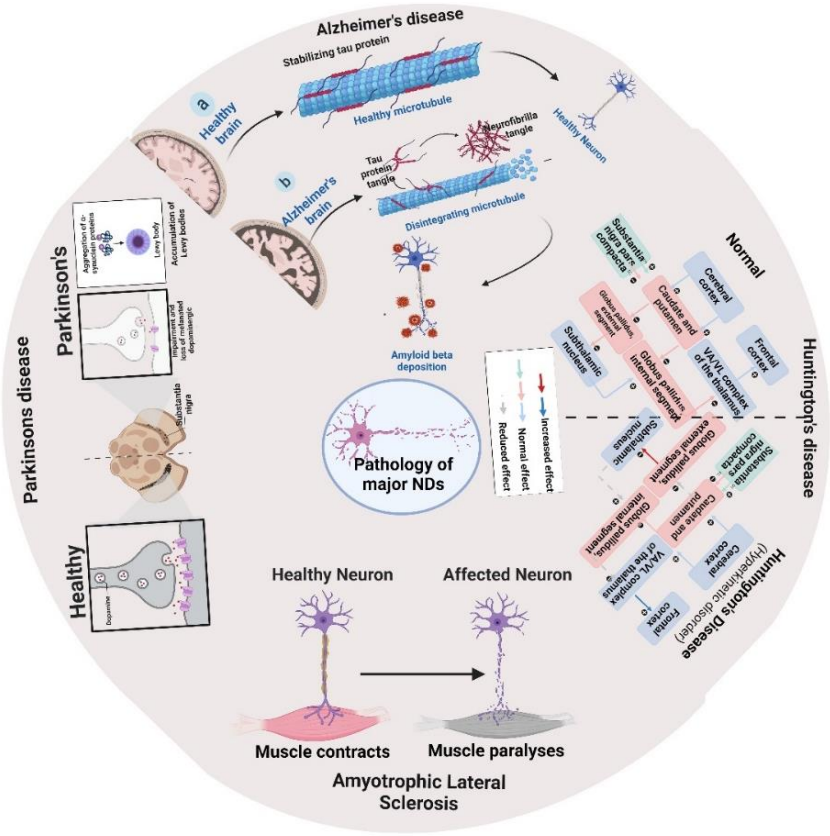

Fig 1.4 The pathophysiology of four major NDs - AD, PD, HD, and ALS. Created with BioRender.com

1.1.2. Oxidative stress and neurodegenerative disorders

Oxidative stress contributes a significant role in the aetiology of common NDs [35]. The disparity between reactive oxygen species (ROS) production and poor antioxidant defence potential results in oxidative stress causing cellular damage, system impairment, mitochondrial dysfunction, and DNA repair. These abnormalities encourage the neurodegenerative action and advancement of NDs [36]. The cellular damages include lipids, proteins, and nucleic acid damage that leads to excessive uptake of calcium (Ca^{2+}) through the mitochondrial membrane, which triggers the ROS production, deteriorates energy (ATP) production, and releases cytochrome c (cyt c, a proapoptotic factor) into the cytoplasm that causes neuronal cell death [37].

1.1.2.1. Neuroinflammation: Furthermore, neuroinflammation plays an important role in the pathophysiology of NDs [38]. Neuroinflammation, known as an inflammatory response (innate and adaptive immune system) within CNS and contributes to neurodegeneration. Microglia are the most abundant macrophage (innate immune effector cells) in the CNS and participate in homeostasis of CNS while neuronal development and ageing [39]. The nervous system in response to any pathological changes rapidly secrete different inflammatory molecules viz. chemokines, cytokines, and toxic components (glutamate, ROS, cyclooxygenase, prostaglandins, etc) [38,39]. The release of these inflammatory molecules or mediators is stimulated by astrocytes that ultimately trigger secondary inflammatory responses that encourage the survival of neurons [38].

1.1.2.2. Mitochondrial dysfunction: Mitochondria signify the energy (ATP) powerhouse and protecting guard of the cell. Mitochondria function as the site of oxidative phosphorylation, and cellular respiration, and maintain low calcium (Ca^{2+}) concentration in cytosol [37]. The consequences of mitochondrial dysfunction are dire, so it is considered a critical organelle for determining cell fate (death/survival) by controlling autophagy and apoptotic signals [37]. Activation of multiple signals (autophagic or apoptotic) stimulates mitochondrial permeability transition (MPT) that causes the release of proapoptotic proteins (cyt c) from the intermembrane space, which activates caspase for apoptosis or stimulates autophagy [37]. In the cytosol, cyt c activates a cascade of caspases (caspase 3, 6, and 7) after binding to apoptotic protease-activating

factor-1 (apaf-1) and procaspase-9 and forming apoptosome complex. The caspase activation eventually leads to apoptotic neuronal cell death [40].

1.1.2.3. Release of Cytochrome c: Rationally, the release of cyt c into the extracellular space can occur during the incidence of cell damage, there it acts as a danger-associated molecular pattern (DAMP) i.e., translocation of self-molecules in an inappropriate compartment [41]. Therefore, the anti-inflammatory/pro-inflammatory activity of cyt c depends on their location. In normal cells, cyt c is present inside the mitochondria. Emigration of cyt c into the cytosol triggers the apoptotic pathway (non-inflammatory). However, translocation of cyt c into the extracellular space induces inflammation and can be measured in the serum as a marker of severe mitochondrial damage or cell death (Fig 1.5) [40,41].

1.1.2.4. Apoptosis: Apoptosis of neuronal cells contribute to neurodegeneration, which results in NDs. Apoptosis is known as programmed cell death, which is characterized by chromatin condensation, shrinkage of the cells, and DNA fragmentation. This process is an energy-dependent mechanism that requires ATP for translation (protein synthesis) and signal activation [13]. Apoptosis can occur via two pathways, i.e., intrinsic and extrinsic pathways. In the extrinsic pathway, death ligands bind and activate death receptors, which induce death signals via a cascade of protein and protein interactions. Whereas the intrinsic pathway is triggered by the release of proapoptotic (cyt c) mediators from the intermembrane space of mitochondria through MPT and induces a caspase-dependent/caspase-independent pathway [42,43]. Targeting the aforementioned mechanism of action may hold good promise for the treatment and prevention of NDs. To deal with NDs, various probable therapeutic targets can be explored.

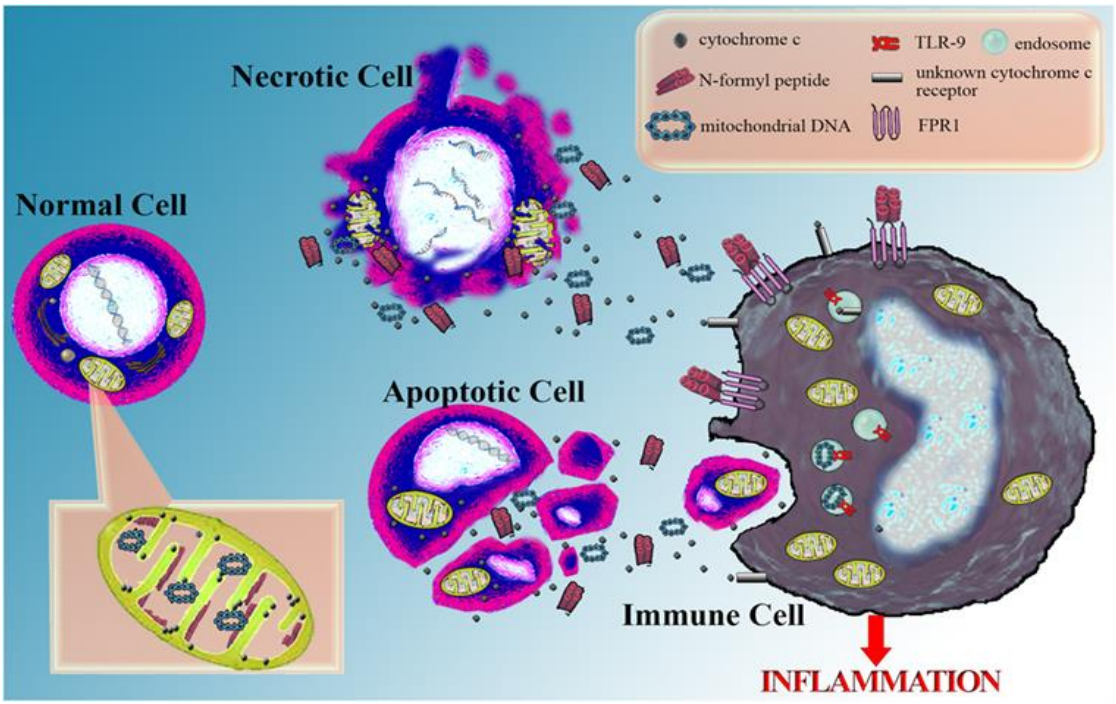

Fig 1.5 The potential cytochrome c (a marker of apoptosis) as a danger-associated molecular pattern (DAMP). This figure is adapted from Eleftheriadis et al. (2016) [40].

1.1.3. Challenges associated with current therapy

Management of NDs is disease-specific. Currently accepted strategies for the management either aim at the pathogenesis of the disease or challenge the recovery of the symptoms experienced [8]. Present therapy can potentially control the disease progression rather than eliminate the root causes of NDs. The blood-brain barrier (BBB) is one of the major concerns for successfully treating neurodegenerative diseases, where most of the drugs under clinical trials fail. The BBB is known as a diffusion barrier that thwarts the transport of ingredients into the brain, aided in maintaining the homeostasis and normal functioning of the brain. The effective intervention of NDs is restricted due to the unsuccessful delivery of adequate formulations to the brain. The poor permeability of most drugs and the advanced properties of BBB account for the lack of appropriate treatment opportunities for NDs [8].

The pharmacokinetic characteristics of systematically conducted drugs determine their efficacy [44]. In most cases, the therapeutic molecules are unfavorable for delivering to the target site. The plasma proteins in the human circulation system are the initial point of attention. Some medicinal drugs have a high affinity towards these proteins, thus restricting the extent of the drug in circulation and eventually reducing the availability of

the unbound pharmaceutical and their transportation to the brain [8,45]. Furthermore, the elimination rate of some drugs by the liver and kidney (clearance organ) is limited and releases few into the blood.

Moreover, the drug-target cell interaction confines the amount of drug absorption. Unambiguously, drug molecules can block the channels (alter the membrane potential), which affects the cell conformation. The administration and absorption of drug molecules are limited by this transient effect [46]. Small lipophilic therapeutic drug molecules with limited interactions with plasma proteins aid brain delivery [8].

The multifactorial nature of NDs promotes the search for multi-target drugs (hybrids and co-drugs), which target more than one pathophysiological symptom. This approach is promising for treating NDs [47].

1.2. Neurotrophins: Role in neurological disorders and prospects

1.2.1. Neurotrophins and their receptors

Neurotrophins are a group of endogenous soluble proteins with similar structures and functions, which profoundly affect neuronal development in vertebrates. The first neurotrophin, viz. nerve growth factor (NGF), was identified in 1951 by Levi-Montalcini and their colleagues [48]. The neurotrophins family consists of NGF [48], brain-derived neurotrophic factor (BDNF) [49], neurotrophin- 4/5 (NT-4/5) [50] and neurotrophin-3 (NT-3) [51]. Other than these neurotrophins, two neurotrophins named neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7) have been discovered in teleost fish [52]. These neurotrophins are grouped based on structural similarity to the NGF, which is involved in neuronal growth, development, and maintenance [53,54].

The mechanism of neuritogenesis involves the binding of neurotrophins to transmembrane receptors belonging to the tyrosine kinase receptors family, for example, tropomyosin-related kinase A (Trk A), tropomyosin-related kinase B (Trk B), and tropomyosin-related kinase C (Trk C) [55,56]. Neurotrophins also bind to the receptor of the Tumor Necrosis Factor (TNF) superfamily, the p^{75NTR} neurotrophin receptor [55,56], and mediates pro-neurotrophin signalling (converting precursors to mature neurotrophins) [57]. NGF and NT-3 show high-affinity binding (K_d value~10^{-11} M) [58-61] with Trk A and Trk C receptors; respectively, although, brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5) exclusively bind with high-affinity (K_d

value~10^{-11} M) to the Trk B receptor [58,61]. However, all the neurotrophins show low-affinity binding (K_d value~10^{-9} M) with p^{75NTR} [58,59]. Moreover, p^{75NTR} can also regulate the selective and specific binding of neurotrophins (NT) to the correct tropomyosin-related kinase (Trk) receptors [62].

1.2.2. Snake venom neurotrophins

Venoms are exciting sources of special molecules that are being enhanced in evolution and also have unique characteristics such as low molecular mass, pharmacological activity, stability, and high potency, along with selectivity and affinity in mammalian systems for many targets. Animal venoms, therefore, have a great potential to generate therapeutic agents, and many venom toxins have been applied clinically and used as templates for drug design [63].

Nerve growth factor (NGF), a prominent member of the neurotrophin family, is one of the intriguing non-enzymatic proteins found in snake venoms. The proteomic analysis has shown that venoms of all the 'Big Four' venomous snakes of India contain several isoforms of NGF; however, in a small proportion (Fig 1.6) [64-67], although its role and significance in snake venoms remain unclear [68].

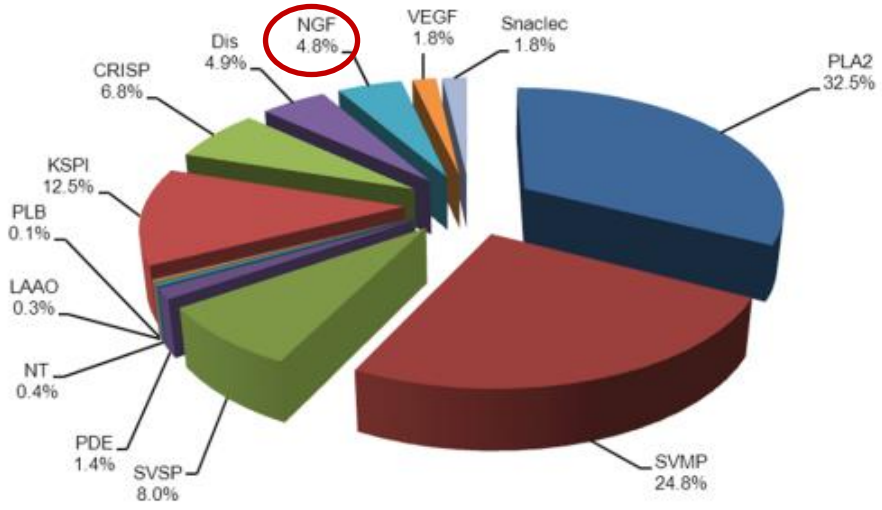

Fig 1.6 Protein family composition of western India (WI) Russell's viper venom (RVV). Nerve growth factor (NGF) constituting 4.8% of WI RVV proteome identified by tandem mass spectrometry analysis. This figure is adapted from [67].

1.2.3. Neurotrophins signalling pathways

After binding to the respective Trk receptors, neurotrophins promote their dimerization, followed by autophosphorylation of intracellular tyrosine residues of the receptor, which activates a cascade of events through two adapter proteins—Src and Shc. The Trk receptor-induced cascade of signalling pathways includes the Ras-induced Mitogen-Activated Protein Kinase (MAPK) pathway [54,69], MAPK-extracellular signal-regulated kinase (ERK) pathway [70], phosphatidyl inositol 3-kinase (PI3K) stimulation of protein kinase B (Akt) and phospholipase Cγ (PLCγ)-dependent secretion of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3)-mediated pathway (Fig 1.7)[54,69]. Neurotrophin and Trk interaction-induced signalling pathways resulted in neuronal proliferation, survival, and differentiation [70,71].

Notably, the p^{75NTR} can function antagonistically to the Trk receptor [57]. For example, the interaction between neurotrophin and Trk results in cell survival, whereas the binding of p^{75NTR} with neurotrophins precursor leads to neuronal cell death by apoptosis [62]. The mechanism of such cell death involves that p^{75NTR} receptor activation stimulates the c-Jun N-terminal kinase (JNK) signalling pathway, which activates the tumour suppressor (p53) protein that causes apoptosis [72,73]. The binding of the p^{75NTR} receptor by NGF or neurotrophins also stimulates the expression of the Fas ligand responsible for activating the Fas receptor resulting in apoptosis [72,73]. When the Trk A receptor is absent, the pro-apoptotic function is reported in cells where the p^{75NTR} receptor is expressed [74]. The TrkA receptor performs all the neuritogenesis functions of NGF independent of the appearance of the p^{75NTR} receptor [75,76]. p^{75NTR} receptor induces both negative and positive signals, which play a significant role in neural development and other higher-order functions viz. learning and memory (Fig 1.7).

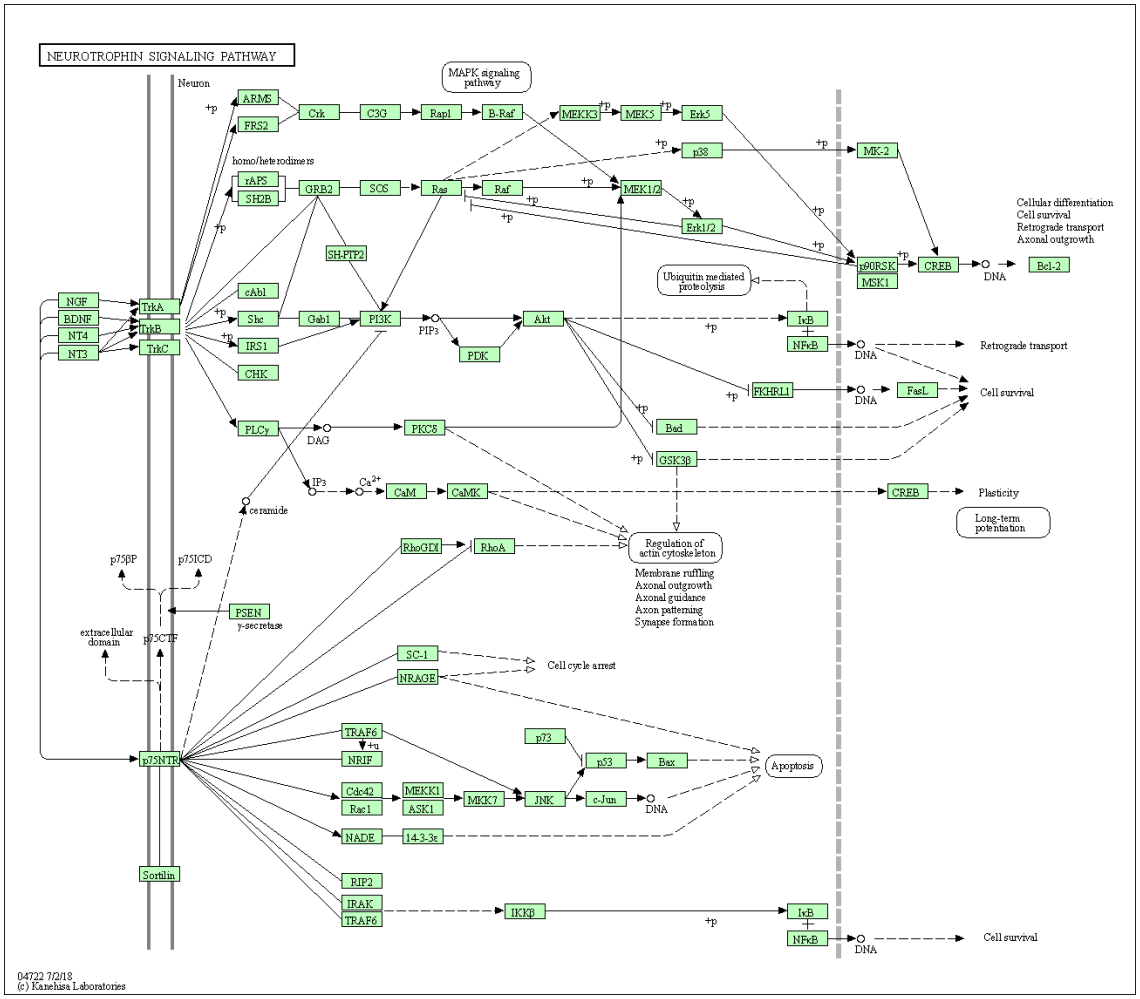

Fig 1.7 Kegg pathway. Human neurotrophin signalling pathways.

1.2.4. Neurotrophins as therapeutics in NDs

Since the discovery of NGF, the crucial role of neurotrophins to regulate numerous essential neuronal functions, such as promoting neurite regeneration, avoiding degeneration, and increasing synaptic plasticity has been elucidated by *in vitro* laboratory studies and in *in vivo* experimental models [77]. Because of their prominent effects on neuronal development by systemic administration of exogenous neurotrophin molecule proteins, NGF molecules can be used as drug prototypes to treat various NDs. Over the years, the neuroprotective effect of neurotrophic factors has been well documented, leading to the hypothesis of their therapeutic application [77-79].

1.2.5. Limitations of neurotrophins as a therapeutic in neurological diseases

Despite the high pharmacotherapeutic potential of neurotrophins, their poor pharmacological properties, marginal permeability of the blood-brain barrier, short half-life, activation of multiple receptors, and pleiotropic effects have limited their therapeutic applications [54,80]. Therefore, there is an urgent requirement to resolve these impediments for the successful therapeutic application of neurotrophins. Several strategies focusing on the better pharmacokinetics of native neurotrophins, such as - (a) systemic or intraventricular administration of neurotrophins, (b) transplantation of cells producing neurotrophins, (c) neurotrophin expression via viral vectors and cell-based delivery systems, (d) combinatorial strategy using combination of neurotrophins enhanced neuroprotection, and (e) small molecule or synthetic peptide development that binds to specific receptors or neurotrophins mimetics have been suggested [81,82].

1.3 Peptidomimetics and small molecules therapeutics

A peptidomimetic is a compound with pharmacophore similarity that mimics natural protein-fragment, peptide, or whole protein and which also possesses the ability to interact with the specific target and generate similar biological effects [83]. Peptide mimetics provide an obvious way to tackle the disadvantages of natural peptides. The molecule which mimics the biological activity of a natural peptide and has a molecular weight of less than 700 Da is referred to as peptide mimetics. Peptide mimetics also have significantly improved patient compliance and cost savings.

Moreover, peptide mimetics are less expensive to produce than natural peptides. Also, natural peptides not encountered with peptide mimetics have issues with peptide storage, stability, and immunoreactivity [84]. In recent years, peptide mimetics have emerged as a new generation of promising drugs due to the rapid screening of small molecule libraries and rational design approaches [84,85].

1.3.1 Neurotrophin's mimetics role in neurological diseases

The word 'mimetic' in neurotrophin mimetic is broadly used to illustrate a modulator with similar structural features of neurotrophic factor and stimulating the property of neurotrophin molecules. Mimetic group of members might act as receptor agonists [86-88] or antagonists [86,89-91]. However, some group of mimetics binds in a non-competitive manner at neurotrophin receptor to upregulate or downregulate the activity

of receptor to change the expression of different cellular proteins to induce neuritogenesis [92,93].

In recent years attention has been paid to the characterization of novel neurotrophin peptidomimetics in clinical trials of different neurological diseases due to their therapeutic approach to neuroprotective effect, synaptic and neuronal plasticity, neurogenesis, better pharmacokinetics than the parent neurotrophin [86,94,95]. Neurotrophin mimetics may trigger the change in different prospects of signalling pathways in a manner that is unique from the native neurotrophin-triggered pathways [82].

1.4. Model organism for neurobiological studies

1.4.1. Rat pheochromocytoma (PC-12) cell as an *in vitro* model

The rat pheochromocytoma cell line (PC-12 cells) is considered to be an appropriate *in vitro* model for neuronal differentiation, development, and neurological diseases [96-100]. PC-12 cell lines have advantages in being derived from neural crest cells that have similar structures and functions, and they are easy to grow and maintain [99].

1.4.2. *Caenorhabditis elegans* (*C. elegans*) as *in vivo* model

Caenorhabditis elegans, a tiny microscopic nematode, is a good choice of *in vivo* model for its wide acceptability in neuronal research to understand the development of neural lineages and neuronal differentiation. The advantages of using *C. elegans* as a model organism are its transparent body, small size, short life cycle, and prominent and well-developed nervous system; consequently, they serve as a widely-used model organism for neuronal research [101-104]. Unlike experimental rodents, they do not require any room for growth, their maintenance is easy and cost-effective, and they can save the life and high expenses of using laboratory experimental animals. A transgene strain (BZ555; Pdat-1::gfp) has dopaminergic (DAergic) neurons expressing green fluorescence protein (GFP), and has been used to study neurodegeneration.

Another *C. elegans* strain (NL5901; Punc-54:: α-synuclein:: YFP+unc-119) expressing the human *α*-synuclein protein tagged with yellow fluorescence protein (YFP) in the muscles (one of the critical proteins involved in PD), can easily live imaged by *a* confocal microscope. Therefore, neuronal damage caused by toxic substances and their

regeneration by a therapeutic molecule can be assessed speedily in *C. elegans* model [105-108]. Further, the genome of this worm is wholly sequenced and shows 60-80% similarity with human genes, which is an added advantage of using them as *in vivo* model organisms [109-111].

In 1993, Lee et al. reported the first miRNA, lin-4, discovered in *C. elegans*, which targets lin-14 and regulates the aging process in *C. elegans* [112]. Since then, *C. elegans* has been used as a model system for researching miRNAs related to neuronal development and their target genes [113-115]. *C. elegans* as a model system has proven several advantages, including highly conserved miRNAs during its evolution, well-known neural networks, and neuroanatomy [116]. Moreover, it is in high demand to understand the role of miRNAs in neurobiology and their dysfunction related to medical implications.

1.4.3. Micro-RNAs

MicroRNAs (miRNAs) are small non-coding RNAs with an approximate length of 22–24 nucleotides that act as transcriptional repressors primarily by binding to the 3′-untranslated region (3′-UTR) of target mRNAs [116]. Micro-RNAs regulate a range of biological functions, such as ageing, proliferation, development, differentiation, apoptosis, inflammation, immune response, and neurodegeneration [117,118] by targeting the genes involved in these processes [119]. Altered expression of miRNAs ends up in various diseases, including NDs [116-118]. Although some miRNAs, for example, miR-64, miR-81 [120], and miR-128 [121], etc., have been reported to be involved in regulating specific biological processes; however, the function of a large number of miRNAs has yet to be revealed [116].

In the field of neuroscience, accumulated evidence progressively revealed the potential of miRNA in regulating neurodevelopment, neurite outgrowth synaptic plasticity, memory process, neurodegeneration, and nervous system morphogenesis [122,123]. Numerous miRNAs are reported as biomarkers in the pathogenesis of NDs, which imparts targets for ingenious therapies [124-127]. An individual miRNA can affect multiple target genes; therefore, the entire phenotype of the disease can be improved by modifying a single miRNA. This property makes RNA molecules very captivating from therapeutic prospectives. Moreover, the identification of dysregulated miRNAs in ND or

other disease cases may help in early diagnosis or monitoring of the disease progression [127].

1.5. Gap in the study

Despite the success of mammalian NGFs in diseased animal models for treating neurogenerative disorders, their clinical trials need to look more promising as drug prototypes. The failure of applying large neurotrophin polypeptides as drugs (poor pharmacological agents) can be attributed to various reasons, such as undesired pleiotropic effect, short half-life, proteolytic degradation, and poor pharmacokinetics. To overcome the impediments associated with the isolation, purification, and therapeutic application of high molecular weight neurotrophins, research on developing low-molecular-weight mimetics of the neurotrophins possessing innate neurotrophic activity and improved pharmacokinetic role to replace the traditional neurotrophins for treating the neurodegenerative disorders has gained tremendous momentum in recent years. There is a dearth of knowledge on snake venom neurotrophins and their low-molecular-weight mimetics in treating neurological disorders.

1.6. Objectives of this study

By *in silico* analysis, our laboratory has synthesized four custom peptides from the TrkA receptor binding region of Indian Russell's viper (*Daboia russelii*) and Indian cobra (*Naja naja*) venom NGF molecules. These peptides were characterized further to develop as potential drug prototypes to treat neurodegenerative disorders. The specific objectives of the present research work are mentioned below.

1. To study the interaction of synthetic custom peptides with mammalian TrkA receptor and TrkA homolog in *C. elegans* by computational (*in silico*) analysis.

2. To study the *in vitro* mechanism of neuritogenesis and neuroprotective role, of custom peptides in pheochromocytoma of the rat adrenal medulla (PC-12) cells.

3. To study the *in vivo* neuroprotective mechanism of custom peptides in *C. elegans*.

4. To study the microRNA expression profile in custom peptides-treated cultured *C. elegans*.

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