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Motor Neuron Diseases and Newer Therapeutic Approaches

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ABSTRACT

Motor neuron disease (MND) is a rapidly progressive adult onset neurodegenerative disease of unknown origin [1, 2]. MND takes several forms of which Amyotrophic Lateral Sclerosis (ALS) is most common and the leading cause of death [3]. Etiology of MND remain unclear, but the mainstay was found to be mutation in Cu/Zn Superoxide Dismutase 1 (SOD1) gene, which occurs for about 2%[1,4]. Various pathologic processes that can damage the motor neurons include mitochondrial dysfunction, protein misfolding, oxidative damage, defective axonal transport, excitotoxicity, insufficient growth factor signaling, and inflammation [4]. Genetic concepts over the past 3 to 4 years have changed the concept of triggering the disease [5].The discrepancy of familial and sporadic ALS based on family history may become outmoded over time as new genes are discovered [5]. Diagnosing ALS was difficult earlier but recent new diagnostic concepts have been anticipated to increase sensitivity of diagnosis [1]. Once diagnosed the survival is about 3-5 years [5]. 'El Escorial criteria' helps in diagnosing and ruling out the differentials of ALS [2, 5]. Riluzole a glutamate antagonist is the only treatment available from 1996 for MND patients and other therapies are symptomatic [5]. Once damaged, the motor neurons cannot be repaired and currently gene and stem cell therapy have proven to be effective in slowing the disease progression [5,6]. Restoration of cellular integrity using stem cell grafts may also help in increasing the survival rates of ALS, they have also shown that non-neuronal cells that could genetically release neurotrophic factors will also be a milestone [7].

INTRODUCTION

Motor neuron disease (MND) is a neurodegenerative condition that affects the brain and spinal cord, which primarily affects the motor neurons that ultimately, leads to loss of voluntary movements [2, 3, 5]. MND was first described by a French neurologist Jean Martin Charcot and Joffroy in the 19th century [4]. MND can be hereditary or acquired [5]. Most MNDs affect only the lower motor neurons, which are situated in the spinal cord anterior horn and in the brain stem that innervates skeletal muscles, so called as anterior horn cell diseases. Whereas, the upper motor neurons are located in the motor cortex and they form corticospinal and corticobulbar tracts [5]. MND have a very rare presentation, about 2-3 in 1, 00,000 cases were identified per year. Most commonly occurring is sporadic ALS (SALS) which peaks between 50 – 75 years and only 5 to 10% are familial ALS (FALS), it is very rare under 20 years [8]. MND are diversely classified based on whether the Upper Motor Neurons (UMN) or Lower Motor Neurons (LMN) getting damaged.

(i) Amyotrophic Lateral Sclerosis – most common adult onset neurodegenerative disease that involves both UMN and LMN. It is a fatal disorder that manifests as progressive skeletal muscle weakness and wasting/atrophy that terminates in respiratory paralysis. Average life expectancy is 3 to 5 years for an ALS patient. Most commonly occurring is sporadic ALS (SALS) which peaks at a mean age of 60 years and only 5 to 10% are familial ALS (FALS). Mutation of c9orf72 gene causes 30 - 40% and approximately 20% of familial ALS is because of mutation in Cu/Zn Superoxide Dismutase (SOD1) gene [1, 5, 3]. Studies show at least 16 genes and genetic loci are involved in ALS pathogenesis [3].

(ii) Progressive Bulbar Palsy – progressive degenerative disorder of motor nuclei in medulla (involving cranial nerves XI & XII) that produces atrophy, fasciculation of lingual muscles, dysarthria and dysphagia it mainly affects the muscles of speech and swallowing [2,4].

(iii)Progressive Muscular Atrophy – degeneration of LMN in the spinal cord with absence of UMN and bulbar effects presented mainly as muscle wasting, weakness, twitching and loss of weight [4].

(iv) Primary Lateral Sclerosis-Affects the UMN, it will cause stiffness and limb paralysis [4].

(v) Spino-Muscular Atrophy - Hereditary disease affecting the LMN, an autosomal recessive disease caused by mutation in SMN_1 gene that makes a protein important for the survival of motor neurons.

ETIOPATHOGENESIS:

Motor Neuron Disease is a fatal disorder with no properly proposed etiology. Various studies have shown there are environmental and occupational exposure risks for ALS [10]. Exposure to high-grade physical activity as in case of athletes and military workers who get unprotected to a wide range of chemicals. Electromagnetic fields shared by power production plants, electric and military workers have increased risk of motor neuron damage, because of increased oxidative stress and glutamate excitotoxicity. Cigarette smoke having numerous toxic and chemical substances have an impact on increasing the risk for ALS in genetically susceptible individual by direct neurotoxic effect on the motor neurons by increasing the oxidative stress [10]. Apart from these risk factors soccer players are at a high risk of ALS due to high levels of herbicide and fertilizers that are used to maintain the football grounds that could cause mitochondrial dysfunction contributing to MND.

Currently, studies have shown almost 16 genes are involved in the pathogenesis of ALS. Sporadic and familial ALS occur due to mutation in SOD1, FUS, TARDP, VABP which are the main genes involved in cellular functions as axonal transport, oxidation, DNA & RNA processing and apoptotic signaling, all which culminates in neurodegeneration [6]. ALS-associated to chromosome 21 mutation and accounts for about 20% of FALS and 2-3% SALS [6]. SOD1 is an abundant cytosolic homodimeric protein and each of its subunits has Cu and Zn ions in active site. SOD1 reduces the superoxide radical to hydrogen peroxide which is reduced by catalase to avert oxidative damage [6]. Two other superoxide dismutase are found as SOD2 which is a manganese SOD which is existent in the mitochondria and SOD3 an extracellular SOD which binds to Cu and Zn ions. Mutation in SOD1 occurs when an alanine is replaced by valine in the 4^{th} position that bases 50% of ALS cases. SOD1 activity is reliant on catalytic copper, free copper is highly responsive and lethal [4,9].Mutant SOD1 misfolding happens and form abnormal protein aggregates proposing the toxic gain-of-function mechanism [9].

Accumulation of Transactive Response DNA Binding Protein (TARDBP) which plays an active role in RNA processing accounts for about 5% of FALS and frontotemporal dementia associated with this accumulation is also evident [5]. Fused in Sarcoma (FUS) is a protein with two RNA binding domain and terminal nuclear localization signal and mutation accumulates a nuclear protein. Chromosome 9 open reading frame 72 (C9orf72) may predispose ALS if there is a hexanucleotide (GGGGCC) repeat expansion [5]. Genetic level motor neuron degeneration could be a collective final expression of disturbance in number of cellular systems [12].

Hypoxia and reduced vascularization also have a vital role in ALS. Vascular Endothelial Growth Factors (VEGF) originally discovered in 1983, a neurotrophic factor for motor neurons and stimulate angiogenesis which is useful in neuroprotection during hypoxia [4]. Low levels of VEGF are found in patients with early ALS. Angiogenin which is coded by ANG gene is present in the CNS and motor neurons that have an intranuclear RNase activity and neovascularization. Mutation in ANG gene could lead to defective angiogenin that is found in ALS patients [4, 15]

Glutamate excitotoxicity can induce neuronal damage by inappropriate activation of glutamate receptors, which results in increased intracellular calcium and glutamate levels that could lead to damage and death of motor neurons by excess production of reactive oxygen species [17]. Unlike other neurons, motor neurons have a low expression of Ca^{2+} buffering proteins and capacity and a high number of Ca^{2+} permeable AMPA receptors resulting from a low expression of GluR2 subunit which is essential for normal functioning of motor neurons [17]. At pathologic circumstances, motor neurons gets overwhelmed with glutamate and increased Ca^{2+}. A drug that could interfere with the glutamate release and inhibition of Ca^{2+} permeable AMPA receptors could be useful in treatment. Hydrogen peroxide produced by mutant SOD1 and neuronal toxicity causes inactivation of excitatory amino acid transporters (EAAT) terminates the reuptake of glutamate [4, 9, 17].

Inflammation is an innate immune response executed by a variety of phagocytic cells as neutrophils, macrophages and monocytes. Resident macrophage cells in the CNS are the microglial cells that execute neuroprotective action and immunological defense. Repeated insults make the microglial cells active and stimulate secretion of inflammatory mediators [19].

Autoimmunity plays a role in pathogenesis, which is still controversial. In the spinal cord of ALS patients activated microglia and T cells are found and IgG antibodies against the motor neurons.

Activated microglial cells produce many inflammatory products that mediates motor neuron injury [19]. Antibodies against voltage-gated calcium channels are found in patients with sporadic ALS that may disrupt the calcium homeostasis and increase intracellular calcium levels that damage the motor neurons [9, 19].

Neurofilaments having heavy, medium and light subunits has a role in axonal transport, and deciding the cell shape and caliber of axons can be targeted by SOD1 induced toxicity. Large caliber and neurofilament-rich motor neurons are mainly affected by SOD1 mutations and results in aggregation [4, 9]. Errors in expressions of intermediate filament subunit called peripherin, which is found in the motor neurons, its level increases in response to cellular injury and inflammation [9, 20]. Increased expression of peripherin after neuronal injury can cause MND by forming toxic aggregates composed of medium and heavy subunits sparing the light subunits [9]. Elevated homocysteine levels in the plasma stimulate production of free radicals, increase intracellular calcium level, impair mitochondrial function, depletes ATP levels and cause DNA breakage because of transmethylation of DNA and apoptosis. Neurodegeneration occurs mainly because of ATP depletion [19].

Various stages of MND are useful in predicting the prognosis of the disease

Preclinical stage - asymptomatic phase which takes long duration may be months or years.

Diagnostic stage - selective involvement of motor neurons in the anterior horn and spinal cord.

Progression stage - weakness in one leg followed by the other, patient may necessitate a wheelchair, difficulty in swallowing, respiratory muscle weakness which progress as dyspnea.

Terminal stage - respiratory insufficiency symptoms as tachypnea, orthopnea, excessive daytime sleepiness, poor concentration, memory and appetite, and confusion. In the terminal stage, patient may need assistance for respiration as a mechanical ventilator. Respiratory symptom is common in MND that develops because of weakness in respiratory muscles Progressive motor weakness and bulbar dysfunction lead to premature death, frequently from respiratory failure [3]. impaired bulbar function causing aspiration, dyspnoea may be due to infection [9].

Based on the clinical, laboratory, electro and neurophysiological studies patient are classified into three criteria certainly as “probable”, “possible” and “definite”. Revised El Escorial World Federation of Neurology was used widely for diagnosis of MND [2, 5].

Definite ALS	Upper and lower motor neuron signs present in at least 3 body regions (upper limb, lower limb bulbar, thoracic)
Probable ALS	Upper and lower motor neuron signs in atleast2 regions with some extra upper motor neuron signs.
Clinically probable ALS: laboratory support ALS	Clinical signs of upper and lower motor neuron dysfunction in one or more region, upper motor signs are found in one body part and EMG defines the lower motor neuron signs.
Clinically possible ALS	Upper and lower motor neuron signs are found in one region, UMN signs in at least two regions, UMN and LMN signs in two regions without UMN signs rostral to LMN signs

Diagnosis of ALS is usually reliable on the UMN and LMN signs with disease progression, EMG and nerve conduction studies also important. The signs of upper and lower motor neurons differ and they are presented as [3, 5]. Electromyography (EMG), and nerve conduction studies should be done for confirming the diagnosis of motor neuron abnormalities [2, 9].

UPPER MOTOR NEURON SIGNS	LOWER MOTOR NEURON SIGNS
Increased tone, Sparse wasting, active reflexes Babinski sign, Spasticity	Atrophy Flaccidity Hyporeflexia or areflexia Fasciculations

There is no specific test for MND, various investigations are done. In typical MN, motor neuron conductivity is normal and EMG reveals fasciculation and fibrillation. Lumbar puncture is done to rule out other pitfalls during diagnosing MND.

INVESTIGATIONS DONE IN A SUSPECTED MND:

Pure upper motor neuron syndrome:

1. Based on the signs MRI of brain and spinal cord
2. Transcranial magnetic stimulation techniques are used to study the central conduction time.
3. Lumbar puncture.
4. B12/ folate.

Pure lower motor neuron syndrome:

1. Genetic testing in case of slowly progressive disease.
2. EMG / Nerve conduction studies.
3. Thyroid function and routine blood analysis.

Mixed upper and lower motor neuron syndrome:

1. EMG and nerve conduction study
2. Routine blood test, thyroid function
3. HIV testing
4. MRI of brain and spinal cord.

MND prognosis is variable to every patient, most commonly patient experience progressive respiratory failure, dysphagia, difficulty in using their limbs. Slow progression is shown in some predominantly lower motor neuron dominance. Prognosis is worse with bulbar involvement and there occurs compromise in respiration [1].

MANAGEMENT:

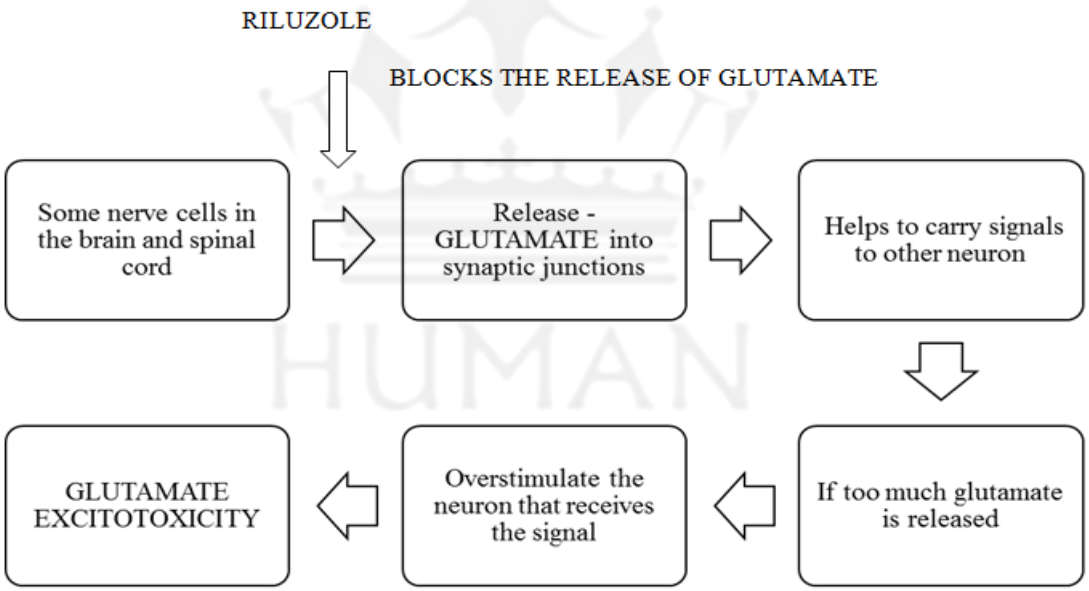
Precise and proper evaluation of the disease progression and patient symptoms are mandatory for providing the treatment guidelines. People with MND have complex needs that could be managed only by a multidisciplinary team [1]. Treatment options provided should be pharmacologic, symptomatic and supportive to modify the acquired risk factors [6, 19]. Currently, there is no proposed cure for neurodegenerative disorders. Recent advances have proven to be successful in halting the degeneration of motor neurons as gene therapy, stem cell therapy and growth factor

therapies [1, 4, 15]. Even ALS is not curable all the symptoms need to be treated, respiratory and nutritional therapy is necessary for improving the quality [3].

Pharmacologic treatment: - RILUZOLE

Riluzole is the only FDA approved treatment for ALS till date which has been available to the patients from 1996, which has revealed a survival lead of 2-3 months. It acts by blocking the glutamate release. Riluzole is useful in patients with definite and probable ALS as diagnosed by World Federation of Neurology (WFN), symptoms present for less than 5 years, FVC > 60% predicted and no tracheostomy (monograph). Contraindicated in hepatic and renal disease, elevated baseline transaminases, pregnancy and breastfeeding (mono).

Mechanism of action:



Pharmacokinetics:

Absorption:	High fat meal decreases absorption.
Bioavailability:	60%
Tmax:	0.75 hour
Protein binding	99.6% mainly to albumin
Volume of distribution	3.4 liters
Excretion	90% in feces , 5% in urine

Metabolism occurs mainly in the liver by CYP450 dependent hydroxylation and glucuronidation. Riluzole is hydroxylated at primary amine group to N-hydroxy Riluzole and glucuronide conjugation of riluzole is very slow in human liver and converted to O- and N-glucuronides.

Adverse effects:

Respiratory: Respiratory depression (10.2 – 15%)

Hepato-biliary: Abnormal ALT, AST

Cardiovascular: Hypertension, tachyarrhythmias

Gastrointestinal: Nausea, abdominal pain, vomiting, diarrhea

Neurologic: Asthenia, dizziness, somnolence

Gene therapy:

Gene therapy involves a viral vector like lentivirus or adeno-associated virus helps to transfer the genes that enhance motor neuron survival [13]. Gene products approach as a successful treatment in neurodegenerative disorders and it delivers them without the need of recombinant preparations. Genes encoding the neurotrophic growth factors or anti-apoptotic genes are considered as potentially therapeutic. Gene replacement restores beneficial protein or nucleic acid levels that can reduce mutated gene expressions. Efforts have been taken for degrading the mutant SOD1 by using siRNA to catalyze the degradation of mRNA [13]. It is considered as an authenticated research tool and it applies to reduce number of problems [4, 13]. For having increased stability of siRNA it is been given as a short hairpin RNA which is converted to a functional RNA inside host cell and successfully delivers siRNA. The provided siRNA helps in silencing the mutated gene and reduces damage [13]. Safety protocols in developing these viral vectors are done and proceeds to the clinic for ALS patient [1, 13].

Cellular therapy:

Motor neuron dysfunction and death occurs most commonly seen in ALS and SMA than other types of MND. Once the motor neurons are died one main therapy to replace the neuron will be cellular replacement. In both ALS and SMA directly replacing the motor neuron cell is a perplexing thought, it carries enormous risks as the transplanted cell should become a motor

neuron which should be able to connect to host CNS and that should also be able to produce axonal connections and form neuromuscular junction. In ALS to renovate the motor circuit replacement of corticospinal tracts is obligatory. Neural stem cells (NSC) can be used to isolate and expand multipotent cells from multiple regions of fetal or adult nervous system. These stem cells are used as a biological tool to understand the disease and it could be used to halt the rate of damaging neurons, facilitate axonal growth or glial function. They have the ability to differentiate into oligodendrocytes and myelinate the damaged neurons and host axons [21].

Motor neurons generated by exposing mouse embryonic stem cells to retinoic acid that helps to neutralize and establish a caudal position identity for the pluripotent stem cells and development of morphogen sonic hedgehog (Shh) or their chemical agonists. When these cells are transplanted to a human mammalian nervous system, they survived and few has shown the ability to form neuromuscular junction with the skeletal muscle and could partially restore the function [4, 21].

Growth factor therapies:

Neurotrophic factors have dominant effects in treatment of ALS in animal models, and it has evidenced that increased levels of factors would benefit the motor neurons. Insulin-like Growth Factor (IGF) and Vascular Endothelial Growth Factor (VEGF) have shown efficacy in animal models after they have been introduced inside them. Efficacy achieved by delivering the growth factors by two approaches

I Viral vectors

II Infusing the tropic molecules directly into the brain or spinal cord

For the earlier method, the gene encoding IGF1 was inserted into adeno-associated virus and injected into the animals. Results of these have proven that injection into the muscle yielded a viral expression within the motor neurons for about 1 year and has slowed the disease progression [4]. VEGF has an ability to protect hippocampal neurons from glutamate excitotoxicity[15]. It has increased angiogenesis in the chamber that produces neuroprotective effects and enriched Schwann cell proliferation and migration. However, considering VEGF therapy, careful dosing and optimization is mandatory to avoid hemangioma [15].

Symptomatic treatment:

Despite the treatment available, aim of this symptomatic therapy is palliative and aim to improve the quality of life.

Cramps	May reduce with time, rarely needs treatment
Spasticity	Baclofen,Tizanidine
Sialorrhoea	Hyoscine patches, botulism toxins into salivary ducts
Depression	Amitriptyline
Emotional liability	Tricyclic antidepressants, selective serotonin reuptake inhibitors
Constipation	Lactulose, Senna
Respiratory symptoms	Ventilator aids (Mechanical ventilation or tracheostomy)
Dysphagia	Percutaneous Endoscopic Gastrostomy (PEG)
Anxiety	Benzodiazepines
Weight loss	Monitored by a dietician and appropriate nutrition

Various other attempts are being done for improving the quality. Methylcobalamin (MeCbl) reduces the elevated homocysteine levels which are proven to be toxic to the motor neurons. It is also useful in reducing the oxidative stress, glutamate toxicity and apoptosis that could ultimately reduce the progression of the disease [13, 22]. Protandim another moiety which is obtained from five different plants*: Silybummarianum* (milk thistle), *Cameliasinensis* (green tea), turmeric, *Withaniasomnifera* (Ashwagandha), and *Bacopamonniera*. This oral tablet can activate a nuclear factor erythroid 2-related factor (Nrf2) an intracellular molecule activating Antioxidant Response Element (ARE) that upregulates 200 or more antioxidant and anti-inflammatory genes [5].

Gluten, a protein found in wheat, rye and barley can cause allergic reactions in 5% of people. Some can also have neurologic symptoms, gluten-related disorders are thought to be auto-immune and have alterations in T-lymphocytes and involve certain antibodies. Unfortunately, GRD antibodies that target TG6 antigen is found on spinal motor neurons, which points out that gluten induced autoimmunity can cause a motor neuron disease. Gluten free diet can reduce damage to motor neurons [24]

THERAPY FOR ALS:

Glutamate antagonists	Riluzole
Antioxidants	Vitamin E Creatine Selenium Coenzyme Q Acetylcysteine
Neurotrophic factors	Brain-Derived Neurotrophic Factor Insulin-like Growth Factor Thyrotropin Releasing Hormone Glial-Derived Neurotrophic Factor
Immunomodulatory approaches	Plasmapheresis Interferon Gangliosides Intravenous immunoglobulin
Antiviral agents	Amantadine Tilorone

CONCLUSION

Motor neuron diseases are a heterogeneous group of disorders that may be caused because of hereditary, environmental or sporadic causes. Mutations in the genes can cause many pathologic abnormalities that could damage the motor neuron. Isolation of different gene mutations that cause ALS pave way for future treatment options in improving the quality of life of MND patients. Symptoms of MND depend on the type of motor neuron involved and vary accordingly. Treatment rely on the symptoms and usually supportive. Multidisciplinary team care is needed for management of prognosis of MND.

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