

Amyotrophic Lateral Sclerosis – A Comprehensive Review

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Abstract: Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, generally said to affect people with an age between 50 years and 70 years. Signs of upper motor neuron and lower motor neuron damage, which are not explained by any other disease process, and are the reasons behind ALS. It attacks the neurons in the brain and spinal cord. These neurons transmit messages from brain and spinal cord to the voluntary muscles. At first, it causes mild muscle problems. Some people show symptoms like difficulty in walking, running, writing and speech. Eventually, the patient may lose the strength and cannot move. Due to the depletion of neuronal transmission to muscles in the chest, the patient may report difficulty to breath. The loss of lower motor neurons leads to flaccid paralysis, decreased muscle tone, decreased reflexes, muscle weakness, muscle atrophy. The defining feature of ALS is the death of both upper and lower motor neurons in the motor cortex of the brain, the brain stem, and the spinal cord. There are small eosinophilic, hyaline intra-cytoplasmic inclusions that stain positive for cystatin and transferring and are present in 70–100% of cases. Environmental and lifestyle factors play a major role in the development of ALS, but no conclusive evidence is available to support making specific changes to decrease the risk of the disease, Genetic mutations can lead to inherited ALS, which appears nearly identical to the non-inherited form. Patients with ALS generally have higher levels of glutamate in the brain, around the nerve cells in their spinal fluid. ALS patients may experience pain involving more than one type, no rigid treatment program that involves the sole use of a single agent should be employed to treat ALS-associated pain conditions. Opioids have proven to be effective in providing pain relief in advanced disease. Nevertheless, these therapies lack the ability to induce long-term lasting effects without constant administration. Over the last decade awareness of ALS has significantly increased, diagnosis is being achieved in a more timely fashion, and overall care is better. This review gives a comprehensive information about the symptoms, pathophysiology common causes, drugs currently used in the treatment.

Keywords: Amyotrophic Lateral Sclerosis (ALS), Lou Gehrig's Disease, Symptoms, Pathophysiology, Drugs, *Invitro*, *In vivo* Models.

1. INTRODUCTION

Neurodegenerative diseases are defined as the hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. These disorders are also associated with atrophy of the affected central or peripheral nervous system. The framework of health information on neurodegenerative diseases may also include brain diseases, which are pathologic conditions also affecting the brain (composed of the intracranial components of the central nervous system). Cerebral cortex; intracranial white matter, basal ganglia, thalamus, hypothalamus, brain stem, and cerebellum are included. Degenerative nerve diseases include Alzheimer's disease, amyotrophic lateral sclerosis, Friedreich's ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, spinal muscular atrophy, these and all these disorders are related with atrophy of the affected central or peripheral nervous system. Neurodegenerative disorder is the basic term used for the progressive loss of structure or function of neurons, including results in death of neurons. These diseases are mostly incurable, which leads to neurodegeneration or death of neuron cells. Degenerative nerve diseases, decreases many of the body's activities such as balance, movement, talking, breathing and heart function. These diseases are usually genetic. Some of the medical condition such as alcoholism, a tumor, or a stroke also contributes to neurodegenerative disorder. Other causes may include toxins, chemicals and viruses, but sometimes the cause may not be identified. Treatment may be given to improve symptoms, relieve pain, and increase mobility. Recently, the complexity of the diseases and traits have been focused less on individual susceptibility variants and instead the biological pathways and networks were studied. Pathologically, the characteristic of these diseases included are accumulation and aggregation of abnormal or misfolded proteins, as with amyloid- β ($a\beta$) in Alzheimer's disease (AD), α -synuclein in Parkinson's disease (pd),

huntingtin protein in Huntington's disease (HD), and Trans-Active response DNA-binding Protein 43 (TDP-43) in Fronto Temporal Dementia (FTD) and Amyo-Trophic Lateral Sclerosis (ALS).

2. AMYOTROPHIC LATERAL SCLEROSIS

ALS is one among the neurodegenerative diseases by which nervous system gets affected. It was first described in 1869 by a French neurologist Jean-Martin Charcot. It is also known as Lou Gehrig's disease, which affects around 2,000 people per year in England and Wales, with a peak age of onset between 50 years and 70 years(1) . In the United States, when baseball player Lou Gehrig was diagnosed with the disease in 1939. It is also named as Charcot disease in honor of the first person to describe the disease, Jean-Martin Charcot. It causes the gradually break down and death of neurons. A-myotrophic comes from the Greek language. "a" refers no, "myo" means muscle and "trophic" refers nourishment – "no muscle nourishment." when a muscle gets no nourishment, it "atrophies" or wastes away. "Lateral" refers to the areas in a person's spinal cord where the portions of the neurons that signal and control the muscles are located. ALS occurs more in men than in women by a factor of between 1.2 and 1.5 as the muscle degenerates it causes scarring or hardening (sclerosis) in the particular region(2). Degeneration of the motor neurons in ALS eventually leads to the death of the neurons. When it occurs, the ability of the brain to initiate and muscle movement control is lost. Since voluntary muscle action gets affected, the patients may lose their ability to speak, eat, move and breathe. It may also be said that it is neurodegeneration with prodilection for the corticomotoneurons in the motor cortex and the bulbar and spinal motor neurons. Mainly the motor neurons are selectively targeted for degeneration in this disease. In 1993, missense mutations in the gene encoding the antioxidant enzyme Cu/Zn Super Oxide Dismutase-1 (SOD-1) were discovered in subsets of patients diagnosed with familial ALS. The pathogenesis is complex and multifactorial and disease commonly characterized by stiff muscles, muscle twitching, and gradually worsening weakness due to decrease in size of muscle. This leads to difficulty in speaking, swallowing, and eventually breathing. Sometimes spasticity may develop in the weakened atrophic limbs, which affects the manual dexterity and gait. Neurodegeneration is most commonly seen in the anterior horns of the spinal column, which hold the nerve roots which are responsible for control of the muscles of the trunk or head. It causes progressive deterioration of both the upper motor neurons of the corticospinal tract, which helps to conduct impulses from the brain to the spinal cord, and the lower motor neurons, which helps to carry the impulses from the corticospinal tract to the individual muscles in 90% to 95% of cases the cause is unknown. A very high frequency of ALS that often presents with parkinsonism and dementia was seen in the Chamorro tribe, who live on the pacific island of Guam about 5–10% of cases are genetically inherited(3). The incidence of ALS in European populations is usually two to three people per year 100,000 general populations about half of these genetic cases are normally caused due to one of two specific genes (4) namely SOD-1 and TARDBP. It results in the degeneration of the neurons which control voluntary muscles. The disease usually seen around the age of 60 and in inherited cases in the age of 50. The average survival of an individual from onset to death is three to four years and only about 10% of individuals survive longer than 10 years. Most of the ALS cases are sporadic, but 5–10% of cases are familial, and of these 20% have a mutation of the SOD-1 gene and about 2–5% have mutations of the TARDBP gene. Mutations occur in two percent of apparently sporadic patients, and TARDBP mutations also occur in sporadic cases. The main focus of pharmaceutical management is on relieving excess salivation, breathlessness and malnutrition. Life expectancy can be extended by about two to three months by the support of drugs. There is currently one FDA approved drug, riluzole which slowdown the progression of ALS in some cases. The better quality of life can be experienced by ALS people by participating in support groups and attending an ALS association certified treatment center of excellence or a recognized treatment center. The major evidence from multiple population registries is that, after a peak at about age 75 years, the risk plateaus usually decline. However, as in certain cases as older patients are more likely to be referred to non-neurological services, which means ALS may also under-recognized in elderly patients (5,6,7). A clear data about the incidence of ALS Asian continent is not found.

3. SYMPTOMS OF ALS

Symptoms are first noticed in hands, feet or limbs, and then it spreads to the other parts of the body. As the disease progresses, muscles become weaker. Treatment focus mainly on supportive care provided by a multidisciplinary team, that provides more comfort to the patients in response to symptom(8). The weakness affects chewing, swallowing, speaking and breathing ability of the patient. Symptoms usually start in younger people and do not develop until the age 50, later it causes loss of muscle strength that eventually gets worse and makes it impossible

to do routine works such as climbing steps, getting out of a chair, or swallowing. Breathing or swallowing muscles are the first to be affected. It rarely affects urine or bowel function, eye movement, or the ability to think or reasoning. Early signs and symptoms of ALS include difficulty in walking, tripping or difficulty doing normal daily activities, weakness in legs, feet or ankles, hand weakness or clumsiness, slurring of speech or difficulty in swallowing, muscle cramps and twitching in the arms, shoulders, muscle contractions called fasciculations may occur. Muscle weakness that slowly gets worse to paralysis, speech problems, such as a slow or abnormal speech pattern (slurring of words), voice changes, hoarseness, weight loss are also usually seen.

4. PATHOPHYSIOLOGY OF ALS

ALS is a progressive neurodegenerative disease, in which both the upper and lower motor neurons are degenerated. Due to this the anterior and lateral columns of the spinal cord are replaced by fibrous causing harden of these areas.

About 10% are familial ALS cases, usually with an autosomal dominant pattern of inheritance (9,10). Considerable diagnostic delays are caused by the unusual clinical picture together with lack of neurophysiological evidence of denervation in other regions (11). These two variants show slower progression when compared to more typical forms of ALS (12,13). More than 90% of ALS cases are sporadic and they are not associated with any other known gene or abnormalities of SOD-1. The most common disease-causing mutation occurs in the superoxide dismutase (SOD-1) gene, although other mutations are identified (14). The degeneration of the corticospinal upper motor neurons (UMN) that reside in the cerebral cortex specifically the precentral gyrus caused by ALS. Loss of UMN leads to spastic paralysis, hyperreflexia it is the syndrome that is characterized by a child's precocious ability to read significant difficulty in understanding and using verbal language and significant problems during social interactions, stiffness. The degeneration of the Lower Motor Neurons (LMN) that resides in the anterior horn of the spinal cord and in the brainstem caused by ALS. Marked prolongation in the Central Motor Conduction Time (CMCT) is seen in Familial Amyotrophic Lateral Sclerosis (FALS) patients with D90a SOD-1 mutations and patients with the flail arm and flail leg variants (15,16,17) in addition to the presence of subcategories of ALS there are also pathological subtypes of ALS that partially overlap with the clinical phenotype and genetic substrate based on phenotype and genetics. Most patients with ALS have ubiquitinated inclusions that stain for Trans Active Response (TAR) DNA-binding protein 43 (TDP-43). Fasciculations were followed by antidromic impulses in the test unit axon as judged from collision test, and they persisted after lidocaine blockades of the nerve to muscle (18). Flaccid paralysis, decreased muscle tone, decreased reflexes, muscle weakness, muscle atrophy caused due to the lower motor neurons. The death of both UMN and LMN in the motor cortex of the brain, the brain stem, and the spinal cord is the defining feature of ALS. There are small eosinophilic, hyaline intracytoplasmic inclusions that stain positive for cystatin and transferrin, and these are present in 70–100% of cases prior to their destruction, motor neurons help in development of protein-rich inclusions in their cell bodies and axons. This may be partly caused due to the defects in protein degradation. These inclusions often contain ubiquitin, and generally incorporate one of the ALS-associated proteins like SOD-1, TAR DNA binding protein (TDP-43, or TARDBP), and/or fus-proposed hypotheses for the pathogenesis of amyotrophic lateral sclerosis; glutamate excitotoxicity - glutamate is a prime candidate for the cause of motor neuron excitotoxicity because it is the principal excitatory neurotransmitter in the human motor system, including the corticospinal tract, spinal cord interneurons, and cortical association pathways. The concentration of glutamate is 20,000-fold higher intracellularly when compared to extracellularly. Copper mediated conversion of hydrogen peroxide to reactive hydrogen radical, promoting oxidative damage is catalyzed by free-radical-mediated oxidative stress-SOD-1 mutations. The increased oxidative stress is caused by higher levels of protein carbonyl groups and oxidized nucleic acids in brain homogenates from patients with sporadic ALS. In addition, fibroblasts from patients with mutant SOD-1 appear more sensitive to oxidative stress which is caused by hydrogen peroxide. Basic Metabolic Index (BMI) is a generally accepted marker of nutritional status: in a study of 55 ALS patients prospectively followed-up, BMI score < 18.5 kg/m² was an independent prognostic factor of death in both univariate and multivariate analyses (19) a loss of 5% in usual weight at the time of diagnosis is also associated testing antioxidant therapies in amyotrophic lateral sclerosis and developing potential biomarkers of disease provided by this (20). To contribute to mutant SOD-1 mediated damage to mitochondria several mechanisms have been proposed. Disruption of energy metabolism, impaired protein import machinery, and impaired calcium buffering are the mechanisms. Due to the immune or inflammatory reactions increased intracellular calcium and motor neuron degeneration get occurs. Neuroinflammation within the central nervous system are mediated by the microglia of immune cells. Activation of the microglia through the release of cytotoxic and inflammatory substances, such as oxygen radicals, nitrous oxide, glutamate, cytokines

and prostaglandins by CNS injury is one of the important events. In ALS tissue activation and proliferation of microglia in regions of motor neuron loss has been observed. Common feature among neurodegenerative diseases are protein aggregation-intracellular inclusions. Neurofilament abnormality-neurofilament is cytoskeleton elements whose normal production and transport to the termini are critical to the health and integrity of motor neurons. In ALS as neurofilaments accumulate in proximal axons neurofilament abnormality may play a role in the pathogenesis of ALS. Various studies evaluating respiratory status has found that a lower predicted forced vital capacity (FVC) rate (FVC %) at diagnosis was the most relevant prognostic factor in ALS (21). The intermediate protein expressed in spinal motor neurons, peripheral sensory neurons, and autonomic nerves is Peripherin. Impaired axonal transport is dynein and dynactin. Dynein helps in the retrograde transport system. Positioning the endoplasmic reticulum and Golgi as well as in assembly of the mitotic spindle, in neurons also the important roles of dynein. It is the only known mechanism for retrograde transport. Similarly, disruption of the dynactin complex, an activator of cytoplasmic dynein, inhibits retrograde axonal transport, provoking a late-onset, progressive motor neuron disease. A LMN disorder is caused in humans due to a dominant point mutation in the P150 subunit of dynactin. Reciprocal interactions between motor neurons and surrounding astrocytes might play a crucial role in ALS pathogenesis Glial cell abnormality proposed by Rao and Weiss. Damaging reactive oxygen species produced in motor neurons could exit motor neurons and induce oxidative disruption of glutamate transport in surrounding astrocytes according to this model. This would exacerbate excitotoxic stress to motor neurons and result in a vicious cycle. Abnormal RNA metabolism- pathogenesis of ALS being linked to RNA regulation, specifically associated with the role of microRNA(miRNA) has been reported. The miRNA IS involved with mRNA degradation, and it is associated with both Fluid in Sarcoma/translocated in liposarcoma (FUS/TLS)and TDP-43 (proteins with amyotrophic lateral sclerosis).

5. CAUSES OF ALS

There are two different types of ALS sporadic (randomly occurring) and familial. Around 10% of cases sporadic ALS and is inherited, with the offspring of a person with ALS having a 50% chance of developing the condition. Upto 50% Of ALS patients having the symptoms like cognitive impairment and dementia-like symptoms, typically related to frontotemporal executive dysfunction. Parkinsonian symptoms, facial masking, tremor and postural instability may also develop in ALS patients (22). In the western pacific exposure to Beta Methylamino L-alanine(BMAA) has been proposed to explain the high incidence of ALS–parkinsonism dementia complex (PDC) (23). In one meta-analysis, pesticide use was found to be significantly associated with a higher risk of ALS in men (24). In addition to genetic factors contributing to the heritability of familiar ALS, there are different. The speed of disease progression varies considerably between patients (25) some of the body's cells, potentially killing nerve cells are attacked by the immune cells. Higher levels of glutamate, a chemical messenger in the brain, near the motor neurons are seen in the people with ALS. High content of glutamate is known to be toxic to nerve cells. If proteins are not processed correctly by nerve cells, abnormal proteins could potentially accumulate and results in the death of nerve cells. On chromosome 1, and codes for the DNA-binding and RNA-binding protein TDP-43 theTARDBP gene is located. Thus 30 mutations of this gene have been identified in about 5% of patients with familial ALS and in 1% of patients with sporadic ALS (26) environmental and lifestyle factors likely play a role in the development of ALS, mechanical or electrical trauma, military service, high levels of exercise, high levels of agricultural chemicals,high levels of a variety of heavy metals also increase ALS. Gene mutation- various genetic mutations can lead to inherited ALS, which appears nearly identical to the non-inherited form. People with ALS generally have high amount of glutamate, a chemical messenger in the brain, around the nerve cells in their spinal fluid. Some of the nerve cells become toxic due to too much glutamate. It is involved in up to 40% of familial ALS cases in the Us and Europe, and in up to 5–11% of sporadic ALS cases (27). Sometimes a person's immune system begins attacking some of his or her own cells, which may lead to the degeneration or death of nerve cells. The imbalance between the production of oxygen-containing molecules that carry an electrical charge, which can be toxic, and a biological system's ability to readily detoxify them causes oxidative stress. Oxygen-containing charged particles are byproducts of cellular metabolism. The mitochondria are microscopic energy "factories" which are present inside cells. They resemble miniature cells themselves and have their own DNA. Abnormalities of the mitochondria may be seen in ALS causation and/or progression. The immune system, particularly immunologic cells in the nervous system known as microglia, can be both beneficial as well as harmful in ALS. Cell death is caused due to cellular adenosine triphosphate (ATP) production is decreased and calcium homeostasis is disturbed, triggering apoptosis (28) microglia may be protective up to a certain point and then become damaging of cells in the body. Glutamate helps in carrying the signals between neurons

(nerve cells), and there may be too much of glutamate in ALS. Glutamate is one of the neurotransmitter chemicals in the nervous system that carries signals between nerve cells. There is a result that in ALS glutamate accumulates in the spaces around a nerve cell after it has completed its signaling function, causing problems for the nerve cells. Globally, mutations in the *SOD-1* gene are found in 10–20% of familial and in 1–5% of sporadic ALS cases. More than 170 mutations of *SOD-1* have been identified in ALS till today. Most *SOD-1* mutations are inherited, except for the d90a mutation, which is the most common *SOD-1* mutation worldwide, which is inherited in both a dominant and recessive manner (29), cyanobacteria are also found in some bodies of water which is toxic. The heavy metals lead, mercury and arsenic, although they can be toxic to the nervous system. There are some variants of genes that may increase susceptibility to the development of ALS. Smoking is the only probable risk factor for ALS. Intriguingly, smoking may be a risk factor in women, especially postmenopausal women, but not in men. the controversy over the role of smoking in ALS appears to still be unresolved (30).

6. DRUGS USED FOR TREATMENT OF ALS

Only a few drugs are available for the treatment of ALS patients. The details of the drugs used for the treatment are discussed below:

Riluzole

Riluzole (2- amino-6 [trifluoromethoxy] benzothiazole) has complex actions in the nervous system. Riluzole is administered orally and is highly protein bound in nature. Its half-life is about 12 hours and undergoes extensive metabolism in the liver in which both Cytochrome p450- mediated hydroxylation and glucuronidaion are involved, an antiglutaminergic, is the only disease-modifying agent currently licensed for use in ALS thus, periodic monitoring is needed(31). It also functions as a glutamate antagonist, became the only drug that received approval by the us FDA for the treatment of ALS (32,33).

The drug slows the disease's progression in some people, perhaps by reducing levels of brain glutamate which is often present in higher levels in people with ALS. It also non-competitively inhibits the action.

Riluzole tablets should be swallowed although the tablets can be crushed and mixed with soft food for patients for easy swallowing. Crushed tablets should be taken immediately due to stability problems. Riluzole does not dissolve well in water, and should not be administered by a percutaneous endoscopic gastronomy (PEG) feed as it may block the tubing (34) citatory amino acids postsynaptically(35)riluzole has further mechanisms of action including the inactivation of voltage gated sodium channels and activation of g-protein-dependent processes (36).The side effects of Riluzole includes dizziness, gastrointestinal conditions and liver function changes. Other symptoms in ALS patients include muscle cramps and spasms, spasticity, constipation, fatigue, excessive salivation, excessive phlegm, pain, depression, sleep problems, uncontrolled outbursts of laughing or crying. Other methods of medications may include therapy like breathing care, physical therapy, occupational therapy, speech therapy, and nutritional support, psychological and social support.

Baclofen

Baclofen is safe and effective means for treating spasticity following with reduction in frequency of spasms and clonus and there was improved range of joint movement which enable patients to maintain functional status for prolong periods(37). It is a white in colour, mostly odorless crystalline powder, with a molecular weight of 213.66 g/mol. It is insoluble in water and poorly soluble in most organic solvents. Further, it exhibits low partition coefficients into organic solvents.

The dose of Baclofen normally used to treat ALS is 5 mg /, 3 times / day, increasing upto 20 mg / time. It is given small doses, gradually the dose should be increased. Patients can operate autonomously, according to the severity of the symptoms. Jerks flexor and extensor spasm or reduce spasticity, muscle tension etc are reported. Hence the patients can operate autonomously, to minimize the occurrence of adverse reactions. Adult principle, the initial dose of 5 mg each time, three times a day; after careful gradually increase, such as every three days to every 10 mg three times a day, until the results have spasticity. Usually, the appropriate dose of 30 to 70mg. A small number of patients with serious symptoms need to increase to 100 to 120 mg daily.

Tizanidine

Tizanidine is a central alpha-2-adrenergic receptor agonist and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. The effects of tizanidine are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. Tizanidine is an agonist of α_2 adrenergic receptors in the inhibition of motor neurons.

Zanaflex (tizanidine hydrochloride) is a central alpha2-adrenergic agonist. Tizanidine HCl is a white to off-white, fine crystalline powder, which is odorless or with a faint characteristic odor. Tizanidine is slightly soluble in water and methanol; solubility in water decreases as the pH increases. Its chemical name is 5-chloro-4-(2-imidazolin-2-ylamino)-2, 1, 3-benzothiazole monohydrochloride. In an open label study, patients with neuropathic pain received 1 to 4 mg of tizanidine once daily for 7 days, followed by weekly dose escalation of 2 to 8 mg his/her effective or maximum tolerated dose or a maximum of 36 mg over an 8 week period(38).

7. DRUGS UNDER CLINICAL TRIALS

The drug Mastinib is now under clinical trials for the treatment of ALS. It has a mechanism of action which inhibits the receptor tyrosine kinase c-kit which is displayed by various types of tumor. It also inhibits the platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGF) and it has molecular weight of 498.(39,40,41,42). Mycoplasmas are infectious agents involved in several diseases including neurological diseases (rate 50% - 80%). Our results are similar to those reported in the literature regarding the implication of Mycoplasmas in ALS, but in this study, we also reported the presence of antibodies against lamps of Mycoplasmas which may be involved in damage of the host cell. Riluzole had a significant effect on rates of survival and muscular deterioration in this randomized, stratified, double-blind, placebo-controlled study of 155 patients with ALS. The favorable effect of riluzole on survival cannot be explained by other confounding factors. When we considered the previously reported predictive variables that influence survival in ALS, we could not identify any statistically significant difference between the placebo group and the riluzole group at entry. The favorable effect of riluzole on survival seems to depend on the site of onset of disease. A large and significant effect was observed in patients with ALS of bulbar onset, whereas in those with disease of limb onset only a trend toward a positive effect was detected. Clearly, riluzole was less effective in patients with limb-onset disease, but at this point we cannot precisely account for the differences with respect to the pattern of onset. Such a striking difference between subgroups must, however, be interpreted carefully because, as pointed out, such an effect can arise by chance. Therapeutic effect of riluzole seems to be time-related, with a strong effect observed in the first 12 months and an apparent decrease in effect from month 12 to month 21. Although the overall number of patients with at least one adverse reaction was similar in the two study groups, there was a significantly higher proportion of drug-related withdrawal from treatment in the riluzole group. The reason for these withdrawals included asthenia, stiffness, and increases in aminotransferase levels. Adverse drug reactions can worsen the quality of life, but such consequences may be outweighed by the effect of the drug on muscle function. Further clinical trials, such as a study of dose ranges, are needed before riluzole can be offered as a treatment in amyotrophic lateral sclerosis.(39,40,41,42)

8. CURRENT RESEARCH TOPICS IN ALS

The relevant research conducted in ALS states that clinical investigations, however, provide only a small window of insight into the body of clinically. Availability and safety of investigational drugs are dependent on the clinical investigations. Basic and pre-clinical research on ALS has increased dramatically in recent years. More than half of the 8,182 research papers published on ALS between 1945 -2005. Almost a third of all scientific papers on ALS have been published within the past two years.

9. INVITRO MODELS IN ALS

In ALS, the earliest such models were *in vitro* cell culture models, typically in which motor neurons (or cells with similar properties) were subjected to a specific chemical, viral, or bacterial insult which led to their death. Recently, in an *in vitro* study by Shanmukhapuvvada and Vankayalapati, (chitosan nanoparticles were formulated to carry riluzole to the brain(43). Thus, a great number of *in vitro* studies have been based on models in which excitatory compounds are administered to healthy neurons in toxic doses – a model of disease which, despite rather effectively causing motor neuron death, replicates only a portion of the observed biological phenomena in ALS. In the absence of a reliable animal model of disease, *in vitro* models were for a long time the primary disease model for testing possible treatments and despite the availability of animal models for ALS, the use of *in vitro* models has increased exponentially in recent

years with the onset of high-throughput screening programs. *In vitro* results have also been the laboratory basis for at least 6 clinical trials. However, a conflicting report observed that increasing levels of human wild type TDP-43 from 1.9-fold to 2.5-fold above endogenous levels, resulted in neurodegeneration and reduced life span to between 4 and 8 weeks (44). Because of the wide number of *in vitro* cell culture models proposed for ALS, and there is only partial replication of disease pathology, it is nearly impossible to point to any one standard cell culture model in ALS. Typically, researchers have used a variety of assays – not all of which are necessarily ALS specific – in order to identify drugs which, have high level of activity against a range of processes known to cause or exacerbate neurodegeneration. A recent trend *in vitro* models of ALS has been an increasing focus on organotypic co-cultures of motor neurons and supporting cells (astrocytes, microglia, or both.) flag-tagged human wildtype TDP-43 in an adeno associated virus (AAV) 1 vector was injected directly into the side of the dominant hand of the cervical spinal cord of adult cynomolgus monkeys. The AAV used in this study has a preference for infecting terminally differentiated cells, and tagged TDP-43 was only observed in motor neurons (45)

10. IN VIVO MODELS FOR TESTING ALS DRUGS

In vivo models, in which diseases are modeled in complete organisms rather than in cultured cells, provide a much more holistic approach to modelling disease compared to *in vitro* models. The earliest *in vivo* models for neurodegenerative diseases like ALS were general neuronal injury models created by injuring or administering a neurotoxin to the animal being studied, and hereditary models in which animals expressed naturally occurring mutations that caused ALS-like symptoms.

11. NATURALLY OCCURRING RODENT MODELS

A number of naturally occurring mutations have been shown to cause ALS like syndromes in mice. One of the most widely used naturally occurring animal models of ALS – especially prior to the discovering of the involvement of SOD-1 in familial ALS is the mouse, which had been identified in the mid twentieth century but first appeared in the literature on ALS in the early 1980s. The expression of the mutant TDP-43 in these mice was approximately 3- fold greater than endogenous TDP-43 levels for all wild type and mutant TDP-43 lines. In all lines this resulted in a significant down regulation of endogenous TDP-43 levels, between 3 and 10 months of age technically, the Wobbler mouse does not have ALS – instead, it expresses a phenotype that has been compared to both progressive muscular atrophy (a variant of ALS affecting primarily lower motor neurons) and Werdnig Hoffman disease (a form of hereditary infantile spinal muscular atrophy.)

Riluzole, thyrotropin releasing hormone (TRH), insulin like growth factor I (IGFI), hyperbaric oxygen therapy, n-acetyl-Lcysteine, various steroids, 7-nitroindazole, leukaemia inhibitory factor (LIF), and SOD-1 supplementation have all been tested using Wobbler mice, with significant benefit. One group has reported that expression of human wild type TDP-43 at 3-4-fold greater levels than endogenous TDP-43 did not result in any deleterious effects other than mild gliosis and ubiquitin aggregation within the spinal cord. The Wobbler mouse's positive response to a wide range of treatments that have proved futile in clinical trials has cast doubt on its appropriateness as a model for ALS. The variation in the severity and development of deleterious effects of mutant TDP-43 is likely to be due to a combination of the differences in choice of promoter, gender, level and timing of transgenic expression and type of protein(46). However, there may also be confounds associated with the down regulation of endogenous mouse TDP-43 (47, 48).

12. TRANSGENIC & KNOCKOUT RODENT MODELS

Transgenic models of disease provide a promising means of overcoming the limitations of naturally occurring disease models. In transgenic models, the observed disease phenotype is caused by the same gene or genes that cause disease in human patients. Despite crucial genetic, physiological, and immune system differences between mice and humans, the mutated genes that cause diseases in people will frequently cause same diseases in animals. In support of this possibility, induction of transgenic expression from 21 days of age did not result in any behavioral phenotype up to 12 months of age. However, cortical atrophy and pathology were evident, albeit in the absence of mitochondrial aggregation, leading the authors to suggest that abnormal cytoplasmic aggregation of mitochondria was a developmental effect, and that induction of transgenic expression from 21 days of age resulted in pathological hallmarks of TDP-43 proteinopathies(49). Transgenic technology also offers the possibility of accelerating the onset date of late onset diseases – by inserting multiple copies of a disease causing gene. Thus, the researchers can significantly reduce the time taken for the mutation to cause disease and increase the pace of research. The first

transgenic mouse model for ALS was created through over expression of the human neuron filament heavy (NFH) subunit. Involvement of cytoskeleton components in ALS pathogenesis is supported by several mouse models of motor neuron disease with neuron filament abnormalities and with genetic defects in microtubule-based transport(50).

13. CONCLUSION

Clinically this review clearly indicates that ALS is prevailing in almost all countries, but least awareness and studies are carried out in developing countries. The clinical symptoms were closely associated with other neurodegenerative disorders much more complicates the accurate diagnosis and consequently the quality of treatment for this disease. Pain in ALS is a commonly overlooked, understudied, underrated, and potentially undertreated aspect of the disease. Family respondents also identified that there was a need for more guidance and support to deal with the pain of the patient and nearly 30% of respondents believed that medical staff was reluctant to medicate. These pain-related barriers to medical care echo earlier reports investigating pain among the elderly where nearly 50% of dying patients lack adequate pain treatment at the time of death. Further, the gaps and scopes for basic and applied research on the causes as well as pathophysiology of ALS were highlighted in this review. Nevertheless, these therapies lack the ability to induce long-term lasting effects without constant administration. Over the last decade, awareness of ALS has significantly increased, diagnosis is being achieved in a more timely fashion, and overall care is better. Epidemiological studies of ALS are complicated due to variability of signs and symptoms and low incidence, so it is difficult to include a high number of patients.

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DOI: <https://doi.org/10.15379/ijmst.v10i5.3569>

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