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Duchenne Muscular Dystrophy; A Mechanistic Approach

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ABSTRACT

Muscular dystrophy is known to be an X-linked rare inherited disorder that belongs to a group of a disorder called dystrophinothies, it is characterized by muscles weakness and reduced muscle tone, which is caused by a mutation in the gene which encodes the protein namely dystrophin, it is essential for maintaining the muscle cell integrity. Duchenne muscular dystrophy is commonly seen in most of the population. It mainly affects the male child individual under the age of 3-6 years, it has an incidence of one in 5000 no. male individual. Untreated patients become wheelchaired and they died at the age of 12th to 15th years, death is mainly due to complications of cardiorespiratory. There is no proper treatment available and it is believed that improvement in patient care and disease management reduce the progression of the disease. Further research is going on to develop effective treatment of muscular dystrophy. Gene addition, exon skipping, and stop codon readthrough can be found to restore the functional protein. In this review pathophysiology associated with DMD, Investigation, Hypothesis in DMD, and Management approach is reviewed.

INTRODUCTION

Muscular Dystrophy is characterized by a group of genetic disorders called dystrophinothies. It is considered a genetically inherited disorder caused by a mutation in the number of a gene that makes essential protein for the stability of a muscle cell. In this diseased state, individuals lose control over the muscle and felt weak, and progressive degeneration of muscles occurs. There are approximately 9 types of muscular dystrophy found such as Duchenne, Becker, Congenital, myotonic, Emery-Dreifuss, Facioscapulohumeral, Oculopharyngeal, and limb-girdle muscular dystrophies, among them Duchene Muscular dystrophy most commonly seen. Duchenne muscular dystrophies is named afterFrench neurologist Duchenne de Boulonge. It is an X-linked inherited muscle disorder; it has been found in one case in every 5000 no. boys [1]. Due to the localization of the dystrophin gene on the X-chromosome, DMD is predominantly found in males. Each disease has its severity of the action, the difference in the occurrence of age group, the difference in inheritance pattern and they affect different muscles and other organs. Duchenne muscular dystrophy is commonly seen in early childhood at the age of 3-6 years and it is characterized by symptoms like proximal muscles weakness, gross motor delay, cardiomyopathy, arrhythmia, facial weakness with drooping of eyes lid, gait abnormalities, Scoliosis, difficulty in rising from the ground [2]. Pain and Swallowing difficulties may also be seen. The impairment in the cardiac and respiratory systems leads to the main cause of death of patients. Diagnosis of these devastating diseases may require a patient medical history, age of the patient, family history, investigation of serum creatinine phosphokinase, electromyography, and muscle biopsy. If treatment is not provided then it causes the patient weaker and finally dependent on a wheelchair by the age of 12thand after the progression of the disease, patients die in their late teens. In myotonia dystrophy, the endocrine system is also affected. Successful treatment of Duchenne muscular dystrophy is still unknown but advances in management therapy significantly improved the patient's condition and improve quality of life [3].

Prevalence and epidemiology

Concerning DMD, epidemiology is expected seen to be similar globally because no specific population is observed among which this disease is at high risk. Variations may be observed due to differences in study design and quality. The prevalence of DMD is less than 10 cases per 100,000 males. In addition, the prevalence rate of BMD is less than 8 cases per 100,000 live male birth. Due to a lack of data, the change in the prevalence rate over the time of DMD

is not known. DMD in females is rarely seen in less than one patient per million. The survival of a patient with DMD is improved with optimal care and owing to the guideline of health care management, a study report of France found that median life expectancy was 25.77 years for those born before 1970 and 40.95 years for those born after 1970.

Pathophysiology Associated with DMD

It is thought that the cause of the development of Duchene muscular dystrophy is the responsible gene and its product, Dystrophin protein. Dystrophin is a large structural protein that forms a link between an extracellular matrix constituent called laminin and the cytoskeleton of muscle fiber. Dystrophin belongs to the spectrin superfamily of protein, in which spectrin, the alpha actinins, and dystrophin and its related protein are considered. These are closely related proteins from the dystrophin-related protein family. Chromosome 6 is mainly associated with the encoding of a dystrophin-related protein called utrophin. The absence of Dystrophin protein leads to muscular degeneration, and this mechanism is still unclear [4]. Mutation in the gene that encodes the protein dystrophin is mainly considered the cause of DMD [5]. The dystrophin protein molecule forms a complex structure when it is localized to the vicinity of a large no. of the protein molecule and this formed structure is called the Dystrophin-associated protein complex structure [6]. The complex structure formed a mechanical link between the cytoskeleton and extracellular matrix [7]. Mutation in the gene of DMD is due to its large size, there are approx. 79 exon present this will make a larger size molecule that is easily susceptible to mutation. Mutation seen in the DMD gene is mostly intragenic deletion, as it includes 65-70% of total mutation. Duplication mutation is also seen (5-15%), it is seen in about 7-8% of patients, and the remaining consist of point mutation (20%) or deletion/insertion. Inpatient with BMD 60-70% mutation is deletion, 20% are duplication and 5-10% are point mutation, small deletion, or insertion.

Mutation in the DMD gene leads to expressing prematurely truncated, unstable protein. The phenotypic difference seen between DMD and BMD (Becker muscular dystrophy) is described by Reading Frame Rule. The mutation that disrupts the open reading frame, results in the expression of truncated dystrophin protein molecule that will ultimately cause Duchenne muscular dystrophy, whereas mutation which maintains the open reading frame expressing the partly functional dystrophin protein molecule that leads to cause BMD [8].

In inpatients with Duchenne muscular dystrophy, necrotic and regenerating muscle fiber often observed in the cluster is characteristically demonstrated in muscle biopsy. These

necrotic fibers are surrounded by macrophages and CD4+ lymphocytes. Myoblast fiber is a small, nucleated fiber also observed, it denoted the muscle regeneration [9]that results in a balance between the necrotic and regenerative process. This situation can be present in the early phase of the disease and later the regenerating capacity of muscles is reduced and is replaced by connective and adipose tissue, this causes an imbalance between necrosis and regenerative process and this ultimately leads to the occurrence of Duchenne muscular dystrophy [10]. Muscle dystrophy also occurred when the mutation occurred in the dystrophin-associated protein complex component that leads to disassembling of the complex structure and disruption is seen between the extracellular matrix of fiber and cytoskeleton.

Pathophysiologic Hypothesis In DMD

Certain Hypothesis is suggested in favor of the occurrence of Duchenne muscular dystrophy. 2 hypotheses are mainly considered in the Development of DMD. 1. Mechanical Hypothesis 2. The Impaired Calcium Homeostasis. In addition, certain more hypotheses have also been proposed like the vascular hypothesis, gene regulation hypothesis, and glycosylation hypothesis [11,12].

Mechanical Hypothesis

Muscle Membrane integrity is balanced by the dystrophin-associated protein complex, The absence of one of the components compromises the muscle membrane integrity of fiber, this can lead to drastically reduced inability of a muscle to sustain eccentric contraction [13-17]. (i.e., Contraction with forced lengthening)**(Figure 1)**. On the cytoplasmic face of Sarcolemma, a rib-like lattice is formed which provides support to the extracellular matrix and cytoskeleton, this lattice is formed by a dystrophin-associated protein complex and this phenomenon is called Customers [18]. Customer is considered as a frequency distributor which distributes contractile forces generated in sarcomere laterally through the sarcolemma to the basal lamina and thereby maintains uniform sarcomere length along fiber [19]. The mutation leads to the absence of dystrophin or mutated dystrophin protein which leads to complete loss of dystrophin-associated protein complex along with the complete disruption in a customer lattice formation and thus this leads to muscle fragility [20-22].

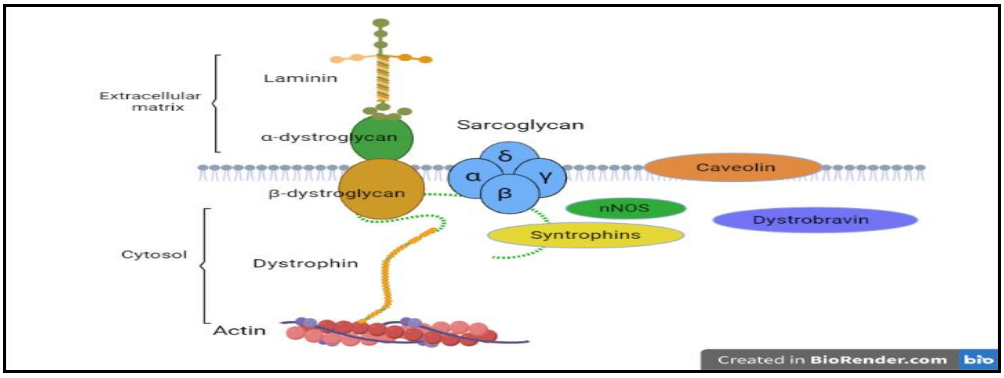

Figure 1: organization of the dystrophin-associated complex. Schematic representation of the organization of dystrophin-associated protein complex provides support to the membrane integrity by the interaction of dystrophin protein molecule between actin and extracellular matrix component.

Impaired Calcium Hypothesis

Calcium homeostasis is also considered to be an important aspect of muscle function [23]. In muscle biopsy presence of calcium accumulation and hypercontracted fiber has led evidence to investigate that calcium possesses a possible role in the development of DMD [24-26]. Increased influx of calcium in the cell through mechanosensitive voltage-independent calcium channel **(Figure 2)** [27]. Sustained increase in cytosolic calcium concentration led to activation of proteases specially calpains, which destroys membrane constituents, which results in more calcium influx, and finally, excessive calcium led to cell death [28, 29]. By Considering the calcium homeostasis hypothesis, several treatment processes by using calcium blocker (diltiazem)are also conducted but demonstrated no clinical benefits seen [30, 31]. Other approaches are also used by overexpression of Calpastatinto to reduce necrosis [32, 33].

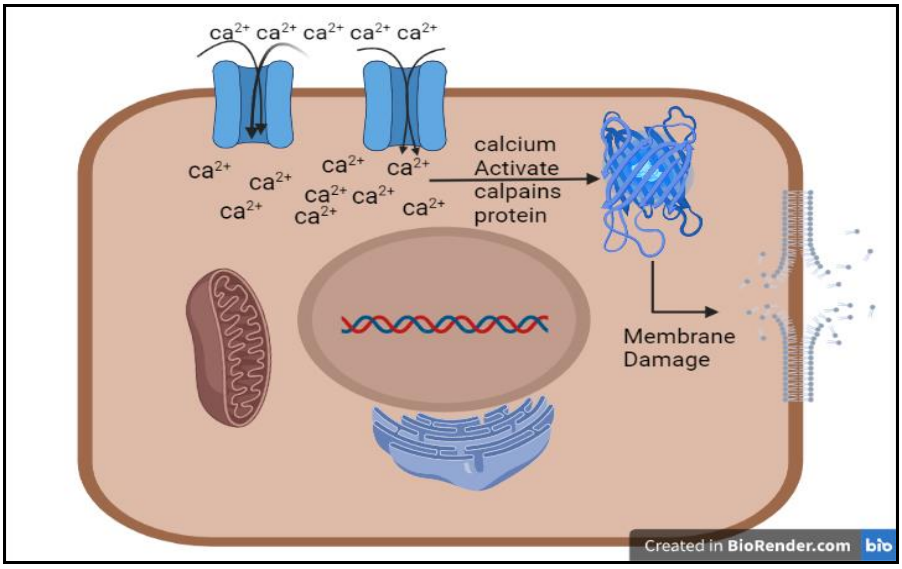

Figure 2: Diagrammatic representation show calcium influx activates the protease and this leads to cell membrane damage.

Vascular Hypothesis:

In this hypothesis, mouse model studies are considered. In Duchenne muscular dystrophy, Necrotic fibers were observed in the cluster, in the early pathophysiologic hypothesis role of the muscle vasculature is considered for the development of DMD, but structural studies found no blood vessel abnormalities [34, 35]. Recently study on the role of local vasodilator nitric oxide in skeletal muscles may have relevant relation to DMD pathophysiology [36]. Nitric oxide is produced in muscle cells by a neuronal isoform of NO synthase, this is normally bound to the dystrobrevin & synotrophin. When dystrophin is not produced, then muscles become dystrophin-deficient and n-NOS (a neuronal isoform of NO synthase) is delocalized from its subsarcolemmal site and freely floats in the cytoplasm**(Figure 3)** [37, 38]. When oxygen demand is increased during exercise, Muscle ischemia may develop in DMD [39, 40]. But it is seen that n-NOS knockout mice do not develop muscle disease [41]. This suggests that there is no direct involvement of n-NOS in the progression of DMD, but it is suggested to indirectly contributions of n-NOS in the progression of DMD, because of intracellular irregulating of pH found in mice [42].

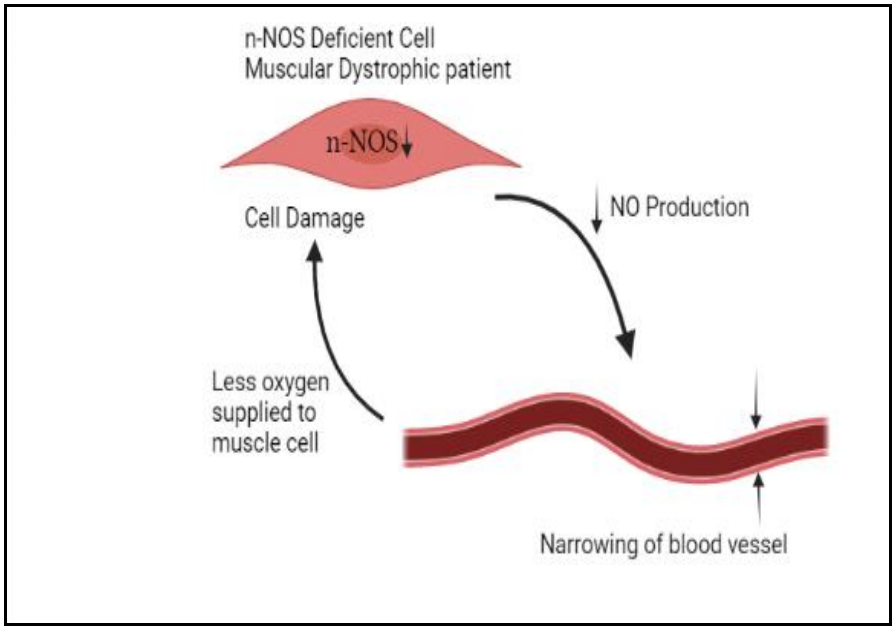

Figure. 3: Schematic representation shows narrowing of a blood vessel by the reduction of n-NOS, which leads to cell damage.

Gene Regulation Hypothesis

Dystrophin protein is associated with membrane stabilization by forming a link between extracellular matrix and cytoskeleton, Dystrophin associated protein complex is also involved in the other process like mechanotransduction, mutation in the DMD gene leads to loss of dystrophin and it results in a decrease in dystrophin-associated complex, that causes selective regulation of various gene**(Figure 4, 5)** [43]. It is seen that when a stem cell is given in the dystrophin-deficient cell, restoration of the dystrophin-associated complex occurs, and also physiologic gene expression is restored [44].

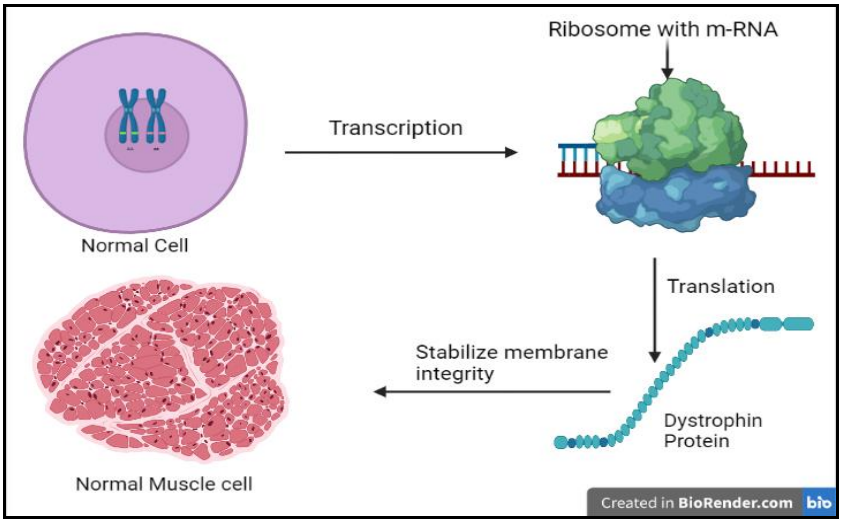

Figure 4: Schematic representation show normal cell produce dystrophin protein which stabilizes the membrane integrity.

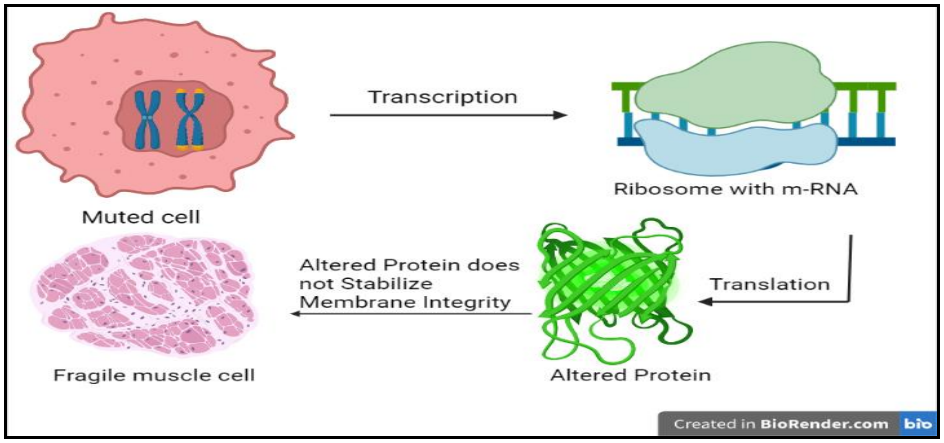

Figure 5: Schematic representation show mutation causes the production of altered protein which does not stabilize membrane integrity.

Glycosylation Hypothesis

For The proper functioning of muscle protein, it has to be gone through post-translational processes like glycosylation, Glycosylation of muscle protein is very important for proper functioning and correct assembling. Glycosylation of components of the dystrophin-associated protein complex, like alpha-dystroglycan, leads to control interaction with extracellular matrix components. Disruption in glycosylation of alpha-dystroglycan protein molecule leads to uncoupling of muscle fiber, this ultimately led to muscle degeneration. Disruption of glycosylation is due to mutation in a gene that encodes for glycosyltransferase [45].

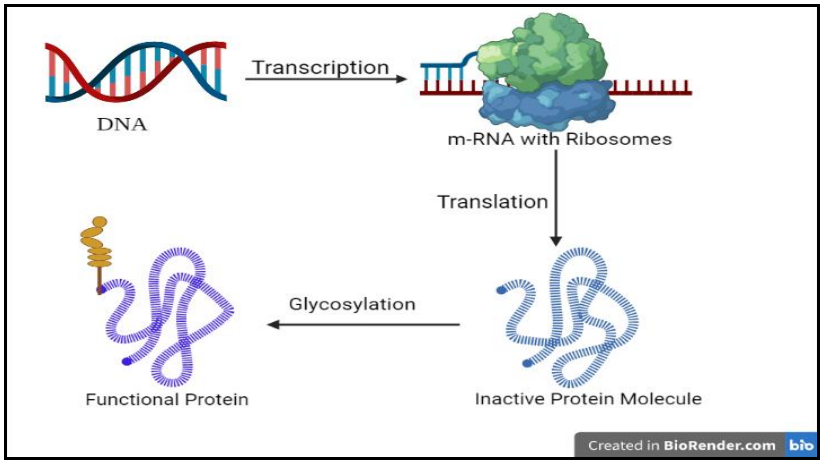

Figure 6: Diagrammatic representation of glycosylation process of protein molecule which makes it fully functional.

Clinical Features:

DMD is mostly seen in boys aged between 3-and 6 years. Symptoms like delayed gross motor, gait abnormalities, difficulties in walking, and difficulty in rising from the ground are seen. Weakness is generally associated with proximal lower limb and truncal weakness, and latter involvement of upper limb and distal muscles also occurs [2]. Almost individuals with DMD are never able to walk. Various symptoms like calf enlargement, waddling gait, standing on the ground with the help of support, scoliosis, and bone deformities are associated. Increased level of enzyme-like serum creatine kinase and hepatic transaminase is also observed in the inpatient. Dilated cardiomyopathy, arrhythmia, and tachycardia were also seen. Cardiomyopathy is the first evident after 10 years of age, which affect one-third of the patient by age of 14 years. Cardiac involvement is highly noted but many patients are asymptomatic due to physical inactivity [46, 47]. Chronic respiratory insufficiency is also seen, vital capacity is increased up to the 12^{th} year and then decreases up to 4-8%. [(42)]. Sleep disorder breathing is seen in the 1^{st}decade, caused by obstructive sleep apnoea [48]. Bone deformities like bone fracture, joint contraction, and scoliosis are developed in almost all patients. Spinal deformities create an impact on respiration vital capacity [49].

Investigation

The investigation is carried out to know about the cause of the development of disease and the factor responsible for it. In DMD investigation is done to find out the occurrence of the disease.

Serum Muscle Enzyme

Serum creatinine kinase enzyme is a marker enzyme for the development of Duchenne muscular dystrophy, an increase in the level of this enzyme indicates that the occurrence of DMD is seen at the age of 5 years. Serum alanine transaminase and aspartate transaminase are also observed to be high and correlated with CK levels [2].

Muscle Biopsy

Muscle biopsy is taken into consideration when genetic testing does not satisfy, it is performed when genetic testing gives negative results [2]. In this method, muscle fiber degeneration and necrosis with mononuclear cell invasion, cluster of the small regenerating cell is taken into account that gives an idea about disease progression. Determination of the absence of dystrophin protein is done by western blot analysis or immunostaining [50].

Genetic Testing

Genetic testing involves the testing of molecules of genetic material and it forms the mainstay of diagnosis. High-resolution chromosomal microarray and multiplex ligation-dependent probe amplification (MLPA) is used for the determination of deletion, and duplication of genetic material. Determination of mutation in protein dystrophin is done by chromosomal microarray method but it is also required for the confirmation and is done by the MLPA method. Point mutation is found by direct sequence analysis of the DMD gene [51].

Management Approach

Symptomatic and rehabilitative management of the patient is the only option to decrease complications, an increase in management effort will also increase the chance of survival of the patient and improve the quality of life [52].

Corticosteroid Therapy

Administration of corticosteroids has proven very helpful in the management of DMD, satisfactory results were seen by using corticosteroids in DMD patients with a decrease in the number of symptoms and complications. In a randomized controlled clinical trial, it is found to be very effective in improving muscle strength and respiratory functions. Long-term use of corticosteroids has proven very helpful in reducing the risk of progressive scoliosis and improving quality of life [53]. Prednisoneand deflazacort is considered the main

corticosteroid used in the treatment of DMD. Management of DMD with corticosteroid also depend upon the cost, formulation, availability, and side effect of the drug. Daily administration gives more beneficial results rather than alternate use of the drug [54, 55].

Respiratory Management

Sleep-disordered breathing, nocturnal hypercapnia, diurnal hypercapnia, acute respiratory infections, and decrease in vital capacity is the major respiratory problem raised in DMD, early management of respiratory complications leads to reducing the complications associated with the disease. Respiratory evaluation should be done annually to prevent and detection of problems [56]. The use of antibiotics for acute respiratory infection and the use of various vaccine-like pneumococcal and influenza vaccines are very important in the management of DMD.

Cardiac Management

Cardiomyopathy, arrhythmia, and tachycardia are some common complications that can be seen in the early phase of the disease, early detection, and prevention of these complications is very essential in the management of DMD complications. The use of electrocardiogram and echocardiogram in cardiac management are necessary, they help in the diagnosis of complication before it reaches to severity level. Cardiac surveillance is continuously run for 10 years [54].

Bone Health Management

Management of bones is very helpful because bone deformities like contracture and scoliosis occur in DMD. Bone health assessment includes monitoring of calcium level, phosphate level, and alkaline phosphatase level. For the maintenance of these extra supplement is taken [54].

Future Prospective of DMD

With the use of corticosteroids like prednisolone, the symptom of DMD is seen to be decreased but these treatments are not able to eradicate the symptom [58]. Knowledge of the nature of genetic patterns or dystrophic genesallowsthe researcher to develop novel therapies like Somatic Gene Therapy, Gene addition Therapy, Exon skipping, Genome editing, stop codon read-out through, and Stem cell Therapy which represents the most promising approach for the treatment of DMD. Exon Skipping induced by antisense strategies and

corrective gene therapy via functional dystrophin protein gives a better outcome and is considered to be the most promising strategy for treatment and several results are seen in ongoing clinical trials [59]. In addition to this, Gene delivery is seen to be very effective, In this method mini or micro functional dystrophin gene is delivered to the muscle fiber in vivo and muscle stem cell ex-vivo. AAV-based vector shows efficient systemic gene delivery to skeletal muscle directly.

CONCLUSION

Duchenne muscular dystrophy is a devastating disorder that creates a burden on society and family. Recently no proper treatment is available, only prevention and management therapy give beneficial results which decrease the progression and complications of the disease and improve the quality of life. A certain drug like corticosteroid is administered which gives many beneficial results in reducing DMD symptoms. The development of novel therapy will give result in much more effective treatment in the future.

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