"DUCHENNE MUSCULAR DYSTROPHY, IT'S SYMPTOMS, DIGNOSIS AND TREATMENT"

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ABSTRACT:

Duchenne muscular dystrophy (DMD) is an X-linked inherited neuromuscular disorder caused by mutations in the dystrophin gene, leading to the absence of dystrophin protein. This results in progressive muscle weakness, especially in the proximal muscles of the limbs, muscle wasting, and cardiomyopathy. Dystrophin is essential for maintaining the structural stability of striated muscle fibers. Diagnosis typically involves clinical evaluation, family history, and examination, with symptoms including developmental delays, proximal muscle weakness, and elevated serum creatine kinase levels. Confirmatory tests such as muscle biopsy and genetic analysis are often required. Early intervention with physiotherapy, nutritional support, and lifestyle management can improve patient outcomes. In advanced cases, gene therapy using viral vectors or plasmids may correct the underlying mutation. Antisense oligonucleotides are also employed for exon skipping, while stem cell or myoblast-based therapies show promise for muscle regeneration. These therapeutic strategies aim to slow disease progression and enhance the quality of life.

KEYWORDS: Duchenne muscular dystrophy, Gower's sign, dystrophy, cardiomyopathy, gene mutation, muscle wasting and stem cells, serum creatine kinase.

INTRODUCTION:

Duchenne muscular dystrophy (DMD) is a rare genetic disease that affects the muscles and tends to show early signs of progressive muscle weakness. It is a chronic condition that is passed down through the X chromosome and affects approximately 1 in every 3,500 male babies. The disease was named after two doctors, Guillaume Benjamin Am and Duchenne, who discovered it in 1860[1]

Usually, patients with DMD disease start experiencing symptoms at around 5-6 years old. As they get older, the symptoms become more severe. DMD primarily affects the cardiac muscles and respiratory systems, and it is more commonly observed in boys. By the age of 13, most

patients are unable to walk anymore. If the condition is not treated promptly, the cardiac and respiratory systems may become infected and dysfunctional[2]

Dystrophin plays an important role in the structure of muscle cells, safeguarding them from strain during muscle contraction and relaxation. When dystrophin is not present, it leads to problems in both skeletal and cardiac muscles, ultimately resulting in the loss of motor function in human activity [3]

Patients have been found to have thousands of various mutations in DMD[4,5] Deletions account for around 60-70% of these mutations, while duplications make up 5-15%, and point mutations, small deletions, or insertions make up the remaining 20%[4,6]

About two-thirds of DMD mutations are passed down to the sons of mothers who carry dystrophin mutations without even realizing it. The rest of the cases occur due to spontaneous mutations in the reproductive cells[7]

DMD patients need to have accurate DNA diagnostic analysis. This analysis is crucial because it helps healthcare providers offer the best care and assists families in making informed decisions regarding family planning. Additionally, it provides valuable information about eligibility for mutation-specific treatments[8]

Physical therapists play an important role in the treatment of individuals with DMD. They assess the severity of the condition, the range of motion, and the ability to move effectively. They also implement various therapies and guide how to position oneself, stretch properly, and modify activities for better mobility.[9,10]

Until now, there has been no cure for DMD, causing most patients to succumb to cardiac and respiratory issues by the age of thirty. However, there is hope for the treatment of Duchenne muscular dystrophy through genetic interventions and stem cell therapy.[11]

DEFINITION:

Duchenne muscular dystrophy (DMD) is one of the earliest genetic disorder scientist have studied to determine if, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology can correct the underlying genetic mutations that cause the condition.

Duchenne muscular dystrophy (DMD) is one of the most severe types of muscular dystrophy. It occurs when mutations are in a large X-linked dystrophin gene. This gene is quite extensive, covering approximately 2.3 megabases[12]

DMD, which is the most common lethal monogenic disorder, mainly impacts boys because they have a single X chromosome. Interestingly, about two-thirds of DMD mutations are passed down from mothers who are carriers of dystrophin mutations without even realizing it. On the other hand, the remaining cases occur due to spontaneous germline mutations.[13]

SYMPTOMS:

1. Sarcolemma weakening.

2. Progressive muscle degeneration.[14]

3.Curved spine due to weak back muscles

4. Muscle weakness in arms, neck and other area.[15]

5. Hypertrophy

6. Frequent falls

7. Difficulty in climbing stairs

8. Gower's sign[16]

9. Loss of calcium level or muscle fibre in the body.

10. Exhibits inflammatory changes in the muscle.[17]

11. Impairment of motor function

12. loss of ambulation in the patient

13. Nervous system developmental delay

14. Cardiomyopathy[18]

DIAGNOSIS :

Doctors can confirm the diagnosis of DMD by conducting various laboratory tests. These tests include checking the blood for high levels of creatine kinase, performing multiplex PCR, and conducting high-throughput DNA sequencing[19, 20]

In most cases, doctors usually send patients to neuromuscular specialists. These specialists then ask for a genetic analysis of the DMD gene to confirm if the patient has DMD[21]

It is important to consider several factors when diagnosing DMD. These include the age at which the first symptoms of the disease appear, the specific type of myodystrophic lesion (whether it affects the distal and proximal muscles), the location of muscle atrophies, the presence or absence of
pseudohypertrophies, fasciculations, sensory disorders, cramps, skeletal deformities, and joint muscle
contractures. Additionally, the condition of muscle tone and tendon reflexes and the course and progression of the disease should be considered.[22]

1)Laboratory test

The laboratory tests consist of a biochemical blood test that determines the levels of creatine phosphokinase (CPK), Alanine transaminase, Aspartate transaminase, and lactate dehydrogenase (LDH)[23]

CPK, an enzyme found inside cells, is vital in providing energy to the body. It is most abundant in the heart and skeletal muscles. When the cells are destroyed, CPK is released into the blood, indicating its presence. An elevated CPK level is an early sign of DMD. A diagnostic significance is attributed to a greater increase in CPK levels. In the early stages of the myodystrophic process,
DMD is characterized by a significant enzyme level increase of 10-100 times[24]

Upon these Findings (delayed muscle function, speech delay and high CK), Patients are generally referred to neuromuscular specialists, who Request genetic analysis of the DMD gene to confirm whether the patient has DMD[25]

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location of muscle atrophies, the presence or absence of pseudohypertrophies, fasciculations, sensory disorders, cramps, skeletal deformities, and joint muscle contractures.[26]

2)Magnetic imaging and neurofunctional findings

Magnetic imaging and neurofunctional findings include techniques like EMG, Ultrasound examination and ECG.

Electroneuromyography (EMG) is a straightforward yet highly informative diagnostic method that focuses on recording and analyzing the bioelectric activity of the neuromuscular system while at rest and during activation. EMG consists of two main methods: stimulation and needle. In the diagnosis of DMD, stimulation EMG does not hold much significance, as needle EMG provides more valuable information. Needle EMG serves as the primary research method for suspected DMD. This technique can determine the primary muscular changes in the motor unit potentials, such as a decrease in duration and amplitude. Additionally, it helps assess the spontaneous activity of muscle fibres, which can be observed through acute wave potentials and fibrillation potentials. These indicators reflect the level of activity in each specific muscle. Pronounced spontaneous activity is a distinctive feature of DMD, setting it apart from other hereditary primary muscular diseases. It becomes evident even during the early stages of the disease, alongside detecting fibrillation potentials, acute wave potentials, and high-frequency discharges [27,28]

Assessing the condition of muscles through ultrasound examination is appealing because it does not involve radiation and is relatively inexpensive. Moreover, this non-invasive method allows for high accuracy (86% to 91%) in distinguishing between a healthy patient and one with neuromuscular pathology. In conditions like DMD and BMD, muscle degeneration is evident through the presence of adipose or fibrous tissue replacing the muscle tissue.

Magnetic resonance imaging (MRI) has become the preferred method for imaging muscles due to its lack of side effects (such as ionizing radiation), excellent resolution, and ability to provide clear contrast of soft tissues in full-body scans. In the case of patients with DMD and BMD, MRI can be used not only for diagnostic purposes but also to monitor the progression of muscle tissue degeneration over time.[29]

The ECG helps identify arrhythmias, conduction disturbances, ventricular hypertrophy, and dilatation. EchoCG, on the other hand, is the most reliable method for diagnosing problems related to the structure and function of the heart muscle. It is crucial to detect cardiac issues early before they become clinically apparent. Some common indications of abnormalities on the ECG include irregular heart rate and rhythm, as well as irregular electrical conduction within the heart.[30]

3)Genetic Analysis:

We obtained blood samples from patients with suspected DMD in EDTA vacutainers, totalling three millilitres. Then, we extracted genomic DNA using a simple desalting method. The DNA was subsequently stored at -20˚C until it was required for future use. The molecular diagnostic analysis of the genomic DNA was performed according to the provided algorithm.DNA was isolated from the patient. Samples were subjected to the following steps: Step I involved multiplex PCR, step II involved MLPA, and Step III involved NGS (Next.
Gene sequencing)[31]

i)mPCR:

mPCR was created to identify any deletions in the area of the DMD gene, which includes 30 exons.

In simple terms, we conducted multiplex PCR analysis for the 30 exons located in the central and 5' end of the DMD gene[32]

ii)**Multiplex ligation-dependent probe amplification (MLPA)**

Patient samples that were negative for mutations in the DMD gene by mPCR, or where the borders of the identified mutations were unclear (BNC: borders not clear), were subjected to MLPA analysis.

We typically assess the number and occurrence of the 79 DMD exons by utilizing a widely used technique called multiplex ligation-dependent probe amplification (MLPA).

MLPA is capable of identifying deletions and duplications in both patients and carriers. When there are small mutations within an exon, they can hinder the probe from binding to that specific exon. Consequently, these mutations may appear as single-exon deletions when utilizing the MLPA technique [33]

MLPA determines the exons affected by the deletion or duplication but does not provide information about where the intronic breakpoints are located.

In most patients, MLPA can detect the causative mutation, as around 75% have DMD deletions or duplications[34]

iii) **Next-generation sequence analysis (NGS)**

Those samples had previously tested negative for mPCR and MLPA. The goal was to identify the specific gene variant responsible for the condition. To achieve this, targeted next-generation sequencing (NGS) was employed. The team at MedGenome Labs prepared targeted sequencing libraries using the Roche Nimblegen SeqCap kit from Pleasanton, CA, USA. This kit included biotinylated oligonucleotide capture probes, also known as baits, specifically designed for the targeted exons in our muscular dystrophy and the congenital myopathy gene panel for NGS analysis. Through hybridization, these baits enriched the region of interest (targeted gene regions[35]

Next-generation sequencing (NGS) encompasses a wide range of technologies, each with its unique biochemical approach. To fully assess the DMD gene, conducting a molecular quantitative analysis for detecting copy number variations and genomic sequencing is necessary, both of which NGS can facilitate. Additionally, NGS enables the identification of remaining rare mutations that the previously mentioned methods may have missed through more targeted transcript analysis. A study demonstrated that NGS could accurately diagnose up to 92% of DMD/BMD patients. Furthermore, by combining next-generation high throughput DNA sequencing techniques with MLPA, the accuracy of results can be further enhanced.[36]

4)**Muscle Biopsy**

Another way doctors diagnose DMD is by performing a muscle biopsy to study the histology of the skeletal muscles. This type of biopsy and dystrophin analysis can help confirm a DMD diagnosis if genetic testing comes back negative or if a genetic variation of unknown significance is discovered.[37]

All dystrophies have histological findings that show abnormal variations in muscle fibre size and necrosis. Tissue macrophages are also present, and the degree of muscle mass replacement by connective and adipose tissue depends on the stage of the disease.

To accurately identify the type of muscular dystrophy, it is necessary to conduct an open muscle biopsy. This procedure guarantees that there is a sufficient amount of muscle tissue available for examination. Nonetheless, if the differential diagnosis solely involves DMD, a needle biopsy may be considered as an alternative[38]

After acquiring the muscle sample, the primary analysis for DMD involves conducting immunocytochemistry and immunoblotting tests to detect the presence of the dystrophin protein.

Differentiating between the complete or partial lack of the dystrophin protein is vital to definitively distinguish between Duchenne muscular dystrophy (DMD) and other types of dystrophinopathy[39]

Patients who have Duchenne muscular dystrophy (DMD) will not show any dystrophin on a western blot test or very little. On the other hand, patients with Becker muscular dystrophy (BMD) will have reduced dystrophin levels that can vary in size. It can be 80% smaller, 15% normal, or even 5% larger. If less than 5% of normal dystrophin is found in DMD patients, it confirms the diagnosis. However, in BMD patients, a range of 20-100% of normal dystrophin levels is considered diagnostic[40]

5)Measurement of Range of Motion

Range of motion (ROM) refers to the extent of movement a joint or body part can perform. Physical therapists commonly measure ROM using a goniometer, an instrument that helps evaluate joint flexibility during assessments or therapy sessions. This tool is especially useful for assessing joints such as the knee, shoulder, and hip.

In research settings, goniometry is also employed to assess motor function in both the upper and lower limbs of patients with conditions like muscular dystrophy.

For example, the full range of motion of the knee joint includes:
Full knee flexion: approximately 135 degrees
Full knee extension: 0 degrees
Knee internal rotation: about 10 degrees
Knee external rotation: around 30–40 degrees [41,42]

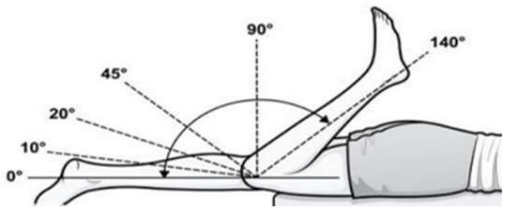

Fig. 1. Full range of motion of the knee joint

https://images.app.goo.gl/FKXpni8X1b6wzjj89

6)Prenatal Screening

With the emergence of new and advanced technologies, genetic counselling and prenatal diagnosis are being used more frequently. Nowadays, it is possible to detect nearly all fetal muscular dystrophies before delivery through these technologies. Prenatal screening methods, such as amniocentesis and chorionic villus sampling, as well as "Rhesus Hemolytic Disease of the Newborn" (RHDO) analysis of maternal plasma, which involves sequencing the fetus genome from cell-free fetal DNA fragments in the mother's plasma, are used for this purpose [43]

The use of non-invasive prenatal screening, which involves analyzing cell-free DNA in the mother's blood, is becoming more common in routine prenatal care. However, this approach presents new challenges. It can detect fetal aneuploidies, such as trisomy 21, 13, and 18, and it can also uncover variations in the number of copies of certain genes in the mother's DNA, known as copy-number variations (CNVs). The implications of these CNVs need to be carefully evaluated. Among the most frequently observed CNVs in mothers are those related to the DMD gene, although the significance of some of these CNVs remains unknown [44]

7) Timed Test

These tests include the 6-min walking test, 10-m walking test, stair climbing test, chair-rise test, 30-min walking test, and 2-min walking test. The 6-min walking test measures endurance and functional capacity by evaluating the distance walked in 6 min [45, 46]

The 10-m walking test provides insights into mobility and gait by assessing walking speed over a 10-m distance. The stair-climbing test evaluates lower limb strength and balance by assessing stair ascent and descent. The chair-rise test measures lower limb strength and mobility by timing the rise from a chair without using hands. The 30-min walking test evaluates endurance and ambulation abilities over a longer duration. The 2-min walking test offers a quick assessment of mobility by evaluating walking speed over a shorter duration. These tests play a crucial role in evaluating functional limitations and disease progression in muscular dystrophy. They help monitor physical changes, assess treatment effectiveness, and guide personalized rehabilitation planning. The 6-min walking test commonly utilizes formula (1) to evaluate individuals' endurance.

Score = Distance Covered/ Expected Distance for Age and Gender*100 …… (1)

In the 30-min walking test, the formula that corresponds to it is as follows:

Score = Distance Covered /Body Weight *100…… (2)

Treatment:

1)Glucocorticosteroid treatment

Boys with DMD who have stopped or started to decline need to be treated with the Glucocorticosteroids Prednisone or Deflazacort, according to the most recent recommendations, and they strongly recommend that they continue to receive therapy throughout their lives[47] .The exact mechanism by which glucocorticosteroids retard disease progression in DMD is yet to be determined since glucocorticoid receptor activation has pleiotropic effects. However, it has been shown that glucocorticosteroids reduce inflammation

and improve total muscle mass and strength in patients with dmd by the stimulation of insulin-like growth factors, decreased cytokine production, decreased lymphocyte reaction, increased myoblast proliferation, and upregulation of synergistic molecules[48]

2)Stem Cell therapy

Stem cell therapy has been regarded as one of the most promising therapies for treating muscular dystrophy. The use of human induced pluripotent stem cells (HIPSC) with the ability to produce unlimited numbers of myogenic and non-myogenic mesenchymal stem cells are among the promising strategies for stem cell therapy to regenerate skeletal muscle with the ability to achieve greater self-renewal and regenerative capacity. Mesenchymal stem cells (MCSC) secrete soluble substances for skeletal muscle development and regeneration. MCSC has also been shown to regulate immune responses and can reduce the severity of fibrosis[49]

3) Gene Therapy

DMD gene therapy involves restoring the damaged dystrophin by providing a functional copy of DMD or repairing it. Gene addition therapy uses viral vectors to deliver a cDNA copy of functional DMD to affected tissues. Although most viruses do not have a natural tropism for skeletal muscles and the heart, adeno-associated viruses (AAVS) are an exception and can easily infect these tissues. However, AAV only has a small carrying capacity of 4.7 kb, whereas dystrophin's dp427 muscle isoform is encoded by an 11.4 kb c-DNA[50]
In addition, AAV administration results in an anti-AAV capsid-neutralizing antibody, which prevents retreatment. Moreover, some patients already have pre-existing AAV-neutralizing antibodies, which prevents them from receiving this therapy. Plasmapheresis, alternative AAV serotypes, and immune-modulating drugs are being investigated as ways to counter this antibody response[51]

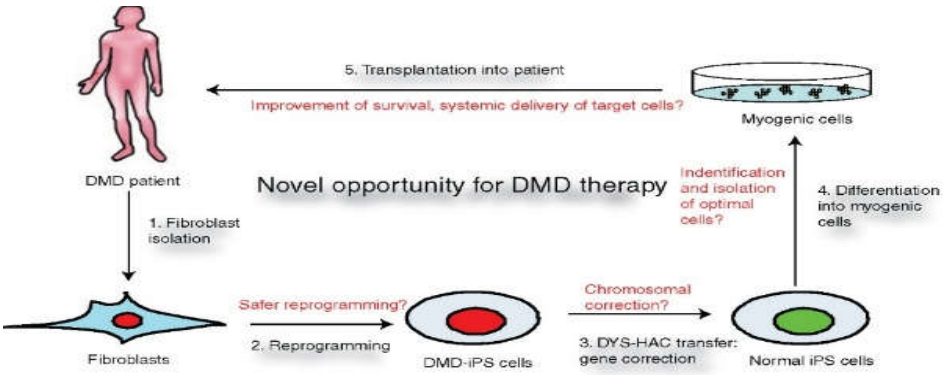

Fig 2. DMD Therapy

[https://images.app.goo.gl/6TQMckuAcAkEwJFe8]

4)Exon Skipping:

The exon-skipping scheme is based on the fact that out-of-frame mutations result in severe DMD.

Antisense oligonucleotides (AON), small pieces of modified RNA that specifically bind to a target exon during pre-mRNA splicing, can restore the reading frame. This binding prevents the exon from being included in m-RNA. AON is very small (20–30 nucleotides) and can be delivered in humans using a single injection[52]

ASOS are mutation-specific strategies, and different dystrophin proteins will be formed after skipping different exons for different mutations. Skipping the same exon can form different dystrophins; exons 47–50, 48–50, 49–50, and 52 can be restored by exon 51 skipping. However, the dystrophins produced after exon 51 skipping will differ. Exon 51 or 53 skipping can also be deleted, resulting in two different dystrophins.[53]

Exon skipping using Antisense Oligonucleotide (AON)only modifies dystrophin mRNA, leaving the original mutation intact in the dystrophin gene. For AON-based therapies to be effective, the DMD patient must undergo lifelong biweekly administration of this drug. In contrast, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)gene editing isn't likely to be "one and done" since it corrects the root cause of the genome mutation[54]

Table I .Therapies used for Duchenne muscular dystrophy

Sr.No	Main Mechanism	Therapy
1	Drugs acting on biochemical, metabolic, and oxidative stress	Laevadosin, Allopurinol, Vitamin D and Calcium, Creatine and Glutamine, Coenzyme Q10, Idebenone
2	Drugs acting on growth, height and muscular function	Mazindol, Growth Hormone, Isaxonine, Anabolics (Sinestrol and Oxandrolone), Albuterol
3	Drugs producing changes in the sarcolemma and calcium aggregate	Verapamil, Flunarizine, Nifedipine, Diltiazem
4	Drugs interfering with the muscle blood flow	Methysergide, Tadalafil
5	Conjunctive tissue proliferation	Penicillamine, Pentoxifylline
6	Inflammatory reaction	Azathioprine, Cyclosporine
7	Restoration of premature termination codon	Gentamicin, Ataluren
8	Corticosteroids	Prednisone, Prednisolone, Deflazacort

CONCLUSION

Duchenne muscular dystrophy (DMD) is a devastating genetic disorder that results in progressive muscle degeneration and weakness. This disorder affects primarily boys, who are most seen in early childhood. DMD is caused by mutations in the dystrophin gene, resulting in the absence or dysfunction of the dystrophin protein, which is essential to maintaining muscle cell integrity. People with DMD have difficulty walking and breathing and eventually develop life-threatening injuries as the disease progresses. Despite advancements in supportive care, there is no cure for DMD, highlighting the need for continued education and therapeutic interventions to improve patients' quality of life and extend their lives.

DMD is being treated with gene therapy and molecular therapy, with ongoing clinical trials examining potential avenues to address the condition's root cause. To improve our understanding of DMD and translate scientific findings into effective therapies, researchers, healthcare professionals, and advocacy organizations are vital. Although challenges persist, the development of novel therapies and increased awareness of Duchenne muscular dystrophy contribute to a common desire for improved outcomes and, ultimately, a better future for those affected by the condition.

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