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DUCHENNE MUSCULAR DYSTROPHY

Summary

Duchenne muscular dystrophy (DMD) is an X-linked recessive, progressive and incurable disease that mainly affects skeletal muscles. Dystrophin, a structural protein that is related to the stabilization of muscle contraction, is absent or altered in DMD. Patients affected by this dystrophy present loss of muscle mass, impairing the ability to run, climb stairs and jump, culminating in a wheelchair confinement, on average at 12 years of age. Due to the immobility and inactivity of the respiratory muscles, these patients die due to respiratory complications. Several therapeutic strategies have been studied in order to improve the quality of life of these patients and their prognosis. The present study consists of a literature review, addressing the main aspects of the disease and pointing out some of the various current therapeutic strategies. Keywords: Duchenne Muscular Dystrophy, Pathology, Dystrophin

Abstract

Duchenne muscular dystrophy (DMD) is a recessive X-linked disease, a progressive and incurable disease that primarily affects the skeletal muscles. The dystrophin, a structural protein that is related to stabilization of muscle contraction is absent or altered in DMD. Patients with this dystrophy exhibit muscle wasting, impairing the ability to run, jump and climb ladders, culminating in a confinement to a wheelchair, on average 12 years old. Due to inactivity and immobility of the respiratory muscles, these patients will die due to respiratory complications. Several therapeutic strategies have been studied to improve the quality of life of these patients and their prognoses. The present study consists of a literature review, covering the main aspects of pathology and pointing out some of the different therapeutic strategies.
Key words: Duchenne Muscular Dystrophy, Pathology, Dystrophin.

Introduction

Duchenne muscular dystrophy is the most common human dystrophy, affecting an average of 1 in 3,500 live male births [1]. Two-thirds of cases are hereditary and one-third are new mutations [2]. DMD is a recessive, X-linked, progressive disease that affects skeletal muscles,

often leading to death before the age of 20. In addition to skeletal muscle degeneration, these patients suffer from heart problems and can die due to cardiorespiratory failure [3].

There are few reports of women with DMD because it is an X-linked recessive disease in which, in addition, affected boys die before producing offspring. The clinical picture of these women is milder and usually manifests later than in men, but even so, most of them present cardiac anomalies. When the X chromosome carrying the mutant DMD allele in female carriers is predominantly active, they will present signs of DMD [1]. Women carrying the mutant gene with Turner syndrome (X0), or with a chromosomal translocation involving the dystrophin gene may also present signs and symptoms of the disease [4].

Dystrophin is a structural protein whose function is to connect the cytoskeleton of the skeletal fiber with the extracellular matrix proteins, stabilizing muscle contraction [5]. In DMD, dystrophin is absent or altered, which implies an alteration in membrane permeability, facilitating the entry of large amounts of Ca^{++} in muscle fibers, leading to degeneration [6].

The clinical picture of the pathology is characterized by loss of muscle mass and function. The loss of muscle strength occurs most frequently in the proximal muscles of the limbs, being more exacerbated in the lower limbs, affecting the ability to run, jump, climb stairs and, in a more advanced stage, the ability to walk [7]. The clinical manifestations, although present since neonatal life, become more exuberant at 3 to 5 years of age, and by 12 years of age, most of those with the aforementioned dystrophy are already confined to a wheelchair [8].

A very common clinical feature in children with DMD is an increase in calf muscle size. This is considered pseudo-muscular hypertrophy, as it is caused by the abnormal proliferation of interstitial tissue in the fibers of the gastrocnemius muscle. This hypertrophy occurs in an attempt to compensate for the imbalance of the anterolateral leg muscles [9].

Patients with DMD have difficulty getting up from the floor due to atrophy of the muscles responsible for knee, hip and trunk extension, presenting as a characteristic the Gowers sign or myopathic getting up [9,10].

When standing up, the patient assumes a typical posture of hyperlordosis in the lumbar region, with a protruding abdomen and shoulders back, in an attempt to maintain an upright posture despite the atrophy of the hip extensor muscles [9].

During the walking phase, the patient presents an anserine gait, in which there is a large movement of the hip associated with accentuated lordosis, resulting from the weakness of the pelvic girdle. Due to tendon retractions of the posterior portion of the thigh and ankle, the child can walk on tiptoe [10].

As in other muscular dystrophies, respiratory muscle weakness occurs, which leads to reduced expansion, hypoventilation and inefficient coughing. These characteristics make patients extremely vulnerable to atelectasis and lung infections [11].

Although most people with DMD die due to pulmonary complications, it has been found that 20% of deaths occur due to cardiac causes, often as a result of ventricular dysfunction [7]. The literature reports that cardiac involvement occurs in parallel with involvement of skeletal striated muscles [12,13].

Another less discussed finding is mental retardation (MR), present in approximately 30% of patients. This incidence is considerably high when compared to the prevalence in the general population, which is 10%. Psychiatric comorbidities, such as attention deficit hyperactivity disorder, are also observed [14].

As it is a disease that has no cure, treatment must involve a multidisciplinary approach, because despite being mainly palliative, it provides an improvement in quality of life, preventing early complications and encouraging maximum independence in the daily activities of these patients.

Methodology

This is a systematic review of the literature from the last 10 years. The survey of published data was carried out through research of articles in the following databases: Lilacs, PubMed, ScienceDirect, Scielo and ISI Web of Knowledge, as well as using textbooks, magazines and scientific journals.

1. Etiology and Pathogenesis

Several mechanisms are involved in the pathophysiology of this disease. The genetic abnormality in DMD is present in band 1 of region 2 of the short arm of the X chromosome (band Xp21). This gene is currently the largest ever discovered, measuring approximately 2.4 megabases (Mb) of DNA, that is, about 1% of the total X chromosome. The gene has more than 2.6 million base pairs and 79 exons, encoding the protein called dystrophin. Due to the large size and complexity of the gene, the rate of mutation, deletions or duplications is quite high, which result in misreading and/or premature cessation of gene transcription and/or abnormal encoding of this protein [8,2].

Dystrophin is a protein with a relative molecular mass of 427 kilodaltons (kDa), present on the cytoplasmic surface of the sarcolemma, forming part of the subsarcolemmal cytoskeleton, which connects the myofilaments to a complex of glycoproteins of the cell membrane. Biochemical studies report its absence or low concentration in dystrophic muscles of patients with DMD [6]. The expression of muscular dystrophin is regulated according to the stages of development and is expressed first in the embryonic somites, participating in the process of myogenesis [15].

The dystrophin-glycoprotein complex – CDG, expressed in high concentration in skeletal striated muscle, connects the muscle fiber cytoskeleton (actin) to the extracellular matrix and is composed of sarcolemmal proteins [16]. Dystrophin is considered one of the central components of CDG, and its absence modifies the expression of several others, suggesting the interdependence of dystrophin with this complex [5].

It is suggested that dystrophin, bound to its constituent proteins, stabilizes and prevents the formation of gaps in the sarcolemma during muscle contraction and relaxation cycles, thus maintaining the integrity of the muscle fiber. Thus, a deficiency in its expression compromises the expression and organization of other proteins, promoting fiber deterioration in the event of injury [16]. A deficiency in dystrophin synthesis would promote muscle fiber fragility, making it susceptible to injury and necrosis. Microlesions in the membrane facilitate the influx of calcium, leading to the activation of proteases that promote self-digestion of the sarcoplasm. Subsequently, macrophages reach the tissue and remove the necrotic material by phagocytosis. After phagocytosis, satellite cells are activated and proliferate, inducing muscle fiber regeneration [17]. The regenerative capacity, however, declines sharply from the age of 3, when the necrotic fibers begin to be replaced by fibro-adipose tissue. When this process affects the respiratory muscles, especially the diaphragm, a large proportion of patients die from respiratory failure [9,18].

In addition to being expressed in all muscle types, dystrophin is also expressed in the central nervous system. This is because the transcription of the dystrophin gene is controlled by three independent and tissue-specific promoters, the brain promoter, the muscle promoter and the Purkinje promoter [5]. These promoters transcribe different dystrophin isoforms, Dp427m, Dp427c and Dp427p, respectively [19]. There are also other internal promoters of the dystrophin gene that lead to partial size isoforms, 260 kDa (DP260), 140 kDa (DP140), 116 kDa (DP116), and 71 kDa (DP71) [5].

Cognitive deficits and behavioral problems are more frequent in patients with mutated Dp140 and Dp71 isoforms. These isoforms are expressed in the brain in high quantities, especially Dp71, and are structural components of neurons, glial cells and Schwann cells [20].

The function of dystrophin in the central nervous system has not yet been fully elucidated. However, it is suggested that this protein plays a role in neurogenesis, neuronal migration and cell differentiation during the formation of the central nervous system and that it modulates the integrity of synaptic terminals, synaptic plasticity and cellular signal integration [21].

2. Different responses of skeletal striated muscle to DMD

In DMD, both the trunk and appendicular muscles are compromised by necrosis of muscle fibers. However, the intrinsic muscles of the larynx, with the exception of the cricothyroid, and the extraocular muscles (EOMs) are protected from myonecrosis [22].

The EOMs and the intrinsic muscles of the larynx present anatomical and physiological differences in relation to other skeletal muscles, such as rapid contraction-relaxation time, extraocular myosin heavy chain, addition

continuous myonuclei and differentiated expression of CDG proteins [23].

One of the possibilities to explain the protection against myonecrosis of dystrophic EOMs may be directly related to specific properties of these muscles, such as the small fiber diameter, embryological origin, muscle fiber type, innervation pattern and organization of the neuromuscular junction, preservation of beta-dystroglycan (b-DG) and increase in utrophin, which has a structure and function homologous to dystrophin [24]. Since utrophin and dystrophin have the same associated proteins (dystroglycans, sarcoglycans and syntrophins), it has been suggested that utrophin could compensate for dystrophin deficiency [25].

Another possible explanation for this protection is the intrinsic ability of these muscles to maintain Ca homeostasis$_{2+}$, through the increase of calcium regulatory proteins, such as calmodulin, SERCA1 and calsequestrin [22]. However, the mechanisms by which the extraocular and intrinsic muscles of the larynx maintain calcium homeostasis are still unclear.

3. Diagnosis

The diagnosis is established through the clinical picture, family history, laboratory, genetic, molecular tests and muscle biopsy [9].

The appearance of the first symptoms, such as progressive muscle weakness, and family history provide essential information for the diagnosis of DMD, together with elevated serum levels of the enzyme creatine kinase (CK). There is the possibility of diagnosis immediately after birth, through blood analysis of this enzyme collected from the newborn's umbilical cord, indicating impairment of the skeletal muscles [26].

Creatine kinase is present in muscle tissue, heart and brain. Its increase in circulatory flow is related to the degeneration of muscle fibers, a progressive and continuous process in DMD. Other enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH) and alkaline phosphatase (ALP) also increase in serum levels at the onset of the disease. Among the enzymes, the most important continues to be CK, and all tend to show a decrease in serum levels as the disease progresses, due to the progressive loss of muscle mass [27].

Immunohistochemical staining can also be performed using an anti-dystrophin monoclonal antibody (which does not cross-react with β-spectrin, α-actinin, and utrophin). This antibody will bind to the sarcolemma of normal muscle fibers and staining will occur. However, in cases of DMD, there will be no staining because the antibody will not bind. Determination of the amount and distribution of dystrophin by immunohistochemical staining can confirm the presence of dystrophinopathy [28,29].

With the evolution of diagnostic techniques and the discovery of the dystrophin gene, molecular biology allows for much safer and more accurate diagnoses, including the confirmation that the most common mutations in dystrophin are intragenic deletions that correspond to 65% of the alterations and that, in addition to the deletions, there are duplications that can occur, not in a *locus* specific, but at virtually any point in the dystrophin gene [28,30].

Laboratory testing can be used to confirm a clinical diagnosis of DMD and prenatal testing. Diagnostic testing for dystrophin involves a variety of methodologies, including multiplex PCR, Southern blot, MLPA, DOVAM-S, and SCAIP; however, these methods are laborious, costly, and do not detect duplications in the dystrophin gene. High-resolution genomic hybridization (CGH) has been shown to detect deletions and duplications in the dystrophin gene [30]. Noninvasive prenatal diagnosis of DMD is possible by massively parallel sequencing technology performed from fetal DNA present in maternal plasma [31].

4. Experimental models in the study of DMD

There are more than 60 natural and laboratory animal models used for studies on DMD worldwide [32]. Currently, the most widely used animal model of DMD is the mdx mouse [33].

Despite presenting some different characteristics in relation to human muscular dystrophy, in terms of severity and persistence of the myopathy, *mdx* is easy to maintain and available, and is the most widely used experimental model of DMD to study the biology of dystrophic skeletal muscles, mechanisms of dystrophinopathies and development of therapies [33,34]. Just like patients with

DMD, mdx mice exhibit progressive damage to the cardiac muscle, with cardiomyopathy, however, less severe than that observed in humans [35].

THE*mdx*It was discovered more than 30 years ago and much of what is known today about the pathophysiology of DMD is due to studies carried out with these animals [36].

Tropical zebrafish are increasingly being used in muscular dystrophy studies because they have important characteristics such as compact size, external fertilization and external embryo development, high reproductive capacity, genetic tractability, embryo transparency and low daily maintenance costs. In addition, zebrafish have 70% of their genes similar to human genes. Thus, this animal model brings great advantages in the identification of DMD genes and in the study of possible therapies [8,37].

5. Treatment

Drug treatment

The great advantage of pharmacological treatment is that the drugs have a systemic action, reaching all of the patient's muscles, which is a very important factor in the treatment of Duchenne muscular dystrophy. However, many of the drugs used have several side effects and the development and testing of new drugs is a complex task [38].

Corticosteroid therapy

Drug treatment aims to prolong the patient's survival, slowing the progression of the disease. Corticosteroids have been used to improve muscle strength, slow the rate of muscle degeneration and increase lung and heart capacity [39]. Prednisone and defrazacort are the most commonly used glucocorticoids in the treatment of DMD [40].

The mechanism of action of corticosteroids is still unclear, but theories suggest that they probably participate in the modulation of several cellular events such as inflammation, apoptosis, regulation of intramuscular calcium concentration and myogenesis. There are also theories that they increase the expression of dystrophin, utrophin, muscle levels of creatine and taurine, alter the mRNA levels of immune system genes and interfere with neuromuscular transmission [20,40].

The major disadvantages of corticosteroids are the serious side effects, including osteoporosis, cataracts, weight gain, insulin resistance, behavioral changes, Cushingoid facies, growth disorders and high blood pressure [41].

Gentamicin

Gentamicin is an antibiotic from the aminoglycoside class that is capable of increasing dystrophin levels in the body. This antibiotic reduces the cell's ability to detect mutations in RNA and, in this way, the dystrophin gene mutated in DMD patients becomes capable of translation [42].

Clinical studies have shown that its prolonged use can present some side and toxic effects, including kidney damage [43].

Utrofina

Utrophin is a protein very similar to dystrophin in both structure and function, with 80% similarity in amino acid sequence [44]. It is expressed in the sarcolemma during fetal development and is progressively replaced by dystrophin. In adults, utrophin is present in the neuromuscular junction, the myotendinous junction and also in the sarcolemma of regenerated myofibers. In patients with DMD, its concentration is naturally increased in areas of muscle regeneration [45]. Increased utrophin expression through genetic and pharmacological therapies is considered a very promising treatment because it leads to improved muscle contractile function and reduced muscular dystrophy [8].

Stem cell therapy

Stem cell therapies for DMD treatment can be autologous or allogeneic. In autologous therapies, stem cells are taken from the DMD patient and genetically altered.*in vitro*to restore dystrophin expression and

reimplanted into the same patient. In allogeneic therapies, stem cells are taken from an individual with normal dystrophin expression and then implanted into a patient with DMD [20].

Stem cell populations that have been most studied for DMD therapy include embryonic stem cells, satellite cells, muscle-derived stem cells, peripheral blood cells, bone marrow-derived stem cells, mesangioblasts, adipose-derived stem cells, muscle- or blood-derived CD133+ cells, and pericytes [8]. The most promising multipotent progenitor is the mesangioblast, because it is easily isolated from blood vessels and because of its ability to transmigrate arterially [46].

Gene therapy

Gene therapy aims to introduce the missing dystrophin gene or replace the defective gene using various vectors [20]. The most promising vector is the adeno-associated virus (AAV), because it is non-pathogenic, stable in cells that do not usually replicate, such as muscles, and has serotypes that have tropism for muscles (AAV1, AAV6, AAV8 and AAV9). However, the dystrophin gene is very large and exceeds the transport capacity of AAV. The development of minidystrophins and microdystrophins through the deletion of non-essential codon regions was a solution found to this problem [46].

Antisense oligonucleotide therapy/ Exon skipping therapy

Exon skipping is a molecular treatment in which antisense oligonucleotides are introduced into the patient and bind to specific sites of the pre-messenger RNA of the dystrophin gene, resulting in the removal of mutated exons [47]. In this way, the final dystrophin protein sequence will be incomplete but more functional [20].

Physiotherapy treatment

Physiotherapy treatment is essential for maintaining and even improving muscle strength and range of motion, reducing the development of contractures, preventing respiratory complications, postural changes, deformities and promoting independence in carrying out activities of daily living [48,49].

There is no consensus in the literature regarding the indication of physical exercises for patients with DMD, as there is a debate about their beneficial and harmful effects on muscle fiber. High-intensity physical exercises lead to an increase in calcium influx and production of free radicals, accelerating the degenerative process of muscle fiber in patients with DMD [50]. On the other hand, low-intensity and regular exercises decrease oxidative stress, stimulate protein synthesis in the muscle and mitochondrial biogenesis, helping to prevent atrophy, contracture and loss of muscle function [51,52].

The combination of active-assisted and passive stretching of muscles and tendons daily minimizes contractures and deformities and helps maintain muscle length [53,54].

Physiotherapy can also help patients with DMD with orthoses, especially for the lower limbs (knee-ankle-foot and hip-knee-ankle-foot). These orthoses are intended to minimize the appearance of contractures and prolong the patient's functional independence [49].

6. Conclusion:

Since it is considered an incurable genetic disease, it is very important that the diagnosis is made as early as possible. A rigorous individual assessment and daily monitoring of these patients by a multidisciplinary team is necessary, ideally composed of doctors, pediatric nurses, social workers, physiotherapists, occupational therapists, psychologists, speech therapists and nutritionists, since the complications of the disease occur in several areas.

Depending on each case, the administration of drugs that can work together with palliative care can improve the patient's quality of life. Therefore, this team must establish an appropriate treatment program that aims to prolong survival, but with a good quality of life.

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