A review of diagnosis of Duchenne and Becker muscular dystrophy

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Abstract

Duchenne muscular dystrophy (DMD and Becker muscular dystrophy (BMD) are progressive serious neuromuscular disorders. We have reviewed contemporary data on diagnosis of DMD and BMD. Searches were carried out from 2010 to 2020. This article discusses clinical signs, features in biochemical blood analysis, findings on instrumental investigation, various mutations causing DMD/BMD, indications for morphological examination of muscles available for setting up the diagnosis for children suspected of DMD/BMD.

Key words: Duchenne muscular dystrophy, Becker muscular dystrophy, diagnostic methods

Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are the most common forms in the structure of orphan diseases with damage to the neuromuscular system. They represent one of the important problems of clinical neurogenetics [1].

DMD is a severe type of muscular dystrophy with manifestation at the age of 2-5 years, progressive malignant course with a complete loss of motor activity at the age of 13-16 years and death, usually from heart or respiratory failure at the age of 20 years [2,3]. The disease is named after the French neurologist Guillaume-Benjamin-Amand Duchenne, who was the first person described it as "hypertrophic paralysis" in 1868. The incidence among newborn boys is 1 in 3,000-5,000 [4].

In 1955, German physician Peter Becker described "mild" type of muscular dystrophy, which was later named after him. This disorder occurs 6 times less often (1 case in 18,000-31,000), usually debuts later, the loss of motor activity is noted after 20 years, the life expectancy of patients can reach the fourth decade [5].

The diseases are caused by mutations in the dystrophin gene, the largest human gene located at the Xp21.2 locus [6]. At DMD this mutation results in a severe lack of dystrophin (<5%); at BMD there is an abnormal dystrophin formation or dystrophin deficiency. Dystrophin performs structural functions, protecting the muscle cell from tension at the time of contraction and relaxation of the muscle fiber. The absence of dystrophin causes impairment of skeletal and cardiac muscles, also the motor component of human activity is lost [7].

Since DMD/BMD is inherited in an X-linked recessive manner, it is usually affecting boys who have received the mutant X chromosome from a phenotypically healthy mother or mother with 'mild" clinical manifestation [8]. In this case, their daughters will be carriers of the defective gene and will pass it to their male children - thus, the disease manifests only through a generation.

The prevalence of DMD and BMD in world populations is characterized by variability, the approximate average figure according to the meta-analysis of 31 studies is 4.78 (95% CI 1.94-11.81) and 1.53 (95% CI 0.26-8.94) per 100,000 men, respectively [9,10]. The results of an epidemiological study conducted in 6 American states (Arizona, Colorado, Georgia, Hawaii, Iowa and New York) showed a prevalence of DMD and BMD of 10,2 per 100,000 men aged 5 to 24 [11]. Also, the prevalence of DMD and BMD is higher than in the world, in Norway (16.2 per 100,000 men under 18 years old), in Canada (10.3 per 100,000 men under 24 years old), in France (10.9 per 100 000 male population) [12,13]. In some regions of the Russian Federation, the prevalence of DMD and BMD is different: in the Republic of Dagestan - 6.0 per 100,000 men, in the Samara region - 10.4 [14,15].

Currently, despite the developed diagnostic criteria, despite advances in diagnostic technology, the early diagnosis of DMD and BMD is a challenging issue. Patients consulted with many doctors before setting up the clear diagnosis [16].

Purpose: to review the literature on the diagnosis of DMD and BMD for the last 10 years.

Materials and methods

Searches were carried out from 2010 to 2020 in databases: PubMed and Google scholar. The search language: English and Russian. Key words: Duchenne muscular dystrophy, Becker muscular dystrophy, diagnostic methods. In both databases there were found 511 free full-text articles. Exclusion criteria: Studies published in non-English and non-Russian languages, studies published prior to 2010 and after 2020, animal studies, studies on epidemiology, clinical scales, treatment issues, life quality of patients. After reading of topic and abstract 32 articles were applicable for our aim. They were taken for analysis.

Results and Discussion

According to Clinical protocol of diagnosis and treatment on "Progressive Duchenne/Becker muscular dystrophy" of the Ministry of Health of the Republic of Kazakhstan dated by April 19, 2019, the modern diagnostic pattern of DMD and BMD includes the following methods: clinical manifestation, laboratory, instrumental, molecular-genetic tests and morphological examination of biopsy.

When establishing the DMD and BMD diagnosis the age of onset of the first clinical symptoms of the disease, the type of myodystrophic lesion (distal and/or proximal), the localization of atrophies, the presence or absence of pseudohypertrophies, fasciculations, sensory disorders, cramps, skeletal deformities, joint muscle contractures, the condition of muscle tone and tendon reflexes, disease course and progression of the disease is important [17].

As the dystrophin is localized in the central nervous system it's lack affects the brain's cognitive function. Almost one from three patients with DMD and BMD diagnosis has the cognitive impairment. However, this cognitive impairment proceeds without progression. There is a high incidence with autism spectrum disorders, obsessive compulsive disorder and attention-deficit hyperactivity disorder. Children might have delayed cognitive development, problems with reading, learning and speech delay [18,19]. These cause the intervention of multidisciplinary team (language specialists, neuropsychologists) to diagnostics and treatment [20].

Laboratory tests

The list of laboratory tests includes a biochemical blood test with the determination of the level of creatine phosphokinase (CPK), Alanine transaminase, Aspartate transaminase and lactate dehydrogenase (LDH) [21].

CPK is an intracellular enzyme that performs an energy function in the body. It is found in the greatest amount in the heart and skeletal muscles. Since the enzyme is contained inside the cells, so its increase in the blood indicates the destruction of these cells. An increase in CPK is the obligate early preclinical sign of DMD and BMD. An increase in the CPK level by 5 or more times is diagnostically significant. DMD is characterized by a significant increase in the level of enzymes by 10-100 times already in the early stages of the myodystrophic process [22].

Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes predominantly accumulate in hepatocytes, but are also largely concentrated in muscle cells. It is recognized that an increase in ALT and AST levels can signal the cytolysis of muscle cells, therefore, muscular dystrophies can lead to hypertransaminasemia [23]. Sometimes hypertransferasemia can be the only clinical and laboratory finding, more often it occurs with a parallel increase in CPK. Unlike other myopathies in DMD, there is a high degree of hyperenzymemia already in the early stages of the process development. Thus, the literature describes clinical cases of DMD and BMD with an increase in ALT to 477 IU/L, AST - to 497 IU/L [24].

LDH is an intracellular glycolytic enzyme that is involved in the reversible conversion of lactate to pyruvate and is found in most body tissues, and is most active in skeletal muscle. In diseases accompanied by tissue damage and cell destruction, the LDH activity in the blood increases, and therefore, it is an important marker of tissue destruction. Despite the fact that an increase in enzyme activity does not indicate any specific disease, its determination in combination with other laboratory tests helps in the diagnosis of muscular dystrophy. An elevated LDH level is not the obligate sign of DMD, whereas for BMD it is an early and diagnostically significant symptom. In a comparative analysis of 17 patients with DMD and 38 patients with PMD of unspecified etiology, the most significant differences between the groups were in the levels of enzymes of muscle breakdown (muscle cytolysis), namely, the LDH level was significantly higher by 4 times in patients with DMD [25].

One of the novels both diagnostic and therapeutic targets of DMD picture is the identification of miRNAs types in DMD. Its amount in blood and in muscle biopsy may be as biomarker of early disease or disease stage. miRNAs have correlation with muscle fibrosis due to partial connection to myogenesis [26].

Magnetic imaging and neurofunctional findings

Electroneuromyography (EMG) is a fairly simple but highly informative diagnostic method based on the registration and study of the bioelectric activity of the neuromuscular apparatus at rest and during its activation. EMG includes two main methods - stimulation and needle. In the diagnosis of DMD, stimulation EMG does not play a special role, needle EMG is more informative. It serves as the main research method for suspected DMD. The implementation of this technique allows us to identify the primary muscular type of changes in the motor unit potentials (decrease in the duration and decrease in the amplitude of motor unit potentials) and the spontaneous activity of muscle fibers (in the form of acute wave potentials, fibrillation potentials), indicating the degree of activity of the process in each specific muscle. The spontaneous activity recorded in DMD is always significantly pronounced, which distinguishes DMD from other hereditary primary muscular diseases. It is observed in the very initial stages of the disease, when, along with fibrillation potentials, acute wave potentials, and high-frequency discharges are detected [27,28].

The problem of involvement of the heart in the pathological process in DMD is well known [29]. Damage to the cardiovascular system (cardiomyopathy) develops in 73% of sick children, while the level of detection of cardiac disorders and the prevention of severe complications in the early stages are still very low [30]. Deficiency of dystrophin in cardiomyocytes leads to progressive atrophy and their replacement by fibrous tissue [31]. Cardiomyopathy for first time is diagnosed at the age of 6-7 years; by the age of 20, 95% of patients have it [32]. Patients with DMD are required to undergo electrocardiography (ECG) and echocardiography (EchoCG). The ECG is an important screening tool for detecting arrhythmias and conduction disturbances, ventricular hypertrophy or dilatation. EchoCG is considered the gold standard for diagnosing structural and functional disorders of the myocardium and contributes to the identification of cardiac pathology at the preclinical stage. Typical signs on the ECG are abnormalities in the heart rate,

rhythm and conduction of the heart, on the EchoCG - are signs of systolic dysfunction, left ventricular dilatation, myocardial hypertrophy, mitral regurgitation [33].

Ultrasound examination for assessing the condition of the muscles is attractive due to the absence of a radiation effect and relatively low cost. Also, the method is not invasive and allows with a high degree of probability (from 86% to 91%) to distinguish a healthy patient from a patient with neuromuscular pathology. Pathology in DMD and BMD is manifested by signs of muscle degeneration: replacement of muscle tissue with adipose or fibrous tissue [34].

Magnetic resonance imaging (MRI) has become the method of choice for muscle imaging due to the absence of side effects (ionizing radiation), good resolution and soft tissue contrast for full body scans [35]. For patients with DMD and BMD, the study can be used not only for diagnostic purposes, but also to assess the degree of muscle tissue degeneration in dynamics [36,37].

Genetic analysis

DMD/BMD are caused by mutations in the gene encoding dystrophin (DMD gene) [38]. The presence of genetic confirmation of the mutation is important for patients as it is important for the prognosis of the disease, neuropsychiatric involvement, genetic counseling and assessment of each patient's compliance with the criteria for new genetic therapies [39-42]. Determination of large mutations in the DMD gene in DMD/BMD is carried out firstly by the MLPA method. "Next-generation" sequencing technology and Sanger sequencing are proceeded when MLPA result is negative [43]. Next-generation sequencing is the way of choice as a DMD gene single-point detection technique, so some authors recommend to use it as routine strategy [44]. In accordance with international clinical guidelines, genetic testing for DMD/BMD is carried out in the presence of clinical signs of hereditary neuromuscular disease, as well as the patient's relatives and children. The biological material for research is DNA isolated from peripheral blood leukocytes, fibroblasts, chorionic villi, cultured amniocytes and other human tissues.

There are more than 3 thousand various mutations that might cause DMD, however the huge number of these mutations are localised within hotspots regions of the dystrophin gene. That is 6-7, 43-46 and 50-53 exons [45]. Genetic diagnosis is not easy due to the large size of the dystrophin gene (79 exons) and the complex spectrum of mutations. Large deletions (60-68%) and duplications (10-11%) are most common, but small mutations (20-30%) are also found [46,47].

The presence of the mutation is a molecular genetic confirmation of the clinical diagnosis of DMD/BMD and allows prenatal diagnosis in the family [48-50].

Morphological data

If genetic testing does not confirm a clinical diagnosis of DMD, the muscle biopsy sample should be tested for the presence of the protein dystrophin [51]. The indication for biopsy is the signs of muscle damage: muscle weakness, discomfort, cramps, pathological muscle fatigue; increased CPK level; myopathic lesion according to EMG data; differentiation between segmental demyelination and axonal degeneration; identification of inflammatory neuropathies; the presence of a systemic disease with myopathic manifestation. An open muscle biopsy is necessary if a differential diagnosis is performed, which considers DMD as one of the possible options among other muscular dystrophies, since it allows you to obtain the necessary tissue volume for further analysis. Needle biopsy does not require open surgery and may be warranted if DMD alone is considered. When examining muscle biopsy data, two types of tests are usually performed: immunohistochemical and immunoblotting analysis (a method for studying of protein antigens). These tests determine the presence or absence of dystrophin in quantitative form; with their help, it is possible to distinguish DMD from the milder form of myodystrophy - BMD. The morphological criteria of muscle pathology are: violation of the muscle fibers types distribution; change in the size of muscle fibers; violation of the structure of muscle fibers and their elements; pathological inclusions and mass formations in muscle fibers; pathological changes in skeletal muscle tissue in general. The histopathological peculiarities of DMD are: abnormal diameter variation of the muscle fibers due to atrophy or hypertrophy, focal necrosis, regenerative fibers and replacement of muscle tissue with fat and connective tissue (visually that is looks like pseudohypertrophy of muscles (usually calf muscles) [52].

Conclusion

To sum up, the diagnosis of DMD and BMD is complex. It includes the clinical manifestations, laboratory findings, genetic testing, instrumental tests and muscle biopsy. The majority of diagnostic technics are broadly available and feasible. The results of molecular-genetic analysis may correlate with the severity of clinical signs, it is valuable issue in confirmation of diagnosis and important in choosing of further pathogenetic therapy drug. Immunohistochemical and immunoblotting analysis of muscle biopsy data give the possibility to differentiate the number of dystrophin protein in order to diagnose the DMD/BMD or control the treatment with gene therapy in the future.

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