

RESULTS OF MEDICO-GENETIC STUDY OF PATIENTS WITH DUCHENNE/BECKER PROGRESSIVE MUSCULAR DYSTROPHIES IN UZBEKISTAN



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ABSTRACT

The purpose of study was to analyze clinical and genetic polymorphism of Duchenne/Becker progressive muscular dystrophies among patients with neuromuscular diseases in Uzbekistan. 106 male patients with progressive pseudohypertrophic forms of muscular dystrophy were retrospectively and prospectively analyzed in the period from 2004 till 2014: 93 patients with Duchenne PMD aged from 3 years to 18 years and 13 patients with Becker PMD aged from 10 years to 25 years, who had been examined in the medico-genetic consulting department of the Republican Center "Mother and Child Screening" of Tashkent city. Comprehensive clinical, neurophysiological, biochemical and genetic study of patients as the integral part in the differential diagnosis of Duchenne/Becker progressive muscular dystrophies allows creating the national database on D/B PMD to prevent the birth of children in families burdened by this disease.

UDC CODE & KEYWORDS

■ UDC: 616-007 ■ Duchenne/Becker progressive muscular dystrophies ■ Electroneuromyography ■ Dystrophin gene ■ Children

INTRODUCTION

According to Zinchenko (2001), Rakhmonov (2004) and Kirilenko (2005), annually more than two million children with hereditary diseases are born in the world, amounting from 2.5 to 15 patients per 1000 live births. Early death or disabilities occur in more than half of them. The others need constant medical and social care.

Ginter and Zinchenko conventionally considered hereditary diseases with the prevalence more than 1:50000 of population as frequent hereditary diseases. They include such hereditary diseases as myotonic dystrophy, Huntington's chorea, Duchenne/Becker myodystrophy, and motor-sensory neuropathies (Zinchenko, 2001).

In the Republic of Uzbekistan, there has been realized the State Program "Mother and Child Screening" on creating and developing the state system of early detection of congenital and hereditary diseases through conducting mass neonatal newborn screening and prenatal investigation of pregnant women at risk, which was adopted by the Cabinet of Ministers No.140 dated from August 4, 1998. Researches on the analysis of acquired genes of progressive muscular dystrophies (PMD) are of particular relevance due to their significance for the solution of many problems of care for patients suffering from these very severe and debilitating genetic diseases. Primarily, they naturally focus to the most common and clinically important types of PMD, including Duchenne/Becker and Erb-Roth muscular dystrophies (Krakhmaleva et al., 1999; Zinchenko, 2001; Rakhmonov, 2004; Koren, 2005).

Considering the high frequency, severe degree of disability and early mortality, the studying clinical polymorphism of Duchenne/Becker progressive muscular dystrophy (D/B PMD) in our region with further creation of an algorithm of differential diagnosis and registry of patients is relevant and timely for prevention of births of children with defective dystrophin gene in families burdened by D/B PMD.

The purpose of study: To analyze clinical and genetic polymorphism of Duchenne/Becker progressive muscular dystrophies among patients with neuromuscular diseases in Uzbekistan.

Patients and Methods

Between 2004-2014, we retrospectively and prospectively analyzed 106 male patients with progressive pseudohypertrophic forms of muscular dystrophy: 93 patients with D PMD aged from 3 years to 18 years and 13 patients with B PMD aged from 10 years to 25 years, who had been examined in the medico-genetic consulting department of the Republican Center "Mother and Child Screening" of Tashkent city. Diagnosis based on clinical manifestations of the disease, biochemical studies (determination of the level of activity of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in blood serum), electroneuromyography using concentric needle electrodes, as well as molecular diagnostics performed by direct PCR on the applicator "Tertsik" (Russia) using diagnostic kit DMD-del. Motor functions were evaluated by Modified Rank in Scale (MRS).

Results and Discussion

Genealogical analysis revealed the presence of X-connected recessive inheritance of D/B PMD in 23 (27.7%) families, while in 60 (72.3%) families identified the only cases.

According to clinical findings, in patients with D/B PMD muscle strength was 5 points in 1.6% (n=1); 4 points in 49.2% (n=31); 3 points in 30.1% (n=19); 2 points in 14.3% (n=9); 1 point in 4.7% (n=3) of patients, respectively.

Depending on mean age of patients, results on MRS scale were as follows: in group with 5 points - 3 years old; 4 points - 5 years and 10 months; 3 points - 6 years and 9 months; 2 points - 12 years and 3 months; 1 point - 15 years and 6 months, respectively.

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CPK average levels in different points of muscle injury were as follows: in the group with 5 points - 5025 u/l; 4 points - 4523 u/l; 3 points - 5219 u/l; 2 points - 3188 u/l; 1 point - 1258 u/l (Table 1).

Table 1: Muscular strength in patients with PMD, depending on age and creatine phosphokinase (CPK) levels

Muscular strength (in points)	Number of patients (in brackets)	Mean age of patients	Average CPK level in blood (u/l)
5	1.6 % (1)	3 years	5025
4	49.2 % (31)	5 years and 10 months	4523
3	30.1 % (19)	6 years and 9 months	5219
2	14.3 % (9)	12 years and 3 months	3188
1	4.7 % (3)	15 years and 6 months	1258

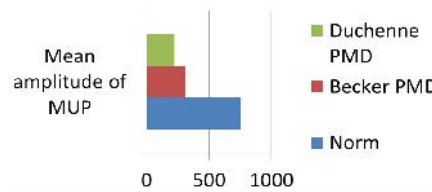
Source: Author

Electroneuromyography with using concentric needle electrodes (needle EMG) is known to be one of the most important methods in the diagnosis of D/B PMD. This simple, highly informative and widely available method allows not only to identify the level of involvement of the motor unit, but also on the basis of EMG monitoring to evaluate the severity of the pathological process at different stages of the disease.

We examined 32 patients with diagnosis of D/B PMD aged from 6.5 to 24 years, including 4 patients with Becker PMD. All patients had complaints of weakness of different degrees in limbs and gait disturbance. To clarify the nature of the lesion and to identify the degree of denervation, in all patients we used needle EMG with concentric needle electrodes on the apparatus "Neuron-Spectrum-4/VPM". EMG was performed in the most atrophied muscles, exactly in the top group of inferior limb muscles (anterior, lateral and medial femoral muscles). During the investigation, we conducted the test to identify for spontaneous activity in the muscles and to determine MUP (motor unit potentials). Spontaneous activities in the form of fibrillations, fasciculations and positive sharp waves were recorded with subsequent data processing. To identify MUP the patient was recommended to fight back against the direction of force for the minimal activation of muscle. Appeared potentials were recorded followed by data processing. When analyzing MUP, we measured duration, shape and amplitude. To identify mean indicators we recorded 20 PDE from different parts of the examined muscles.

Thus, in all patients duration of MUP was less than 10 ms and on the histogram corresponded to stage II of denervation-reinnervation process (DRP) (Figure 1). In all cases, the form of MUP was changed as polyphase curves. All surveyed noted decrease in the mean duration of MUP: on 36.3% in case of D PMD and on 18.9% in B PMD, that corresponded to stage II of DRP.

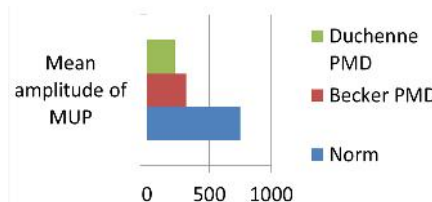
Figure 1: Mean duration of motor unit potentials (MUP) in patients with PMD (ms)



Source: Author

In patients with D PMD the mean amplitude of MUP decreased on 70.3%, while in patients with B PMD – on 58%. In 2 patients the mean amplitude of MUP increased because of pseudohypertrophy of muscle fibers (Figure 2).

Figure 2: Mean amplitude of motor unit potentials (MUP) in patients with PMD (mkW)

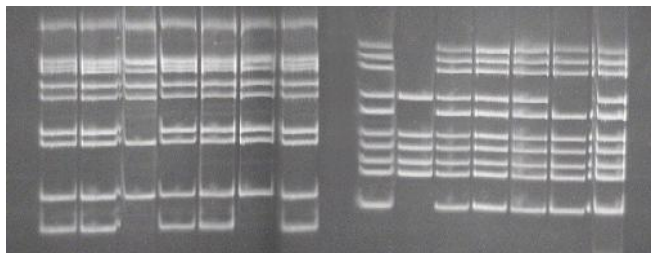


Source: Author

In 24 patients, the survey observed spontaneous activities in the form of fibrillation potentials (FP), which varied by quality and quantity. In 8 patients with significant changes and rapid progression the spontaneous activity was not determined. Thus, in patients with the most long-lasting process (the earliest age) the spontaneous activity was revealed in the least and low amplitude than patients with relatively recent process. In 9 patients the spontaneous activities were also noted as positive sharp waves; in this case, this phenomenon was observed in patients with the most long-lasting process.

We have carried out a direct molecular genetic diagnosis in 47 patients from 35 families, which were registered at the Republican Center "Mother and Child Screening" and diagnosed with D/B PMD. Analysis was performed by 20 exons of the dystrophin gene - the promoter region, exons 3, 4, 6, 8, 13, 17, 19, 32, 42, 43, 44, 45, 47, 48, 50, 51, 52, 53, and 60. The study has found extensive deletions in patients from 19 families (54.3%). The more frequent deletions in a 50 (27.2%) 51 (27.2%) 52 (27.2%) 19 (18.2%) exons. There were no deletions in the analyzed exons in patients from 17 families (48.6%).

Figure 3: PCR study of dystrophin gene in patients with D/B PMD



Source: Author

According to the literature, approximately 30% of all cases are due to de novo mutations in the egg of mothers of sick patients, and the remaining 70% are due to the maternal heterozygosity by pathological mutations in the dystrophin gene. However, with pedigrees with clearly traced illnesses in the family, according to our data, were about 27.7%.

Summary

After data of clinical and biochemical studies at the time of initial refer had been analyzed, a direct correlation only between age and the muscular system injury degree was found. At the same time, the serum CPK level reflected motor impairment degree only in the terminal stages of the disease and was not a marker of the effectiveness of therapeutic interventions.

Based on data of electroneuromyography, we can conclude that in patients with D/B PMD characteristic changes of MUP and severity of spontaneous activity reflects the nature and degree of severity of the process, as well as the ability to forecast the quality of life and outcomes of the disease.

According to our study, the number of deletions and the ratio of "hot spots" in sporadic and familial cases of the dystrophin gene in Uzbek patients correspond to data of international studies. However, the frequency of cases of primarily identified cases of D/B PMD in families is about 2/3 of the total number of cases found, while according to the literature the familial mutations in the dystrophin gene are found in two thirds of cases.

Conclusion

Thus, the comprehensive clinical, neurophysiological, biochemical and genetic study of patients is the integral part in the differential diagnosis of Duchenne/Becker progressive muscular dystrophies, which also allows creating the national database on D/B PMD to prevent the birth of children in families burdened by this disease.

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