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Klinik Araştırma

The evaluation of muscle biopsy findings in children with neuromuscular disorders

Nöromuskuler hastalığı olan çocuklarda kas biyopsi bulgularının değerlendirilmesi

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ABSTRACT

Objective: The studies considering the clinical and histopathological features of childhood muscle disease are scarce in number worldwide. This study aims to examine the histopathological profile of the children's muscle biopsies evaluated at the Behcet Uz Children's Hospital (BUCH) and determine their correlation with clinical diagnosis.
Methods: We retrospectively reviewed 323 children who underwent muscle biopsy examination between 2004 and 2011 at pathology laboratory of Izmir BUCH. All patients were clinically diagnosed as neuromuscular disorders and biopsy specimens were collected from 5 different centers of neurological disorders.
Results: The mean age of the patients was 6.15 years (3 weeks-18 years). Only 22 cases (6.8%) were older than 14 and 39 (12.07%) were younger than twelve months. Spinal muscular atrophy (n=11; 28.2%) was the most common disease in the infantile group. Dystrophies were diagnosed in 148 (45.7%) patients and 102 of them were dystrophinopathies. Other disorders were 23 primary, 16 inflammatory and 19 metabolic/mitochondrial myopathies and 28 neuropathies. Seventy three (22.9%) patients had similarly affected family members. Consanguinity rate was 25.1%. Serum enzymes alterations, EMG pathologies and fiber type disproportion were determined in 109 (33.7%), 188 (58.2%) and 85 (26.3%) of the patients respectively. There were statistical significant correlations between the types of disease and serum enzyme levels (p= 0.001), and age (p=0.015) of the patients.
Conclusion: The present study revealed the panorama of the childhood muscle diseases in western Turkey. We conclude that if avoidance from unnecessary diagnostic and therapeutic interventions was desired, we must be aware of the limitation and diagnostic value of muscle biopsy evaluations in childhood muscle diseases.

Key words: Muscle biopsy, childhood, histopathological evaluation

ÖZET

Amaç: Dünyada çocukluk dönemi kas hastalıklarının klinik ve histopatolojik özelliklerini irdeleyen çalışmalar çok az sayıdadır. Bu çalışma Dr. Behçet Uz Çocuk Hastanesi (BUÇH) patoloji laboratuarında değerlendirilen çocuk kas biyopsilerinin histopatolojik profilini incelemeyi ve klinik ön tanılarla kas biyopsi sonuçlarının uyumunu değerlendirmeyi amaçlamıştır.
Yöntemler: Dr. BUÇH patoloji laboratuarında 2004 ile 2011 yılları arasında incelenen 323 kas biyopsisi retrospektif olarak değerlendirildi. Tüm hastalar klinik olarak nöromuskuler hastalığa sahipti ve kas biyopsi materyalleri 5 farklı nörolojik hastalıklar bölümünden gönderildi.
Bulgular: Hastaların ortalama yaşı 6,15 idi (3 hafta- 18 yaş). Yalnızca 22 olgu (%6,8) 14 yaşın üstünde, 39 (%12,07) olgu 12 aydan küçüktü. Spinal muskuler atrofi (n=11, %28.2) infantil grupta en sık gözlenen patolojiydi. Muskuler distrofi tanısı 148 (%45,7) hastaya kondu ve bunların 102'si distrofinopatiydi. Diğer hastalıklar; 23 primer, 16 inflamatuvar, 19 metabolik/mitokondrial myopati ve 28 nöropatiydi. Yetmiş üç hastada (%22,6) aile öyküsü mevcuttu. Akraba evliliği oranı %25,1 bulundu. Serum enzim yüksekliği, EMG patolojisi ve myofiber tip dağılım bozukluğu sırasıyla 109 (%33,7), 188 (%58,2) ve 85 (%26,3) hastada saptandı. Hastalık tipiyle CPK düzeyleri (p=0,001) ve yaş (p=0,015) arasında istatistiksel olarak anlamlı ilişki vardı.
Sonuç: Bu çalışma Türkiye'nin batısındaki çocukluk çağı kas hastalıklarının panoramasını gözler önüne sermiştir. Gereksiz tanısal işlemler ve tedavilerden sakınmak isteniyorsa, çocukluk çağı kas hastalıklarının değerlendirilmesinde kas biyopsisinin sınırlılığının ve tanısal değerinin farkında olmak gerektiğini düşünmekteyiz.

Anahtar kelimeler: Kas biyopsisi, çocukluk çağı, histopatolojik değerlendirme

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INTRODUCTION

Skeletal muscle biopsy plays an important role in guiding the management of a child thought to have a neuromuscular disorder [1]. But it must be kept in mind that the muscle biopsy is only one facet of the overall diagnostic evaluation of the children with neuromuscular disorders [2]. Therefore, the differential diagnosis requires coordination of the clinician, surgical team and pathologists. The clinician must first arrive at a possible diagnosis by synthesizing information obtained from the clinical history, physical examination, laboratory findings and electrodiagnostic studies [3]. This information is used to determine the details of biopsy procedures such as choosing the right time for biopsy and selection of muscle to be biopsied. Surgeon must have skills requisite to provide a nontraumatized specimen [1-3].

After the biopsy specimens arrive in the pathology laboratory, they undergo a complex series of studies [4,5]. Clinical features of neuromuscular disease are highlighted because knowledge of the clinical history is crucial to correctly interpret the histological findings in a skeletal muscle specimen [3]. Interpretation of a muscle biopsy specimen can be difficult, because few individual histological findings are specifically diagnostic of a single disorder. Most biopsy specimens exhibit numerous findings in varying degrees, each of which is consistent with an assortment of diagnoses and these findings must be synthesized to arrive at a pathological diagnosis. The muscle pathologist uses knowledge of the clinical features to assist in interpretation of the constellation of pathologic findings in the biopsy, judges the clinical significance of each finding and helps to determine whether additional studies are needed for each patient [4-6].

In this article, we aimed to reveal the diagnostic value of muscle biopsy in children with neuromuscular disorders, to emphasize the importance of collaboration with the clinician and muscle pathologist and to exhibit the panorama of the childhood muscle diseases in western Turkey.

MATERIAL and METHODS

The study was performed at the Pathology Laboratory of the Izmir Dr. Behcet Uz Children's Hospital from January 2004 through December 2011. Three hundred and twenty three children with neuromuscular disorders were included in this study. Individual patient database was reviewed in all cases and detailed clinical information of the patients was recorded including age, status of functional ability associated complaints like muscle weakness and respiratory distress, detailed family history and consanguinity. Neurological examination and laboratory findings were also evaluated. Laboratory evaluation included analyses of serum creatine kinase (CK), serum aspartate aminotransferase (AST), and nerve conduction and electromyographic (EMG) tests. Most of the muscle biopsy speciments were from gastrocnemius muscle (n=310). The remaining biopsy specimens were from the deltoid muscle.

Specimens were frozen in isopentane cooled in liquid nitrogen and 8 to 12-micron sections were cut using cryostat. Slides were stained with hematoxylin-eosin (HE), Gomori's trichrome, modified Gomori's trichrome (Engel-Cunningham modification), oil red-O, Periodic Acid Shiff (PAS), D-PAS, crystal violet stains. In enzymatic histochemical techniques nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), succinate dehydrogenase (SDH), cytochrome oxidase (COX) and combined COX-SDH stains were used. Besides, spectrin (Novo-castra, UK, NCL-spec1), dystrophin N-terminus (Novo-castra, UK, NCL-dys3), adhalin (Novo-castra, UK, NCL-a-sarc), other sarcoglycans (beta, delta, gamma; Novo-castra, UK, NCL-b-d-g-sarc), laminin alpha-2 chain (Novo-castra, UK, NCL-merosin), myotilin (Novo-castra, UK, NCL-myotilin), collagen VI (Novo-castra, UK, NCL-COLL-VI), β-dystroglycan (Novo-castra, UK, NCL-b-DG), HLA Class 1 (Novo-castra, UK, NCL-HLA-ABC), NCAM (ThermoScientific, CA, USA, CD56), nitric oxide Synthase-1 (Novo-castra, UK, NCL-NOS-1), emerin (Novo-castra, UK,

NCL-emerin), caveolin 3 (Novus Biologicals, CA, USA, NB110-5029), calpain 3 (Abcam, Cambridge, UK, ab103250) and dysferlin (Novo-castra, UK, NCL-Hamlet-2) antibodies were used for immunohistochemical analyses. Myosin heavy chain fast (Novo-castra, UK, NCL-MHCf) antibody was used for discriminating fiber type, and myosin heavy chain neonatal (Novo-castra, UK, NCL-MHCn) antibody was used for identification of pathological immature fibers.

Spearman's correlation analysis and chi-square test for the comparison between groups were performed for statistical analysis. P values less than 0.05 were considered to be statistically significant.

RESULTS

The mean age of the children was 6.15±4.6 years ranging from 3 weeks to 18 years. Detailed clinical characteristics of the patients were presented in Table 1. The majority of the patients presented some degree of muscle weakness. Others were suspected to have a neuromuscular disorder with high creatine kinase (CK) and/or transaminase levels. In floppy infants, muscle biopsies were performed for differential diagnosis. Seventy three patients (22.6%) had similarly affected family members. Consanguinity rate was 25.1% (n=81). Physical examination at the time of diagnosis revealed muscle weakness in all patients.

Table 1. Clinical Characteristics of children.

Features	Patient Number	Percent
Positive family history	73	22.6%
consanguinity	81	25.1%
Associated congenital anomalies	18	5.5%
Increased enzym level	109	33.7%
Pathological EMG findings	188*	72.3% (of 260 patients)
Pathological genetics findings	20**	60.2% (of 33 patients)

** EMG could not be performed in 63 (19.5%) patients and most of them were in infantile age group. **Genetics investigations were performed in only 33 patients (10.2%)*

One hundred and nine patients (33.7%) had at least one abnormal muscle serum enzyme level. Needle electromyogram was performed in 260 patients (80.5%) and was classified as myopathic in 182 (56.3%), neuropathic in 6 (1.9%) and normal in 72 (22.3 %) cases. EMG could not be performed in some patients, especially in the infantile age group. Final diagnosis was assigned on the basis of muscle biopsy findings (Table 2). There were only minimal number of abnormal findings in the muscle biopsy specimens of 89 children (27.6%). These biopsies were reported as normal or nearly normal striated muscle tissues. Other 234 children (72.4%) had a muscle disease. In 23 patients (7.1%), nonspecific myopathic changes such as alterations of myofiber size and shape, increased number of internal nuclei, fiber degeneration, and fiber type disproportions were determined and they were simply classified as congenital myopathy. Myofibrillar myopathy (MFM) and centronuclear myopathy (CNM) were also diagnosed in muscle biopsies which were also included in this group. In patients with metabolic and mitochondrial diseases, diagnoses were confirmed with electron microscopic and/ or genetic investigations. Type 1 fiber atrophy was determined in 12 patients (3.7%), while type 2 fiber atrophy in 72 (22.3%). In addition in one case (0.3%), there was no fast myosin staining and it was diagnosed as myopathy with myosin heavy chain defect. In the other 238 patients (73.7%) fiber type distribution was normal.

Muscular dystrophies (MD) were the most common disease (45.8%) in this series and in 102 patients

Table 2. Profile of Muscle biopsy diagnosis (n=323).

Disorder	Patient Number	Percent
Minimal changes	89	27.6%
Congenital myopathy	23	7.1%
Muscular dystrophy, dystrophin negative	102	31.6%
Muscular dystrophy, dystrophin positive	46	14.2%
Neuropathy/ spinal muscular atrophy	28	8.7%
Metabolic / mitochondrial myopathy	19	5.9%
İnflammatory myopathy	16	5%

with muscular dystrophy, sarcolemmal dystrophin defects were determined. The distribution of the dystrophin positive muscular dystrophies was shown in Table 3. Frequency of neuromuscular disorders exhibited differences according to the age (Table 4 and Table 5). Dystrophin positive muscular dystrophy was the most common disease in the older (18.2%) age group and neuropathy (28.2%) was the most common disease in the infantile age (0-1 years) group.

In Spearman's correlation analysis, there were statistical significances between the types of neuromuscular disease and the elevation of muscle enzymes (p=0.001) and age of the children (p=0.015).

Table 3. Subtypes of dystrophin positive Muscular dystrophies (n=46).

Type of Dystrophine positive Muscular Dystrophy	Patient Number	Percent
Limb Girdle MD Type 2C (Gamma Sarcoglycan deficiency)	15	32.6%
Limb Girdle MD Type 2B (Dysferlin deficiency)	2	4.3%
Congenital Muscular dystrophy, (merosin positive or negative)	10	21.8%
Limb Girdle MD Type 2E (Beta sarcoglycan deficiency)	2	4.3%
Limb Girdle MD Type 2D (Alpha sarcoglycan deficiency)	1	2.2%
Emery Dreifuss MD (Emerin deficiency)	4	8.7%
Not otherwise specialized MD	12	26.1%

Table 4. Profile of Muscle biopsy diagnosis in infantile group (n=39).

Disorder	Patient Number	Percent
Minimal changes	16	41%
Congenital myopathy	3	7.7%
Muscular dystrophy, dystrophin negative	2	5.1%
Muscular dystrophy, dystrophin positive	3	7.7%
Neuropathy/ spinal muscular atrophy	11	28.2%
Metabolic / mitochondrial myopathy	4	10.3%

Table 5. Profile of Muscle biopsy diagnosis in older age group (n=22).

Disorder	Patient Number	Percent
Minimal changes	8	36.4%
Congenital myopathy	2	9.1%
Muscular dystrophy, dystrophin negative	3	13.6%
Muscular dystrophy, dystrophin positive	4	18.2%
Neuropathy/ spinal muscular atrophy	3	13.6%
Metabolic / mitochondrial myopathy	1	4.5%
inflammatory	1	4.5%

DISCUSSION

Relatively little information about the frequency of neuromuscular disorders (NMDs) in childhood has been published [6-12]. Strehle et al. reported that the NMDs affect approximately one in 3500 children wordwide and X-linked dystrophinopathy has the highest incidence among them [11]. Knowledge of NMDs has expended dramatically during the last 4 decades thanks to modern pathological techniques and genetics. Currently the dystrophinopathies and most limb girdle muscular dystrophies (LGMDs) can be diagnosed immunohistochemical staining on the muscle tissues [4-6,13]. With occasional exceptions, evaluation of muscle biopsy is an essential element in the assessment of a child with suspected myopathy [13-18]. Muscle biopsy is also indicated for the diagnosis of various systemic disorders and the evaluation of suspected mitochondrial dysfunction [1-3,19]. In this study, we determined that the muscular dystrophies (MDs) especially caused by dystrophin deficiency was the most common NMD in the children. Some type II LGMDs were also diagnosed by immune histochemical staining. In addition, a centronuclear myopathy case with a novel genetic mutation was determined with muscle biopsy [17,18].

The patient's history and the profile of the disease are often informative in the diagnostic procedures of neuromuscular disorders. For example, a rapid onset of symptoms is suggestive of an inflammatory myopathy, whereas insidious progression favors other noninflammatory myopathies such as metabolic myopathy, muscular dystrophy and most of congenital

myopathies [1-3]. Inflammatory myopathies are infectious in origin or immune mediated [2]. Recently the percent of inflammatory disorders which are diagnosed with muscle biopsies is decreased, because the laboratory findings aid in differential diagnosis. Therefore evaluation of muscle biopsy is rarely required. In our series, only 16 children (5%) have inflammatory myopathies.

Pathologic alterations in muscle fibers are conventionally classified as myopathic or neuropathic [3-6]. The disorders of neuromuscular junction are generally diagnosed with electrophysiological studies and muscle biopsy is not performed [20]. The distinction between neurological diseases and congenital muscular dystrophies may be difficult in infantile cases without histopathological examination [7]. Especially in the floppy infants who require persistent mechanical ventilator support, EMG couldn't be performed. Patient's history may not be suggestive, either. Because both the neurogenic processes and congenital myopathies have the similar insidious progression, muscle biopsy can help to determine whether a neonate has a neurogenic or myogenic disorder, which is a relatively common issue prompting performance of a biopsy [6,7,20]. In this series, muscle biopsies were also very useful for the differential diagnosis of neuropathies, especially in infantile cases. In 72.4% of the children with clinically suspected muscle disease, the presence of muscle disease was confirmed histopathologically. This high percentage was an indicator of good clinicopathologic correlation.

The patients with mitochondrial diseases may have several mutations in the mitochondrial DNA. However, most mutations are organ specific and could not been demonstrated in blood samples [4,19]. Electron microscopy is also of limited use in the diagnosis of mitochondrial disease. Because in normal muscle, there are abnormal fibers which accumulate with aging and it's critical to know the percentage of abnormal fibers [4]. Mitochondria are more numerous in type 1 fibers than type 2 fibers and they are normally found in four locations within the muscle fiber: in intermyofibrillar region adjacent to the Z discs, beneath the sarcolemma, at the poles of nuclei and at motor end plates. The subsarcolemmal mitochondrial masses are particularly prominent in the muscle of children and the point which they become abnormal is sometimes hard to judge [4]. On the other hand the ragged red fibers were being noticeable with the Modified Gomori's trichome staining in chronic mitochondrial diseases and in muscle biopsy of children with the mitochondrial myopathies, no ragged red fibers were shown with histochemical stains [19]. Therefore enzyme activity revealed on muscle biopsy is an important diagnostic tool in mitochondrial diseases especially in early childhood [19,21,22].

In summary, it must be kept on mind that the histopathologic examination of muscle tissue is only one component of the total diagnostic effort and it mustn't be isolated from the patient's history, physical examination and relevant laboratory tests. Paradoxically, in some disorders such as mitochondrial dysfunction, spinal muscular atrophy and most of the muscular dystrophies, differential diagnoses are very difficult without the evaluation of muscle biopsy specimens.

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