Annals of Diagnostic Pathology 47 (2020) 151532

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

The spectrum of muscle pathologies: Three decades of experience from a reference laboratory in Saudi Arabia

Ali H. Alassiri[a,b,c], Ali A. Alyami[b], Mohammed I. Alshabibi[b], Abdullah M. Alhusain[b], Majed H. Nasradeen[b], Abdulelah T. Barri[b], Bader H. Shirah[d,e], Hussein A. Algahtani[d,e,f,*]

[a] Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia
[b] College of Medicine, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
[c] King Abdullah International Medical Research Center, Riyadh, Saudi Arabia
[d] King Abdullah International Medical Research Center, Jeddah, Saudi Arabia
[e] King Saud bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia
[f] King Abdulaziz Medical City, Jeddah, Saudi Arabia

ARTICLE INFO

Keywords:
Muscle biopsy
Neuromuscular disorders
Neuropathology
Reference laboratory
Saudi Arabia

ABSTRACT

Background: When investigating patients with a suspected neuromuscular disorder, a muscle biopsy is considered an instrumental tool to reach a definitive diagnosis. There is a paucity of publications that assess the diagnostic utilization and yield of muscle biopsies. We intend to present our experience in this regard over an extended period of more than three decades.

Methods: This is an observational retrospective cohort study in which we collected pathology reports for muscle biopsies diagnosed at our reference lab between 1986 and 2017.

Results: We identified a total of 461 cases of muscle biopsy performed, which fulfilled the inclusion criteria. Pediatric cases defined as ≤14 years of age constituted a significant proportion of cases (n = 275, 60%). Normal biopsies were reported in 27% of cases (n = 124), and in 4%, the biopsies were non-diagnostic. The most common pathologies reported were non-specific myopathy (n = 72, 16%), dystrophy (n = 71, 15%), and neurogenic disorders (n = 60, 13%).

Conclusion: In conclusion, the muscle biopsy will continue to play a crucial role, as a gold standard or as a complementary investigation, in the diagnosis of certain neuromuscular disorders. Increasing the yield and accuracy of muscle pathology should be the main concern and priority to neuropathologists reporting muscle biopsies. In addition, utilizing next-generation sequencing and other molecular techniques have changed the location of muscle biopsy in the algorithm of the diagnosis of neuromuscular disorders. This paper is an urgent call to establish the Saudi Neuropathology Society and the muscle pathology and neuromuscular disorders registry.

1. Introduction

The first muscle biopsy was taken by Duchenne in 1860 while investigating a patient with a suspected myopathy. It was not until a century later when Victor Dubowitz in 1970 revolutionized the role of muscle biopsy in the diagnosis of different muscle pathologies using histochemical methods. This was followed a few years later by the introduction of immunohistochemical methods for the diagnosis of various subtypes of dystrophies [1]. Over the past 20 years, spectacular progress was made by the utilization of muscle biopsy with the use of molecular methods. The number of muscle biopsies requested is rising since the application of these new techniques. This goes in parallel with the dramatic change in the treatment of neuromuscular disorders and the discovery of promising genetic therapeutic approaches [2].

When investigating patients with a suspected neuromuscular disorder, a muscle biopsy is considered an instrumental tool to reach a definitive diagnosis. This is especially correct if various morphological, ultrastructural, and biochemical analyses were utilized [3]. Although muscle biopsy is an invasive procedure, it is considered safe whether a needle or open biopsy is used [4]. There is a paucity of publications that assess the diagnostic utilization and yield of muscle biopsies. In addition, data from institutional studies focusing on biopsy-proven skeletal muscular diseases from Arab countries is scarce. We intend to present our experience in this regard over an extended period of more than

* Corresponding author at: King Abdulaziz Medical City/King Saud bin Abdulaziz University for Health Sciences, P.O. Box: 12723, Jeddah 21483, Saudi Arabia.
E-mail address: gahtaniha@ngha.med.sa (H.A. Algahtani).

https://doi.org/10.1016/j.anndiagpath.2020.151532

three decades. This data may aid the diagnostic process in clinical practice and prioritize therapeutics. In addition, we review the topic and provide important practical information to the readers.

2. Methods

This is an observational retrospective cohort study in which we collected pathology reports for muscle biopsies diagnosed at our reference lab between 1986 and 2017. Our institution is a governmental 1501-bed tertiary care center with an advanced central laboratory equipped with the necessary tools to process and analyze muscle biopsies. In our institution, the open muscle technique is mostly used under general anesthesia. A sample from the belly of the selected moderately affected muscle is obtained and wrapped in a gauze that is lightly moistened in saline. It is then delivered immediately in the fresh state to the muscle lab to be handled by an experienced specially-trained histotechnologist. Rapid freezing is performed to retain muscle enzymatic activities for routine histochemical staining. After orienting the muscle biopsy longitudinally, a cylindrical piece is cut out and placed vertically in a hollowed-out space in Gum Tragacanth mounted on a disposable cork pad. The specimen is plunged quickly and completely for 30 s into a small container of isopentane, which is placed in another larger container of liquid nitrogen. The latter step allows the temperature of the isopentane to drop to −160 °C that is necessary for rapid freezing and avoiding freezing artifact. Then the biopsy is transferred to the cryostat for immediate transverse sectioning and preparation of the unstained frozen section slides. Additionally, a tiny fragment about one cubic millimeter is submitted in glutaraldehyde fixative to be processed for electron microscopic examination. The remainder of the muscle biopsy is routinely processed using formalin fixation and paraffin embedding. The following panel of histochemical stains is used: modified Gomori trichrome stain, Periodic Acid Schiff (PAS), Oil red O, Congo red, non-specific esterase, NADH, SDH, COX, myophosphorylase, and ATPases at pH 4.3, 4.6, and 9.4.

Non-probability consecutive sampling technique was used to collect data from the archives of the anatomical pathology division. Essential information from the full pathology reports was extracted to a data collecting sheet including the following variables; age, gender, biopsy site, diagnosis, and any relevant blood tests. The inclusion criteria were Saudi patients, available full pathology reports, and in-house diagnosed cases. Accordingly, cases of non-Saudi nationals or lacking detailed pathology reports were excluded. In addition, muscle biopsies in which the pathology is secondary to adjacent diseases or diagnosed outside our institution were also excluded. This study is conducted under the permission of the Institutional Review Board at King Abdullah International Medical Research Center. The resultant data were analyzed using version 22 of the SPSS software. Descriptive statistics were used for statistical analysis.

3. Results

We identified a total of 461 cases of muscle biopsy performed over a period of 31 years (1986 to 2017), which fulfilled the inclusion criteria. Male patients represented 52% of cases (n = 239) while female represented 48% of cases (n = 222). Pediatric cases defined as ≤14 years of age constituted a significant proportion of cases (n = 275, 60%). The most commonly biopsied site was thigh muscles (n = 309, 67%), and in 22% of cases, the site was unspecified (Table 1). Normal biopsies were reported in 27% of cases (n = 124), and in 4%, the biopsies were non-diagnostic (Fig. 1). The most common pathologies reported were non-specific myopathy (n = 72, 16%), dystrophy (n = 71, 15%), and neurogenic disorders (n = 60, 13%). The most common form of muscular dystrophy reported was Duchene muscular dystrophy (n = 18, 25%) followed by limb-girdle muscular dystrophy, type 2B (dysferlinopathy) and merosin-deficient congenital muscular dystrophy both equal in number (n = 7, 10%) (Table 2). In 39% (n = 28) of cases, features were diagnostic of muscular dystrophy. However, no specific subtype was given (muscular dystrophy not otherwise specified). Among the neurogenic disorders category, denervation atrophy was the most common pathology (n = 42) followed by spinal muscular atrophy cases (n = 18). Inflammatory myopathy category comprised 7% (n = 32) including 13 cases of inflammatory myopathy not otherwise specified (n = 13), polymyositis (n = 12), dermatomyositis (n = 6), and myositis secondary to systemic lupus erythematosus (n = 1) (Table 3). Less common pathologies described in our patients' cohort include cases with type l fibers predominance (n = 9), type II predominance (n = 8), and rare entities such as GNE myopathy (n = 1) (Table 4). Congenital myopathy category included the following five subcategories; centronuclear, congenital fiber-type disproportion, central core, multi-mini core, and nemaline myopathies (Table 5). Metabolic myopathy category included glycogen storage disease not otherwise specified (n = 4), mitochondrial myopathy (n = 2), glycogen storage disease type II (acid maltase deficiency) (n = 1) and lipid storage myopathy (n = 1) (Table 6). Finally, the non-diagnostic included 13 unsatisfactory muscle biopsies due to various pre-analytical reasons and 6 end-stage muscle diseases defying further classification. Histologic illustrations of some muscle pathologies are shown in Fig. 2.

Table 1
Frequency of muscle biopsies based on site.

Biopsy sites	Frequency	Percent
Unspecified site	101	22
Thigh muscle (not otherwise specified)	309	67
Gastrocnemius muscle	14	3
Deltoid muscle	20	4
Gluteus muscle	1	0.2
Neck muscle (not otherwise specified)	1	0.2
Triceps muscle	6	1
Hip muscle (not otherwise specified)	2	0.4
Back muscle (not otherwise specified)	1	0.2
Biceps muscle	2	0.4
Cheek muscle (not otherwise specified)	1	0.2
Upper limb (not otherwise specified)	1	0.2
Thoracic muscle	1	0.2
Gastrocnemius muscle and thigh muscle	1	0.2

4. Discussion

Skeletal muscular diseases can be a source of significant morbidity and mortality with significant impairment of the quality of life and sometimes may lead to death. The overall incidence is ranging from 0.07 to 26.5 per 100,000 per year. They are broadly classified as either acquired (e.g. endocrine myopathies, inflammatory myopathies, drug-induced myopathies, and toxic myopathies) or inherited (e.g. congenital myopathies, metabolic myopathies, muscular dystrophies, and channelopathies). Myopathies can affect any part of the skeletal muscles including the channel, structure, or metabolism of skeletal muscles. They can be distinguished from other neuromuscular disorders by characteristic clinical and laboratory features. In certain myopathies, the role of muscle biopsy on the diagnosis remains irreplaceable. This is especially true if there is an agreement between clinical data, biochemical data, neurophysiology, and muscle biopsy [5]. Ahlstrom et al. [6] found that the combined prevalence rate for myotonic disorders, muscular dystrophies, and myositis is 50 per 100,000 population even though in their study, they included only patients below the age of 19 years. In a regional study by El-tallawy et al. [7], they identified a 76.6 per 100,000 point prevalence of primary muscular diseases in Assiut city of Egypt. Among the different categories of primary muscle diseases, Deenen et al. [8] identified dystrophies as the most common one with myotonic dystrophy, limb-girdle muscular dystrophy, and facioscapulohumeral dystrophy being the top three, respectively. In the same study, inclusion body myositis ranked the first within the

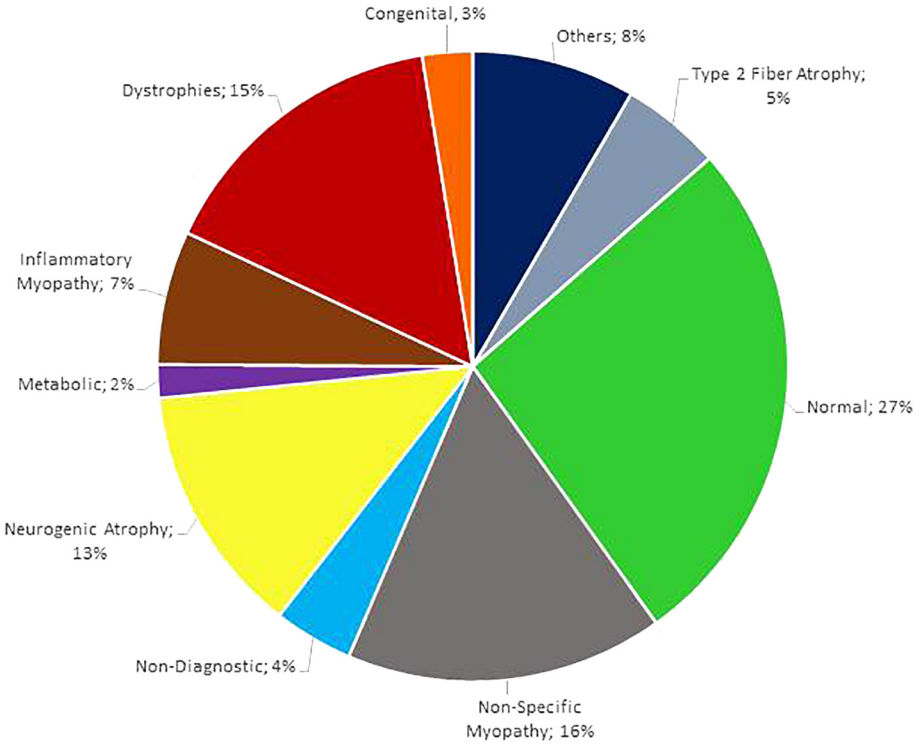

Fig. 1. Pie chart showing the frequency of different categories of muscle biopsy findings.

Table 2
Subcategories of muscle dystrophies.

Dystrophy type	Frequency	Percent
Limb-girdle muscular dystrophy, type 2B (dysferlinopathy)	7	10
Congenital muscular dystrophy, not otherwise specified	2	3
Congenital muscular dystrophy (Walker Warburg syndrome)	1	1
Duchenne muscular dystrophy	18	25
Limb-girdle muscular dystrophy type 2C (gamma sarcoglycanopathy)	1	1
Limb-girdle muscular dystrophy, type 2D (alpha sarcoglycanopathy)	3	4
Merosin-deficient congenital muscular dystrophy	7	10
Merosin-positive congenital muscular dystrophy	4	6
Muscular dystrophy, not otherwise specified	28	39

Table 3
Inflammatory myopathy subcategories.

Muscle pathology (inflammatory myopathy subcategories)	Frequency	Percent
Dermatomyositis	6	19
Inflammatory myopathy, not otherwise specified	13	41
Myositis secondary to SLE	1	3
Polymyositis	12	37

inflammatory category. In a local study by Salih et al. [9], they found that 48% of their cases belonged to different forms of muscular dystrophies and hence considered the most common category in their cohort of pediatric neuromuscular patients.

Neuromuscular diseases are considered infrequent with a prevalence ranging between 1 and 10 cases per 100,000 of the population [10]. Although this descriptive study does not allow for recognition of incidence and prevalence of the spectrum of neuromuscular pathologies, it serves as a tool for raising awareness and helping the specialist to recognize the yield of muscle biopsies in neuromuscular disorders. The spectrum of neuromuscular diseases may vary from one country to

Table 4
Other uncommon muscle pathology findings.

Muscle pathology (others' subcategories)	Frequency	Percent
Changes related to steroid treatment	2	5
Congenital myopathy, not otherwise specified	1	3
Destructive myopathy	1	3
Fatty infiltration and atrophic changes	1	3
Lymphocytic vasculitis associated with myopathic changes	1	3
Minimal myopathic changes with focal collection of macrophages	1	3
Necrotizing myopathy	2	5
Type I fibers predominance	9	23
Brucellosis-related myositis	1	3
Hereditary inclusion body myopathy (GNE myopathy)	1	3
Vacuolar myopathy	1	3
Type II fibers predominance	8	20
Amyloid myopathy	1	3
Atrophic changes	1	3
Critical care myopathy	2	5
Marked muscular atrophy	1	3
Mitochondrial myopathy	1	3
Glycosylation type 2L	1	3
Myopathy with abnormal mitochondria	1	3
Non-specific changes	1	3
Pre-pathological spinal muscular atrophy	1	3

Table 5
Congenital myopathies.

Muscle pathology (congenital subcategories)	Frequency	Percent
Central core myopathy	2	17
Centronuclear myopathy	6	50
Congenital fiber type disproportion	2	17
Multi-minicore myopathy	1	8
Nemaline myopathy	1	8

Table 6
Metabolic myopathies.

Muscle pathology (metabolic subcategories)	Frequency	Percent
Glycogen storage disease type II (acid maltase deficiency myopathy)	1	12
Glycogen storage disease, not otherwise specified	4	50
Lipid storage myopathy	1	12
Mitochondrial myopathy	2	25

another and from one time to another in the same country. This difference can be explained by the availability of specialized tertiary care hospitals, different methods and sources to obtain data, and the presence of different genetic neuromuscular diseases in certain regions. In addition, differences between the results of studies done in the same area on different time setting may reflect an improvement of diagnosing possibilities and increased accuracy of diagnosis. In our study, 20% of pathologies were related to hereditary conditions. There is evidence from the literature that consanguinity is associated with higher rates of neuromuscular diseases. Although this study was not meant to determine the exact prevalence of consanguinity, it is likely to be a major risk factor. In a study done by Warsy et al. [11], the rate of consanguinity in Saudi Arabia is high ranging from 25 to 65%.

Muscle biopsy is a minimally invasive procedure that is well-tolerated. Although it is part of the paraclinical workup for patients with neuromuscular diseases, it is considered the last investigation to be utilized after detailed clinical examination, laboratory testing, neurophysiological assessment, and molecular studies. Due to recent advances in molecular techniques and serological markers over the past two decades, the utilization of muscle biopsy for the diagnosis of neuromuscular disorders has decreased. In addition, the diagnostic strategy of the management of several neuromuscular disorders has been modified, and the applicability of muscle biopsy in the diagnostic algorithm has been changed [12]. When performing muscle biopsy, the clinician can determine if the muscle is abnormal with specific histopathological features, abnormal with non-specific histopathological features, and completely normal muscle tissues [3]. Unfortunately, a normal biopsy does not exclude the presence of a neuromuscular disorder. In our study, 47% of the muscle biopsies were inconclusive (27% of normal biopsies, 16% of non-specific myopathy, and 4% of non-diagnostic because of either the muscle biopsies were unsatisfactory or because of end-stage muscle disease). Detailed clinical examination and performing multiple paraclinical non-invasive investigations such as electromyography and nerve conduction studies prior to the muscle biopsy help identify the most affected group of muscles and, therefore, the best site for muscle biopsy [4]. Also, performing multiple simultaneous biopsies was recommended in one study where it increased the chance of accurate diagnosis from 78% to 95% [13].

In an epidemiological study about in the Netherlands in 2016, data were collected over approximately eight years through the nationwide Computer Registry of All Myopathies and Polyneuropathies (CRAMP). About 25,000 individuals with neuromuscular disorders have been registered. Around 14,159 were males, while 11,233 were females. The most prevalent disorder was amyotrophic lateral sclerosis (2057 cases) and the least prevalent was Emery-Dreifuss muscular dystrophy (9 cases) [8]. In Saudi Arabia, no registry is available for either clinical or histopathological documentation of different neuromuscular disorders. In addition, there is no society for neuropathologists to exchange their experience, collect their data, and build up the registry.

In another middle-eastern study, based upon a screening questionnaire distributed, 448 cases were positive and referred to Qena University Hospital to undergo further investigations and confirm the diagnosis. Out of the 448 individuals, 426 cases were proven to have a neuromuscular disorder (408 neuropathies and 18 muscle diseases) [14]. In our center, a muscle biopsy is not part of the workup for neuropathies. When comparing our results with this study conducted in Egypt, congenital myopathies, muscular dystrophies, and inflammatory myopathies were higher in our center.

Limitations in our study are several including paucity of research about muscle pathology and neuromuscular disorders originating from Saudi Arabia with only one study in the literature including a small sample size. Another limitation is the difficulty in comparing the results to other studies due to the differences in the methodology and non-standardized investigation tools. In addition, our sample size was small in comparison to most studies published in the literature supported by registries. Finally, the age range of our patients is large including pediatric and adult patients, while most of the publications were focusing on a special age group.

5. Conclusion

In conclusion, the muscle biopsy will continue to play a crucial role, as a gold standard or as a complementary investigation, in the diagnosis of certain neuromuscular disorders. In a standard muscle pathology laboratory, the results can be available within 48 h with a reliable diagnosis for the clinician to start the treatment of treatable

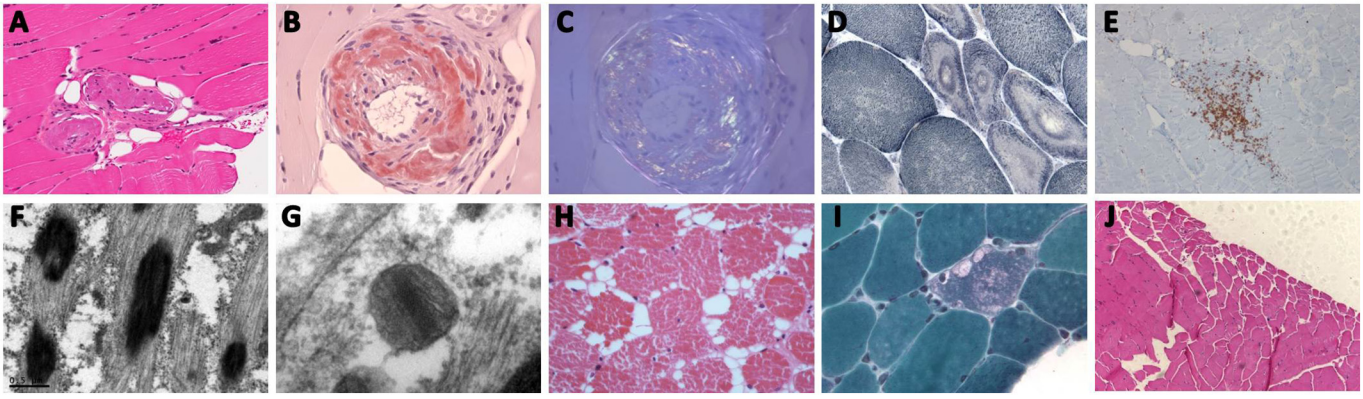

Fig. 2. Histologic illustrations of some muscle pathologies showing: (A) homogeneous amphophilic deposits within the vascular walls in a case of amyloid myopathy. (B) Positive Congo red staining of the deposits in (A). (C) Under polarized light, the apple-green birefringence of the Congo red-stained deposits in (B) is depicted. (D) Target fibers are shown by NADH stain from a case of neurogenic atrophy. (E) The T lymphocytic marker CD3 highlights infiltration of the myofibers and endomysium in a case of inflammatory myopathy. (F) Electron micrograph depicts the dense nemaline rods from a case of nemaline myopathy. (G) Abnormal mitochondrion with crystalline inclusion was identified in the same case in (F). (H) Vacuolar change from a case of glycogen storage disease (Pompe disease). (I) Rimmed vacuoles in a case of myopathy with rimmed vacuoles. (J) Perifascicular atrophy in a case of dermatomyositis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

neuromuscular disorders. Increasing the yield and accuracy of muscle pathology should be the main concern and priority to neuropathologists reporting muscle biopsies. In addition, utilizing next-generation sequencing and other molecular techniques have changed the location of muscle biopsy in the algorithm of the diagnosis of neuromuscular disorders. This paper is an urgent call to establish the Saudi Neuropathology Society and the muscle pathology and neuromuscular disorders registry.

Acknowledgment

We would like to acknowledge the contribution of Aamir Omair in data collection and statistical analysis.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

References

[1] Dubowitz C, Sewry CA. Dubowitz C, Sewry CA, editors. Muscle biopsy. A practical approach. Elsevier, Saunders: Philadelphia, USA; 2007.
[2] Sundaram S, Uppin MS. Approach to the interpretation of muscle biopsy. Muscle biopsy. InTech; 2012.
[3] Thavorntanaburt S, Tanboon J, Likasitwattanakul S, et al. Impact of muscle biopsy on diagnosis and management of children with neuromuscular diseases: a 10-year retrospective critical review. J Pediatr Surg 2018;53(3):489–92.
[4] Mikell CB, Chan AK, Stein GE, Tanji K, Winfree CJ. Muscle and nerve biopsies: techniques for the neurologist and neurosurgeon. Clin Neurol Neurosurg 2013;115(8):1206–14.
[5] Deenen JC, Horlings CG, Verschuuren JJ, Verbeek AL, Van engelen BG. The epidemiology of neuromuscular disorders: a comprehensive overview of the literature. J Neuromuscul Dis 2015;2(1):73–85.
[6] Ahlström G, Gunnarsson LG, Leissner P, Sjödén PO. Epidemiology of neuromuscular diseases, including the postpolio sequelae, in a Swedish county. Neuroepidemiology 1993;12(5):262–9.
[7] El-tallawy HN, Khedr EM, Qayed MH, Helliwell TR, Kamel NF. Epidemiological study of muscular disorders in Assiut, Egypt. Neuroepidemiology 2005;25(4):205–11.
[8] Deenen JC, Van doorn PA, Faber CG, et al. The epidemiology of neuromuscular disorders: age at onset and gender in the Netherlands. Neuromuscul Disord 2016;26(7):447–52.
[9] Salih MA, Mahdi AH, Al-jarallah AA, et al. Childhood neuromuscular disorders: a decade's experience in Saudi Arabia. Ann Trop Paediatr 1996;16(4):271–80.
[10] Vill K, Blaschek A, Gläser D, et al. Early-onset myopathies: clinical findings, prevalence of subgroups and diagnostic approach in a single neuromuscular referral center in Germany. J Neuromuscul Dis 2017;4(4):315–25.
[11] Warsy AS, Al-jaser MH, Albdass A, Al-daihan S, Alanazi M. Is consanguinity prevalence decreasing in Saudis?: a study in two generations. Afr Health Sci 2014;14(2):314–21.
[12] Nilipour Y. The art of muscle biopsy in the new genetic era: a narrative review. Iran J Child Neurol 2019;13(4):7–17.
[13] Shaibani A, Jabari D, Jabbour M, Arif C, Lee M, Rahbar MH. Diagnostic outcome of muscle biopsy. Muscle Nerve 2015;51(5):662–8.
[14] Khedr EM, Fawi G, Abbas MA, et al. Prevalence of neuromuscular disorders in Qena governorate/Egypt: population-based survey. Neurol Res 2016;38(12):1056–63.