Diagnostic Value of Dystrophin Immunostaining in the Diagnosis of Duchenne and Becker Muscular Dystrophy Patients

Shinta Andi Sarasati1,2, Kristy Iskandar3, Maria Alethea Septinastiti3, Rusdy Ghazali Malueka1,4, Ery Kus Dwianingsih1,4,*

1Department of Anatomical Pathology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; 2Department of Child Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; 3Department of Neurology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; 4Dr. Sardjito General Hospital, Yogyakarta, Indonesia; 5Academic Hospital, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

BACKGROUND: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive muscular disorders caused by the absence or reduction of the muscle cytoskeletal protein dystrophin. Standard procedures to detect deletion and duplication of the DMD gene use multiplex ligation-dependent probe amplification (MLPA). However, genetic testing, such as MLPA, is not covered by the national insurance scheme in Indonesia. Immunohistochemical (IHC) staining of dystrophin from muscle biopsy in the form of formalin-fixed paraffin-embedded (FFPE) specimens can be an alternative method to detect dystrophin expression in protein levels to establish the diagnosis of DMD or BMD.

AIM: The objectives of the study were to determine sensitivity, specificity, and accuracy of IHC analysis of dystrophin in DMD/BMD patient in comparison with the standard genetic testing, MLPA.

METHODS: Twenty-six patients enrolled in this study were clinically diagnosed as DMD/BMD in Dr. Sardjito Hospital and Universitas Gadjah Mada Academic Hospital. Genomic DNA was isolated from 3 mL of EDTA peripheral whole blood samples. The deletion and duplication of DMD genes were detected by MLPA. IHC examination was performed using a specific antibody dystrophin (Dys2). Complete loss of dystrophin staining indicated DMD, while partial loss of dystrophin staining indicated BMD. MLPA result was used as the gold standard to determine sensitivity, specificity, and accuracy of IHC technique using a 2 × 2 table.

RESULTS: MLPA results revealed 18 (18/26; 69.3%) patients with deletion and 3 (3/26; 11.5%) patients with duplication. Five (5/26; 19.2%) patients who showed no deletion nor duplication were excluded from the analysis. Among 21 patients with deletion or duplication, 18 (18/21; 85.7%) patients were out-of-frame (DMD) and 3 (3/21; 14.3%) patients were in-frame (BMD). Six patients showed a discrepancy between the IHC and MLPA results with 9.5% (2/21) false positive and 19% (4/21) false negative. The sensitivity of dystrophin IHC was 77.78%, specificity 33.33%, positive predictive value 87.5%, negative predictive value 20%, and accuracy 71.43%.

CONCLUSION: Muscle biopsy followed by IHC can be one of the diagnostic tools to diagnose BMD or DMD, with high sensitivity. The protein-based strategy is probably the most efficient way to approach the diagnosis of Duchenne and Becker muscular dystrophy in limited health-care settings.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive muscular disorder caused by the absence or reduction of dystrophin, a cytoskeletal protein. The causative gene, DMD, is the largest human gene and is located on chromosome Xp21. The products of DMD provide muscle fiber stability during contractions and maintain membrane stability. Muscle fibers with negative expression of dystrophin are prone to damage since they connect the sarcomeric structure to the extracellular matrix [1]. DMD is characterized by progressive muscular weakness that eventually can progress to respiratory and cardiac failure in some patients which are critical factors that determine their survival [2]. Patients with mutations of the DMD gene may show variable symptoms, ranging from the severe phenotype (DMD) to the milder form, Becker muscular dystrophy (BMD). In DMD, the out-of-frame mutation causes the disruption of the translational reading frame leading to the complete absence of dystrophin. In the milder phenotype, an in-frame mutation still maintains the translational reading frame so that a short but functional dystrophin is able to be produced [3]. When the mutation can maintain the open reading frame, it allows the production of internally deleted, partially functional, dystrophin proteins [4]. DMD and BMD are distinguished by the reading frame hypothesis, because the mutations that cause the distortion of the reading frame may generate a premature termination and loss of dystrophin production, promoting the severe phenotype, DMD [5].

Some methods are available and have been routinely applied to detect deletions and duplications in
the DMD gene for diagnostic purposes. The multiplex ligation-dependent probe amplification (MLPA) has been a general method to determine the copy number of up to 45 nucleic acid sequences in one single reaction [6]. In the amplification process, multiple pairs of oligonucleotides comprising universal primer sequences, variable lengths of stuffer sequences, and genomic target sequences that hybridize at adjacent positions are ligated with a thermostable ligase enzyme. MLPA has been demonstrated to be a reliable and faster method for quantitative identification of all DMD genes to detect the deletions and duplications [7].

In patients with DMD, the immunohistochemical (IHC) analysis from muscle biopsy specimens shows complete absence of dystrophin, while in BMD, patients may have 10–40% of the normal protein, partly expressed in the sarcolemma of the muscle fibers [1], [8].

The national health insurance scheme in Indonesia does not cover genetic testing, so muscle biopsy with IHC staining becomes an important diagnostic tool to diagnose DMD/BMD. The immunostaining of fresh frozen muscle samples is still the standard method to detect the expression of proteins in patients with DMD/BMD using commercially available antibodies [9]. While the snap frozen technique has some advantages, this method needs cryostat and an immunofluorescence microscope to produce fresh frozen sections that are not widely available in all health institutions, especially in developing countries, such as Indonesia. In our institution, formalin-fixed paraffin-embedded (FFPE) specimens are more readily available to be used for analysis. However, the successful IHC staining of sarcolemma membrane-associated proteins in FFPE muscle samples has rarely been described. As a diagnostic tool, it is important to establish the accuracy of the method to ensure the diagnostic reliability. The objectives of this study were to report the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the accuracy of IHC in diagnosis of Duchenne and Becker muscular dystrophy compared with the MLPA technique as a gold standard.

**Materials and Methods**

**Samples and data collection**

Twenty-six patients were recruited in the pediatric and neurological departments of Dr. Sardjito and Universitas Gadjah Mada (UGM) Hospital, Yogyakarta, from 2017 to 2018. The oldest was 21 years old and the youngest was 6 years old. Patients who showed progressive muscle weakness, calf hypertrophy, and elevated serum creatine kinase levels were enrolled in this study. Routine histological analysis of muscle biopsies revealed a dystrophic myopathy in all cases and was followed with immunostaining of dystrophin to confirm the protein expression. Written informed consent for genetic examination was obtained from patients’ parents. The study protocol was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, UGM (KE/0615/05/2021).

**Immunohistochemistry staining**

Muscle biopsy specimens were investigated in our routine diagnostic pathology laboratory. Paraffin blocks were cut in 3 μm thickness and placed on charged slides (Superfrost Plus slides; Thermo Scientific (Newcastle upon Tyne, United Kingdom). They were dried at 60°C for 30 min, and then dewaxed, rehydrated, and underwent a heat-mediated antigen retrieval method. Sections were pre-treated with Tris-EDTA in pH 9.0 at 95°C for 10 min using a pressure cooker, incubated in room temperature for 10 min, and washed with phosphate buffer saline for 30 min. Endogenous peroxidase blocking was done by adding 1–2 drops of 5% hydrogen peroxidase. After dipping the slide in distilled water, non-specific background blocking was executed using bond primary antibody diluent (Leica) at room temperature for 30 min. Mouse monoclonal antibody of Dys2 (Novocastra, Leica) was introduced with dilution 1:20, followed with UltraTek Anti-Polyvalent and UltraTek HRP incubation each for 10 min. Immunoreactions were visualized using 3,3′-diaminobenzidine tetrahydrochloride hydrate with subsequent counterstaining of Mayer’s hematoxylin. The slides were then dehydrated, cleared, and mounted. Complete loss dystrophin staining resulted in the diagnosis of DMD, while partly loss dystrophin staining revealed BMD as diagnosis.

**Genomic DNA extraction**

Each patient provided 3 mL of EDTA peripheral whole blood sample. Genomic DNA was extracted using Qiagen® QIAamp DNA Mini Kit, according to the manufacturer’s protocol. DNA of each sample was purified and 100 ng of it was used for MLPA analysis.

**MLPA**

Mutation in DMD gene was detected using MLPA (SALSA MLPA Probes mix P034/P035, MRC Holland) according to manufacturer’s protocol. All reactions were performed on a thermal cycler PCR Applied Biosystem Veriti 96. One hundred nanograms of sample DNA were diluted in 5 μl deionized water and denatured at 98°C for 15 min before addition of 3 μl MLPA probe mix and buffer. The reaction mixture was denatured at 95°C for 1 min and incubated for 16–18 h at 60°C to secure the specific hybridization of the probes with the target sequences. In ligation step, after the hybridization, ligase mixture (32 μl) was added and incubated at
54°C for 15 min to maintain the ligation reaction. This ligation process was stopped by heating at 98°C for 5 min. Ten microliters of this product were mixed with 30 μL of PCR buffer and put in a thermocycler at 60°C. Subsequently, a 10 μL reaction mix was added, which contained dNTPs, Taq polymerase, and one unlabeled and one labeled PCR primers that complementary to the universal primer sequences on the MLPA probes. PCR was done for 35 cycles (95°C for 30 s; 60°C for 30 s, and 70°C for 30 s). The fragments were analyzed on an ABI model 3500 capillary sequencer (Applied Biosystems) with the GeneScan software using GeneScan 600 Liz size standards (Applied Biosystems). Each of the peaks that represent each exon was analyzed based on the difference in migration relative to the size standards and compared to control samples. Deletion and duplication were identified and analyzed for their reading frame status, to determine whether they are in-frame or out-of-frame mutations. Patients with MLPA result of no deletion or no duplication were excluded from this study.

**Data analysis**

The primary aims of our study were to calculate the sensitivity, specificity, PPV, NPV, and accuracy for IHC staining of dystrophin based on MLPA as the gold standard. To calculate sensitivity and specificity, 2 × 2 tables were generated to analyze IHC results from muscle biopsy (absent or patchy) and genetic results (out-of-frame or in-frame status) from MLPA.

**Results**

Among 26 male patients, the mean of patients' ages was 9 years old, the youngest was 6 years old, while the oldest was 21 years old. Most of the patients (57.7%) were aged 9 years old or older. The IHC results in normal muscle were localized at the sarcolemma of the fibers (Figure 1).

Data of all patients are summarized in Table 1. Among the 26 patients with DMD studied, a totally negative immunostaining was observed in most patients, in as many as 20 (20/26; 77%). In the remaining 6 patients (6/26; 23%), a patchy staining of the sarcolemma was seen in a proportion which was varied from 4% to 30% of scattered fibers. In addition, there was no patient with complete dystrophin expression.

![Figure 1](image)

**Table 1: Immunohistochemistry and MLPA results of 26 male samples**

| No. | Code     | IHC       | MLPA       | Type of exon deletion or duplication |
|-----|----------|-----------|------------|-------------------------------------|
| 1   | DMD-4-MFARH | DMD       | No del no dup | del 53-54                           |
| 2   | DMD-5-MHH   | DMD       | Out-of-frame | del 17-43                           |
| 3   | DMD-8-NSA   | DMD       | Out-of-frame | del 51                              |
| 4   | DMD-10-AP   | BMD       | Out-of-frame | del 46-51                           |
| 5   | DMD-11-NPP  | BMD       | No del no dup | del 52                              |
| 6   | DMD-14-RPP  | DMD       | Out-of-frame | del 48-50                           |
| 7   | DMD-15-AM   | DMD       | Out-of-frame | del 45-52                           |
| 8   | DMD-16-AAM  | DMD       | Out-of-frame | del 52                              |
| 9   | DMD-17-MDZ  | DMD       | Out-of-frame | del 2-62                            |
| 10  | DMD-19-RKS  | DMD       | Out-of-frame | dup 2-62                            |
| 11  | DMD-20-PBT  | BMD       | In-frame    | del 7-43                            |
| 12  | DMD-21-STA  | DMD       | Out-of-frame | dup 2-62                            |
| 13  | DMD-27-FWVA | DMD       | No del no dup | del 47                              |
| 14  | DMD-28-SNF  | DMD       | In-frame    | del 47                              |
| 15  | DMD-30-DNM  | DMD       | Out-of-frame | del 51-54                           |
| 16  | DMD-34-BWCN | DMD       | Out-of-frame | del 49-50                           |
| 17  | DMD-35-OCIS | DMD       | Out-of-frame | del 14-17                           |
| 18  | DMD-36-ANA  | DMD       | Out-of-frame | del 18-47                           |
| 19  | DMD-37-NFA  | DMD       | Out-of-frame | del 56-74                           |
| 20  | DMD-38-AS   | BMD       | In-frame    | del 45-49                           |
| 21  | DMD-39-GR   | BMD       | Out-of-frame | del 18-34                           |
| 22  | DMD-53-GAP  | DMD       | Out-of-frame | del 51                              |
| 23  | DMD-64-ASA  | DMD       | No del no dup | del 48-50                           |
| 24  | DMD-65-SNR  | DMD       | Out-of-frame | del 38-43                           |
| 25  | DMD-66A-PCCA| DMD       | Out-of-frame | del 38-43                           |
| 26  | DMD-67-ML   | DMD       | No del no dup | del 2-62                            |

DMD: Duchenne muscular dystrophy; BMD: Becker muscular dystrophy; del: Deletion; dup: Duplication.

MLPA results revealed 18 (18/26; 69.3%) patients with deletion and 3 (3/26; 11.5%) patients with duplication. Five (5/26; 19.2%) patients who showed no deletion nor duplication were excluded from the analysis. Among 21 patients with deletion or duplication, 18 (18/21; 85.7%) patients were out-of-frame (DMD) and 3 (3/21; 14.3%) patients were in-frame (BMD). Therefore, 21 patients (21/26; 80.8%) were analyzed with a 2 × 2 table, as shown in Table 2.

**Table 2: Analysis result dystrophin immunohistochemistry expression and MLPA**

| Immunostaining/MLPA | MLPA result | Total |
|---------------------|-------------|-------|
|                     | Out-frame   | In-frame |
| Dystrophin immunostaining | 14 (66.7) | 2 (9.5) | 16 |
| DMD, n (%) (complete loss) | 4 (19) | 1 (4.8) | 5 |
| BMD, n (%) (partial expression) | 18 (85.7) | 3 (14.3) | 21 |

The complete absence of dystrophin staining was matched with out-of-frame mutation by MLPA in 14 patients (14/21, 66.7%). However, 4 patients (4/21; 19.05%) with out-of-frame mutation showed patchy staining of dystrophin. One patient (1/21; 4.76%) with in-frame mutation also showed patchy expression of dystrophin.

**Table 3: Sensitivity, specificity, PPV, NPV, and accuracy between immunohistochemistry and MLPA result to detect dystrophin**

| Method          | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|-----------------|-----------------|-----------------|---------|---------|--------------|
| IHC             | 77.7            | 33.3            | 87.5    | 20      | 71.4         |

PPV: Positive predictive value, NPV: Negative predictive value.
The sensitivity of dystrophin IHC was 77.78%, specificity 33.33%, PPV 87.5%, NPV 20%, and accuracy 71.43% (Table 3).

Discussion

The fresh frozen analysis from muscle biopsy is the standard examination for muscular dystrophy, but this service is not widely available in countries with limited facilities to perform immunofluorescence analysis. The successful immunostaining of sarcolemma membrane-associated proteins in a previous study inspired the use of IHC for the diagnosis of common muscular dystrophy. When it is combined with clinical and histopathology findings of the muscle biopsy, especially in cases when frozen muscle sections cannot be obtained, IHC is beneficial for the diagnosis and screening of DMD/BMD [8].

IHC analysis in dystrophinopathies routinely involves the use of antibodies against C-terminal (Dys2), rod domain (Dys1), and optionally N-terminal (Dys3) of dystrophin protein [10]. In this study, IHC was performed using the Dys-2 antibody since successful immunostaining of Dys-2 using FFPE muscle sections had been reported previously [11], [12]. The Dys-2-binding domain is encoded by exons 77–79 of dystrophin in the C-terminal [13]. Since no deletions nor duplications were found in exons 77–79 in this study, the use of Dys-2 antibody alone is sufficient for analysis.

There were six patients with a discrepancy of the IHC and MLPA results with 9.5% (2/21) false positive and 19% (4/21) false negative. The false-positivity results may occur due to non-immunological binding or protein substrate reaction products, or endogenous enzymes reaction such as pseudoperoxidase, endogenous peroxidase, or endogenous biotin [14], [15]. This study had eliminated endogenous enzymes reactions using Tris-EDTA pre-treatment in pH 9.0 for 10 min. Another reason that may explain false positivity is revertant fibers, which are muscle fibers that express a smaller, but functional, dystrophin protein due to exon skipping and clonally expand in size along with increasing age through the process of muscle degeneration or regeneration [16]. The precise mechanisms by which revertant fibers arise and expand are poorly understood [17].

Common causes of false negativity are poor tissue fixation, over diluted antibody, and poor optimization of the epitope retrieval method [17]. Another reason for the discrepancy is the reading frame theory in BMD, because BMD has higher proportions of duplications, various mutation distribution and higher exceptions to the reading frame rule. Mutations that maintain the reading frame generally result in abnormal but partly functional dystrophin [14]. However, when alternative splicing of the mutant exon restores the reading frame, it appears to be excluded at both the mRNA and protein levels. Some mechanisms that may modulate this discrepancy are ribosomal frameshift, unexpected alternative splicing, exon skipping of mutated exon, and somatic mosaicism [15].

The MLPA technique has increased the mutation pick-up rate [18]. Moreover, the technique also enables the identification of carrier individuals. The MLPA test has proved to be a powerful tool in detecting deletions or duplications in the DMD gene [19]. Studies on comparing IHC staining with genetic mutation using MLPA in patients with DMD have received relatively little investigation.

Sensitivity, specificity, PPV, NPV, and accuracy of dystrophin immunostaining were 77.78%, 33.33%, 87.5%, 20%, and 71.4%, respectively. High sensitivity of dystrophin immunostaining showed that the method was reliable to detect dystrophin protein in tested samples and imply that any positivity of dystrophin expression will exclude the diagnosis of DMD [20]. Although this study showed low specificity, high sensitivity of dystrophin staining can be alternative method to differentiate DMD from BMD, especially in developing country, such as Indonesia, where genetic testing is not covered by insurance while muscle biopsy is. Unlike sensitivity and specificity, PPVs/NPVs are chiefly subjected to disease prevalence in the examined population. Prevalence influences PPV and NPV differently. PPV is increasing, while NPV declines with the increased prevalence of the disease in a population. The change in PPV is more significant, while NPV is only slightly affected by the disease prevalence [21].

A study from India, comparing the sensitivity and the pattern of mutations by both mPCR and MLPA in the same cohort of DMD, revealed that 36.4% of MLPA negative cases were confirmed to be diagnosed with DMD by immunostaining with high accuracy [7]. A comparative study of PCR-based deletion detection and IHC in Brazil stated that immunostaining can be a gold standard technique to diagnose DMD/BMD in developing countries. However, since muscle biopsy is an invasive procedure, it should only be performed in cases when PCR-based mutation detection using blood is unsuccessful to detect deletions [22].

Conclusion

Muscle biopsy followed by IHC staining provides diagnostic tools for DMD or BMD with highly sensitivity. The protein-based strategy can be the most efficient way to approach diagnosis of DMD/BMD in health-care centers with limited setting. Immunohistochemistry Dys-2 can be an alternative method to distinguish BMD.
and DMD. The combination of direct dystrophin analysis and genetic testing will give optimum diagnostic and prognostic accuracy.

References

1. Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. Muscle Nerve. 2000;23(10):1456-71. doi.org/10.1002/1097-4598(200010)23:10<1456:AID-MUS2>3.0.CO;2-T
PMid:11003781

2. Van Putten M, Hulsker M, Nadarajah VD, van Heiningen SH, van Huizen E, van Iteron M, et al. The effects of low levels of dystrophin on mouse muscle function and pathology. PLoS One. 2012;7(2):e31937. doi.org/10.1371/journal.pone.0031937
PMid:22356942

3. Malik V, Rodino Klapac LR, Violett L, Mendell JR. Analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat. 2015;36(4):395-402. doi.org/10.1002/humu.22758
PMid:25604253

4. Bladen CL, Salgado D, Monges S, Foncuberta ME, Kekou K, Kosma K, et al. The TREAT-NMD DMD global database: Analysis of measures of diagnostic and genetic testing will give optimum diagnostic and prognostic accuracy.

5. Nadifi S, Bellayou H, Hamzi K, Rafai MA, Karkouri M, Slassi I, et al. Duchenne and Becker muscular dystrophy: Contribution of a molecular and immunohistochemical analysis in diagnosis in Morocco. J Biomed Biotechnol. 2009;2009:325210. doi.org/10.1155/2009/325210
PMid:19461958

6. Janssen B, Hartmann C, Scholz V, Jauch A, Zschocke J. MLPA analysis for the detection of deletions, duplications and complex rearrangements in the dystrophin gene: Potential and pitfalls. Neurogenetics. 2005;6(1):29-35. doi.org/10.1007/s10977-004-0204-1
PMid:15655674

7. Manjunath M, Kiran P, Sanpapant S, Tubthong N, Pinpradap K, Cripe L, et al. A comparative study of mPCR, MLPA, and muscle biopsy results in a cohort of children with Duchenne muscular dystrophy: A first study. Neurol India. 2015;63(1):58-62. doi.org/10.4103/0028-3886.152635
PMid:25751470

8. Surilyonplengsaeng C, Dejthevapon C, Khongkhathithum C, Sanpapant S, Tubthong N, Pinpradap K, et al. Immunohistochemistry of sarcomembranemembrane-associated proteins in formalin-fixed and paraffin-embedded skeletal muscle tissue: A promising tool for the diagnostic evaluation of common muscular dystrophies. Diagn Pathol. 2017;12(1):1-10. doi.org/10.1186/s13000-017-0610-y
PMid:28219397

9. Bushby K, Finkel R, Birmkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy. Part 1: Diagnosis, and pharmacological and psychosocial management. Lancet Neurol. 2010;9(1):77-93. doi.org/10.1016/S1474-4422(09)70271-6
PMid:19945913

10. Vogel H, Zamecnik J. Diagnostic immunohistology of muscle diseases. J Neuropathol Exp Neurol. 2005;64(3):181-93. doi.org/10.1093/jnen/64.3.181
PMid:15804049

11. Yoshino S, Okoshi N, Watanabe M, Shoji S. Immunohistochemical staining of dystrophin on formalin-fixed paraffin-embedded sections in Duchenne/Becker muscular dystrophy and manifesting carriers of Duchenne muscular dystrophy. Neuromuscul Disord. 2000;10(6):425-9. doi.org/10.1016/S0960-8966(99)00116-9
PMid:10899449

12. Sheriffs IN, Rampling D, Smith VV. Paraffin wax embedded muscle is suitable for the diagnosis of muscular dystrophy. J Clin Pathol. 2001;54(7):517-20. doi.org/10.1136/jcp.54.7.517
PMid:11429422

13. Shamsa A, Katoaoka N, Takeshima Y, Yagi M, Awano H, Ota M, et al. Chemical treatment enhances skipping of a mutated exon in the dystrophin gene. Nat Commun. 2011;2(1):308. doi.org/10.1038/ncomms1306

14. Gibbs EM, Barthalémy F, Douine ED, Hardiman N, Shieh PB, Khanol N, et al. Large in-frame 5' deletions in DMD associated with mild Duchenne muscular dystrophy: Two case reports and a review of the literature. Neuromuscul Disord. 2019;29(11):683-7. doi.org/10.1016/j.nmd.2019.09.009
PMid:31672265

15. Nadkarni J, Dasrur G, Gaitonde P, Khadilkar S. Becker muscular dystrophy in Indian patients: Analysis of dystrophin gene deletion patterns. Neurol India. 2008;56(3):374. doi.org/10.4103/0028-3864.40961
PMid:18974567

16. Nowak KJ, Davies KE. Duchenne muscular dystrophy and dystrophin: Pathogenesis and opportunities for treatment. EMBO Rep. 2004;5(9):872-6. doi.org/10.1038/sj.embor.7400221
PMid:15470384

17. Echigoya Y, Lee J, Rodrigues M, Nagata T, Tanihata J, Nozohourmehrabad A, et al. Mutation types and aging differently affect revertant fiber expansion in dystrophic Mdx and Mdx52 mice. PLoS One. 2013;8(7):e69194. doi.org/10.1371/journal.pone.0069194
PMid:23894429

18. Schwartz M, Dune M. Improved molecular diagnosis of dystrophin gene mutations using the multiplex ligation-dependent probe amplification method. Genetic Testing. 2004;8(4):361-7. doi.org/10.1089/gte.2004.8.361
PMid:15684864

19. Todorova A, Todorov T, Georgieva B, Lukova M, Guergueltcheva V, Kremensky I, et al. MLPA analysis of PCR-deletion detection and immunohistochemistry of dystrophin in Brazilian Duchenne and Becker muscular dystrophy patients. Neuromuscul Disord. 2008;18(8):667-70. doi.org/10.1016/j.nmd.2008.06.369

20. Grunau G, Linn S. Commentary: Sensitivity, specificity, and predictive values: Foundations, pliabilities, and pitfalls in research and practice. Front Public Health. 2018;6:1-4. doi.org/10.3389/fpubh.2018.00256

21. Wong HB, Lim GH. Measures of diagnostic accuracy: Sensitivity, specificity, PPV and NPV. Proc Singapore Healthc. 1992;20(4):316-8.

22. Werners L, Scola RH, Henriq C, Maegawa B. Comparative analysis of PCR-deletion detection and immunohistochemistry in Brazilian Duchenne and Becker muscular dystrophy patients. Am J Med Genet. 2001;103(2):115-20. doi.org/10.1002/ajmg.1508
PMid:11568916

Open Access Maced J Med Sci. 2021 Dec 01; 9(A):1137-1141. 1141