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Chapter

Duchenne Muscular Dystrophy (DMD) Diagnosis: Past and Present Perspectives

Nahla O. Mousa, Ahmed Osman, Nagia Fahmy, Ahmed Abdellatif, Suher Zada and Hassan El-Fawal

Abstract

Duchenne muscular dystrophy (DMD) is a fatal X-linked disorder, characterized by progressive skeletal muscle wasting. The disease is caused by various types of mutations in the dystrophin gene (DMD). The disease occurs at a frequency of about 1 in 5000 male births, making it the most common severe neuro-muscular disease. In addition to clinical examinations of muscle strength and function, diagnosis of DMD usually involves a combination of immunological assays using muscle biopsies, typically immunohistochemistry and western blotting, and molecular techniques such as DMD gene sequencing or Multiplex Ligation Dependent Probe Amplification (MLPA) using blood samples. In fact, precise molecular diagnosis is a prerequisite for determining the appropriate personalized therapeutic approach such as exon-skipping, gene therapy or stem cell-based therapies in conjunction with gene editing techniques like CRISPR-Cas9. However, the quest for reliable biomarkers with high sensitivity and specificity for DMD from liquid biopsy is still a hotspot of research, as such non-invasive biomarker(s) would not only facilitate disease diagnosis but would also help in carrier detection, which will eventually result in better disease management. In this chapter, we will illustrate the detailed current and prospect strategies for disease.

Keywords: DMD, diagnosis, biomarkers

1. Introduction

Dystrophin protein is present in myocytes in skeletal, cardiac, and smooth muscles, acting to connect the actin microfilaments, via N-terminus of the protein, to the extracellular matrix by binding membrane—bound (sarcolemma) glycoprotein complex (dystrophin associated glycoprotein complex; DGC) to the C-terminal end of the protein, and thus, plays an important role in normal muscle function [1]. Inactivating mutations occurring in DMD gene causes immature termination of protein translation, giving rise to C-terminally truncated protein product that fails to transmit muscle impulses, which causes increasing intracellular Ca^{2+} influx and thus, activating apoptotic machineries and eventually causes cell death and muscle atrophy/necrosis [2]. Death usually occurs in the third decade of life as a result of respiratory or heart failure [3].

2. Methods for DMD diagnosis

2.1 Clinical picture

Affected DMD boys are usually normal at birth but in early childhood they suffer from inability to get up from floor or climb stairs or run and they fell very often. Also, enlarged calf muscles (pseudo hypertrophy) are always noticed [4]. From the age of 7–12, the cases become more deteriorated, and the patients start to suffer from scoliosis [5], and joint contracture [6]. Also, patients will have an apparent reduction in bone-mineral density and will have hypocalciuria and osteoporosis [7].

Because the disease affects proximal as well as distal muscles, thus, in early teenage, DMD boys usually get respiratory infections and sleep apnea [8], and later, the patient will develop cardiomyopathy and eventually heart failure [9].

2.2 Circulating blood biomarkers

2.2.1 CK levels and other proteins/enzymes

One of the dystrophin protein main functions is to stabilize the muscle tissue, since it exists and binds to sarcolemma. The absence of dystrophin will eventually lead to the increased permeability of the muscular tissue and consequently the release of the muscle proteins [10], of which the creatine kinase (CK) enzyme that is responsible for the production of phosphocreatine and ADP from creatine and ATP as part of energy homeostasis. In normal condition, normal myocytes turnover, serum levels of CK ranges from 20 to 200 U/L, however, it can be slightly increased in some neurological disorders. On the other hand, in case of DMD boys, due to the accelerated muscular destruction, it may reach higher levels reaching several thousands of units/L, and in severe muscle damage it can reach 200,000 U/L [11–13]. However, CK levels sometimes can be misleading because in advanced stages of DMD, CK levels may come within normal range due to progressive muscular atrophy [14].

CK is considered one of the most used serum biomarkers in DMD diagnosis, however, many studies were performed to detect alterations in other muscle related proteins using immunoassay and MS-based detection to screen for other potential diagnostic biomarkers (**Table 1**).

2.2.2 MicroRNA

MicroRNAs (miRNAs) are a tissue—specific class of small, non-coding RNA molecules that function as gene regulators/silencers and consequently they are considered sensitive indicators for different cellular contexts. MiRNAs act through binding to a specific region in the 3′-UTR in the target mRNA molecules, thus, inducing mRNA degradation and inhibiting the translation process [42]. The circulating levels of miRNAs in serum reflect the intracellular status and hence, they are excellent biomarkers for many pathological conditions as they can be detected from liquid biopsies and/or tissue specimens [43]. Many studies attempted to study the modulation in the levels of different miRNAs (**Table 2**).

2.2.3 Lipids, metabolites, amino acid, and organic acid

In addition to the previously mentioned biomarkers, lipid profile and metabolites in the blood or urine are also very important parameters that reflect the status

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
Alkaline phosphatase (AP)-A	Elevated in Grade 1 and Grade 2 patients	Serum	Measuring enzyme activities	[15]
AP-B	No change			
Gly-AP	Elevated in Grade 1 and Grade 2			
Ala-AP	Elevated in Grade 1			
Ser-AP	Elevated in Grade 1,2,3			
Leu-AP	Elevated in Grade 1			
Met-AP	No Change			
Phe-AP	Elevated in Grade 1,2,3			
Trp-AP	Elevated h in Grade 1,2,3			
Gly-pro-AP	Elevated in Grade1 Reduced in Grade 3			
Gly-Pro-Leu-AP	Reduced in Grade1 and Grade 2			
Trypsin	Reduced in Grade 1			
Cathepsin C	Reduced in Grade 1 and Grade 2			
Sulphatase	No change			
Phosphatase	No change			
Acetyl-choline esterase	Reduced in Grade 2			
Esterase	Elevated in Grade 1,2,3			
RNase	Reduced in Grade 1 and Grade 2			
Angiotensin Converting enzyme	Reduced in Grade 3			
Myostatin (Growth and differentiation factor 8; GDF8)	Elevated in DMD patients	Serum	ELISA	[16]
Interleukin 17	Elevated in Emery-Dreifuss MD and Limb-Girdle MD 1B	Serum	ELISA	[17, 18]
TGF-β2	Elevated in Emery-Dreifuss MD and Limb-Girdle MD 1B			
IL6	Variable			
Skeletal troponin I (sTnI),	Elevated in DMD, BMD, LGMD2B	Serum	ELISA	
Myosin light chain 3 (Myl3),	Elevated in DMD, BMD, LGMD2B			
Fatty acid binding protein 3 (FABP3)	Elevated in DMD, BMD, LGMD2B			
Muscle-type creatine kinase (CKM)	Elevated in DMD, BMD, LGMD2B			

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
N-terminal α Dystroglycan (αDG-N)	Reduced in DMD patients	Serum	ELISA	[19]
Fibronectin	Elevated in DMD Normal in BMD	Serum	ELISA	[20]
Basic fibroblast growth factor	Elevated in DMD patients	Serum	ELISA	[21]
cardiac myosin light chain I	Elevated in DMD patients (correlated with CK levels)	Serum	Immunoradiometric assay	[22]
Troponin I, fast skeletal muscle	Elevated in DMD	Serum	SOMAscan assay "Aptamer-based proteomic technology"	[23]
Carbonic anhydrase 3	Elevated in DMD			
Fatty acid-binding protein, heart	Elevated in DMD			
Troponin I, cardiac muscle	Elevated in DMD			
Creatine kinase M-type	Elevated in DMD			
Mitogen-activated protein kinase 12	Elevated in DMD			
Alanine aminotransferase 1	Elevated in DMD			
Myoglobin	Elevated in DMD			
Fibrinogen	Elevated in DMD			
Phospholipase A2, membrane associated	Elevated in DMD			
Acidic leucine-rich nuclear phosphoprotein 32 family member B	Elevated in DMD			
Hepatoma-derived growth factor-related protein 2	Elevated in DMD			
40S Glucose-6-phosphate isomerase ribosomal protein S7	Elevated in DMD			
Heparin cofactor 2	Elevated in DMD			
Persephin	Elevated in DMD			
Calcium/calmodulin-dependent protein kinase II α	Elevated in DMD			
Malate dehydrogenase, cytoplasmic	Elevated in DMD			
l-lactate dehydrogenase B chain	Elevated in DMD			
Aminoacylase-1	Elevated in DMD			
Proteosome subunit α type-2	Elevated in DMD			

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
C-X-C motif chemokine 10	Elevated in DMD			
cAMP-dependent protein kinase catalytic subunit α	Elevated in DMD			
Heat-shock 70 kDa protein 1A/1B	Elevated in DMD			
Proto-oncogene tyrosine-protein kinase receptor Ret	Reduced in DMD			
Growth/differentiation factor 11	Reduced in DMD			
Complement decay-accelerating factor	Reduced in DMD			
Cadherin-5	Reduced in DMD			
Tumor necrosis factor receptor superfamily member 19 L	Reduced in DMD			
Gelsolin	Reduced in DMD			
Wnt inhibitory factor 1	Reduced in DMD			
Contactin-5	Reduced in DMD			
Prolyl endopeptidase FAP	Reduced in DMD			
Jagged-1	Reduced in DMD			
Netrin receptor UNC5C	Reduced in DMD			
Kunitz-type protease inhibitor 1	Reduced in DMD			
Protein SET	Reduced in DMD			
Disintegrin metalloproteinase domain-containing protein 9	Reduced in DMD			
Cell adhesion molecule L1-like	Reduced in DMD			
Osteomodulin	Reduced in DMD			
WAP, Kazal, Ig, Kunitz and NTR domain-containing protein 1	Reduced in DMD			
Bone sialoprotein 2	Reduced in DMD			
Interleukin-34	Reduced in DMD			
Neurogenic locus notch homolog protein 3	Reduced in DMD			
Cytoplasmic aspartate aminotransferase	Elevated in DMD	Serum	Measuring enzyme activity	[24]
mitochondrial aspartate aminotransferase	Elevated in DMD			

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
Alanine transaminase (ALT)	Elevated in DMD	Serum	ELISA	[25]
Aspartate transaminase (AST)	Elevated in DMD			
Muscle-specific enolase (MSE, beta beta and alpha beta enolases)	Elevated in DMD and another progressive muscular dystrophies	Serum	Enzyme immunoassay	[26]
Serum carbonic anhydrase III (CA-III)	Elevated in DMD, limb-girdle dystrophy, facioscapulohumeral dystrophy and congenital dystrophy	Serum	Enzyme immunoassay	[27]
Creatine kinase (CK) isoenzymes (MM, MB, and BB)	Elevated in DMD	Serum	Sensitive enzyme immunoassay	[28]
Matrix metalloproteinase-9 (MMP-9)	Elevated in DMD	Serum	ELISA	[29]
Tissue inhibitors of metalloproteinase-1 (TIMP-1)	Elevated in DMD			
Osteopontin (OPN)	Normal			
MT-1-MMP	Elevated in autosomal dominant EDMD	Serum	ELISA and zymography	[30]
MMP2	Elevated in autosomal dominant EDMD and in X-linked EDMD			
MMP9	Non-significant elevation			
TIMP-1	Normal in AD-EDMD Elevated in X-linked EDMD	Serum	ELISA sandwich immunoassay	[31]
TIMP-2	Non-significant decrease AD-EDMD/X-EDMD cases			
TIMP-3	Reduced in AD-EDMD/X-EDMD			
Carbonic anhydrase III (CA-III, EC 4.2.1.1)	Elevated in DMD, congenital (Fukuyama-type), limb-girdle, also elevated in: polymyositis myotonic dystrophy amyotrophic lateral sclerosis spinal progressive muscular atrophy or Kugelberg-Welander disease and in carriers of DMD	Serum	Radioimmunoassay	[32]
Vitamin D binding protein (GC)	Reduced in DMD	Serum	2D-HPLC off-line coupled to LC-MALDI-TOF-MS verified with ELISA	[33]
Fibulin-1 (FBLN1)	Elevated in DMD			

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
Gelsolin (GSN)	Reduced in DMD			
Carbonic anhydrase 1 (CA1)	Elevated in DMD			
Apolipoprotein B100	Reduced in DMD			
ALT, AST, LDH, and ALP	Elevated in DMD	Serum	Enzymatic assay	[34]
ALT, AST, and LDH	Elevated in BMD and LGMD			
FSHD and EDMD	lack of abnormal serum enzyme levels			
ALP	Highly elevated in LGMD2B Elevated in non-LGMD2B			
Vascular endothelial growth factor	Highly elevated in BMD Elevated in Bedridden DMD, spinal muscular atrophy, myotonic dystrophy	Serum	ELISA	[35]
Creatine kinase MB fraction	Elevated in DMD	Serum	Multiplex, microsphere-based immune-fluorescent assay	[36]
Tissue-type plasminogen activator PLAT	Slightly elevated in DMD			
Myoglobin	Slightly elevated in DMD			
Epidermal growth factor	Slightly elevated in DMD			
Chemokine (C-C motif) ligand 2	Slightly elevated in DMD			
CD 40 ligand	Slightly elevated in DMD			
Vitronectin	Slightly elevated in DMD			
Carboxyterminal propeptide of type I procollagen	No significant alteration	Serum	Radioimmunoassay	[37]
Aminoterminal propeptide of type III procollagen	No significant alteration			
Laminin P1	No significant alteration			
Creatine kinase	Elevated in DMD and BMD	Serum	Measuring enzyme activity	[38]
Pyruvate kinase	Elevated in DMD and BMD			
Myosin light chain—3	Elevated in DMD	Serum	affinity proteomics-based screening approach using an antibody suspension bead array	[39]
Carbonic anhydrase III	Elevated in DMD			
Electron transfer flavoprotein A	Elevated in DMD			

Tested marker	Levels (high or low) in DMD patients and/or other MDs	Location (serum/muscle)	Detection method	Ref.
Mitochondrial malate dehydrogenase 2	Elevated in DMD			
Electron transfer flavoprotein B	Reduced in DMD			
Fast skeletal muscle troponin T	Elevated in DMD			
Matrix metalloproteinase 9	Elevated in DMD	Serum	Immunoassay	[40]
Matrix metalloproteinase 2	Reduced in BMD			
Myostatin (GDF-8)	Reduced in DMD			
Follistatin (FSTN)	Elevated in DMD and BMD			
N-terminal fragment of titin	Elevated in DMD patients	Urine	ELISA	[41]

Table 1.
List of potential protein biomarkers that could be utilized in the diagnosis of Duchenne muscular dystrophy.

of the muscles and thus, they could be measured to indicate the extent of muscular dystrophy and can serve as good candidates for diagnostic purposes (**Table 3**).

2.3 Muscle imaging

Magnetic resonance imaging (MRI) is now used to visualize the composition of skeletal muscles and detect structural abnormalities in the of DMD patients [61]. The produced images can reveal the presence of fat infiltration of muscle tissue, a characteristic consequence of DMD, and thus, can be used for monitoring disease progression and response to treatment [62].

2.4 Genetic diagnosis

2.4.1 RFLP

Detecting the mutation, especially non-sense point mutations, in the 2.4 Mb gene represents a challenging task. In this context, restriction fragment length polymorphism (RFLP) analysis could be used by digesting the genomic DNA using specific restriction endonucleases followed by Southern blotting using DMD-specific DNA probes (genomic or cDNA probes). At 1985, Bamkan et al. developed 11 RELP markers that are present in the X chromosome and can be used for diagnosis. However, RFLP can detect only small percentage of the mutation and hence it cannot be used as gold standard technique in the diagnosis process [63–65].

2.4.2 Multiplex PCR

Multiplex PCR is one of the modified PCR protocols that allows the co-amplification of multiple products using different primer pairs that specially bind complementary regions in the target segment. This method showed a great potential

microRNA	Status	Disease	Location	Ref.
miR-133a	Upregulated	DMD, BMD, LGMD, FSHD	Serum and skeletal muscles	[44–47]
miR-206	Upregulated	DMD, BMD, LGMD, FSHD	Serum and skeletal muscles	
miR-1	Upregulated	DMD, BMD, LGMD, FSHD	Serum and skeletal muscles	
miR-499	Upregulated	DMD	Serum	[45]
miR-208a	Upregulated	DMD	Serum	
miR-208b	Upregulated	DMD	Serum	
miR-95	Upregulated	DMD	Serum	[48]
miR-539	Downregulated	DMD	Serum	
miR-30c	Upregulated	DMD	Serum	[49]
miR-181a	Upregulated	DMD	Serum	
miR-21	Downregulated	DMD	Urine	[50]
miR-29	Downregulated	DMD	Urine	
miR-23	Downregulated	DMD	Urine	
miR-181a	Upregulated	DMD	Serum	[51]
miR-4538	Upregulated	DMD	Serum	
miR-4539	Upregulated	DMD	Serum	
miR-606	Upregulated	DMD	Serum	
miR-454	Downregulated	DMD	Serum	
miR-483	Upregulated	DMD	Serum	[52]
hsa_miR_146b, hsa_miR_368, hsa_miR_381, hsa_miR_487b, hsa_miR_495, hsa_miR_376a, hsa_miR_299_5p, hsa_miR_155, hsa_miR_382, hsa_miR_199a, hsa_miR_379, hsa_miR_335, ambi_miR_5021, hsa_miR_432, hsa_miR_199b, hsa_miR_369_5p, hsa_miR_21, hsa_miR_34a, hsa_miR_199a*, hsa_miR_154, hsa_miR_221, hsa_miR_214, hsa_miR_518a_2*, hsa_miR_409_3p, hsa_miR_452, ambi_miR_2537, hsa_miR_127, hsa_miR_493_3p, hsa_miR_130a, ambi_miR_4983, ambi_miR_13145, hsa_miR_148a, hsa_miR_210, hsa_miR_485_5p, hsa_miR_299_3p, hsa_miR_134, hsa_miR_222, hsa_miR_181d, ambi_miR_13258	Upregulated	DMD	Serum	[53]
hsa_miR_423, hsa_miR_361, hsa_miR_197, hsa_miR_92, hsa_miR_26a, ambi_miR_7075, hsa_miR_30b, hsa_miR_30e_5p, hsa_miR_29a, ambi_miR_13156, hsa_miR_30a_5p, hsa_miR_193b, hsa_miR_331, hsa_miR_486, hsa_miR_30d, hsa_miR_29b, hsa_miR_101, hsa_miR_30c, hsa_miR_22	Downregulated			

Table 2.
List of different microRNAs that could be used as potential biomarkers in the diagnosis of DMD.

Tested marker	Levels (high or low)	Location (serum/ muscle)	Ref.
24,25(OH)2D3	Reduced in DMD	Serum	[54]
1,25(OH)2D3	No change		
25(OH)D3	No change		
Creatinine	Reduced in DMD, BMD, LGMD2A and LGMD2B	Serum	[55]
Imidazole acetic acid	Reduced in DMD and LGMD2B		
5α Dihydrotestosterone glucuronide // androsterone glucuronide // Etiocholan-3alpha-ol-17-one 3-glucuronide	Reduced in DMD		
DL-p-Hydroxyphenyllactic acid // Isohomovanillic acid	Reduced in DMD		
Creatine	Elevated in DMD, DM1, LGMD2Aand LGMD2B		
Guanidinoacetic acid	Reduced in DMD, BMD, DM1 and LGMD2A		
p-Coumaric acid	Reduced in DMD		
Citrulline	Reduced in DMD		
5-Methoxyindoleacetate // Indoleacetic acid	Reduced in DMD		
L-Aspartic acid	Reduced in DMD		
Ornithine	Reduced in DMD		
2-Hydroxycaproic acid	Reduced in DMD		
L-Serine	Reduced in DMD		
Dehydroisoandrosterone 3-sulfate	Reduced in DMD		
Erythrose	Reduced in DMD, BMD, FSHD		
Glutamine	Reduced in DMD, BMD, LGMD-2B, FSHD and elevated in DM-1	Serum	[56]
Acetate	Elevated in DMD, BMD, FSHD, LGMD-2B and DM-1		
Tyrosine	Elevated in BMD		
Lysine	Reduced in FSHD, LGMD-2B and DM-1		
Citrate	Reduced in FSHD Elevated in LGMD-2B		
Lactate	Reduced in LGMD-2B		
Histidine	Reduced in FSHD		
Serum creatinine	Elevated in BMD Decreased in DMD	Serum	[57]
3-Methylhistidine	Deduced in DMD and LGMD	Urine	[58]
N epsilon,N epsilon-dimethyllysine	No alteration		
N epsilon, N epsilon, N epsilon-trimethyllysine	No alteration		
NG,NG-dimethylarginine	Elevated in DMD and LGMD		
NG,N'G-dimethylarginine	No alteration		

Tested marker	Levels (high or low)	Location (serum/ muscle)	Ref.
Tetranor PGDM (PGD2 metabolite)	Elevated in DMD	Urine	[59]
Nitric oxide	Reduced in DMD	Serum	[60]

Table 3.
List of metabolites that can be used as potential biomarkers in DMD diagnosis.

to diagnose DMD since the multiple primers covered commonly mutated locations across the entire DMD gene, hotspot regions [66–68]. This technique was first developed by Chamberlain et al. [69] through utilization of 6 primer sets that were modified to 9 sets and later to 10 by Beggs et al. [70] (to amplify exons 45, 48, 19, 17, 51, 8, 12, 44, 4). If no amplification take place, this will confirm deletion of this exon. The developed primer sets were successfully able to detect deletion mutations in the hot spot regions. One of the limitations of such technique was its inability to diagnose all cases with other deletion mutation in other regions, or patients with SNPs or deep intronic mutation.

2.4.3 Multiplex ligation dependent probe amplification (MLPA)

In order to simultaneously investigate the status of the 79 exons of the DMD gene, a PCR-based technique was developed to diagnose DMD in a multiplex PCR reaction. The assay uses multiple probes to target different exons in the DMD gene. Each probe consists of two oligonucleotides; one consists of a 5′-adapter and a 3′-exon-specific region, and vice versa for the second oligonucleotide, where the 3′-end of the first primer and the 5′-end of the second hybridize to two adjacent nucleotides in the target exon. Hybridized probes are subjected to ligation reaction, thus, only hybridized probes get ligated, amplified by PCR using adapter-specific primers and separated by capillary electrophoresis. Positive PCR product indicates the presence of the target exon, while deleted exon(s) will not produce corresponding product(s). In this assay, it is also possible to detect exon duplication, which will be detected as larger peak [71, 72]. However, this assay cannot detect non-sense nor in/del point mutations.

2.4.4 Microarray

High-throughput methods such as DNA microarrays were adopted using specific oligonucleotide probes that cover the entire 2.4 mbp DMD gene (targeted high density comparative genomic hybridization (CGH) microarray). Such method could effectively be used to detect known as well as novel intronic mutations [73–75].

2.4.5 Next generation sequencing (NGS)

The development of NGS and the massively parallel sequencing allowed the sequencing of 100 s of millions of independent short reads (100–300 bp) at the same time. Such approaches generate huge amount of data that uses bioinformatic analysis for annotations and alignments of the generated sequences to produce sequence information for large genes such as DMD and titin and delineate the exact locations of mutations [76]. One major advantage of resorting to NGS for DMD diagnosis is that it could be used for the analysis of MLPA-negative samples that could have small deletions/duplications or single nucleotides variants [77].

Also, RNA sequencing by NGS (RNA-seq) is very useful in detecting the splicing pattern that occur in the DMD transcripts in the muscles through different developmental stages, muscle breakdown or muscle regeneration [78–80].

2.4.6 Muscle biopsy

In some cases, muscle biopsy is required to fully characterize the phenotypic effect of the mutation. The muscle tissue is used in immunoassays, using different antibodies targeting different regions of dystrophin protein (C-terminal, Rod and N-terminal domains), such as western blotting [81–83] or immunohistochemistry [83]. Uchino et al. [83] developed a multiplex western blotting assay to analyze the expression of other muscle proteins like dysferlin, merosin, different forms of sarcoglycan (alpha, beta, gamma, delta), and calpain in addition to dystrophin protein, due to the frequent epigenetic changes incited in these proteins as a consequence to the alteration in dystrophin expression.

3. Conclusion

In this chapter, we have presented a comprehensive review for the methods that have been used in the diagnosis of DMD. Because of the nature of the disease, an X-linked disorder, DMD symptoms of the first affected male births of asymptomatic carrier mothers are usually go unnoticed until the age of 5, where the progressive muscle weakness becomes obvious and fibrotic fatty tissue infiltration is prominent. However, it is well known that early diagnosis and treatment results in better disease management and improve the clinical outcomes. In fact, some studies have pointed out to the fact that initiating corticosteroids therapy early enough has delayed the loss of ambulation in most cases by about 2 years [84]. In addition, with the fast-paced progress in molecular/personalized therapies such as exon-skipping and gene-editing based approaches, precise diagnosis and mutation detection becomes a necessity. Moreover, the genetic testing has been extensively used in prenatal diagnosis and has assisted in decreasing disease burden by aborting affected male pregnancies. In a retrospective study conducted in the Netherland, the authors reported 145 abortions of male fetuses over 26 years that had been found to carry inactivating mutations of the DMD gene [85]. Furthermore, identifying female carriers, is gaining momentum to decrease the possibility of giving birth to affected males and consequently contributes to the overall disease management.

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