

# An overview of Diagnostic Approaches for Detection of Duchenne Muscular Dystrophy (DMD)/ Becker Muscular Dystrophy (BMD)



Gaurava Srivastava and Preeti Srivastava\*

Genetic Centre, Government Medical College and Hospital, India

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\*Corresponding author: Preeti Srivastava, Genetic Centre, Government Medical College and Hospital, Sector-32, Chandigarh-160032, India, Tel: 9988949868; Email: preetibtbhu@gmail.com

## Abstract

D/BMD is an X-linked recessive disorder characterized by progressive muscle weakness, which predominantly affect males. This study intended to elucidate overview of available diagnostic approaches utilized for detection of D/BMD. Till date no absolute cure of the disease is available to be used in clinical practice. Early diagnosis and timely management are requisite for D/BMD and it enhances the quality of life of patients. Various diagnostic approaches for D/BMD includes Clinical features, biochemical analysis, molecular analysis, muscle biopsy, electrodiagnostic approach, imaging tests, NCV, Pedigree and family history, IQ measurement, genetic counseling of families for carrier detection and prenatal screening. Each diagnostic approach has its own usefulness for detection of D/BMD. Defining alternative, less invasive and objective outcome measures to assess disease progression and response to therapy will aid drug development and clinical trials in DMD. Most of the diagnostic approaches are discussed in this review, with particular emphasis on the most recent approach i.e., molecular diagnosis for D/BMD patients. The advantages and shortcomings of each approach and challenges are outlined in detail. Singly or in combination, all of these diagnostic strategies hold great promise for early and better detection of this devastating childhood disease.

**Keywords:** Duchenne muscular dystrophy; Becker muscular dystrophy; Diagnostic approaches; Molecular methods; Muscle biopsy

**Abbreviations:** DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; NCV: Nerve Conduction Velocity; IQ: Intelligence Quotient

## Introduction

Duchenne muscular dystrophy is most common fatal X-linked recessive disorder of childhood muscle wasting, which alone accounts for approximately 80% of all the myopathies, with an incidence of about 1 in 3,600-9,337 live male births worldwide [1,2]. Predominantly males are affected with DMD due to X linked inheritance pattern and family history may or may not affect the occurrence of disorder [3]. It is allelic muscle wasting condition, arising from mutations in large DMD gene at Xp21.2 [4]. It is generally caused by protein truncating mutations in large DMD gene. Dystrophin is a vital protein for my fiber function and muscle-fiber plasma membrane integrity, its expression and biological activity diminishes due to mutation in this gene [5,6]. Dystrophin gene is the one of the largest gene with 30,000 genes, which encodes for proteins of human genome: 79 exons cover 2.6million bp and protein product is of size 427KDa [6]. Clinically, the disease is characterized by progressive muscle dysfunction, leading to loss of ambulation by 8-15y of age and early death due to complications of respiratory, orthopedic, and cardiac problems [1,7]. The difference between the severe DMD and the allelic, milder Becker muscular dystrophy (BMD)

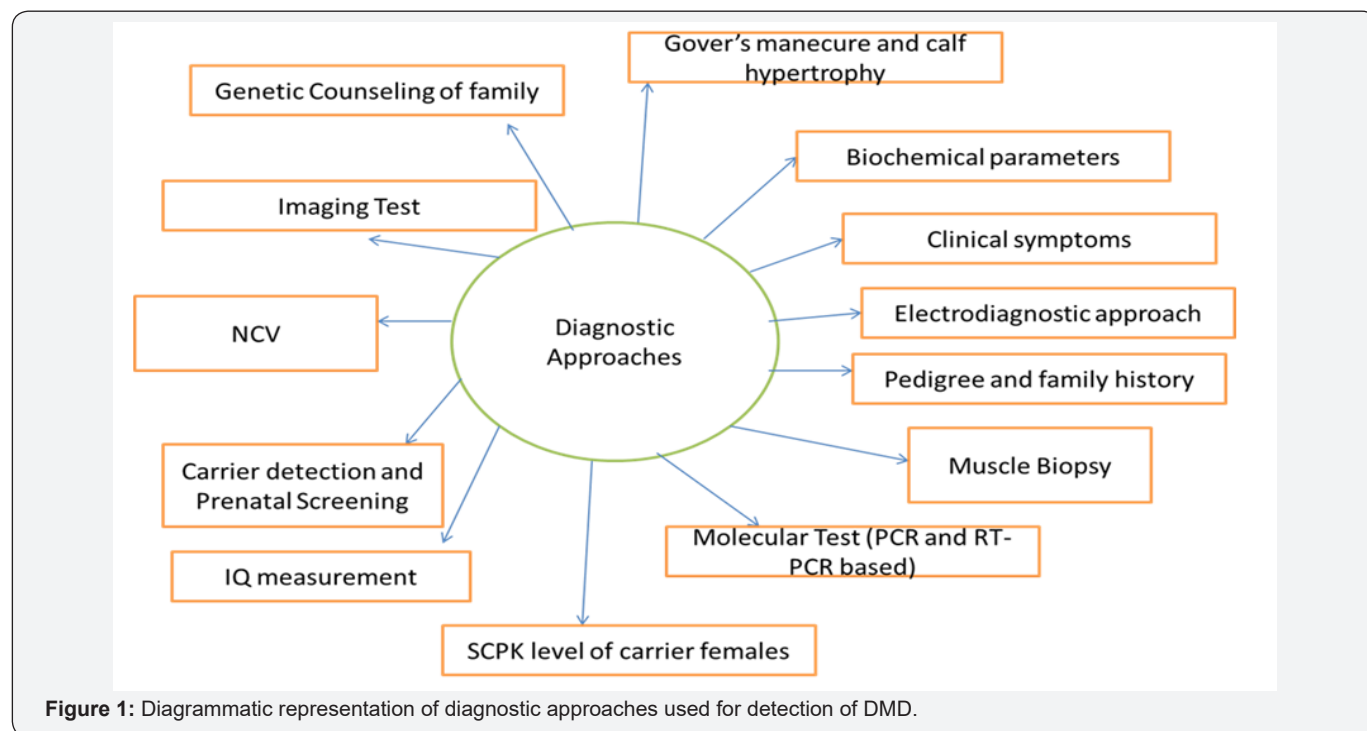
occurs due to alterations in gene mutations. DMD is principally caused by out-of-frame deletions or duplications, which lead to complete loss of protein, whereas BMD is mostly caused by in-frame deletions or duplications, which lead to altered-size, but still partly functional protein [8]. This disorder is basically characterized by progressive skeletal muscle weakness and loss of muscle integrity accompanied by cardiac and smooth muscle dysfunction. Thus, DMD affected patients have weaker skeletal, cardiac, nervous and retinal functions with deteriorating evolution until death around second decade of life. The affected children also have difficulties in climbing stairs as well as in running.

## Diagnosis strategies in D/BMD

Diagnosis of any disorder is comprising several steps and similarly in case of D/BMD. The muscular dystrophies are varying in age of onset, degree of severity, mode of inheritance and the muscle group that are primarily affected. D/BMD is manifested with muscular weakness, hypertrophy of the calf muscles and positive Gower's sign. Although skeletal muscle weakness is

predominant symptom, progressive cardiomyopathy is common and can be severe. In addition to muscle pathology, intellectual impairment of varying degree is present in about 30% of all patients with DMD. DMD affected boys are usually wheelchair bound by the age of 13 years and die early in their 3<sup>rd</sup> decade of life [1]. Many studies reported delays in diagnosis of D/BMD [9-12]. Serum creatine kinase (CK) or serum creatinine phosphokinase (CPK) is the main biochemical marker of muscle necrosis and affected individuals may have very high CK/CPK levels (range: 15,000 – 35,000 IU/L). Thus serum CK/CPK can be utilized as a biomarker for identification and detection of affected individual at birth [1]. Along with CK/CPK levels, liver enzymes also play critical role for identification of DMD. The affected patients have high levels of liver enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and lysosomal enzymes of muscles such as Aldolase, Aspartate aminotransferase [13]. For earlier diagnosis through non-invasive method using biochemical enzymes, is efficient and quick method. Dystrophin protein detection through immunohistology in muscle biopsy samples are being performed [14]. Most powerful and important

tool for diagnosis of D/BMD is molecular/genetic test. Through molecular techniques mutation in DMD gene is easily identified. In DMD, deletion accounts for 65%, 5-8% by duplication and the remaining are caused by point mutation and small insertions [15]. Other diagnostic tests include electrodiagnostic approach, imaging tests, NCV, Pedigree and family history, IQ measurement and other advance test. Since last two decades, analyses of both the DMD gene and dystrophin protein have improved the diagnosis of DMD [16-18] (Figure 1). Knowing the exact mutation in a proband, today one can determine the possibility of a life span up to their 4<sup>th</sup> decade [1]. The most common cause of death is cardiac and respiratory failure but improvements in health care with use of steroids and assisted ventilation have extended their life span [19]. Approximately, 60% of dystrophin mutations are large insertions or deletions that lead to the frame shift errors downstream, whereas approximately 40% are point mutations or small frame shift rearrangements [20]. Around one-third of the DMD patients originate through new mutations while the rest are inherited through carrier mothers or arise from germinal mosaicism.



**Figure 1:** Diagrammatic representation of diagnostic approaches used for detection of DMD.

Through multiplex PCR simultaneously different set primers can be evaluated in a single lane to give specific bands. With advancement of molecular tools now-a-days Multiplex ligation-dependent probe amplification (MLPA) is used for identification of deletion, duplication as well as point mutation of whole 79 exons. With more advancement of scientific techniques in recent era for detection of virtually all mutations of DMD/BMD disorders-SSCP (DOVAM-S), single condition amplification/internal primer sequencing (SCAIP), comparative genomic hybridization (CGH) microarray, next-generation sequencing can be implemented.

### Clinical symptoms

The major clinical symptoms for initial identification of D/BMD include delayed walking, repeated falls or awkward manner of walking, stepping, or running [1]. For identification of DMD in maximum cases the mean age is at around 5 years. Delay in detection of D/BMD is one of the major drawbacks due to its variable and nonspecific behavior, which can be overcome by implementing newborn screening [21]. Early diagnosis in the 1970s became possible through detection of elevated creatine kinase (CK) in dried blood spots [22]. Cyrus et al. [23] reported the total of 264 male infants was screened for DMD and

screening was feasible in a pediatric office. Gower's maneuver and/or calf hypertrophy are clinical screening symptoms for muscle modification, typically seen in D/BMD patients [24]. The presence of Gower's sign in a male child should trigger the diagnostic investigation of D/BMD, especially if the child also has a waddling gait. Walking ability has lost in around 90% of DMD cases due to development of scoliosis, at varying age of onset between 11-16 [25-27]. Around 50% of DMD affected male's attained scoliosis between 12-15 years [28,29] with a sharp deterioration at 13 years or one to two years after wheelchair confinement [26]. In the D/BMD presence of a positive family history, in particular a maternal history is useful and inexpensive tool to identify individual at high risk who may require specific consideration [30-32].

### Biochemical parameters

A variety of constituents are present in skeletal muscle including Serum creatine phosphokinase (CPK), myoglobin (Mb), lactate dehydrogenase (LDH), serum glutamic pyruvic transaminase (SGPT) or alanine transaminase (ALT), serum glutamic oxaloacetic transaminase (SGOT) or aspartate transaminase (AST) and calcium (Ca) etc. Various tissues as well as cells of our body expressing enzyme serum creatine phosphokinase (CPK), also known as creatine kinase (CK) or phosphocreatine kinase. Serum CPK, is a sensitive indicator of skeletal and cardiac muscle injury and is a fast and reliable means of determining muscle disease [33-38]. It is reported CPK can reach 25-200 times in D/BMD cases [39-42]. Due to wide diversity among ethnic groups and sources, the comparison of available data is doubtful [43]. Serum myoglobin reflects a balance between intravascular release of myoglobin from muscle and

renal clearance [44]. CPK and LDH, both are significant enzymes of skeletal muscle and their molecular mass as well as level vary and easily determined in various patients for identification [43]. Ca<sup>2+</sup> has a major role in variety of physiological pathways as well it is universal intracellular signaling molecule [45-47]. In D/BMD patient's abnormal calcium levels are present which affect physiological mechanisms [48]. Thus for detection of D/BMD at earlier stage biochemical parameters viz., SCPK, LDH, Myoglobin and Ca levels are deciding parameters.

### Molecular tests

DMD gene is one of the known largest gene with 79 exons (0.6% of gene) including 8 promoters and rest 99.4% of gene constitutes large intron area. Thus mutation detection of B/DMD gene was not easy task in past but with advancement of molecular tools detection of B/DMD is more precise and accurate. Majorly in around 65% of DMD and 85% of BMD cases, deletion of one or more exons accounts as major molecular defect whereas only 6-10% duplications and around 2% due to point mutations, small insertions/deletions or splice site changes in DMD gene occur [49]. Around 66% mutations in this gene are maternally inherited where as 33% are de novo. For mutation analysis in DMD gene, multiplex PCR observed as most convenient tool. In DMD gene M-PCR, is utilized for amplification of most commonly deleted and hotspot region exons. Various centers are being using PCR multiplex sets of Chamberlain et al. [50], Beggs et al. [51,52] & Kunkel et al. [53] and also their improvements. Many scientific groups from all over the world reported M-PCR as convenient tool for detection of DMD from past to till date (Table 1). Earlier reports based on use of M-PCR for detection of DMD are as follows:

**Table1:** Reports available based on application of M-PCR for detection of DMD.

Author	No. of subjects	% Deletion	M-PCR Exons	Region of Study
Banerjee & Verma [54]	160	64.4	27	Northern India
Dastur et al. [55]	382	78.7	32	Mumbai
Swaminathan et al. [40]	112	73	27	South India
Saktivel et al. [56]	150	68.7	30	Chennai
Kohli et al. [57]	180	50	22	New Delhi
Basumatary et al. [58]	69	71	17	Northeast India
Rao et al. [41]	88	73.68	26	Gujarat
Datkhile et al. [59]	50	94	21	South Western Maharashtra
Hung et al. [60]	84	13	19	Taiwan
Lai et al. [61]	63	69.8	18	Hong Kong
Takeshima et al. [15]	442	61		Japan
Lee et al. [62]	29	72.4	20	Korea
Nouri et al. [63]	74	51.3	19	Iran
Kerr et al. [64]	128	31	24	Johannesburg, South Africa

With the advancement of various molecular methods, along with deletion, duplications, insertions, small mutations can also be detected. Applications of molecular methods and its role for DMD detection in male and/or female are mentioned in below

(Table 2). Thus by development and advancement of these molecular tools detection of DMD become more accurate and convenient [54-76].

**Table2:** Various molecular methods, their applications for detection of DMD disorder.

Methods	Importance	Applications in Male	Applications in Female	Reference
Genetic Testing for Deletion/Duplication				
Multiplex PCR	Specific exon deletions	+		Rao et al. [41]
Southern Blotting	Most exon deletions & some duplications	++	+	Prior and Bridgeman[65]
Quantitative PCR	Most exon deletions & some duplications	++	+	Abildinova et al. [66]
MLPA	Most exon deletions & duplication	+++	++	Stuppia et al. [67]
Dystrophin Array CGH	Most exon deletions & duplication	+++	+++	Gaudio et al. [68]
Genetic Testing for Small/Point Mutations				
Mutation Scanning (DDGE, SSCP, DHPLC)	Scans coding region for potential sequence changes. Followed by sequencing to identify the specific change	++	++	Muscarella et al., Hofstra et al. [69-70]
Sequence Analysis	Determines sequence for coding region	+++	+++	Okubo et al. [71]
Resequencing Array	Array (chip) based sequencing Includes known intronic mutations	+++	+++	Hedge et al. [72]
Genetic Testing for Others				
SCAIP: combines testing for large deletions, and small deletions and point mutations				Flanigan et al. [73]
Targeted/familial mutation testing: Targeted sequence test for known familial mutation				Bogue and Ramchandren [74]
mRNA / cDNA: For those without mutation identified by other tests -Functional testing for rare mutations -Requires muscle biopsy				Dunnen et al. [75]
Linkage: Tests regions in/near gene -does not identify mutation -May require one or more males with DMD -Risk of recombination				Towbin et al. [76]

### Muscle biopsy

Muscle biopsy is executed with enormous importance, when no mutation confirms or genetic testing is negative for the mutation. Several neurologists, mostly in India, diagnose children with DMD, by performing muscle biopsies. Manjunath et al. [77] reported the patients with negative mutational finding by m-PCR and MLPA, are being performed for muscle biopsy whereas more advanced molecular tool such as next generation sequencing (NGS) to diagnose the MLPA negative cases will be more preferred and completely non-invasive. It is reported worldwide that in at least 4% of case, underlying mutation is not detectable with existing genetic testing due to probable difference between mutation study and the clinical phenotype [78,79]. Patients with no mutation are subjected to muscle biopsy for IHC and western blotting [78,79]. The crucial tests through the muscle biopsy for D/BMD types are immunohistochemistry (IHC) and immunoblotting for targeting dystrophin protein [80]. A muscle biopsy can provide information on the amount and molecular size of dystrophin, as long as the protein is present [81]. IHC

can be used to confirm the results of genetic diagnosis of D/BMD in prenatal diagnostic techniques (PNDT) [82]. According to Hedge et al. [72], with advancement of molecular techniques such as NGS detection of complex rearrangements and large scale intronic alterations, become more accurate than MLPA and other exon based tests. However, it is apparent from the majority of genetic studies that all mutations cannot be identified with standard molecular analysis. For such small number of cases, a muscle biopsy may be helpful for protein studies and muscle RNA analysis to establish an accurate diagnosis [77]. Nevo et al. [83] reported that always there are some cases in which molecular methods fail to provide a definitive diagnosis and in such cases fetal muscle biopsy found as an option to avoid prenatal diagnostic dilemmas.

### Electrodiagnostic approach

During the diagnosis as well as management of DMD, electrodiagnostic (EDX) assays play an important role as an extension of the physical examination [84]. However, in presence of molecular analysis, these indices are not always needed to



diagnose a D/BMD. The patients with positive family history for D/BMD have very limited utility for EDX test, whereas conclusive diagnosis requires genetic testing at times muscle biopsy. Comparative electrodiagnostic pattern and myopathic features of DMD and BMD patients were studied [85]. For timely managements or rehabilitation of D/BMD patients as well as carrier females, early diagnosis of cardiac involvements may be very useful [86]. Histopathologic or molecular studies, or both should be followed by EDX assays. Ultimately, this approach usually allows the clinician to make the correct diagnosis and real time analysis of condition [87].

### Imaging tests

Imaging techniques such as ultrasonography [88], computed tomography [89] or magnetic resonance imaging (MRI) [90] have developed to evaluate muscle internal damage and quality non-invasively [91]. Thus these techniques are beneficial in observing muscle loss, cardiac function and other vital components in DMD patients, but major limitations associated are often costly, laborious and low throughput.

### Conclusion

The present study reviewed diagnostic approaches utilized for D/BMD treatment. The early diagnosis and timely management with available treatment options along with frequent monitoring have led to vast improvements in the quality of life of D/BMD patients. The longer lifespan although, has left DMD patients exposed to increased risk of various health problems but with proper management, the quality of life will be improved as well. If in clinical trials any treatment option seems adequate, then the major target to provide early treatment to patients before development of any muscle pathology. Early intervention will require early diagnosis. There is a requisite for further comprehensive efforts for the accurate molecular diagnosis, carrier detection, prenatal diagnosis, genetic counseling and rehabilitative measures along with screening the population either at early stage such as newborn or later stage in India. Thus early diagnosis using various approaches for better treatment as well as for management of D/BMD patients are today's need.

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