ORIGINAL ARTICLE

Clinical and Molecular Profile of Duchenne Muscular Dystrophy (DMD): Case-Record Analysis From Uttar Pradesh, India

Ankur Singh,[1] Minketan Sidar,[1] Akhtar Ali, [2] Abhishek Abhinay,[1] Rajniti Prasad,[1] Om Prakash Mishra[1]

[1]Department of Pediatrics and [2]Centre for Genetic Disorders, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

ABSTRACT

Objectives: To assess the clinical and molecular profile of patients with Duchenne Muscular Dystrophy (DMD) presenting to a tertiary center in Eastern Region of Uttar Pradesh, India.

Methods: In this retrospective study, case records of all patients diagnosed as DMD were analyzed to ascertain the clinical phenotype and molecular profile. Multiplex polymerase chain reaction (PCR) technique, Multiplex Ligation Dependent Probe Amplification (MLPA) and Next Gen Sequencing (NGS) were used for establishing the molecular diagnosis. Leiden Open Variation Database (LOVD) frame checker online tool was used to predict clinical severity of the cases.

Results: Records of 112 children with DMD were analyzed. The median (IQR) age of onset and clinical presentation of disease was 60 (12, 132) months and 90 (33, 156) months, respectively. The most common clinical presentations were difficulty in standing from sitting position (n = 107), difficulty in climbing stairs (n = 106), and difficulty in walking (n = 99). Bilateral calf muscle hypertrophy and a positive Gower's sign was seen in 110 and 108 patients at presentation. The median (IQR) creatinine phosphokinase (CPK) levels at diagnosis were 6296.5 (4320, 7432.5) U/L. The genetic mutations in 111 patients were reported as deletion (n = 105), duplication (n = 3), and point variation (n = 3). 22 patients could benefit from the available exon skipping therapy. Exondys (exon 51 skipping) could be used in 14 patients.

Conclusion: Deletion mutations were recorded in a much higher proportion of patients compared to previous studies from India. There were 22 patients who could have been benefitted by present available exon skipping therapy.

Keywords: *Deletion, DMD, Duplication, Dystrophin gene, Exondys*

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INTRODUCTION

Duchenne Muscular Dystrophy (DMD OMIM#310200) is the most common of all muscular dystrophies inherited as a X-linked recessive disorder with an incidence of 1:3500 live male births. It is caused by mutation (deletion/ duplication/point variation) in the DMD gene which encodes dystrophin and is located at position Xp21.1 of the X chromosome [1]. It is the largest known gene in the human body, spanning ~ 2.3 MB of DNA with 79 exons and 78 introns [2]. Frequency of genetic mutation in DMD patients is recorded as deletion (60-65%), duplication (5-6%) or point variation. Most of the deletions occur in exons 45-53, while the duplications occur in exons 2-20 [3]. Numerous diagnostic techniques are available to identify the disease. Deletion and duplication are identified by using multiplex polymerase chain reaction (mPCR) (Hot spot exons) in the DMD genes or Multiplex Ligation Dependent Probe Amplification (MLPA). Next generation sequencing (NGS) is useful for diagnosing patients who are negative by MLPA [4].

Progressive muscle weakness is followed by a loss of ambulation; respiratory, cardiac and orthopedic complications occur as the disease progresses and patients succumb in the second decade of life without intervention. Exon skipping is considered a promising therapeutic approach for DMD. Hence, this study was planned to assess the clinical, biochemical and molecular spectrum of patients diagnosed with DMD at a tertiary centre in Eastern region of Uttar Pradesh, India, and to assess the prevalence of genotypes which are amenable to treatment by exon skipping.

METHODS

The retrospective study was conducted in the Division of Genetics and Metabolism of the Department of Pediatrics in Institute of Medical Sciences, Varanasi, Uttar Pradesh,

Correspondence to: Dr Ankur Singh, Professor, Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, 221005, India. *ankur@bhu.ac.in*
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India, between January 2020 and December 2023. The records of children with a confirmed diagnosis of DMD who were being followed up in the Pediatric Genetics Clinic of the institute were analyzed. The study was approved by the Institutional Ethics Committee.

Records of boys with a confirmed diagnosis of DMD were included. Diagnosis of DMD was confirmed by genetic tests using at least one of the following techniques: mPCR, MLPA, and NGS, or histopathological evaluation of muscle biopsy. mPCR was performed using 10 exon primers [3, 6, 13, 43, 47, 49, 50, 52 and 60, Pm (muscle-specific promoter)]; for each exon, forward primers and reverse primers were used [5]. The samples which were negative for DMD on mPCR were subjected to MPLA testing and subsequently by NGS if needed. Muscle biopsy followed by immunohistochemistry for dystrophin protein was used if necessary. Demographic details, clinical features (like the age of onset, features at presentation, family history, distribution of muscle involvement, developmental milestones, degree of ambulation) and genetic tests were collected and documented in a predesigned performa.

Statistical analysis: Descriptive statistics were used to present the data like frequency, mean (SD), median (interquartile range) and range using SPSS version 21.0. Severity of disease was predicted using online prediction tool (Leiden Open Variation Database), which can predict outcome of deletion or duplication in patients with muscular dystrophy [6].

RESULTS

Out of 137 patients registered at the clinic with suspected DMD, we analyzed records of 112 confirmed cases of DMD; 25 children were excluded from the study due to incomplete records. The median (IQR) age of onset of symptoms and presentation to the hospital was 60 (12,132) months and 90 (33, 156) months respectively. The age range of this cohort was 2.7 to 13 years. All cases hailed from Eastern Uttar Pradesh. DMD was diagnosed on the basis of multiplex PCR, MLPA, NGS and muscle biopsy in 50, 58, 3 and 1 patient respectively. **Table I** depicts the sociodemographic and clinical profile of study participants. 24 (21.4%) participants had a positive family history; maternal uncles were affected in 12 families; first degree siblings were affected in 11 families and one family had a maternal uncle and siblings affected. Prenatal counselling was offered to 10 families for future pregnancy; 8 mothers gave birth to normal unaffected children. All children were offered physiotherapy and prescribed oral deflazacort (1 mg/kg/day) and multivitamins. Sibling screening and carrier testing of proband-related females was advised. **Table II** highlights the molecular profile of study participants (one patient had biopsy proven DMD). Single exon deletion was the commonest exon deletion (36/105; 34.28%). LOVD frame checker was applied on 58 patients (detected by MLPA), which showed 41 out-of-frame deletions and 17 in-frame deletions. **Table III** describes the amenability of patients to available exon skipping therapy. There were 95 patients who had deletions in the hot spot region. Mostly deletions were present in distal hot spot region (n = 84, 88.42%) and 11 patients (11.57%) had deletions in the proximal hot spot region. 22 participants (19.6%), were amenable to available exon skipping therapy. One child had cardiomyopathy and had exon 43 deletion.

DISCUSSION

The study included 112 confirmed cases of DMD with age ranging from 2.7-13 years. Another study from Uttar Pradesh included 121 children with much younger age profile (1.5 to 5 years) [6]. The variation in age of onset of disease may be due to differences in genetic profile suggesting a need to correlate clinical and genetic profile. Similar to previous studies, a positive family history was noted in nearly one-fourth of the patients, although a delay in motor milestones and loss of ambulation were less frequently encountered in the current study [7-9]. Most of patients presented to us were late and were still ambulatory (93.75%). This again could be due to different genetic profile and epigenetic factors present in this region of patients.

Table I Sociodemographic and Clinical Profile of Patients with Duchenne Muscular Dystrophy (n = 112)

Age (months) at onset[a]	60 (12, 132)
Age (months) at presentation[a]	90 (33, 156)
Family history of similar illness[b]	24 (21.43)
Delayed milestones[b]	24 (21.43)
Gower's sign[b]	108 (96.43)
Calf muscle hypertrophy[b]	110 (98.21)
Loss of ambulation[b]	7 (6.25)
Contracture[b,c]	2 (2.9)
Serum creatinine phosphokinase (U/L)[a]	6296.5 (4320, 7432.5)
Difficulty in walking[b]	99 (88.39)
Difficulty in standing[b]	107 (95.53)
Difficulty in going upstairs[b]	106 (94.64)
History of frequent falls[b]	93 (83.03)
Cardiomyopathy[b,d]	1 (0.89)
Religion[b]	
Hinduism	104 (92.86)
Islam	8 (7.14)

Values presented as [a]median (IQR), [b]n (%)
[c]Details available for 69 patients, [d]This patient has exon 43 deletion

Table II Molecular Profile of Patients with Duchenne Muscular Dystrophy (*n* = 111)

Type of genetic defect	*Number of patients (%)*
Duplication	3 (2.7)
Point variation	3 (2.7)
Deletion	105 (94.59)
Single exon	36 (34.28)
Two exons	24 (22.85)
Three exons	22 (20.95)
Four exons	13 (12.38)
Five exons	7 (6.66)
Six exons	1 (0.95)
Nine exons	1 (0.95)
Sixteen exons	1 (0.95)

This study recorded the highest percentage of deletions (94.59%) compared to other Indian studies [7-9]. Previously, Dey et al reported deletions in 90.12% of the patients with DMD from Eastern India [8]. This study also emphasizes the role of MPLA or mPCR in the diagnosis of DMD especially where NGS is not feasible due to higher costs or non-availability. Single exon deletion was present in 36 cases; single exon 47 was the commonest deletion (13 patients). Multiexon deletion was present in 69 cases; exons 49 and 50 were the commonest exons deleted. There was one child with 16 exon deletions; most of deletions were in the hot spot region (distal hot spot in 84 and proximal hot spot in 11). This finding is comparable to some other studies from India [7,8,10]. We applied LOVD deletions/ duplications reading frame-checker to patients who had been diagnosed by MLPA technique (n = 58). Out-of-frame deletion (n = 41) was more common than in-frame deletion (n = 17). Reading frame rule is valid in 90% of DMD cases and 94% of BMD (Becker Muscular Dystrophy) cases [11]. This tool has its limitations like the change in DNA did not match the changes in RNA because of incorrect splicing and inefficient splicing.

Cardiac manifestation of DMD patients reported are dilated cardiomyopathy (myocardial fibrosis, heart failure and arrhythmia) and supraventricular arrhythmias. Kummitha et al also concluded that conventional criteria of cardiomyopathy (reduced ejection fraction) could only be picked up in 7 patients against 58 patients picked by non-conventional methods (Global longitudinal strain by speckle tracking echocardiography) [12]. Our study could pick only one patient with cardiomyopathy, based on conventional criteria. This patient was 7 years of age, with exon 43 deletion (out of frame deletion) with positive family history. Cardiac MRI is imaging of choice in children, aged more than 7 years [13]. This was not done due to high cost, sedation problem and unwillingness of parents. The under reporting of dilated cardiomyopathy (DCMP) in our cohort could be due to use of conventional echocardiography and also because only 35 patients underwent echocardiography. Under-reporting may also have been due to the retrospective design of the study. Goyal et al had reported DCMP in two out of 116 patients, with both cases having distal hot spot deletions [7].

Table III Deletion Pattern, Number of Patients and Amenability to Exon Skipping Therapy of DMD Patients (*n* = 105)

Deleted exon	*Number of patients*	*Hot spot region*	*Amenable to available exon skipping therapy (name of exon)*
6	2	Proximal	No
13	1	Proximal	No
21	1		No
43	4		Yes (Exon 44)
44	1		Yes (Exon 43, Exon 45)
45	9	Distal	Yes (Exon 44)
47	13	Distal	No
48	1	Distal	No
50	1	Distal	Yes (Exon 51)
52	3	Distal	Yes (Exon 51, Exon 53)
3,6	4	Proximal	No
8,9	2	Proximal	No
13,43	1		No
47,49	3	Distal	No
47,50	1	Distal	No
49,50	9	Distal	Yes (Exon 51)
50,52	3	Distal	No
53,54	1	Distal	No
47,49,50	15	Distal	No
49,50,52	6	Distal	Yes (Exon 53)
3,6,13	1	Proximal	No
47,49,50,52	12	Distal	No
8,9,10,11	1	Proximal	No
46,47,48,49,50	3	Distal	No
48,49,50,51,52	1	Distal	Yes (Exon 53)
13,43,47,49,50	1	Distal	No
45,46,47,48,49	1	Distal	No
22,23,24,25,26	1		No
16,17,18,19,20,21	1		No
45,46,47,48,49,50, 51,52,53	1	Distal	No
35,36,37,38,39,40, 41,42,43,44,45,46, 47,48,49,50	1		Yes (Exon 51)

WHAT THIS STUDY ADDS?

- The study characterises the clinical (n = 112) and molecular profile of (n = 111) of DMD patients, highlighting the need for accessibility to exon skipping therapy to 22 patients, who can be benefitted, if it becomes available.

In our study, 22 patients were amenable to exon skipping therapy which can delay the natural course of the disease. Exondys (exon 51 skipping therapy) was given in 14 patients of DMD in this cohort. Percentage of DMD patients, being benefitted by exon skipping therapy has been demonstrated previously [14]. Out of ten families who were offered prenatal testing, 8 mothers gave birth to normal unaffected children.

Although, this study was not intended to assess the performance of various genetic tests. The study highlighted limitations of using only 10 set of primers as 45 cases were missed by using these 10 sets of primers. By extending the set of primers according to the common deletions present in the region, it was possible to detect 85.58% (95/111) of cases by mPCR. This highlights that mPCR is still good enough tool for resource constrained centers.

Our study also had some limitations. We did not quantify the motor weakness in our patients during study course making it difficult to correlate it with the genetic variations. Other limitation was the retrospective study design and lack of follow-up. Compilation of rare disease data, high number of patients, molecular data, regional data and information about the number of patients benefitted with exon skipping therapy, were some of the strengths of the study. Therefore, to conclude, this study gives a fairly good idea of the most common genetic myopathy presenting to a tertiary center in pediatric population. This will help policy makers in decision making while formulating diagnostic and therapeutic funding of this rare disease in future.

Contributors: AS: Searched literature, wrote the manuscript; AbA: Searched literature, analyzed data; MS: Curated and analyzed the data; AkA: Performed molecular testing, data collection; RP: Data collection; OPM: Critical inputs. All authors approved the final version.

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