

Duchenne Muscular Dystrophy: Genetic and Clinical Profile in the Population of Rajasthan, India

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive muscular dystrophy that affects young boys and is caused by mutation of the dystrophin gene located over X chromosome. **Materials and Methods:** In this prospective study, 120 clinically diagnosed DMD patients were tested for exon deletions, duplication or point mutation. **Results:** Of the 120 clinically suspected DMD patients, the diagnosis of DMD was confirmed by the genetic study or muscle biopsy in 116 patients. The mean age of onset was 3.2 years and the mean age at presentation was 7.2 years. 110/120 cases were confirmed by genetic testing and six were by absence of staining for dystrophin on muscle biopsy. DMD gene deletion was present in 78.5%, duplication in 5.3% and point mutation in 11.2% cases. 70.3% of patients had deletion located at a distal hot spot region. Single exon deletion was found in 16.5%. Distal hotspot exons 47, 48 and 50 were the commonly deleted exons. **Conclusions:** In our study, 94.8% cases showed genetic change in the DMD gene. Muscle biopsy was the choice of investigation in earlier days. Detection of DMD by DNA based method eliminates the need to do an invasive procedure for diagnosis. Hence the genetic testing should be the investigation of choice in suspected cases of DMD. The pattern of deletion, obtained in the population of Rajasthan was similar when compared with other ethnic groups of the Indian population. It would be helpful for researchers to develop drugs specific to exons or for ongoing mutation-specific therapies.

Keywords: Deletion, Duchenne muscular dystrophy, dystrophin gene, muscular dystrophy

INTRODUCTION

Duchenne Muscular Dystrophy (DMD OMIM#310200) is the most common form of muscular dystrophy, inherited as X-linked recessive disorder. It is reported with an incidence rate of 1:3500 live births and is caused by mutation in the DMD gene, leads to loss of function of dystrophin protein.^[1] DMD gene is one of the longest genes, located on xp21.1 locus and contains 79 exons and 78 introns, spanning approximately 2.4 Mb of genomic DNA.^[2-4] Deletion in the DMD gene is responsible for 60-65% cases, duplication for 5-6% and point mutation including missense, splice site, nonsense and frame shift mutation for the remaining cases.^[5-7] In clinical practice, deletion and duplication are identified by using either primers targeting 18 hot spot exons in the DMD gene or MLPA (Multiplex Ligation Dependent Probe Amplification). Genetically unconfirmed cases can be diagnosed by muscle biopsy with immune histochemistry.

The majority of DMD patients have a history of change in physical activity and are diagnosed around 5 years of age.^[8] Progressive muscle weaknesses results in loss of ambulation at around 10 years and is usually wheelchair bound by the age of 13 years. Disease is progressive in nature causing respiratory, orthopedic and cardiac complications and they often die around the second decade of life without intervention.^[8]

There are various studies describing the genotype and phenotype of DMD patients in various populations including India.^[9-14] The current study illustrating the clinical and genotype profile of DMD in a cohort from Rajasthan.

MATERIALS AND METHODS

A total of 120 clinically suspected DMD patients, who had consulted at the Rare Diseases clinic, Department of Pediatrics, J K Lon hospital, SMS Medical College, Jaipur, India between 2017 and 2019 were reviewed. Institute's ethical clearance was obtained. Boys with characteristic pattern of proximal muscle weakness with positive Gower's sign and/or valley sign, calf hypertrophy/pseudohypertrophy, elevated serum creatine kinase (CPK) and/or a positive electromyographic study were included in the study. Patients with normal CPK levels and having unrelated co-morbidities like trauma, infection were excluded from the study. Clinical data on the age at onset, presentation, family history, distribution of muscle involvement and ambulation were collected. Pretest counseling was done and informed consent

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was obtained from parents. The blood sample was collected in EDTA vial and sent to molecular genetics lab for MLPA. Copy number changes in targeted regions of the DMD gene were identified by hybridizing with MLPA probes at out-sourced lab [Figure 1]. Complete DMD gene sequencing was performed in cases where deletion or duplication not detected on MLPA under research project at same lab. Genotype phenotype correlation was done. Descriptive statistics was used.

RESULTS

Out of 120 clinically suspected DMD patients, 86.6% were belonging to the Hindu community and 13.4% from Muslim community, with a positive family history in 20 patients (17.2%). The mean age for onset of disease was 3.2 years and the mean age at presentation was 7.2 years. All ambulatory boys presented with complaints of frequent fall and need of support while standing from sitting posture, particularly with lower limb weakness. Gower’s sign was observed in all walking patients and non-ambulatory patients had clinical history suggestive of Gower’s sign.

In our study, 97 out of 120 patients were confirmed by MLPA and 13 by the DMD gene sequencing. Remaining ten genetically undiagnosed cases were re-examined and counseled for muscle biopsy. Two of them refused to undergo muscle biopsy. Six out of 8 cases proved to be DMD by the absence

of staining for dystrophin. In this study, mutations could not be detected in ten patients, which may be due to rearrangements in introns or the 3’ or 5’ untranslated regions (UTRs). Thus, authors included a total of 116 genetically or biopsy proven DMD cases in the present study. The clinical details are given in Table 1.

Seventeen out of 116 (14.6%) DMD patients were non-ambulatory at the time of presentation. All had contracture at ankle joint with weakness of upper limb muscle. 98/116 patients (84.4%) had calf-muscle hypertrophy and 8/116 (6.8%) had pseudo calf-hypertrophy. Mild mental retardation was seen in 16 (13.7%) of patients.

The highest & lowest serum CPK value was 19400 IU and 412 IU respectively. Serum CPK, transaminase (SGOT/SGPT) and LDH were significantly high.

The detailed genetic pattern is presented in Figure 2. Gene deletion was present in 91/116 cases (78.5%), duplication in 6 cases (5.2%) and point mutation in 13 cases (11.1%). Majority (70.3%) of the patients had deletion located at the distal hot spot region. Single exon was deleted in 16.5% and exon 47 was the most commonly involved exon followed by exon 50 and exon 48. The most common exon deletion pattern was in deletion of 48-51 exons. The pattern of gene deletion is depicted in Table 2 Largest deletion was extending from exon 21 to 54 and from exon 12 to 41. The spectrum of identified small mutation is depicted in Figure 3.

Most of our patients with loss of ambulation had deletion of distal exon. We did not find any difference in number of involved exons and loss of ambulation.

Association with reading frame

The change that will result in DMD or BMD phenotype is predicted by reading the frame rule (Monaco rule). Of the 91 deletions in the entire cohort, 19 (20%) were in-frame deletions, and 72 (80%) were out-of-frame deletions. The larger out-frame deletions involved exons 18-41, 8-34, 46-63 (onset at 3-4 years of age), 12-41 (onset at 4 years of age).

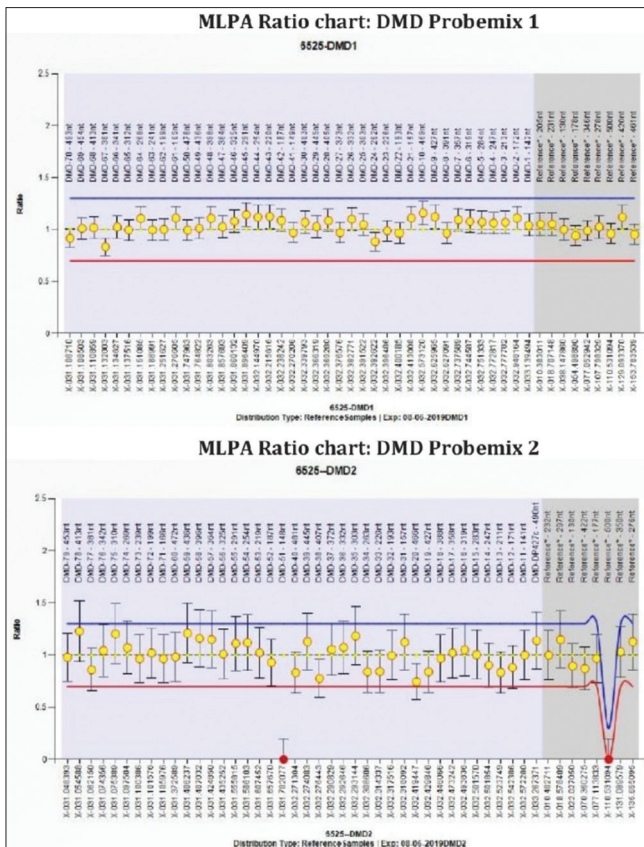


Figure 1: MLPA showing hemizygous deletion of exons 48-52

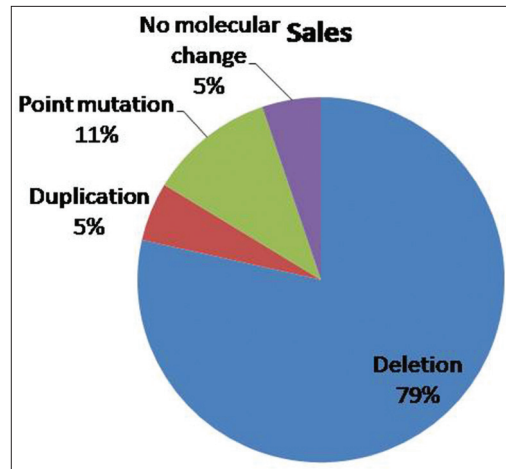


Figure 2: Molecular characterizations of DMD patients

Table 1: Clinical Features of the Patients

| Features | n=116 |
|--|-------------|
| Mean age of onset (years) | 3.2±1.3 |
| Mean age at presentation (years) | 7.2±2.4 |
| Consanguinity | 23 (19.8%) |
| Positive family history | 20 (17.2%) |
| Delayed motor milestones | 68 (62%) |
| Progressive lower limb muscle weakness | 110 (94.8%) |
| Gower's sign | 99 (85.3%) |
| Calf hypertrophy/pseudohypertrophy | 106 (91.3%) |
| Toe walking | 52 (44.8%) |
| Loss of ambulation | 17 (14.6%) |
| Tendoachilles Contracture | 17 (14.6%) |
| Mild Intellectual disability (IQ: 38-63) | 16 (13.7%) |

Table 2: Deletion Pattern of the DMD Patients

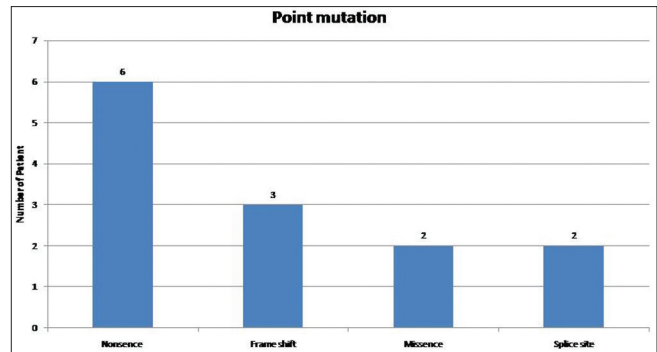
| Type of deletion | N=91 (%) |
|----------------------------|---------------|
| Proximal hot spot deletion | 15/91 (16.4%) |
| Distal hot spot deletion | 64/91 (70.3%) |
| Single exon involvement | 15/91 (16.5%) |
| 2-5 exons | 39/91 (42.8%) |
| 5-10 exons | 28/91 (30.8%) |
| >10 exons | 9/91 (9.9%) |
| Distal exon 45 | 21/91 (23%) |
| Distal exon 47 | 39/91 (42.8%) |
| Distal exon 48 | 33/91 (36.2%) |
| Distal exon 49 | 32/91 (35.1%) |
| Distal exon 50 | 35/91 (38.4%) |
| Distal exon 51 | 25/91 (27.4%) |
| Distal exon 52 | 20/91 (21.9%) |

Among duplications, 3 were in-frame duplications, and 2 were out-of-frame duplications. One DMD child showed duplication of exons 68 to 79, whose effect may be difficult to predict using the Leiden deletions/duplications reading frame checker. This child was evaluated at 5 years of age with history of onset of symptoms at 3 years of age. These duplications were equally distributed towards the 5' and 3' ends of the dystrophin gene.

Intellectual Disability (ID) was defined by IQ less than 70 or Development Quotient (DQ) lower than 85. DQ, most frequently used with preschool children and the optimum time for testing a child's IQ is between ages 5 and 8 years of age. IQ tests performed in 20 DMD children showed mild ID in 5 children and moderate ID in 2 children. The children with below average intelligence had deletions toward the distal part of the gene.

DISCUSSION

DMD is a condition mainly affecting boys and remains undiagnosed for a long time due to lack of diagnostic facilities and awareness. The diagnosis of DMD cases is based on clinical, biochemical and histopathological examinations and confirmed by molecular analysis. Multiplex PCR allows

**Figure 3: Spectrum of identified point mutation**

detection of about 98% cases with deletion, which account for 65% of all mutations. MLPA is one of the widely used techniques and can accurately analyze all 79 exons of the dystrophin gene. It has a sensitivity rate of 63% to 79.5% to identify deletion and duplication.^[15] Limitation of MLPA is its inability to detect small point mutations because it is a probe-amplification based method and the probe cannot be combined with small mutations of the DNA. Next Generation Sequencing (NGS) can detect all mutation types. Hence the combination of MLPA and NGS is the most efficient method for diagnosis of DMD. However, the higher cost of NGS makes it unaffordable for most of the families. In our study we did MLPA first to detect deletions/duplications, and NGS was then applied on MLPA negative cases.

This study presents the clinical and molecular pattern of a cohort of 116 patients with DMD from a tertiary referral center of Rajasthan. A family history was present in 17.2%% cases in our study, comparable to a previous study showing family history of 18.5%.^[15] Other studies showed a slightly more prevalence of family history of 20-27%.^[10,13,16,17] Most of the DMD cases presented late even with a positive family history.

The mean age of onset of symptoms and mean age at presentation was 3.2 years and 7.2 years respectively. This is comparable to the studies from the southern and eastern part of India showed age of onset at 3.1 and 3.9 year of age respectively.^[10,13] The delay in the presentation could be due to lack of awareness and knowledge in the rural area of Rajasthan hence misdiagnosed with weakness and deficiency of nutrients and treated accordingly.

Progressive lower limb weakness dominated in our study with toe walking, gait change, difficulty in running, frequent falls and difficulty in getting up from the sitting position. It is reported as the lower limb predominant phenotype in previous published literature. In our cohort, 94.8% boys presented with progressive proximal muscle weakness particularly of the lower limbs and 5.2% (6/116) were examined at an early age in the asymptomatic phase. These asymptomatic boys had a history of motor delay. The distribution of weakness pattern, calf-hypertrophy and Gower's sign presented in our cohort was similar to the other studies.

Developmental delay was present in 62% of DMD cases. A study from southern India found that 63% experienced developmental delay, which is similar to our cohort.^[13] Study from eastern India reported a lower rate (46.9%) of development delay among genetically confirmed cases.^[10]

The mean age of wheelchair-bound in our patients occurred at about 11.2 year and most of them had loss of ambulation at around 9 year of age. Study by Parker *et al.*^[18] found loss of ambulation at approximately 10 years and wheel chair dependency at 11 years of age.

Cognitive impairment or ID was observed in 13.7% of cases. Presence of ID may cause delay in diagnosis and might present difficulty in managing these children. The mean IQ score in our cohort was 82 which are comparable with study by Magri *et al.*,^[19] reported mean IQ score of 83.2 in their study.

Cardiac involvement in DMD is most commonly manifesting as a cardiomyopathy and/or cardiac arrhythmias. It is caused by the replacement of myocardium by connective tissues.^[20] Cardiac evaluation by Transthoracic Echocardiography was done to all DMD patients on their diagnosis. Cardiac visits then repeated yearly when normal. For patients with established cardiac abnormalities, more frequent visits were advised.

In our study, two patients had cardiac involvement in the form of hypertrophy cardiomyopathy, detected on echocardiography. Previous studies reported deletion and duplication of exon 48 and 49 were more commonly associated with early cardiac involvement.^[21] Our both cases had deletion at a distal hot spot.

In the current study, 78.5% of cases resulted from deletions and 5.3% were detected with duplication. Small mutations were identified in 11.2% cases. Studies from various parts of India like east, north, south and west showed deletion rates of 63%, 73%, 73.1% and 72% respectively.^[12,22] Our detected rate of dystrophin gene deletion mutations is relatively higher than that reported in the studies including from neighboring areas like Pakistan (40.7%), China (66.25%) and Africa (61.1%).^[23-25] This study specifically looks for the spectrum of mutation along with the distribution spectrum in the proximal and distal hot spot regions of dystrophin gene among Rajasthani population.

Distal exon involvement was present in 70.3% cases, which is comparable to previous studies from eastern and southern India showed 79 and 78% respectively.^[10,11] Single exon was deleted in 16.5% of DMD cases and most frequent single exon deleted were 45 and 47. Among multiexon deletions, 45-52 were most commonly involved. Similarly, Vengalil *et al.*^[15] found that the most prevalent exons deletions involved exons 45 – 52 in the Indian population.

The proportions of deletion in different regions of India have been published and show a wide variation in the distribution. The varying rate of deletion in different regions of the country may be due to selection bias of groups, and ethnic variations in this population. The location and size of deletion, duplication

or small mutations found in the dystrophin gene does not clearly make a correlation with severity or progression of DMD. Biochemical indices such as CPK, SGOT, SGPT and LDH have the role to suspect and screen but not capable of diagnosing the disease.

In our study, 94.8% cases showed genetic change in the DMD gene. Muscle biopsy was the diagnosis of choice for such cases. Detection of DMD by DNA based method eliminates the need to do invasive procedure for diagnosis. Hence the genetic testing should be the investigation of choice in suspected cases of DMD.

Genotype-Phenotype correlation

The severity of the phenotype depends on the reading frame rule, postulating that mutations destroying the reading frame causes premature termination of protein synthesis resulting in the absence of dystrophin in the skeletal muscle and the severe DMD phenotype. Whereas mutations preserving the reading frame permit the expression of semi functional dystrophin and BMD phenotype. The reading frame rule has been reported for 96% and 93% of the mutations in DMD and BMD patients' respectively.^[26]

Adjacent exons that can maintain an Open Reading Frame (ORF) in the spliced mRNA despite a deletion event would give rise to the less severe BMD phenotype. Adjacent exons that cannot maintain an ORF because of frame shift would give rise to the more severe DMD phenotype due to the production of a truncated, nonfunctional dystrophin protein.

In our study, there were 79% of DMD and 70% of BMD cases that were consistent with the reading frame rule. A systemic review of dystrophinopathy data examined 135 DBMD patients with in-frame deletion of exon 51. Among them the majority ($n = 81$) had BMD whereas 16 patients had a more severe phenotype and 6 had no definitive phenotype.^[27] In our study, all patients with exon 51 deletion had milder DMD phenotype. Tuffery-Giraud *et al.*^[26] reported a case with deletion of a single exon 48 had no neuromuscular sign till 9 years of age. However 2 cases in our study with deletion of exon 48 presented at 4 and 5 years of age respectively and had the DMD phenotype. In our cohort variability of clinical features were also found in cases with identical deletion/duplication, even within the same pedigree. Mechanisms such as recording after ribosomal frameshift, unusual alternative splicing and usage of the potential promoter within the intron. However, it is difficult to predict clinical phenotypes at the genomic level using the reading frame rule. Therefore, further studies are required to determine precise genotype/phenotype correlation, and reading frame theory should be further refined.

Treatment options include physical therapy uses different exercises and stretches to keep muscles strong and flexible, respiratory therapy can help if the child is having trouble breathing. Although therapeutic strategies such as gene therapy and regenerative medicine have been extensively developed for DMD, definite treatment for patients with DMD are lacking at

our center. Oral Steroid (Prednisolone @0.75 mg/kg/day or Deflazacort 0.9 mg/kg/day) was started for ambulatory children.

Motor function tests such as a 6 minute walking test, 10 meter running time, 4 steps stair climbing test and Gower's time (time to rise from the floor) as well as motor function evaluation by the North Star Ambulatory Assessment (NSAA) was performed by a physiotherapist for ambulatory DMD patients. Forced vital capacity was evaluated yearly to ambulatory and 6 monthly in early non ambulatory DMD children. It has been associated with improvements in muscle strength and prolongation of independence and is the current standard of care.^[28]

Treated children showed a significant improvement in motor and pulmonary functions of all outcomes in comparisons of the period from the start of treatment. Side effects such as weight gain, facial fullness, blood pressure, bone health, cataracts, gastrointestinal symptoms, behavior were noted at each visit.

Exon-Skipping therapies for DMD

It is observed that not all of the 79 dystrophin exons are essential for the functional protein, came the principal of Exon-skipping therapy.^[29] DMD patients, who have in-frame deletions typically feature a milder BMD phenotype.^[30]

Antisense Oligonucleotides (AONs) are engineered to resist nuclease degradation and make Synthetic (AONs). These AONs are used to target mRNA of the dystrophin gene for removal, so can restore the reading frame and promote the production of partially functional dystrophin protein.^[31] Thus exon skipping has been shown to delay DMD progression.

Currently, many exon-skipping therapies are in clinical testing.^[32] AON; Exondys 51 (Eteplirsen) and Golodirsen (Vyondys 53) was developed by Sarepta therapeutics and approved in the US in 2016 and 2019, respectively. Eteplirsen binds to exon 51 of dystrophin gene to enable exclusion of the exon during expression of the dystrophin gene (exon skipping).^[33] Golodirsen designed to skip exon 53.^[34]

A newly approved AON, viltolarsen received approval in Japan for the skipping of exon 53.^[35] It was conditionally approved by the U.S. Food and Drug Administration (FDA) in August 2020.^[36] Phase II trial strengthened the evidence that viltolarsen can stabilize or improve muscle strength and functionality based on timed tests as compared with other drugs.

PTC drug trial for DMD male patients with nonsense point mutation in the dystrophin gene was going on in the department of Neurology, AIIMS New Delhi. Two patients from our cohort were enrolled. We are waiting for the results from AIIMS.

Early diagnosis of patients can be useful for genetic counseling and to give recurrence risk in future pregnancy. Further, the birth of a baby with DMD can be prevented by prenatal genetic testing.

CONCLUSION

In our study, 94.8% cases showed genetic change in the DMD gene. Muscle biopsy was the choice of investigation in earlier

days. DNA based method eliminates the need of painful and invasive procedure for diagnosis. Hence, the genetic studies should be the investigation of choice in suspected cases of DMD and muscle biopsy should be reserved for cases where genetic study couldn't confirm the disease.

In our study, maximum deletion was reported in mid-distal hot spot regions of dystrophin gene and exons 47, 48 and 50 were mostly deleted. We conclude that pattern of deletion, obtained in the population of Rajasthan was similar when compared with other ethnic groups of the Indian population. It would be helpful for researchers to develop drugs specific to exons or for ongoing mutation-specific therapies.

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Conflicts of interest

There are no conflicts of interest.

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