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ORIGINAL ARTICLE

Clinical and genetic profile of patients of Duchenne muscular dystrophy: experience from a Tertiary Care Center in Delhi

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ABSTRACT

Background: Duchenne muscular dystrophy (DMD) the most common muscular dystrophy affecting mainly males, is caused by a mutation of DMD (dystrophin) gene. The present study aimed at reporting the phenotypic and mutation spectrum of clinically suspected DMD children by molecular testing from the tertiary hospital, Delhi.

Methods: In this retrospective study, molecular testing was done in 73 clinically suspected DMD patients to identify DMD deletions, duplication, or point mutation.

Results: Among 73 clinically suspected DMD patients confirmed by genetic testing, *DMD* gene deletion was present in 79.45 %, duplication in 1.3%, and point mutation in 19.17 % of cases. In 89.6 % of patients, deletion was located at the distal hot spot region. Single exon deletion was found in 18.9 %. Distal hotspot exons 44, 45, and 52 were the commonly deleted exons.

Conclusion: Positive results by genetic testing prove to be an investigation of choice for definitive diagnosis, to offer genetic counseling and prenatal diagnosis and it also helps in further discovery of mutation-specific therapeutic interventions.

Keywords: Duchenne muscular dystrophy, dystrophin, deletion.

Introduction

Duchenne muscular dystrophy (DMD, OMIM: 310200) with a reported incidence of 1 in 3500 male children worldwide, is a common fatal muscular dystrophy affecting mainly males and is inherited as X-linked recessive manner. It occurs due to mutations in the *DMD* gene located on Xp21 (1) which encodes the dystrophin protein essential for anchoring the cytoskeleton to the plasma membrane (2). These mutations result in the absence or dysfunction of dystrophin leading to membrane instability, muscle fiber damage, and eventual cell death leading to progressive muscle degeneration and weakness. Clinically DMD manifests in a wide range of symptoms, including motor developmental disorder, difficulty in walking, muscle weakness in early childhood, speech delay, learning disability, and cognitive impairment, necessitating early and effective management and finally leading to ambulatory loss by adolescence and life-threatening problems like cardiomyopathy and respiratory failure in later stages (1,3,4).

Previous studies have shown that the spectrum of *DMD* gene mutations includes large deletions (68%) and duplications (11%) affecting two hot spots (exon 2-20 and exon 45-55) resulting in prematurely truncated proteins, nonsense mutations (11%), small (32) deletions (5%), small insertions (2%), and splice site mutations (3%) (5,6). Advances in genetic testing have helped in getting more definitive diagnosis, carrier detection, and prenatal screening. Additionally, genotype-phenotype correlations provide important insights into the disease's clinical variability and progression.

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Currently, multiplex ligation-dependent probe amplification (MLPA), next generation sequencing (NGS), or a combination of multiplex polymerase chain reaction (PCR) and Southern blotting are used to detect deletions, which make up 65% of all mutations. If PCR results are negative, NGS analysis can be performed to identify point mutations, small deletions, duplications, or insertions (7). Combining PCR with exome sequencing offers an accurate diagnosis, aiding early and effective therapeutic intervention, particularly in developing countries. Recent potential therapeutic interventions including gene therapy, exon skipping, and stem cell therapy are based on altering the disease's natural history. Though many population-based studies have described the phenotype and genotype of DMD patients, despite these studies, DMD remains a significant therapeutic challenge, underscoring the need for further research into the molecular mechanisms driving disease pathology and potential interventions. Here, we are providing clinical and genetic insights into DMD identified by molecular testing in 73 clinically suspected DMD children from the tertiary hospital, New Delhi, India.

Subjects and Methods

The study team reviewed data from 73 clinically suspected DMD patients who visited the genetic clinic at the Department of Pediatrics, Lok Nayak Hospital, Maulana Azad Medical College, New Delhi, India, between 2018 and 2021. Ethical clearance was obtained from the institute of Maulana Azad Medical College and Associated Hospital with Approval No F.No.17/IEC/MAMC/2018/17 on date 26.10.2018.

Boys with frequent falls, proximal muscle weakness, positive Gower's sign, and/or calf hypertrophy/pseudo hypertrophy were included. Patients with unrelated comorbidities like trauma or infection were excluded from the current study. Clinical data on age at onset, manifestation pattern, family history, muscle involvement, and ambulation for eligible patients were recorded. Blood samples were collected after genetic counseling and parental consent, and MLPA was conducted at an outsourced laboratory. Patients without identified deletions or duplications underwent NGS for the *DMD* gene at the same laboratory. Genotype-phenotype correlation was performed, and descriptive statistics were used. Oral steroid treatment was initiated for all ambulatory children (Prednisolone @ 0.75 mg/kg/day or Deflazacort 0.9 mg/kg/day), and their side effects, motor function, and cardiopulmonary function were assessed.

Results

Among 73 clinically suspected DMD patients, the mean age of disease onset was 4.5 years, with a mean age at presentation of 7.5 years. Symptoms began before age 5 in 80% of patients. Common symptoms included frequent falls, lower limb weakness, calf hypertrophy, and Gower's signs are depicted in Table 1. Overall, genetic testing showed a diverse mutation pattern associated with DMD, with implications for disease phenotype and management. Gene deletion was present in 58/73 cases (79.45 %), Gene duplication in 1/73 cases (1.3%), and point mutation in 14/73 cases (19.17 %). The majority 52/73 (71.2 %) of the patients had deletion located at the distal hot spot region, 4/73 (5.47 %) had proximal deletions, and 2/73 (2.7%) had proximo-distal deletions. 1/73 (1.3%) had proximal duplication and 14 (19.17%) had point mutations. Single exon was deleted in 11/58 (18.9 %) and exon 52 was the most commonly involved exon followed by exon 44 and exon 45. The most common exon deletion pattern was in the deletion of 45-50 exons. The largest deletion was extending from exon 12 to 49 and from exon 17 to 43. The children with respiratory distress, abnormal body movements, and contractures had the majority of the deletions toward the distal part of the gene (Figure 1).

In association with the reading frame rule of the 58 deletions in the entire cohort, 8 (13.4 %) were in-frame deletions, and 50 (86.9%) were out-of-frame deletions. The larger out frame deletions involved exons 46-53, 45-56, 44-54, and 12-49. The Proximal duplication was out of frame.

Small mutation spectrum: 14/73 small mutations were identified. Nonsense mutation was identified in 13/73 (17.8 %) and missense mutation was detected in 1/73 (1.3 %).

Table 1. *Clinical details.*

Clinical features of the patients	*n* = 73
Mean age of onset (years)	4.5
Mean age at presentation (years)	7.5
Consanguinity	1
Delayed motor milestones	5 (6.8%)
Progressive lower limb muscle weakness	65 (89.0%)
Gower's sign	62 (84.9%)
Calf hypertrophy/pseudohypertrophy	65 (89.3%)
Abnormal gait	16 (21.9%)
Contracture	10 (13.6%)
Respiratory distress	31 (42.4%)
Abnormal body movements	22 (30.1%)

Discussion

DMD arises from mutations in the *DMD*, one of the largest human genes, located on the X chromosome at Xp21.2, responsible for encoding Dystrophin muscular protein, dysfunction of which can lead to progressive and fatal muscular disease. It is often misdiagnosed or undiagnosed in developing countries due to limited access and awareness or the high cost of diagnostic facilities (MLPA and NGS). While these tests are routine in developed countries, and replaced the need for muscle biopsies in diagnosing suspected cases of DMD, their cost limits their use in many areas.

The present study, conducted at a tertiary referral center in New Delhi, highlights the clinical and genetic spectrum of 73 DMD patients. The mean age of symptom onset was 4.5 years, with presentation occurring at 7.5 years. This aligns with findings from studies in eastern India and Bangladesh, which showed a mean age of onset at 3.93 and 4.62 and a mean age at a presentation at 7.74 and 7.1 years of age, respectively (8,9). Delays in presentation in our study could be due to a lack of awareness and misdiagnosis of nutritional deficiency in the peripheral regions of New Delhi.

In the present study, progressive proximal muscle weakness, primarily in the lower limbs (89.0%), was the predominant phenotype, consistent with previous literature. Distribution of weakness, calf hypertrophy, and Gower's sign were also common complaints, aligning with other studies (10). Delayed milestones were found in only 6.8% of cases, lower than reported elsewhere, possibly due to recall bias (10,14). Regarding cognitive function, IQ assessment revealed two patients with intellectual disability, posing challenges for diagnosis and management. Previous literature also shows the mean IQ score of 82 and 83.2 among DMD patients (10).

Cardio - respiratory involvement in DMD is the most common cause of lethality (11). Cardiac evaluation in two patients via 2D Echocardiography showed that one patient had dilated cardiomyopathy, and one

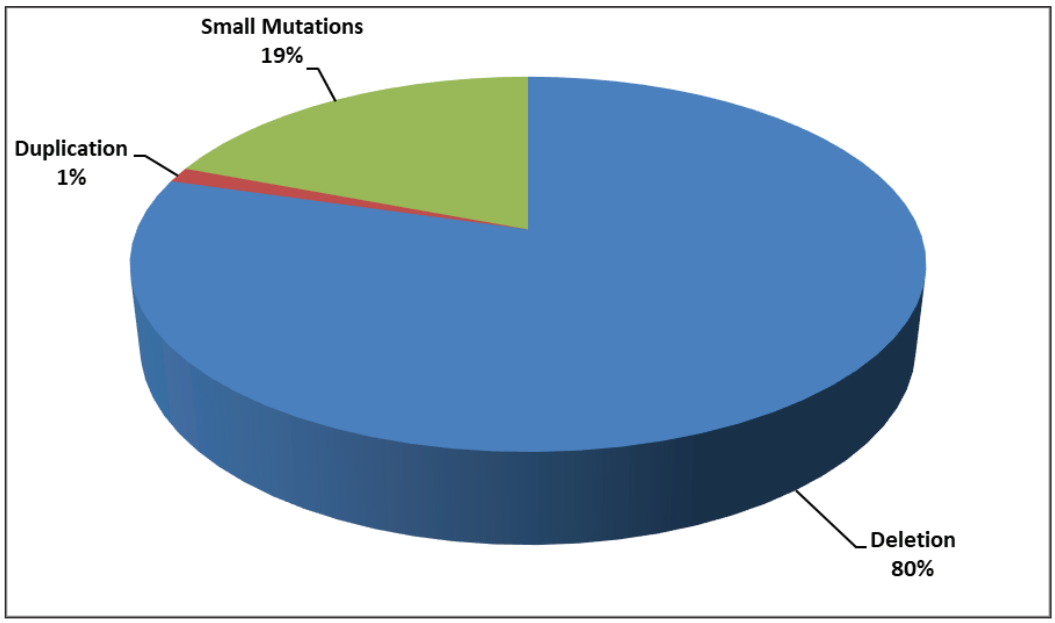

Figure 1. *Mutation patterns in dystrophin gene (DMD) patients.*

Table 2. *Deletion pattern of the DMD patients.*

Type of deletion	*N* = 58 (%)
Proximal hot spot deletion	4/58 (6.89 %)
Distal hot spot deletion	52/58 (89.6 %)
Proximo- distal deletions	2/58 (3.4%)
Single exon involvement	11/58 (18.9 %)
2-5 exons	24/58 (41.3%)
5-10 exons	19/58 (32.7 %)
>10 exons	5/58 (8.6%)
Small mutation spectrum	*n* = 14 (%)
Nonsense mutation	13/14 (92.8 %)
Missense mutation	1/14 (7.14%)

had hypertrophic cardiomyopathy. These findings are in concordance with previous studies showing an association between specific gene mutations and cardiac complications. Goyal et al. (10) reported two patients showing hypertrophy cardiomyopathy and Dey et al. (8) reported 17.1% of the patients having arrhythmias and 37.5% having dilated cardiomyopathy (10). Goyal et al. (10) reported deletion and duplication of exons 48 and 49 more commonly associated with early cardiac involvement. Furthermore, the current study showed both cases with cardiac involvement had a deletion at a distal hot spot. Previous literature indicates that respiratory failure typically occurs after the loss of ambulation in DMD (12). However, in our study, 20 patients experienced respiratory distress before losing ambulation. Therefore, pulmonary function tests were conducted for patients over 8 years old, and cardio-pulmonary surveillance frequency depended on the test results. Our study also examined the mutation spectrum of the dystrophin gene in the Delhi population. We found gene deletions in 79.45%, duplications in 1.3%, and point mutations in 19.17% of cases. Comparisons with literature from north, south, east, and west India showed deletion rates of 73%, 82%, 63%, and 72%, respectively, (8,13-15) and from Pakistan, China, and Africa revealed the lower percentage of deletions 40.7%, 66.25%, and 61.1%, respectively, than our study (16,17,23). The varying deletion rates, are likely influenced by selection criteria, population demographics, and ethnic differences.

In our study, distal exon deletion was present in 91.3% of cases, higher than rates reported in studies from eastern India, Bangladesh, and southern India showing 79%,81.8%, and 78% rates, respectively (8,9,18). Single exon deletions were found in 18.9% of cases, with exons 52, 44, and 45 most frequently affected. Among multi-exon deletions, exons 45-50 were commonly involved, although a study in Rajasthan found exons 45-52 as the most common (10). We observed duplications in 1.3% and point mutations in 19.17% of cases, including 13 nonsense mutations and 1 missense mutation. These rates differ from those reported in other Indian studies showing duplication in 5.2% and point mutation in 11.1% and Bangladesh study showing 3.44% had duplication, 10.3% had a stop-gain mutation, 6.89% had a frameshift deletion, and only 3.44% had a splice-site mutation indicating variability in mutation patterns across populations (9,10). Region-based mutation spectrum suggests a potential in-house customized panel for DMD. It also helps in implementing personalized treatment such as in our study high number of nonsense mutations provides clinicians the option of Ataluren an oral read through to the stop signal to make a full-length protein for managing DMD due to non-sense mutations. Our study highlighted the wide spectrum of clinical severity among patients with deletions/duplications, consistent with previous research (19). We did not find a clear correlation between disease severity and the location, number, or size of mutations like in other studies (14,20). Additional features such as delayed milestones, respiratory distress, and abnormal body movements shown by some patients in our study suggest a role for biochemical, epigenetic, and environmental factors and highlight the need for detailed neurodevelopmental evaluation by physicians and further studies with larger samples to better understand the correlation between disease severity and genotype. Previous studies have shown that in-frame mutations typically result in a less severe Becker muscular dystrophy (BMD) phenotype, while out-of-frame mutations lead to a more severe DMD phenotype. Our study confirms this pattern with all patients having out-of-frame deletions consistent with the reading frame rule and Tuffery Giraud et al. (21) report for 96% and 93% and Goyal et al. (10) report for 79% and 70% of the mutations in DMD and BMD patients', respectively, consisting with the reading frame rule.

However, findings from Waldrop et al. (22) for 135 DMD/BMD patients with inframe deletion of exon 51 showed inconsistent results regarding phenotype. This highlights the challenge of predicting clinical phenotypes solely based on the reading frame rule at the molecular level and underscores the need for further studies to refine this theory. In terms of strengths, the current study contributes to the diagnostic understanding of DMD through genetic testing, facilitating the development of new therapeutic strategies. The information regarding genetic spectrum not only aids clinicians in making early diagnoses and implementing personalized treatment approaches for deletion and duplications such as Exon skipping therapies like Exondys 51 (Eteplirsen) and Golodirsen (Vyondys 53), but it also allows them to have the option of Ataluren an oral read through to the stop signal to make a full-length protein for nonsense mutations. The small sample size in our study remains a limitation that prevented a clear correlation between mutation spectrum and disease severity. Therefore, we recommend future studies with larger sample sizes to address this limitation and further refine our understanding of DMD.

Conclusion

Genetic tests should be the primary diagnostic tool for suspected cases of DMD, with muscle biopsy reserved for cases where DNA-based tests cannot confirm the diagnosis. This study adds valuable insights to the limited literature on DMD mutations and phenotypic spectrum, particularly in developing countries. It aids in counseling regarding prognosis, carrier assessment, and prenatal diagnosis, and provides essential data for research aimed at developing mutation-specific drugs, gene editing technologies, and the genetic factors that modulate disease fate in improving outcomes for patients with DMD.

List of Abbreviations

DMD	Duchenne muscular dystrophy
MLPA	multiplex ligation-dependent probe amplification
NGS	next generation sequencing
PCR	polymerase chain reaction

Conflict of interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Consent to participate
Informed consent was obtained from the patients.

Ethical approval
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