Mutation spectrum of Duchenne muscular dystrophy patients in Indian population

Priyanshu Mathur[1], Ansh Agarwal[2], Kashish Goyal[3], Avisha Mathur[4]

From [1]Assistant Professor, [2]Intern, [3]Senior Resident, Department of Pediatrics, [4]Junior Specialist, Department of Ophthalmology, Sawai Man Singh Medical College and Hospital, Jaipur, Rajasthan, India

Correspondence to: Dr. Ansh Agarwal, Sawai Man Singh Medical College and Attached Group of Hospitals, C-29, Mahesh Nagar, Near 80 feet road, Jaipur - 302 015, Rajasthan, India. E-mail: agarwal_ansh@ymail.com

Received - 12 February 2020 Initial Review - 03 March 2020 Accepted - 25 May 2020

ABSTRACT

Background: Duchenne muscular dystrophy (DMD) is the most common X-linked neuromuscular disorder in children. Since the novel, DMD therapies are mutation-specific, so detection of mutation is of paramount importance in planning the treatment of DMD patients. Objective: The objective of this study was to find different mutations present in DMD patients in Indian population. **Materials and Methods:** This study was a hospital-based retrospective observational study conducted from December 2018 to December 2019 in the pediatric department of a tertiary hospital of western India. A total of 52 children age 2–16 years, presenting with progressive muscle weakness, were included in the study. DMD multiplex ligation-dependent probe amplification (MLPA) for 79 exons was done for detection of deletion/duplication for all patients. Whole DMD gene sequencing was done for those who were found MLPA negative for DMD gene mutation (deletion/duplication). **Results:** Our study states that most of the DMD patients presented with deletions (84%) or duplications (11%) in the dystrophin gene, and remaining due to point mutation. The study shows that most of the mutations occur due to deletions (67.30%) in DMD gene at distal hotspot 45–52 exons and deletions (15.38%) in DMD gene at proximal hotspot 10–19 exons. In addition, to expanding the mutational spectrum of DMD, these results establish improved mutations data in the Indian population. **Conclusion:** The novel developed therapies for DMD are mutation-specific, so molecular diagnostic tests are very important in diagnosis and categorization for the prevalent mutations in the Indian population.

Key words: *Deletions, Duchenne muscular dystrophy, Duplications, Dystrophin protein, Neuromuscular disorder*

Duchenne muscular dystrophy (DMD) is a fatal X-lined neuromuscular disorder affecting around one in 3500 male births that are characterized by progressive muscular deterioration. It is caused by loss of functional mutations in the DMD gene (located in the region Xp21 and contains79 exons and approximately 2.2 Mb of genomic DNA) coding for dystrophin protein, a cytoskeletal protein that stabilizes the plasma membrane of muscle fibers of skeletal and heart muscles. The total absence of dystrophin protein observed in DMD patients is generally caused by mutations that disrupt the reading frame of DMD gene, and about 95% of cases are due to gene deletions and duplications. There are hotspot regions present on the proximal and mid–distal sides of the gene which contains 1–19 exons (about 20% deletions) and 45–52 exons (about 80% deletions), respectively [1,2].

Deletions lead to a shift in the reading frame (out of frame) and decreased production of functional dystrophin protein. Altered dystrophin protein leads to increased muscle degradation and protein destruction and leads to an increase in serum creatine phosphokinase (CPK) level. DMD is characterized by muscle weakness and usually begins around the age of 2 years in boys and worsens quickly [3]. Typically, muscle loss occurs first in the thighs and pelvis followed by arms. It can result in trouble in standing up. Most are unable to walk by the age of 12. The affected muscles may look larger due to increased fat content. Genetic testing can often make the diagnosis at birth. Since the novel DMD therapies are mutation-specific, so deletion of mutation is of paramount importance in planning the treatment of DMD patients. The objective of this study is to find the different mutations present in DMD patients in the Indian population.

MATERIALS AND METHODS

This study is a hospital-based retrospective observational study, conducted during December 2018–December 2019 in the Rare Disease Clinic of the Pediatric department of a tertiary hospital of western India. A total of 52 children age 2–16 years, presenting with progressive muscle weakness (delayed ability to sit, stand, or walk), were included in the study. The exclusion criteria included children diagnosed with muscular weakness due to any central nervous system disease, children with autoimmune diseases, and children who did not have parental consent for the study.

The detailed history included progressive muscle weakness showing difficulty in walking, climbing stairs, standing from sitting

position, running and vigorous physical activities, wheelchair confinement, developmental, and family history. The physical examination included checking for Gower's sign (patient taking support while standing from sitting position), muscle wasting, calf hypertrophy, waddling gait, neurological examination (higher functions, cranial nerve involvement, power/strength in extremities, reflexes, sensations, presence of any involuntary movements, or fasciculation), musculoskeletal examination (spinal deformities and muscle contractures) and 6 min walk test, for all patients with serum CPK level, electromyography, nerve conduction study, electrocardiogram, and echocardiography for heart muscle involvement. DMD multiplex ligation-dependent probe amplification (MLPA) for 79 exons was done for detection of deletion/duplication for all patients. Whole DMD gene sequencing was done for those who were found MLPA negative for DMD gene mutation (deletion/duplication).

The data collecting sheet was checked for completeness and stored securely. Data from the collecting sheet were entered into a computer using Microsoft Excel 2012. It was then exported into statistical program SPSS version 17. $p<0.05$ was considered statistically significant.

RESULTS

A total of 52 male patients aged 2–16 years were included in the study, and final diagnosis of DMD was made after a clinical evaluation (Gower's sign positive), significantly elevated serum CPK levels and DMD MLPA showing the presence of specific mutation with location and number of exons involved (Table 1). Out of 52 cases, 22 cases had a positive family history. Most of the DMD patients presented with deletions (84%) or duplications (11%) in the dystrophin gene and remaining due to point mutation (Fig. 1). The study shows that most of the mutations occur due to deletions (67.30%) in DMD gene at distal hotspot 45–52 exons and deletions (15.38%) in DMD gene at proximal hotspot 10–19 exons (Fig. 2).

DISCUSSION

DMD is characterized by muscle weakness and muscle wasting of the voluntary muscles being first affected, especially those of the hips, pelvic area, thighs, shoulders, and calves. Symptoms of DMD can be abnormal walking due to calf tenderness, frequent falls, and fatigue. Lumbar hyperlordosis can be present as the disease progresses. It can eventually lead to loss of ability to walk and trouble getting up from lying or sitting position by the age of 12 years and wheelchair confinement [4]. Cardiomyopathy, particularly dilated cardiomyopathy, is common, but the development of congestive heart failure or arrhythmia is only occasional. Respiratory disorders include pneumonia and swallowing difficulty of food or fluid passing into the lungs in the later stages of the disease.

DMD can be diagnosed clinically by the above presentation and a positive Gower's sign reflecting the more severe impairment of the lower extremities muscles. Family history is important.

Table 1: Mutational analysis in DMD patients

Type of mutation	Exon location	Number of exons	Number of patients
Hemizygous deletion	10–12	3	2
	12	1	3
	17–45	29	1
	19	1	2
	30–43	14	1
	45	1	5
	45–50	6	7
	45–52	8	3
	46–50	5	3
	46–52	7	4
	46	1	3
	48–50	3	1
	49–50	2	2
	50	1	4
	50–52	2	2
	52	1	1
Hemizygous duplication	2–13	12	2
	3–11	9	3
	20–37 and 39–41	24	1
Point mutation	39	1	2
Total			52

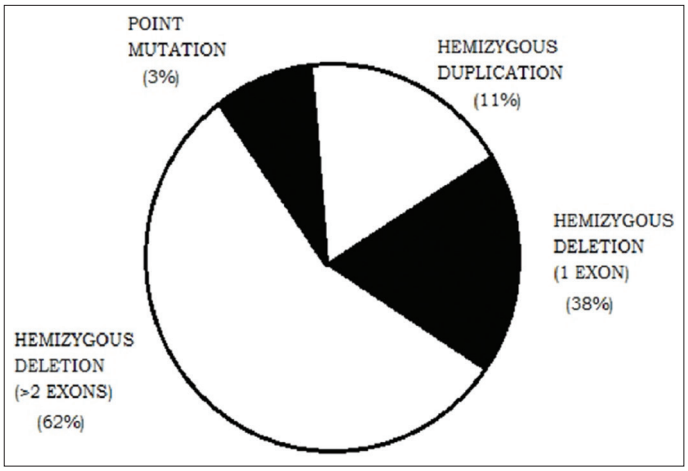

Figure 1: Frequency distribution of different mutations in Duchenne muscular dystrophy patients

Elevated CPK may help in making a diagnosis; however, the gold standard for DMD diagnosis is molecular genetic testing. In the present study, the mutational analysis showed that most of the mutations occurred due to deletions (67.30%) in the DMD gene at distal hotspot 45–52 exons, and deletions (15.38%) in DMD gene at proximal hotspot 10–19 exons.

In a study done by Agarwal *et al*. [5], the authors showed that the majority of the deletions 66.66% were located in the distal hotspot region 45–55 exons and 15.38% of deletions were located at the proximal hotspot region 2–19 exons. Kumari *et al*. [6] showed that deletions were observed in both proximal and distal hotspot regions with the maximum deletion localized in the distal hotspot region of the gene. The study by Dey *et al*. [7] showed

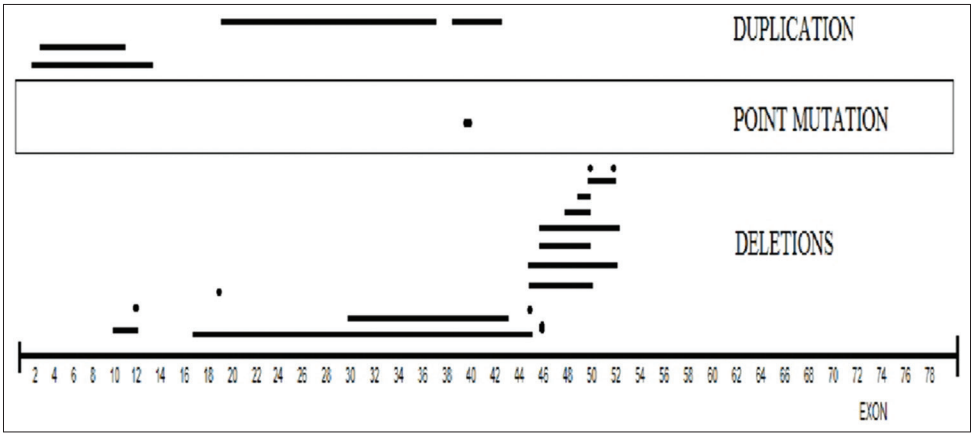

Figure 2: Different types of mutation in Duchenne muscular dystrophy patients

that there were 72.6% deletion in distal exons, 16.4% deletion in both proximal and distal exons, while 10.9% had only proximal deletion.

The goal of DMD treatment is to control the symptoms and related complications caused by severe progressive muscle weakness. The dilated cardiomyopathy can be treated with medications, but in severe cases, a heart transplant may be necessary. Assistive devices for breathing difficulties may be needed, especially at night and as the disease progresses. Physical therapy may be helpful to maintain muscle strength and function. Orthopedic devices (such as braces and wheelchairs) may improve the ability to move. Steroids such as prednisone and deflazacort may improve the strength and function of muscles in people with DMD, including lung function, as mentioned by Falzarano *et al*. [8].

The latest researches are going on to find the medications that either return the ability to make dystrophin or urotrophin. The researches include exon-skipping, stem cell replacement therapy, analog up-regulation, or gene replacement. Exon-skipping allows the faulty parts of the dystrophin gene to be skipped at the time of transcription of RNA, permitting a still-truncated but more functional version of the protein to be produced [9]. It is also known as non-sense suppression therapy [10]. Drugs drisapersen, Exondys 51 (eteplirsen), and Vyondys 53 (golodirsen) have been approved [11]. Exondys 51 (eteplirsen) is targeted to skip exon 51. Vyondys 53 (golodirsen) is targeted to skip exon 53.

Another drug ataluren (Translarna), which was also known as PTC124, is used in patients with DMD who have a non-sense mutation in the dystrophin gene, can walk, and is more than 5 years old [12]. Ataluren makes ribosomes less sensitive to premature stop codons by promoting the insertion of near-cognate tRNA at the site of non-sense codons with no apparent effects on downstream transcription, mRNA processing, stability of the mRNA, or the resultant protein, thereby making a functional protein [13]. It seems to work particularly well for the stop codon "UGA" [14,15].

Another drug morpholino, which is phosphorodiamidate morpholino oligomer, is used in patients with DMD and is been approved. Morpholino is the safest anti-sense oligonucleotide for therapy in DMD patients using synthetic DNA like molecules for exon-skipping therapy. Morpholino treatment involves splicing out the frame-disrupting segment of the dystrophin mRNA, which restores the reading frame and produces a truncated yet functional dystrophin protein.

Another therapy is gene therapy for DMD, which is currently in an experimental phase. Gene therapy includes the gene-editing method to correct a mutation that leads to DMD. The technique is called CRISPR/Cas9-mediated genome editing, which can precisely remove a mutation in the dystrophin gene in DNA, allowing the body's DNA repair mechanisms to replace it with a normal copy of the gene [16,17]. The benefit is that it can permanently correct the defect in a gene rather than just transiently adding a functional gene. Biostrophin is a delivery vector for gene therapy in the treatment of DMD [18]. However, this study tells the mutation spectrum of DMD gene in the Indian population which allows the better and more specific treatment of DMD gene mutations. The patients who were diagnosed with DMD were started with physiotherapy and deflazacort 0.9 mg/kg body weight of the child and were called for monthly follow-up.

The study had few limitations. There was the availability of small sample size and limited follow-up data received from patients after starting the treatment.

CONCLUSION

Our study results establish the improved mutations data for the DMD gene in the Indian population. The most common mutation in DMD gene was deletion (84%) involving distal hotspot region 45–52 exons (67.30%) and proximal hotspot region 10–19 exons (15.38%). The novel developed therapies for DMD are mutation-specific, so molecular diagnostic tests are very important in diagnosis and categorization, for the prevalent mutations in the Indian population.

REFERENCES

1. Koenig M, Hoffman EP, Bertelosn CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) DNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 1987;13:509-17.
2. Den Dunnen JT, Grootscholten PM, Bakker E, Blonden LA, Ginjaar HB, Wapenaar MC, *et al*. Topography of the Duchenne muscular dystrophy

(DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. Am J Hum Genet 1989;13:835-47.
3. NINDS. NINDS Muscular Dystrophy Information Page. Bethesda: NINDS; 2016.
4. NINDS. Muscular Dystrophy: Hope through Research. Bethesda: NINDS; 2016.
5. Agarwal R, Chaturvedi S, Chhillar N, Pant I, Sharma A. Duchenne muscular dystrophy: A immunohistochemical profile and deletion pattern in dystrophin gene in North Indian population. Asian J Med Sci 2017;8:13-8.
6. Kumari P, Joshi D, Shamal SN, Singh R. Study of dystrophinopathy in Eastern Uttar Pradesh population of India. J Pediatr Neurosci 2018;13:182-8.
7. Dey S, Senapati AK, Pandit A, Biswas A, Guin DS, Joardar A, *et al*. Genetic and clinical profile of patients of Duchenne muscular dystrophy: Experience from a tertiary care Center in Eastern India. Indian Pediatr 2015;52:481-4.
8. Falzarano MS, Scotton C, Passarelli C, Ferlini A. Duchenne muscular dystrophy: From diagnosis to therapy. Molecules2015;20:18168-84.
9. Dunckley MG, Manoharan M, Villiet P, Eperon IC, Dickson G. Modification of splicing in the dystrophin gene in cultured Mdx muscle cells by antisense oligoribonucleotides. Hum Mol Genet 1998;7:1083-90.
10. Finkel RS. Read-through strategies for suppression of nonsense mutations in Duchenne/Becker muscular dystrophy: Aminoglycosides and ataluren (PTC124). J Child Neurol 2010;25:1158-64.
11. FDA. FDA Grants Accelerated Approval to First Drug for Duchenne Muscular Dystrophy. USA: FDA; 2016.
12. UK Electronic Medicines Compendium. Translarna-Summary of Product Characteristics. United Kingdom: UK Electronic Medicines Compendium; 2017.
13. Roy B, Friesen, WJ, Tomizawa Y, Leszyk JD, Zhuo J, Johnson B, *et al*. Ataluren stimulates ribosomal selection of near-cognate tRNAs to promote nonsense suppression. Proc Natl Acad Sci U S A 2016;113:12508-13.
14. Andrea P, Silvestre B, Palumbo PA, Ivana P. Recent advances in the chemistry of 1, 2, 4-oxadiazoles. In: Eric FV, Christopher R, editors. Advances in Heterocyclic Chemistry. United States: Academic Press; 2015. p. 127.
15. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, *et al*. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447:87-91.
16. Cohen J. Gene Editing of Dogs Offers Hope for Treating Human Muscular Dystrophy; 2018.
17. Narayanan PS, Nareshwaran G, Nian LM, Roslina H, Shaik FK, Zubaidah Z. CRISPR/cas9 in stem cell research: Current application and future perspective. Curr Stem Cell Res Ther 2018;13:632-44.
18. Khurdayan VK, Bozzo J, Prous JR. Chronicles in drug discovery. Drug News Perspect 2005;18:517-22.

Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Mathur P, Agarwal A, Goyal K, Mathur A. Mutation spectrum of Duchenne muscular dystrophy patients in Indian population. Indian J Child Health. 2020; 7(6):247-250.

Doi: 10.32677/IJCH.2020.v07.i06.002