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Mutational Analysis Among Patients with Duchenne Muscular Dystrophy

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MUTATIONAL ANALYSIS AMONG PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT

Background and objective:

Duchenne Muscular Dystrophy is caused by mutations in dystrophin gene that include deletion, duplication and small mutations. Introduction to newer drug therapies in DMD is based on the type of mutation. The objective of this study was to assess distribution and percentage of different mutations among DMD patients.

Methods:

This retrospective cross-sectional study was conducted in Pediatric Neurology department of UCHS & Children Hospital Lahore. All the cases of Duchenne Muscular Dystrophy confirmed through genetic analysis from January 2022 to June 2022 through MLPA method were enrolled in the study. Data was recorded on detailed history and physical examination of the subjects and all lab investigations along with genetic study was reviewed to collect the data on given Performa. All the collected data was saved for final analysis.

Results:

Total 60 patients were enrolled in the study. The most common mutation was deletion, detected in 32 (56%) cases, duplication was detected in 10 (17.5%) cases and genetic study of 15 (26.5) patients was turned out to be normal. The most common deletion was found to be at 45-52 (43%) exons.

Conclusion:

The most common mutation among genetically confirmed cases of DMD was found to be deletion at 45-52 exons. This mutational evaluation is the first step toward trial of new medicines while treating DMD, as mechanism of action of new drugs is based on the type of mutation.

Key words: Duchenne Muscular Dystrophy (DMD), Multiplex Ligation Dependent probe amplification technique (MLPA), Deletion, Duplication.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is an inherited X-link recessive neuromuscular disorder caused by the mutation in dystrophin gene resulting in absent or insufficient nonfunctional dystrophin protein which is a cytoskeleton protein responsible for stability of structure and function of myofibrils.^{1,2} Its global prevalence has been reported as 7.1 cases (95% CI: 5.0 -10.1) per 100,000 live male birth and 2.8 cases (95% CI: 16.6-23.6) per 10000 live male birth in general population.¹ The disease usually starts at 3-4years of age and progressive in nature resulting from ongoing muscular damage and degeneration leading to progressive muscular weakness, loss of ambulation, contractures, scoliosis, respiratory and

cardiac insufficiency.^{1,3} Supportive laboratory parameters to suspect DMD are elevated muscle enzymes due to leakage into the bloodstream mainly creatine kinase (CK 50-100times normal), but also transaminases such as aspartate transaminase and alanine transaminase.^{2,3} Confirmation of the diagnosis is done by qualitative and quantitative immunohistochemistry and western blot analysis of dystrophin protein in muscle biopsy and genetic analysis.^{1,4}

Genetic analysis reveals mutation in dystrophin encoding DMD gene.⁴ It is the largest known gene which has relatively high rate of mutation resulting in large variation of mutations that has been identified in

the patients with DMD.^{4,5} Almost one in three cases of DMD has de novo mutation and the majority of the Patients have a deletion (68%) or duplication (11%) of one or more exons , small mutations are found as well (20% of patients) including 13% of the patients have nonsense mutations and 3% splice site mutation.⁵ Genetic analysis is mostly done through Multiplex Ligation Dependent probe amplification technique (MLPA).^{4,6} This technique covers all 79 exons in dystrophin gene and detects deletions and duplications of one or more exons by determining chromosomal DNA copy number change for each exon in a single multiplex-PCR based reaction.^{5,6}

Previously no curative treatment was available for DMD, and the care was principally limited to symptomatic treatment through Glucocorticoid therapy.^{6,7} Now globally promising genetic therapies have been developed to restore dystrophin protein in myocytes that include gene therapy that uses virus as a vector for the delivery of missing gene, read through approach that inhibits translational termination at nonsense mutation to restore dystrophin gene expression and exon skipping with antisense oligonucleotides to splice out selected exon to generate partially functional dystrophin protein.⁶⁻⁸ So, for the introduction of new therapies it is important to know the distribution of different mutations in target population as these advanced therapies are based on the type of mutation.^{8,9} The clinical outcome of these mutations in reference to disease progression can also vary depending upon the number of nucleotide in exon that are deleted or duplicated.⁷⁻⁹ In Pakistan, only palliative therapy with steroid is available which sometimes halts the progression of the disease but still no curative therapy is available. Other newer therapies based on genetic modifications like exon skipping medicines are FDA approved and read through medicines are approved by European medical Agencies, but not available in Pakistan. The purpose of this study was to assess mutational spectrum among patients with DMD in Pakistan to take initiative toward selection and introduction of newer therapies. In future, on the basis of already collected data of mutational defects, we may proceed toward drug trial of advanced therapies^{9,10}. In this study there is description of different genetic mutation of dystrophin gene in relation to their frequencies and percentages that exist in the targeted pool of patients with DMD in Pakistan.

METHODS

Study design: Retrospective cross-sectional study.

Place and duration of study: The study was performed in the Department of Pediatric Neurology, UCHS & Children Hospital Lahore. Duration of study was six months from January 2022 to June 2022.

Sample size: The prevalence of DMD is about 3 per 100,000 of population in Pakistan. So the calculated sample size by using WHO calculator for sample size determination in health studies is 60 keeping 5% margin of error and 95% confidence level.

Sampling technique: Non-probability consecutive sampling.

Inclusion criteria: All new and old cases of Duchenne Muscular Dystrophy, diagnosed clinically on the basis of waddling gait, calf muscle hypertrophy and positive Gower sign with supportive evidence of raised CPK and genetic analysis through MLPA method were included in the study.

Exclusion criteria: . All those clinically suspected cases of muscular dystrophy and not having supportive evidence of raised creatinine kinase level were excluded from the study.

Operational definitions:

DMD : An inherited X-linked recessive neuromuscular disorder due to mutation of gene encoding dystrophin cytoskeleton protein resulting in progressive muscular degeneration.

MLPA: Multiplex-ligation dependent probe amplification (MLPA) is a method that employs a pool of numbers at an exon-level resolution.

Deletion mutation: A deletion mutation is a mistake in the DNA replication process which removes nucleotides from the genome.

Duplication mutation: Duplication is a type of mutation that involves the production of one or more copies of a gene or region of a chromosome.

Nonsense mutation: A nonsense mutation occurs when the sequence of nucleotides in DNA is changed in a way that stops the normal sequence of amino acids in the final protein.

Data collection: Departmental data of detailed history and physical examination of the subjects were recorded and all lab investigations and genetic study through MLPA technique were reviewed. Data were collected on given pro forma that included all required details about onset of illness, duration of treatment, family history of affected sibling and effected maternal uncle. All the data were recorded and saved for the final analysis.

Data analysis: The data was analyzed by using SPSS version 25. Patients were stratified by age, onset of symptoms, CPK levels, type of mutation that included deletions and duplications. Patients with strong clinical suspicion and very high CPK level but normal genetic analysis through MLPA method were considered to have small mutations. Frequency of deletion, duplications and small mutations were calculated. Data was analyzed for the identification of most common site for deletions and duplications.

Ethical considerations: The study was approved by the institution's Ethical Review Committee.

RESULTS

Total 60, all male patients were enrolled in the study. Mean age of onset of symptoms was 3.9 years (SD ± 2.2988). Mean current age at the time of study was 8.3 years ($\pm SD 3.14051$). Families with more than one effected siblings were seven (11%) and five (8%) patients had history of affected maternal uncles. The most common mutation was found to be deletion, detected in 32 (56%) cases. Duplication was detected in 10 (17.5%) cases. Genetic study of 15 (26.5%) patients through MLPA method turned out to be normal and they were considered to have small mutation (Figure 1). The most common deletion was found to be at 45-52 (43%) exons, and the most common duplication was found to be at 3-7 (30%) exons. This distribution of frequencies was statistically significant with P value of < 0.05 .

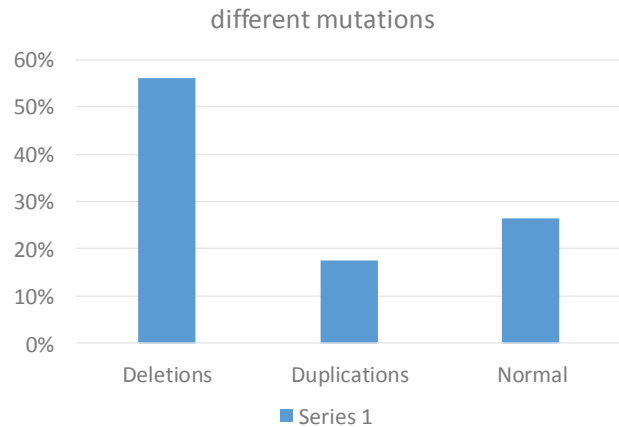


Figure 1: Distribution of different types of mutations in the study population

DISCUSSION

Standard treatment for DMD in Pakistan is administration of corticosteroid that may prolong normal motor functions but cannot stop progression and their long term use is linked to debilitating side effects.³⁻⁵ It is, therefore, required to introduce appropriate , alternative mutational based advanced therapy. Based on the genetic mutation, the available therapies are targeted to restore dystrophin gene expression and these therapies include vector mediated gene therapy, Read through approach for nonsense mutation and exon skipping with synthetic antisense oligonucleotides.¹⁰⁻¹² As these therapies are based on genetic defect, it's important to know the mutational distribution for introduction and selection of appropriate therapy. So, this study was done to assess distribution of different mutations among included cases. Total 60 male patients were included in the study on the basis of progressive difficulty in walking, proximal muscle weakness, calf muscle hypertrophy and raised CPK enzymes. Multiple ligation dependent probe amplification (MLPA) can quickly and accurately identify deletions and duplications among 79 exons in dystrophin gene, so we used this method to describe large rearrangements in dystrophin gene.^{13,14} But in addition to deletions and duplications 25 to 35% of DMD patients may have single nucleotide variant, small deletion, single base change and splice site change and out these small mutations, 20-30% may have nonsense mutations.^{15,16} In case of deletions and duplications exon skipping therapy would be treatment of choice and in case of nonsense mutation, read through approach is applicable.^{16,17}

According to this study the most common mutation was found to be deletion and among all, deletions at 45-51 (43 %) exon were found to be most common. Those patients who had normal genetic study through MLPA were considered to have minor mutations of dystrophin gene or may have muscular dystrophy other than DMD. So, for the confirmation of genetic defect in these patients we need to perform genetic panel for muscular dystrophy or whole exome sequencing. This is the limitation of this study and the other limitation is demographic distribution of all patients as all the patients belonged to different areas of Punjab that may not reflect the genetic distribution of DMD patients in all of Pakistan. It means in order to assess actual picture of genetic defects we need to perform more studies like this in all provinces of Pakistan. This is very important step toward introduction of curative therapies. One study with same objective was done and published in 2019 in Karachi and the results of that study were also compatible with this study showing 45% deletion and 7% duplications.¹⁸ One more study done in Pakistan showed 5% detection of non sense mutation through whole exome sequencing among 18 clinically suspected cases of dystrophinopathy.¹⁹ MPLA identification of dystrophin gene mutation in India also showed 91.6% deleting and 8.3% duplications and 45-52 region was found to be the hot spot for deletions.²⁰ One Russian study showed spectrum of dystrophin gene mutation that revealed 49% gross deletion, 14.5% duplications and 36.5% minor

mutations and among which 19.3% turned out to be nonsense mutations.²¹ Identification of this nonsense mutation is important for the therapeutic usage of read through medicines. Mutational analysis among 68 families of Kuwait revealed 66% deletion, 4.4% duplications and 5.8% nonsense mutations.²² The deletion rates among Arab, Chinese and North Indian population were 63.4%, 59.4%, 70.2% respectively.²³

These results are in agreement of present study that also showed higher rates of deletion in target population. So, this is just the distribution of different mutations among clinically suspected cases of DMD only using MLPA method. It's just the beginning for actual mutational analysis among these patients. First , we need to apply more extensive genetic tests like whole exome sequencing or SSG for detailed description of genetic defects and second these type of studies should be done all across Pakistan to describe actual mutational pool with demographic distribution.

CONCLUSION

The most common mutation among genetically confirmed cases of DMD was found to be deletion at 45-52 exons. DMD is caused by mutations in dystrophin gene and confirmation of genetic defect is important to predict disease progression, genetic counselling and evaluation of patient's eligibility for emerging genetic therapies.

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Authors' contribution:

Shumaila Rafique; Concept, data analysis, manuscript writing, manuscript revision

Muhammad Asif Ibrahim; Concept, data collection, data analysis, manuscript writing,

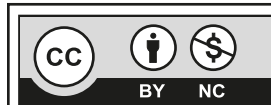
Natasha Ghani; Data collection, manuscript writing, manuscript revision

Muhammad Athar Khalily; Data collection, data analysis, manuscript writing

Syed Zubair Shah; Data collection, data analysis, manuscript writing

Tipu Sultan; Concept and design, manuscript revision

All the authors have approved the final version of the article, and agree to be accountable for all aspects of the work.



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