RESEARCH PAPER

Genetic and Clinical Profile of Patients of Duchenne Muscular Dystrophy: Experience from a Tertiary Care Center in Eastern India

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Objectives: To study the genetic pattern, clinical profile and to find any correlation between them in patients of Duchenne muscular dystrophy.

Methods: Patients were selected from Neurogenetic clinic on the basis of clinical features, elevated serum CPK level and electromyographic features. After history and clinical examination, molecular genetic testing was performed by Polymerase Chain Reaction (PCR) technique.

Results: Among 100 patients, 73 patients had genetically confirmed disease while 8 cases were proven by biopsy, and thus a total 81 cases were further taken up for the study. Mean age of onset of clinical symptoms was 3.9 yrs; Valley sign and calf hypertrophy were most consistent features, while about 51% had facial weakness. Out of 73 genetically confirmed cases 53 (72.6%) showed deletion in distal exons and 12 (16.4%) showed deletion in both proximal and distal exons while 8 (10.9%) had only proximal deletion. There was no correlation between genetic pattern and clinical features.

Conclusion: The positivity of PCR- based diagnosis is higher in our study possibly related to highly selective group of patients. Phenotype and genotype correlation was not seen.

Keywords: *Diagnosis, Muscular dystrophy, Polymerase chain reaction*.

Duchenne muscular dystrophy (DMD) is the most common form of all muscular dystrophy with an incidence rate of 1:3500 live male births [1]. The gene responsible for DMD is one of the longest genes; deletions account for 60-65% cases in DMD, duplications for 5-6% and point mutations for the remaining cases [2]. Using primers targeting 18 hotspot exons in the dystrophin gene, 98% of deletions can be detected. The proximal hotspot encompasses exons 1-20 and the distal hotspot exons 43-52 [3]. There is data available from various regions, including India [4-9]. The main objective of this study was to look for clinical profile of DMD patients with their genetic pattern as well as to find any correlation between clinical profile and genetic pattern among patients of DMD from Eastern India.

METHODS

Participants were enrolled between April 2010 and February 2013 from Neurogenetics clinic of the Bangur Institute of Neurosciences after Institutional ethical committee clearance. After taking detailed history, each patient was examined by one of the investigators (Neurologist) and verified by a senior Neurologist with particular emphasis on atrophy and hypertrophy of muscles and power of individual muscle, the presence of Gower's sign and valley sign. Patients were also subjected for psychometry and assessment of IQ using Binet-Kamath Test of Intelligence [10]. CPK estimation and electromyography (EMG) study was performed in all patients. The chest radiograph, ECG and echocardiography were done in some of the patients.

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We selected male children with following features: proximal muscle weakness with positive Gower sign and valley sign, supported by an elevated serum CPK level and a positive EMG finding of myopathic pattern. The patients clinically suspected but having normal CPK level and with no historical evidence of X-linked inheritance; or having unrelated co-morbidity which may influence the course and progression of the disease were excluded from the study.

After selection, the patients were subjected to genetic analysis after pretest counseling of parents and obtaining necessary informed consent from them. Muscle biopsy was performed in patients where the genetic analysis was negative.

Muscle biopsy: This was carried out after taking written informed consent from parents. Muscle biopsies were

obtained by the open method from quadriceps or biceps muscles. The specimen was subjected to microscopic examination after hematoxylin and eosin staining and also for Immunostaining with monoclonal antibodies to dystrophin (1-3).

Molecular genetic testing and analysis: After written informed consent, genomic DNA was isolated from blood by phenol-chloroform method. Multiplex polymerase chain reactions (PCR) were carried out for 18 exons which include Chamberlain-set and Beggs-set [3]. PCR products were resolved on 1% Agarose gels, and the gels were analyzed for exonic deletions by the presence or absence of a corresponding band.

Statistical analysis: All statistical analysis was done by using SPSS software version 19. Comparisons of variables were done using independent t–test. Correlation of variables was done using Spearman and Pearson's test wherever applicable with *P* value <0.05 considered statitstically significant.

RESULTS

A total 100 of clinically suspected DMD patients were included in this study. Of them 73 patients (73%) were confirmed by genetic analysis. Nine (33.33%) out of 27 genetically undiagnosed cases underwent muscle biopsy. Eight of them proved to be cases of DMD by absence of dystrophin staining, while one patient proved to have congenital dystrophy. Rest of the cases (*n*=18) could not be confirmed as they refused to undergo muscle biopsy. We included only genetically or biopsy proven DMD cases (*n*=81) for further study of genetic pattern, clinical profile and their correlation. The demographic and clinical profile is given in ***Table* I**.

In majority of the cases (80%), the onset of the disease symptoms was 5 yrs or below. Seventy three (90.12%) patients showed positive Valley sign while 76 (93.82%) patients showed calf hypertrophy and 46 (56.79%) showed hypertrophy of extensor digitorum brevis (EDB). Gower's sign was observed in 57(70.37%) cases. Non-ambulatory patients had historical suggestion of Gower's sign but could not be confirmed.

The patients with inability to get up without support, inability to walk and or wheel-chair bound and with severe contracture, and marked wasting were considered in advanced stage of the disease. Twenty patients (24.69%) were non-ambulatory at the time of presentation.

Among non-ambulatory patients, the youngest patient presented to us was 8.5-year-old. The mean (SD) age of onset of the symptoms in non-ambulatory patients was

TABLE I DEMOGRAPHIC AND CLINICAL FEATURES OF STUDY PATIENTS (*N*=81)

Features	
Age of onset, Mean (SD), y	3.93 (1.13)
Age at presentation, Mean (SD), y	7.74 (2.11)
Consanguinity	10 (12.3%)
Delayed motor milestones	38 (46.9%)
Family history (siblings)	22 (27.1%)
Progressive lower limb weakness	81 (100%)
Toe walking	42 (51.8%)
Contractures (n=57)	
Ankles	49 (85.9%)
Hamstrings	20 (35.1%)
Iliopsoas	12 (21.0%)
Hypertrophy	
Quadriceps	20 (24.7%)
Calf	76 (93.8%)
Extensor digitorum brevis	46 (56.8%)
Neck muscles weakness	79 (97.53)
Flexors	76 (93.8%)
Extensors	32 (39.5%)
Facial weakness	41(51.3%)
Weakness – upper limbs	
Deltoid	44 (54.3%)
Biceps	31(38.2%)
Triceps	30 (37.0%)
Distal	18 (22.2)
Weakness – lower limbs	
Gluteus maximus/Iliopsoas	81 (100%)
Hip abductors/adductors	59 (72.8%)
Quadriceps	73(90.1%)
Hamstrings	58(71.6%)
Wasting of different muscles (n=17)	
Wasting of shoulder girdle	15 (88.2%)
Wasting of thigh	11 (64.7%)
Wasting of leg	8 (47.0%)
Electrocardiography (n=35)	
Abnormal Q-waves	6 (17.1%)
Right ventricular dominance	7 (20.0%)
Echocardiography (*n*=16)	
Dilated cardiomyopathy	6 (37.5%)
Myopathic pattern on EMG	81(100%)
Mild intellectual disability (IQ 38-63)	21 (32.3%)

4.71 (0.72) y, while the mean age of presentation to our clinic was 10.23 (1.15) y. The mean (SD) age of loss of ambulation in our patients was 8.81 (0.81) y. We also observed mild facial weakness in 41(51.3%) cases. Although there was an increase incidence of facial weakness in older children, there was no correlation of facial weakness with age (P=0.057). Similarly the facial weakness was equally common in ambulatory and non-ambulatory patients.

The highest and lowest serum CPK values among these 100 patients are recorded as 38160 IU/L and 388 IU/L, respectively.

Detailed genetic pattern is shown in ***Table* II**. Exon 48 was the most commonly deleted exon in our study. Although no specific pattern of deletion was found, most of our patients with severe clinical features had deletion of distal exons. We also did not find any difference of size and pattern of exon deletion with loss of early ambulation (P=0.089) in our patients.

DISCUSSION

This study presents the clinical and genetic pattern of confirmed (genetically or biopsy proven) DMD cases from a tertiary referral centre of eastern India. The age of onset of DMD was distributed randomly among this small group of patients similar to earlier studies [11]. The distribution of weakness, inter-individual variability of weakness pattern [12], and presence of calf hypertrophy and Valley sign were similar to other studies [13]. Similar to our study, mental retardation has been reported in about one-third of DMD patients [14].

The frequency of dystrophin gene deletions is reported to vary from 22% to 86% [6-9, 15-19], which is in agreement with our study. Possibilities in the remaining cases were either point mutation and duplication or other rare variants of congenital dystrophy which mimic DMD in clinical presentation.

TABLE II EXON DELETION PATTERN IN DUCHENNE MUSCULAR DYSTROPHY (N=73)

Deletion	*No. (%)*
Proximal hotspot	8 (10.9%)
Distal hotspot	53 (72.6%)
Both proximal and distal hot spot	12 (16.4%)
Single exon	15 (20.5%)
Two or more consecutive exons	46 (63.0%)
Three or more consecutive exons	29 (39.7%)
Distal exon 45 involvement	22 (30.1%)
Distal exon 47 involvement	20 (27.4%)
Distal exon 48 involvement	43 (65.7%)

Our observation of dystrophin gene deletion mutations is relatively higher than that reported in most of the other studies including geographically contiguous neighbouring areas like Pakistan (40.7%), Sri Lanka (62.5%) and China (66%) [15-17]. Low rate of deletion in frequency in some studies may be due to screening of fewer exons than recommended and small group of patients studied. However, studies from various regions of India like South [5], North [8] and West [9] showed similar deletional rates of 73.1%, 72% and 72% respectively, while previous study from eastern India by Basak, *et al*. [4] reported lower rate of deletion (65.7%). The varying rate of deletion in different parts of country may be due to highly selective group, different population group and ethnic differences in these populations.

In our study, 72.6% cases showed distal exon involvement, which is in agreement with literature [4-7]. The patients with advanced clinical features showed variable genetic pattern with distal exon deletion being the most common pattern. It is difficult to correlate clinical severity with deletion type within DMD as patients are being seen at different points in the natural history of their disease making assessment difficult, especially in the young isolated case. Any particular deletion pattern is rarely seen in DMD as DMD deletions are varied in position and extent. Our data clearly shows that DMD deletions are inhomogeneously distributed among our patients. Our observation was similar to other studies like Banerjee, *et al*. [20] and Swaminathan, *et al*. [5] where they could not establish any phenotypic and genotype correlation considering the size of exon deletion and pattern of deletion. Moreover, Baumbach, *et al*. [21] and Lindlof, *et al*. [22] found no significant correlation between clinical phenotype and number of exon deletion.

The diagnosis of DMD is based on clinical, biochemical and histopathological studies and further confirmed by molecular analysis. Other than multiplex PCR, detection of duplication can be done by Southern blot analysis [23], dosimetric PCR based methods [24] or techniques such as multiplex ligation dependent probe amplification [25] (MLPA), but all these methods can not identify point mutation. We could not analyze point mutation or duplication in our laboratory as they require sophisticated facilities and are being performed in very few places and for research purpose only. The identification of female carrier or prenatal diagnosis is important for preventing the birth of children affected by DMD, when the index case is alive.

The availability of genetic testing makes it possible to confirm the diagnosis early without going for any invasive procedure. Hence, the genetic studies should be

WHAT THIS STUDY ADDS?

- No definite correlation was observed between genotype and phenotype of DMD both in respect of size and pattern of deletion.

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.

Contributors: SD: data collection, statistical analysis and manuscript writting; AKS, AP, AB and GG: study design, data collection and manuscript writing; DSG: data collection and manuscript writing; AJ and SR: genetic testing.

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