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The Genetic, Functional, and Clinical Characteristics of Dystrophinopathy in a Single Tertiary Pediatric Center in Israel

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Abstract

Objectives: To evaluate the genetic and clinical characteristics of boys with Dystrophinopathy at the neuromuscular multidisciplinary clinic of a single tertiary pediatric center in Israel.

Methods: One hundred and sixteen boys with Dystrophinopathy were retrospectively analyzed. The diagnosis of DMD or BMD was based on clinical findings and the dystrophin mutation.

Results: The mean age of the entire cohort was 11.3 ± 4.9 years. Exon deletions were detected in 90/116 (77.6%) children, while 20/116 (17.2%) had single nucleotide variants (SNV), and 6/116 (5.2%) had exon duplications. Twenty-three of 116 (19.8%) children had an affected sibling. Children with DMD and BMD were diagnosed at the average age of 3.6 ± 2.4 and 5.8 ± 4.4 years respectively. Sixty-six/79 (83.5%) of children with DMD and 14/37 (37.8%) of childhood BMD were treated with corticosteroids. Angiotensin-Converting Enzyme inhibitors as cardioprotective agents were prescribed for 32/39 (82%) of DMD and 15/23 (65%) of BMD patients. Thirty patients were non-ambulatory. Children with DMD (n=25) lost ambulation at the age of 11.3, compared to 17.2 years of BMD (n=5), ($p<0.01$).

Conclusion: The clinical and genetic characteristics of an Israeli cohort of DMD and childhood-onset BMD are presented. In our cohort early-onset, BMD is associated with a relatively severe phenotype. Recently, prenatal female carrier screening for DMD was included in the national governmental health program and its impact on early genetic diagnosis should be investigated in future studies.

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Introduction

Duchenne muscular dystrophy (DMD) is the most common childhood-onset muscular dystrophy affecting 1:3600-6000 live male births worldwide [1]. DMD is associated with almost absence of dystrophin protein. Dystrophinopathy clinically milder allelic form, Becker Muscular Dystrophy (BMD), is caused by reduction in quantity and function of dystrophin protein [2-3].

DMD most frequently occurs when deletion interrupts the translational reading frame, resulting in "out of frame deletion." In contrast, when the translational reading frame is preserved despite the deletion, it is referred to as "in-frame deletion" and causes BMD in most cases [3-4].

While the clinical course of DMD is severe, the clinical phenotype of BMD is variable. Severe cases may overlap with DMD. However, milder cases may present with cardiomyopathy, muscle cramps, or only hyperCKemia without severe muscle weakness [5].

Functionally, DMD children present with walking difficulties between the age of 2 to 5 years, while the initiation of symptoms in most BMD patients starts later in childhood or early adulthood [1]. The skeletal muscle degeneration is progressive and results in DMD with loss of ambulation by 13 years [6]. Loss of ambulation in BMD may occur at a significantly later age (≥16 years of age) [7].

Serum Creatine Phosphokinase (CPK) which is a commonly used biomarker for muscle damage is significantly elevated in DMD patients compared to BMD and normal children [8].

Current guidelines suggest initiating steroid treatment for children with DMD before clinical walking worsening, usually around 4–6 years of age [1]. Corticosteroid management delays deterioration of muscle strength, daily activities, walking abilities, pulmonary and cardiovascular function, improves the quality of life and prolongs survival [9-10].

In BMD due to variability in the course of the disease, guidelines and solid data regarding the efficacy of steroid treatment are unavailable [11]. In a population-based cohort study by Matthews et al [12], there was a wide variation in corticosteroid use from an annual mean percent of 8.4% to 80.2% across individual clinics. The variation in physician prescribing practices appears to be a strong determinant of corticosteroid usage.

Heart management in DMD/BMD relies mainly on cardiac protective drugs, including Angiotensin-Converting Enzyme (ACE) inhibitors and beta-blockers [13]. Initiation of ACE inhibitor is recommended towards the age of ten years [14], as suggested that early treatment effectively reduces cardiomyopathy in DMD when started even before signs of abnormal functioning [15].

Boys with DMD/BMD have a higher risk of developing osteoporosis [16]. Therefore, vitamin D and Calcium supplementation are standard treatments for DMD/BMD children as part of the physician and dietitian evaluation [17].

DMD is associated with an increased risk of cognitive impairment [18]. Cognitive impairment is associated with mutations in the second part of the gene leading to the involvement of specific dystrophin isoforms, Dp71 and Dp140 which are expressed in the brain [19]. Mental retardation was observed in about 20%-50% of children with DMD, much higher than in the general population [20].

In addition, a high prevalence between 11–32% of attention-deficit hyperactivity disorder (ADHD) has been described in DMD compared to the general population [21]. As Methylphenidate (Ritalin) treatment reduces ADHD-related symptoms, including hyperactivity, impulsivity, and inattentive behavior, it is commonly prescribed to DMD/BMD boys [21-22]. Autism spectrum disorders are also common in DMD, with incidence up to 15%, and obsessive-compulsive disorder (OCD) up to 60% as summarized in a recent review [23]. In addition, verbal fluency difficulties, expressive and receptive language disorders, reading, and verbal learning problems are more common in children with DMD (18).

In this study, the genetic, and clinical characteristics of dystrophinopathy were evaluated in 116 children between 3-26 years old attending the multidisciplinary clinic in a single tertiary pediatric medical center in Israel.

Material and methods

The study was based on a retrospective analysis of individuals' medical records attending the neuromuscular multidisciplinary clinic at Schneider Children's Medical Center of Israel. The study protocol was approved by the local IRB Committee (approval No. IRB: 0722-17).

Inclusion criteria included children with dystrophinopathy confirmed by a dystrophin gene mutation who had at least one visit to the Neuromuscular multidisciplinary clinic.

The diagnosis of DMD and BMD was based on genetic data of the type of the mutation, (LOVD exonic deletions/duplications reading - frame checker software (https://databases.lovd.nl. [24]). The SNV mutation sequence was tested using the 'Varnomen' database (http://varnomen.hgvs.org. [25-26].

Data of the last clinic visit of the patient were obtained manually directly from the medical record software system, with the followings data records:

1. Individuals' characteristics included age (years), age of diagnosis (years), and age at loss of ambulation (years).
2. Genetic data included the type of mutation (exon deletions, exon duplications, and single nucleotide variants (SNV). The SNV mutations were subdivided into point mutation/stop codon variant (none sense mutations), splice site, single nucleotide insertion, or single nucleotide deletion (frameshift mutation). The specific number of exons and mutation sequence was also documented and analyzed for mutation type prevalence. The exon deletions and duplications were tested for 'out / in' frame mutation.
3. The last score of the six-minute walk test (6MWT) distance (meters) and the North star ambulatory assessment (NSAA) score (points) were documented. Results of children with DMD who had at least two repeated tests within the study period were also obtained.
4. Clinical data included CPK level (u/l), corticosteroid treatment type (Prednisone, Deflazacort, or Vamorolone), and dosage (daily, weekly, or weekends only). Preventive care such as ACE inhibitors, beta-blockers, Vitamin D, calcium, and Ritalin medication. Education status (regular or special class) and paramedical treatments (physiotherapy and hydrotherapy treatments).

Statistical Analysis

Children's clinical and genetic records were organized using Microsoft Excel 2018 and analyzed using SAS statistical software and Prism Graph Pad Software 9.3.1. Several parameters were compared between DMD and BMD patients.

The analysis included the frequencies of all variables subdivided into DMD, BMD, ambulation status, and steroid treatment.

All variables were first tested for normality, using the D'Agostino & Pearson test. If yes, an unpaired parametric T-Test (for differences between two groups) was performed. For more than 2 groups comparison, a One-way ANOVA with post-hoc Turkey's, multiple comparisons test was performed. If not, an equivalent non-parametric Mann Whitney test was performed or for more than 2 groups comparison a non-parametric Kruskal-Wallis ANOVA test was performed with post-hoc Dunn's multiple comparisons tests.

Normally distributed variables included the 6MWT and NSAA.

Skewed variables included the CPK level

Pearson correlation was performed for normally distributed variables. For the CPK a log-transformation was performed for normal distribution and correlation with age.

Data are presented as mean ± SD, with a p-value of ≤ 0.05 considered significant.

Results

Patients general description

Clinical and genetic data of 116 patients with dystrophinopathy was obtained from medical files of the neuromuscular clinic of Schneider Children's Medical Center of Israel. Our cohort consists of 79 (68.1%) children with Duchenne muscular dystrophy (DMD) and 37 (31.9%) with Becker muscular dystrophy (BMD). Fifty-three (67.1%) of DMD boys were ambulatory (ADMD) and 26 (32.9%) non-ambulatory (NADMD) boys. Thirty-two (86.5%) of BMD boys were ambulatory (ABMD) and 5 (13.5%) non-ambulatory (NABMD) boys.

The average age of children with DMD in our cohort was 10.7 ± 4.5 (range: 3.6-26.2) years and 12.5 ± 5.4 (range: 3.0-25.8) years for children with BMD. DMD and BMD boys were diagnosed by molecular genetic testing at the average age of 3.6 ± 2.4 and 5.8 ± 4.4 years respectively.

Children with DMD were significantly younger when lost ambulation compared to children with BMD (DMD (n=25): 11.3 ± 2.7, BMD (n=5): 17.2 ± 4.4 years old, (p<0.01).

Genetic classification in DMD and BMD patients

The most common mutation type in our cohort were exon deletions that accounted for 77.6% (90/116) of cases, followed by single nucleotide variants (SNV) in 17.2% (20/116) and duplications in 5.2% (6/116).

Among children with DMD, exon deletions were accounted for 73.4% (58/79) of cases, SNV for 21.5% (17/79) of cases, and duplication for 5.1% (4/79) of patients.

SNV in children with DMD was subdivided into point mutation/stop codon variants (none sense mutations) in 58.8% (10/17) of cases, splice site variants in 29.4% (5/17), and single nucleotide insertions in 11.8% (2/17), causing frameshift mutation.

Among children with BMD, 86.5% (32/37) of the cases resulted from exon deletions, SNV mutations were detected in 8.1% (3/37), and duplications in 5.4% (2/37). The SNV in children with BMD was point mutation, splice site variant, and single 2-nucleotides deletion causing frameshift mutation, each in a single patient **(Figure 1)**.

Forty-nine of 116 (42.2%) of the entire cohort had a confirmed carrier mother. A total of 23/116 (19.8%) affected siblings were found.

Within the overview of therapeutic exon skipping deletions reported by Leiden DMD (LDMD) [27-28], the six leading skippable exons are 44, 45, 46, 50, 51, and 53. These top 6 potential skippable exons accounted for 46.8% of our cohort a little bit lower than LDMD database (54.8%). In our cohort mildly lower prevalence of potential exon 45, 50, and 51 skipping, and a slightly higher prevalence of exon 46, 53, and 44 skipping compared to LDMD database was detected, **Table 1**.

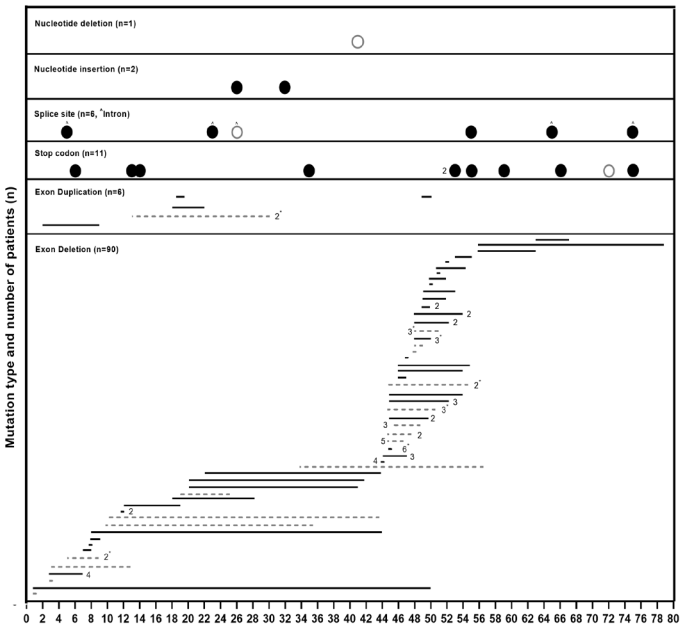

Figure 1: Individuals' DMD and BMD genetic mutations summary (n=116). Solid Line; DMD (n=79), Dashed line; BMD (n=37), Black circle; DMD single nucleotide variant. Empty circle; BMD single nucleotide variant. ^Intronic mutaion. *Number of siblings with similar mutaion.

Table 1: Deletions amenable to exon skipping in our DMD cohort compared to Leiden DMD (LDMD) exon skipping database*

Exon skipp	Current study	LDMD	Differences (%)
45	6/79; 7.6%	11.20%	-3.60%
50	1/79; 1.3%	5.20%	-3.90%
51	9/79; 11.4%	17.50%	-6.10%
46	6/79; 7.6%	5.60%	2%
53	8/79; 10.1%	7.50%	2.60%
44	7/79; 8.9%	7.80%	1.10%
Total	**37/79; 46.8%**	**54.80%**	**-8%**

*Leiden DMD (LDMD) exon skipping database [27-28].

Functional tests measurements in ambulatory DMD/BMD boys

The six-minute walk test (6MWT) was documented in 43 DMD and 21 BMD boys, and the North Star Ambulatory Assessment (NSAA) test was documented in 42 DMD and 22 BMD boys. Even though our DMD cohort was on the average younger than BMD, children with DMD (mean age 9.0 ± 3.0 yr.) had significantly lower scores in 6MWT and NSAA tests compared to children with BMD (mean age 11.6 ± 4.1); 6MWT; DMD: 381.4 ± 96.1 meters, range: 100-548 vs. BMD: 472.6 ± 96.1 meters, range: 293-632, p<0.001. NSAA; DMD; 24.3 ± 5.9 points, range: 8-33 vs. BMD; 29.8 ± 4.4 points, range: 10-34, p<0.001.

We found a significant correlation between 6MWT distance and NSAA score in the entire ambulatory cohort (n=85, r = 0.81; p<0.001) and a significant correlation between 6MWT distance and NSAA score in each group separately (ADMD; n=56, r = 0.74; p<0.001, ABMD; n=29, r = 0.88, p<0.001).

The individual's analysis of 6MWT and NSAA tests, conducted in 34 DMD patients from our cohort with at least two repeated tests, showed an increase in 6MWT and NSAA score below seven years of age, stability around 7 to 12 years of age, and then decrease after the age of 12 years **(Figure 2)**.

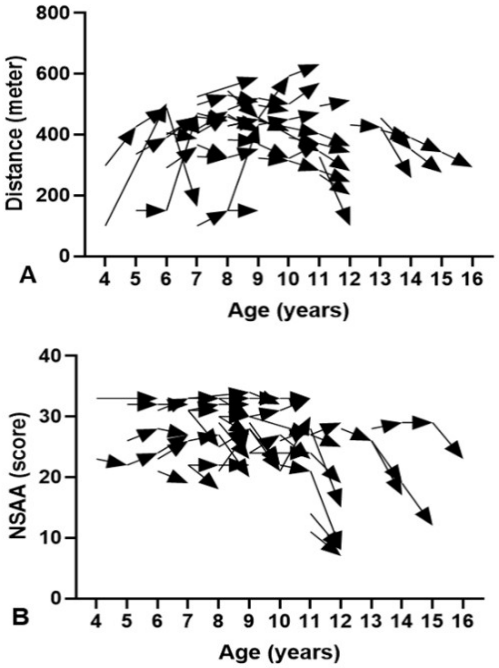

Figure 2: Individuals' course of 6MWT **(A)** and NSAA **(B)** in children with DMD (n=34). Sub-group analysis of ambulatory DMD patients having at least two retrospectives repeated tests.

Creatine phosphokinase (CPK) results

A significantly elevated CPK level was found in children with DMD (n=76) with a median of 7,833 (interquartile range (IQR); 3,764-13,263 U/L) compared to children with BMD (n=34, media of 2,942, IQR; 959.8-6,810 U/L, p < 0.001).

A significant decrease in CPK level was found with age in children with DMD after the age of 7 years (n=58, r = -0.72, p < 0.0001). Weaker correlation was found in children with BMD (n=28, r = -0.38, p = 0.04), **Figure 3**.

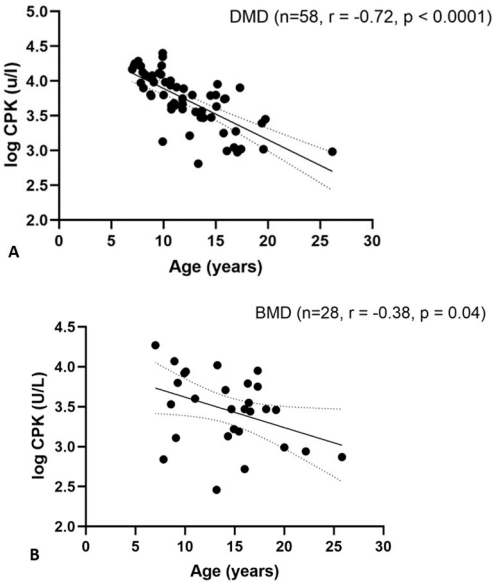

Figure 3: Pearson correlation between CPK level (u/l) and age in DMD **(A)** and BMD **(B)** boys (n=86). Data presented after log transformation of CPK level due to non-normal distribution.

Corticosteroids treatment management in DMD and BMD

Corticosteroids treatment data is presented in **Table 2**. A total of 66/79 (83.5%) children with DMD were treated with corticosteroids, which included 53/66 (80.3%) Prednisone, 9/66 (13.6%) Deflazacort, and 4/6 (6.1%) Vamorolone. Out of all treated DMD children, 61/66 (92.4%) were treated daily and 3/66 (4.6%) on alternate-day and 2/66 (3%) only on weekends.

A total of 14/37 (37.8%) children with BMD were treated with corticosteroids, which included 10/14 (71.4%) Prednisone and 4/14 (28.6%) Deflazacort. All treated BMD children, 14/14 (100%) were treated daily.

Table 2: Steroid treatment distribution in DMD/BMD children.

Steroid treatment	DMD	BMD
Steroid treatment: yes (n, %)	66 / 79 (83.5%)	14 / 37 (37.8%)
Prednisone: (n, %)	53 / 66 (80.3%)	10 / 14 (71.4%)
Deflazacort: (n, %)	9 / 66 (13.6%)	4 / 14 (28.6%)
*Vamorolone: (n, %)	4 / 66 (6.1%)	0 / 14 (0%)
Treatment: every day (n, %)	61 / 66 (92.4%)	14 / 14 (100%)
Treatment: alternate-day (n, %)	3 / 66 (4.6%)	0 / 14 (0%)
Treatment: weekend (n, %)	2 / 66 (3.0%)	0 / 14 (0%)

DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy, *Vamorolone – Corticosteroid in clinical trial.

Preventative healthcare in DMD and BMD patients

Cardiac medical treatment included ACE inhibitors, as a single cardioprotective agent, in 25/33 (75.8%) children with DMD above the age of 10 years. All children started treatment after the age of ten years (13.2 ± 2.9). A single child with DMD was treated at the age 9 years. Twelve/15 (80%) of children with BMD above the age of ten years were treated with Ace inhibitors (age 13.7 ± 2.2 years).

A combination of ACE inhibitors and Beta-blockers treatment was used in 7/33 (21.2%) of these DMD and 3/15 (20%) children with BMD (initiation age of 17.3 ± 3.1 years). Beta-blockers were used as a single cardioprotective agent in only a single

child with BMD (starting age 13.5 years). Six out of 39 (15.4%) DMD and 7/23 BMD (30.4%) patients above the age of ten were not treated with any cardioprotective agent.

Children with DMD and BMD were also treated with Vitamin D (DMD n=71 (89.9%), BMD n=21 (56.8%)), calcium (DMD n=40 (50.6%), BMD n=13 (35.1%)), and Ritalin (DMD n=9 (11.4%); BMD n=3 (8.1%).

Education, physical therapy, and hydrotherapy

A total of 55 children with DMD out of 79 (69.6%) studied in regular education while 23/79 (29.1%) DMD boys study were in special education classes. Thirty-one out of 37 (83.8%) BMD boys were in regular education, and only 4/37 (10.8%) were in a special education class. Additionally, 69/79 (87.3%) of children with DMD received physiotherapy treatment regularly, 50/79 (63.3%) Hydrotherapy, whereas 18/37 (48.6%) and 15/37 (40.5%) of children with BMD received these treatments, respectively.

Discussion

The clinical and genetic characteristics of 116 children with dystrophinopathy attending a multidisciplinary clinic in a single tertiary center in Israel are described.

In this cohort the average age of DMD diagnosis was 3.6 years. Even though this cohort includes high rate of families with more than one affected sibling (18.3%); the age of diagnosis of families without affected child was almost similar, 3.7 years. The high rate of families with multiple affected patients reflects in part the presence of certain populations not interested in genetic counselling and therefore in prenatal or very early diagnosis.

The age of onset in Israel is comparable to a recent report from Italy, in which the average age of diagnosis was 3.5 years [29]. This is at an earlier age compared to 4.9 years in a previous report from the US in 2009 [30], and from a Newcastle UK study from 2014 with an average age of diagnosis of 4.3 of boys without family history diagnosed with DMD in the last 10 years (n=20) [31]. The earlier diagnosis in this study compared to previous other studies a decade ago may reflect earlier accessibility and reduced cost of genetic testing in recent years as well as relative abundance of pediatric neurologists in Israel. In addition, recently prenatal female carrier screening for DMD was applied in Israel. The results of the screening process will be evaluated in the next years.

Children with BMD in this cohort were diagnosed, at a relatively early age of 5.8 ± 4.4 years, younger than previous studies with an average age around 10-11 years [32-36]. This reflects the fact that this cohort is based on a pediatric center while adult-onset BMD are seen at other medical centers.

Early genetic diagnosis for DMD/BMD has implications towards both genetic counseling and early treatment when applicable for improving prognosis and quality of life for both the child and family [6,17].

The spectrum of genetic mutations in our cohort includes 77.6% (90/116) exon deletions, followed by single nucleotide variants (SNV) of 17.2% (20/116) and duplications 5.2% (6/116). These deletions and duplications occur throughout the gene, with several hot spots concentrated between exons 45–55 and exons 2–10 for deletions and duplications, respectively, as previously described by others [1, 3], **(Figure 1)**.

The prevalence of exon deletions in previous reports is extremely variable; in Eastern Europe (29%) [37], Spain (46%) [38], Kuwait (66%) [39], and China (66%) [40-41], southern regions of India (73.1%) [42], north India (72%) [43], east India (72%) [44], and the U.S up to 79% of all DMD/ BMD cases [45]. Even previous reports from Israel showed much lower incidence of deletions 23/62; 37% [46], and 28/81; 35% [47]. These variations may represent sampling errors as well as a previous diagnosis by PCR which did not include all DMD gene exons.

The exon deletions potentially skippable by the top 6 exons skipping (44, 45, 46, 50, 51, and 53) [27-28], was 46.8% in our cohort, somewhat lower than Leiden DMD (LDMD) database (54.8%) [27-28]. We detected a mildly lower prevalence of exon skipping 45, 50, and 51, and a slightly higher prevalence of exon skipping 46, 53, and 44 compared to the LDMD database [27-28]. In any case in our cohort exon skipping similarly is a significant potential treatment for a substantial number of DMD patients.

Functionally, the 6MWT results in 43 children with DMD demonstrated an average distance of 381.4 ± 96.1 meters (average age 9.2 ± 2.8 years), which were about 60% from predicted healthy age-matched standards (621 ± 68 meters) [48]. Twenty-one children with BMD (average age 11.6 ± 4.1 years), achieved an average of 472.6 ± 96.1 meters, which is much better than children with DMD as expected.

When 6MWT was repeated at consecutive clinic visits, we detected an increase in the distance achieved at the age of 5-7 years, followed by stability between 7-12, and a decline above 12 years of age **(see Figure 2)**. Similar results were presented in other previous reports and are significant in designing therapeutic interventions for DMD/BMD patients [49-50].

It should be pointed out that a previous study demonstrated that from a baseline level, in 6MWT distance of around 350 m, there was a slight increase in walking distance (+34.11 ± 88.8 meters), in children with DMD below age 7 years during the first two years of follow-up, with a small decrease (-2.9 ± 117.7 m) following the third year. Though a significant reduction ($p<0.0001$) of −104.2 ± 146.2 meters, on average, was found over 3 years in children above age 7 years [51]. As for the NSAA, a function test for DMD/BMD boys DMD boys, in our cohort, had on the average age of 9.3 years, a score of 24.3 points (maximum 34 points) [52].

BMD boys on average age; 11.5 years achieved at this study an average score of 29.8 points, higher than children with DMD as expected [53]. Overall, we found a significant correlation between 6MWT distance and NSAA score in our entire ambulatory cohort (n=85), $r = 0.81$; $p<0.001$.

Clinically, as anticipated and documented in a previous study, significantly high CPK levels were observed in DMD compared to BMD ($p<0.0001$) [8]. CPK levels significantly decrease with age in advanced stages of DMD as muscle mass decreases.

Eighty-four % of DMD and 38% of BMD were treated with corticosteroids, mostly with daily prednisone. The fact that almost 40% of our BMD patients were treated with steroids further confirms that the clinical course of relatively early onset BMD is more severe than late onset BMD.

The study limitation includes the facts that this is a retrospective study and the fact that it includes pediatric dystrophinopathy patients only.

Conclusion

An Israeli cohort of children with dystrophinopathy presented quite similar clinical and genetic characteristics to those previously reported in DMD/BMD boys. Interestingly a previous report from Israel showed some different data. Genetically, we found a high prevalence of exon deletion (78%), a low prevalence of exon duplication (5%), and a total of 17% of SNV in this cohort. Early clinical and genetic diagnosis followed by steroid medications were noted in most of our patients. Early-onset BMD is associated with a relatively severe phenotype which re-emphasizes the fact that dystrophinopathy is a continuum and genetic classification of DMD and BMD may be misleading in predicting prognosis and to delineate management. In our cohort, 38% of BMD children were treated with steroids.

Recently, prenatal female carrier screening for DMD was included in the national governmental health program and its impact on early genetic diagnosis for DMD/BMD should be investigated in future studies.

Highlights

- Early diagnosis followed by steroid medications were noted in most patients.
- Exon deletions detected in 78%, duplications in 5% and nucleotide variants in 17%.
- Early-onset BMD is associated with a relatively severe phenotype.
- The nomenclature DMD and BMD may occasionally be misleading in predicting prognosis.
- Recently, prenatal female carrier screening for DMD was applied in Israel.

Declaration of Competing Interest

The authors declare no conflict of interest.

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