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ORIGINAL PAPER

Evaluation of Cardiac, Autonomic Functions in Ambulant Patients with Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder caused by dystrophin gene mutation resulting in muscle weakness, motor delays, difficulty in standing, and inability to walk by 12 years. As disease progresses, it leads to cardiac and respiratory failure. Evaluation of cardiac autonomic status and echocardiography in DMD patients at a young age can be a potential biomarker to assess disease progression. This study aimed to investigate the younger DMD population of 5–11years of age with mild to moderate cardiac involvement for early detection using non-invasive and cost-effective tools. Genetically confirmed male DMD patients, aged 5–11 years ($n = 47$), screened from the outpatient department of a tertiary neuroscience institution were subjected to heart rate variability and echocardiographic analysis, and values were correlated with their clinical variables. DMD patients showed a significantly higher difference in HR, interventricular septum, E m/s, and E-wave to A-wave (E/A) ratio than normal values ($p < 0.001$). Significantly higher HR indicates initial sinus tachycardia and decreased IVD (d), and increased E m/s and E/A ratio mark the onset of cardiac symptoms in DMD patients even though its chamber dimension remains normal and are associated with cardiac muscle fibrosis.

Keywords Duchenne muscular dystrophy · Heart rate variability · Sinus tachycardia · Echocardiography

Abbreviations

DMD	Duchene muscular dystrophy
HRV	Heart rate variability
HR	Heart rate
IVD (d)	Interventricular internal diameter end diastole
E	Peak E-wave velocity
A	Peak A-wave velocity
E/A	Ratio of E-wave to A-wave
BPM	Beats per minute
SDNN	Standard deviation of normal to normal intervals
RMSSD	Root mean square of standard deviation
LF	Low frequency
HF	High frequency
LVEF	Left ventricular ejection fraction
TAPSE	Tricuspid annular plane systolic excursion
LA	Left atrium
RA	Right atrium
LVID (d)	Left ventricular internal diameter end diastole

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked genetic disorder caused by mutation in dystrophin gene, primarily affecting muscles with its onset of symptoms shown around

the age of 4, and worsens overtime in the next two decades [1]. Muscle loss typically occurs first in the thighs and pelvis followed by the arms, resulting in trouble standing up and inability to walk by the age of around 12 years. Delayed motor development, toe walking or waddling gait, using hands to get up off the floor (Gower's maneuver), and enlarged calf muscles were some of the symptoms seen in these patients. Later stages of the disease lead to progressive heart enlargement (cardiomyopathy) and breathing difficulty due to weakness in diaphragm, and scoliosis and tight joints (contractures) also develop due to the progression of muscle loss and weakness [2, 3].

Patients with DMD initially have a structurally normal heart that gradually progresses into fibrosis of the inferobasal wall of the heart, which is the earliest sign of myocardial involvement. Subsequently, this leads to progressive fibrosis, left ventricular (LV) dysfunction, and dilation leading to end-stage heart failure [4]. Incidence of cardiac complication including dilated cardiomyopathy (DCM) starts at an early age having statistics of one third of patients by 14 years of age, which reaches half of the patients by 18 years, and almost all patients would have one or the other cardiac issues above the age of 18 [5]. Despite the high incidence of DCM, the majority of children with DMD are relatively asymptomatic until later stages of disease progression, probably because of their inability to perform physical activities or exercises (as DMD symptoms worsen when muscle activity increases). Dilated cardiac cavities, myocardial thickening, and right and left ventricular abnormalities were revealed by echocardiographic studies [6]. Standard echocardiographic parameters despite being normal in some patients showed a reduction in circumferential and longitudinal strain measurements with speckle-tracking echocardiography (STE). In young patients with DMD who had global normal systolic function, systolic deformation parameters were reduced and also early diastolic myocardial velocities which could be detected particularly in the basal inferolateral LV. There was a peak decrease in systolic strain of posterior wall despite normal standard ECG as shown by the myocardial strain imaging. Patients with DMD had frequently abnormal wall thinning during early diastole and even when conventional ECG findings were normal [7–10]. STE demonstrated subclinical myocardial dysfunction with decreased average circumferential and longitudinal strain [11]. A prospective multicenter study performed using STE analysis in all three (longitudinal, radial, and circumferential) displacements had shown that global left ventricular (LV) strain mean measures significantly worsened in the DMD group for all the three displacements. Basal area of LV inferior-lateral and anterolateral segments were highly impaired even though right ventricular functions were normal both in DMD and in healthy individuals [12]. Fifty percent of DMD patients showed left ventricular systolic dysfunction (LVSD), and early LVSD could be detected using myocardial strain analysis [13]. Several other studies detected that radial strain, epicardial longitudinal strain, strain rate, and parasternal short axis view were lower in DMD patients. Circumferential strain in endocardium, myocardium, and epicardium significantly decreased. Myocardial circumferential strain deterioration can act as an indicator for predicting cardiomyopathy [14]. A study has demonstrated yearly rate of decline of left ventricular ejection fraction and LVDV 2% and 4%, respectively. Around a mean age of 14.4 years, DMD patients had less LV dilation and closer to normal LVFS, but after 2 years, LV dilation increased without changing LVFS [15]. The present study deals with conventional cardiac parameters in younger population with its structural cardiac parameters with its functional abnormality.

Autonomic evaluation was carried out by heart rate variability (HRV), and these measures revealed a sympathetic dominant system in several studies in DMD. These studies showed a decrease in HRV parameters such as high frequency (HF) power, percentage of normal to normal RR intervals with adjacent difference of >50 ms (pNN50), standard deviation of normal to normal intervals (SDNN) [16], and low coefficient of variation of RR interval (<3%) indicating respiratory insufficiency, diminished vagal influence, and sympathetic dominance with the progression of cardiac and respiratory dysfunction [17–20]. In DMD patients of 6–8 years of age, greatest percentage of autonomic attenuation indicating early blood pressure changes is an appropriate prognosis for therapeutic intervention in DMD patients [21]. Thus, age-related autonomic parameters could play a key role in understanding early functional autonomic changes in these patients. Moreover, cardiac arrhythmias are considered to be the most common signs seen in young DMD patients indicating progress in cardiac abnormalities. Arrhythmias increase with declining ejection fraction irrespective of age, and development of arrhythmia could be predicted according to age of the patients [22].

Hence, an early detection of cardiac abnormalities is essential to come up with a required treatment. Since a comprehensive study involving all these echocardiographic and HRV parameters was lacking, this study aimed to investigate the younger DMD population of 5–11 years of age with mild to moderate levels of cardiac disease as an early detection biomarker for the disease progression using non-invasive and cost-effective measures.

Methods

Patients diagnosed as DMD were screened for recruitment into study from the outpatient department of NIMHANS with a sample size of 47. Genetically confirmed male cases of DMD are in the age group of 5–11 years with elevated

serum creatinine kinase and clinical signs consistent with DMD like progressive loss of function, delayed walking, frequent falls or difficulty with running and climbing stairs, positive Gower sign, and calf pseudo-hypertrophy. Patients in ambulatory phase and able to walk independently for at least 75 m and patients/families ready to give consent for enrollment in the study were included in this study. Patients in non-ambulatory phase with advanced symptoms of cardiomyopathies and respiratory complications; patients with other muscle dystrophies and myopathies like Becker muscular dystrophy, congenital muscular dystrophies, myelopathies, congenital myopathies, and metabolic muscle diseases; patients with any neurological disorders such as any significant head injury and epilepsy; patients with evidence of malignancy and any other condition which the investigator thinks may jeopardize the study; and patients/families unwilling or unable to comply with the protocol study procedures were excluded.

The subjects who fulfill the inclusion criteria were appraised of the investigational nature of the study, and informed consent of the patients was obtained for their willingness to participate in the study. All the participants were encouraged to visit the Autonomic Function Test Laboratory and cardiopulmonary laboratory, NIMHANS, where recordings were performed to enhance the familiarity to the set-up before consenting and recording. The genetic profile used to confirm the cases are used to analyze the link between different exon mutation and disease status in terms of heart rate variability. As DMD gene has 79 exons and different patients present with different genetic mutations, we grouped them based on a break point of in-frame and out-of-frame deletion before exon 44. Hence, there would be partially functional protein synthesis and any deletion after 44 would not create the dystrophin protein, respectively.

Since this study was carried out during the COVID-19 pandemic, healthy subjects did not accept to undergo echocardiographic recording nor electrocardiographic recordings within hospital settings. Hence, we have taken previous control data from previous projects of our laboratory for HRV analysis and echocardiographic recording normal values from previously published studies [23–28]. And then, we have done one-sample *t* test to check whether our data from patients is different from normative data.

Experimental Procedure

The study was carried out in the Department of Neurophysiology in collaboration with the Department of Neurology, NIMHANS. Ethical clearance for the study was obtained by the Institutional Ethics Committee, and informed consent and assent were obtained before recruiting the subjects for the study. Data available in our laboratory from 2017 has been taken along with their age of presentation and other clinical history. Together, all patients' clinical data, HRV, and echo data were compared using appropriate statistical tests in consultation with the statistician of the team (PPV).

Table 1 Clinical history of the DMD patients

Variables (*n*)	Present	Absent
Consanguinity (16)	3 (18.81%)	13 (81.3%)
Family history (31)	10 (32.2%)	21 (67.7%)
Neonatal intensive care unit admission (16)	9 (56.3%)	7 (43.8%)
Developmental delay (32)	21 (65.6%)	11 (34.4%)
Loss of ambulation (13)	3 (23.1%)	10 (76.9%)
Difficulty in walking (18)	17 (94.4%)	1 (5.6%)
Difficulty in getting up (19)	19	0
Difficulty in climbing stairs (16)	16	0
Repeated falls (14)	14	0
Calf hypertrophy (17)	17	0
Ankle contracture (17)	17	0
Knee contracture (17)	17	0
Hip contracture (9)	5 (55.6%)	4 (44.4%)

Various clinical parameters collected from patients provided in parentheses and this particular variable either present or absent as number and %

Results

Subjects in the Study

Forty-seven patients were recruited in this study of which fifteen of them are from previous data from our laboratory pooled together. Patient's minimum age is 5 and maximum age is 11. Table 1 shows the clinical history of patients in terms of consanguinity and family history, and 13 such clinical variables show the division of our studied cohort with either presence or absence of such factors. Table 2 shows the timeline of the onset of various symptoms with their mean and SD values. Table 3 shows HRV parameters compared between cases and controls. It shows

Table 2 The patient symptoms onset

Variables	*N*	Mean (SD)
Age of presentation	31	7.61 (1.723)
Age of onset of symptoms (years)	41	4.427 (1.81)
Onset year for difficulty in walking	16	3.344 (1.589)
Onset year for difficulty in getting up	25	4.7 (1.51)
Onset year for difficulty in climbing stairs	7	4.29 (2.43)
Onset year for repeated falls	5	2.6 (1.34)
Onset year	23	4.348 (1.64)

Table 3 HRV parameters compared between cases and controls

HRV	Cases ($n = 47$) Median (Q1, Q3)	Controls ($n = 17$)	U	p value
Heart rate (bpm)	99.47 (91.73, 105.88)	86.16 (76.2, 90.75)	145	<0.001
SDNN	44.23 (32.39, 56.69)	50.21 (36.22, 59.19)	350	0.452
RMSSD	40.43 (28.89, 58.74)	50.29 (37.99, 67.39)	322	0.239
Total power	1921.86 (1018.68, 3253.93)	2013.64 (1202.01, 3559.79)	377	0.732
LF	444.98 (299.72, 869.83)	588.28 (324.07, 1117.53)	361	0.558
HF	621.04 (336.17, 1392.17)	802.66 (543.05, 1650.56)	340	0.366
LF(NU)	36.76 (25.01, 46)	35.75 (22.94, 47.32)	388	0.861
HF(NU)	50.40 (40.16, 61.98)	61.69 (44.01, 72.52)	298	0.123
LF/HF	0.73 (0.45, 1.08)	0.58 (0.32, 0.97)	357	0.518

HRV heart rate variability, *bpm* beats per minute, *SDNN* standard deviation of normal to normal intervals, *RMSSD* root mean square of standard deviation, *LF* low frequency, *HF* high frequency, *NU* normalized units

significantly higher HR in DMD patients. The condition of DMD patients worsens as muscle activity increases, thus affecting the onset of symptoms and age of presentation; we performed a comparison between age of onset of symptoms and HRV parameters shown in Table 4 and found a negative correlation between age of presentation onset and HRV status (total power).

Tables 5 and 6 shows echocardiographic data of the patients comparing it with the standard values from the previous studies. It showed that heart rate, intraventricular internal diameter end diastole [IVD (d)], E m/s, and E/A ratio were significantly different from normal values. Furthermore, we looked into the genetic profile of the patients.

Table 4 Correlation between onset of symptoms with HRV parameters

HRV	Age of onset of symptoms (years) ($N = 41$) rho (p value)	Age of presentation ($N = 31$) rho (p value)
Heart rate (bpm)	−0.057 (0.723)	−0.191 (0.304)
SDNN	−0.090 (0.577)	−0.282 (0.124)
RMSSD	−0.101 (0.530)	−0.216 (0.243)
Total power	−0.085 (0.599)	**−0.364* (0.044)**
LF	−0.103 (0.522)	−0.326 (0.074)
HF	−0.087 (0.589)	−0.285 (0.120)
LF (NU)	0.008 (0.962)	0.134 (0.471)
HF(NU)	0.083 (0.604)	0.044 (0.814)
LF/HF	−0.003 (0.987)	0.142 (0.446)

The asterisk sign and bold emphasis reflects statistical significant difference have been found in that variable

HRV heart rate variability, *bpm* beats per minute, *SDNN* standard deviation of normal to normal intervals, *RMSSD* root mean square of standard deviation, *LF* low frequency, *HF* high frequency, *NU* normalized units

As DMD gene has 79 exons and different patients present with different genetic mutations, we grouped them based on a break point of in-frame and out-of-frame deletion before exon 44. Hence, there would be partially functional protein synthesis and any deletion after 44 would not create the dystrophin protein, respectively. However, these groups when compared with HRV data showed no difference between the groups. Table 7 shows comparison of age of onset of symptoms and presentation with HRV measures in these groups. We found a negative correlation between these variables in Tables 4 and 7. A significant difference in correlation noted between total power and LF values with age of presentation implies that with early intervention, the status of the patients is better. We could see a difference in the onset of symptoms between the groups with early onset in before 44 group compared to after 44 group, but the difference was not significant probably because of the outlier in the group with highest number of 8. Thus, there might be a difference caused by complete absence of the protein versus partial protein synthesis, but it was not evident in cardiac, autonomic changes as these changes usually start later in life of DMD patients.

Discussion

The main aim of this study was to evaluate the cardiac and autonomic dysfunction in DMD patients of 5–11 years of age and to assess the utility of this cardiac measure as a predictor for the morbidity in these patients. In this study, echocardiography was used as a measure of cardiac structural changes and HRV measures as a functional assessment for understanding autonomic regulation in DMD patients.

DMD patients tend to have autonomic and cardiac structural changes as the disease progresses. The major cause of death in these patients is dilated cardiomyopathy leading to end-stage heart failure [5]. Thus, parameters such

Table 5 Echocardiography parameters compared with Bornaun et al. (2018)*

Serial number	Variables	Mean ± SD (cases from the study) ($n = 31$)	DMD cases (from the paper mentioned above*)	Two-tailed p value comparing our data	Controls (from the paper mentioned above*) ($n = 17$)	p value of our data with controls
1	Age	7.61±1.7	9.0 (7–12)	–	8 (6.75–11)	–
2	Heart rate	97.3±13.9*	72 (69–76)	**<0.001#**	73 (70–75)	**<0.001#**
Conventional echocardiography:						
1	LVEF%	60 $	59 (58.75–68)	–	69 (66.00–71.00)	–
2	IVS (d) (mm)	7.03±0.31	7 (0.64–0.78)	0.57	7.3 (0.66–0.80)	**<0.001#**
3	E m/s ($n = 24$)	0.85±0.11	0.71 (0.64–0.88)	**<0.001#**	0.79 (0.68–0.89)	**0.014#**
4	A m/s ($n = 24$)	0.49±0.09	0.46 (0.42–0.52)	0.09	0.50 (0.43–0.56)	0.84
5	E/A ratio	1.77±0.4	1.53 (1.45–1.70)	**0.007#**	1.60 (1.40–1.77)	**0.04#**

Due to COVID-19, we took normative data and comparison is done with previous studies

*Recorded during echocardiographic recording, <0.05 significance

$All patients had same value, hence no SD and one-sample t test cannot be computed

#Significance

as autonomic and cardiac changes in early stage of disease assessing its utility as a predictor were tested. In this study, DMD patients with a mean age of 7.61±1.7 years showed a mean heart rate that is significantly different from that of normal healthy controls. This observation was similar with previous work from our laboratory (17) showing increased heart rate in DMD patients with 46.8% of them having sinus tachycardia. This is also evident from previous work that in spite of absence of cardiomyopathy symptoms, initial increase in heart rate could be observed in DMD [23].

The echocardiographic study did not show any enlargement of cardiac chambers. However, increase in the baseline heart rate indicating functional changes has started before the structural effects. In young patients with DMD who had global normal systolic function, systolic deformation parameters were reduced and also early diastolic myocardial velocities which can be detected particularly in the basal inferolateral LV. There was a peak decrease in systolic strain of posterior wall despite normal standard ECG as shown by the myocardial strain imaging. Patients with DMD have frequently abnormal wall thinning during early diastole and even when conventional ECG findings were normal [7–10]. Studies performed in older patients (10–20 years) showed cardiac autonomic involvement of increased sympathetic and decreased vagal activities as measures by HRV measures (16–20). Thus, we can conclude that DMD patients eventually experience dilated cardiomyopathy in later stages of life, whereas earlier, decreased myocardial strain could not be appreciated here in our study as the dimensions of the chambers were unaffected until they reach 10 years of age. However, cardiac muscles undergo fibrotic changes which can be appreciated by other functional measures such as 46.8%

Table 6 Echocardiographic measures

Echo parameters	Mean ($n = 31$)	Normative data based on historical data (as indicated by the reference)
LVEF%	60	56–78 (Tissot et al., 2018)
LA (mm)	23.29±2.29	20–40 (Heller et al., 2008)
LVID (s) mm	21.03±2.04	35–56 (Heller et al., 2008)
LVID (d) (mm)	32.97±2.91	20–40 (Heller et al., 2008)
PA pressure (mm Hg)	23.16±2.43	≥25 mmHg at rest (Widlitz & Barst, 2003)
TAPSE (mm)	20	1.9–24.7 (neonates to 18 years old) (Koestenberger et al., 2009)
IVC (mm)	8.87±0.88	4.6 to 22.6 (Patil et al., 2016)

LVEF left ventricular ejection fraction, *TAPSE* tricuspid annular plane systolic excursion, *LA* left atrium, *RA* right atrium, *LVID (d)* left ventricular internal diameter end diastole, *IVS* interventricular septum

Table 7 Correlation of onset of symptoms within study groups divided based on the loci of genetic mutation (before and after exon 44)

HRV measures	Before 44 [rho (*p* value)]		After 44 [rho (*p* value)]	
	Age of onset of symptoms (years) ($N = 12$)	Age of presentation ($n = 8$)	Age of onset of symptoms (years) ($N = 12$)	Age of presentation ($n = 8$)
Heart rate (bpm)	−0.131 (0.686)	−0.205 (0.627)	−0.108 (0.579)	−0.157 (0.475)
SDNN	−0.455 (0.137)	−0.217 (0.606)	0.019 (0.924)	−0.378 (0.075)
RMSSD	−0.339 (0.282)	−0.205 (0.627)	0.014 (0.944)	−0.271 (0.212)
Total power	−0.123 (0.702)	−0.084 (0.843)	−0.041 (0.832)	−0.405 (0.055)
LF	−0.123 (0.702)	−0.133 (0.754)	−0.096 (0.621)	**−0.416* (0.049)**
HF	−0.258 (0.419)	−0.157 (0.711)	−0.038 (0.847)	−0.322 (0.134)
LF (NU)	0.123 (0.702)	0.072 (0.865)	−0.061 (0.753)	0.105 (0.632)
HF (NU)	0.049 (0.879)	0.169 (0.69)	0.176 (0.360)	0.036 (0.872)
LF/HF	0.081 (0.802)	−0.06 (0.887)	−0.077 (0.690)	0.133 (0.547)

The asterisk sign and bold emphasis reflects statistical significant difference have been found in that variable

HRV heart rate variability, *bpm* beats per minute, *SDNN* standard deviation of normal to normal intervals, *RMSSD* root mean square of standard deviation, *LF* low frequency, *HF* high frequency, *NU* normalized units

of our patients in the study who had sinus tachycardia. This increased heart rate could be a marker of the fibrotic changes occurring in the heart as myocardial fibrosis is associated with altered cardiac conduction, slowing conduction, and even blocking of the conduction [24–26]. There were experimental and computational studies showing that fibrosis in the cardiac muscle modulates the cardiac after potential formation mainly early after potential triggering atrial fibrillation [27] and ventricular fibrillation [28]. Thus, tachycardia could be a marker for fibrosis occurring in the heart and this sinus tachycardia could be seen mainly in patients aged 8 or above compared with younger age groups [29]. In our study, the mean age of participants was 7.61±1.7 which was consistent with previous studies.

The SDNN of DMD group showed no difference from healthy controls. This trend was similar with other time domain HRV parameters like RMSSD which was the best predictor of parasympathetic activity. It was the same as normal healthy controls indicating normal break for the system which is functioning normally unlike older patients (10–20 years) with longer disease duration (16–20). Even frequency domain component of HRV such as LF and HF power showed no difference in DMD group compared to the healthy control and also total power is varied and falls under normal range. These results showed optimal sympatho-vagal balance in these patients; it can also be appreciated by having no change in LF/HF ratio indicating maintained balance in the system.

Echocardiographic measures such as LVEF, TAPSE, LA, RA dimensions, LVID (d), LVID (s), and IVC were all under normal range showing no change in ventricular function and chamber dimensions. Interestingly, PA pressure was slightly decreased than normal. When we compared IVS (d) with previous data, mean (average) of it showed to be significantly reduced from normal healthy data. IVS was inversely correlated with heart rate [30]. Also, mitral annular E and E/A ratio was significantly higher than that in healthy children. The E/A ratio is the ratio of the early (E) to late (A) ventricular filling velocities. Peak E/A ratio has been the single most important variable used to help characterize the overall mitral flow velocity pattern E/A ratio [31], and values above 2.5 may be indicative of elevated filling pressure in an abnormal heart [32]. Increased abnormality in E/A ratio indicates dysfunctional ventricular filling progressing cardiac dysfunction in these patients. These were the signals observed in the early stages of the disease causing some changes in cardio-autonomic and echocardiographic parameters in the initial stage of disease. There is a need to further examine this issue based on age-wise and genetic mutations of these patients and to draw a meaningful conclusion.

DMD gene, being one of the largest genes in human genome within X-chromosome, comprises 79 exons plus seven promoters linked to unique first exon and the remaining ones being introns totally containing 2–5 million base pairs of genetic sequence comprising 0–1% of total human genome [33–35]. This genome transcribes 14,000 base pairs of mRNA that is predominantly expressed in skeletal and cardiac muscles as well as small amounts in brain [36–38]. Dystrophin is a large rod- shaped protein with a molecular weight of 427 kDa having four domains. Mutation in DMD gene can occur by deletion and duplication mutations. Deletion of DMD gene is more frequently found (60–65%) than

duplication (5–15%) [39] and the rest being small mutations involving frame-shift mutation, nonsense mutations, and insertion of repetitive sequences [40]. Two types of hotspots are observed in deletion category, one in the 5′ end and the other in central region, the latter majorly including in exons 45–55 with genomic break points. The 5′ end mutations hotspot includes exons 2–19 breakpoints, commonly found in introns 2–7, and goes downstream [41–44].

The clinical feature seen and its relation to the size or position of the mutation occurred in the gene are complex. The classical DMD symptoms can be observed with deletion of small exons such as exon 44. However, 50% of genes involved large mutation size corresponding to partial symptoms baring BMD [45–47]. The mutation in rod-shaped central and distal regions shows complex clinical features; there might be patients with isolated increase in CK or only myalgia and muscle cramps but no typical weakness present. These patients are categorized under in-frame deletion in exons 32–44, 48–51, and 48–53 and surprisingly had normal to near normal concentration of dystrophin [41, 48–50]. To investigate which genetic factors [amount of deletion or location or the mutation (occurred in disrupting reading frame or not)] correspond to the symptoms in patients, we divided patients based on their genetic profile and analyzed clinical history, features as well as their cardiac, autonomic profile to understand the type of mutations causing more morbidity in patients, thus facilitating the disease management in these patients. Based on our findings, we can conclude that mutation before exon 44 deletion results in no dystrophin protein formation, and in mutation after exon 44, partial dystrophin proteins could be produced. Thus, we kept 44 as our breakage point and checked whether our HRV/ECHO measures and clinical history could predict any story related to this phenomenon. However, there is no significant difference observed between the group before 44 and after 44, implying that irrespective of genetic makeup, if the protein formed was partially or completely absent, the autonomic changes happen irrespective of genetics. When looked at the age of presentation and age of onset of symptoms in the patients, there exists a difference in onset of symptoms between the groups before 44 that has early onset compared to after 44 group although not significant because of the outlier in the group which has the highest number of 8. Thus, there might be a difference caused by complete absence of the protein versus partial protein formed, but it was not evident in cardiac, autonomic changes as the changes usually starts later in life of the patients which needed to be investigated further in longitudinal studies.

Conclusion

HRV and echocardiography measures could be used as a biomarker in early stages of DMD before cardiac involvement. Sinus tachycardia and echocardiographic changes such as decreased IVD (d) and increased E m/s and E/A ratio hold as a marker in these patients even though its chamber dimension remains normal. Further longitudinal studies are required to underpin the significance of these findings in the management of DMD patients.

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Author Contribution AN collected and analyzed neurophysiological data and drafted the initial manuscript, taking inputs from all the authors. AG and RR collected and interpreted the ECHO data; MB and AG did the genetic analysis regarding the DMD sample. PV provided the statistical support, and NA, STN, and KU provided the supervision. All authors read, edited, and approved the final manuscript.

Data Availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics Approval The study was carried out in the Department of Neurophysiology in collaboration with the Department of Neurology, NIMHANS. The study obtained approval from the Institute Human Ethics Committee (IHEC): NIMH/DO/14th Ethics sub-committee (BS&NS) dated 26.10.2020. Since the age group of the subjects falls between 5 and 11 years, we have taken an assent form from the parents.

Consent to Participate Written informed consent and assent was obtained before recruiting the subjects for the study.

Consent for Publication All authors acknowledge their contribution and provide consent for the publication.

Conflict of Interest The authors declare no competing interests.

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