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ORIGINAL ARTICLES

Ambulatory Duchenne muscular dystrophy children: cross-sectional correlation between function, quantitative muscle ultrasound and MRI

Hala Abdulhady[1], Hossam M. Sakr[2], Nermine S. Elsayed[3], Tamer A. El-Sobky[4], Nagia Fahmy[5], Amr M. Saadawy[2], Heba Elsedfy[3]

[1] Department of Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [2] Department of Diagnostic and Interventional Radiology and Molecular Imaging, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [3] Department of Medical Genetics, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [4] Division of Pediatric Orthopedics, Department of Orthopedic Surgery, Faculty of Medicine, Ain Shams University, Cairo, Egypt; [5] Neuromuscular Unit, Department of Neuropsychiatry, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Duchenne muscular dystrophy (DMD) is a progressive genetic muscle disease. Quantitative muscle ultrasound (US), muscle MRI, and functional tools are important to delineate characteristics of muscle involvement. We aimed to establish correlations between clinical/functional and above-named imaging tools respecting their diagnostic and prognostic role in DMD children. A cross-sectional retrospective study of 27 steroid-naive, ambulant male children/adolescents with genetically-confirmed DMD (mean age, 8.8 ± 3.3 years). Functional performance was assessed using motor function measure (MFM) which assess standing/transfer (D1), proximal (D2) and distal (D3) motor function, and six-minute walk test (6MWT). Imaging evaluation included quantitative muscle MRI which measured muscle fat content in a specific location of right rectus femoris by mDixon sequence. Quantitative muscle US measured right rectus femoris muscle brightness in standardized US image as an indicator of muscle fat content. We found a highly significant positive correlation between the mean MFM total score and 6MWT (R = 0.537, p = 0.007), and a highly significant negative correlation between fat content by muscle US and MFM total score (R = -0.603, p = 0.006) and its D1 subscore (R =-0.712, p = 0.001), and a significant negative correlation between fat content by US and 6MWT (R = -0.529, p = 0.02), and a significant positive correlation between muscle fat content by mDixon MRI and patient's age (R = 0.617, p = 0.01). Quantitative muscle US correlates significantly with clinical/functional assessment tools as MFM and 6MWT, and augments their role in disease-tracking of DMD. Quantitative muscle US has the potential to act as a substitute to functional assessment tools.

Key words: dystrophinopathies, quantitative magnetic resonance imaging, muscle ultrasonography, motor function measure, 6-minute walk test

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Correspondence
Hossam M. Sakr
Department of Diagnostic and Interventional Radiology and Molecular Imaging Faculty of Medicine, Ain Shams University, Abbassia square, 11381 Cairo, Egypt.
E-mail: drhossam_sakr@med.asu.edu.eg

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Introduction

Duchenne muscular dystrophy (DMD) is a severe, progressive X-linked inherited disease that affects 1 in 3600-6000 live male births [1]. DMD oc-

curs as a result of mutations (mainly exon deletions) in the dystrophin gene (DMD; locus Xp21.2). The most common presenting feature is secondary deterioration of the motor milestones typically recognized at mid childhood. With time, untreated progressive muscle weakness, joint contractures, and cardio-pulmonary compromise affect quality of life. The disease eventually runs a fatal course by approximately 20 years of age [1,2]. This is especially true for untreated or steroid- naïve patients, whereas steroid-administered patients may show some amelioration of their clinical course. The management of DMD requires a multidisciplinary approach to improve the quality of life [2-4].

Therapeutic interventions have recently demonstrated intense upswings in modifying the natural history of DMD. These interventions include; therapies that slow the decline in muscle strength and function as glucocorticoids and therapies that target the pathology of DMD or improve muscle growth and regeneration [1,5]. Furthermore, gene therapies as gene-addition, exon-skipping, stop codon read through and genome-editing therapies aim at improving the expression and functionality of dystrophin protein. Other therapies work on a cellular level and aim at replacement of damaged muscle tissue. Both of the above therapies yielded encouraging preliminary results [6,7]. Nevertheless, these therapeutic gains have accentuated the need for consistent, valid and reliable assessment tools/ outcome measures to monitor responses to treatment and to act as prognostic indicators. Widely used clinical scales that measure the motor function and activities of ambulatory and non- ambulatory patients with DMD exist [8-10]. Recently significant interest in the use of magnetic resonance imaging (MRI) of muscles as whole-body MRI, quantitative MRI and magnetic resonance spectroscopy (MRS) to monitor disease progression has evolved [11-13]. MRI is a valuable non-invasive tool to reveal distinct pathologic patterns of various hereditary muscular diseases such as facioscapulohumeral muscular dystrophy [14], sarcoglycanopathies [15], congenital myopathies [14], muscular dystrophies [16], including DMD [14,17]. The use of quantitative muscle MRI and whole-body MRI have also been shown valuable to draw clinical-imaging-genetic correlations and identify ideal sites for muscle biopsy [11,14,16,17]. The combined use of quantitative muscle US and MRI showed promising results in regard to delineating disease stage and its relevance to functional status in facioscapulohumeral muscular dystrophy patients [18,19]. In DMD quantitative muscle MRI [20-22], and quantitative muscle ultrasound (US) [23,24], were found to be a satisfactory substitute to clinical/functional assessment tools as timed function tests and so forth in regard to monitoring disease progression.

Nevertheless, we are not aware of studies that investigated the clinical utility of quantitative muscle US and quantitative muscle MRI when used simultaneously with clinical functional tests in patients with DMD. The objectives of this study are: a) to assess the role of quantitative muscle US and muscle MRI as a potential diagnostic and prognostic tool in a series of ambulatory children with genetically-confirmed DMD; b) to draw relevant correlations between these two imaging modalities and clinical outcomes namely 6-minute walk test and motor function measure.

Materials and methods

This was a cross-sectional retrospective study. Twenty-seven steroid-naive, ambulant male children/adolescents with genetically-confirmed DMD were enrolled. The mean age of patients was 8.8 ± 3.3 years (range, 3.1 to 18). Exception to the reading frame rule occurred in one patient (18 years) with frameshift deletion of exon 3-7, who presented with Becker phenotype. This was likely due to either the presence of an alternative translation initiation site in exon 8 that was activated by the mutation, or due to genetic modifiers [25-27]. The functional performance of patients was assessed using the motor function measure (MFM) [28], and six-minute walk test (6MWT) [8]. MFM consists of 32 items (20 items for children < 7 years). It assesses all three dimensions of motor performance including standing and transfer (D1) subscore, axial and proximal motor function (D2), and distal motor function (D3). 6MWT is a commonly used timed functional test that also sufficiently monitors changes in muscle function. The imaging evaluation included quantitative muscle MRI, which measured muscle fat content in the right rectus femoris at the junction of the proximal 1/3 - distal 2/3 of thigh by mDixon sequence, and quantitative muscle US which measured muscle brightness in standardized US image as an indicator of muscle fat content. Twenty-five patients completed *both* functional tests; 21 patients completed the muscle US examination and 16 completed the muscle MRI examination. The study was approved by the Medical Ethics Research Committee of Faculty of Medicine, Ain Shams University, Egypt, number FMASU R 110 / 2021. Informed consent from participants was waived by the regulatory authority.

Assessment tools

The imaging and functional evaluation took place over two consecutive days where imaging evaluations were done prior to the functional tests. The patients who had sufficient cognitive ability to comply with verbal commands pertaining to the functional tests, were evaluated by the same examiner and approximately at the same time-point of the day. The functional tests were performed uniformly in the following order: MFM followed by 6MWT.

Motor function measure (MFM)

Items of the MFM-32 and MFM-20 are classified in three domains: D1: standing and transfers (13 items for the MFM-32 and 8 items for the MFM-20); D2: axial and proximal motor function (12 items for the MFM-32 and 8 items for the MFM-20); D3: distal motor function (7 items for the MFM-32 and 4 items for the MFM-20). Scores ranged from 0 to 3 as follows: (0) cannot perform the task, or cannot maintain the starting position, (1) initiated the task, (2) performs the movement incompletely, or completely but imperfectly (compensatory movements, position maintained for an insufficient duration of time, slowness, uncontrolled movement) and (3) performs the task fully and "normally" namely the movement is controlled, mastered, directed and performed at constant speed. The calculation of scores was expressed as a percentage in relation to the maximum score (for furthers details see https://mfm-nmd.org/).

Six-minute walk test

6MWT was performed according to the ATS guidelines, modified by having two examiners, one recording time and distances, and one staying close to the patient for safety issues [29].

Quantitative muscle MRI

mDixon sequence was taken using Achieva 1.5-T MR machine (Philips Medical Systems, The Netherlands) with the following parameters; (Slice thickness 10 mm, Spacing 5 mm, Number of phase encoding steps: 268, Acquisition matrix 272/0/0/268, Flip angle 15), the images were transferred to workstation (ViewForum R 6.3). mDixon sequence was done and the workstation was used to generate the four sequences (fat, water, in phase and out of phase). The mean fat and water signal of a region of interest (ROI) within the right rectus femoris muscle were measured at the junction between the proximal 1/3 and distal 2/3, between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella).

Quantitative muscle US

US in axial plane of the right rectus femoris muscle was taken at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella), using GE Logiq p7 machine (GE Healthcare, Waukesha, Wisconsin, USA) with high resolution linear probe 7-12 MHz. All the imaging parameters (including the probe frequency, depth, and gain) were constant, the images were transferred to a personal PC and mean grayscale (i.e. muscle echogenicity) was calculated using image histogram analysis software (ImageJ), and was used as an indication of muscle fat content. The quadriceps is a large proximal muscle which is frequently and severely involved by fatty infiltration in DMD and at a relatively early stage. Technique-wise its examination is practical [17].

Statistical methods

Data was revised for its completeness and consistency. Data entry was done on Microsoft Excel workbook. Quantitative data was summarized by mean, standard deviation while qualitative data was summarized by frequencies and percentages. The program used for data analysis was IBM SPSS statistics for windows version 23 (IBM Corp., Armonk, NY, USA). Chi-square test, student t test, and Pearson correlation coefficient were used in analysis of this study. Kappa test was done to measure level of agreement. A "P value" of less than 0.05 was considered statistically significant.

Results

Six patients (22%) were < 7 years of age. 19 patients (70%) had exon deletions, 2 (7%) had exon duplications, and 6 (22%) had small variants, two of which were nonsense and four were small deletions. Comparatively, patients with exon deletions had a lower mean MFM score without statistical significance. Tables I and II show the descriptive statistics of all clinical and imaging assessment tools used. We found a

Table I. Descriptive statistics of functional assessment tools.

	N	Mean	SD	IQR	Median	Min	Max
Age	27	8.7	3.4	6.6-10.2	8.6	3.1	18
MFM							
Total %	25	74.3	17.6	63.9-88.0	78.3	26.0	96.8
D1 %	25	57.7	26.8	37.1-76.9	61.5	0	97.4
D2 %	25	86.2	17.3	83.3-97.2	94.4	30.5	100
D3 %	25	84.8	13.5	75.5-95.2	85.7	42.8	100
6MWT (meters)	25	291.4	112.4	241.5-370	304.0	40	525

N: Number of patients; MFM: motor function measure; 6MWT: 6-minute walk test; SD: Standard Deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum

highly significant positive correlation between 6MWT and the mean total MFM score (R = 0.537, p = 0.007) and its D1 subscore (R = 0.751, p = 0.000) (Figs. 1,2). We found a highly significant negative correlation between fat content by muscle US and total MFM score (R = -0.603, p = 0.006) and its D1 subscore (R = -0.712, p = 0.001) (Figs. 3-5). Additionally, we found a significant negative correlation between fat content by muscle US and 6MWT score (R = -0.529, p = 0.02) (Tab. S3). This denotes that the higher the fat replacement in muscles of the thigh, the lower the scores of MFM and its D1 subscore and the lower meters achieved by patients in 6MWT. We found a significant positive correlation between patient's age and muscle fat content by mDixon MRI (R = 0.617, p = 0.01). However, we did not find a statistically significant correlation between both muscle fat content and muscle water content by mDixon MRI and neither total MFM score nor 6MWT (Tab. S4) (Figs. 6,7) (Fig. S9). Statistical correlations between age and both mean total MFM scores and subscores, and 6MWT scores were non-significant. For additional information, see (File S1). A graphical abstract of results is shown in Figure 8.

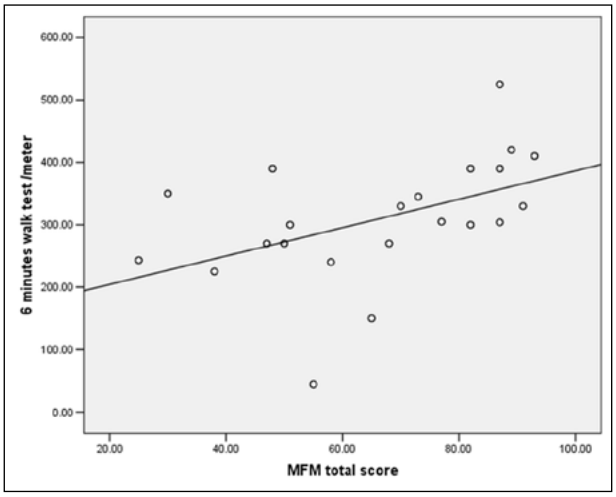

Figure 1. Correlation coefficient between Motor Function Measure (MFM) total score and 6-minute walk test showed a -p < 0.01- a highly significant **positive** correlation.

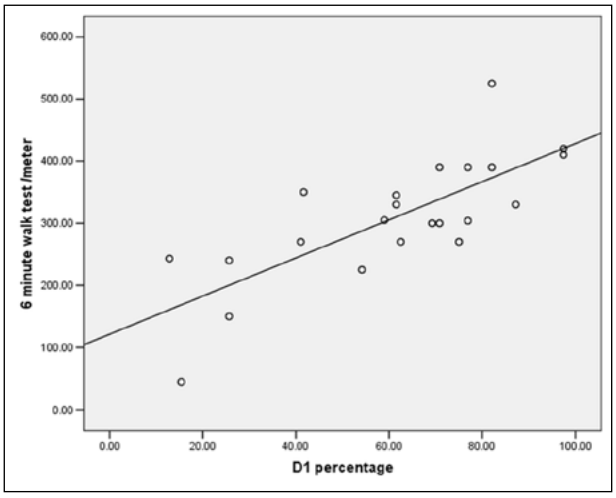

Figure 2. Correlation coefficient between motor function measure (MFM) D1 subscore percentage and 6-minute walk test showed -p < 0.01- a highly significant **positive** correlation.

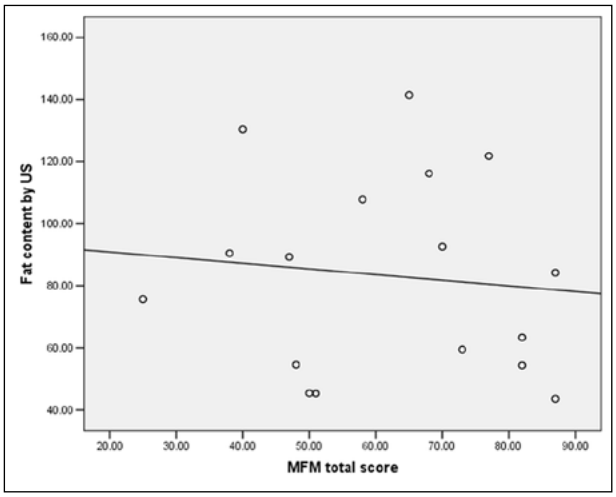

Figure 3. Correlation coefficient between motor function measure (MFM) total score and fat content by muscle ultrasound examination Showed a -p < 0.05 - a significant **negative** correlation.

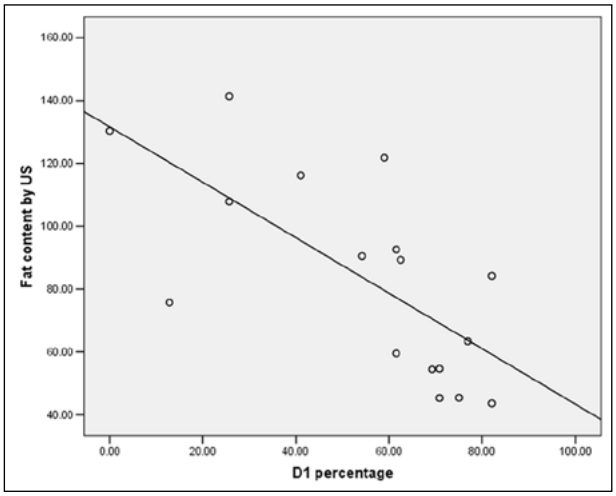

Figure 4. Correlation coefficient between motor function measure (MFM) D1 subscore percentage and fat content by muscle ultrasound examination -p < 0.01- a highly significant **negative** correlation.

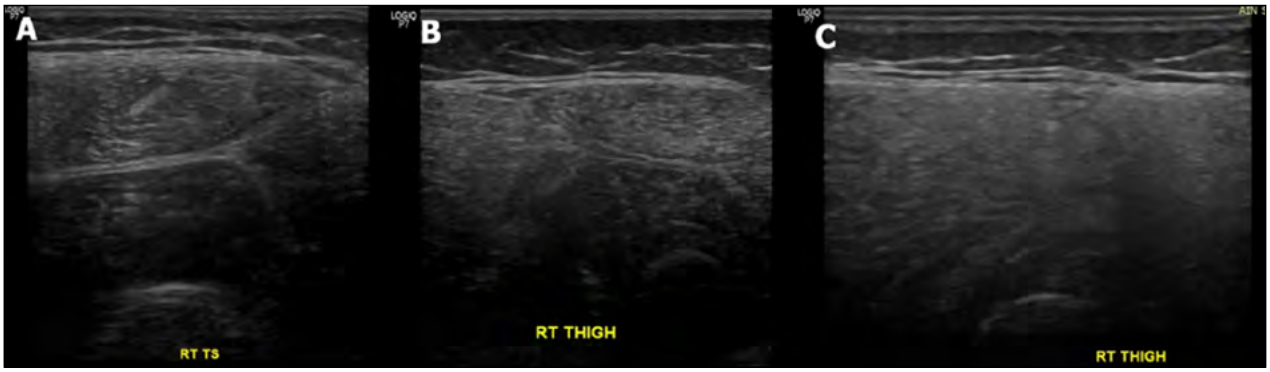

Figure 5A-C. Transverse US image of the right thigh of three different patients (A, B & C) showing different degrees of fatty infiltration of the quadriceps muscle as per increased echogenicity and blurred cortical surface of the femur. The mean histogram is 53, 76 and 80 respectively.

Table II. Descriptive statistics of imaging assessment tools.

	N	Mean	SD	IQR	Median	Min	Max
US	21	83.5	29.1	57.0-108.6	84.2	43.6	141.4
mDixon fat	16	77.9	60.7	44.1-86.5	61.6	30.2	272
mDixon water	16	375.1	145.7	321.2-468.7	431.3	52.1	535.7

N: Number of patients; SD: Standard Deviation; IQR: Interquartile range; Min: Minimum; Max: Maximum

Discussion

Corticosteroids remain the mainstay of treatment in DMD children whereas gene therapeutic modalities are emerging [30,31], among others [32]. Some of these gene therapeutics have received regulatory approval in the USA, Europe and Japan [33]. Both treatment modalities aim at improving the child's functional status. Recent advances in therapeutics for the treatment of DMD children have sparked significant interest in finding reliable clinical and imaging assessment tools to monitor responsiveness to these treatment modalities. The current study has incorporated various clinical and imaging assessment tools to explore their diagnostic and prognostic role in genetically-confirmed ambulatory and steroid-naïve DMD children and adolescents. Whereas quantitative US depends on measuring muscle echogenicity using the mean gray scale of a ROI within a selected muscle [23], quantitative MRI depends on measuring muscle fat replacement [22,34]. Both quantitative imaging tools have proved to aid clinical assessment fundamentally.

Our study implications are twofold. Firstly, it confirmed the widely acknowledged role of MFM and 6MWT as reliable and clinically meaningful assessment tools in DMD children [8,21,28]. Secondly, our study introduced statistically significant positive correlations between scores of these clinical assessments tools, namely MFM and 6MWT on the one hand and quantitative muscle US in DMD children. This highlights the potential clinical utility of quantitative muscle US for DMD monitoring, and underscores the role of quantitative muscle US as an important complement to clinical functional assessment tools. This role has been elucidated in both cross-sectional [35] and longitudinal [23] study designs capable of monitoring disease progression in DMD children. Contrastingly, quantitative muscle fat content measured by US did not show a statistically significant correlation with patient's age.

Quantitative muscle MRI -as per T2 mapping and mDixon- has been found helpful for delineating upper [12,36] and lower extremity [22,37,38] muscle involvement, and to be a satisfactory predictor of both strength of the investigated muscles or of the overall function of the investigated extremity in DMD children. However, our results did not establish a similarly significant correlation between the scores of these clinical assessments tools, namely MFM and 6MWT and quantitative muscle MRI namely muscle fat content and muscle water content by mDixon MRI. This may be attributed to the limitations of our study. Including the genotype profile in the analysis would have been insightful for interpreting the clinical-imaging results. However, this was beyond the scope and objectives of this study, and is reserved for a separate study. Missing data of some tests were sporadic and attributed to patient-related transport barriers among others.

North Star Ambulatory Assessment and Performance of the Upper Limb Module are reliable outcome tools which are designed to assess functional mobility [39] and upper limb performance [40,41] among DMD children. Both

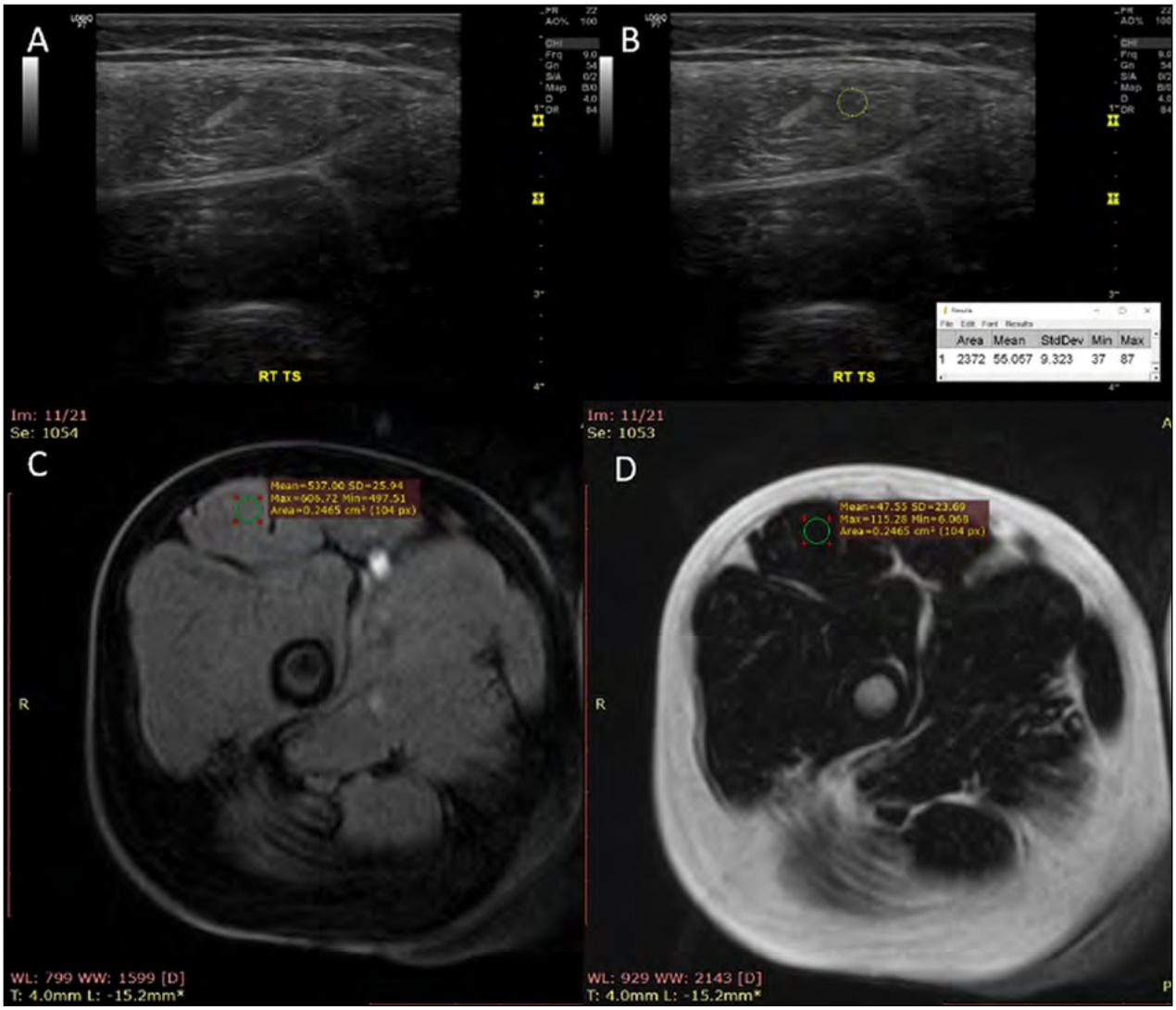

Figure 6A-D. A patient with Duchenne muscular dystrophy. A) Transverse US image of the right thigh taken at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella) showing fatty infiltration of the quadriceps muscle as shown by increased echogenicity and blurred cortical surface of the femur; B) the same image in (A) analyzed by ImageJ software with the ROI is within the rectus femoris muscle, mean histogram, 55; C,D) axial MRI image (mDixon sequence) with the region of interest (ROI) within the right rectus femoris muscle at the junction between the proximal 1/3 and distal 2/3 between its origin (from the anterior inferior iliac spine) and its insertion (at the upper pole of the patella), mean fat & water signals were 66 and 546 respectively.

outcome tools have been used to better characterize natural history, responsiveness to treatment and genotype-phenotype correlations in ambulatory [39,40] and non-ambulatory DMD children [41]. Although these outcome tools were not implementedin our cross-sectional study, we believe they represent valid substitutes for such.

Study limitations

Our study contained multiple clinical and imaging assessment tools (dependent variables). Additionally, its retrospective and cross-sectional nature does not allow for complete bias control in terms of standardization of patient characteristics among others. Moreover, longitudinal study designs have a greater potential to consolidate evidence for the use of both quantitative muscle US and muscle MRI as a substitute or a supplement to functional assessment tools in disease tracking and monitoring response to treatment of DMD patients. Further, the diversity of clinical assessment tools in general and functional tools in specific used across

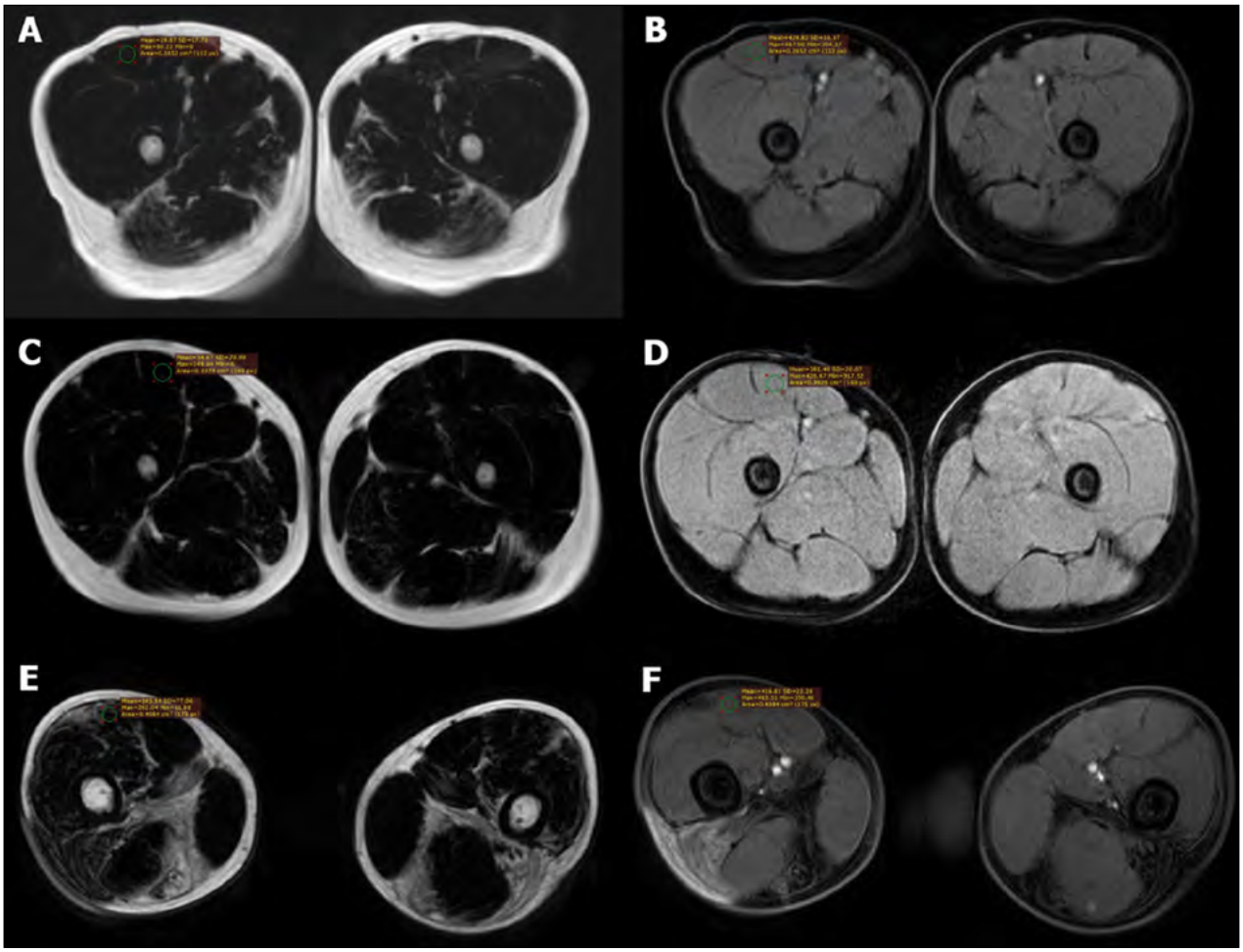

Figure 7A-F. Axial MRI image (mDixon sequence) in three different patients with DMD showing fat (A, C & E) and water (B, D & F) signal. The mean fat signals were 29, 35 and 143 respectively while those of water were 430, 351 and 416 respectively.

studies, need to be considered when interpreting the imaging-clinical correlation results [42]. Interestingly, quantitative muscle fat content measured by mDixon MRI showed a statistically positive correlation with patient's age. This is a clinically meaningful correlation, and it supports the assumption that the above-noted non-significant correlations between the clinical MFM and 6MWT scores, and quantitative fat content by mDixon MRI is mainly due study limitations. Additionally, of note is that statistical significance does not necessary equate to clinical significance. Consequently, all findings should be interpreted cautiously, and within clinical context. Results from the assessment of a single muscle by quantitative MRI and US, may not be fully representative of the overall muscle pathology.

Conclusions

Quantitative muscle US correlates significantly with clinical/functional assessment tools as MFM and 6MWT, and augments their promising role in disease tracking in DMD children. In that regard, quantitative muscle US has the potential to act as a complement to functional assessment tools. The presence of multiple clinical and imaging assessment tools (dependent variables) and study design-related limitations may have underpowered our statistical correlations of quantitative muscle MRI.

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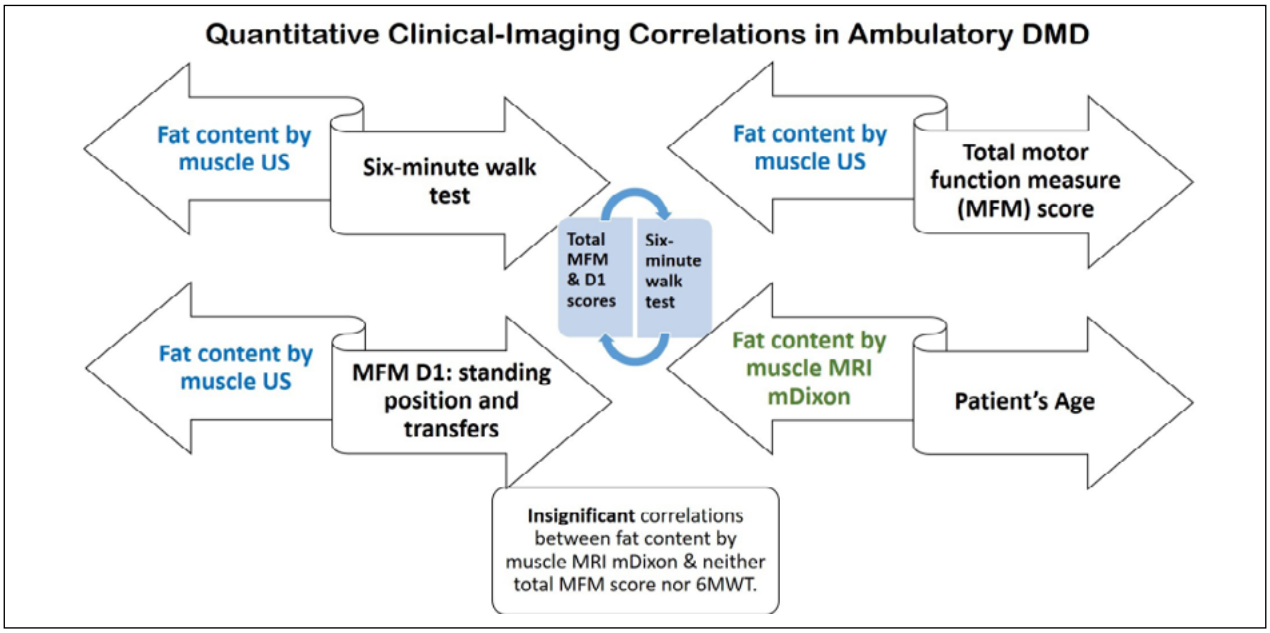

Figure 8. Graphical abstract: divergent arrows represent negative correlations while convergent the arrow represents a positive correlation.

The current manuscript has been posted on a preprint server (https://doi.org/10.1101/2021.08.17.21262119).

Conflict of interest statement

None of the Authors has any conflict of interest to disclose. No benefits in any form were received or will be received from a commercial party related directly or indirectly to the subject of this article.

Author's contributions

Conceptualization and design of study: HA, TAE, HMS, NSE; Acquisition and analysis of data: HA, NSE, HMS; Interpretation of data: NSE, HMS, NF, AMS, HE; Drafting the work: TAE assisted by NSE, HMS; revising it critically for important intellectual content; HA, HMS, NSE, NF, AMS, HE; All Authors approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical consideration

This study was approved by the Institutional Ethics Committee of the Faculty of medicine, Ain Shams University (FMASU R110/2021).

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

Informed consent from participants was waived by the regulatory authority

References

1 Falzarano MS, Scotton C, Passarelli C, et al. Duchenne muscular dystrophy: from diagnosis to therapy. Molecules 2015;20:18168-18184. https://doi.org/10.3390/molecules201018168

2 Suthar R, Sankhyan N. Duchenne muscular dystrophy: a practice update. Indian J Pediatr 2018;85:276-281. https://doi.org/10.1007/s12098-017-2397-y

3 Apkon SD, Alman B, Birnkrant DJ, et al. Orthopedic and surgical management of the patient with Duchenne muscular dystrophy. Pediatrics 2018;142(Suppl 2):S82-S89. https://doi.org/10.1542/peds.2018-0333J

4 Salera S, Menni F, Moggio M, et al. Nutritional challenges in Duchenne muscular dystrophy. Nutrients. 2017;9:594. https://doi.org/10.3390/nu9060594

5 Liew WKM, Kang PB. Recent developments in the treatment of Duchenne muscular dystrophy and spinal muscular atrophy. Ther Adv Neurol Disord 2013;6:147-160. https://doi.org/10.1177/1756285612472386

6 Verhaart IEC, Aartsma-Rus A. Therapeutic developments for Duchenne muscular dystrophy. Nat Rev Neurol 2019;15:373-386. https://doi.org/10.1038/s41582-019-0203-3

7 Barthélémy F, Wein N. Personalized gene and cell therapy for Duchenne Muscular Dystrophy. Neuromuscul Disord 2018;28:803-824. https://doi.org/10.1016/j.nmd.2018.06.009

8 Mcdonald CM, Henricson EK, Abresch RT, et al. The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: longitudinal natural history observations over 48 weeks from a multicenter study. Muscle Nerve 2013;48:343-356. https://doi.org/10.1002/mus.23902

9 Mayhew A, Cano S, Scott E, et al. Moving towards meaningful measurement: rasch analysis of the North Star Ambulatory Assessment in Duchenne muscular dystrophy. Dev Med Child Neurol 2011;53:535-542. https://doi.org/10.1111/j.1469-8749.2011.03939.x

10 Bushby K, Finkel R, Birnkrant D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77-93. https://doi.org/10.1016/S1474-4422(09)70271-6

11 Warman Chardon J, Díaz-Manera J, Tasca G, et al. MYO-MRI diagnostic protocols in genetic myopathies. Neuromuscul Disord 2019;29:827-841. https://doi.org/10.1016/j.nmd.2019.08.011

12 Willcocks RJ, Triplett WT, Forbes SC, et al. Magnetic resonance imaging of the proximal upper extremity musculature in boys with Duchenne muscular dystrophy. J Neurol 2017;264:64-71. https://doi.org/10.1007/s00415-016-8311-0

13 Senesac CR, Lott DJ, Forbes SC, et al. Longitudinal evaluation of muscle composition using magnetic resonance in 4 boys with Duchenne muscular dystrophy: case series. Phys Ther 2015;95:978-988. https://doi.org/10.2522/ptj.20140234

14 Leung DG. Magnetic resonance imaging patterns of muscle involvement in genetic muscle diseases: a systematic review. J Neurol 2017;264:1320-1333. https://doi.org/10.1007/s00415-016-8350-6

15 Tasca G, Monforte M, Díaz-Manera J, et al. MRI in sarcoglycanopathies: a large international cohort study. J Neurol Neurosurg Psychiatry 2018;89:72-77. http://dx.doi.org/10.1136/jnnp-2017-316736

16 Sakr HM, Fahmy N, Elsayed NS, et al. Whole-body muscle MRI characteristics of LAMA2-related congenital muscular dystrophy children: an emerging pattern. Neuromuscul Disord 2021;31:814-823. https://doi.org/10.1016/j.nmd.2021.06.012

17 Polavarapu K, Manjunath M, Preethish-Kumar V, et al. Muscle MRI in Duchenne muscular dystrophy: evidence of a distinctive pattern. Neuromuscul Disord 2016;26:768-774. https://doi.org/10.1016/j.nmd.2016.09.002

18 Mul K, Horlings CGC, Vincenten SCC, et al. Quantitative muscle MRI and ultrasound for facioscapulohumeral muscular dystrophy: complementary imaging biomarkers. J Neurol 2018;265:2646-2655. https://doi.org/10.1007/s00415-018-9037-y

19 Janssen BH, Pillen S, Voet NB, et al. Quantitative muscle ultrasound versus quantitative magnetic resonance imaging in facioscapulohumeral dystrophy. Muscle Nerve 2014;50:968-975. https://doi.org/10.1002/mus.24247

20 Sherlock SP, Zhang Y, Binks M, et al. Quantitative muscle MRI biomarkers in Duchenne muscular dystrophy: cross-sectional correlations with age and functional tests. Biomark Med 2021;15:761-773. https://doi.org/10.2217/bmm-2020-0801

21 Nagy S, Schädelin S, Hafner P, et al. Longitudinal reliability of outcome measures in patients with Duchenne muscular dystrophy. Muscle Nerve 2020;61:63-68. https://doi.org/10.1002/mus.26690

22 Bonati U, Hafner P, Schädelin S, et al. Quantitative muscle MRI: a powerful surrogate outcome measure in Duchenne muscular dystrophy. Neuromuscul Disord 2015;25:679-685. https://doi.org/10.1016/j.nmd.2015.05.006

23 Zaidman CM, Wu JS, Kapur K, et al. Quantitative muscle ultrasound detects disease progression in Duchenne muscular dystrophy. Ann Neurol 2017;81:633-640. https://doi.org/10.1002/ana.24904

24 Jansen M, van Alfen N, Nijhuis van der Sanden MW, et al. Quantitative muscle ultrasound is a promising longitudinal follow-up tool in Duchenne muscular dystrophy. Neuromuscul Disord 2012;22:306-317. https://doi.org/10.1016/j.nmd.2011.10.020

25 Aartsma-Rus A, Ginjaar IB, Bushby K. The importance of genetic diagnosis for Duchenne muscular dystrophy. J Med Genet 2016;53:145-151. https://doi.org/10.1136/jmedgenet-2015-103387

26 Vo AH, McNally EM. Modifier genes and their effect on Duchenne muscular dystrophy. Curr Opin Neurol 2015;28:528-534. https://doi.org/10.1097/WCO.0000000000000240

27 Winnard AV, Mendell JR, Prior TW, et al. Frameshift deletions of exons 3-7 and revertant fibers in Duchenne muscular dystrophy: mechanisms of dystrophin production. Am J Hum Genet 1995;56:158-166. PMID: 7825572

28 Vuillerot C, Girardot F, Payan C, et al. Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. Dev Med Child Neurol 2010;52:60-65. https://doi.org/10.1111/j.1469-8749.2009.03316.x

29 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-117. https://doi.org/10.1164/ajrccm.166.1.at1102

30 Rivera SR, Jhamb SK, Abdel-Hamid HZ, et al. Medical management of muscle weakness in Duchenne muscular dystrophy. PLoS One 2020;15:e0240687. https://doi.org/10.1371/journal.pone.0240687

31 Frank DE, Schnell FJ, Akana C, et al. Increased dystrophin production with golodirsen in patients with Duchenne muscular dystrophy. Neurology 2020;94:e2270-e2282. https://doi.org/10.1212/WNL.0000000000009233

32 Nagy S, Hafner P, Schmidt S, et al. Tamoxifen in Duchenne muscular dystrophy (TAMDMD): study protocol for a multicenter, randomized, placebo-controlled, double-blind phase 3 trial. Trials 2019;20:637. https://doi.org/10.1186/s13063-019-3740-6

33 Duan D, Goemans N, Takeda S, et al. Duchenne muscular dystrophy. Nat Rev Dis Primers 2021;7:13. https://doi.org/10.1038/s41572-021-00248-3

34 Burakiewicz J, Sinclair CDJ, Fischer D, et al. Quantifying fat replacement of muscle by quantitative MRI in muscular dystrophy. J Neurol 2017;264:2053-2067. https://doi.org/10.1007/s00415-017-8547-3

[35] Shklyar I, Geisbush TR, Mijialovic AS, et al. Quantitative muscle ultrasound in Duchenne muscular dystrophy: a comparison of techniques. Muscle Nerve 2015;51:207-213. https://doi.org/10.1002/mus.24296

[36] Forbes SC, Arora H, Willcocks RJ, et al. Upper and lower extremities in Duchenne muscular dystrophy evaluated with quantitative MRI and Proton MR spectroscopy in a multicenter cohort. Radiology 2020;295:616-625. https://doi.org/10.1148/radiol.2020192210

[37] Naarding KJ, Reyngoudt H, van Zwet EW, et al. MRI vastus lateralis fat fraction predicts loss of ambulation in Duchenne muscular dystrophy. Neurology 2020;94:e1386-e1394. https://doi.org/10.1212/WNL.0000000000008939

[38] Yin L, Xie ZY, Xu HY, et al. T2 mapping and fat quantification of thigh muscles in children with Duchenne muscular dystrophy. Curr Med Sci 2019;39:138-145. https://doi.org/10.1007/s11596-019-2012-8

[39] Muntoni F, Domingos J, Manzur AY, et al. Categorising trajectories and individual item changes of the North Star Ambulatory Assessment in patients with Duchenne muscular dystrophy. PLoS One 2019;14:e0221097. https://doi.org/10.1371/journal.pone.0221097

[40] Mayhew AG, Coratti G, Mazzone ES, et al. Performance of upper limb module for Duchenne muscular dystrophy. Dev Med Child Neurol 2020;62:633-639. https://doi.org/10.1111/dmcn.14361

[41] Demir G, Bulut N, Yılmaz Ö, et al. Manual ability and upper limb performance in nonambulatory stage of Duchenne muscular dystrophy. Arch Pediatr 2020;2:304-309. https://doi.org/10.1016/j.arcped.2020.06.007

[42] Senesac CR, Lott DJ, Willcocks RJ, et al. Lower extremity functional outcome measures in Duchenne muscular dystrophy – a Delphi survey. J Neuromuscul Dis 2019;6:75-83. https://doi.org/10.3233/JND-180337

Supplemental material

Table S3. Correlation coefficient between age, functional assessment tools namely MFM and 6MWT and muscle fat content by ultrasound.

	US fat content
MFM total score	**R = -0.603** **P = 0.006****
D1 subscore	**R =-0.712** **P = 0.001****
D2 subscore	R = -0.127 P = 0.6
D3 subscore	R = 0.101 P = 0.6
Age	R = 0.237 P = 0.3
6MWT	**R = -0.529** **P = 0.02***

MFM: motor function measure; 6MWT: 6-minute walk test

Table S4. Correlation coefficient between age, functional assessment tools namely MFM and 6MWT and muscle fat and water content by mDixon MRI.

N = 16	**mDixon Fat**	**mDixon water**
Age	**R = 0.617** **P = 0.01***	R = -0.208 P = 0.4
MFM total score	R = -0.140 P = 0.6	R = -0.005 P = 0.9
D1 sub score	R = -0.235 P = 0.3	R = 0.056 P = 0.8
D2 sub score	R = 0.164 P = 0.5	R = -0.239 P = 0.4
D3 sub score	R = 0.152 P = 0.5	R = -0.228 P = 0.4
6MWT	R = -0.208 P = 0.4	R = 0.224 P = 0.4

*****Statistically significant; MFM: motor function measure; 6MWT: 6-minute walk test

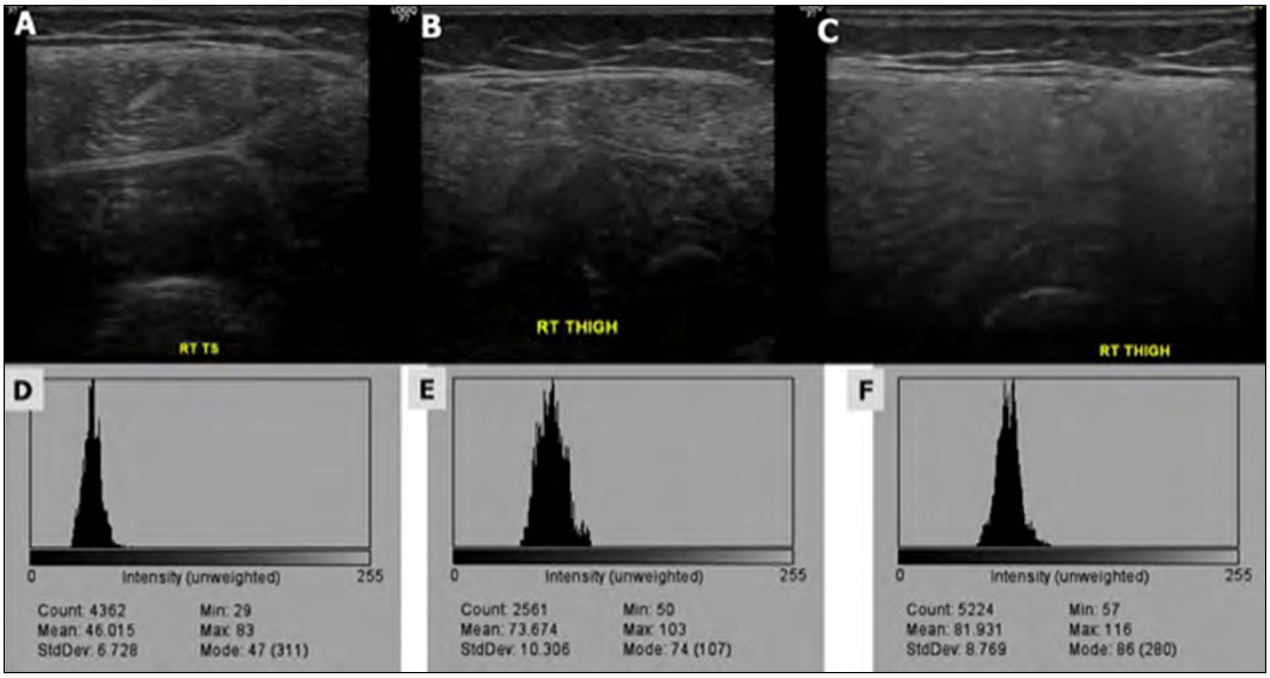

Figure S9. Transverse US image of the right thigh of three different children with DMD.

File S1. Tables S5 to S225

Table S5. Age distribution of studied patients.

N = 27	No.	%	
Age			
Below 7 years	7	25.9	
7 years or more	20	**74.1**	
	Mean	**SD**	**Range**
Age (years)	8.7	3.4	3.1-18
Median age	**8.6**		

This Table shows that 74.1% of the patients were 7 years or more of age. The mean age of patients was 8.7 years while the median age is 8.6 years.

Table S6. Distribution of type of mutation by molecular findings.

N = 27	No.	%
Deletion	19	**70.4**
Duplication	2	7.4
Small mutation	6	22.2

This Table shows that 70.4% of the cases had gene deletion and 22.2% had small mutation while 7.4% of the patients had duplication.

Table S7. Comparison between gene abnormality and the mean age of patients.

Age		Mean	SD	F	P
Deletion	N = 19	**9.3**	3.9		
Duplication	N = 2	7.5	0.07	0.7	0.4
Small mutation	**N = 6**	7.6	1.4		

P > 0.05 not significant. This Table shows a higher mean age among patients with deletion compared to other groups with no significant difference statistically. This table shows a lower mean age among cases with duplication or small mutation compared to deletion group with no statistically significant difference.

Table S8. Comparison between age of patients and type of gene mutation.

Age	Deletion No. %	Duplication No. %	Small mutation No. %	X2	P
Below 7 N = 7	5 71.4	0	1 28.6	0.8	0.6
7 or more N = 20	14 70.0	2 **10.0**	4 20.0		

P > 0.05 not significant. This table shows a higher percentage of small mutation among cases in group of 7 or above compared to cases below 7 years of age but the difference is not significant statistically.

Table S9. Comparison between the age groups in regard to the mean MFM D1 subscore.

D1 percentage	Mean	SD	t	P
Below 7 N = 7	62.5	12.5	0.5	0.8
7 or more N = 20	55.8	30.7		

P > 0.05 not significant. MFM: motor function measure. This table shows no statistically significant difference between the two age groups as regards D1 subscore of the motor function measure. A higher mean D1 subscore in younger age group compared to older age group with no statistically significant difference.

Table S10. Comparison between the age groups in regard to the mean MFM D2 subscore.

D2 percentage	Mean	SD	t	P
Below 7 N = 7	81.5	17.1	0.8	0.4
7 or more N = 20	88.1	17.5		

P > 0.05 not significant. MFM: motor function measure. This table shows a higher mean D2 subscore among older age group with no statistically significant difference.

Table S11. Comparison between the age groups in regard to the mean MFM D3 subscore.

D3 percentage	Mean	SD	t	P
Below 7 N = 7	79.7	9.4	1.1	0.2
7 or more N = 20	86.7	14.5		

P > 0.05 not significant. MFM: motor function measure. This table shows a higher mean D3 sub score among older age group patients with no statistically significant difference.

Table S12. comparison between the age groups in regard to the mean MFM total score.

MFM total score (%)	Mean	SD	t	P
Below 7 N = 7	73.5	13.2	0.1	0.8
7 or more N = 20	74.7	19.3		

P > 0.05 not significant. MFM: motor function measure. This table shows no significant difference statistically between the two age groups as regards the mean total motor function measure percentage.

Table S13. Comparison between the age groups as regards the 6-minute walk test (6MWT).

6MWT	Mean	SD	t	P
Below 7 N = 7	302.0	54.8	0.2	0.7
7 or more N = 18	287.3	129.3		

P > 0.05 not significant. This table shows no significant difference statistically between the two age groups as regards the mean meters of the 6-minute walk test. The table shows a lower mean value for 6-minute walk test among cases above seven years of age compared to younger age group with no statistically significant difference.

Table S14. Correlation coefficient between the age and MFM scores i.e. total score and subscores.

	Age
MFM total score	R = 0.044 P = 0.8
D1 subscore	R = -0.007 P = 0.9
D2 subscore	R = 0.063 P = 0.7
D3 subscore	R = 0.163 P = 0.4

P > 0.05 not significant. This table shows no significant correlation between the total MFM score or the subscores and the age of DMD patients.

Table S15. Distribution of rapid decline of ambulation according to the 6-minute walk test. Cut-off value 350 meters.

N = 25	No. %	95% CI
No Rapid decline	**7** **28**	**12.0-49.0**
Rapid decline	**18** **72**	**50.6-87.9**

This Table shows that 72% of the patients have rapid decline in ambulation in the next 48 months as predicted by the results of the 6-minute walk test below 350 m while 28% have no rapid decline in ambulation.

Table S16. Distribution of rapid decline of ambulation according to the 6-minute walk test. Cut-off value 300 meters.

N = 25	No. %	95% CI
No Rapid decline	**15** **60**	**38-78**
Rapid decline	**10** **40**	**21-61**

This Table shows that 40% of the patients have rapid decline in ambulation in the next 48 months as predicted by the results of the 6-minute walk test below 300 m while 60% have no rapid decline in ambulation.

Table S17. Correlation coefficient between the MFM results, age, 6-minute walk test and mDixon fat and water findings of MRI.

N = 16	mDixon Fat	mDixon water
Age	**R = 0.617** **P = 0.01***	R = -0.208 P = 0.4
MFM total score	R = -0.140 P = 0.6	R = -0.005 P = 0.9
D1 sub score	R = -0.235 P = 0.3	R = 0.056 P=0.8
D2 sub score	R = 0.164 P = 0.5	R = -0.239 P = 0.4
D3 sub score	R = 0.152 P=0.5	R = -0.228 P = 0.4
6MWT	R = -0.208 P = 0.4	R = 0.224 P = 0.4

*P < 0.05 significant. This table shows a significant positive correlation between mDixon fat values and age of patients. This Table shows no correlation between mDixon fat values and MFM, 6-minute walk test values. This table shows no correlation between mDixon water content and MFM, 6-minute walk test.

Table S18. Correlation coefficient between the MFM results, age, 6-minute walk test and MRS Press and MRS steam findings of MRI**.**

N = 16	MRS Press	MRS steam
Age	R = 0.058 P = 0.8	R = 0.047 P = 0.8
MFM total score	**R = 0.651** **P = 0.009****	**R = 0.660** **P = 0.01***
D1 sub score	**R = 0.594** **P = 0.03***	R = 0.469 P = 0.09
D2 sub score	**R = 0.538** **P = 0.03***	R = 0.393 P = 0.1
D3 sub score	**R = 0.534** **P = 0.04***	**R = 0.791** **P = 0.001***
6MWT	R = 0.373 P = 0.1	R = 0.310 P = 0.2

*P < 0.05 Significant, **P < 0.01 highly significant.
This Table shows a highly significant positive correlation between MRS press values and total MFM score.
This Table shows a significant positive correlation between MRS press values and D1, D2, D3 subscores of MFM. This Table shows a significant positive correlation between MRS steam and total MFM score. This table shows a highly **significant positive correlation** between D3 subscore and the MRS steam findings of MRI.

Table S19. Correlation coefficient between the MFM results, age, 6-minute walk test and DTI finding of MRI results**.**

N = 14	DTI. FA	DTI. ADC
MFM total score	R = -0.438 P = 0.1	R = 0.417 P = 0.1
D1 sub score	**R = -0.640** **P = 0.01***	**R = 0.617** **P = 0.01***
D2 sub score	R = -0.283 P = 0.3	R = 0.246 P = 0.3
D3 sub score	R = 0.004 P = 0.9	R = 0.032 P = 0.9
age	R = 0.402 P = 0.1	R = -0.404 P = 0.1
6MWT	**R = -0.701** **P = 0.004****	**R = 0.782** **P = 0.001****

*P < 0.05 Significant, **P < 0.01 highly significant. This Table shows a significant negative correlation of D1 subscore and the DTI FA findings. Which denotes the higher D1 scores associated with lower DTI FA scores. This Table shows a significant positive correlation of D1 subscore and the DTI ADC findings. Which denotes the higher D1 scores associated with high DTI ADC scores.

Table S20. Comparison between type of gene mutation and the screening of patients with 6-minute walk test. Cut-off value 300.

300	No rapid decline No. %	Rapid decline No. %	X2	P
Deletion N = 18	9 50.0	9 **50.0**	2.9	0.08
Others N = 7	6 85.7	1 14.3		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with deletion 50% compared to 14.3% among patients with other gene mutation with border line significance.

Table S21. Comparison between type of gene mutation and the screening of patients with 6-minute walk test. Cut-off value 350.

350	No rapid decline No. %	Rapid decline No. %	X2	P
Deletion N = 18	4 22.2	14 **77.8**	1.0	0.3
Others N = 7	3 42.9	4 57.1		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with small mutation or duplication 85.7% compared to 50% among patients with deletion with border line significance.

Table S22. Correlation coefficient between the ultrasound findings of fat content and the MRI findings of patients with DMD.

	US fat content
mDixon Fat	**R = 0.743** **P = 0.002****
MRS steam	R = -0.139 P = 0.6
MRS press	R = 0.294 P = 0.3
DTI FA	R = 0.396 P = 0.1
DTI ADC	R = -0.342 P = 0.2

*P < 0.05 Significant, **P < 0.01 highly significant. This table shows a highly significant positive correlation between ultrasound fat content and the mDixon fat content among patients with DMD. This table shows no correlation between ultrasound fat content and MRS steam, MRS press, DTI FA and DTI ADC.

Table S23. Comparison between type of gene mutation and the ultra sound grouping of patients (fat content of muscles). Cut-off value 84 (median value of US).

	Low fat content **No.** **%**	**High fat content** **No. %**	**X2**	**P**
Deletion N = 13	6 46.2	7 53.8	0.02	0.8
Others N = 8	4 50.0	4 50.0		

P > 0.05 not significant

Table S24. Comparison between US fat content and the screening of patients with 6-minute walk test. Cut-off value 300 for screening test.

US	**No rapid decline** **No.** **%**	**Rapid decline** **No.** **%**	**X2**	**P**
Low fat N = 10	7 70.0	3 30.0	2.5	0.1
High fat N = 9	3 33.3	6 **66.7**		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with high fat content in US 66.7% compared to 30% among patients with low fat content and the difference is border line significant.

Table S25. Comparison between US fat content and the screening of patients with 6-minute walk test. Cut-off value 350 for screening test.

350	**No rapid decline** **No.** **%**	**Rapid decline** **No.** **%**	**X2**	**P**
Low fat N = 10	3 30.0	7 70.0	1.0	0.3
High fat N = 9	1 11.1	8 88.9		

P > 0.05 not significant. This Table shows a higher percentage of rapid decline among patients with high fat content 88.9% compared to 70% among patients with low fat content with no significant difference statistically.