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Original Article

Semi-quantitative assessment of lower limb MRI in dystrophinopathy

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Abstract: Dystrophinopathy is a group of inherited muscular disorders, including Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XLDCM), and manifesting/non-manifesting DMD/BMD carriers, which predominantly affects lower limbs. We studied the clinical and lower limb MRI data to assess muscle preservation and muscle choice of dystrophinopathy. Forty-two patients with dystrophinopathy underwent clinical, laboratory, and MRI examinations. Semi-quantitative data of muscle strength and muscular MRI were collected and statistical analyzed. Logistic regression was used to assess MRI scores and clinical variables. MRI changes in thigh were more significant than in lower leg ($P < 0.001$). In addition, the anterior group was more severe than the posterior group in thigh, whereas in lower leg, the posterior was more severe than the anterior. At thigh level, the vastus lateralis was predominantly involved, and the gracilis was less involved. At lower leg level, the long fibular muscle was more involved than the anterior tibial muscle. BMD patients had similar MRI characteristics as DMD. The logistic regression analysis showed that there are three variables (age, weight, proximal muscle strength) affected thigh muscle injury scores. MRI provides a simple, non-invasive means of detecting subtle, subclinical changes in individual muscles that reflects the progression of dystrophinopathy. The selective muscle involvement observed in lower limb MRI can provide diagnostic evidence for DMD and support the argument to initiate therapy at earlier ages.

Keywords: Dystrophinopathy, duchenne muscular dystrophy, becker muscular dystrophy, skeletal muscle biopsy, muscle MRI

Introduction

Dystrophinopathy is caused by mutations in the *DMD* gene, which encodes the protein dystrophin, and is located on the X-chromosome (Xp21). The dystrophinopathy spectrum encompasses Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XLDCM), which includes asymptomatic hyper-creatine kinase (CK) emia, cramps, myalgia, and quadriceps myopathy), XLDCM, and manifesting/non- manifesting DMD/BMD carriers [1]. DMD is the most severe dystrophinopathy that begins in early childhood and affects 1 in every 3,500-4,000 male births. *DMD* gene mutations lead to defects or deletion of the protein dystrophin, an important cytoskeletal muscle component. This abnormality results in structural fragility, membrane permeability, metabolic crisis, and progressive myocyte degeneration. Boys with DMD experience progressive muscle weakness and wasting that results in wheelchair dependence in later teenage years, cardiorespiratory compromise, and eventually death during young adulthood. BMD has a slower progression and a longer life expectancy. BMD patients ambulate independently until at least 16 years of age, with a mean age of 30 years.

Muscle involvement in DMD is characterized by repetitive cycles of injury, inflammation, and repair that results in progressive degeneration, necrosis, and regeneration of myocytes by fat and connective tissue. One of the hallmarks of DMD is a significant loss in bulk of the proximal muscles, including those of the pelvis and thighs. To precisely diagnose DMD, the clinician is required to make appropriate judgments regarding the distribution of the affect-

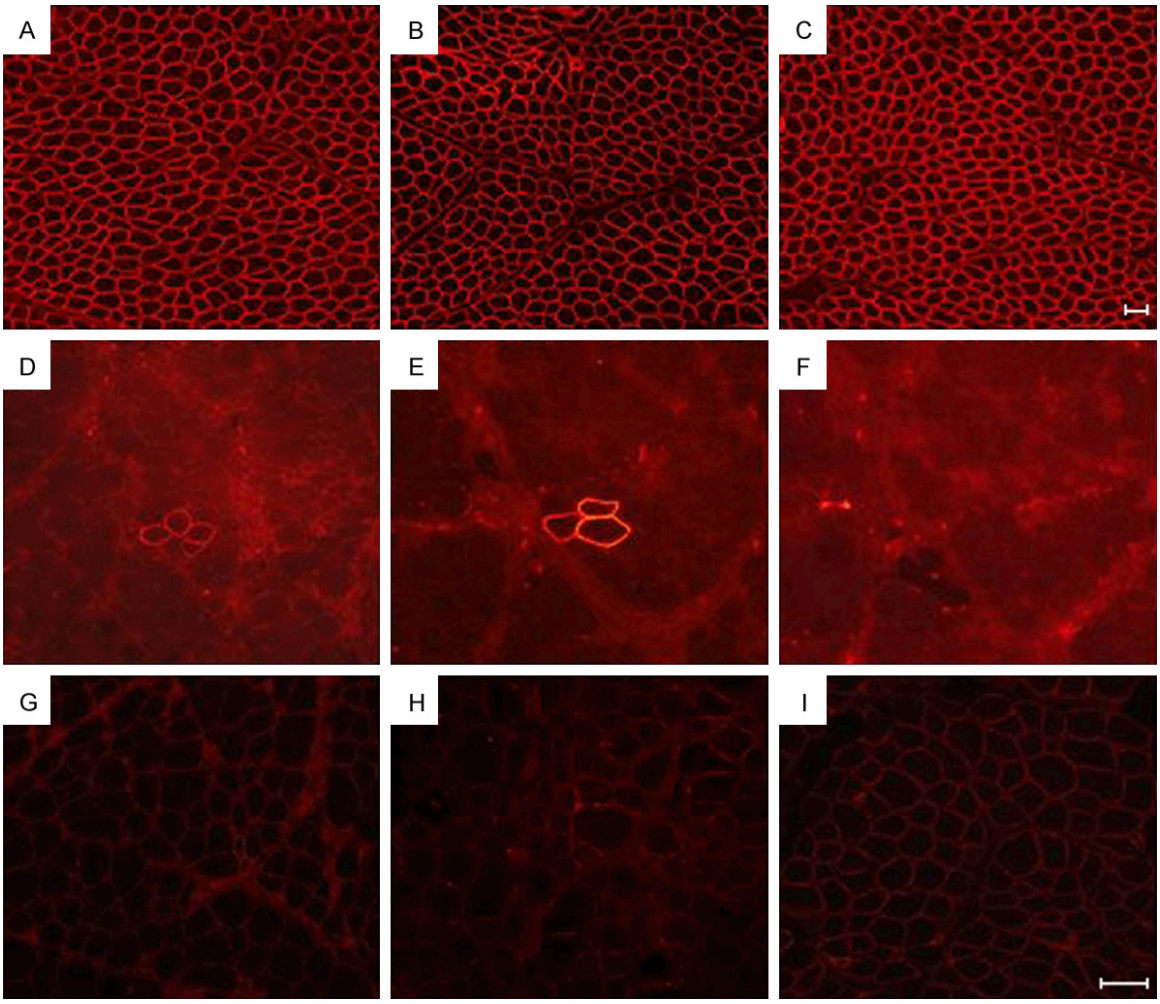

Figure 1. Immunofluorescence staining of DMD and BMD patients: Normal control (A-C): normal dystrophin presents on the sarcolemma; DMD (D-F): dystrophin is absent on the sarcolemma; BMD (G-I): decreased dystrophin on the sarcolemma. (Bar = 100 μm).

ed skeletal muscles, muscular strength, and capacity. Ultrasound (US) has been used as a well-established and validated diagnostic imaging method for evaluating muscle involvement, but its application is limited to superficial muscle groups because it is difficult to display deeper structures and multiple muscle groups that overlap. Computed tomography (CT) has also been widely used to evaluate the presence and extent of changes in myopathy, but its high radiation dose and limited soft tissue contrast makes the application obsolete, particularly in children [2]. MRI provides a high soft tissue contrast, thereby allowing excellent assessment of muscles shape, volume, and tissue architecture [3]. The inflammatory changes and repair observed during the initial stages of the disease are followed by fatty tissue infiltration. This progressive fatty tissue infiltration and muscle weakness reportedly leads to loss of ambulation between the ages of 8-12 years [4, 5].

Few pelvis and thigh MRI studies in DMD patients have shown a characteristic pattern of fatty infiltration that spares the gracilis, sartorius, and semimembranosus muscles [6, 7]. However, lower leg muscles frequently show enlargement known as pseudohypertrophy. Research has indicated that the gastrocnemius and soleus muscles are predominantly affected [8]. Torriani et al. [9] showed that marked involvement of peroneal muscles is a characteristic feature of boys with DMD. In addition, increased contractile content in both peroneal muscle and medial gastrocnemius with age are

suggestive of compensatory hypertrophy [10]. Furthermore, the degree of adiposity of the lower leg muscles measured by MRI correlated with clinical manifestation, considered quantitative and objective measures of disease severity. We collected muscle MRI data in the thigh and lower leg of dystrophinopathy patients and analyzed the MRI characteristics of the lower limb.

Materials and methods

The Research Ethics Committee of the Third Hospital of Hebei Medical University of China approved this study. Written informed consent was obtained from each participant's parent or guardian, and each child agreed to participate in this study.

Participants

Forty-two patients with dystrophinopathy (age range 4.0-25.0 years) participated in this study. Patients included 33 boys with DMD, 7 boys with BMD, 1 boy with asymptomatic hyperCKemia, and 1 female manifesting carrier (MC). Dystrophinopathy diagnoses were based on a history of progressive muscle weakness, physical symptoms, and significantly elevated serum CK levels. Muscle biopsy showed muscular dystrophy pathologic changes and there was decreased or absent dystrophin immunohistochemical staining on the sarcolemma using the dystrophin-N, -C, and -R antibodies (**Figure 1**). Knee flexion and extension were evaluated as proximal strength, whereas ankle plantar flexion and dorsiflexion were evaluated as distal strength. The proximal and distal lower limb muscle strength was evaluated using the Medical Research Council (MRC) scale, as follows [11]: grade 5, normal strength; grade 4, slight-to-moderate weakness; grade 3, muscle can move the joint against gravity but not against any added resistance; grade 2, muscle cannot move the joint against gravity but only in absence of it; grade 1, a trace of contraction; and grade 0, no visible evidence of contraction.

MR imaging procedures

The imaging data were acquired using a 3.0T scanner (GE, USA) with an 8-channel receive-only phased array torso coil (USA Instruments, Aurora, OH). Participants lay supine with the lower limbs fixed snugly using a belt and foam pads to restrict leg movement. T1-weighted axial images were obtained using the following parameters: TE: ranged from 13.9 to 18.6 ms, TR: 500 ms, FOV: 38, 18 sections, 6.0/3.0 mm (thickness/gap). This session lasted for 150 seconds. Next, the T1-weighted axial images of fat suppression were obtained using the same parameters. T2-weighted axial images were acquired using the following parameters: 3620/85 ms (TR/TE), 20 sections, 6.0/3.0 mm (thickness/gap), and the T2-weighted axial images of fat suppression were acquired with the same parameters.

For the muscle MRI of the lower limb, sections were generally analyzed within the mid to upper section of the thigh and lower leg, because the muscle bulk in this section is greatest and muscle abnormalities can be more clearly visualized. The muscles listed below were assessed in 4 grades.

Thigh: rectus femoris, vastus medialis, vastus lateralis, vastus intermedius, semitendinosus, semimembranous, biceps femoris, gracilis, and adductor magnus.

Lower leg: anterior tibial muscle, extensor digitorum longus, extensor hallucis longus, long fibular muscle, caput mediale musculi gastrocnemii, caput laterale musculi gastrocnemii, and musculus soleus.

Fatty infiltration of the lower limb musculature was graded using a semi-quantitative method, which entailed consensus scoring by two experienced musculoskeletal radiologists blinded to patient data to minimize bias. At the largest cross-sectional area of each muscle, we used the scale described by Mercuri E et al. [12] as follows: Stage 0: Normal appearance; Stage 1: Numerous discrete areas of increased density less than 30% of the muscle volume; Stage 2: Numerous discrete areas of increased density with early confluence, 30%-60% of the muscle volume; Stage 3: Numerous discrete areas of increased density with confluence, more than 60% of the muscle volume; Stage 4: Severe appearance, muscle entirely replaced by areas of increased density (**Figure 2**).

Data analyses

The clinical and MRI data were not normally distributed, Spearman's rank correlation model was used to evaluate the correlation between

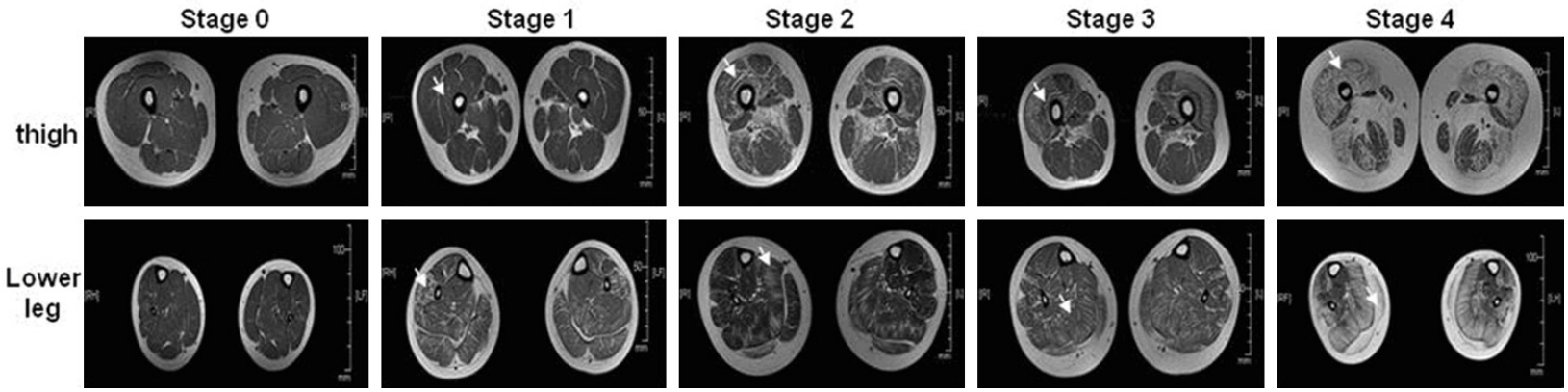

Figure 2. Different degrees of MR images of DMD patients: Good correlation between muscle MRI in DMD scoring of the thigh and lower leg, showing the selective muscles injured (arrows).

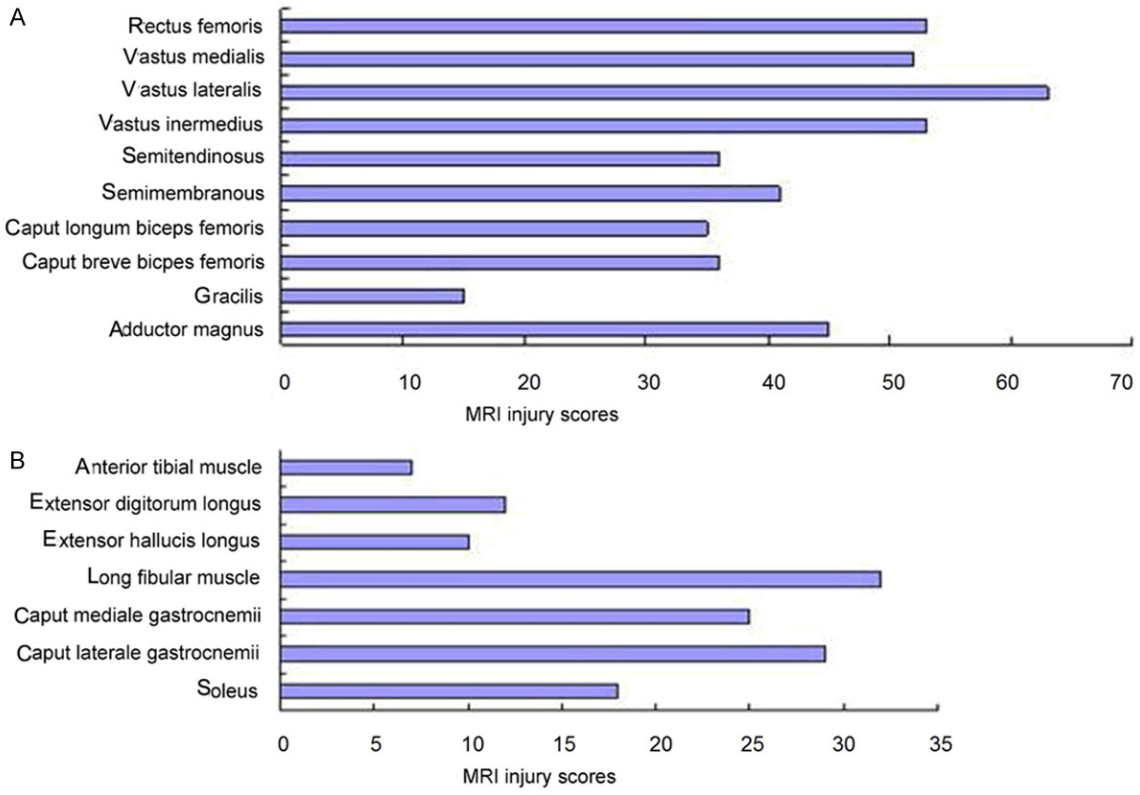

Figure 3. MRI injury scores of the thigh (A) and lower leg muscles (B) of DMD patients. (A) The vastus lateralis is the most involved and the gracilis is the least; (B) The long fibular muscle is the most injured and the anterior tibial muscle is the least.

muscle semi-quantitative MRI scores and clinical variables (age, muscle strength, and serum CK level). A positive correlation was considered significant if the $P < 0.05$. The non-parametric test was used to analyze the MRI scores of the thigh and lower leg muscles. Logistic regression was used for MRI scores and clinical variables (age, weight, course of disease, muscle strength, and serum CK level), and variables with $P < 0.05$ were included in the final model. Statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 13.0).

Results

Clinical findings

Thirty-three boys with DMD between 2 and 12 years of age participated in the study. Fourteen of the boys underwent muscle MRI examination at their first visit to hospital, and the remaining boys (n = 19) underwent the MRI when receiving glucocorticoid therapy. Negative correlations existed between proximal and distal muscle strength and patient age (proximal: $r = -0.550$, $P = 0.00 < 0.05$; distal: $r = -0.400$, $P = 0.021 < 0.05$), indicating muscle strength gets worse with age. There was a significant positive correlation between proximal muscle and distal muscle strength ($r = 0.642$, $P < 0.01$).

Muscle MRI findings

The extent of fatty infiltration in MRI of the thigh was more severe than in the lower leg ($P = 0.000 < 0.001$). In addition, the anterior group was more severe than posterior group in thigh, whereas in the lower leg, the posterior was more severe than the anterior. Of the thigh muscles, the vastus lateralis was the most involved, and the gracilis was the least involved. In the lower leg muscles, the long fibular muscle was the most involved, and the anterior tibial muscle was least involved (**Figure 3**). BMD and DMD patients had similar characteristics in muscle MRI, and female MC showed asymmetric muscle involvement. Although the patient with asymptomatic hyperCKemia was slightly affected (stage 1) in the quadriceps femoris

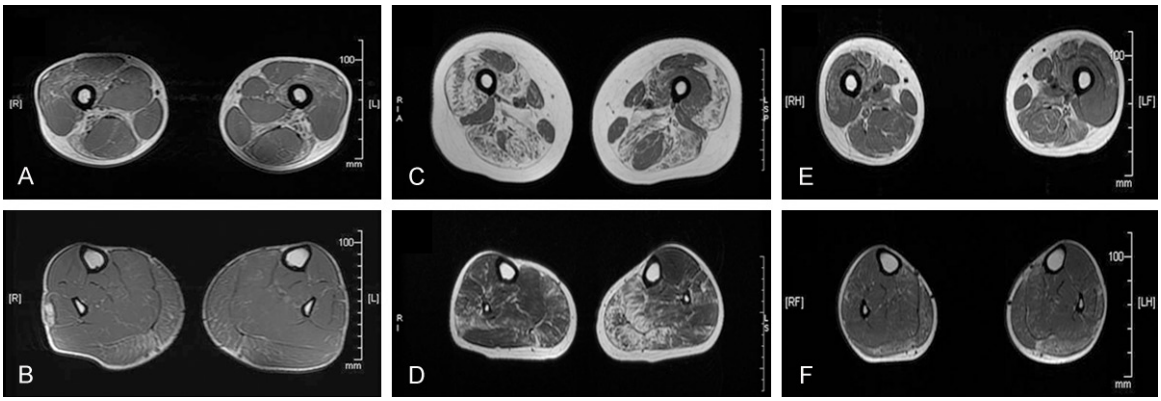

Figure 4. Lower limb MRI of BMD (A, B), female MC (C, D) and symptomatic hyperCKemia (E, F). (A, B) The vastus lateralis and gastrocnemius are slightly involved, and the long fibular muscle is more noticeably involved in BMD patients. (C, D) The female MC has asymmetric muscle involvement. (E, F) The quadriceps femoris and gastrocnemius are slightly affected in the patient with asymptomatic hyperCKemia.

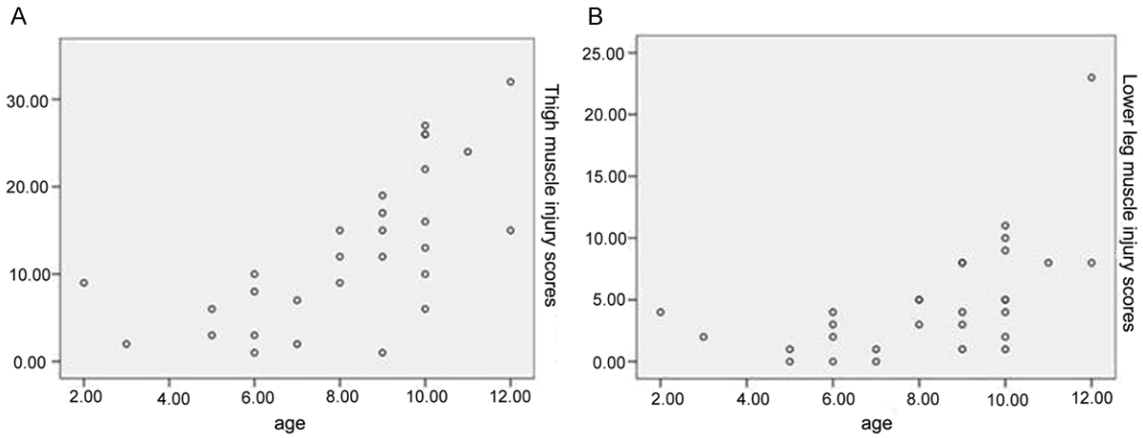

Figure 5. Correlation between the MRI thigh and lower leg muscle injury scores and patient age: Positive correlation between the MRI thigh and lower leg muscle injury scores and patient age (A: $r = 0.720$, $P < 0.05$; B: $r = 0.631$, $P < 0.05$).

and gastrocnemius, the remainder of their musculature was well preserved (**Figure 4**). Due to the small number of cases, there was no statistical significance.

Relationship between muscle MRI and clinical data

There was a significant positive correlation between the MRI muscle injury scores in the thigh and lower leg and patient age (thigh: $r = 0.720$, $P = 0.009$; lower leg: $r = 0.680$, $P < 0.01$) (**Figure 5**). There was a strong negative correlation between proximal and distal muscle strength compared with the degree of muscle injury in the thigh and lower leg in MRI analyses (thigh: $r = -0.611$, $P < 0.01$; lower leg: $r = -0.462$, $P = 0.012$). However, there was no statistically significant correlation between serum CK levels and MRI muscle injury scores ($P = 0.266$).

Logistic regression

Logistic regression analysis were used for the five clinical variables (age, weight, course of disease, proximal/distal muscle strength, and serum CK level) screened and MRI scores in thigh/lower leg, there are three variables (age (X1), weight (X2), proximal muscle strength (X3)) included in the final regression equation only in thigh (The main parameters in **Table 1**).

LogitP = -34.995+3.004X1+1.097X2-3.126X3

Table 1. Results of Logistic regression analysis of clinical variables and MRI scores in thigh

Variables	B	S.E	OR	Wald	95% *CI*	*P* value
Age	3.004	4.348	1.203	17.536	1.104-1.312	0.049
Weight	1.097	0.709	1.396	0.083	0.747-2.015	0.022
Muecle strength	-3.126	2.297	0.044	1.852	0.000-3.961	0.047
Constant	-34.995	34.634	1.000	1.021		

Discussion

Dystrophinopathy is caused by defects in dystrophin present in the sarcolemma and is characterized by progressive muscle weakness and wasting. Clinical strength examinations are established, accepted methods used to assess the functional status of DMD patients. Pathologic studies of DMD have revealed muscle fiber degeneration upon microscopic analyses, regeneration and necrosis, increased connective tissue, and replacement of fatty elements. The histological dystrophic changes in muscle are consistent with these MRI abnormalities [6]. The T1-weighted sequence of images showed muscle bulk and selective muscles were affected. The increased signal intensity on T1 images may reflect increased fibrous tissue or adipose tissue [13], and fat suppression imaging and three-point Dixon values of muscle adiposity may reflect fatty infiltration of muscles and disease severity [14]. Furthermore, the increased signal intensity on water sensitive sequences, such as the T2-weighted images with fat suppression sequence, suggests that muscle edema or inflammation might occur prior to the replacement of muscle tissue by fatty or fibrotic tissue [9]. Therefore, these data provide increasing evidence that non-invasive quantitative methods to determine muscle fat accumulation provide important information about the progression of dystrophinopathies. Skeletal muscle MRI can assist in understanding the *in vivo* pathophysiology of the disease, and provide an additional means of evaluating a patient's condition, in conjunction with the clinical examination.

The majority of this research focused on lower leg and thigh muscles. Thigh muscles are involved earlier and more severely than lower leg muscles. Among the thigh muscles, the anterior group muscles are more severely affected than the posterior, with the vastus lateralis the most seriously affected. The gracilis is the least affected. In the lower leg, the posterior is more severely affected, and the long fibular muscle is the most seriously affected. The anterior tibial muscle is the least affected. All of the injured selective muscles are in agreement with the clinical manifestation of progressive weakness of ankle dorsiflexor, hip flexor, and extensor muscle groups, followed by hip adductor and extensor [15]. This pattern of selective muscle involvement may be related to DMD gene mutations or special dystrophin components in muscles and the different exercise modes of muscle groups; however, its exact reason is unclear. The seemingly asymmetric affected muscles in female carriers are consistent with the hypothesis that random X-autosomal translocations lead to skewed X inactivation of the normal dystrophin gene and somatic mosaicism in muscles [16-18]. The logistic regression analysis showed that there are three variables (age, weight, proximal muscle strength) affected thigh muscle injury scores. The greater age and weight and the larger proximal muscle worse, thigh muscle injury in MRI more severe, these indicated the "progression" process. Although the clinical presentation of muscle weakness and muscle involvement in MRI from DMD patients positively correlated with age, among the same aged patients, the muscle strength and muscle injury degree in MRI were different. In the ten-year old group, the thigh muscle injury score ranged from 6 to 32, and the lower limb muscle injury score ranged from 1 to 10. These differences may be related to the sites of gene mutation, duration of steroid therapy, and habits.

Muscle MRI has increasingly been used as a diagnostic and differential diagnostic tool for various inherited neuromuscular disorders showing a specific pattern of muscle involvement. For boys with proximal limb weakness and wasting, gastrocnemius pseudohypertrophy, and hyperCKemia, the diagnosis is difficult before muscle biopsy, particularly the identification of BMD (dystrophinopathy), LGMD2A (calpainopathy), and LGMD2C-2F (sarcoglycanopathy). Skeletal muscle MRI is helpful because of the different muscle involvement in each of these diseases. Dystrophinopathy and sarcoglycanopathy often significantly afflict the

anterior group muscles rather than the posterior in the thigh [6, 19, 20], but calpainopathy predominantly involves the posterior compartment muscles, such as semimembranosus, biceps femoris, and adductor muscles in the thigh [21]. In the lower legs, dystrophinopathy often presents with early and marked changes in gastrocnemius, whereas sarcoglycanopathy does not involve these muscles. The medial gastrocnemius and soleus muscles are more likely to be involved in calpainopathy. Therefore, muscular MRIs of both the thigh and lower leg are essential for the diagnosis of inherited myopathy [2].

DMD patients present with ankle dorsiflexor and hip flexor weakness at the early course, but plantar flexor muscle strength is preserved [15]. Contracture in the Achilles tendon gradually occurs and is common in patients that are 6-7 years old. Achilles tendon contracture causes standing and walking on toes, and at the same time, the long-term overload of gastrocnemius may result in pseudohypertrophy. However, the mechanism of this phenomenon remains unknown. Pseudohypertrophy predominantly occurs as an accumulation of fat and fibrous tissue that is two to three times greater than healthy controls [22]. In our study, all of the cases older than 6 years had contracture of the Achilles tendon (n = 25), and their lower leg muscles involvement in MRI was more obvious than the younger cases (n = 4). Therefore, we suggest that boys with DMD should start Achilles tendon stretching exercises at 6 years old. DMD patients who underwent aponeurotomy of the iliotibial band and Z-shaped Achilles' tendon lengthening at the age of 6 years were able to walk longer than controls at the age of 9 years [23]. Muscle MRI can evaluate lower leg muscle involvement and help clinicians select the timing of Achilles tendon lengthening surgery.

Muscle MRI is also useful for understanding the mechanisms of action of medications and monitoring therapies. This study demonstrates that MRI is important for understanding the disease process and for monitoring subclinical changes that assist the medical therapy. The severity of disease reflected in MRI may provide additional information to the clinicians regarding when to initiate steroid medication. Many studies have indicated that small doses of glucocorticoid (prednisone 0.5-0.75 mg/kg.d) increases the number of regenerated muscle fibers [24] and induces muscle formation [25]. These data suggest that glucocorticoids have a role in preserving muscle strength and prolonging ambulation. Many experts suggest that patients should initiate medical therapy with corticosteroids, such as prednisone, at younger ages before extensive, irreversible changes occur in and around the muscles [26].

However, some interpretations of our study may be limited due to the moderate sample size. Because of technical reasons, quantitative muscle involvement data from MRI were not obtained. Further studies are needed to increase the number of cases evaluated and to perform dynamic MRI contrast analyses during hormone therapy.

Conclusions

Results from lower limb MRI showed selective muscle involvement in dystrophinopathy, which is essential for the differential diagnosis of progressive muscular dystrophy. The severity of muscle involvement in MRI can reflect clinical severity and guide glucocorticoid treatment. MR imaging provides a non-invasive means of detecting subtle, subclinical changes in individual muscles that reflects the progression of dystrophinopathy.

Disclosure of conflict of interest

None.

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