Muscle Magnetic Resonance Imaging for the Differentiation of Multiple Acyl-CoA Dehydrogenase Deficiency and Immune-mediated Necrotizing Myopathy

Ya-Wen Zhao, Xiu-Juan Liu, Wei Zhang, Zhao-Xia Wang, Yun Yuan
Department of Neurology, Peking University First Hospital, Beijing 100034, China

Abstract

Background: Clinically, it is difficult to differentiate multiple acyl-CoA dehydrogenase deficiency (MADD) from immune-mediated necrotizing myopathy (IMNM) because they display similar symptoms. This study aimed to determine whether muscle magnetic resonance imaging (MRI) could be used for differential diagnosis between MADD and IMNM.

Methods: The study evaluated 25 MADD patients, confirmed by muscle biopsy and ETFDH gene testing, and 30 IMNM patients, confirmed by muscle biopsy. Muscles were assessed for edema and fatty replacement using thigh MRI (tMRI). Degrees and distribution patterns of fatty infiltration and edema in gluteus maximus and thigh muscles were compared.

Results: Total fatty infiltration and edema scores (median, [Q1, Q3]) were 4.00 (1.00, 15.00) and 0 (0, 4.00) in MADD and 14.50 (8.00, 20.75) and 22.00 (16.75, 32.00) in IMNM, respectively, which were significantly more severe in IMNM than that in MADD (P = 0.000 and P = 0.004, respectively). Edema scores for gluteus maximus, long head of biceps femoris, and semimembranosus were significantly higher in IMNM than in MADD (all P = 0.000). Fatty infiltration scores for anterior and medial compartments were significantly more severe in IMNM than that in MADD (all P = 0.000).

Conclusion: Different patterns of muscle involvement on tMRI can contribute to differential diagnosis between MADD and IMNM when clinical suspicions alone are insufficient, thereby reducing the need for muscle biopsy.

Key words: Immune-mediated Necrotizing Myopathy; Multiple Acyl-CoA Dehydrogenase Deficiency; Muscle Edema; Thigh Magnetic Resonance Imaging

Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD) is the most common subtype of lipid storage myopathy.[1] The late-onset type is characterized by acute or subacute proximal weakness in adulthood with elevated serum creatine kinase (CK) levels and myogenic damage on electromyography.[1-4] Idiopathic inflammatory myositis (IIM), especially polymyositis or immune-mediated necrotizing myopathy (IMNM), usually appears in adulthood with symptoms similar to those of MADD.[5-8] Thus, it is very difficult to achieve a differential diagnosis between MADD and IIM based on clinical features alone. While several studies investigated over diagnosis of polymyositis among patients with “clinical” polymyositis, only 5% of patients have “histological” polymyositis.[9] Misdiagnosis of MADD as IMNM is not uncommon and usually leads to inappropriate corticosteroid therapy before a muscle biopsy is taken.[1,3,6]

A muscle biopsy plays an important role in differential diagnosis between the two diseases. In muscle biopsies, MADD showed numerous small vacuoles in type I muscle fibers filled with lipid droplets positive for Oil Red O (ORO) staining,[1,3] while IMNM exhibited necrotizing fibers without inflammatory infiltration.[5-7] However, a muscle biopsy is an invasive test not always accepted by patients and does not always show typical myopathology.[8] Thus, it

Address for correspondence: Dr. Wei Zhang, Department of Neurology, Peking University First Hospital, Beijing 100034, China
E-Mail: neurozw@163.com

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is necessary to establish a noninvasive test for differential diagnosis between these two diseases. With this goal, we compared thigh magnetic resonance imaging (tMRI) in patients with IMNM and MADD to achieve a differential diagnosis between the two diseases.

Muscle MRI is used to examine unique patterns in various neuromuscular disorders because of its high sensitivity for detecting muscle edema, atrophy or hypertrophy, and fatty replacement, all potentially correlated with clinical characteristics. In general, muscle MRI shows typical changes in similar muscle diseases, such as Duchenne muscular dystrophy and collagen VI-related myopathies. Consequently, changes observed with muscle MRI are not particularly specific for diagnosis. Isolated studies showed that IMNM patients had more edema and mild fatty infiltration at the later stage. Meanwhile, MADD patients showed no muscle edema but had increased fatty infiltration in the posterior, compared with the anterior or medial, compartments of the thigh muscles. Therefore, muscle imaging is potentially useful for differential diagnosis between MADD and IMNM. In our study, we compared the differences observed by muscle imaging between MADD and IMNM cases and identified statistically significant indicators for differentiating MADD from IMNM.

**Methods**

**Ethical approval**

The procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation and approved by the Institutional Review Board of Peking University First Hospital. Informed consent for all examinations was obtained from the patients or their guardians.

**Patients**

Twenty-five patients with MADD and 30 patients with IMNM, all treated at the Department of Neurology at Peking University First Hospital from October 2013 to October 2016, were included in the study. Diagnosis of MADD was confirmed by muscle biopsy and genetic analysis of the ETFDH gene. Diagnosis of IMNM was confirmed by muscle biopsies, in which 25 patients showed anti-SRP antibody positivity and 5 patients had statin-related myopathy. Demographic and clinical features were collected for all patients [Table 1].

**Muscle magnetic resonance imaging**

All patients underwent tMRI (3.0 T). Eight patients with IMNM had been taking steroids for 16.0 (8.5, 27.0) months and 7 patients with MADD had been misprescribed steroids for 8.0 (4.5, 11.0) months. The other 22 patients with IMNM and 18 patients with MADD had not received steroid treatment. T1-weighted MRI was performed to evaluate the degree of fatty infiltration, according to the modified Mercuri scale (0–5 scale). Short T1 inversion recovery sequences were used to assess the degree of edema (0–5 scale). Edema and fatty infiltration scores were calculated in the gluteus maximus and thigh muscles (vastus intermedius, vastus medialis, vastus lateralis, rectus femoris, biceps femoris, semitendinosus, semimembranosus, adductor magnus, sartorius, long adductor, and gracilis). We calculated and summed the total fatty infiltration and edema scores of the gluteus maximus and thigh muscles in the 25 patients with MADD and 30 patients with IMNM to compare fatty infiltration, edema severity, and distribution in the two diseases.

**Statistical analysis**

Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features were indicated by medians (Q1, Q3). Pairwise comparisons of categorical variables between groups were performed using the Chi-square test or Fisher’s exact test, as appropriate. The Mann–Whitney U-test was used to compare continuous variables among groups and correlations were examined using coefficients. Correlations between MRI changes and various clinical and muscle pathological parameters (onset age, duration,

| Table 1: Clinical characteristics of patients with MADD and IMNM |
|---------------------------------------------------------------|
| **Characteristics**                                            | **MADD (n=25)** | **IMNM (n=30)** | **Z/χ²** | **P** |
| Age (years)                                                   | 36.0 (24.5, 46.0) | 47.0 (27.0, 54.3) | -1.674*  | 0.094 |
| Onset age (years)                                             | 30.0 (23.0, 37.0) | 46.5 (26.5, 53.0) | -2.587*  | 0.010 |
| Gender (male, n (%))                                          | 19 (76.0)         | 13 (43.3)         | 5.981†   | 0.014 |
| Duration (months)                                             | 36.0 (7.5, 132.0) | 5.0 (2.0, 24.0)   | -3.747*  | 0.000 |
| Dysmnesia, n (%)                                              | 18 (72.0)         | 4 (13.3)          | 19.556ujący | 0.000 |
| Neck flexor weakness, n (%)                                   | 14 (56.0)         | 24 (80.0)         | 3.628*   | 0.055 |
| Myalgia, n (%)                                                | 10 (40.0)         | 5 (16.7)          | 3.743*   | 0.053 |
| CK (U/L)                                                      | 990.0 (490.5, 2191.5) | 4618.0 (3124.8, 7303.0) | -4.564*  | 0.000 |
| EMG, n (%)                                                    | 11 (44)           | 27 (90)           | 14.401*  | 0.001 |

Values were shown as median (Q1, Q3), or n (%). *Z value; χ² value. MADD: Multiple acyl-CoA dehydrogenase deficiency; IMNM: Immune-mediated necrotizing myopathy; CK: Creatine kinase; EMG: Electromyography.
CK, modified Rankin Scale, muscle fiber necrosis, muscle fiber regeneration, fat droplet deposition, major histocompatibility complex class-I (MHC-I) expression, and membrane attack complex deposition) were evaluated by the Spearman’s rank test, and \( P < 0.05 \) was considered statistically significant. Forward multiple logistic regression was used to select the tMRI features that were most informative for clinical data. A likelihood ratio test significance of 0.01 was selected to include variables in the model and maintain a manageable number of items in each formula.

All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). To account for the number of statistical tests performed, a two-sided \( P \leq 0.001 \) was considered statistically significant for univariate analyses, while \( P < 0.05 \) was considered statistically significant for multivariate analyses.

**RESULTS**

**Patients with multiple acyl-CoA dehydrogenase deficiency**

The patients with MADD included 19 males and 6 females with a median onset age of 30.0 (23.0, 37.0) years. Proximal weakness appeared with a median duration of 36.0 (7.5, 132.0) months. Muscle biopsies showed numerous small vacuoles in Type I muscle fibers filled with lipid droplets positive for ORO staining [Figure 1a and 1b]. Three patients presented with angular fibers and small-type grouping indicating neurogenic changes. Two patients received a sural nerve biopsy that showed chronic axon neuropathy. Genetic analysis showed heterozygous or homozygous mutations in the ETFDH gene [Supplementary Table 1].

The tMRI revealed fatty infiltration in 22 patients and edema in 11 patients [Figure 2a and 2b]. The median total fatty infiltration score was 4.00 (1.00, 15.00). The median total edema score was 0 (0, 4.00) [Table 2]. Fatty infiltration mainly appeared in the gluteus maximus (2.00 [1.00, 3.00]), long head of the biceps femoris (1.00 [0, 2.00]), and semimembranosus (1.00 [0, 2.00]) [Figure 3a and 3b]. There were no correlations between total edema and total fatty infiltration scores and onset age, disease duration, or muscle pathological parameters.
Table 2: Thigh magnetic resonance imaging scores of patients with MADD and IMNM

| Scores of fatty infiltration, median (Q1, Q3) | MADD (n=25) | IMNM (n=30) | Z    | P     |
|---------------------------------------------|-------------|-------------|------|-------|
| Total muscles                               | 4.00 (1.00, 15.00) | 14.50 (8.00, 20.75) | −2.898 | 0.004 |
| Anterior group                              | 0 (0, 3.00)  | 3.00 (0, 4.00)  | −2.551 | 0.011 |
| Internal group                               | 1.00 (0, 3.50) | 4.00 (3.00, 7.00) | −4.005 | 0.000 |
| Posterior group                             | 3.00 (1.50, 8.00) | 7.00 (4.00, 9.25) | −1.921 | 0.055 |
| Scores of edema, median (Q1, Q3)            |             |             |      |       |
| Total muscles                               | 0 (0, 4.00)  | 22.00 (16.75, 32.00) | −6.259 | 0.000 |
| Anterior group                              | 0 (0, 0)     | 7.00 (4.00, 12.00) | −6.365 | 0.000 |
| Internal group                               | 0 (0, 0)     | 6.00 (4.00, 8.25)  | −6.262 | 0.000 |
| Posterior group                             | 0 (0, 2.00)  | 10.00 (6.50, 12.25) | −5.832 | 0.000 |

MADD: Multiple acyl-CoA dehydrogenase deficiency; IMNM: Immune-mediated necrotizing myopathy.

Patients with immune-mediated necrotizing myopathy

The patients with IMNM included 13 males and 17 females with a median onset age of 46.5 (26.5, 53.0) years. Proximal weakness of all limbs appeared with a median duration of 5.0 (2.0, 24.0) months. Muscles biopsies mainly revealed necrotic and regenerating fibers without inflammatory infiltration [Figure 1c]. Some patients showed mild or moderate lipid droplet deposition with ORO staining. In some
patients, there were diffuse or multifocal MHC-I-positive fibers [Figure 1d] and complement deposition in muscle fibers or blood capillaries.

The tMRI results showed various extents of fatty infiltration and edema among the thirty patients [Figure 2c and 2d]. The median total fatty infiltration score was 14.50 (8.00, 20.75). The median total edema score was 22.00 (16.75, 32.00) [Table 2]. Fatty infiltration mainly appeared in the gluteus maximus (2.00 [1.00, 2.25]), adductor magnus (2.00 [1.00, 3.00]), long head of the biceps femoris (2.00 [1.00, 2.00]), and semimembranosus (2.00 [1.00, 3.00]) [Figure 3c]. Muscle edema appeared mainly in the gluteus maximus (3.00 [2.00, 4.00]), adductor magnus (2.50 [1.75, 3.25]), long head of biceps femoris (2.50 [1.75, 4.00]), and semimembranosus (2.00 [1.00, 3.00]) [Figure 3d]. There was a significant positive correlation between total edema score and CK level ($r = 0.383$, $P = 0.037$). Total fatty infiltration score was positively correlated with onset age ($r = 0.399$, $P = 0.029$) and negatively correlated with muscle regeneration ($r = −0.376$, $P = 0.041$).

Comparison of the two diseases

Total edema scores in patients with MADD were significantly lower than those in patients with IMNM ($P = 0.004$). Edema scores in the three muscle subgroups differed significantly between MADD and IMNM ($P = 0.000$).

Total fatty infiltration scores in patients with MADD were significantly lower than those in patients with IMNM ($P = 0.000$). Fatty infiltration scores in the anterior and medial subgroups differed significantly between MADD and IMNM ($P = 0.000$). There were no significant differences among fatty infiltration scores in the posterior subgroup ($P = 0.15$).

Multivariate analysis revealed that IMNM exhibited significantly more extensive edema and fatty replacement than that of MADD ($P < 0.05$) independent of age, duration of illness, and sex [Table 3]. Total muscle edema, edema in the internal group, and edema in the posterior group were more prevalent in IMNM than that in MADD [Figure 4]. After selecting the most balanced cutoff values for the logistic regression formulas, using Youden’s index, we estimated that the positive (100%) and negative (96.7%) predictive values of the formulas were suboptimal.

Table 3: Multivariate analysis of the extent of several thigh magnetic resonance imaging features, comparing patients with MADD and those with IMNM by forward multiple logistic regression

| Items                      | OR     | 95% CI (range) | $P$  |
|----------------------------|--------|----------------|------|
| Total edema scores         | 1.895  | 1.101–3.261    | 0.021|
| Medial group edema scores  | 8.798  | 1.280–60.466   | 0.027|
| Posterior group edema scores| 1.901  | 0.922–3.919    | 0.082|

MADD: Multiple acyl-CoA dehydrogenase deficiency; IMNM: Immune-mediated necrotizing myopathy; OR: Odd ratio; CI: Confidence interval.

Figure 4: Multivariate analyses in patients with MADD and IMNM. Total muscle edema was more prevalent in IMNM than that in MADD (a), while total muscle fatty infiltration was similar between MADD and IMNM (b). Edema in the internal group (c) and the posterior group (d) was more prevalent in IMNM than that in MADD. MADD: Multiple acyl-CoA dehydrogenase deficiency; IMNM: Immune-mediated necrotizing myopathy.
DISCUSSION

Our study showed that mild fatty infiltration mainly occurred in the posterior compartment of thigh and gluteus muscles in MADD patients, while edema was rarely seen. These findings were similar to a previous report on muscle imaging changes in South Chinese patients with MADD.[23] Interestingly, all our patients originated from northern region of China, suggesting that tMRI of MADD patients may not show regional differences.

The patients with IMNM had moderate or severe muscle fatty infiltration and edema in the posterior compartment by tMRI in our study. A previous study on 12 patients positive for anti-SRP antibodies showed that fatty infiltration was most severe in the hamstring and adductor magnus, while edema was most severe in the vastus lateralis, rectus femoris, biceps femoris, and adductor magnus.[14] In addition, a tMRI study examining 101 patients in the USA revealed extensive edema and early muscle damage in IMNM.[15] Our study further confirmed such previously reported tMRI features in IMNM patients.

While patients with both MADD and IMNM had fatty infiltration and edema in the posterior compartment of the thigh muscles, those with IMNM had more common and more severe muscle edema and fatty infiltration than those with MADD. We also observed a correlation between serum CK and muscle edema in IMNM, but not in MADD. This finding suggested a reason why the progression of disease over time in metabolic myopathy (including lipid storage myopathy) is not as severe as that in IIM but, instead, varies in duration. While serum CK levels and muscle edema have been used to assess disease activity in patients with IIM,[16-19] our findings suggested that muscle edema would be a reliable marker to evaluate disease activity in patients with IMNM but not in those with MADD. Furthermore, muscle edema was strongly associated with IMNM in logistic regression models, also strengthening the hypothesis that muscle edema in tMRI would be an effective indicator to achieve a differential diagnosis between the two diseases.

Similar fatty infiltration distribution patterns, predominantly involving the posterior compartment of the thigh, were also reported in mitochondrial diseases[20] and limb-girdle muscular dystrophy (LGMD).[21-23] LGMD-2A showed more severe fatty infiltration in the adductor major, adductor longus, and semimembranosus, with sparing of the anterior compartment of the thigh, at the early stage of disease.[21,22] Similarly, LGMD-2B presented with large variations in fatty infiltration between the anterior and posterior compartments of the thigh.[23] However, the edema distribution patterns between LGMD-2B and IMNM or MADD were different. LGMD-2B presented with muscle edema mainly in the vastus lateralis, medialis, and intermedius.[23]

In conclusion, we confirmed that muscle fatty infiltration and edema occurred mainly in the posterior compartment of the thigh in MADD and IMNM. IMNM was characterized by a higher proportion of thigh muscle edema, compared with in MADD. Muscle edema detected by tMRI might, therefore, be a useful indicator to differentiate MADD from IMNM.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest
There are no conflicts of interest.

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| MADD patient number | *ETFDH* mutation                              | Homozygosis/heterozygous                      |
|---------------------|----------------------------------------------|-----------------------------------------------|
| P1                  | Exon7 c.770 A>\>G p.Tyr>Cys; Exon11 c.1395 T>G p.Tyr>Term | Compound heterozygous                         |
| P2                  | Exon7 c.691T >A/T p.Phe231Ile; Exon7 c.770A>\>G p. 257 Tyr>Cys | Compound heterozygous                         |
| P3                  | Exon8 c.872T>G p.Val29Gly; Exon10 c.1227A>\>C p.Leu409Phe | Compound heterozygous                         |
| P4                  | Exon7 c.770 A>\>G p.Tyr>Cys; Exon10 c.1211 T>C/T p.Met>Thr | Compound heterozygous                         |
| P5                  | Exon10 c.1123C>A p.P575T; Exon6 c.617A>T p.H206L | Compound heterozygous                         |
| P6                  | Exon1 c.3G>C p.M1I; Exon2 c.152G>A p.R51Q | Compound heterozygous                         |
| P7                  | Exon6 c.617A>T p.H206L; Exon8 c.872T>G p.Val29Gly | Compound heterozygous                         |
| P8                  | Exon3 c.389A>T p.Asp130Val; Exon12 c.1597C>T p.Gln533Termin | Compound heterozygous                         |
| P9                  | Exon7 c.691T>A/T p.Phe231Ile; Exon10 c.1211 T>C/T p.Met>Thr | Compound heterozygous                         |
| P10                 | Exon5 c.535A>G p.K179E; Exon9 c.1044A>C p.L348F | Compound heterozygous                         |
| P11                 | Exon2 c.242T>C p.L81P; Exon2 c.295C>T p.R99C | Compound heterozygous                         |
| P12                 | Exon8 c.1028T>C p.M343T; Exon8 c.1044A>C p.L348F | Compound heterozygous                         |
| P13                 | Exon10 c.1227A>C p.L409F; Exon11 c.1395T>G p.Y465* | Compound heterozygous                         |
| P14                 | Exon8 c.872T>G p.Val291Gly; Exon10 c.1211T>C p.Met404Thr | Compound heterozygous                         |
| P15                 | Exon10 c.1227A>C p.L409F; Exon10 c.1281_1282del p.T427fs | Compound heterozygous                         |
| P16                 | Exon1 c.53C>G p.A18G; Exon5 c.587A>G p.Y196C | Compound heterozygous                         |
| P17                 | Exon1 c.61T>C p.S21P; Exon10 c.1271C>G p.T424S | Compound heterozygous                         |
| P18                 | Exon3 c.315G>A p.M105I | Homozygosis                                   |
| P19                 | Exon3 c.250G>A p.Ala84Thr | Single heterozygous                           |
| P20                 | Exon11 c.1395 T>G/T p.Tyr>Term | Single heterozygous                           |
| P21                 | Exon10 c.1227A>C p.Leu409Phe | Single heterozygous                           |
| P22                 | Exon10 c.1227A>A/C p.Leu409Phe | Single heterozygous                           |
| P23                 | Exon11 c.1454C>G p.Thr485Ser | Single heterozygous                           |
| P24                 | Exon3 c.176-2A>G p.I72T | Single heterozygous                           |
| P25                 | Exon2 c.92C>T p.Thr31le | Single heterozygous                           |

MADD: Multiple acyl-CoA dehydrogenase deficiency.