Metabolic Characterization of muscular dystrophy, inflammatory and mitochondrial myopathy using in vitro MRS

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OBJECTIVE

To investigate the utility of in-vitro NMR in distinguishing muscular dystrophy, inflammatory and mitochondrial myopathy.

INTRODUCTION

Adult myopathies comprise a wide range of clinically heterogeneous disorders which can broadly be categorized in three groups i.e. muscular dystrophies (MD), inflammatory myopathies (IM) and other myopathies. Other myopathies group encompass more heterogeneous disorders like congenital myopathies, metabolic myopathies and mitochondrial myopathies (MM). Accurate diagnosis of these disorders is essential for appropriate therapeutic strategy and predicting the prognosis. Conventionally clinical, electrophysiological and histopathological evaluation enable diagnosis of muscle disorders. However, histopathology showed variable sensitivity and specificity in different disorders especially inflammatory myopathies, mitochondrial myopathies and fascio-scapulo humeral muscular dystrophy (1-2). Hence, newer techniques, which can supplement traditional procedures in increasing the diagnostic accuracy and better understanding of the pathophysiology and biochemistry of muscle disorders are required. We earlier reported metabolic differences in patients with Duchenne Muscular Dystrophy and Limb Girdle Muscular Dystrophy compared to controls (3,4). We report here the results of in-vitro proton MR spectroscopy investigation carried out on skeletal muscle tissue to evaluate whether metabolic profile distinguish among patients with MD, IM and MM.

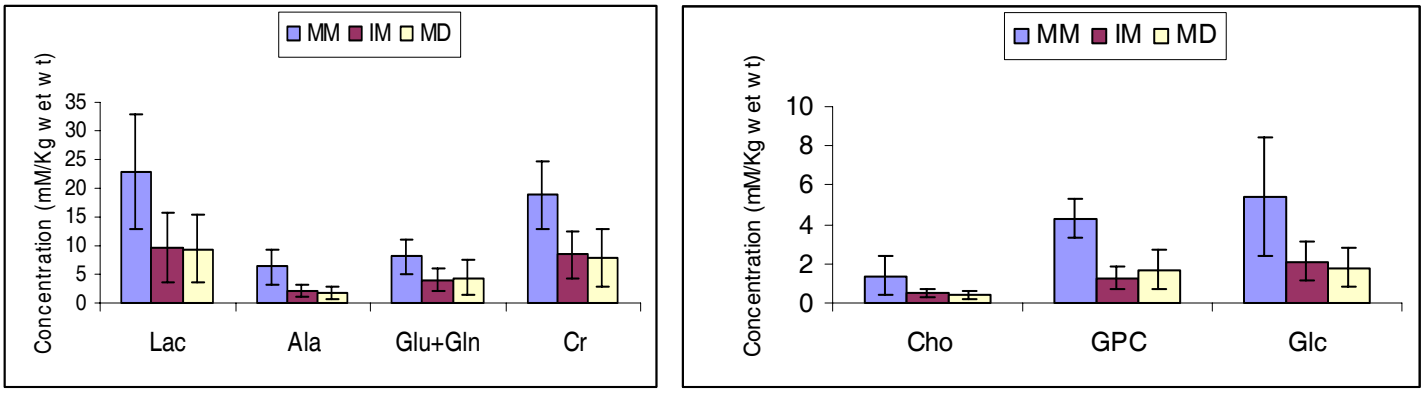
METHODOLOGY

A total of 77 patients (age range 13-62 yrs, mean age 30 ± 13 yrs, M:F 2:1) were recruited for the study. After clinical, electrophysiological and histopathological evaluation, 43 patients (56%) were diagnosed to have MD, 14 (18%) IM and 9 (12%) MM. Muscle tissues were obtained by open surgical biopsy procedure under regional anesthesia and NMR spectroscopy was performed in a subgroup of patients [MD (n=20), IM (n=10), MM (n=9)]. Perchloric acid extracts of muscle tissues were prepared and various 1D and 2D NMR experiments were performed at 400 MHz. The concentration of metabolites were determined using the procedure described earlier (3,4). One way ANOVA was used for statistical analysis and probability values of (5%) were considered as significant ($p < 0.05$).

RESULTS

In all 44 metabolite resonances comprising of amino acids (Glu, Gln, Ala, Lys etc.), sugars (Glc,and mI), membrane metabolites (GPC and Cho), organic acids (Lac, fumarate, etc.,) and nucleotides were unambiguously assigned using 2D NMR. MM patients showed higher concentration of Glc, Lac, Ala, Glu+Gln and Cr ($p < 0.05$) compared to MD and IM patients (Fig. 1). Significantly higher concentration of Ala, Cr and Glc in IM patients differentiates them from patients with MD (Fig. 1).

Fig. 1. Comparison of the concentration of metabolites in MM,IM and MD patients.



DISCUSSION

Mitochondrial myopathies are a diverse group of disorder with great genotypic and phenotypic variability. No definite conclusive test is, yet, available for reliable diagnosis. Our results demonstrate significantly higher concentration of Lac in MM patients compared to patients with IM and MD which could be attributed to abnormal mitochondrion because of which these patients may have accelerated rate of anaerobic glycolysis to maintain ATP concentrations resulting in higher values of Lac. Biochemical investigations and ^{31}P MRS revealed lactic acidosis in patients with mitochondrial myopathy (5). Higher concentration of Gln and Ala reflect that the glucose requirement of the muscle cells is met by gluconeogenic pathway. Patients with IM showed higher concentration of Cho, Cr and Glc compared to patients with MD which is probably related to less degeneration of muscle tissue compared to patients with MD. Our results demonstrate potential usefulness of in vitro MRS in understanding the metabolism in muscle disorders and indicate the diagnostic utility of technique.

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