Clinical Neurophysiology 128 (2017) 227–232

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Quantitative muscle ultrasound as a biomarker in Charcot-Marie-Tooth neuropathy

N. Shahrizaila [a,b,*], Y. Noto [a], N.G. Simon [c], W. Huynh [a], K. Shibuya [a], J.M. Matamala [a], T. Dharmadasa [a], E. Devenney [a], M.L. Kennerson [d], G.A. Nicholson [d], M.C. Kiernan [a]

[a] Brain and Mind Centre, University of Sydney, Camperdown, Australia
[b] Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[c] St Vincent's Clinical School, University of New South Wales, Darlinghurst, Australia
[d] ANZAC Research Institute and Sydney Medical School, University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Accepted 6 November 2016
Available online 21 November 2016

Keywords:
Muscle ultrasound
Charcot-Marie-Tooth
Nerve ultrasound
Outcome biomarkers
CMT Neuropathy Score

HIGHLIGHTS

- Charcot-Marie-Tooth (CMT) patients have increased muscle echogenicity of hand and lower leg muscles.
- In CMT, the volume and thickness of the hand and lower leg muscles correlate with muscle strength.
- CMT patients with worse muscle strength have reduced muscle thickness but increased echogenicity.

ABSTRACT

Objective: The utility of quantitative muscle ultrasound as a marker of disease severity in Charcot-Marie-Tooth (CMT) disease subtypes was investigated.
Methods: Muscle ultrasound was prospectively performed on 252 individual muscles from 21 CMT patients (9 CMT1A, 8 CMTX1, 4 CMT2A) and compared to 120 muscles from 10 age and gender-matched controls. Muscle ultrasound recorded echogenicity and thickness in representative muscles including first dorsal interosseus (FDI) and tibialis anterior (TA).
Results: Muscle volume of FDI and thickness of TA correlated with MRC strength. Muscle echogenicity was significantly increased in FDI (65.05 vs 47.09; $p < 0.0001$) and TA (89.45 vs 66.30; $p < 0.0001$) of CMT patients. In TA, there was significantly higher muscle thickness (23 vs 18 vs 16 mm; $p < 0.0001$) and lower muscle echogenicity (80 vs 95 vs 108; $p < 0.0001$) in CMT1A compared to CMTX1 and CMT2A. This corresponded to disease severity based on muscle strength (MRC grading CMT1A vs CMTX1 vs CMT2A: 59 vs 48 vs 44; $p = 0.002$).
Conclusion: In CMT, quantitative muscle ultrasound of FDI and TA is a useful marker of disease severity.
Significance: The current findings suggest that quantitative muscle ultrasound has potential as a surrogate marker of disease progression in future interventional trials in CMT.

1. Introduction

Therapeutic clinical trials depend on valid markers of disease activity that can reliably monitor disease progression. In slowly progressive disorders such as Charcot-Marie-Tooth disease (CMT), detecting small changes in disease activity and thereby potential responsiveness to therapy, remains challenging. Presently, responsive outcome measures in CMT are lacking (Lewis et al., 2013).

Clinical scoring tools such as the CMT Neuropathy Score (CMTNS) have been identified as poor primary outcome measures, particularly in terms of responsiveness, and may result in the introduction of false negatives in treatment trials (Pareyson et al., 2011; Piscosquito et al., 2015). In a recent study, the high responsiveness of MRI outcome measures in monitoring intramuscular fat accumulation in CMT patients suggested that muscle imaging may have a role in clinical trials (Morrow et al., 2016). Muscle ultrasound of thickness and echogenicity represents a further measure

* Corresponding author at: Neurology Unit, Department of Medicine, Faculty of Medicine, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia.
E-mail address: nortina@um.edu.my (N. Shahrizaila).

http://dx.doi.org/10.1016/j.clinph.2016.11.010
1388-2457/

of quantitative muscle imaging. Quantitative muscle ultrasound including muscle thickness and echogenicity has been demonstrated to be reproducible (Simon et al., 2015). In healthy subjects extensor digitorum thickness correlated with peroneal compound muscle action potential (CMAP) (Seok et al., 2016) whereas in denervated muscles of the hand including first dorsal interossesus (FDI), severity of electromyograph abnormalities correlated with both muscle echogenicity and muscle thickness (Simon et al., 2015). Ultrasound also has the advantage of being accessible, cost effective and well-tolerated (Zaidman et al., 2014). Presently, the role of quantitative muscle ultrasound in CMT has not been evaluated.

In the present study, the utility of quantitative muscle ultrasound was investigated across a range of CMT patients, with the specific objectives to study the relationship between muscle ultrasound and conventional functional measures, nerve conduction parameters and nerve ultrasound in CMT patients. The performance of ultrasound at detecting and differentiating disease severity between the CMT subtypes was also explored.

2. Materials and methods

2.1. Subjects

Between November 2015 and March 2016, CMT patients with a diagnosis of CMT1A, CMTX1 and CMT2A were invited to participate in the study at the Forefront Neurology Clinic, Brain and Mind Centre, University of Sydney. Inclusion criteria were patients with genetic confirmation of *PMP22* duplications in CMT1A, point mutations in *GJB1* in CMTX1 and point mutations in *MFN2* in CMT2A patients. Exclusion criteria included the presence of a concomitant illness that may result in peripheral neuropathy or myopathy. A total of 21 CMT1A, 35 CMTX1 and 30 CMT2A were invited. 24 patients (10 CMT1A, 9 CMTX1and 5 CMT2A) agreed to participate. Of these, 3 CMT patients were excluded due to the presence of concomitant diabetes mellitus. The final number of patients included were 21 of which 9 were CMT1A, 8 CMTX1 and 4 CMT2A. Results were compared with 10 age- and gender-matched healthy control subjects, recruited from staff of Brain and Mind Centre and their relatives. The Sydney University Human Research Ethics Committee approved the study and all subjects provided written informed consent.

2.2. Clinical assessment

A full medical history and neurological examination were performed in all patients. Muscle strength examination included assessment of distal hand muscles (thumb abduction, abduction of index and little fingers) and leg muscles (plantar flexion, dorsiflexion of foot and great toe, plantar inversion and eversion) as well as grip strength with hand-held dynamometry. The CMTNS was also assessed in all patients (Murphy et al., 2011).

2.3. Nerve conduction studies (NCS)

NCS was performed by NS applying standard techniques of supramaximal percutaneous stimulation and surface electrode recording (Oh, 2003). Sensory studies were performed using the orthdromic technique in the median and ulnar nerves, and the antidromic technique in the radial and sural nerves. Motor studies were performed bilaterally acquiring the corresponding compound muscle action potentials (CMAPs) and F waves as follows: (i) median nerve stimulating distally at the wrist and proximally at the elbow, recording over abductor pollicis brevis muscle; (ii) ulnar nerve stimulating distally at the wrist, proximally at two sites: below elbow and above elbow, recording over abductor digiti minimi. Distal stimulation at the wrist recording over FDI muscles were also performed; (iii) peroneal nerve stimulating distally just lateral to the anterior tibial tendon at the ankle, proximally at two sites: below fibular head and lateral popliteal space recording over extensor digitorum brevis. Proximal stimulation recording tibialis anterior (TA) muscles was also performed; (iv) tibial nerve stimulating distally posterior to the medial malleolus at the ankle and proximally at the popliteal space, recording abductor hallucis muscle. Skin temperature was maintained above 33 °C throughout the procedures.

2.4. Neuromuscular ultrasound

Nerve and muscle ultrasound studies were performed by NS using MyLab Alpha System (Esaote, Genoa, Italy). A 6–18 MHz probe (SL2325) was used for nerve ultrasound whereas a 3–11 MHz probe (AL2442) was used for muscle ultrasound. The device setting was uniformly set with gain at 70% and depth of 4 cm for all muscle studies. Ultrasound was performed in both upper and lower limbs.

Nerve ultrasound of the ulnar and peroneal nerves was performed in the CMT patients only to allow for correlation analyses with their respective muscles (FDI and TA). Nerve cross-sectional area (CSA) of the ulnar nerve was assessed at the distal crease of the wrist for correlation with FDI and the peroneal nerve at the fibular head for correlation with TA. CSA measurements were traced manually inside the hyperechoic rim of the nerve using an electronic tracer.

A range of proximal and distal muscle images were obtained from subjects in the supine position in both upper and lower limbs by lightly applying the transducer to the skin, avoiding muscle compression. Muscles assessed in the upper limbs were biceps brachii, brachioradialis and FDI. In the lower limbs, the rectus femoris, vastus medialis and TA muscles were assessed. Supplementary Table S1 lists the localisation of the individual muscle image captured. In all muscles, except FDI, muscle thickness was calculated by using electronic calipers to measure the diameter across each muscle belly from the cross-sectional views of the muscles. In FDI, CSA was measured instead as the entire muscle could be visualised in a single image. CSA measurements of FDI were traced along the muscle rim using an electronic tracer. Two images were obtained for each muscle assessed and the mean value was obtained.

Muscle echogenicity was assessed using the ImageJ software (National Institutes of Health, Bethasda, Maryland) which analyses muscle gray scale values (Simon et al., 2015). The gray scale values ranged from 0 (lowest echogenicity) to 255 (highest echogenicity). The points of acquisition of regions of interest were fixed in each participant and traced freehand just within the hyperechoic superficial muscle fascia and mean gray scale values were measured using the histogram function (Supplementary Fig. S1). The gray scale analyses were performed by a single investigator (NS). To assess the reliability of this muscle gray scale analyses, images were also analysed by a second assessor (YN).

2.5. Statistical analysis

Normality of data was tested using the Shapiro–Wilk normality test, SPSS version 21. Inter-rater reliability of muscle echogenicity analyses were ascertained by calculating the intraclass correlation coefficient (ICC). Demographic data, clinical, neurophysiological and neuromuscular ultrasound parameters were compared between CMT and control groups using the independent *t*-test for parametric and Mann Whitney test for non-parametric variables respectively. Comparative studies between CMT subtypes