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Nerve high resolution ultrasonography in Tangier disease.

Authors: Antonella Di Pasquale[1], MD, PhD, Luca Leonardi[1], MD, Laura Fionda[1], MD, Fiammetta Vanoli[1], MD, Matteo Garibaldi[1], MD, Maurizio Inghilleri[2], MD, PhD, Marco Ceccanti[2], MD, Chiara Cambieri[2], MD, Emanuela Onesti[2], MD, PhD, Giovanni Antonini[1], MD.

[1]Department of Neuroscience, Mental Health and Sensory Organs (NESMOS), Sapienza University of Rome, Sant'Andrea Hospital, Rome, Italy.

[2]Human Neuroscience Department, Sapienza University of Rome, Rome, Italy

Number of words in abstract: 126

Number of words in manuscript: 1464

Corresponding author: Giovanni Antonini, giovanni.antonini@uniroma1.it

Running title: Nerve ultrasound in Tangier disease.

Ethical standards: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interests: None of the authors has any conflict of interest to disclose. No external funding was received for this study.

Abstract:

Introduction: Tangier disease (TD) is an autosomal recessive disorder characterized by severe reduction in high-density lipoprotein and accumulation of cholesterol esters in peripheral nerves and other tissues. The aim of this study was to evaluate whether nerve high-resolution ultrasonography (HRUS) can detect morphological nerve changes in TD. **Methods:** Three related patients of a previously reported Italian family with Tangier disease, carrying Y1698X mutation in *ABCA1*, underwent clinical, neurophysiological and quantitative nerve HRUS evaluation. Nerve HRUS data were compared with normal controls. **Results:** Despite neurophysiological abnormalities, no quantitative HRUS abnormality was detected in peripheral nerves. **Discussion:** Normalcy of HRUS in neurophysiologically abnormal nerves suggest possible subtle abnormalities that escape quantitative HRUS detection. Systematic studies in larger TD cohorts with different mutations are needed to confirm our findings.

Key words: Tangier disease; Nerve imaging; Nerve High Resolution Ultrasonography; Inherited neuropathies; Clinical-morphological correlations; Neurophysiological-morphological correlations.

Introduction: Tangier disease (TD) is an autosomal recessive disorder caused by loss-of-function mutations in the ATP-binding cassette transporter A1 gene (*ABCA1*) [1-3] and characterized by severe reduction in high-density lipoprotein (HDL) and accumulation of cholesterol esters in many tissues through-out the body including tonsils, liver, spleen, lymph node, cornea, thymus, intestinal mucosa and nerves, causing complex clinical pictures [4-10]. Heterozygous carriers may present intermediate phenotypes [11]. Though neurological involvement in TD is widely heterogeneous, three different, often overlapping, phenotypes, have been reported: a) mononeuritis multiplex with upper limb predominance and frequent facial involvement, in which both axonal and demyelinating features have been described; b) syringomyelia-like syndrome, with non-length dependent loss of small fibres; c) widespread nerve involvement with diffuse axonal loss [4-10]. Recently, an Italian TD family, carrying a novel Y1698X nonsense mutation in *ABCA1,* has been reported [12]. Nerve high resolution ultrasound (HRUS) is a non-invasive inexpensive tool for evaluating peripheral nerve morphology that has been applied in acquired and inherited neuropathies [13-16]. Herein, we assessed peripheral nerve morphology through HRUS in three patients of the reported Italian family and compared morphological and neurophysiological findings.

Methods

Patient and clinical assessment: Three siblings of unaffected consanguineous parents, all carrying the Y1698X mutation in *ABCA1*, in homozygosity in two patients (Pt. 1 and 2) and in heterozygosity in one patient (Pt. 3), have been studied. All patients underwent a neurological examination performed by a neuromuscular disease expert (MI). The Medical Research Council (MRC) scale was used to measure muscle strength. The detailed family pedigree is displayed in the previously mentioned publication (12). High-density lipoprotein levels were non-detectable in patients. 1 and 2, and 44 mg/dl (normal >45mg/dl) in patient. 3.

Neurophysiological evaluation: Neurophysiological evaluation was performed by an examiner (MC) non-blinded to the patient diagnosis. Nerve conduction studies (NCS) were performed on ulnar,

median, common peroneal, tibial, facial and sural nerves bilaterally and included evaluation of sensory nerve action potentials (SNAP), sensory conduction velocities (SCV), compound muscle action potentials (CMAP), motor conduction velocities (MCV), and F-wave latencies. Sensory NCS were performed orthodromically in the median and ulnar nerves and antidromically in the sural nerves; SNAP amplitude was measured peak-to-peak; CMAP of the facial nerve was recorded from the orbicularis oculi muscle. Skin temperature was monitored and maintained between 29°C and 32°C.

Ultrasonographic evaluation: Nerve HRUS was performed by a non-blinded examiner (ADP). A General Electric Voluson E6 imaging system (GE Healthcare, Waukesha, WI) with a broadband linear transducer (frequency band 10–18 MHz) was used. Median, ulnar, and common peroneal nerves were scanned bilaterally at the axilla, arm, elbow, forearm, wrist, and popliteal fossa, keeping the probe perpendicular to the nerves. Brachial plexus and cervical roots were evaluated bilaterally at the supraclavicular fossa and paravertebral space. Nerve cross-sectional area (NCSA) was measured by tracing the nerve just inside the hyperechoic rims, using the "ellipse formula" or "tracing technique" for nerves of irregular shape [16]. Moreover, we evaluated the single nerve fascicles within each scanned nerve. We defined as being abnormal those nerves with a NCSA higher than normal values or, regardless their NCSA, with at least 3 nerve fascicles with cross-sectional area >2 mm^2 [16]. Our laboratory reference values for NCSA were used as normative data, while literature values were used for brachial plexus and nerve roots [17, 18]. Normal values are expressed as mean ± 2 SD. Echogenicity and Power-Doppler indices in the nerve segments were not evaluated.

Written informed consent was obtained from all involved subjects. Our institutional review board approved this study.

Results

Clinical and neurophysiological features

Patient 1

Patient 1 (58-year-old woman, height 1.65 m, weight 55 kg) complained of numbness in her face (lips and chins on both sides) and weakness in the right hand. Neurological examination showed bilateral horizontal nystagmus and mild postural hand tremor; pain sensation was reduced over the face on both sides, over the neck, both upper limbs, chest, back and abdomen between dermatomes C3-T12; strength was reduced in upper and lower facial muscles with Bell's phenomenon on both sides, and distal muscles in right upper limb (MRC 3/5). NCS (Table 1) showed severe asymmetrical reduction of SNAP in right median and ulnar nerves, CMAP reduction by 90% in right the median nerve and MCV reduction in upper limb nerves (about 60% and 80% of normal on the right- and left side respectively). The compound muscle action potential was reduced in both facial nerves. EMG showed fibrillation potentials in the right first dorsal interosseous, and polyphasic long-duration motor unit potentials (MUPs) with reduced recruitment in the orbicularis oris, right extensor radialis carpi and right first dorsal interosseous. Cervical MRI was unremarkable. We considered these results to be indicative of asymmetric axonal loss.

Patient 2

Patient 2 (54-year-old man, height 1.74, weight 70 kg) was neurologically asymptomatic. He presented with mild bilateral orbicularis oculi weakness, reduced sensation to painful stimuli in upper limbs, and bilateral *pes cavus*. NCS (Table 1) showed reduced MCV (80% of normal) and normal CMAP amplitude in right median nerve; reduced MCV and CMAP amplitude (78% and 86% of normal, respectively)in left median nerve; slight increase of F-wave latency in both median nerves; normal SCV with symmetrical reduction of SNAPs in both ulnar and median nerves bilaterally and in the right sural nerve. Bilateral facial nerve involvement was detected by NCS in this patient as well. EMG showed slight reduction of recruitment without denervation in tibialis anterior, abductor pollicis brevis and orbicularis oculi on both sides, with polyphasic long-duration MUPs.

Patient 3

Patient 3 (66-year-old woman, height 1.69 m, weight 61 kg) complained of bilateral lower limb paraesthesia and showed mild bilateral weakness in orbicularis oculi and oris, reduced sensation to temperature in upper limbs, and bilateral *pes cavus*. NCS showed SNAP reductions in the right and left median nerves (Table 1). EMG in first dorsal interosseous and orbicularis oris bilaterally showed polyphasic long-duration MUPs, with reduced recruitment, without abnormal spontaneous activity.

High-resolution ultrasonography features

Nerve HRUS results are summarized in Table 2. NCSA of all nerve trunks, brachial plexus and roots were within the normal values. Similarly, no change in the single nerve fascicle measures was observed.

Discussion: HRUS usually is able to detect nerve morphological abnormalities in acquired and hereditary chronic demyelinating neuropathies, which are characterized by pathological changes that can cause nerve trunk enlargement [13-15, 16]. Conversely, nerve HRUS is usually normal in axonal polyneuropathies [13-15]. The studied members of this family with TD presented with heterogeneous neurophysiological phenotypes. Patient 1 had a picture suggesting an asymmetric axonal loss limited to upper limbs, clinically associated with a syringomyelic-like pattern. Conversely, patient 2 demonstrated neurophysiological findings that could be consistent with some degree of demyelinating changes in both median nerves, considering normal CMAP and lowered MCV, even though SNAP amplitude was reduced and SCV in the right median was near-normal. Finally, patient 3 showed neurogenic EMG changes and slight SNAP reductions in the median nerves.

A non-length-dependent mononeuritis multiplex pattern, as well as polyneuropathic and syringomyelic-like forms of presentation, have been reported in TD. A demyelinating pattern with a multifocal distribution also has been reported [7, 8, 19].

Few nerve pathological studies in TD are available in the literature. The main nerve pathological hallmarks reported in TD are lipid accumulation in Schwann cells, formation of scattered onion bulbs

and small tomaculae, mostly in small myelinated fibres [3-5, 7]. Moreover, Cai Z. *et al.* reported a TD patient with neurophysiological features of a demyelinating neuropathy in whom lipid storage in myelinated Schwann cells physically separated the myelin sheath from the axon at the juxtaparanodes, and who also demonstrated focal swellings and formation of myelin folding (small tomaculae) in the paranodal and juxtaparanodal regions. Quantitative morphometric evaluation demonstrated fibre and axon diameters to be smaller than normal [19]. Based on the reported pathological studies [3-5, 7, 19], we would expect that nerve HRUS could detect morphological nerve changes in TD. However, quantitative nerve HRUS in the studied sample was normal, even in those nerve trunks with electrophysiological abnormalities. We cannot exclude that morphological changes in these TD patients might consist of subtle abnormalities below the level of detection of HRUS. There are other limitations of this study: the sample size is small; all patients have the same genetic mutation and may not be representative of the disease as a whole; investigators were not blinded; alternative HRUS measures such as nerve echogenicity and vascularity were nor employed. Systematic studies in larger TD cohorts, with different types of mutations, are needed to further investigate our findings.

List of abbreviation: HRUS: high-resolution ultrasonography; TD: tangier disease; HDL: high-density lipoprotein; ABCA1: ATP-binding cassette transporter A1 gene; MRC: Medical Research Council; NCS: nerve conduction study; SNAP: sensory nerve action potential; SCV: sensory conduction velocity; CMAP: compound muscle action potential; MCV: motor conduction velocity; EMG: electromyography

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Table 1. Nerve conduction studies

	Patient 1		Patient 2		Patient 3		NV
Sensory	**R**	**L**	**R**	**L**	**R**	**L**	**-**

Nerves								
SNAP (μV)	**Sural**	12.6	8.3	2.4*	7.1*	28.4	21.4	>10
	Ulnar	Abs*	3.6*	4.8*	5.6*	8.4	10.9	>8
	Median	Abs*	2.8*	9.8*	8.2*	14.6*	11.2*	>15
SCV (m/s)	**Sural**	52	50	48	45	54	50	>41
	Ulnar	-	54	50	55	50	50	>50
	Median	-	44*	48*	50	57	60	>50
Nerves		**R**	**L**	**R**	**L**	**R**	**L**	-
cMAP (mV)	**Tibial (A/K)**	5.7/4.7	7.4/4.9	13.1/7.8	12.5/7.8	6.4/4.9	12.5/7.8	>3
	Peroneal (A/FH/K)	6.1/5/4.6	6.1/5.1/4.7	3.5/3.5/3.5	1.2/1.1/1.1*	9.7/9.7/9.2	7.8/5.8/5.6	>2.5
	Ulnar (W/BE/AE/EP)	0.4/0.3/0.3/0.3*	6.6/6.6/6.6/4.3	11.1/9/7.4/7.4	7.8/7.4/7.2/7.2	16.6/16.6/13.2/12.8	12.5/12.5/12/12	>5
	Median (W/E/EP)	0.3/0.3/0.3*	1.8/1.8/1.8*	5.3/4.5/4.3	4.3/3.3/3.3*	14.3/14.3/13.4	6.1/6/6	>5
	Facial (OO)	0.8*	0.5*	0.5*	0.8*	1.8	2	>1.5
DML (ms)	**Tibial**	3.9	4.2	3.9	5.1	3.2	3.7	<6
	Peroneal	3.2	2.9	4.2	5.6*	4.4	3.2	<5.5
	Ulnar	5.7*	1.8	2.4	2.3	2.8	2.8	<3.4
	Median	6.1*	4.2*	4.2	3.7	2.4	3.9	<4
	Facial	2.4	2.9	2.7	2.9	2.9	2.9	<3
MCV (m/s)	**Tibial**	46	50	45	47	47	54	>41
	Peroneal	48	45	40*	39*	51	45	>41
	Ulnar	30*	43*	55	50	59	61	>50
	Median	27*	43*	40*	39*	62	57	>50
F-wave	**Tibial**	52.7	51.3	53.2	57.4*	42	43.2	<55
	Peroneal	47.6	46.7	54.7	Abs*	41.7	43.5	<55
	Ulnar	Abs	25.4	26.3	26.9	24.4	25.6	<32
	Median	Abs	29.5	30.1	31.5	25.9	24.4	<32

SNC: sensory nerve conduction; SNAP: sensory nerve action potential; MNC: motor nerve conduction; DML: distal motor latency; MCV: motor conduction velocity; NV: normal values; cMAP: compound motor action potential; Pt: patient; R: right; L: left, A: ankle; K: knee; FH: fibular head; K: knee; W: wrist; BE: below elbow; AE: above elbow; EP: Erb's point; E: elbow; OO: orbicularis oculi. Abnormal values are marked with asterisks (*).

Table 2. Nerve high resolution ultrasonography

Nerve	Site	Side	Pat 1	Pat 2	Pat 3	NV
Median	**Wrist**	**R**	9	8	7	≤12
		L	10	8	6	

	Forearm	**R**	5	7	5	≤9
		L	6	6	5	
	Elbow	**R**	9	10	8	≤10
		L	9	8	6	
	Arm	**R**	8	10	9	≤11
		L	8	8	6	
	Axilla	**R**	9	9	5	≤11
		L	7	6	5	
Ulnar	**Wrist**	**R**	3	3	5	≤6
		L	4	3	4	
	Forearm	**R**	4	4	4	≤8
		L	4	4	4	
	Elbow	**R**	11	8	7	≤12
		L	8	8	7	
	Arm	**R**	7	5	8	≤8
		L	4	5	6	
	Axilla	**R**	8	4	5	≤9
		L	8	4	7	
Peroneal	**Pop fos**	**R**	9	11	9	≤13
		L	6	10	10	
Bra plex	**Sup cla**	**R**	47	66	50	≤82,67
		L	54	63	61	
C5, 6, 7	**Int sca**	**R**	8/9/11	9/10/12	9/9/8	≤ 11/15/15
		L	9/9/11	10/10/11	8/9/10	

Values of NCSA (expressed in mm^2) of all studied segments; NV: normal values; NR: not recorded; Pop fos: popliteal fossa; Bra plex: brachial plexus; Sup cla: supraclavicular space; Int sca: interscalenic Space; Pat: patient. Abnormal values are marked with asterisks (*).