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ELECTROPHYSIOLOGICAL EVALUATION IN MYOTONIC DYSTROPHY

Correlation with CTG length expansion

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ABSTRACT - In myotonic dystrophy (MD), disease severity has been correlated with expansion of CTG repeats in chromosome 19. The aims of this study were to evaluate efficacy of electromyography in the diagnosis of MD, access the frequency and the characteristics of peripheral involvement in the disease and to verify whether the CTG repeats correlated with the electrophysiological abnormalities. Twenty-five patients and six relatives at risk of carrying the MD gene were examined. Electrical myotonia (EM) was scored. Sensory and motor conduction velocity (CV) were studied in five nerves. Leukocyte DNA analysis was done in 26 subjects. Myopathy and myotonia were found in 27 cases. EM was most frequent in muscles of hand and in *tibialis anterior*. No significant correlation was found between EM scores and length of CTG expansions. EM scores correlated significantly with the degree of clinical myopathy, expressed by a muscular disability scale. Peripheral neuropathy was found in eight subjects and was not restricted to those who were diabetics.

KEY WORDS: myotonic dystrophy, electromyography, myotonia, myopathy, peripheral neuropathy, CTG repeat.

Avaliação eletrofisiológica na distrofia miotônica: correlação com a expansão de tripletos CTG.

RESUMO - Na distrofia miotônica (DM) a severidade da doença tem sido correlacionada com a expansão de tripletos CTG, no cromossomo 19. Foram objetivos do estudo avaliar a eficácia da eletromiografia no diagnóstico, verificar a freqüência e tipos de neuropatias periféricas e determinar se a expansão CTG correlacionava-se com as anormalidades eletrofisiológicas nesta doença. Examinamos 25 pacientes e seis familiares sob risco de herdar a doença. A miotonia elétrica (ME) foi graduada, cinco velocidades de condução sensitivas e cinco motoras estudadas. O DNA leucocitário foi analisado em 26 indivíduos. Encontrou-se miopatia miotônica em 27 pacientes, sendo a ME mais freqüente nos músculos da mão e no tibial anterior. Não houve correlação significativa entre os graus de ME e o número de repetições CTG. Os graus de ME correlacionaram-se significantemente com a severidade da miopatia clínica, expressa por escala de disfunção muscular. Neuropatia periférica foi encontrada em oito indivíduos, não exclusivamente em diabéticos.

PALAVRAS-CHAVE: distrofia miotônica, eletromiografia, miotonia, miopatia, neuropatia periférica, repetição de tripletos.

Myotonic dystrophy (MD) is a systemic disorder, with a variable phenotype including myopathy and myotonia[1]. Disease severity has been correlated with unstable expansion of CTG repeats in the gene encoding myotonin protein kinase in chromosome 19[2,3]. Electromyography (EMG) is a master tool for the diagnosis, but the incidence of EMG myotonia has varied in different reports because of selection and number of muscles examined and interpretation of electromyographic findings, as well as diagnostic criteria[4]. Electroneuromyography may also detect peripheral neuropathy (PN) in MD patients[5,6].

The aims of this paper were to study prospectively the efficacy of EMG in MD diagnosis, access the frequency and the characteristics of peripheral neuropathy in the disease and to correlate the CTGn with abnormalities documented in EMG.

METHOD

Thirty one subjects from 20 different families participated, by writing informed consent; 25 with clinical hypothesis of MD and six relatives at risk of carrying the MD gene. Inclusion criteria were presence of clinical myopathy with myotonia and others systemic features of MD

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and asymptomatic subjects or with minimal MD signs or symptoms belonging to MD families. They were submitted to neurological examination, EMG protocol, routine blood analyses to detect common causes of PN and blood sample for DNA analysis. Clinical and EMG results were established before the genetic study. They were classified according to Mathieu et al.[7], into a muscular disability rating scale, as follow: Grade 1 – no clinical muscular impairment (diagnosis made by EMG, slit-lamp examination or DNA analysis); Grade 2 - minimal signs (myotonia, jaw and temporal wasting, facial weakness, sternomastoids wasting / weakness, ptosis, nasal speech, no distal weakness except isolated *digitis flexor* weakness); Grade 3 - distal weakness (no proximal weakness, except isolated *triceps brachii* weakness); Grade 4 - mild or moderate proximal weakness; Grade 5 - severe proximal weakness (confined to wheelchair for short and long distances).

EMG protocol studied the anatomical distribution of electrical myotonia (EM), in 13 muscles and was scored according to Streib and Sun[4]: Grade 1 - occasional short run of positive waves following needle movement (interpreted as nonspecific); Grade 2 - myotonic discharges of more than 0.5 sec duration in two or more muscle areas; Grade 3 - myotonic discharges in most needle locations; Grade 4 - myotonic discharges in each movement in all examined areas. Score for individual muscle: sum of the grades divided by the number of times the muscle was examined. Total EMG myotonia score for a subject: summed grades of EMG-myotonia, divided by the number of muscles examined.

H reflex (tibial nerve), CV in five motor and in five sensory nerves were evaluated in each patient. CV techniques and normal values were considered according to Kimura[8]. Investigations were performed using a Neuropack equipment (Nihon Khoden Co.), superficial electrodes for stimulation and recording and monopolar needle for EMG. Temperature was controlled by a Cole Palmer Digi Sense Termometer and maintained over 32°C.

Routine blood examination included hemogram, creatine kinase, alanine and aspartate aminotransferase, total calcium, potassium, sodium, lactate dehydrogenase, cholesterol total, HDL and LDL-cholesterol, creatinine, glucose, triglycerides, urea nitrogen, thyroxine free and thyrotropin, serum B12 and folic acid.

Genomic DNA was prepared from peripheral blood leukocytes [9,10]. The size of the CTG-repeat expansion was analysed by PCR-Elongase. PCR analyses were performed in a volume of 30 μml, containing 100 ng template DNA,60 mM Tris-SO4 (ph 9.1), 18 mM (NH4)2SO4, 1.1 mM Mg SO4, 10 mM dNTP mix, 10% Dimethylsulfonamide (DMSO), 1 μml Elongaseââ (mixture of Taq and *Pyrococcus* species GB-D DNA polymerases from Life Technologies, 10480-010), 0.2 μmM of primers 101 and 102 (101: 5'-CTTCCCAGGCCTGCAGTTTGCCCATC - 3'; 102: 5'-GAACGGGGCTCGAAGGGTCTTGTAGC 3'). PCR involved heating to 94°C for 6 min, 65°C for 1 min, 72°C for 1 min and then 30 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 1 min. Amplified product (2 μml) was mixed with 2 μml of formamide loading buffer, heated for 5 min at 100°C, subjected to electrophoresis in a 2% agarose gel, blotted on to nylon membrane and probed with the 3'-end-labelled 101 primer.

Statistical analyses were based on linear correlation coefficient of Spearman (non-parametric); Kruskal-Wallis test was used to verify homogeneity between myotonic score and the degree of muscular disability.

RESULTS

Myotonia and myopathy were found in 27 subjects, with mean age of 34,4 years. None of them presented congenital MD. The mean grade of muscular disability was 2,77. Total EMG-myotonia score, the number of CTG repeats, grade of muscular disability and age of each case is presented in Table 1.

Six subjects (Cases 1,2,3,4,13,21,Table 1), belonging to three different families with MD, were initially considered relatives at risk to have the abnormal gene. They were clinically normal (force grade five in all muscles, no clinical myotonia, no muscular atrophy, no other clinical signs of MD). In cases 1,2,3 and 4, EMG and muscle enzimes were also normal. They were daughter (Case 1), son (Case 2) and sisters (Cases 3 and 4) of one patient (Case 26). Cases 13 and 21 were also clinically normal, had mild abnormalities in EMG, and DNA confirmed MD. Case 13 was sister of patients (Cases 22 and 25). The deceased mother of case 21 had a definitive diagnosis of MD.

The frequency of EM and composite score for each muscle examined is shown in Table 2.

A significant correlation (0,617), at 0,05 level with Spearman's test, was found between myotonic score and the grade of muscular disability. There was not a significant correlation (0,232), between CTG expansion and the score of myotonia, neither with the degree of muscular disability (0.026).The p-value in Kruskal-Wallis test was 0,012 for the score of myotonia and muscular disability.

The electroneurography detected PN in eight subjects (seven patients and one relative at risk). Their data are listed in Table 3. Diabetes mellitus was diagnosed in four patients and ,in the other four individuals, no etiology for PN was defined. Six patients presented mild to moderate sensory-motor axonopathy and one of them had also left carpal tunnel syndrome; one had mild motor axonopathy. A relative at risk had a mild sensitive axonopathy with symptomatic right carpal tunnel syndrome.

Table 1. Casuistic in relation to age, DNA and scores.

Case number	Age*	DNA	Score of muscular disability	EMG myotonia score
1	20	R	N	0
2	23	R	N	0
3	37	R	N	0
4	44	R	N	0
5	12	5/110	3	0.38
6	14	10/110	2	2
7	19	R	2	1.23
8	20	5/180	2	1.07
9	20	5/200	3	1.38
10	24	5/200	2	1
11	24	5/200	2	2.23
12	27	7/110	3	1.84
13	27	5/200	1	0.15
14	28	7/110	2	0.3
15	35	5/110	4	3.23
16	36	7/200	3	2.15
17	36	5/200	4	2
18	38	15/400	4	2.61
19	39	5/200	4	2.07
20	40	7/100	4	2.15
21	40	5/200	1	0.6
22	42	5/200	3	2.61
23	42	5/100	1	0.15
24	43	7/100	3	1.07
25	45	5/200	3	2.46
26	46	7/150	4	2.15
27	46	5/110	2	2.46
28	46	7/110	2	1.53
29	47	5/80	4	2.15
30	47	5/110	4	2.46
31	56	5/390	3	2.53

*, patient's ages at the time of EMG; N, diagnosed as normal (force grade five in all muscles, no clinical myotonia, no muscular atrophy, no other clinical signs of MD); R, DNA analysis was refused by these patients.

Table 2. Percentage of EM and composite score for each muscle examined.

Muscle examined	Number of patient	Percentage of EM	Composite score
First dorsal interosseous	27	96	2.81
Abductor pollicis brevis	27	100	2.85
Abductor digiti minimi	27	88	2.55
Extensor digitorum communis	27	77	1.85
Biceps brachii	27	70	1.55
Deltoid	26	46	1.07
Vastus lateralis	27	51	0.96
Tibialis anterior	27	88	2
Gastrocnemius	27	66	1.4
Extensor digitorum brevis	27	81	1.74
Lumbar paraspinal	26	65	1.23
Genioglossus	26	57	0.88
Orbicularis oris	27	59	0.8

Table 3. Electroneurographic results in subjects with peripheral neuropathy

	Patients							
	Diabetic				Non diabetic			Relative at risk
Case	25	20	19	29	18	22	17	2
Age	45	40	39	47	38	42	36	23
R Fib DL	4,20	4,56	4,02	5.40	5.70	6.36	5.82	3.90
R Fib A	2.20	2.40	1.73	1.93	0.47	1.30	1.40	4.93
R Fib CV	40.2	42.0	37.6	42.0	31.5	30.5	47.0	45.1
R Fib Fw	50.6	51.6	55.0	51.4	∅	62.0	46.0	50.8
L PT DL	4.32	4.50	3.30	3.84	4.26	5.64	4.86	4.80
L PT A	5.00	6.00	1.53	3.80	0.93	7.00	4.27	9.83
L PT CV	39.2	40.7	34.5	42.7	35.1	34.8	48.4	48.4
L PT Fw	53.6	51.8	55.8	57.0	57.8	62.2	44.4	51.8
R Med DL	3.06	3.72	3.54	3.30	3.48	3.78	3.06	4.02
R Med A	8.17	5.00	6.50	0.83	7.00	10.0	4.67	12.0
R Med CV	53.3	54.2	51.3	50.2	50.6	51.6	56.4	58.9
R Med Fw	27.4	30.2	31.4	∅	30.6	29.4	32.2	27.8
R Uln DL	2.76	2.04	2.58	2.76	2.82	2.76	2.46	2.70
R Uln A	10.0	7.17	11.7	3.33	7.93	10.5	4.93	11.8
R Uln CV	52.4	51.6	50.0	50.6	48.0	45.6	56.8	57.0
R Uln Fw	30.0	29.4	29.6	35.6	31.2	33.2	27.6	28.4
L Med DL		4.80	3.78	3.12	3.60		3.00	
L Med A		2.20	5.83	3.87	5.93		4.93	
L Med CV		48.5	51.3	47.1	49.8		56.8	
L Med Fw		33.8	31.4	31.2	33.4		27.6	
			Sensitive					
R Med DL	2.76	2.40	2.76	2.60	2.80	3.08	2.04	3.16
R Med A	35.3	30.3	17.3	36.0	58.0	44.7	72.7	48.0
R Med CV	53.3	52.1	56.2	57.7	53.6	48.7	61.3	44.3
R Uln DL	2.44	2.56	2.40	2.44	2.60	2.72	1.96	2.60
R Uln A	33.3	23.7	13.3	30.7	44.7	26.0	50.0	34.7
R Uln CV	53.3	60.5	52.5	55.3	51.9	46.0	53.6	51.9
R Rad DL	2.12	1.96	2.28	2.24	3.36	2.36	1.76	2.08
R Rad A	28.0	40.3	15.7	32.7	24.0	30.0	44.0	40.3
R Rad CV	59.0	66.3	57.0	60.3	43.2	53.0	54.0	57.7
R Sur L	3.40	2.84	2.96	2.68	4.64	4.20	2.68	3.32
R Sur A	13.0	14.3	7.67	10.5	18.0	13.0	24.0	17.3
R Sur CV	45.6	52.8	47.3	52.2	37.7	35.7	48.5	43.7
L Sur L	3.60	3.16	3.08	2.48	4.80	4.28	2.72	3.76
L Sur A	12.0	13.3	7.00	14.0	18.1	15.3	27.0	15.0
L Sur CV	40.3	49.1	45.6	50.4	37.5	49.1	46.0	42.6
R H reflex	∅	∅	∅	∅	33.0 Small A	33.2 Small A	∅	29.5
L H reflex	∅	∅	∅	33.6 Small A	36.8 Small A	∅	∅	30.8

Nerves: FIB, Fibular; PT, Posterior tibial; MED, Median; ULN, Ulnar; RAD, Radial; SUR, Sural. Temperature over 32° C. L, Left; R, Right;
DL, distal latency (ms), measured to the onset of the evoked response; A, amplitude (mV -motor; μmV- sensitive) of the evoked response, measured from baseline to negative peak.
CV, conduction velocity (m/s); FW, F wave latency (ms) to recording site (wrist or ankle); H reflex latency (ms), POSTERIOR TIBIAL NERVES; ∅, not obtained.

DISCUSSION

Despite the discovery of the gene defect in DM the molecular pathological mechanism in the disease is still not completely known[10-13]. Currently explanations include: deficiency of the DM protein kinase[14]; effect of expanded CTG repeats on neighboring genes[15-18]; interference of the mutant DM protein kinase transcripts with others transcripts (trans RNA interference)[19]. Studies that are in progress look for new transgenic models that may more closely resemble clinical DM and that provide also a model for anticipation[20,21].

The role of DNA analysis to the diagnosis of DM is well establish. In one of the studies that described the genetic mutation in MD[13] it was showed that over 98% of MD patients had abnormal CTG repeats in the DMPK gene in chromosome 19. A relationship between the size of the CTG repeat in leukocyte DNA and the disease severity, in its multisystemic aspects, has been reported in several studies[2,3,22]. However, the disease is heterogeneous, with clinical variability in all ages considered[7] and a given triplet size[2]. The somatic instability of the CTG repeats has also been reported and raises the question whether expansions in different tissues might account for the lack of precise correlation between the number of CTG repeats in leukocyte DNA and clinical findings[23]. The CTG expansions are larger in skeletal muscle than in leukocytes[24,25], but the pattern of weakness and atrophy in MD do not seem to be related to the differences in CTG-repeat length between different muscles[25].

In MD it may be difficult to access the degree of muscular disability using a functional scale, because it may be influenced by multiple and complex factors[7]. We used the muscular disability rating scale constructed by Mathieu et al[7], based on the clinical muscular involvement and concordant with the usual distal to proximal progression of the muscular involvement in MD. In accordance with this author's observations, we found that the scale allowed registration of significant changes in the degree of muscular deficit. We did not demonstrated a significant correlation between CTG expansion in leukocytes and the EM score, neither with muscular disability. However, patients with CTG equal or over 200 had a tendency to have higher scores of EM. The number of patients examined and clinical variability in all ages are possible explanations for our results.

The pathophysiology responsible for the muscle wasting, weakness and for myotonia is not yet completely defined[26,27]. Deficiency in muscle anabolism due to multiple causes[1], deficiency of MD protein kinase leading to dysregulation of the calcium metabolism in muscle fiber[26] and reduction of type 2 muscle fiber numbers[27] are proposed to explain weakness and wasting. Alteration in the function of slow potassium channels can produce myotonia[26]. Recently, evidences based on a transgenic mice, support a possible mechanism of RNA gain of function in the pathogenesis of MD[15].

In classical cases, myotonia is often widespread and easy to elicit, but in some patients it may be subtle, limited and difficult to be recognized in clinical level [1]. Electromyography is important specially in these cases and in the evaluation of subjects at risk from MD families[4]. Detailed EMG was diagnostic in two asymptomatic relatives in our data, in whom DNA analysis was confirmatory. Twenty-five cases of MD diagnosed clinically had positive EMG for myopathy and myotonia. DNA study was done in twenty-four of them and CTG expansion was documented in all. Concordance between presence of myotonia on clinical examination and the EMG results was similarly referred by others authors[4,28]. The selection and number of muscles examined are fundamental for unequivocal EMG diagnosis, as the sensitivity of diagnosis by EMG exam may vary according to this parameters[4]. However, in patients with minimal MD, molecular analysis is the gold standard for diagnosis.

EM was encountered more frequently in hand muscles (88 to 100%) and *tibialis anterior* (88%) and less in deltoid (46%). The composite score, showing intensity and frequency of EM, was also greater in hand muscles and *tibialis anterior*. These data are in agreement with the observations of Streib and Sun[4]. In our series, EM correlated significantly with the degree of myopathy, in accordance with Streib and Sun[4], although we used a different muscular disability scale[7]. Clinical myotonia may be difficult to elicit in patients with severe wasting and in congenital cases, in the first two years of life[29]. It should be noticed that no patient in our study had score five for muscular disability and we did not included congenital cases.

PN have been reported as part of the clinical picture of MD, like polyneuropathy[1,29]. In electroneurography, slowing of motor and sensory CV, increase of minimal latency of F wave and delay or absence of the H reflex, reduction of sensory and motor amplitudes of recorded potentials have been de-

scribed[6,30]. Pathological studies of peripheral nerve showed sensory involvement[5,31,32]. The abnormalities are probably primary axonal and they do not parallel the severity of muscular involvement[6]. Although the cause of the polyneuropathy remains uncertain, it seems to be one of the manifestations of the disease[6], but in some patients diabetes mellitus can be associated. Our findings included seven cases of PN out of 27 patients with MD and one case in a relative at risk, in whom the EMG did not diagnosed MD. Six patients presented a mild to moderate sensory-motor axonal polyneuropathy; four were diabetics, and in two, no other evident etiology was found. Two electrophysiological exams showed a carpal tunnel syndrome, one asymptomatic and associated with axonal sensory-motor neuropathy. The other, in a relative at risk, the carpal tunnel syndrome was symptomatic and associated with a mild axonal sensory neuropathy in lower limbs. Mondelli et al.[6] concluded that PN is not rare in MD, involving about 46% of their patient. In our casuistic, 25,9% had PN, with type and intensity similar the encountered in the literature[5,6,31,32].

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