Ital J Neurol Sci (1998) 19:75-80

ORIGINAL

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Positive correlation of CTG expansion and pharyngoesophageal alterations in myotonic dystrophy patients

Received: 6 August 1997 / Accepted in revised form: 2 December 1997

Abstract Alteration of the pharyngoesophageal musculature is a common finding in patients with myotonic dystrophy (MD), regardless of the presence of dysphagia. The aim of the present study was to determine whether a specific pattern of swallowing abnormalities could be identified in MD patients, and the possible correlation with the size of CTG repeats. Fifteen MD patients, 8 of whom were asymptomatic for dysphagia, underwent a videofluoroscopic study of swallowing. Alterations of the pharyngoesophageal phase of swallowing were detected in 12 of 15 patients, 6 without clinical evidence of dysphagia. Incomplete relaxation of the upper esophageal sphincter (UES) and esophageal hypotonia were the most common alterations. We found a significant correlation between the number of radiological alterations and the size of CTG repeats. A typical radiological pattern of swallowing has also been identified. The role of videofluoroscopy in evaluation of MD patients is briefly discussed.

Key words Myotonic dystrophy • Pharyngoesophageal motility • Videofluoroscopy • CTG expansion

This paper was supported by the grant n. 1061 from Theleton

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Introduction

Myotonic dystrophy (MD) is an autosomal dominant disorder associated with the abnormal expansion of CTG repeats in the 3' untranslated region of the gene encoding a myotonin-protein kinase on chromosome 19q13.3 [1, 2]. Clinically, MD is characterized by muscular weakness and myotonia, as well as by systemic alterations such as cataracts, endocrine abnormalities, and cardiac conduction defects [3].

The size of CTG expansion correlates significantly with the muscular disability and inversely with the age at onset [4-6]. The finding of unstable CTG repeats in MD helped to understand some peculiar features of the disease, such as the marked intrafamilial phenotypic variability as well as the earlier onset and the increased severity in offspring of successive generations (i.e. anticipation) [7].

The involvement of the pharyngoesophageal musculature is a common finding in MD, which may lead to the development of dysphagia. Radiological evidence of pharyngoesophageal alterations in MD has previously been described [8-17]. Most of the studies, however, are relatively old and were performed with radiological techniques less sensitive than those available nowadays. Using videofluoroscopy, we evaluated the dynamic swallowing in MD patients to determine whether a specific pattern of alterations was present and if it correlated with the clinical evidence of dysphagia. We also investigated the possibility of a correlation between the size of CTG expansion and the pharyngoesophageal alterations, as detected by videofluoroscopy.

Patients and methods

MD patients followed at the Neuromuscular Center of the University of Padua were considered. The diagnosis was established by accepted clinical and laboratory criteria [18], and by genetic and electromyographic findings. The severity of the clinical

weakness and myotonia was scored with the Muscular Disability Rating Scale (MDRS) [19] and graded from 1 to 5 as follows:

1. No clinical muscular impairment (diagnosis made by EMG, slit lamp examination, or DNA analysis);
2. minimal signs (myotonia, jaw and temporal wasting, sternomastoid wasting/weakness, ptosis, nasal speech, no distal weakness except isolated digital flexor weakness);
3. distal weakness (no proximal weakness except isolated triceps brachii weakness);
4. mild or moderate proximal weakness; and
5. severe proximal weakness (confined to wheelchair).

Patients were asked detailed questions about their eating habits and swallowing capability to identify possible overlooked mild signs of dysphagia. The degree of dysphagia was classified as:

- *Normal*, normal eating habits;
- *Fair*, early eating problems;
- *Moderate*, dietary consistency changes; and
- *Poor*, oral intake is inadequate, but patients still take most food orally. Supplemental tube feeding needs to be considered.

All the procedures described below were performed under patients' informed consent.

Videofluoroscopy

Videofluoroscopy [20, 21] was carried out during swallowing of fluid and semisolid contrast medium (barium mixture of Prontobario HD, Bracco, Milan, Italy). A water-soluble contrast medium (Gastrografin, Schering, Milan, Italy) was given when the risk of aspiration was suspected. The patient was seated upright and observed with a four-views evaluation (antero-posterior, lateral, and oblique right and left projections).

The following alterations were evaluated: oral involvement; lateral pharyngeal wall hypotonia; incomplete or inadequate velopharyngeal closure; vallecular, pyriform or hypopharyngeal stasis; incomplete epiglottic inversion; and presence of aspiration. The functionality of upper esophageal sphincter (UES) and lower esophageal sphincter (LES), and the pharyngeal and esophageal motility were also assessed. All videofluoroscopic studies were recorded on videotape Sony U-Matic VO 5800 PS using Sony XBR cassettes (Sony, Tokyo, Japan) and the results evaluated in blinded fashion.

Genetic analysis

Genomic DNA (10 µg) extracted from patients' peripheral blood leukocytes was digested to completion with EcoRI or BamHI (Bio-Rad, Italy). The digestion products were separated by electrophoresis on 0.8% agarose gels and transferred onto nylon membranes (Hybond N, Amersham, UK). Southern blots were probed with the 1.4 kb BamHI fragment of the DMY 1 cosmid containing the variable CTG repeat [5]. Hybridime (HT Biotechnology, UK) was included at 0.25 mg/ml in both the prehybridization and hybridization solutions. Blots were washed in 1X sodium chloride/sodium citrate (SSC) and 0.1% sodium dodecyl sulphate (SDS) at 65°C, and then exposed to AGFA XAR film for 1-4 days. Polymerase chain reaction (PCR) was performed using 101 and 102 flanking primers, according to Brook et al. [4]. The number of CTG repeats was established for each patient by length variation on Southern blotting (EcoRI and/or BamHI) using specific software (Image-Quant, Molecular Dynamics, USA) after densitometric analysis on Personal Densitometer (Molecular Dynamics, USA).

Statistical analysis

Data were analyzed with commercial statistic packages (SIGMA-STAT and EXCEL). Multiple linear regression and Spearman's test were used to explore the relationship between CTG expansion, clinical parameters and number of analyzed subjects.

Results

Fifteen MD patients (10 males and 5 females), with a mean age of 36.66 years (range, 21-60 years) were identified (Table 1). The degree of dysphagia was normal in 8 patients, fair in 3, moderate in 2, and poor in 2. A younger age at onset of the disease was present in patients with moderate and poor swallowing (mean age 24 and 17.5 years, respectively), when compared to the patients with fair and normal deglutition capability (mean age 30.3 and 28.75 years).

Table 1. Clinical features of 15 MD patients

Patient	Sex	Age at onset (years)	Duration of the disease (years)	Swallowing capability	MDRS score	CTG repeats
1	M	15	10	Normal	2	638
2	M	22	14	Normal	2	900
3	M	15	6	Normal	2	420
4	M	45	5	Normal	1	70
5	F	52	6	Normal	2	205
6	F	16	13	Normal	3	935
7	M	50	10	Normal	1	70
8	M	15	2	Normal	2	100
9	M	25	15	Fair	3	415
10	F	24	3	Fair	3	850
11	F	42	0	Fair	1	100
12	M	23	6	Moderate	3	624
13	F	25	24	Moderate	5	1356
14	M	10	22	Poor	4	1370
15	M	25	5	Poor	3	931

We found a direct correlation between the mean duration of the disease and the degree of dysphagia: 14.25 years in patients with moderate and poor swallowing, and 7.25 years in patients with fair and nomal deglutition capability. None of our patients had dysphagia as the presenting symptom. There was no clear correlation between the muscular disability and the degree of dysphagia. In fact the patients with moderate and poor deglutition had mean MDRS scores of 4 and 3.5, respectively.

Five patients (no. 3, 4 , 6 , 7 and 15 who correspond, respectively, to no. 4, 3, 7, 5 and 6 in the pedigree in Fig. 1) were members of the same family, and patients no. 11 and 12 were mother and son.

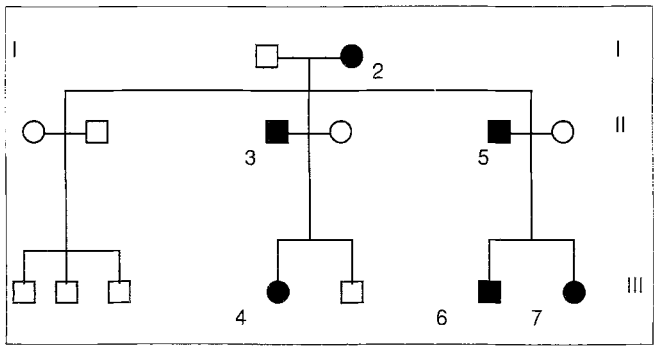

Fig. 1. II 3= patient 4 who presented CTG 70 and a normal videofluoroscopy. II 5= patient 7 who presented CTG 70 and a normal videofluoroscopy. III 4= patient 3 who presented CTG 420 and two alterations at videofluoroscopy. III 6= patient 15 who presented CTG 935 and three alterations at videofluoroscopy. III 7= patient 6 who presented CTG 935 and three alterations at videofluoroscopy

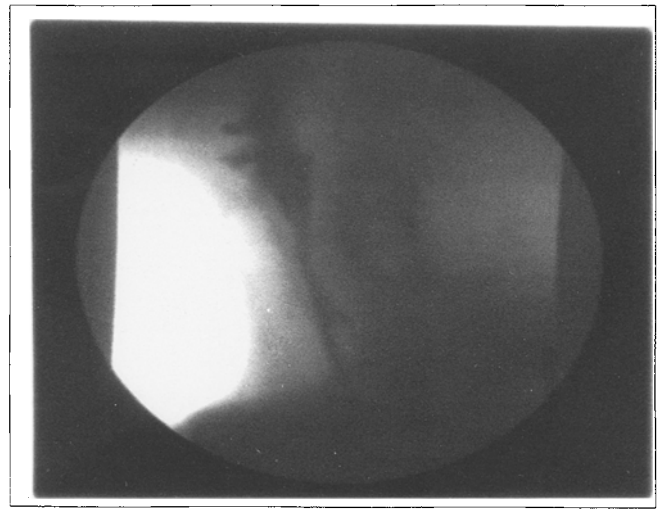

Fig. 2. Videofluoroscopy shows an incomplete relaxation of upper esophageal sphincter (patient 8)

Videofluoroscopy

Radiological abnormalities of the pharyngoesophageal function were found in 12 of 15 patients (Table 2). Incomplete UES relaxation (Fig. 2) and esophageal hypotonia often associated with reduced esophageal peristalsis (Fig. 3), were the most common alterations, detected in 8 of 15 patients. Pharyngeal wall hypotonia with vallecular and pyriform barium stasis (Fig. 4) was present in 6 patients. Aspiration was observed in 1 dysphagic and in 2 non-dysphagic patients, but in all three cases was clinically silent.

Genetic analysys and correlation studies

An expanded number of CTG repeats, ranging from 70 to 1370, was observed in all patients (Table 1). A significant correlation was found between the size of CTG expansion and the severity of muscular involvement, as expressed by the MRDS score (Spearman's Rho=0.74, $p<0.002$). A significant correlation was also found between the whole of pharyngoesophageal abnormalities, detected by videofluoroscopy, and the size of CTG repeats (Spearman's Rho=0.56, $p<0.05$). An increased size of CTG expansion was found in

Table 2. Videofluoroscopic abnormalities present in each patient compared to the swallowing capability.

Patient	Swallowing capability	Oral involvement	Pharyngeal hypotonia	Velopharyngeal insufficiency	Hypopharyngeal stasis	Aspiration	Incomplete epiglottic inversion	Defective UES relaxation	Esophageal hypotonia	Defective LES opening
1	Normal	-	+	+	+	-	-	-	-	-
2	Normal	+	+	+	-	+	-	+	-	-
3	Normal	-	+	-	+	-	-	-	+	-
4	Normal	-	-	-	-	-	-	-	-	-
5	Normal	-	-	+	-	-	-	+	+	-
6	Normal	-	-	+	+	-	-	+	+	-
7	Normal	-	-	-	-	-	-	-	-	-
8	Normal	-	-	-	-	+	+	+	-	-
9	Fair	-	-	+	+	-	-	+	+	-
10	Fair	-	-	-	-	+	-	-	+	-
11	Fair	-	-	-	-	-	-	-	-	-
12	Moderate	-	+	+	+	-	-	+	-	+
13	Moderate	-	+	-	+	-	-	+	+	-
14	Poor	-	-	-	+	-	-	+	+	-
15	Poor	-	+	-	+	-	-	-	+	-

+, absent; –, present

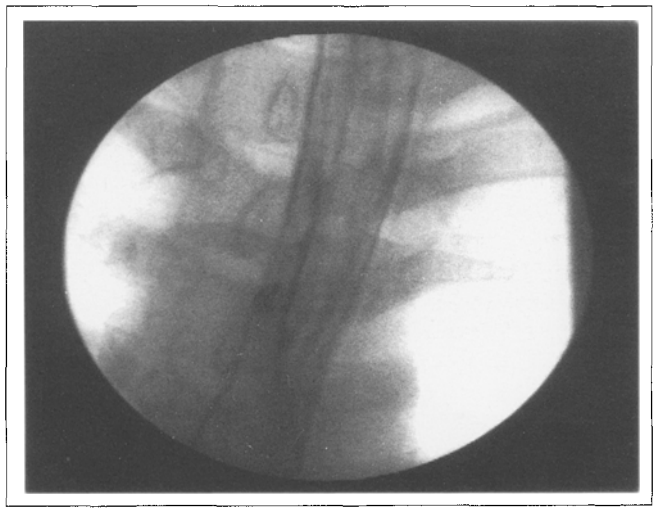

Fig. 3. Videofluoroscopy shows an atonic and dilatated esophagus (patient 13)

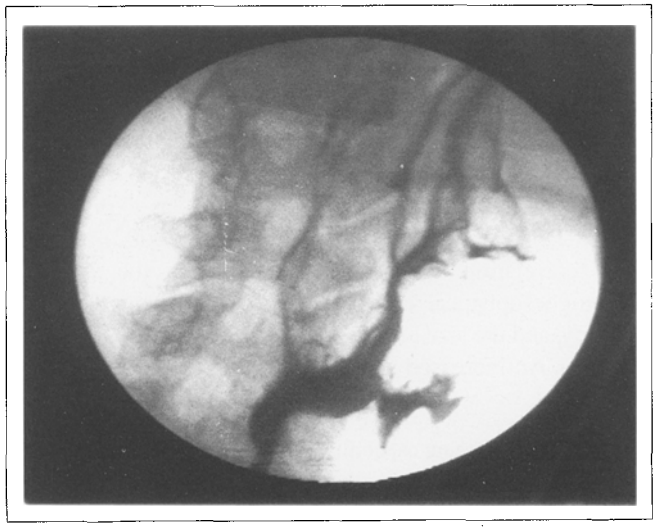

Fig. 4. Videofluoroscopy shows pharyngeal hypotonia with barium stasis in vallecula and piriform sinuses after swallowing (patient 12)

members of the same family in succeeding generations and it was associated with a more severe radiological pattern (Fig. 4).

Discussion

The results of our study confirmed and extended previous reports about the presence and degree of pharyngoesophageal alterations in MD patients, and also showed a correlation with the size of CTG expansions. We were able to identify a most common pattern of swallowing abnormalities, characterized by the presence of an incomplete UES relaxation and a hypotonic or atonic esophagus, in which the contrast medium progresses merely by gravity. An atonic pharynx with barium stasis was often, but not constantly present. In our experience, this pattern is quite specific for MD, since we did not find it in patients with dysphagia due to other neuromuscular diseases. Moreover, our parallel study conducted in 23 motor neuron disease patients [22] showed that the oral phase of deglutition was the most compromised, followed by the pharyngeal phase. In all patients without clinical evidence of dysphagia, subclinical videofluoroscopic alterations were present in a pattern similar to that found in the dysphagic group.

The function of LES resulted normal at videofluoroscopy in all our patients, except for one. These findings, however, need to be considered with caution since videofluoroscopy, while elective for the study of oropharyngeal tract, is less specific than manometry in the study of esophageal body function. Consistently, a parallel manometric evaluation performed in some of the patients enrolled in the present study out of 15 evidenced clear manometric alterations of the LES in 10 patients [23], confirming previous reports about involvement of smooth muscle in MD [24-29].

Our study also provided evidence of subclinical oropharyngeal abnormalities with potentially important clinical implications. The finding of silent aspiration in three patients suggests the risk of aspiration pneumonia, and raises the need for investigations of the oropharyngeal function in all MD patients.

Velopharyngeal hypomobility was present in 6 patients, 4 of whom were without dysphagia. Interestingly, Hillarp et al. [12] recently reported 4 cases of MD where velopharyngeal insufficiency was the presenting symptom which prompted further evaluation until diagnosis of MD.

The lack of correlation between the degree of clinical dysphagia and radiological alterations has been previously observed [30] and may be explained by the presence of compensatory mechanisms which are known to develop in patients with neurogenic dysphagia, especially of gradual onset [31].

The genetic studies confirmed the correlation between the CTG repeat size and severity of disease, and also provided additional information. We found, in fact, a significant correlation between the number of radiological abnormalities, as detected by videofluoroscopy both in dysphagic and non-dysphagic patients, and the size of CTG expansion. This correlation probably reflects a greater severity of muscle involvement, which may not be fully expressed on a clinical basis for the presence of compensatory mechanisms. Interestingly, in the two families studied, the siblings presented a greater number of videofluoroscopic alterations when compared to the parents, even in absence of clinically evident dysphagia.

Given the critical role of videofluoroscopy in the early identification of oropharyngeal abnormalities with possible

clinical implications, we suggest a videofluoroscopic study of swallowing as part of the evaluation of MD patients. Moreover, videofluoroscopy might help to identify atypical MD cases where oropharyngeal alterations are the sole presenting symptoms, sometimes preceding by several years the classical manifestations of the disease [9, 12, 15, 31-34].

Sommario *È frequente riscontrare nei pazienti affetti da Distrofia Miotonica (DM) delle alterazioni nella muscolatura faringo-esofagea, indipendentemente dalla presenza o meno di disfagie. L'obiettivo di questo studio è stato quello di identificare l'eventuale pattern di alterazioni nella funzione deglutitoria, tipico della DM e la loro possibile correlazione con il numero di triplette CTG. Quindici pazienti affetti da DM, 8 dei quali non disfagici, sono stati sottoposti a videofluoroscopia con studio della deglutizione. In 12 su 15 pazienti valutati (6 dei quali asintomatici per disfagia) sono state riscontrate delle alterazioni nella fase faringo-esofagea della deglutizione, le più comuni delle quali sono state un completo rilassamento dello sfintere esofageo superiore (UES) e ipotonia esofagea. È stata trovata una correlazione significativa fra il numero delle alterazioni videofluoroscopiche e l'entità dell'espansione CTG e identificato un pattern radiologico tipico della DM. È stato, inoltre, discusso il ruolo della videofluoroscopia nella valutazione dei pazienti DM.*

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