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Cardiac involvement in chronic progressive external ophthalmoplegia

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ABSTRACT

Myocardial involvement has not been extensively investigated in mitochondrial myopathies. The aim of the study was to assess the myocardial morpho-functional changes in patients with chronic progressive external ophthalmoplegia (PEO).

Twenty patients with PEO and 20 controls underwent standard echocardiography with tissue Doppler imaging (TDI) and integrated backscatter (IBS) analyses. These techniques are capable of providing non-invasively the early, subtle structural and functional changes of the myocardium. TDI myocardial systolic (S_m) and early (E_m) and late (A_m) diastolic velocities of left ventricular walls were determined. The systo-diastolic variation of IBS was also determined.

Patients with PEO exhibited lower S_m, lower E_m, and higher A_m, and a reduced E_m/A_m ratio than controls ($p < 0.001$ for all) at interventricular septum and lateral wall levels. In PEO patients, septal and posterior wall cyclic variations of IBS were significantly lower than those in controls ($p < 0.001$).

Patients with PEO showed myocardial wall remodeling characterized by increased fibrosis and early left ventricular systo-diastolic function abnormalities. Although cardiac involvement in PEO is generally considered to be limited to the cardiac conduction system, left ventricular dysfunction may be present and should receive more attention in the management of these patients.

1. Introduction

Mitochondrial diseases are disorders caused by the impairment of the mitochondrial respiratory chain [1]. The genetic error can affect mitochondrial DNA (mtDNA) or nuclear DNA (nDNA). Clinical phenotypes are polymorphic and range from pure myopathies to multisystemic disorders. The estimated prevalence of mitochondrial disorders is 1–2 in 10,000 [2], which makes them among the commonest inherited neuromuscular disorders.

Chronic progressive external ophthalmoplegia (PEO), which includes Kearns–Sayre syndrome, is a mitochondrial disorder with large deletions of mitochondrial DNA [3]. The most common clinical presentation of these syndromes is adult-onset progressive ophthalmoplegia, due to weakness of the external eye muscles. PEO is accompanied by a proximal myopathy with ragged-red fibers and mild reduction in the activities of respiratory-chain enzymes [4].

Different syndromes have been identified, but the range of their clinical presentation is quite wide with multiorgan involvement including cardiac abnormalities [5]. Cardiac involvement of patients with PEO is generally considered to be limited to the cardiac conduction system [6,7]. Hypertrophic or dilated cardiomyopathy is much less frequent and seems to be a late-onset phenomenon [6,7].

To clarify the characteristic clinical features of cardiac involvement and the effect of mitochondrial mutations on the heart, we evaluated cardiac structure and function in patients with PEO by standard echocardiography with tissue Doppler imaging (TDI) and integrated backscatter (IBS) analyses, two techniques capable of detecting and quantifying non-invasively the early, subtle structural and functional changes of the myocardium [8,9].

2. Materials and methods

We studied 20 patients (15 females, 5 males; mean age 55.3 ± 12.9 years) with PEO, previously diagnosed patients in neurology clinic. Nine patients were also affected by proximal myopathy. Eleven patients harbored multiple mtDNA deletions, seven patients harbored a mtDNA single deletion, one the mtDNA A1555G mutation, and one the mtDNA A3243G mutation. Mitochondrial disease was diagnosed based on clinical signs and symptoms, as well as standard biochemical and molecular analyses (*e.g.*, muscle respiratory chain activities, mtDNA gene analysis) [10]. Biochemical respiratory chain analysis on muscle sample

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homogenate was performed in 16 patients with standard methods [11]. Patients with mtDNA multiple deletions underwent nDNA gene sequencing for research of *ANT1*, *Twinkle*, *POLG1* and *2* mutations, linked to mitochondrial disorders caused by defect in intergenomic signaling [1]. Only patients 4 and 17, two sisters, resulted positive for *ANT1* mutation [12].

In the other cases with multiple deletions, mutations in *POLG1*, *POLG2*, *Twinkle* and *ANT1* were not found. *OPA1* is at present under analysis. All patients who were taking pharmacologic supplements with antioxidant activity (*e.g.*, ascorbate, vitamin E, α-lipoic acid, coenzyme Q10) interrupted such therapies at least 1 month before the beginning of the study.

In addition, 20 healthy volunteers (15 females, 5 males; mean age 54.9 ± 13.5 years) were recruited to form the control group.

The entire study population's demographic characteristics, biochemical parameters, lipid values and ECGs were obtained. Exclusion criteria were as follows: overt hypothyroidism or hyperthyroidism, acute coronary syndrome, prior myocardial infarction and coronary artery disease, congestive heart failure, left ventricular (LV) hypertrophy, reduced LV ejection fraction (<50%), chronic obstructive pulmonary disease, significant valvular heart disease, pacemaker implantation, atrial flutter or fibrillation, hypertension (resting blood pressure ≥ 140/90 mm Hg), diabetes mellitus, medications known to alter cardiac conduction, peripheral vascular diseases, neurological disease, pericarditis, congenital heart disease, alcohol abuse, renal or hepatic disease and poor echocardiographic imaging. Approval for the study was obtained by the local ethics committee. All subjects included in the study signed an informed consent with careful explanation of the study procedures.

2.1. Echocardiographic analysis

2.1.1. Conventional echocardiography

All patients were evaluated by transthoracic M-mode, two dimensional (2D), pulsed-wave (PW), continuous wave (CW), color flow and tissue Doppler imaging (TDI). All examinations were performed with the *Sonos 5500* ultrasound system (Philips Medical Systems, Andover, MA) with a 2–4 MHz transducer at a depth of 16 cm. During echocardiography, continuous single-lead ECG recording was obtained. All patients were imaged in the left lateral decubitus position. 2D and conventional Doppler examinations were obtained in the parasternal and apical views according to the guidelines of the American Society of Echocardiography [13]. LV diameters and wall thickness were measured by M-mode echocardiography. The mitral valve inflow pattern [E-wave, A-wave, E/A ratio and isovolumic relaxation time (IVRT)] was measured using the pulsed wave Doppler. LV mass was calculated according to the "Penn convention" [14].

2.1.2. Tissue Doppler echocardiography

Tissue Doppler echocardiography has become an established component of the diagnostic ultrasound examination. It permits an assessment of myocardial motion using Doppler ultrasound imaging. The technique uses frequency shifts of ultrasound waves to calculate myocardial velocity: this is similar to routine Doppler ultrasound to assess blood flow, but its technological features focus on lower velocity frequency shifts.

TDI was performed by transducer frequencies of 3.5 to 4.0 MHz, adjusting the spectral pulsed Doppler signal filters to acquire the Nyquist limit of 15 to 20 cm/s was reached and using the minimal optimal gain. Myocardial TDI of systolic and diastolic velocities of the basal lateral segment and of the basal interventricular septum in the apical 4-chamber view was measured at the end of echocardiographic studies. The sample volumes were placed in the center of myocardial segments. The acoustic power and filter frequencies of the echocardiographic system were set to the lowest values possible to minimize noise. From the obtained patterns, the systolic myocardial velocity (S_m), the early diastolic myocardial velocity (E_m), the late diastolic myocardial velocity (A_m) at the time of atrial contraction, and the ratio of E_m/A_m of both the left ventricular walls were determined. In addition, the isovolumetric relaxation time (IVRT) of each segment was measured as the interval from the aortic component of the second heart sound to the peak of the early diastolic wave. All parameters were measured during three consecutive cardiac cycles and their mean value was calculated.

2.1.3. Integral backscatter analysis

Ultrasonic tissue characterization by IBS provides an approach for defining the physical state of cardiac muscle tissue that complements the assessment of ventricular wall motion and chamber dimensions by conventional two-dimensional echocardiography [15]. The hypothesis underlying its use is that pathologic changes of myocardial structure and function result in alterations in the fundamental physical properties of tissue that can be quantified with indexes dependent on frequency-dependent ultrasonic attenuation and backscatter.

IBS analysis was performed using a special software package, available as an option of the *Sonos 5500*, as previously described [15]. Briefly, this system is capable of providing either conventional two-dimensional envelope-detected echocardiographic images or IBS images in which the gray level is displayed proportional to the integrated backscattered power. The backscatter can be measured in dB from an operator-defined region of interest (ROI). A maximum of 60 frames displayed at a real-time frame rate of 30 Hz (30 frames/s) are captured into cine-loop memory and subsequently stored in an optical disk in a digital format with the same resolution as the scan converter memory (512 × 512, 8 bits). This system has the possibility to display the transmit power, log compression and time-gain compensation values on a screen; this permits to adjust the system to the same values at every examination. Conventional B-mode images of parasternal long-axis view were obtained in each subject. The IBS was measured by placing an elliptic ROI at the center of the mid-anterior septum and of the mid-posterior wall, and time–intensity curves of backscatter were derived. The average power of the IBS contained within the ROI was measured and displayed in dB for a total of 60 time frames. The magnitude of the cyclic variations of IBS (CV-IBS) was calculated as the average in three consecutive cardiac cycles of the difference between the end-diastolic IBS value, coinciding with the peak of the R wave at ECG, and the value at the end-systole, typically corresponding to the end of the T wave. All measurements were made offline, by a single observer blinded to the patient details.

2.2. Reproducibility and reliability of data

Our laboratory has established reference data for inter- and intra-observer variations for standard and TDI echocardiographic parameters: inter-observer variation was 1.4 ± 4.6%, and intra-observer variation was 0.9 ± 2.6%. We previously determined inter-observer variability of cIBS values in 10 echocardiographic recordings that were measured by two observers at randomly selected cross-sections. The inter-observer variability of CV-IBS values was 1.1 ± 3.0%. Likewise, we determined the intra-observer variability of CV-IBS values in 10 echocardiographic recordings that were measured two times by one observer at randomly selected cross-sections. The intra-observer variability of CV-IBS values was 0.5 ± 3.2%.

2.3. Statistical analysis

All data were expressed as mean ± standard deviation (SD). The analysis of variance (one-way ANOVA) and the linear correlation analysis were used. Differences were considered significant when $p < 0.05$. All statistical procedures and curve fitting for regression analysis were performed by means of personal computer using StatView version 5.0 (SAS Institute Inc., Cray, NC, USA).