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Endothelial function and sympathetic nervous system activity in patients with Takotsubo syndrome☆,☆☆,★

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ABSTRACT

Background: Takotsubo syndrome (TTS) is an acute cardiomyopathy associated with intense physical or emotional stress. The precise mechanisms of the disease remain unclear. The aim of this study was to study alterations in endothelial function, vascular compliance and structure and muscle sympathetic activity in the stable phase of the disease.
Methods: In this prospective observational study, patients with TTS and controls matched for age, sex, cardiovascular risk factors and medications were recruited. Flow-mediated vasodilatation (FMD) as a measure of endothelial dysfunction was the primary endpoint. Secondary endpoints included measurements of arterial stiffness, carotid atherosclerosis, quality of life and laboratory parameters. In a subset of patients, muscle sympathetic activity was measured before and after stress tests.
Results: The study included 22 TTS patients and 21 matched controls. A significant increase in endothelial dysfunction was seen in TTS compared to controls (FMD 3.4 ± 2.4% vs. 4.8 ± 1.9%, $p = 0.016$). No significant differences in arterial stiffness, intima-media thickness, quality of life and laboratory markers including endothelin-1 were noted. TTS patients showed a reduced carotid total plaque area compared to controls (TPA 17.3 ± 15.1 vs 24.7 ± 12.8 mm^2, $p = 0.02$). A trend of increased muscle sympathetic activity at rest was observed in TTS patients vs. controls (53.5 ± 28.4 vs. 29.4 ± 16.5 bursts/100 heart beats, $p = 0.09$) with no significant differences in muscle sympathetic activity in response to stress.
Conclusions: Our findings underscore the importance of endothelial dysfunction in patients with TTS which may be involved in the pathophysiology of this syndrome.
ClinicalTrials.gov identifier: NCT01249599

1. Introduction

Takotsubo syndrome (TTS), also known as stress cardiomyopathy or apical ballooning syndrome is a transient but potentially lethal disease mimicking acute myocardial infarction [1]. In the majority of patients, it is induced by intense emotional or physical stress [2]. First described in Japan in 1990 by Sato et al. [3], TTS is characterized by acute onset of severe chest pain and/or acute left ventricular failure with dyspnoea, ECG changes, typical left ventricular angiographic findings and in most cases resolution of the morphological and clinical manifestations [4,5].

The pathogenesis of the disorder is not well understood and possibly involves excess of catecholamines [6] and endothelin-1[7], myocardial stunning and coronary microvascular dysfunction [8]. Endothelial dysfunction, a pathological state of the endothelium characterized by an imbalance between vasoconstricting and vasodilating factors [9], may represent an important link between stress and myocardial dysfunction in TTS. It is a key factor in the development of cardiovascular disease, particular in atherosclerosis [10] but also in microvascular dysfunction and is a potent predictor of clinical outcome [11].

We have previously shown that mental stress can induce endothelial dysfunction [12]. Pre-existing vascular dysfunction may predispose TTS patients to myocardial dysfunction in the presence of mental or physical stress and abnormal reactivity of the sympathetic nervous system may be involved in this process. Systematic assessments of vascular function

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and structure and sympathetic nervous system activity in TTS beyond case reports are still scarce. It was thus the aim of this study to assess the extent of vascular dysfunction and sympathetic nervous system activity in patients with stable TTS compared to matched controls.

2. Methods

2.1. Study population

Patients with a history of TTS according to standard clinical criteria [13] as well as controls matched for age, cardiovascular risk factors and pharmacological therapy were included in this prospective observational study. Exclusion criteria were use of long-acting nitrates or phosphodiesterase 5 inhibitors, alcohol or drug abuse, malignancy, disease with systemic inflammation (e.g. rheumatoid arthritis, Crohn's disease) and pulmonary hypertension. Patients were recruited in the University Heart Center at the University Hospital Zurich, Switzerland. Informed consent was obtained from and signed by all participants. The study was approved by the local ethics committee (KEK-ZH-No. 2010–0210/1) and was done in accordance with the Declaration of Helsinki. The trial was registered at ClinicalTrials.gov (Identifier: NCT01249599). All patients were studied in the morning, in fasting state, 12 h after refraining from caffeine, smoking and strong physical activity. Assessments of endothelial function, arterial stiffness, carotid atherosclerosis and laboratory parameters were performed before conducting the microneurography stress tests.

2.2. Endothelial function (flow-mediated dilatation)

Flow-mediated dilatation (FMD) as a measure of endothelial function was performed according to the current guidelines [11,14]. In brief, a B-mode ultrasound scan of the left brachial artery was obtained by highly trained and experienced sonographers in a longitudinal section between 2 and 10 cm above the elbow, using a high-resolution 10 MHz linear array transducer and ultrasound system Siemens ×300 (Siemens Switzerland AG, Zurich, Switzerland). The analogue video signal was acquired with a video processing system that computed the artery diameter in real-time (FMD Studio, Pisa, Italy). The high reproducibility of this system has been previously demonstrated [15]. The baseline vessel size was considered as the mean of the measures obtained during the first minute. Flow-mediated dilatation was calculated as the maximal percent increase in diameter above the baseline. Endothelium-independent dilatation was measured after sublingual glycerol trinitrate (GTN; 0.4 mg, Nitrolingual Spray, Pohl-Boskamp, Hohenlockstedt, Germany) by recording arterial diameter continuously for 6 min. The response to GTN was calculated as the maximum percent increase in vessel size above the baseline. The reproducibility of our laboratory was published previously [16].

2.3. Arterial stiffness (pulse wave analysis and velocity)

Arterial stiffness was assessed by pulse wave velocity (PWV) and pulse wave analysis (PWA) using the SphygmoCor system (AtCor Medical, Sydney, Australia) [17]. PWA was conducted at the level of the radial artery and plotted as augmentation index (AIX), adjusted for a heart rate of 75 beats per minute. PWV was obtained by measuring the difference in time delay and distance of the pulse wave at the carotid and femoral artery site synchronized to an electrocardiogram according to current guidelines [18,19].

2.4. Carotid atherosclerosis (intima-media thickness and total plaque area)

Carotid intima-media thickness (IMT) [20] and total plaque area (TPA) [21] were measured according the current guidelines. IMT was measured from the media-adventitia interface to the intima-lumen interface at the level of the common carotid artery [22] as mean of three measurements taken 1 cm segment in the distal common carotid artery, 1 cm proximal to dilation of the carotid bulb. Presence of plaque was determined by a transverse scan applied to the left and right carotid artery with a high resolution ultrasound linear transducer using a 7.5–12.0 MHz probe, which identified all plaques defined by intimal thickening ≥1.0 mm. The complete length of the common carotid artery, the visible parts of the internal and external carotid arteries and the bulb were scanned. Once the largest extent of a plaque was found by panning around the artery, the frozen longitudinal images were used to trace the plaque directly on the screen. The longitudinal area of the plaque was then automatically displayed and the areas of all such plaques were summed up to compute the value for TPA in mm^2. All TPA measurements were made by a single investigator (IS).

2.5. Markers of oxidative stress and vascular health

8-epi-prostaglandin-F2alpha (8-isoprostane), prostaglandin E2 and thromboxane B2 was measured in plasma with an 8-Isoprostane Express Enzyme Immunoassay (EIA) kit, prostaglandin E2 EIA Kit and thromboxane B2 EIA Kit respectively (Cayman Chemicals, Ann Arbor, USA). Endothelin-1 (ET-1) was measured in plasma using an Endothelin-1 Quantikine ELISA Kit (R&D, Minneapolis, USA).

2.6. Muscle sympathetic nervous activity (microneurography)

Multifiber recordings of muscle sympathetic activity (MSA) was obtained from the peroneal nerve posterior to the fibular head with tungsten microelectrodes (200-μm) shaft diameter, 1 to 5 μm uninsulated tip; (Medical Instruments, University of Iowa, USA) as described previously [23]. A reference electrode was inserted subcutaneously 1 to 2 cm from the recording electrode. Electrodes were connected to a preamplifier (gain 1.000) and amplifier (variable gain 10 to 50). Neural activity was fed through a band-pass filter (bandwidth 700 to 2000 Hz) and a resistance-capacitance integrating network (time constant 0.1 s) to obtain a mean voltage neurogram with the typical pulse-wave–triggered bursts. The signal was displayed on an oscilloscope, amplified, and connected to a loudspeaker to further identify the characteristic signal and exclude artefacts. MSA, blood pressure (Finometer Midi, Finapres Medical Systems, Amsterdam, Netherlands), respiration and surface ECG was continuously recorded in a computer with a Ponemah System (Data Sciences International, New Brighton, MN, USA). The signals was sampled at 500 Hz and stored with 12-bit accuracy. Signal processing was done with MATLAB (MathWorks, Natick, MA, USA). MSA was quantified in a computer-assisted evaluation of the frequency and the amplitude of the sympathetic bursts. The results were expressed as bursts per minute (bursts/min and bursts/100 heart beats), whereas changes in MSA were expressed as percent of baseline values.

Mental stress (MS) was performed with a highly reproducible and observer-independent 2-min mental task, the validated Bondet test. Patients were asked to respond as fast as possible to colour lights flashing in random order by pressing a push-button of the corresponding colour. A vacuum pillow was used as "arm support" in order to avoid the muscular work needed to keep the arm in the appropriate position to perform the test. This test has been used for several years in our laboratory and applies for an excellent reproducibility.

Physical stress was tested with the cold face test (CFT) by the application of three cold packs (0.5 °C) to the face and the cold pressor test (CPT) by asking the patients to immerse one hand in ice water (0 °C) up to the wrist for 2 min.

2.7. Questionnaires

Self-reported quality of life was assessed using the German version of the European Quality of Life Questionnaire EQ-5D (www.euroqol.org). Perceived stress, anxiety and depression was measured using the Hospital anxiety and depression scale (HADS) [24].

2.8. Statistics

The difference in FMD between TTS patients and controls was defined as the primary endpoint while the other outcomes were secondary outcomes in the study. The hypothesized FMD delta was 1.3% while the inter-subject variability of change was estimated at 1.5% standard deviation based on prior data in our laboratory. For a significance level of 5%, 21 patients per group were projected to reach a statistical power of 80%. Data was checked for normal distribution. Values are expressed as mean ± standard deviation. Results were compared using the unpaired Student *t*-test for parametric and the Mann-Whitney Test for non-parametric data as appropriate. All tests were two-sided and analysis was per protocol. A *p*-value of <0.05 was considered statistically significant. Statistics were performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, USA).

3. Results

A total of 22 patients with TTS (median age 67.9 ± 10.4 years; 21 female) and 21 controls (median age 68.2 ± 8.2 years; 20 female) were included in the study. Their clinical characteristics, laboratory parameters and concurrent drug therapy are presented in Table 1. Both study groups were well matched with no significant differences in age, sex, cardiovascular risk factors and concomitant drug therapy. TTS patients were in the stable phase of the disease and were measured at a mean of 3.45 ± 1.5 years after initial diagnosis. Measurements of vascular function and structure are shown in Table 2. Patients with TTS showed a significantly reduced endothelial function compared to matched controls as determined by FMD (3.4 ± 2.4% vs. 4.8 ± 1.9% respectively, $p = 0.016$; Fig. 1). No significant difference in endothelium-independent vasodilatation was seen as determined by GTN (14.7 ± 8.0% vs. 16.3 ± 8.2%, $p = 0.49$). Arterial stiffness was similar between TTS patients and matched controls (AIX 42.5 ± 8.4% vs. 39.8 ± 10.0%, $p = 0.35$ and PWV 6.9 ± 2.3 vs. 7.4 ± 2.2 m/s, $p = 0.41$ respectively).

With regard to measurements of carotid atherosclerosis, no significant differences in intima-media thickness were observed between the groups. However, patients with TTS had significantly lower total plaque area than controls (TPA 17.3 ± 15.1 vs. 24.7 ± 12.8 mm^2, $p = 0.02$). There were no significant differences in markers of vascular