

Association between Endothelial Function and Autonomic Modulation in Patients with Chagas Disease

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Abstract

Background: Under homeostatic condition, the autonomic nervous system (ANS), through the release of vasoconstrictor neurotransmitters, and the endothelium, through the release of vasodilating substances, interact to maintain blood vessel tone. However, the association between those two systems in patients with Chagas disease in its indeterminate phase (IChD) has not been studied.

Objective: To assess the association between autonomic modulation parameters and endothelial function in patients with IChD.

Methods: Thirteen patients with IChD (59.2 ± 11.23 years) and no risk factors for cardiovascular disease were assessed for autonomic modulation by using the blood pressure oscillometric method (Finapres) and the heart rate variability technique (HRV) in the frequency domain. Endothelial function was assessed by use of the brachial artery flow-mediated dilation (FMD) method with high-resolution ultrasound images.

Results: In the dorsal decubitus position, correlation of FMD was observed with normalized high-frequency ($r = 0.78$; $p = 0.007$) and low-frequency spectral components ($r = 0.68$; $p = 0.01$), as well as with sympathovagal balance ($r = -0.78$; $p = 0.004$).

Conclusion: Our study indicates the existence of a relationship between the changes in autonomic modulation and endothelial function in patients with IChD. (Arq Bras Cardiol. 2013;100(2):135-140)

Keywords: Chagas Disease / physiopathology; Heart Failure; Endothelium; Autonomic Nervous System.

Introduction

Despite the celebration of the centennial of the first description of Chagas disease (ChD), it still is a medical-scientific challenge, being the third most important parasitic disease, following malaria and schistosomiasis. There are approximately 10 million people infected worldwide¹, 25% to 30% of whom evolve to chronic Chagas heart disease (CCHD)².

The pathophysiology of both ChD in its indeterminate phase (IChD) and CCHD is still little known, but IChD seems to play an important role as it is the link between acute myocarditis and CCHD³.

A failure in the regulation of the autonomic nervous system (ANS) has been shown to be associated with the development of cardiovascular disease⁴, and observations of sympathetic⁵

and vagal⁶ dysfunction in IChD are conflicting⁷. Some studies have suggested that the autonomic dysfunction^{8,9} results from the greater vagal denervation, which unbalances the neural regulation of heart rate and rhythm, and causes myocardial hypersensitivity to catecholamines¹⁰. Other studies have suggested that dysautonomia is not present¹¹, being the autonomic dysfunction associated with the clinical evolution of the disease¹².

Some studies have shown that endothelial dysfunction precedes the clinical manifestations of atherosclerotic lesions¹³ and cardiovascular disease¹⁴. The few studies investigating endothelial function in IChD have reported contradictory results. While one study has found venous endothelial dysfunction¹⁵, another has shown no endothelial dysfunction¹⁶; thus, this subject requires further investigation.

Under normal conditions, blood vessel tone balance is kept by the functional antagonism between vasoconstricting factors of the sympathetic system and vasodilating factors of the endothelium¹⁷. That balance is broken in some diseases, such as hypertension¹⁸, atherosclerosis¹⁹, and insulin resistance²⁰, where a causal relation is observed between endothelial dysfunction and increased sympathetic nervous activity.

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However, studies relating the ANS and endothelial function in IChD have not yet been conducted.

The present study aimed at assessing autonomic modulation and endothelial function, and at testing the association between those mechanisms in patients with IChD.

Methodology

Case series

This cross-sectional study was approved by the Committee on Ethics and Research of the Fundação Universitária de Cardiologia (FUC) of the state of Rio Grande do Sul (protocol 4360-09). Thirty-four patients were consecutively selected at the ChD outpatient clinic of the Instituto de Cardiologia of the state of Rio Grande do Sul (IC-RS), from January to December 2010. All patients selected had routine tests ruling out associated diseases. Of the 34 patients, 13 (mean age of 59.2 ± 11.2 years; 8 (62%) females) met the following inclusion criteria: 1) birth in an endemic area of ChD; 2) two positive serologic tests, one highly sensitive (ELISA) and the other highly specific (indirect immunofluorescence); 3) normal left ventricular function and anatomy (ejection fraction $> 60\%$ assessed on two-dimensional Doppler echocardiography); 4) lack of symptoms and signs suggesting cardiovascular disease; 5) normal biochemical profile; and 6) normal electrocardiogram (ECG). The exclusion criteria were as follows: smoking; diabetes mellitus²¹; hypertension²²; hypercholesterolemia²³; menopause; use of medication with effects on the cardiac ANS (beta-blocker, clonidine, and alpha methyl dopa); and brachial artery diameter lower than 2.5 mm or greater than 5 mm.

Methods

The patients were examined at the ChD outpatient clinic of the IC-RS for the diagnosis of IChD, and the indication of laboratory tests, ECG and color flow Doppler echocardiography. The biochemical and inflammatory profile was analyzed according to the protocol of the IC-RS clinical analysis laboratory. Blood (5 mL) was collected after a 12-hour fasting to measure the following: total cholesterol (TC), triglycerides (T) and glycemia (G) (enzyme method - Roche Laboratories, Basel, Switzerland); high-density lipoprotein (HDL-C - isolated by use of the heparin-2M MnCl₂ procedure and measured with the same enzyme kit used for total serum cholesterol); low-density lipoprotein (LDL-C - by use of the Friedewald et al²⁴ formula, in mg/dL); complete blood count (automatic counter, model 818, AVL); high-sensitivity C-reactive protein (hs-CRP - nephelometry); and fibrinogen (Clauss method).

The anthropometric parameters of weight and height were measured by use of a platform scale (Filizola) with a maximum load of 150 kg and 100-g grading, and an inflexible metal pole, graded in cm up to the height of 2 m. The body mass index (BMI) was calculated by dividing weight (kg) by height square (meter). Patients with a BMI ≥ 30 kg/m² were considered obese according to the World Health Organization (WHO)²⁵. Abdominal circumference (AC) was measured, with the patient

standing, at half of the distance between the lower margin of the last rib and the top of the iliac crest.

Autonomic control and endothelial function were assessed on the same day, in a quiet, dark room with controlled temperature (22 °C to 25 °C).

Assessing autonomic control

Heart rate variability (HRV) is a non-invasive method to assess cardiovascular autonomic modulation; it correlates with the standard of measurement, coronary angiography²⁶. After being instructed about the test, patients rested for 15 minutes. Data were recorded with patients in the supine position, followed by seven minutes of active standing position (sympathetic stimulation), and return to the supine position.

Arterial blood pressure and heart rate were continuously monitored beat-to-beat by use of a sensor placed on the middle finger and connected to a transducer (Ohmeda 2300, Monitoring Systems, Englewood, CO, USA). The transducer captured the signals at real time, and sent them to a microcomputer equipped with an analog-to-digital converter. The signs were converted and acquired by use of the WindaQ system (DataQ Instruments, Inc., Akron, Ohio, USA). Three channels were recorded, each with a sampling rate calculated in 1KHz. The signs were stored in CODAS binary format for posterior analysis.

Autonomic control was assessed by use of spectral analysis, calculated with the autoregressive model (AR)²⁶ applied to stationary time series of 300 beats²⁶. The spectral power was decomposed into low-frequency (LF: 0.03 – 0.15Hz) and high-frequency (HF: 0.15 – 0.40Hz) bands, in normalized units to minimize the effects of the changes of the very low-frequency (VLF) band, and into the ratio between those components (LF/HF), which represents the sympathovagal balance²⁷.

Brachial artery flow-mediated dilation (FMD)

The functional assessment of the vascular reactivity of the endothelium and muscle layer followed the Correti et al²⁸ protocol, being always performed by the same observer trained in vascular ultrasonography. Patients were instructed not to use stimulating substances, alcohol, and vitamin C, and not to practice exercise in the 24 hours preceding the test. Women were also instructed not to undergo the test during menstruation.

Brachial artery FMD was measured with the patients in the supine position, by use of: a pneumatic cuff placed 5 cm above the cubital fossa; high-resolution ultrasound (EnVisor Philips Medical Systems; Bothell, WA, EUA), equipped with high frequency (7-12 MHz) linear vascular transducer; and software for imaging and electrocardiographic monitoring. After measuring the longitudinal diameter of the brachial artery at baseline, the artery was occluded for 5 minutes (when the cuff was inflated 50 mmHg above the systolic blood pressure), and a new measurement of the arterial diameter was taken 45-60 seconds after releasing the occlusion. The analyses were performed off-line, and the brachial artery diameter was measured at the end of diastole (at the peak of the R wave of the ECG). Several points along the vessel were analyzed with the aid of computer systems specific to measure echographic images. The responses of flow-dependent vasodilation were

expressed as a percentage variation of the baseline brachial artery diameter²⁹. After a 10-minute rest and re-establishment of baseline conditions, new measures were taken before and 3 minutes after the sublingual administration of nitroglycerin (0.4 mg), aimed at assessing endothelium-independent vasodilation, to evaluate the integrity of the muscular system of the vessels analyzed²⁸.

Statistical analysis

Data were analyzed by use of the program Statistical Package for the Social Sciences (SPSS), version 13.0 for Windows, and expressed as mean \pm standard deviation, median, and interquartile interval. The Kolmogorov-Smirnov test analyzed data distribution. The asymmetric data were normalized by use of logarithmic transformation. The association between variables was assessed by use of Pearson and Spearman correlation coefficients for data with normal and asymmetric distribution, respectively, with a 5% significance level ($p < 0.05$).

Results

Table 1 shows the anthropometric and hemodynamic characteristics and the biochemical profile and acute phase inflammatory proteins of the 13 patients, whose serology was positive for IChD for 12.5 ± 7.4 years.

Regarding the FMD% values (18.1; 3.2 – 36.5) (table 2), 50% of the sample had a percentage dilation of 18.1%, which is over that considered normal (8%)²⁹ for men and women. Only 25% of the sample had a percentage dilation below 3.2%, and 25% of FMD% values were between 18.1% and 36.5%, demonstrating changes in the endothelial function of those patients.

Regarding autonomic control, the patients showed autonomic changes evidenced by the lack of response to sympathetic activation. When changing posture from a supine to a standing position, no significant difference was observed in the patients' sympathetic modulation (LF) ($p = 1$), parasympathetic modulation (HF) ($p = 0.43$), and sympathovagal balance (LF/HF) ($p = 0.98$).

Table 3 shows data from the HRV analysis and its correlations with FMD%. In the dorsal decubitus position, a significant correlation with FMD% was observed in the high-frequency (HF) and low-frequency (LF) spectra, in normalized values, and the sympathovagal balance (LF/HF). No correlation was observed between the autonomic parameters and FMD% in a sympathetic activation situation.

Discussion

The major finding of this study was the significant correlation between endothelial function parameters and cardiac autonomic modulation in patients with IChD. Our study evidenced alterations in FMD and deficiency in the autonomic response to the posture change from a supine to a standing position, being the first to report correlations between the endothelial function regarding both the sympathetic (LF) and the parasympathetic (HF) modulations, and the cardiac sympathovagal balance.

The literature shows that the increase in sympathetic activity influences endothelial function, reducing vasodilation at rest and in face of vasodilation stimuli^{7,30}, and that there seems to be not only one way by which stimulation of the sympathetic nervous system causes vasoconstriction. Vasoconstriction might result from the release of norepinefrine³¹ at sympathetic nerve endings interacting with α_1 adrenergic receptors of endothelial cells or from stimulus to the release of endothelin³¹; such

Table 1 – Characterization of the patients regarding anthropometric and hemodynamic variables, biochemical profile, and acute phase inflammatory proteins

Variables	x \pm dp n = 13
Sex (n/%)	F8 / 62; M 5/38
Age (years)	52.9 \pm 11.2
Weight (kg)	72.4 \pm 15.4
Height (m)	1.62 \pm 0.08
BMI (kg/m ²)	27.4 \pm 4.5
AC (cm)	100.6 \pm 9.8
SBP (mm Hg)	131 \pm 16.3
DBP (mm Hg)	72.7 \pm 12.5
HR (bpm)	67.5 \pm 9.1
Glycemia (mg/dL)	95.1 \pm 11.2
HDL-C (mg/dL)	54.1 \pm 13.9
LDL-C (mg/dL)	137.6 \pm 40.3
TC (mg/dL)	216.4 \pm 49.7
TG (mg/dL)	123.7 \pm 61.9
hs-CRP (mg/dL)	0.23 (0.1 – 0.4)*
Fibrinogênio (mg/dL)	258.6 \pm 52.7

F: female; M: male; BMI: body mass index; AC: abdominal circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; HDL-C: high-density lipoprotein; LDL-C: low-density lipoprotein; TC: total cholesterol; TG: triglycerides; hs-CRP: high-sensitive C-reactive protein (*median and first and third quartiles).

Table 2 – Assessment of the endothelial function

	x \pm sd Median (Q1 – Q3)
Base-D (mm)	0,32 \pm 0,1
RH-D (mm)	0,37 \pm 0,1
FMD (%)	18,1 (3,2 – 36,5)*
Base-flow (cm/s)	10,1 \pm 5,4
RH-flow (cm/s)	128 \pm 119,9
%NTG (%)	18,7 (11,1 – 22,8)

Base-D: baseline diameter; RH-D: reactive hyperemia diameter; FMD: flow-mediated dilation (*median and first and third quartiles); Base-flow: baseline flow; RH-flow: reactive hyperemia flow; NTG: post-nitroglycerin dilation.

Table 3 – Assessment of autonomic control and correlations with endothelial function

Spectral indices	x ± sd median (Q1 – Q3)	r	p
Dorsal decubitus with controlled breathing			
HRV (ms ²)	726.3 (479.1 – 1526.5)	-0.09	0.79
LF n.u.	41.0 (26.2 – 66.7)	0.68	0.01
LF (ms ²)	487.7 ± 665.8		
HF n.u.	47.3 (30.7 - 57.9)	0.78	0.007
HF (ms ²)	737.4 ± 1402		
LF/HF	0.9 (0.5 - 2.3)	-0.78	0.004
SBPV (mm Hg ²)	22.8 (13.2 - 34.0)	-0.19	0.57
LF (mm Hg ²)	6.5 (1.0 - 19.3)	-0.20	0.53
Baroreflex	3.8 (3.1 - 10.1)	-0.29	0.38
Active standing position			
HRV (ms ²)	758.3 ± 53.0	-0.28	0.39
LF n.u.	51.4 (27.1 - 82.7)	-0.41	0.20
LF (ms ²)	335.7 ± 506.8		
HF n.u.	24.2 (9.6 - 56.3)	0.53	0.08
HF (ms ²)	146.3 ± 160.6		
LF/HF	1.3 (0.4 - 8.5)	-0.41	0.20
SBPV (mm Hg ²)	34.6 (13.8 - 44.8)	-0.03	0.91
LF (mm Hg ²)	12.9 (2.31 - 25.4)	0.49	0.12

HRV: heart rate variability; LF n.u. and HF n.u.: power of the low- and high-frequency spectral components in normalized units, respectively; LF/HF: sympathovagal balance; SBPV: systolic blood pressure variability; Q1: first quartile; Q3: third quartile.

sympathetic vasoconstrictor response is attenuated by nitric oxide³², a potent vasodilator.

Sverrisdóttir et al³³, studying ten healthy individuals, have shown an intrinsic relationship between the endothelium and the sympathetic nervous system, with sympathetic activity inversely related to endothelial function. The same has been reported by Lambert et al³⁴, assessing 25 overweight and obese university students. Our results suggest that those mechanisms are present in IChD, and that such association might comprise a compensation between the systems involved. It is worth noting that the evidence of the association between endothelial dysfunction and autonomic dysfunction³⁰ observed in heart failure³⁵ and arterial hypertension³⁶ has been associated with flaws in both systems. It is yet to be proved whether those diseases cause endothelial dysfunction and autonomic dysfunction and/or whether a failure in the control mechanisms of the ANS and of the endothelial function affects negatively the homeostasis, causing them. Any change in the balance between those two systems, endothelial function and ANS, might also lead to deleterious effects on the cardiovascular system³⁷.

An inverse relation was observed between the endothelial function and the sympathovagal balance, showing that the increase in sympathetic predominance reduces the FMD response. The literature has shown that the increase in the sympathetic activity as compared to the parasympathetic one³³

reduces vasodilation at rest and in face of vasodilating stimuli, which is in accordance with our finding.

Secondary findings of this study showed no significant alteration in both sympathetic (LF) and parasympathetic (HF) modulations with a change in posture from a supine to a standing position. This suggests an alteration in vagal withdrawal, because the change in position did not cause the expected shift from vagal predominance to sympathetic predominance. In reality, our results indicate impairment of both sympathetic and parasympathetic modulations. Some studies have shown that, under conditions associated with endothelial dysfunction¹⁶⁻¹⁸, the deficiency in NO synthesis and/or release might contribute to the sustained sympathetic activation observed³⁸. That is not the case of our study, in which the endothelium is preserved. Köberle⁶ and Rassi Jr. et al⁴ have shown the presence of autonomic dysfunction, with parasympathetic predominance in patients with IChD. In the presence of endothelial dysfunction, a deficiency in NO synthesis and/or release might contribute to the sustained sympathetic activation seen in those conditions.

An interesting aspect of that group of patients is the alteration in autonomic control indicated by the lack of autonomic response to the sympathetic activation maneuver²⁷. The reduction in total variability has been proposed to result from an exacerbated sympathetic activity and/or a reduced parasympathetic tone³⁹. Our findings are in accordance with those of the study by Vasconcelos and Junqueira Jr.¹¹, who,

assessing the autonomic function of 17 patients with IChD by using the same method, have reported a clear and distinct severity pattern of cardiac autonomic dysfunction when comparing patients with IChD and patients with CCHD. Those authors have shown that the two groups of patients had reduced HRV, more marked in the CCHD group. The reduction in HRV has also been associated with the aging process. De Resende et al⁴⁰, studying the autonomic function of 28 elderly with IChD by using HRV, have found a reduction in HRV. However, our findings did not show that same association, ruling out age as a contributor to that finding.

According to Sterin-Borda³⁹, the existence of circulating antibodies in ChD, binding to beta-adrenergic and cholinergic muscarinic receptors in lymphocytes and myocardium, is strongly associated with seropositive asymptomatic patients with autonomic dysfunction. This indicates that the presence of those antibodies might partially explain the cardioneuropathy in ChD, in which the sympathetic and parasympathetic systems are affected.

The major objective of this study was not to assess the presence of endothelial dysfunction and/or autonomic modulation dysfunction in patients with ChD, which would require a control group. However, the lack of that control group is a limitation of this study, as is the non-random selection of the sample. It is worth noting that the associations observed in this study do not represent a cause-effect relationship.

Conclusion

This study showed a correlation between endothelial function and cardiac sympathetic and parasympathetic modulations, in addition to a correlation between endothelial

function and sympathovagal balance in patients with IChD. Further studies are required to confirm such findings, and the inclusion of a larger number of patients and a paired group of healthy individuals should be considered. The study of the relationship between ANS and endothelial function might provide a model to better identify the processes involved in the development of cardiovascular disease.

Author contributions

Conception, design of the research, analysis and interpretation of the data: Truccolo AB, Ribeiro RA, Casali KR, Irigoyen MC, Gus I, Plentz RDM; Acquisition of data: Dipp T, Eibel B, Ribeiro RA, Gus I, Plentz RDM; Statistical analysis and obtaining financing: Plentz RDM; Writing of the manuscript: Truccolo AB, Pellanda LC, Plentz RDM; Critical revision of the manuscript for intellectual content: Truccolo AB, Casali KR, Irigoyen MC, Pellanda LC, Plentz RDM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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