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

Adriana Barni Truccolo[1,2], Thiago Dipp[1], Bruna Eibel[1], Rodrigo Antonini Ribeiro[3], Karina Rabello Casali[4], Maria Claudia Irigoyen[1,5], Iseu Gus[1], Lucia Campos Pellanda[1,6], Rodrigo Della Meã Plentz[1,6]

Instituto de Cardiologia do Rio Grande do Sul/Fundação Universitária de Cardiologia[1], RS; Universidade Estadual do Rio Grande do Sul UERGS[2], Alegrete, RS; Instituto de Educação e Pesquisa, Hospital Moinhos de Vento[3], Porto Alegre, RS; Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Universidade Federal do Rio Grande do Sul- UFRGS[4], Porto Alegre, RS; Universidade de São Paulo, USP - Instituto do Coração[5], São Paulo; Universidade Federal de Ciências da Saúde de Porto Alegre UFCSPA[6], Porto Alegre, RS - Brazil

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 (Finapress) 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 worldwide[1], 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 CCHD[3].

A failure in the regulation of the autonomic nervous system (ANS) has been shown to be associated with the development of cardiovascular disease[4], and observations of sympathetic[5] and vagal[6] dysfunction in IChD are conflicting[7]. 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 catecholamines[10]. Other studies have suggested that dysautonomia is not present[11], being the autonomic dysfunction associated with the clinical evolution of the disease[12].

Some studies have shown that endothelial dysfunction precedes the clinical manifestations of atherosclerotic lesions[13] and cardiovascular disease[14]. The few studies investigating endothelial function in IChD have reported contradictory results. While one study has found venous endothelial dysfunction[15], another has shown no endothelial dysfunction[16]; 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 endothelium[17]. That balance is broken in some diseases, such as hypertension[18], atherosclerosis[19], and insulin resistance[20], where a causal relation is observed between endothelial dysfunction and increased sympathetic nervous activity.

Mailing Address: Adriana Barni Truccolo •
Rua Sarmento Leite, 1011/33 - Cidade Baixa – Postal Code 90050-170 – Porto Alegre, RS, Brazil
E-mail: truccolo@cpovo.net, truccoloab@hotmail.com
Manuscript received March 29, 2012; revised manuscript August 03, 2012; accepted September 26, 2012.

DOI: 10.5935/abc.20130026

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 mellitus[21]; hypertension[22]; hypercholesterolemia[23]; menopause; use of medication with effects on the cardiac ANS (beta-blocker, clonidine, and alpha methyldopa); 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 al[24] 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/m^2 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 ^{0}C to 25 ^{0}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 angiography[26]. 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 beats[26]. 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 balance[27].

Brachial artery flow-mediated dilation (FMD)

The functional assessment of the vascular reactivity of the endothelium and muscle layer followed the Correti et al[28] 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 diameter[29]. 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 analyzed[28].

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 ± 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 norepinefrine[31] at sympathetic nerve endings interacting with α_1 adrenergic receptors of endothelial cells or from stimulus to the release of endothelin[31]; such

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

Variables	x ± dp n = 13
Sex (n/%)	F8 / 62 ; M 5/38
Age (years)	52.9 ± 11.2
Weight (kg)	72.4 ± 15.4
Height (m)	1.62 ± 0.08
BMI (kg/m2)	27.4 ± 4.5
AC (cm)	100.6 ± 9.8
SBP (mm Hg)	131 ± 16.3
DBP (mm Hg)	72.7 ± 12.5
HR (bpm)	67.5 ± 9.1
Glycemia (mg/dL)	95.1 ± 11.2
HDL-C (mg/dL)	54.1 ± 13.9
LDL-C (mg/dL)	137.6 ± 40.3
TC (mg/dL)	216.4 ± 49.7
TG (mg/dL)	123.7 ± 61.9
hs-CRP (mg/dL)	0.23 (0.1 – 0.4)*
Fibrinogênio (mg/dL)	258.6 ± 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 ± sd Median (Q1 – Q3)
Base-D (mm)	0,32 ± 0,1
RH-D (mm)	0,37 ± 0,1
FMD (%)	18,1 (3,2 – 36,5)*
Base-flow (cm/s)	10,1 ± 5,4
RH-flow (cm/s)	128 ± 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 HF (ms^2)	47.3 (30.7 - 57.9) 737.4 ± 1402	0.78	0.007
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. LF (ms^2)	51.4 (27.1 - 82.7) 335.7 ± 506.8	-0.41	0.20
HF n.u. HF (ms^2)	24.2 (9.6 - 56.3) 146.3 ± 160.6	0.53	0.08
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 oxide[32], a potent vasodilator.

Sverrisdóttir et al[33], 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 al[34], 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 dysfunction[30] observed in heart failure[35] and arterial hypertension [36] 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 system[37].

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 one[33] 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 dysfunction[16-18], the deficiency in NO synthesis and/or release might contribute to the sustained sympathetic activation observed[38]. That is not the case of our study, in which the endothelium is preserved. Köberle[6] and Rassi Jr. et al[4] 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 maneuver[27]. The reduction in total variability has been proposed to result from an exacerbated sympathetic activity and/or a reduced parasympathetic tone[39]. 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 al[40], 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-Borda[39], 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.

Sources of Funding

This study was funded by CNPq and FAPICC.

Study Association

This article is part of the thesis of master submitted by Adriana Barni Truccolo, from Instituto de Cardiologia do Rio Grande do Sul / Fundação Universitária de Cardiologia.

References

1. Coura JR, Pinto Dias JC. Epidemiology, control and surveillance of Chagas disease - 100 years after its discovery. Mem Inst Oswaldo Cruz. 2009;104(1):31-40.
2. Organización Panamericana de la Salud. (OPAS). Estimación cuantitativa de la enfermedad de Chagas en las Américas. Montevideo (Uruguay): PAHO Publishing; 2006. p.1-28.
3. Schmunis GA, Yadon ZE. Chagas disease: a Latin American health problem becoming a world health problem. Acta Tropica. 2010;115(1-2):14-21.
4. Rassi Jr A, Rassi A, Marin-Neto J. Chagas disease. Lancet. 2010;375(9723):1388-402.
5. Rassi Jr A, Rassi A, Marin-Neto JA. Chagas heart disease: pathophysiologic mechanisms, prognostic factors and risk stratification. Mem Inst Oswaldo Cruz. 2009;104(Suppl 1):152-8.
6. KöberleF. Enteromegaly and cardiomegaly in Chagas disease. Gut. 1963;4:399-405.
7. Correia D, Junqueira LF Jr, Molina RJ, Prata A. Cardiac autonomic modulation evaluated by heart interval variability is unaltered but subtly widespread in the indeterminate Chagas' disease. Pacing Clin Electrophysiol. 2007;30(6):772-80.
8. Rossi MA, Tanowitz HB, Malvestio LM, Celes MR, Campos EC, Blefari V, et al. Coronary microvascular disease in chronic Chagas cardiomyopathy including an overview on history, pathology, and other proposed pathogenic mechanisms. PLoS Negl Trop Dis. 2010;4(8):e674.
9. Junqueira LF Jr. A summary perspective on the clinical-functional significance of cardiac autonomic dysfunction in Chagas' disease. Rev Soc Bras Med Trop. 2006;39(Suppl 3):64-9.
10. Negrão CE, Santos AC, Rondon MU, Franco FG, Ianni B, Rochitte CE, et al. Muscle sympathetic nerve activity in patients with Chagas' disease. Int J Cardiol. 2009;137(3):252-9.
11. Vasconcelos DF, Junqueira LF Jr. Distinctive impaired cardiac autonomic modulation of heart rate variability in chronic Chagas' indeterminate and heart diseases. J Electrocardiol. 2009;42(3):281-9.
12. Villar JC, León H, Morillo CA. Cardiovascular autonomic function testing in asymptomatic *T. cruzi* carriers: a sensitive method to identify subclinical Chagas' disease. Int J Cardiol. 2004;93(2-3):189-95.
13. Vanhoutte PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. Acta Physiol (Oxf). 2009;196(2):193-222.
14. Vanhoutte PM. Endothelial dysfunction: the first step toward coronary arteriosclerosis. Circ J. 2009;73(4):595-60.
15. Plentz RD, Irigoyen MC, Muller AS, Casarini DE, Rubira MC, Moreno Junior H, et al. Venous endothelial dysfunction in Chagas' disease patients without heart failure. Arq Bras Cardiol. 2006;86(6):466-71.
16. Consolim-Colombo FM, Lopes HF, Rosetto EA, Rubira MC, Barreto-Filho JA, Baruzzi AC, et al. Endothelial function is preserved in Chagas' heart disease patients without heart failure. Endothelium. 2004;11(5-6):241-6.

17. Harris KF, Matthews KA. Interactions between autonomic nervous system activity and endothelial function: a model for the development of cardiovascular disease. Psychosom Med. 2004;66(2):153-64.

18. Raina H, Zhang Q, Rhee AY, Pallone TL, Wier WG. Sympathetic nerves and the endothelium influence the vasoconstrictor effect of low concentrations of ouabain in pressurized small arteries. Am J Physiol Heart Circ Physiol. 2010;298(6):H2093-101.

19. Chumaeva N, Hintsanen M, Hintsa T, Ravaja N, Juonala M, Raitakari OT, et al. Early atherosclerosis and cardiac autonomic responses to mental stress: a population-based study of the moderating influence of impaired endothelial function. BMC Cardiovasc Disord. 2010;10:16.

20. Schwab M, Bloch J, Duplain H, Sartori C, Scherer U. Defective nitric oxide homeostasis: common underlying mechanism between insulin resistance, sympathetic overactivity and cardiovascular morbidity and mortality. Medicina (B. Aires). 2008;68(3):243-50.

21. Sociedade Brasileira de Endocrinologia e Metabologia. Projeto Diretrizes (AMB-CFM). Diabetes Mellitus: classificação e diagnóstico. [Internet]. [Acesso em 2010 jun 10]. Disponível em: http://www.projetodiretrizes.org.br/4_volume/06-Diabetes-c.pdf>

22. Sociedade Brasileira de Cardiologia. Sociedade Brasileira de Hipertensão. Sociedade Brasileira de Nefrologia. Diretrizes brasileiras de hipertensão VI. J Bras Nefrol [online]. 2010;32(supl. 1):S1-S64.

23. Sposito AC, Caramelli B, Fonseca FA, Bertolami MC, Afiune Neto A, Souza AD, et al; Sociedade Brasileira de Cardiologia. IV Diretriz brasileira sobre dislipidemias e prevenção da aterosclerose. Arq Bras Cardiol. 2007;88(supl 1):1-18.

24. Friedewald WT, Levi RI, Fredrickson DS. Estimation of the concentration of low density lipoproteins cholesterol in plasma without use of the ultracentrifuge. Clin Chem. 1972;18(6):499-502.

25. World Health Organization. (WHO). Global database on body mass index. [Access in 2010 Aug 19]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html>

26. Acharya UR, Joseph KP, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Bio Eng Comput. 2006;44(12):1031-51.

27. Montano N, Porta A, Cogliati C, Costantino G, Tobaldini E, Casali KR, et al. Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. Neurosci Biobehav Rev. 2009;33(2):71-80.

28. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65. Erratum in: J Am Coll Cardiol. 2002;39(6):1082.

29. Gokce N, Keaney JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation. 2002;105(13):1567-72.

30. Molina RB, Matsubara BB, Hueb JC, Zanati SG, Meira DA, Cassolato JL, et al. Dysautonomia and ventricular dysfunction in the indeterminate form of Chagas disease. Int J Cardiol. 2006;113(2):188-93.

31. Harris KF, Matthews KA. Interactions between autonomic nervous system activity and endothelial function: a model for the development of cardiovascular disease. Psychosom Med. 2004;66(2):153-64.

32. Verma S, Anderson T. Fundamentals of endothelial function for the clinical cardiologist. Circulation. 2002;105(5):546-9.

33. Sverrisdóttir YB, Jansson LM, Hägg U, Gan LM. Muscle sympathetic nerve activity is related to a surrogate marker of endothelial function in healthy individuals. PLoS One. 2010;5(2):e9257.

34. Lambert E, Sari CI, Dawood T, Nguyen J, McGrane M, Eikelis N, et al. Sympathetic nervous system activity is associated with obesity-induced subclinical organ damage in young adults. Hypertension. 2010;56(3):351-8.

35. Shechter M, Matetzky S, Arad M, Feinberg MS, Freimark D. Vascular endothelial function predicts mortality risk in patients with advanced ischaemic chronic heart failure. Eur J Heart Fail. 2009;11(6):588-93.

36. Treasure CB, Manoukian SV, Klein JL, Vita JA, Nabel EG, Renwick GH, et al. Epicardial coronary artery responses to acetylcholine are impaired in hypertensive patients. Circ Res. 1992;71(4):776-81.

37. Jensen-Urstad K, Reichard P, Jensen-Urstad M. Decreased heart rate variability in patients with type 1 diabetes mellitus is related to arterial wall stiffness. J Intern Med. 1999;245(1):57-61.

38. Thijssen DH, De Groot P, Kooijman M, Smits P, Hopman MT. Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. Am J Physiol Heart Circ Physiol. 2006;291(6):H3122-9.

39. Sterin-Borda LJ, Borda ES. Participation of autonomic nervous system in the pathogenesis of Chagas disease. Acta Physiol Pharmacol Ther Latinoam. 1994;44(4):109-23.

40. De Resende LA, Molina RJ, Ferreira BD, Carneiro AC, Ferreira LA, da Silva VJ, et al. Cardiac autonomic function in chagasic elderly patients in an endemic area: a time and frequency domain analysis approach. Auton Neurosci. 2007;131(1-2):94-101.