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Distinctive impaired cardiac autonomic modulation of heart rate variability in chronic Chagas' indeterminate and heart diseases

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Abstract

Introduction: Cardiac autonomic dysfunction occurs in Chagas' indeterminate and heart disease, but comparison of this disturbance between both forms was not yet performed.
Methods: Time- and frequency-domain 5-minute heart rate variability in supine and standing positions were evaluated in 17 subjects with Chagas' disease with the indeterminate form, 13 with heart disease and 15 controls. Trend of variability indices across the groups was also tested.
Results: In the supine position, reduced time-domain and absolute frequency-domain indices reflecting overall autonomic modulation were observed in both Chagas' disease groups. In the standing position, the coefficient of variation and those frequency-domain indices were also reduced, and the other time-domain indices were reduced only in the cardiac group. Heart rate variability indices hypothesized to reflect relative sympathetic and parasympathetic activity showed no alteration. A significant graded reduction was observed in the altered indices in both postures, from the control to the Chagas' indeterminate and heart disease groups.
Conclusion: Cardiac autonomic dysfunction, with preserved putative measures of sympathetic and parasympathetic modulation in relative terms, was less severe or absent in the indeterminate and pronounced in cardiac form of Chagas' disease.

Keywords: Chagas' indeterminate disease; Chagas' heart disease; Cardiac autonomic dysfunction; Heart rate variability

Introduction

Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, still persists as an important endemic infectious condition chronically affecting more than 11 million people currently in the South American continent.[1] The disease includes an acute phase that may progressively evolve into different chronic clinical forms that are the indeterminate form, in which for a long period, only positive specific serological tests are present without clinically demonstrable organic involvement, and the forms characterized by exclusive or combined cardiac and digestive disease with or without symptoms.[2] The indeterminate form is encountered in about 50% of the infected subjects, persisting for a lifetime without manifestations or manifesting subtle or overt contractile dysfunction and/or cardiac autonomic disturbance, evolving in one third of cases to one overt form of organic disease by up to 30 years after the acute phase.[2] Chagas' heart disease, accounting for 25% to 30% of the chronic cases, is a neurocardiomyopathy characterized by associate or isolate lesions of variable severity and extension of the atrial and ventricular common myocardium, electrical exciting and conduction tissue, and autonomic intrinsic innervation, particularly of the parasympathetic branch.[2,3]

Moderate to severe primary cardiac parasympathetic and sympathetic dysfunction were repeatedly well demonstrated by means of different study methods in subjects with Chagas' disease with exclusive or combined heart or digestive disease.[4-8] In the indeterminate form, the observations using different tests, including heart rate variability analysis, are apparently conflicting, some authors having noted variable autonomic dysfunction,[9-11] and others showed no disturbances.[4,7,12] Unaltered median heart rate variability in subjects with this form of Chagas' disease when considered as a group, but with a individual pattern of subtly widespread from normal to depressed or exacerbated one was also demonstrated in this form.[13]

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The pathophysiology of the cardiac autonomic dysfunction and its functional significance remain incompletely understood in all the chronic forms of Chagas' disease. Evaluation of the severity of cardiac autonomic dysfunction in the different forms of Chagas' disease may be important considering its possible relationship with arrhythmias, sudden death, and the progressive ventricular mechanical dysfunction.[3]

Considering the variable cardiac autonomic dysfunction in the indeterminate and cardiac forms of Chagas' disease and the fact that the comparison between these two forms has not been done yet to this respect, we evaluated cardiac autonomic modulation, assessed by short-term heart rate variability, comparatively in Chagas' disease subjects with both forms of the disease and in healthy control subjects. The objective was to verify whether the expected cardiac autonomic dysfunction in the indeterminate and cardiac forms of Chagas' disease shows a distinctive pattern and severity.

Subjects and methods

Study groups

Three groups of subjects consecutively selected without any order were comparatively evaluated: (*a*) a group of 17 outpatients (5 men and 12 women) aged 28 to 51 years (median = 39) with the indeterminate chronic form of Chagas' disease; (*b*) a group of 13 outpatients (7 men and 6 women) aged 28 to 62 years (median = 44) with the chronic Chagas' heart disease; and (*c*) a group of 15 (9 men and 6 women) healthy control subjects aged 37 to 54 years (median = 43). All subjects with Chagas' disease were recruited from the Brasilia University Hospital Cardiology Ambulatory, where they have been followed up by means of periodic clinical and laboratory examinations, and the healthy control subjects had diverse origins.

Chagas' disease was first diagnosed on the basis of the epidemiological history of exposure to the vector insect and at least 2 positive specific indirect immunofluorescence, hemaglutination and enzyme-linked immunosorbent assay (ELISA) tests, and the forms of the disease were characterized considering current standardized criteria. The indeterminate form was conventionally identified by the absence of any clinical general and organ-specific manifestations and by a normal chest x-ray and 12-lead electrocardiogram (ECG). Chagas' heart disease was defined by the presence of typical electrocardiographic alterations (1st grade atrioventricular block, 8%; complete left bundle branch block, 8%; complete right bundle branch block plus left anterior hemiblock, 15%; complete right bundle branch block, 23%; left anterior hemiblock, 23%; diffuse alteration of ventricular repolarization, 23%); the chest x-ray was normal in all subjects with Chagas' heart disease. Subjects with Chagas' disease who were included had no past or present heart failure or any other cardiovascular or clinical disturbances. Therefore, those subjects that showed any clinical or laboratory manifestation other than Chagas' disease without or with heart disease were excluded. X-ray under contrast of the upper and lower digestive tracts identified 3 subjects with megaesophagus or megacolon, which indicates the presence of associated cardiac and digestive form of the disease. All control and Chagas' disease subjects were in excellent physical and mental condition in regular daily activities and were not using drugs.

This investigation conforms to the Helsinki declaration and the Brazilian Ministry of Health and was approved by the Ethics Committee on Research in Human Beings, Faculty of Medicine, University of Brasilia. All the individuals gave their signed informed consent to participate. The authors declare no conflict of interest.

Experimental session

All subjects were examined 1 to 4 hours after breakfast, between 8:00 and 11:00 AM. They were instructed to avoid ingesting stimulant beverages, tea, coffee, and alcohol drinks and to also avoid exercising, smoking, and ingesting any drug at least 24 hours before the examination.

The clinical and anthropometrical data and lifestyle habits were initially obtained, and the 12-lead ECG was recorded in the supine position in a room without external interference at ambient temperature (25°C-28°C); this initial evaluation lasted around 20 to 30 minutes. Next, after 10 minutes of rest in the supine position, a continuous 5-min ECG strip was recorded in lead II at 25 mm/s using a conventional electrocardiograph with a sampling frequency of 250 Hz and signal filters of 35 and 60 Hz. Subsequently, the subjects were asked to actively stand up at bedside, and after 2 minutes in this active orthostatic position, a new 5-minute ECG tracing was registered. The subjects breathed spontaneously and regularly and had their respiratory rate counted during the registration. Only those with a respiratory rate greater than 9 per minute (0.15 Hz) were examined to avoid the overlapping of the low- and high-frequency spectral areas that occurs in the spectrum of frequency-domain of heart rate variability below that rate. Around 30 to 60 minutes following the experimental session, each subject was submitted to Doppler echocardiographic examination.

Heart rate variability analysis

This analysis was performed as previously described[13] and according to the methodological standards recommended by the Task Force on Heart Rate Variability.[14] For each individual, one electrocardiographic 5-minute series of R-R intervals was automatically recorded in the supine and standing positions and archived real-time to a computer system using an analogical-digital converter by means of a signal-capturing dedicated software while being visually monitored onscreen. Then, each R-R interval series was transferred to another microcomputer system for offline data processing and analysis of variability. The software for the signal capturing and processing and the analyses of the data was developed using the MATLAB version 5.3 platform (The MathWorks, Inc, Natick, MA, USA) and validated at our Cardiovascular Laboratory and the Department of Electrical Engineering of the University of Brasilia.[15]

The R-R interval series were initially checked on a beat-to-beat basis for confirmation of sinus rhythm and detection of nonsinus and ectopic beats, artifacts, and stability. The software precisely identified the peaks of the QRS

complexes by means of a specific module of QRS detection. In addition, the R peaks were also visually verified in each beat. Therefore, no restriction for reliable R-R intervals variability analysis was due to any abnormality in the ECG of the subjects with Chagas' heart disease. Spurious beats were not frequent and when present were deleted from the series with their precedent and following intervals without adding new intervals. Eventual outlier beats were also removed. Artifacts were filtered by a specific module of the proper software. Edited R-R intervals were less than 1% for the series from the Chagas' disease subjects and 0.1% from the controls. As the R-R interval series were recorded in strictly controlled and stable experimental conditions, those qualified for analysis were highly stationary as estimated by the percent differences of the mean and the standard deviation between each pair of 3 segments of the R-R intervals series. In sequence, the series were processed and analyzed for variability in time domain and frequency domain by means of different conventional indices.

The time-domain indices provided include the mean R-R interval series and 2 variability indices reflecting the overall autonomic modulation, which are the standard deviation (SDNN) and coefficient of variation (CV: SDNN/mean) of the R-R interval series. Two other indices were the percentage of successive adjacent R-R intervals greater than 50 milliseconds (pNN50) and the square-root of the mean of the square of successive adjacent R-R intervals differences (rMSSD), which reflect the parasympathetic modulation associated with the respiratory sinus arrhythmia.[13,14,16,17]

For the frequency-domain analysis, the nonuniform 5-minute R-R interval series, without spurious or outlier beats, were initially normalized and resampled at 4 Hz by the cubic splines interpolation method. In sequence, the segments were submitted to filtering with Hanning windowing and then processed by the autoregressive modeling of fixed order equal to 16, for conversion of the signal oscillating components into power spectrum,[17,18] which comprises a very-low-frequency (VLF; 0-0.04 Hz), low-frequency (LF; 0.04-0.15 Hz), and high-frequency (HF; 0.15-0.50 Hz) spectral bands. The frequency-domain indices calculated include (*a*) total power spectral area (0-0.50 Hz), which indicates dominantly the degree of the overall autonomic modulation; (*b*) absolute and relative power areas of the very-low-frequency, low-frequency, and high-frequency bands; (*c*) normalized power areas of the low-frequency and high-frequency bands, which were expressed as percentage and calculated as the absolute power area of each band divided by the sum of both absolute areas, multiplied by 100 (LF (nu%) = LF / (total power − VLF) × 100 or LF (nu%) = LF / (LF + HF) × 100, and HF (nu%) = HF / (total power − VLF) × 100 or HF (nu%) = HF / (LF + HF) × 100); (*d*) ratio of the low-frequency to high-frequency absolute areas, where a ratio less than 1 is hypothesized to indicate a relatively predominant parasympathetic modulation and a ratio more than 1 a higher sympathetic and parasympathetic combined modulation. The basis for this hypothesized relationship is that the low-frequency band area reflects both sympathetic and parasympathetic influences, and the high-frequency band area represents almost exclusively parasympathetic action on the sinus node. Thus, the normalized powers and the ratio of the low-to-high-frequency absolute powers have been proposed to reflect the relative sympathetic and parasympathetic modulation.[13,14,16,17]

Statistical analysis

Because many R-R interval variability variables proved to be nonnormally distributed by the Kolmogorov-Smirnov, D'Agostino-Pearson, and/or Shapiro-Wilk tests, the groups were compared using the Kruskal-Wallis test, and if a significant overall difference became evident, the Dunn's multiple comparison test followed. The Doppler echocardiographic measures were compared between the 3 groups also using the Kruskal-Wallis test. All statistics were reported as medians, interquartile ranges (25th-75th percentiles), and extreme values for each variable. Categorical variables were compared by the χ^2 or Fischer exact tests. The trend of each heart rate variability index across the categorized groups of subjects (control, indeterminate disease, heart disease) was assessed by the Spearman correlation. The significance level for the differences detected was set as a 2-tailed P value < .05. Processing, analysis, and graphic design of the data employed the

Table 1
Baseline anthropometric, clinical and functional data and lifestyle habits of control and Chagas' indeterminate and heart disease subjects

	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*
n	15	17	13	
Age (y)	43 (40-46)	39 (35-45)	44 (35-49)	.22
Sex, men/women (%)	60/40	29/71	54/46	.18
Body mass index (kg/m^2)	24.4 (20.5-26.4)	24.8 (22.9-28.7)	23.4 (20.8-25.8)	.20
Body surface area (m^2)	1.74 (1.59-1.85)	1.65 (1.54-1.81)	1.72 (1.57-1.84)	.58
Systolic blood pressure (mm Hg)	110 (100-118)	120 (114-127)	120 (112-129)	.03
Diastolic blood pressure (mm Hg)	76 (70-84)	82 (78-88)	80 (78-88)	.10
Heart rate (beats/min)	61 (56-70)	64 (60-67	58 (51-68)	.21
Respiratory rate (ripm)	14 (12-16)	16 (16-18)	18 (16-20)	.02
Exercise practicing (%)	20	17.6	53.8	.37
Tobacco smokers (%)	0	11.8	23.1	.15
Alcohol drinkers (%)	100	5.9	7.7	.0001
Beverage drinkers (%)	33.3	52.9	53.8	.28

Continuous variables are given as median (interquartile range) and compared between the groups using the Kruskal-Wallis test. Categorical variables were compared by the χ^2 or Fisher exact test.

Table 2
Median (interquartile range) of Doppler echocardiographic morphological and systolic and diastolic dimensions measures in the rest supine, corrected by the body surface area, in control healthy and in Chagas' indeterminate and heart disease groups of subjects

	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*
n	15	17	13	
AD (mm/m^2)	17.7 (16.5-19.6)	17.9 (16.9-19.5)	17.8 (16.8-19.3)	.91
LA (mm/m^2)	17.4 (15.1-19.0)	19.1 (16.9-20.8)	17.8 (16.3-19.0)	.12
AD/LA ratio	1.1 (0.9-1.1)	0.9 (0.9-1.0)	1.0 (0.9-1.2)	.25
LVESd (mm/m^2)	16.5 (15.9-17.7)	17.3 (16.5-18.4)	18.7 (17.2-19.5)	.07
LVEDd (mm/m^2)	25.8 (25.3-27.5)	27.9 (25.9-29.4)	27.9 (25.7-29.4)	.19
IVSSt (mm/m^2)	7.6 (7.0-8.0)	7.7 (7.3-9.2)	7.9 (7.5-8.3)	.39
IVSDt (mm/m^2)	5.3 (4.7-5.9)	5.6 (5.0-6.3)	5.7 (5.1-6.1)	.38
LVPWSt (mm/m^2)	7.9 (7.6-8.6)	8.7 (8.1-9.7)	8.5 (8.0-9.0)	.08
LVPWDt (mm/m^2)	5.2 (4.8-5.3)	5.4 (5.0-5.9)	5.1 (5.0-5.5)	.22
RVEDd (mm/m^2)	12.8 (9.5-13.9)	12.9 (11.0-15.2)	11.9 (10.1-13.3)	.40
M index (g/m^2)	76.6 (66.5-89.6)	88.5 (74.4-93.4)	86.1 (73.7-110.4)	.22
LVEDv/M (ml/g)	0.71 (0.62-0.74)	0.66 (0.60-0.75)	0.70 (0.59-0.80)	.68

The measures were compared between the groups using the Kruskal-Wallis test. AD indicates aorta root diameter; LA, left atrium; LVESd and LVEDd, left ventricle end systolic and diastolic diameter; IVSSt and IVSDt, systolic and diastolic thickness of the interventricular septum; LVPWSt and LVPWDt, systolic and diastolic thickness of the left ventricular posterior wall; RVEDd, right ventricle end diastolic diameter; M index, ventricular mass adjusted for the body surface area; LVEDv/M, ratio between the left ventricular end diastolic volume and the ventricular mass.

SigmaStat 3.11/SigmaPlot 9.01 for Windows (Systat Software, Inc, USA, 2004) and the Prism 4 for Windows (GraphPad Software, Inc, USA, 2005) software packages.

Results

Table 1 shows comparatively the general baseline data of the 3 groups of subjects. No significant median difference was found for the anthropometrical and lifestyle habits, except for mild social and occasional alcohol intake that was less frequent in both groups with Chagas' disease (P = .0001). Physiological clinical variables were within the normal range in all the groups, although systolic pressure (P = .03) and respiratory rate (P = .02) showed higher values in the Chagas' disease groups. All morphological and functional Doppler echocardiographic measures were normal and comparable in the 3 groups of subjects, as shown in the Tables 2 and 3.

The data from the heart rate variability analysis in the time domain are listed in Table 4. No significant difference was found between groups for the median of mean R-R intervals in both the supine and standing positions (P = .11-.25). All the other time-domain indices showed reduced median values for each group with Chagas' disease in comparison to control group, in the supine position (P = .0002-.0005). In the standing position, the coefficient of variation was significantly lower in the 2 groups of subjects with the disease (P = .002), whereas the SDNN, pNN50, and rMSSD showed reduction only in the heart disease group (P = .009-.01).

The data for the frequency-domain analysis are shown in Table 5. The total and low-frequency and high-frequency absolute power areas had reduced median values in the 2 groups of Chagas' disease subjects in comparison with the control group for both postures (P = .0001-.004). Although more reduced indices were systematically observed in the subjects with cardiopathy, the differences between the 2 groups with Chagas' disease had no statistical significance. The indices suggested to indicate the relative sympathetic and parasympathetic modulation showed no significant difference between the 3 groups, regardless of posture (P = .21-.78).

Table 3
Median (interquartile range) of Doppler echocardiographic systolic and diastolic function measures in the rest supine control healthy and in Chagas' indeterminate and heart disease groups of subjects

	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*
n	15	17	13	
EF (%)	66.7 62.2-68.0)	67.0 (63.6-68.3)	61.7 (54.2-67.6)	.15
Vcf (c/s)	1.2 (1.1-1.3)	1.2 (1.1-1.3)	1.1 (0.9-1.2)	.12
%DΔ	36.2 (32.9-37.5)	37.0 (34.6-37.9)	33.1 (28.1-37.7)	.14
E/A ratio	1.3 (1.1-1.6)	1.3 (1.1-1.5)	1.4 (0.9-1.5)	.63
E-F slope (ms)	190 (180-200)	200 (170-218)	194 (177-226)	.77
e'/a' ratio	1.1 (1.1-1.3)	1.1 (1.0-1.3)	1.1 (1.0-1.2)	.86
E/e' ratio	5.2 (4.7-6.0)	5.4 (5.0-6.0)	4.8 (4.0-5.7)	.32
LVIRT	82.0 (68.7-85.0)	86.7 (71.2-90.0)	83.7 (70.4-90.0)	.60
Tei index	0.40 (0.32-0.48)	0.43 (0.39-0.48)	0.44 (0.34-0.56)	.49

The measures were compared between the groups using the Kruskal-Wallis test. EF indicates ejection fraction; Vcf, velocity circumferential fiber shortening; %DΔ, left ventricular fractional shortening percentage; E/A, ratio between the wave of mitral peak early velocity and the wave of peak atrial contraction velocity; E-F slope, early filling deceleration time of mitral velocity; e'/a', ratio between the wave of early diastolic velocity of the septal tissue Doppler and the wave of end diastolic velocity; E/e', ratio between the E wave and the e' wave; LVIRT, left ventricular isovolumetric relaxation time; Tei index, myocardial performance index.

Table 4
Median (interquartile range) of time-domain short-term heart interval variability indices in healthy control and Chagas' indeterminate and heart diseases groups of subjects in supine and active standing positions

	Supine				Standing			
	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*
n	15	17	13		15	17	13	
Mean RRi (ms)	936 (847-970)	910 (846-959)	973 (872-1040)	.11	753 (668-796)	732 (685-852)	806 742-900)	.25
SDNN (ms)	52.5 (36.7-60.0)	29.3[a] (22.3-41.6)	21.4[a] (12.8-35.0)	.0003	38.8 (30.5-62.4)	26.2 (22.5-39.9)	24.1[a] (19.8-36.7)	.01
CV (%)	5.27 (4.26-6.52)	3.20[a] (2.65-4.54)	2.05[a] (1.33-3.67)	.0004	5.76 (4.51-7.16)	3.83[a] (2.65-4.89)	3.25[a] (2.18-4.28)	.002
pNN50 (%)	9.74 (4.01-34.0)	0.92[a] (0-5.35)	0[a] (0-0.34)	.0002	2.03 (0-17.92)	0 (0-0.56)	0[a] (0-0.14)	.01
rMSSD (ms)	35.6 (23.3-49.7)	23.6[a] (9.4-25.9)	14.3[a] (9.8-19.1)	.0005	20.9 (11.7-39.8)	11.9 (8.2-17.1)	10.2[a] (7.7-13.7)	.009

The groups were compared by the Kruskal-Wallis test followed by the post hoc multiple comparison test of Dunn when a significant difference was observed. See Subjects and methods for definition of the time-domain indices.

[a] Value statistically different ($P < .05$) when pairwised compared with control group by the Dunn test.

A higher reduction of all variability indices was observed in heart disease than in the indeterminate group, as shown by a graded trend decrease of the indices values across the groups, from the control to the indeterminate and heart disease groups. The box-and-whiskers plots in Figs. 1 and 2 illustrate the median, interquartile range, and extreme values of indices, respectively, in the time-domain and frequency-domain for the Chagas' disease and control groups in supine and standing positions. Differences are observed between the indices and their systematically decreasing trend in the Chagas' disease groups as compared to the control group.

Discussion

In the present work, we further evaluated the momentary steady-state cardiac autonomic function in chronic Chagas' disease, which is of utmost pathophysiological importance, simultaneously comparing subjects with the indeterminate and the cardiac forms of the disease and healthy individuals using short-term heart rate variability analysis that has been widely used for that evaluation.[14,16,17] Our most relevant finding was that the expected cardiac autonomic dysfunction shows a clear distinctive pattern and severity in the Chagas' indeterminate and heart diseases.

Although heart rate variability was depressed in Chagas' patients with cardiac disease, the heart rate variability indices believed to reflect sympathovagal balance, that is, the relative modulation of each autonomic component, were not significantly different between the 3 groups, remaining unchanged in both postures in the Chagas' indeterminate and heart disease groups in comparison to the control group. This is not a surprising new functional finding in Chagas' disease because both sympathetic and parasympathetic modulation may be depressed but remain unaltered in relative terms (normalized indices and LF/HF ratio). This could reflect a preservation of the relative activity of both autonomic components but in low absolute level. That is to say, depression of both sympathetic and parasympathetic influences could be proportionally similar. If this were true, it would imply that the lesions of the autonomic intrinsic innervation were more severe in the cardiac than in

Table 5
Median (interquartile range) of frequency-domain short-term heart interval variability indices in healthy control and Chagas' indeterminate and heart diseases groups of subjects in supine and active standing positions

	Supine				Standing			
	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*	Control	Chagas' indeterminate disease	Chagas' heart disease	*P*
n	15	17	13		15	17	13	
Total power spectral area (ms^2)	551 (234-761)	127[a] (87-258)	101[a] (34-230)	.0001	368 (147-770)	118[a] (88-249)	82[a] (46-237)	.004
Low-frequency power spectral area (ms^2)	183 (83-326)	44[a] (18-71)	16[a] (5-60)	.0001	176 (47-481)	40[a] (20-83)	26[a] (9-69)	.002
High-frequency power spectral area (ms^2)	111 (40-261)	30[a] (8-59)	13[a] (6-29)	.0003	46 (8-77)	7[a] (4-16)	9[a] (3-11)	.004
Normalized low-frequency power area (%)	67 (35.2-82.0)	63.9 (45.8-75.4)	60.3 (38.4-78.1)	.96	86.2 (67-91)	87.3 (79.4-89.7)	75 (67.2-88.8)	.48
Normalized high-frequency power area (%)	33 (18-64.8)	31.9 (23.7-50.9)	39.7 (21.8-61.6)	.78	13.8 (9-33)	12.3 (7.4-17.9)	25 (11.2-32.8)	.23
Low- to high-frequency power area ratio	2.02 (0.54-4.45)	2.13 (0.96-3.25)	1.52 (0.62-3.57)	.77	6.27 (2.04-10.7)	7.10 (4.59-13.4)	2.95 (2.06-7.93)	.21

The groups were compared by the Kruskal-Wallis test followed by the post hoc multiple comparison test of Dunn when a significant difference was observed. See Subjects and methods for definition of the frequency-domain indices.

[a] Value statistically different ($P < .05$) when pairwised compared with control group by the Dunn test.

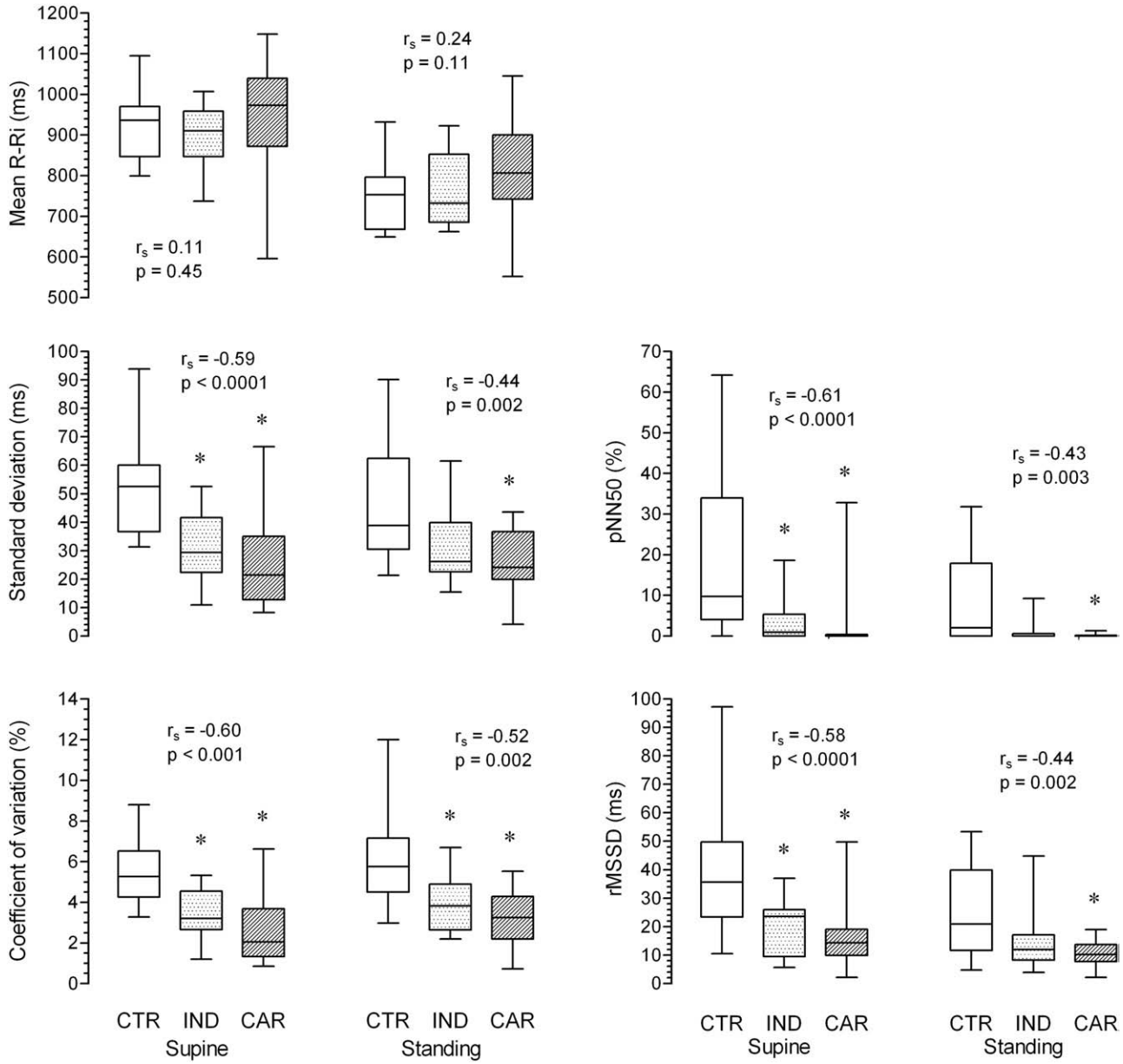

Fig. 1. Median, interquartile range, and extreme values of the time-domain heart rate variability indices of healthy control (CTR, n = 15) and Chagas' indeterminate (IND, n = 17) and heart (CAR, n = 13) disease groups rested in supine and active standing positions. The Spearman's correlation coefficients (r_s) and its P values for the trend of the indices across the groups, from the control to Chagas' disease subjects with the indeterminate and cardiac forms, are shown. *$P < .05$ (Chagasic group vs control group by the Dunn test after the Kruskal-Wallis test).

indeterminate form of disease but remained balanced in both the forms.

In spite of the fact that the differences between the two Chagas' disease groups showed no statistical significance when these groups were compared, subjects with the indeterminate form systematically showed lesser alteration of the time-domain and frequency-domain absolute indices than those with heart disease. In fact, a highly significant negative correlation trend across all the groups, from the control to the Chagas' indeterminate and heart diseases, was observed for the altered indices in both postures, which prove as expected, that cardiac autonomic impairment was more severe in Chagas' heart disease. That is to say, the disturbance was observed to be lesser in the indeterminate form, showing mild-to-moderate severity, which was intermediate between the normality and the marked alteration in the heart disease form.

Prior studies have reported damage of parasympathetic and sympathetic intrinsic innervation of heart, mainly the first.[19,20] Based on these findings, cardiac autonomic impairment has been demonstrated to be a striking aspect of Chagas' disease, which is present in variable intensity in a high number of Chagas' disease subjects according to the form of the disease. Previous demonstration of this disturbance was principally based on the evaluation of the acute heart rate responses to different autonomic tests and scarcely on steady-state heart rate variability analysis. It has been shown that widespread depressed or

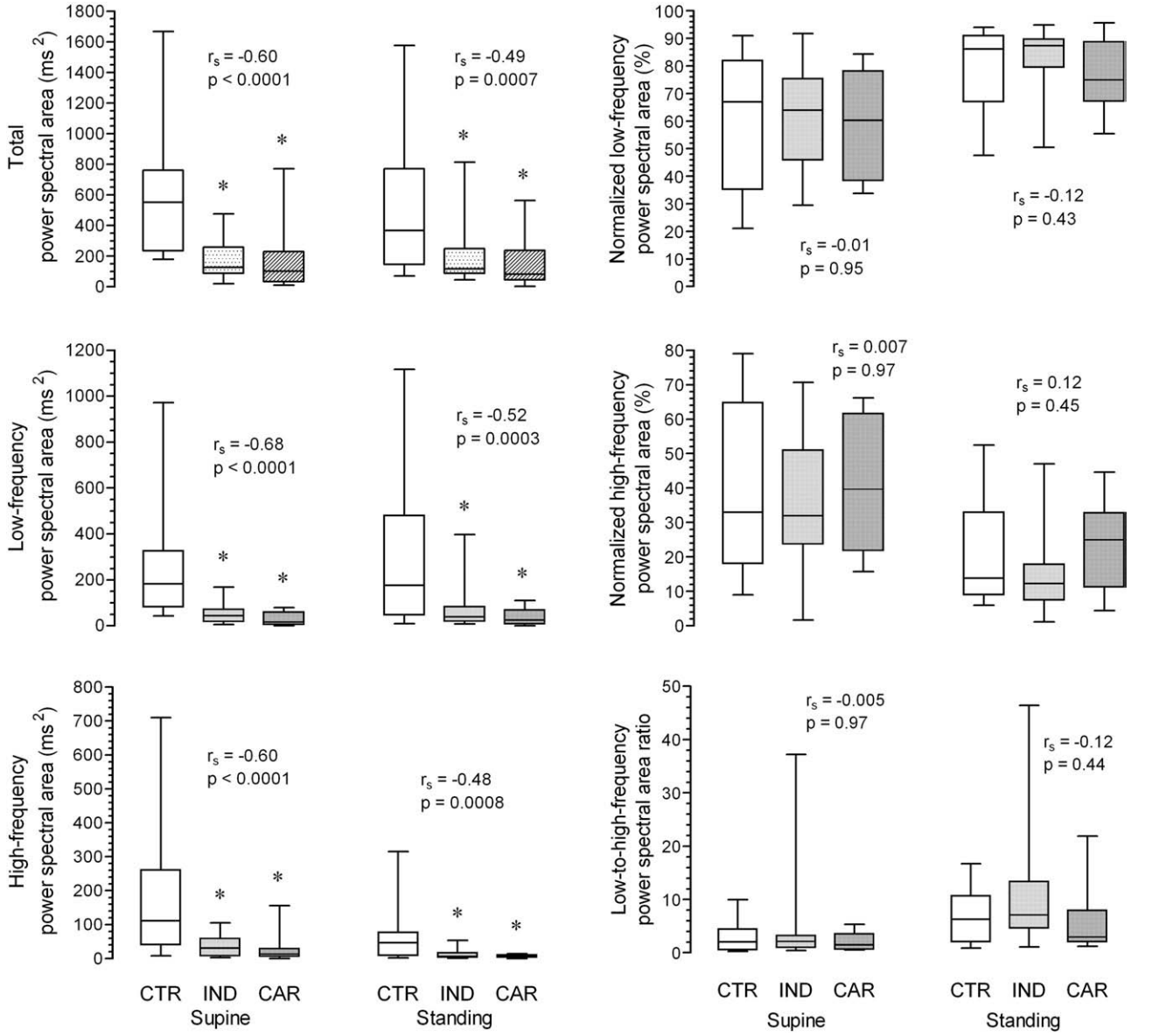

Fig. 2. Median, interquartile range and extreme values of the frequency-domain heart rate variability indices of healthy control (CTR, n = 15) and Chagas' indeterminate (IND, n = 17) and heart (CAR, n = 13) disease groups rested in supine and active standing positions. The Spearman's correlation coefficients (r_s) and its P values for the trend of the indices across the groups, from the control to Chagas' disease subjects with the indeterminate and cardiac forms, are shown. *$P < .05$ (Chagasic group vs control group by the Dunn test after the Kruskal-Wallis test).

exacerbated cardiac autonomic modulation, from normal to mild-to-moderate, appears to occur in the indeterminate form.[4,7,9-13,21] More pronounced disturbance was already noted in the cardiac-digestive and digestive forms and a moderate-to-severe alteration in the exclusive cardiac form without heart failure.[4-7,9-11,20-24] Therefore, our present findings reinforce and extend these previous observations, showing comparatively the autonomic dysfunction between the Chagas' indeterminate and heart disease.

Our findings appears to be reliable and not influenced by the significant differences between the groups in the systolic pressure, respiratory rate, and alcohol ingestion. Notwithstanding the differences, systolic pressure and respiratory rate were within in a close reference range and therefore not sufficient to cause functional disturbances. The control subjects were only mild drinkers, and none had a regular intake, and therefore, it is very improbable that the differences in proportion of alcohol drinkers between this group and the Chagas' disease groups were due to this quantitatively irrelevant factor.

The clinical and functional significance of the cardiac autonomic dysfunction in Chagas' disease is a matter of conjectures.[3] In the Chagas' indeterminate disease, this is a difficult question, considering the mild-to-moderate or subtle alterations of autonomic function and the favorable long-term evolution of this form of the disease.[2,3,12,13] On the other hand, in the cardiac form, it is possible that the autonomic disturbance may have some functional, clinical, or prognostic implication.[3,5,22] Considering the important influence of autonomic modulation on all the heart properties, it is reasonable to speculate that the cardiac dysautonomia may constitute a triggering or risk factor for other disturbances,

such as arrhythmias, progressive ventricular mechanical dysfunction, and even sudden death, or it still could contribute to cardiovascular or overall morbidity and mortality.[3] However, with respect to the possible relationship between ventricular and autonomic function, some findings by others authors have suggest that these disturbances did not have a causal relationship, at least in some occasion in the course of the Chagas' disease[7,8,11,25]; alternatively, a hypothesis suggests that cardiac autonomic dysfunction is a consequence of contractile ventricular dysfunction and/or of other primary disturbances.[22] In addition, the cardiac autonomic impairment may determine inappropriate short-term and long-term cardiovascular adaptations to multiple internal or external stimuli.[3] In fact, evidences indicate unfavorable outcome represented by enhanced cardiovascular and global morbidity and mortality in different clinical conditions, dependent on heart rate variability depression.[14,16,17]

On the other hand, we can only speculate about the clinical significance of the apparently preserved relative sympathetic and parasympathetic balance in the presence of depressed absolute autonomic modulation. Perhaps, the balanced sympathetic and parasympathetic modulations might prevent the functional consequences of autonomic unbalance, such as possibly electrophysiological disturbances and arrhythmias, progressive contractile dysfunction, and even sudden death.[3]

One limitation in our study was the relatively low number of subjects examined in each group. However, this concern was obviated by using nonparametric statistics to compare the groups and by the observation of very low or high levels of the P values, which indicates, respectively, strong statistical evidence or not for the differences observed. It is also possible that the small samples of subjects examined were the reason of the lack of statistically significant differences between the two Chagas' disease groups when they were compared. However, the indices directly correlated with the severity of the form of Chagas' disease, showing a systematic significant trend to be mild to moderately altered, indicating less severe autonomic depression in the indeterminate form, and markedly altered, indicating pronounced autonomic disturbance in the heart disease form.

In conclusion, depressed cardiac autonomic modulation expressed by reduced absolute indices of short-term heart rate variability in the time-domain and frequency-domain was observed in both the indeterminate and cardiac forms of Chagas' disease in supine position and only in the cardiac form in the standing position. A highly significant negative trend across all groups, from the normal autonomic function to less severe autonomic dysfunction in the Chagas' indeterminate disease and to marked dysfunction in heart disease, was observed for the altered indices in both postures. Therefore, absolute overall autonomic depression was systematically mild to moderate or even absent in the indeterminate form and pronounced in cardiac form, which shows that the autonomic dysfunction is correlated with the clinical form of Chagas' disease. However, the heart rate variability measures suggested to reflect the relative sympathetic and parasympathetic modulation remained unaltered in the two forms of the disease in comparison to the control group.

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