Abnormalities in Fractal Heart Rate Dynamics in Chagas Disease

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Background and Methods: In order to study fractal HR dynamics in Chagas disease, we performed detrended fluctuation analysis (DFA)—along with analysis of power-law β slope (β index) and standard deviation of N–N intervals (SDNN)—in edited and unedited (with ventricular premature beats —VPBs, only in DFA analysis) series of R–R intervals from Holter monitoring of healthy controls (Group 0, $n = 27$) and Chagas disease patients with left ventricular (LV) ejection fraction $>50\%$ (Group 1, $n = 137$) and with LV ejection fraction $<50\%$ (Group 2, $n = 23$).

Results: When analyzed from the edited R–R interval data, the long-term scaling exponent α_2 is altered both among the Chagas patients with and without LV dysfunction. The short-term scaling exponent α_1 was higher in Group 1 Chagas patients as compared to controls ($P < 0.01$) and did not differ between Group 2 and controls. In unedited R–R interval series, α_1 was significantly reduced in Group 2 Chagas patients (0.55 ± 0.002) as compared to controls (0.90 ± 0.002) and Group 1 (0.91 ± 0.003) ($P < 0.001$), but did not differ between Group 1 and controls. Similarly α_2 was lower in Group 2 compared to other groups ($P < 0.001$). SDNN did not differ between the groups, but the β index derived from $1/f$ model was reduced both in Group 1 and 2 Chagas patients as compared to controls ($P < 0.01$). There was strong correlation ($r_s = 0.82$; $P < 0.001$) between the β and α_2 index from edited series. There was an inverse correlation ($r_s = -0.63$, $P < 0.01$) between the number of VPBs and α_1 index of unedited series.

Conclusions: The long-term fractal HR dynamics altered in chagasic patients with and without LV dysfunction could be an early sign of autonomic dysfunction. Patients with impaired LV function show marked alterations in short-term fractal HR dynamics toward more random behavior, mainly due to frequent ectopy. Prospective studies are necessary to define the value of these indices as predictors of death in Chagas disease.

A.N.E. 2006;11(2):145–153

ventricular premature beats; heart rate; fractal correlation; nonlinear dynamics, Chagas disease

Detrended fluctuation analysis (DFA) is a nonlinear method that evaluates the self-similarity properties of the heart rate (or R–R interval) time series. The method was described for R–R interval time series by Peng et al. in 1995.¹ Since then, several publications demonstrated that α_1 —a short-term correla-

tion index derived from DFA—is a powerful predictor of death in coronary heart disease, heart failure, aged people, and a predictor of appropriate shocks in patients with implantable defibrillator.^{2–6}

In the presence of ventricular arrhythmia, heart rate variability (HRV) analysis usually requires

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This study was supported by grants from the State of Minas Gerais Research Support Foundation (FAPEMIG), Belo Horizonte, Brazil; College Education Improvement Coordination (CAPES), Brasilia, Brazil; National Council for Scientific and Technological Development (CNPq), Brasilia, Brazil; and Research Department of the Dean's Office of the Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil.

manual edition of ectopic beats, especially if time and frequency domain analysis are performed.⁷ However, it is unknown whether, in addition to time consumption, editing intervention on R-R interval time series might prevent the appraisal of the effects of ectopies and of their contributing mechanisms on R-R dynamics.

When considering DFA, it is not well defined whether this edition is necessary and produces direct effects on DFA parameters. Huikuri et al. observed that when including up to 10% of ventricular ectopic beats, the indices did not change significantly, suggesting that edition was unnecessary² and that α_1 of unedited series was the best predictor of mortality.² Recently, Peltola et al.⁸ analyzed in healthy subjects, the effects and significance of premature beats on fractal correlation properties and suggested that premature ventricular beat editing was not absolutely necessary for risk stratification, although α_1 of unedited series was inferior to that computed on edited series.⁸

Chagas disease is a potential lethal illness that affects nearly 20 million persons in Latin America and thousands of immigrants that are now living in the United States and Europe.^{9,10} The disease presents autonomic dysfunction and anatomic lesions of the autonomous nervous system.^{11,12} In time domain analysis, reduced values of rMSSD and pNN50 are the most consistent findings in Chagas disease.^{13,14} In a recent study, we observed that Chagas disease patients without cardiomyopathy had abnormal nonlinear dynamics of HRV.¹⁵ The purpose of the present study was to analyze the relationship among left ventricular ejection fraction (LVEF), α_1 and α_2 indices as well as to evaluate whether editing of ventricular ectopic beats might affect DFA in Chagas disease patients. The correlation among standard deviation of N-N intervals (SDNN), β index derived from 1/f model, number of premature ventricular beats, and DFA indices was also studied.

METHODS

The study was conducted at the Chagas Disease Outpatient Reference Center of the Federal University of Minas Gerais, Brazil. We have selected consecutive patients from 20 to 70 years old with a definite serologic status for Chagas disease (≥ 2 different positive reactions to *Trypanosome cruzi*). Those who agreed to participate and signed a written informed consent were submitted to a standard

screening protocol that included clinical and laboratory examinations, echocardiogram, and 24-hour Holter monitoring. A control group of healthy volunteers (20–70 years old) with no risk or serologic evidence of Chagas disease was also submitted to the same evaluation. Exclusion criteria were the presence of comorbidities, use of drugs that affect autonomic nervous system, nonsinus rhythm, as atrial fibrillation or pacemaker rhythm, the presence of more than 10% of ectopies or artifacts on Holter monitoring, and the refusal to participate. From 239 patients screened, 38 (16%) were excluded due to comorbidities, presence of supraventricular arrhythmias or amiodarone use, and 14 (6%) were excluded because of technical reasons related to performing or recording the examinations. The final sample was divided into three groups: Group 0 or control group (n = 27); Group 1 with Chagas disease patients with LV ejection fraction $\geq 50\%$ (n = 137); Group 2 of Chagas disease patients with LV ejection fraction $< 50\%$ (n = 23).

Study Protocol

As described previously,¹⁵ the Ethical Committee of the Federal University of Minas Gerais approved the study protocol. Patients were submitted to Doppler echocardiography with color flow using ATL HDI 5000 equipment (Bothell, Washington, USA) according to the recommendations of the American Society of Echocardiography, with a 16-segment model.¹⁵ The LV ejection fraction was obtained through Simpson's method using the software provided with the equipment. It was considered normal if ≥ 0.5 . Twenty-four-hour Holter monitoring was performed using a portable three-channel cassette tape recorder (Dynamis, Cardios, Sao Paulo, Brazil). Subjects were encouraged to continue with their normal everyday activities during the recordings, avoiding physical exercise or drugs that could interfere with autonomic function. Analysis of HRV was performed when > 18 hours of good quality tracings and $> 90\%$ of sinus rhythm beats were available. The recordings were analyzed on a DMI Holter System (Cardios São Paulo/Spacelabs Burdick, Deerfield, Wisconsin) by a semiautomatic technique, with a sampling frequency of 200 Hz. For each tape, the entire recording was carefully reviewed and the QRS complexes were classified as normal beats, artifacts, and ectopies, to create a time series of normal R-R intervals. Ectopies were removed from

edited series without interpolation. The standard deviation of normal interbeat intervals (SDNN, a time-domain index) was calculated in this series of only normal beats (edited series). The β slope of the power law behavior of HRV was obtained from these edited series using an already described methodology.¹⁵ Series of interbeat intervals including ventricular ectopies up to 10% (unedited series) were also created. The indices from DFA method α_1 and α_2 were calculated for both edited and unedited series. The software from Peng et al.¹ is available at the web site <http://www.physionet.org>. The program was tested with known series. The original program uses up to 11,000 points. To analyze longer data sets, maxpoints was increased to 150,000 points to fit 24-hour Holter series. As described by Peng et al., the indices are the slope of the log-log curve: $\text{Log}_{10}[F(n)] \times \text{Log}_{10}(n)$ for windows with n beats, where $F(n)$ is the root mean square fluctuation of the detrended fluctuation time series. α_1 and α_2 are, respectively, the indices for $n < 11$ and $n > 11$ beats in windows like the study of Huikuri et al.² for short- and long-term correlation, respectively.

Statistical Analysis

Data obtained from continuous variables are expressed as mean $\pm$ SD or medians and the interquartil range. Data from categorical variables are expressed as proportions and compared using the chi-square test for contingency tables. Chi-square, Mann-Whitney U, and Kruskal-Wallis tests and Spearman correlation coefficients were used to measure correlation or univariate association among variables. When necessary, mathematical transformation of nonnormal or heteroscedatic

data was performed to allow subsequent analysis. Baseline features and transformed HRV indices of the three groups were compared using analysis of variance with Fisher's multiple comparisons. To perform the covariance analysis, multiple linear regression models were constructed for each HRV measurement, including the classification group as the independent variable and covariates associated ($P \leq 0.10$) in univariate analysis. In order to define which factors were more strongly related to the presence of depressed LVEF ($<50\%$), we performed logistic regression with backward stepwise procedure (probability for stepwise removal = 0.1), considering depressed LVEF as the dependent variable and HRV indices, age, mean R-R value, and 24-hour PVB number as independent ones. When multiple comparisons were made, the Bonferroni's correction was applied. A P value ≤ 0.05 was considered significant at final analysis. For some indices, even when we did mathematical transformation, data did not fit parametric regression models and in these cases we performed nonparametric analysis with Kruskal-Wallis followed by Mann-Whitney U.

RESULTS

The study population consisted of 187 patients with 79 (42.2%) females and 108 (57.8%) males, aged between 21 and 67 years, mean age 41 years. Control group comprised 27 patients; there were 137 Chagas patients with LVEF $\geq 50\%$ (Group 1; range 69–50%) and 23 patients with LVEF $<50\%$ (Group 2; range 49–26%). The general characteristics of the patients are presented in Table 1. The number of ventricular premature beats (VPBs)

Table 1. General Characteristics of the Controls and of the Chagas Disease Patients

Variable	Group 0 n = 27	Group 1 n = 137	Group 2 n = 23	p	
Age (years)	37.3 $\pm$ 10.35	40.9 $\pm$ 9.05	44.1 $\pm$ 9.99	0.039	0 = 1; 1 = 2; 0 $\neq$ 2 ^a
Male sex – n (%)	18 (67)	76 (56)	13 (56)	0.430	0 = 1=2
LV diastolic diameter (mm)	48.8 $\pm$ 4.6	50.4 $\pm$ 4.1	61.0 $\pm$ 5.6	0.000	0 = 1 $\neq$ 2 ^a
LVEF (%)	65 (61–66)	62 (60–65)	40 (35–45)	0.000	0 = 1 $\neq$ 2 ^b
Mean 24-hour RR (ms)	778.7 $\pm$ 93.4	831.9 $\pm$ 113.6	865.0 $\pm$ 134.1	0.042	0 = 1; 0 $\neq$ 2 ^a
VPB (n)	3 (1–6)	62 (4–429)	4119 (913–5841)	0.000	0 = 1 $\neq$ 2 ^b
NSVT – n (%)	0	15 (11%)	14 (61%)	0.000	0 $\neq$ 1 $\neq$ 2

Data are expressed by mean $\pm$ SD = standard deviation, median (Q1–Q3).

^aBonferroni's correction; ^bKruskal-Wallis, followed by Mann-Whitney U.

EF = ejection fraction; HR = heart rate; LV = left ventricle; NSVT = nonsustained ventricular tachycardia; RR = RR interval; VPB = ventricular ectopic beats.

Table 2. HRV Indices in Controls (Group 0) and Chagas Disease Patients (Groups 1 and 2)

Variable	Group 0	Group 1	Group 2	P	
SDNN (ms) ^a	154 (108–172)	150 (117–174)	142 (120–195)	0.958	0 = 1 = 2
β slope index ^a	-0.994 ± 0.037	-1.133 ± 0.017	-1.156 ± 0.040	0.002	0 $\neq$ 1 = 2
α_1 edited ^a	0.984 ± 0.001	1.132 ± 0.001	1.032 ± 0.001	0.008	0 $\neq$ 1; 0 = 2
α_2 edited ^b	0.989 (0.922–1.029)	1.060 (0.989–1.138)	1.055 (0.962–1.117)	0.001	0 $\neq$ 1 = 2
α_1 unedited ^a	0.902 ± 0.002	0.906 ± 0.003	0.548 ± 0.002	0.000	0 = 1 $\neq$ 2
α_2 unedited ^b	0.984 (0.897–1.010)	0.986 (0.864–1.075)	0.820 (0.608–0.918)	0.000	0 = 1 $\neq$ 2

Data are mean $\pm$ SD, adjusted for covariates when indicated^a or medians and interquartile range, compared by Kruskal–Wallis, followed by Mann–Whitney U^b.

was significantly greater in Group 2 patients who also presented a longer mean R-R interval. Non-sustained ventricular tachycardia (NSVT) was more frequently observed in Chagas patients in comparison to controls and among Chagas patients, NSVT was more frequent in Group 2.

Results of time domain analysis of HRV, β index derived from 1/f model, edited and unedited indices of DFA are described in Table 2. In comparison to control, both Chagas disease groups were characterized by a more negative β slope indices and by greater edited α_2 values. When comparing Groups 1 and 2 patients, the latter ones presented decrease unedited α_1 and α_2 values.

The correlation among the different HRV indices and ejection fractions as well as relevant clinical variables is shown in Table 3. Mean R-R interval was strongly associated with SDNN values and, in a lesser extent, to β slope index. Unedited DFA in-

indices were significantly correlated with the number of premature ventricular contractions, in special short-term α_1 index (Fig. 1). LVEF was significantly associated with DFA indices particularly when these were computed on unedited time series. We also observed a weak association among age and most HRV parameters.

Comparing indices from edited and unedited series (Fig. 2), α_1 was significantly different between the two types of series showing the strong influence of editing the time series for VPB in the value of this short-term index. This difference of α_1 values between unedited and edited series was particularly striking in Chagas disease patients with depressed LVEF, in whom we found, as described in Table 1, a high number of VPB. α_2 , as a long-term index, was also influenced by the time series edition, but only in Chagas disease Groups 1 and 2 patients.

Table 3. Spearman Correlation Coefficients Among the Different HRV Indices, Ejection Fraction and Relevant Clinical Variables

		LVEF	Age	Mean RR	24-hour PVB
SDNN	rs	-0.049	-0.220	0.715	0.037
	P	0.517	0.003	<0.001	0.622
β slope index	rs	-0.114	0.228	0.222	0.136
	P	0.129	0.002	0.004	0.070
α_1 edited	rs	0.124	-0.209	0.089	-0.168
	P	0.105	0.006	0.253	0.028
α_2 edited	rs	0.104	0.098	0.121	-0.020
	P	0.157	0.183	0.105	0.783
α_1 unedited	rs	0.290	-0.235	-0.085	-0.630
	P	<0.001	0.002	0.269	<0.001
α_2 unedited	rs	0.280	-0.074	-0.057	-0.375
	P	<0.001	0.317	0.452	<0.001

Correlation is significant at the 0.01 level (2-tailed) after Bonferroni correction (in bold). rs = Spearman correlation index; 24-hour PVB = number of premature ventricular beats in 24 hours; LVEF = left ventricular ejection fraction; Mean R-R = mean R-R intervals in 24 hours.

Figure 1. Correlation between the number of VPB in 24 hour and α_1 unedited index.

Table 4 shows correlation coefficients among different HRV indices, revealing significantly association among most DFA indices. A significant correlation between β index from 1/f model and α_2 index from DFA was detectable (Fig. 3).

In a logistic regression model ($P < 0.001$), only the number of VPB ($P < 0.001$) and β index from 1/f model ($P = 0.005$) were related to depression of LVEF.

DISCUSSION

The main findings of this study were the following: (1) in nonedited series, random short-term HR behavior (reduced α_1) among Chagas disease patients with left ventricular dysfunction, mainly due to frequent ectopy; (2) altered long-term (β and α_2 index from edited series) fractal HR dynamics among the chagasic patients with and without LV

Figure 2. Comparison between α_1 and α_2 indices from edited and unedited in the three groups.

Table 4. Spearman Correlation Among Indices

		SDNN	β Slope Index	α_1 edited	α_2 edited	α_1 unedited
β Slope index	rs	0.012				
	P	0.880				
α_1 edited	rs	0.106	0.021			
	P	0.177	0.793			
α_2 edited	rs	-0.017	0.822	0.323		
	P	0.825	< 0.001	< 0.001		
α_1 unedited	rs	0.052	-0.146	0.551	0.076	
	P	0.503	0.058	<0.001	0.316	
α_2 unedited	rs	0.010	0.300	0.386	0.508	0.702
	P	0.900	< 0.001	< 0.001	< 0.001	< 0.001

Correlation is significant at the 0.01 level (2-tailed) after Bonferroni correction (in bold).

dysfunction, despite normal SDNN; (3) in edited series, patients with normal LV function and Chagas disease had higher values of α_1 than controls.

Short-Term Correlation Properties

This study was conducted in Chagas disease patients, in which both autonomic dysfunction and frequent VPCs are present. Our data showed that, although indices derived from edited and unedited series were correlated, only α_1 index from unedited series was significantly correlated with the number of ventricular ectopies (rs = 0.63). Indeed, there

was an increase in the randomness of short-term interval dynamics with the increase in the number of VPBs. In the work of Peltola et al., a similar difference in values of indices from edited and unedited series was found, but VPBs were artificially introduced in their series.⁸ In a case-control study, it was found that both edited and unedited indices were different in patients who died and those who survived after an acute myocardial infarction. However, in the whole population, only α_1 from unedited series differed significantly between survivors and those who died.²

Figure 3. Correlation between the β index from 1/f model and α_2 index from DFA.

In the present study, when the number of ventricular ectopies was included as covariate in multivariate analysis, there was no difference in the values of α_1 indices among groups. So one could infer that the number of ventricular ectopies might play the role of confounding variable. In edited series, however, the exclusion of ectopic beats caused the loss of interdependent properties of this correlation. This interpretation is supported by the results of a different HRV methodology based on the quantification of cardiac cycle changes after the compensatory pause that follows a premature ventricular beat. Both heart rate turbulence parameters were correlated with ventricular ectopy number, thus confirming these interdependent properties.¹⁶

It is therefore reasonable to speculate that α_1 of unedited series is a measure of complexity of the variability signal that takes into account at least three components: the normal-to-normal interbeat variability; the additional variability caused by premature ventricular beats; and the disturbance caused by cycle changes following a compensatory pause. α_1 of unedited series can therefore aggregate three known predictors of death: the conventional HRV, the number of ectopies, and post-extrasystolic cardiac cycle changes. A finding that could explain the fact that α_1 has been considered as the most accurate parameter to identify high risk patients.²

In our opinion, DFA computed on unedited time series present additional advantages. Premature ventricular beats cause perturbation in the variability signal that cannot be disregarded because they contain information that can be disclosed by non-linear methodology. Patients with Chagas disease represent an unique opportunity of studying this issue for the contemporary presence of autonomic dysfunction, frequent ectopies, and late left ventricular decrease in ejection fraction. The observed results are relevant from both a clinical and physiopathological point of view. Editing continues to be a major problem in HRV analysis. It is time consuming, there is no method to evaluate its accuracy and also in the ideal situation causes a loss of information.

The short-term correlation properties evaluated by α_1 in edited series are consistent with findings of other authors in coronary heart disease.¹⁷ Higher values were found in early stages of disease (without left ventricular dysfunction) and lower values in patients with left ventricular dysfunction when compared to controls.¹⁷ A possible physiologic

explanation was demonstrated by Tulppo et al.¹⁸ They studied α_1 in healthy people submitted to infusion of drugs with sympathetic or vagal effects. α_1 decreased progressively with the increase of the dose of norepinephrine continuous infusion from 0.85 ± 0.25 to 0.55 ± 0.23 ($P < 0.01$). Plasma norepinephrine levels are usually elevated in heart failure¹⁹ possibly explaining the altered short-term HR dynamics toward more random behavior. On the contrary, vagal blockade with atropine increased the value of α_1 (from 0.82 ± 0.22 to 1.24 ± 0.41 ; $P < 0.05$).^{18,20} It is possible that the vagal denervation in the initial stages of Chagas disease explains higher values in Group 1 and, as the left ventricular dysfunction occurs, the sympathetic activation could explain the reduction in the value of the index.

Long-Term Correlation Properties

The results of α_2 indices of edited series are evidences of altered fractal long-term correlation properties of HR variability in Chagas disease independently of LV function. There are no comparable results with DFA method in Chagas disease in the medical literature. Díaz et al. have published results of α_1 index in 17 Chagas disease patients with implantable defibrillators, but they analyzed only short series of internal electrograms.²¹ Our findings on long-term correlation are consistent with the hypothesis that autonomic dysfunction precedes LV dysfunction in Chagas disease.^{12,15} They indicate a breakdown of the fractal long-term correlation of normal R-R interval dynamics.¹ There is evidence that the autonomic nervous system, and in particular the baroreflex control, exerts an important role in the maintenance of this fractal behavior.¹⁸ Gomes et al. found that autonomic blockage induced a significant loss of a deterministic signature underlying the dynamics of HRV in rats.²² Other authors and we have already demonstrated altered HRV in Chagas disease independently of LV function in regard to rMSSD, pNN50, β index, and turbulence of HR.^{13,15,16,23,24}

The correlation between β index and α_2 indices of long-term fractal correlation supports the hypothesis that $1/f$ model can be a measure of fractal dynamics.²⁵ Heneghan and McDarby demonstrated with theoretical simulations and with real Holter time series that α (long-term) index can be obtained from spectral analysis. In their work, they have not used overlap windows, and they

demonstrated perfect correlation between β from 1/f spectrum and α (long-term) index from DFA method.²⁶ Peng et al. suggested this correlation previously.²⁷ These evidences could explain why we have found a strong correlation between β index from 1/f model and α_2 index from DFA (Spearman correlation 0.82) of edited series and in our previous data, among vagal time domain index and β slope.¹⁵

Limitations and Conclusions

The results of this study cannot be compared with studies that utilized different methods from those we described here. The value difference of long-term correlation in patients with Chagas disease was of lower absolute difference than the one obtained in patients with cardiomyopathies of other etiologies.² These discrepancies may indicate that patients with Chagas disease have milder HRV abnormalities than patients with other cardiomyopathies. Alternatively, these differences could be explained by methodological features, such as the younger mean age of this study sample when compared with previous studies.^{2,3} Because patients with Chagas disease with LV systolic dysfunction were, in this sample, significantly older than controls, the adjustment for this covariate is important. As suggested by Coumel et al., analysis of covariance provides a powerful tool for the comparison of HRV indexes when considering covariates such as age.²⁸

Long-term fractal correlation in edited series shows early detection of altered autonomic function in Chagas disease. Short-term fractal correlation in unedited series is strongly altered in patients with left ventricular dysfunction. The short- and long-term fractal correlations are in advantage in comparison with SDNN. In conclusion, fractal analysis of HR dynamics seems to give additional information to standard HR variability measures in assessing the abnormalities in HR behavior in Chagas disease. Patients with left ventricular dysfunction presented significant decrease of α_1 in unedited series, which is inversely correlated with the number of ventricular ectopies. Premature ventricular beats cause perturbation in the variability signal that cannot be disregarded because they contain information that can be disclosed by nonlinear methodology. Prospective studies are necessary to define the value of these indices as predictors of death in Chagas disease.

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