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MORPHOLOGIC AND FUNCTIONAL CHARACTERIZATION OF CHAGASIC HEART DISEASE IN NON-HUMAN PRIMATES

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Abstract. Chagasic heart disease has been documented in non-human primates, but noninvasive characterization of systolic and diastolic function has not been previously reported. Seventeen seropositive (12 females; mean age, 20) and 13 age- and gender-matched seronegative baboons underwent Doppler echocardiography. Systolic function indices included left ventricular (LV) fractional shortening (FS %), velocity of circumferential fiber shortening (VCF, circ/sec), LV mass index, and left and right ventricular ejection fractions (RVEF %). Diastolic function indices included transmitral E-wave, A-wave, E/A ratio, E-deceleration time, and isovolumic relaxation time. Twelve-lead electrocardiographic (ECG) recordings were obtained. There were no significant differences between groups for body size or blood pressure. Seropositive and seronegative groups revealed diffuse non-specific T wave changes precluding differentiation; however, tall "P" waves were seen in four seropositive and two seronegative baboons. Four of the 17 (24%) seropositive baboons had decreased FS (25 ± 8% versus 40 ± 5%, $P < 0.005$) and VCF (1.05 ± 0.36 circ/sec versus 1.84 ± 0.23 circ/sec, $P < 0.0001$), prolonged isovolumic relaxation time (71 ± 16 msec versus 55 ± 9 msec, $P < 0.02$), and reduced RVEF (44 ± 9% versus 54 ± 4%, $P < 0.05$), as compared with the other seropositive baboons. We conclude that chagasic heart disease is present in 24% of the naturally infected baboons in this study. ECG evidence of right atrial enlargement was more common in the seropositive animals. There were systolic and diastolic abnormalities of both ventricles. The LV systolic dysfunction may be segmental or diffuse.

INTRODUCTION

Chagas' disease is caused by the parasite *Trypanosoma cruzi,* which is usually transmitted by blood-sucking triatomine insects though it also can be transmitted by blood transfusions and organ transplantation.[1,2] The natural cycle involves the vector, wild or domestic animals that serve as a reservoir, and humans. Chagas' disease is endemic in many Latin American countries, where an estimated 16 million–18 million people are afflicted.[3] People living in mud huts in rural areas have the highest risk of infection because the bugs live in these dwellings and feed on the inhabitants at night. With the increased flow of Latin American immigrants into this country, the prevalence of Chagas' disease is likely to increase.[4,5] Infection usually occurs in childhood, and some patients may develop acute myocarditis; however, most remain asymptomatic for many years before chronic cardiac and/or gastrointestinal manifestations appear.[6,7] Dilated cardiomyopathy is the most common clinical form of the disease and affects approximately 30% of infected patients.[8]

Non-human primates infected with *T. cruzi* may develop the cardiac manifestations,[9] and acute chagasic disease in the rhesus monkey has been shown to be similar to that in humans.[10–15] Nevertheless, the pathogenesis of chronic chagasic cardiomyopathy is poorly understood, in part because no animal model that adequately mimics the spontaneously acquired form of the disease has been well developed. At the Southwest National Primate Research Center, approximately 6,000 non-human primates live in outdoor open-air caging. A subset has tested positive for antibodies against *T. cruzi*, probably transmitted through bite wounds or by consumption of infected reduviids, and has provided a unique opportunity for a comprehensive non-invasive characterization of their cardiac structure and function.

MATERIALS AND METHODS

Animal preparation. Seropositive baboons (12 females, 5 males; mean age, 20; range 18.5 to 23.7) unselected except for seropositivity status were obtained from an admixed colony of *Papio hamadryas anubis* and *P. h. cynocephalus*. The baboons were designated as seropositive on the basis of concordant results from immunofluorescence and enzyme-linked immunosorbent assays. The exact duration of infection is unknown; however, stored serum samples were examined to determine the minimum length of infection. In addition, 13 age- and gender-matched seronegative baboons served as controls. General anesthesia was achieved by immobilizing the animals with ketamine hydrochloride (10 mg/kg) and valium (5 mg) injected intramuscularly. In addition, the animals were maintained on isofluorane (1.5%) inhalation.

This was done in accordance with the Guidelines on the Care of Laboratory Animals and Their Use for Scientific Purposes. II. Pain, Analgesia, and Anesthesia.[16] The animal's chest and proximal extremities were shaved and the classic 12-lead human electrocardiogram (ECG) was obtained at a standard voltage of 1 mV and 25 mm/s. Blood pressure was obtained with an arm cuff sphygmomanometer. Care and maintenance of the baboons complied with the National Institutes of Health guidelines for the humane use of laboratory animals, and the protocol was approved by the Institutional Animal Care and Use Committee.

Two-dimensional echocardiography. The animals were placed in the left lateral decubitus position, and two-dimensional echocardiography (2-D echo) was performed with a research-dedicated platform (Aspen, Acuson Corp., Mountain View, CA) equipped with harmonic imaging (transmit 1.75 MHz/receive 3.5 MHz). The parasternal long- and short-axes, the apical 4-, 2-, and 3-chambers, and the

subcostal views were recorded on S-VHS videotapes and magnetic optical disks for offline analysis. An average of at least three cardiac cycles was obtained; all measurements were performed by the same person blinded to the serologic results.

The left ventricular (LV) dimensions were obtained at the level of the chordae tendineae; the septum and posterior wall thickness were measured at end-diastole and end-systole, and the LV mass index was calculated from validated methods in humans and baboons.[17,18] Left atrial size was measured at the maximal end-systolic dimension. Ventricular systolic indices included fractional shortening (FS, %), velocity of the circumferential fiber shortening (V_{CF} , circ/sec),[19] and right (RVEF, %) and LV ejection fraction (LVEF, %) using the Simpson's rule method.[20,21] Regional LV function was assessed by a semiquantitative visual scoring system using the 16-segment model similar to that recommended for humans by the American Society of Echocardiography.[22] Wall motion was scored using a 5-point system: normal 1, mild hypokinesis 2, severe hypokinesis 3, akinesis 4, and dyskinesis 5. Segments unable to be scored were marked as "X" and were not counted.

Doppler analysis. Indices of diastolic function were obtained with the pulsed Doppler sample volume at the tip of the mitral leaflets. Peak E-wave, A-wave, E/A ratio, deceleration time and isovolumic relaxation time—measured from the aortic valve closure spike to the beginning of mitral inflow—were obtained. Three mitral inflow patterns were identified following criteria similar to those described in humans: *normal* or *pseudonormal* (E/A ratio > 1.0, E-wave deceleration time 150–220 msec), *abnormal relaxation* (E/A ratio < 1.0, E-wave deceleration time > 220 msec), and *restrictive physiology* (E/A ratio > 2.0, E-wave deceleration time < 150 msec, with a small or absent A-wave).

Pulmonary vein flow was imaged from the apical-4 chamber view with slight superior angulation to obtain a view where part of the aortic valve was visualized. The sample volume was placed in the right upper pulmonary vein 1 cm beyond its left atrial drainage. Pulmonary spectral velocities were measured with and without contrast enhancement.

Spectral Doppler of tricuspid regurgitation, if present, was attained with and without contrast, and the systolic pulmonary artery pressure calculated.[23] Color-flow Doppler from standard apical views was used to assess mitral, aortic, and tricuspid valve regurgitation.

To improve the 2-D echo and Doppler spatial resolution, commercially available intravenous contrast microspheres (Optison® St. Louis, MO) were given via a bolus of 0.5 mL. By enhancing the endocardial border delineation and the Doppler signal-to-noise ratio, 2-D echo and Doppler measurements can be significantly simplified.

Statistical analysis. SPSS statistical software (SPSS, Inc., Chicago) was used for analysis. Data were expressed as mean ±SD. Comparison among multiple groups was performed by analysis of variance with the Sheffé post hoc test. Categorical variables were compared using χ^2 or Fisher's exact test, as appropriate. Univariate relations were assessed by Pearson's correlation coefficients. Independent correlates of continuous measures were identified by multiple linear regression analysis using a stepwise forward procedure with assessment of collinearity diagnostics. A two-tailed $P < 0.05$ was considered significant.

RESULTS

All 30 animals tolerated the anesthesia well and recovered to baseline conditions. Contrast injections improved spatial resolution of the 2-D echo and enhanced Doppler signals in 25% of the animals. 2-D echo evidence of myocardial dysfunction was found in four animals; thus, they were considered cases of chagasic cardiomyopathy. No control animals had segmental wall motion abnormalities. Table 1 shows that the baseline characteristics were similar between groups.

Standard ECG measurements including heart rate, PR interval, and QRS and QT durations were not significantly different between groups. There were no significant ST-segment elevations or depressions. However, most animals in each group showed flattening or inverted T waves in leads II, III, and aVF, some also in leads V4 –V6, and some as a diffuse pattern in all leads. Ten seropositive (59%) and six seronegative (46%) animals had Q-waves across the precordial V_1–V_3 leads. One seropositive animal had a second-degree (Wenckebach) AV block, and two seronegative animals had one premature atrial contraction in their tracing; however, there was no ventricular ectopy. Four seropositive (24%) and two (15%) seronegative animals had tall P waves. Diffuse low voltage was noted in five seropositive (29%) and three seronegative (23%) baboons. Figure 1 illustrates diffuse low voltage despite a standard of 1.0 mV, tall P waves in lead II, Q waves in V_1 and V_2, and diffuse T wave inversion.

When we specifically analyzed the ECG findings in the four animals with echocardiographic evidence of Chagas' heart disease, there were no unique findings differentiating them from the other seropositive animals.

Table 2 shows the echocardiographic features of the two groups. Four of the 17 (24%) seropositive baboons had evidence of wall motion abnormalities, two in the form of regional wall motion abnormalities along the anterior and septal regions and two as global hypokinesis. Relevant findings in these four animals included decreased FS (25 ± 8%), which was significantly lower than that of the other seropositive (40 ± 5%) and the seronegative (35 ± 8%) animals, $P < 0.05$; decreased V_{CF} (1.05 ± 0.36 circ/sec) compared with the other seropositive (1.84 ± 0.23 circ/sec) and the seronegative (1.51 ± 0.33 circ/sec) animals, $P < 0.05$; and reduced RVEF (44 ± 9%) compared with the other seropositive (54 ± 4%) animals, $P < 0.05$.

Relevant Doppler findings in the four seropositive animals with wall motion abnormalities included a prolongation of the

TABLE 1
Clinical characteristics of the study population

	Seropositives (n = 17)	Seronegatives (n = 13)	*P*
Age (yrs)	20 ± 2	20 ± 2	ns
% male	29	31	ns
Weight (kg)	23 ± 5	22 ± 7	ns
Height (cm)	94 ± 8	95 ± 7	ns
CRL (cm)	66 ± 4	65 ± 4	ns
BMI (kg/m^2)	24 ± 4	20 ± 3	0.05
BSA (m^2)	4.8 ± 1.3	4.2 ± 1.3	ns
SBP (mm of Hg)	98 ± 10	93 ± 8	ns
DBP (mm of Hg)	47 ± 11	45 ± 9	ns
Heart rate (bpm)	105 ± 17	106 ± 20	ns

CRL = crown rump length, BMI = body mass index, BSA = body surface area, SBP = systolic blood pressure, DBP = diastolic blood pressure.

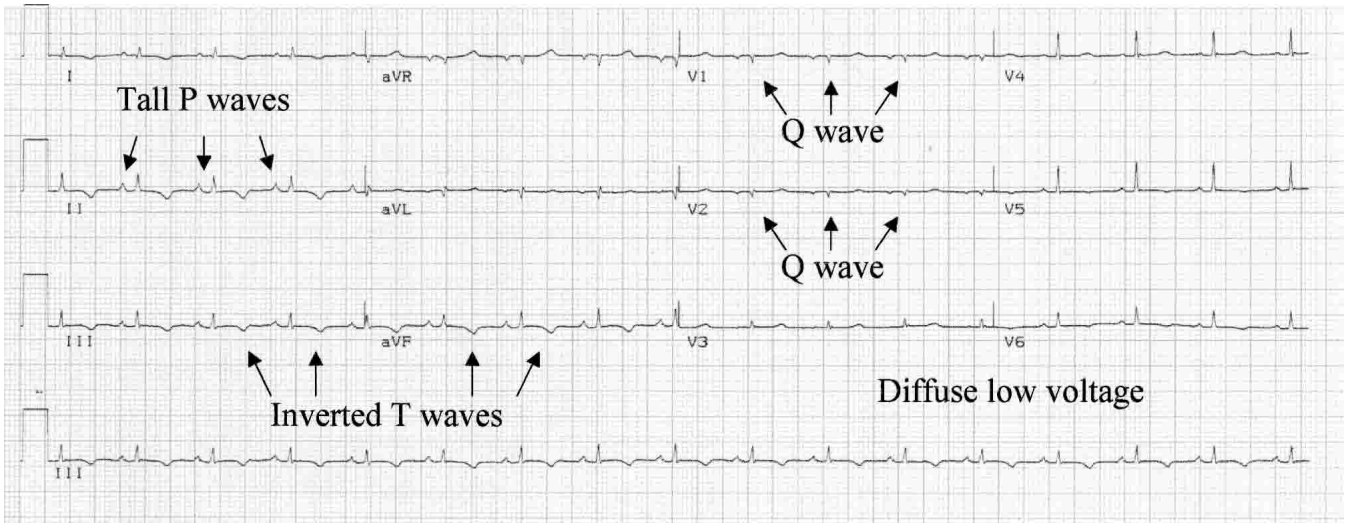

FIGURE 1. Representative 12-lead electrocardiogram illustrating diffuse low voltage despite standardization at 1 mV. Tall P waves are noted in lead II; inverted T waves in leads I, II, III, aVF, V_5 and V_6; and Q waves in leads V_1 and V_2.

isovolumic relaxation time (71 ± 16 msec) compared with that of the other seropositive (55 ± 9 msec) and the seronegative (55 ± 8 msec, $P < 0.05$) animals. All control animals had a normal mitral inflow pattern whereas 20% of the seropositive animals had an impaired relaxation pattern suggesting decreased myocardial compliance. There were no instances of restrictive physiology pattern in either group.

DISCUSSION

This is the first comprehensive non-invasive assessment of cardiac function in non-human primates with naturally acquired Chagas' disease. Our data indicate that 24% of baboons infected with *T. cruzi* developed echocardiographic evidence of Chagasic heart disease involving both the left and right ventricles in the form of systolic and diastolic dysfunction. This was illustrated by the presence of regional and global LV hypokinesis as well as reduced LV systolic VCF indices such as the FS and VCF. In addition, the RVEF also was reduced, indicating a global myocardial involvement. Using equilibrium radionuclide angiography, Marin-Neto et al have documented that right ventricular involvement may be the only detectable cardiac disorder in patients with chronic chagasic disease who have no other clinical signs of heart disease.[24] The segmental wall motion abnormalities noted in this study were indistinguishable from those seen in humans with ischemic heart disease. Prolongation of the isovolumic relaxation time coupled with an abnormal relaxation pattern detected on the mitral inflow is evidence of diastolic dysfunction characterized by decreased myocardial compliance. It is possible, although not proven, that some degree of myocardial fibrosis explains the reduction of these animals' elastic properties.

TABLE 2
Echocardiographic features of the study population

	Seropositives (n = 17)	Seronegatives (n = 13)	*P*
Aorta (cm)	1.8 ± 0.2	1.9 ± 0.4	ns
Left atrium (cm)	2.4 ± 0.3	2.3 ± 0.4	ns
Mitral E-S separation (cm)	0.39 ± 0.23	0.38 ± 0.15	ns
Septal thickness (mm)	6.6 ± 0.9	6.5 ± 1.0	ns
LV diastolic dimension (mm)	32 ± 4	32 ± 6	ns
Posterior wall thickness (mm)	6.9 ± 1.3	6.7 ± 1.4	ns
LV Mass (g)	53 ± 20	55 ± 25	ns
LV Mass/height	51 ± 14	54 ± 20	ns
Relative wall thickness	0.44 ± 0.07	0.42 ± 0.09	ns
Fractional shortening (%)	37 ± 8	35 ± 8	ns
VCF (circ/sec)	1.67 ± 0.42	1.51 ± 0.33	ns
LV ejection fraction (%)	54 ± 6	52 ± 6	ns
RV ejection fraction (%)	52 ± 7	51 ± 5	ns
Cardiac output (l/min)	2.0 ± 0.4	1.9 ± 0.5	ns
Stroke volume (cc)	19 ± 5	19 ± 7	ns
PSWS ($\times 10^3$ dynes/cm^2)	134 ± 22	141 ± 29	ns
ESS ($\times 10^3$ dynes/cm^2)	39 ± 9	51 ± 16	0.01
Midwall fractional shortening (%)	18.5 ± 0.3	18.1 ± 0.6	0.02

E-S = mitral valve e point-septal, LV = left ventricular, VCF = velocity of circumferential fiber shortening, PSWS = peak systolic wall stress, ESS = end systolic wall stress.

Non-human primates have been used as models for Chagas' disease for many years. As observed in humans, there is a wide variety of outcomes in the infected animals. The acute disease has been extensively studied in rhesus monkeys and found to be very similar to that seen in humans.[10,12,15] Histopathologic changes in chagasic myocarditis include edema, inflammatory cell infiltration, and destruction of the myocardial fibers. In the more-advanced stages, myocardial fibrosis takes place either diffusely or in patches. Fibrotic changes and chronic inflammation also can be seen in the conduction system. The pathogenesis of chronic Chagas' disease has been the subject of considerable controversy, and the timing of disease development raises a complex issue. Since this study was cross-sectional rather than longitudinal, the timing of the development of systolic versus diastolic abnormalities is unknown.

Several ECG abnormalities have been described in monkeys with Chagas' disease.[15,25,26] Standard measurements such as PR interval, QRS axis, and QRS and QT durations were similar in seropositive and seronegative animals. Q-waves in the left precordial leads were observed in about half of the seropositive and seronegative baboons. In humans, this finding is commonly associated with septal infarction or pro-

nounced LV hypertrophy with a clockwise rotation; however, no echocardiographic evidence supported either of these possibilities. Therefore, the initial Q-wave deflection commonly seen probably represents lead placement. There were no significant ST-segment elevations or depressions among animals in the two groups. Most animals in both groups showed flattening or inversion of the T waves in leads II, III, aVF, as well as in leads V4 –V6. One seropositive animal had a second-degree (Wenckebach) AV block, and two seronegative animals had one premature atrial contraction in their tracings; however, there was no evidence of ventricular ectopy. Prominent P waves consistent with right atrial enlargement were observed in 24% of seropositive and 15% of seronegative animals. This non-specific finding has been previously described by Falasca et al.[25]

Limitations. Because these animals became infected naturally, rather than experimentally, the exact duration of infection is not known. However, all but two of the animals are known to have been infected for at least 13 years and perhaps as long as 24 years. Chagasic heart disease was presumed in these animals in view of the positive serum complement fixation and indirect hemagglutinin tests, and the presence of segmental wall motion abnormalities on 2-D echo. However, since myocardial biopsies were not performed, pathologic confirmation of chagasic heart disease is lacking.

Conclusions. Our data indicate that 24% of naturally infected baboons develop echocardiographic evidence of chagasic heart disease involving both the left and right ventricles in the form of systolic and diastolic dysfunction. Thus, comprehensive Doppler echocardiography with the help of contrast enhancement is the non-invasive method of choice for assessment of chagasic heart disease not only in humans but also in non-human primates.

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