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Chronic Chagas cardiopathy in Chile. Importance of *Trypanosoma cruzi* burden and clinical evaluation

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ABSTRACT

Currently there are no biological markers to indicate which individuals with chronic indeterminate period of Chagas disease develop heart disease and who will remain all his life in this phase. The aim of this survey was to determine if *Trypanosoma cruzi* burden is related to the presence of heart disease in patients with chronic Chagas disease. 200 patients who had not been treated, 100 with cardiopathy and 100 without, groups A and B respectively, were submitted to clinical study and electrocardiogram, Echo-Doppler was performed for group A in which all important known causes of cardiopathy were discarded. In both groups xenodiagnosis, conventional PCR and quantitative PCR were undertaken. The 100 cardiopaths had 133 electrocardiographic alterations most of them in grade II of the New York Heart Association classification. 98 cardiopaths were classified in grade I by Echo-Doppler and only 2 cases were in grade III due to low ejection fraction. The difference in average parasitemia in patients of group A and B was not significant and no statistically differences were observed between average parasitemia of cardiopaths grade II versus grade I of NYHA. This results allow to characterize same clinical, electrocardiographical and parasitological features in chagasic cardiopaths of Chile.

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1. Introduction

Chagas disease (ChD) is caused by *Trypanosoma cruzi* a zoonotic parasite that affects about 6–7 million people in the American continent (WHO, 2015) causing approximately 12000 deaths per year. The disease has been introduced to Europe, Asia and Oceania through human migration. Only in the United States of America where the disease is not autochthonous there are about 300.000 people with ChD (Bern and Montgomery, 2009). Up to date there are 80–100 million persons at risk to acquire the disease (Salvatella, 2012) ChD is one of the most significant neglected disease despite being the fourth disease in importance in years lost due to incapacity, surpassed only by diarrheas, bronchopulmonary disease and AIDS (Tarleton and Curran, 2012; Apt et al., 2013).

Two periods characterize the natural evolution of the disease, acute and chronic, the latter with persistent infection in the heart and adipose tissue (Wen et al., 2014) may be latent indeterminate or determinate (Rassi et al., 2010; Coura and Viñas, 2010). Fifty to sixty percent of chronic cases have a latent or indeterminate stage without clinical symptoms and with normal routine clinical laboratory tests, 10–30% of them after 10–20 years develop cardiac disease and 8–10% digestive commitment. Chronic Chagas cardiomyopathy (CCC) is the worst form of the disease, due to its high morbidity and mortality (Rassi et al., 2010). Chronic chagasic cardiopathies with heart failure have worse prognosis than other chronic cardiopathies with heart insufficiency (Silva et al., 2008). Patients with CCC present a high frequency of strokes compared to a non Chagas cohort (Da Matta et al., 2012). According to simulated computer models, the annual cost of Chagas cardiomyopathy including both health costs and disability adjusted annual losses, exceeds that which is originated by cervical uterine cancer and rotavirus (Lee et al., 2013).

About 50–60% of the people with the indeterminate phase, remain in this asymptomatic state for life. In Brazil and Chile 1–2% of the patients with latent indeterminate chronic ChD develop cardiopathy each year. It is very important to know which infected persons will develop heart disease and who will not, to apply etiologic therapy to the former and not all the patients with inde-

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terminate chronic ChD. Today there are no biological markers available to answer this questions (Apt et al., 2015).

To determine whether the parasite burden is related to the presence of heart disease in patients with chronic ChD, we performed this study aimed to investigate the parasite burden in chagasic cardiopaths versus chagasic persons without cardiac involvement according to the New York Heart Association Classification (NYHA)

2. Material and methods

2.1. Population

Rural and urban adult patients with Chagas disease who had not been treated from the provinces of Choapa and Limarí, endemic area located between 29° 02′and 32° 16′ South latitude were examined twice a year in outpatients clinics (rural patients) and hospitals (urban patients) by our investigation group. They were submitted to anamnesis, physical examination and ECG of twelve leads. The patients were divided in two groups according to whether or not they presented ECG alterations. 100 persons with chronic chagasic cardiomyopathy were randomly selected (Group A), most of them were from Combarbalá (40%) and Illapel (34%) and lived in rural zones and 100 without cardiomyopathy (Group B), most of these patients were from Salamanca (31%) and Illapel; 60% live in rural zones. Echo-Doppler was performed to patients of Group A to eliminate other important causes of cardiomyopathy, hypertension, valve disease, atherosclerosis, idiopathic cardiomyopathy and congenital malformations. Xenodiagnosis (XD), conventional PCR (cPCR) and quantitative PCR (qPCR) were performed on patients of both groups.

2.2. Ethics statement

The patients participated under Informed Consent approved by the Ethical Committee of the Faculty of Medicine of the University of Chile (Protocol 048-11). Informed Consent from patients was given in written form.

2.3. Conventional serology

IFI and ELISA tests were performed to the 200 individuals. In the IFI test epimastigote forms of *T. cruzi* Tulahuen strain, were used as antigens. They were cultured in Diamond medium supplemented with 5% fetal bovine serum maintained at 28 °C. Parasites were collected in the exponential growth phase by centrifugation at 1300*g* per 10 min at 4 °C (Maya et al., 2007). A test of 1/20 dilution was considered positive. In each determination positive and negative control from patients with and without Chagas disease were included. Chagatek ELISA (Biomerieux, France) was applied. The optical density corresponding to the cutoff value was determined by the average of the negative control plus 0.100. The ELISA OD values for the negative controls fluctuated between 0.001 and 0.09. The plate was read in a spectrophotometer PHOMO of Autobio, by indications of Micro-Elisa system.

2.4. Xenodiagnosis

In our laboratory the colony of *Triatoma infestans* used in xenodiagnosis (XD) has been maintained for more them 50 year fed on chickens, which are refractory to *T. cruzi* (Schenone, 1999) XD was applied using two cylindrical wooden boxes each containing seven uninfected third or fourth instar nymphs of *T. infestans* starved for a period of 3–4 weeks. The insects of the cages were fed by patients (during 20 min) and then maintained at 27 °C and 70% relative humidity without further feeding. The rectal contents of triatomines feed on each patients were obtained by slight abdominal compression in a biology secure hood for examination under an optical microscope at 400×, after 30, 60 and 90 days of incubation. 100 fields were observed to detect mobile trypomastigotes or epimastigotes of *T. cruzi*. A negative XD was the absence of mobile forms of *T. cruzi* in the three periods of microscopic observation (30, 60 and 90 days) (Saavedra et al., 2013).

2.5. Electrocardiographic tracing

The patients were evaluated by a twelve-lead electrocardiogram. Each electrocardiographic trace included the following parameters: P axis, P duration, P-R space, R–R space, R space, QT value, QTc calculation, QRS axis, T axis, ventricular gradients, RV1 intrensicoide deflexion, SV1, RVS, Sokolow index, and an electrocardiographic diagnosis. The final interpretation of this test data was performed by a specialist cardiologist following the double blind protocol recommended by the World Health Organization; the investigator analyzing the ECG traces was unaware of the status of the patients (Maguire et al., 1982).

2.6. Echo-Doppler

The Echo-Doppler was performed in Baquedano Square Medical Image Centre of Santiago, Metropolitan Region, Chile, with a latest generation Philips apparatus. The following parameters were measured with bi-dimensional M mode: systolic diameter of left ventricle, diastolic diameter of left ventricle, septum of left ventricle, posterior wall of left ventricle, left auricular size, hypertrophic sign of left ventricle, mass of left ventricle, and ejection fraction of left ventricle. The Doppler measurements allowed the determination of the status of the different valves and the presence of reflux. The final interpretation of this test was performed by a cardiologist specialist in echography.

2.7. DNA extraction

Five milliliters of venous blood of each patient was mixed with the same volume of a 6 M guanidine hydrochloride 0.2 M EDTA pH 8.0 solution, incubated at 98 °C for 15 min to nick DNA of *T. cruzi* minicircles and stored at 4 °C. DNA extraction was performed in 200 μl of the samples mixture, using the QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The purified DNAwas maintained at −20 °C until use.

2.8. Conventional PCR

Conventional PCR (cPCR) was performed in triplicate using oligonucleotides 121 and 122, which anneal to the four conserved regions present in *T. cruzi* minicircles (Degrave et al., 1988), to obtain 330 bp amplicons. Each sample was tested in a final volume of 20 μl including 5 μl of extracted DNA. The final concentrations of the reagents were as follows: 2.5 mMMgCl2, 0.2 mM of each dNTP, 0.5 μMof each primer, and 1 unit GoTaq DNA polymerase (Promega Corp., USA). The amplification program was performed in a TC- 412 thermal cycler (Techne, UK) which included an initial denaturation at 98 °C for 1 min and 64 °C for 2 min; 33 cycles of 94 °C for 1 min, 64 °C for 1 min and 72 °C for 1 min, and a final extension at 72 °C for 10 min. Each experiment included two negative PCR controls: water instead of DNA and DNA of non-chagasic patients. As positive control purified DNA of *T. cruzi* Tulahuén strain was used. Amplification products were analyzed by electrophoresis in a 2% agarose gel and visualized after staining with RedGel (Biotium Inc.). Five microliter Bench Top 100 bp DNA ladder (Promega Corp.,USA) was

incorporated. A positive result for cPCR was the presence of a 330 bp band specific for *T. cruzi* minicircles.

2.9. *Quantitative PCR for* Trypanosoma cruzi *(qPCR)*

The TaqMan® detection system was applied in a Stratagene MX3000P™ thermocycler (Agilent Technologies) under conditions suggested by the manufacturer and using primers of DNA satellite Cruzi 1, Cruzi 2, and Cruzi 3 (Schijman et al., 2011). The reaction mixture consisted of 2 μl of the sample to be investigated, 10 μl Brilliant Multiplex QPCR Master mix (Stratagene), 0.5 μl of a 1:500 dilution of a reference dye (ROX), 0.5 μl each of Cruzi 1, and Cruzi 2, 0.2 μl Cruzi 3, 0.2 μl BSA (100x) and 6.1 μl Molecular Biology GradeWater (Mo Bio Laboratories Inc.) in a final volume of 20 μl. To obtain a standard curve to perform the quantification, we used a stock of epimastigote forms of *T. cruzi*, Tulahuen strain. The total DNA quantification was carried out using AccueBlue™ High Sensitivity dsDNA Quantitation kit (Biotum Inc.) and the qPCR instrument Mx3000 P ™ (Stratagene, Agilent Technologies Inc.) as detector devices according to Bravo et al. (2012). A *T. cruzi* DNA concentration equivalent to 1×10^6 epimastigotes/ml was adjusted, considering that 1 parasite cell contains 200 fg of DNA (Duffy et al., 2009). The DNA was diluted 1:10. The standard curve of *T. cruzi* was maintained at −20 °C until use. The controls used for qPCR-*T. cruzi* were as follows: negative control, DNA of a non-chagasic patient confirmed by serology (IIF and ELISA), evaluated previously with qPCR equipment and positive controls, DNA of an individual with ChD with confirmed parasitemia by PCR and evaluated previously in qPCR equipment. Control mixture: 20 μl of mixture reaction for *T. cruzi* (without the sample under study). Water control: 2 μl of water free of nucleases (Mo Bio Laboratories Inc.) (replacing the study sample). The control mixture and water control are useful to evaluate contamination in the preparation of the mixture reaction or unspecific qPCR reactions. Each point of the standard curve was performed in triplicate. The samples and controls were included in duplicate. As an internal control of extraction and inhibition of qPCR, we used chromosome 12 (X12) (Bravo et al., 2012). X12 primers were designed by N. Jullien using the AmplifX v.1.5.4 software, and compared with Nucleotide BLAST (National Library of Medicine) to discount any other unspecific amplification (N. Nazal, personal communication). The N1 × 12 forward (5′-GCTGGCTAGACTGTCAT-3′) and N2 × 12 reverse (5′-CTTTGCCGTTGAAGCTTG-3′) and N3 × 12 probe (N3 × 12 5′-/56-FAM/TGGGACTTC/ZEN/AGAGTAGGCAGATCG/3IABkFQ/-3′) primers were used. The standard curve for X12 was prepared with a pool of human genomic DNA of five non-chagasic individuals diluted 1:5 in elution buffer. The reaction mixture was composed of 10 μl Brilliant Multiplex QPCR Master Mix, 0.5 μl of a 1:500 solution of Reference Dye (ROX), 2 μl each N1/N2, 0.8 μl N3, 0.2 μl BSA (100x), 2.5 μl Molecular Biology Grade water (Mo Bio Laboratories, Inc.), and 2 μl of DNA isolate in a final reaction volume of 20 μl. The thermals profiles of qPCR-*T. cruzi* and qPCR-X12 included 10 min of preincubation at 95 °C and 40 amplification cycles (95 °C for 15 s, 60 °C for 1 min). The measurement of emitted fluorescence was performed at 60 °C at the end of each cycle. The MxPro v 4.1 (Agilent Technologies) software delivered automatically the parasites/ml data.

2.10. *Statistical analysis*

The data were analyzed using the SPSS program version 19.0. The description of the data was performed by tables, arithmetic mean, mode, standard deviation, and amplitude of the variable. Chi square and Z test were applied for group comparison of qualitative variables. In the case of quantitative variables, Levene tests were applied to evaluate the homoscedasticity and Student's *t*-test to evaluate the average burden. To compare the distribution of the positive cases by age Kolmogorov-Smirnov (KS) unilateral test was applied. A significance level of 0.05 was used.

3. Results

In group A 68% of the patients were women. Ages of this individuals ranged from 25 to 81 years, average 56.4. In group B the average age was 50.5 ranging from 20 to 77 years, 79% were women. The patients of both groups were examined in the patient clinics and hospitals where they live between 2011 and 2015. The average age of Group A is significantly greater that of group B ($p = 0.0012$). IIF IgG titers fluctuated between 1/40-1/1280 and the OD of ELISA was 0.324–2575. The titers of IIF tests and the OD values of ELISA had brood agreement for patients with strong and weak serological responses which agree with previous studies (Umezawa et al., 1996; Luquetti et al., 2009) (Tables 1–2). Of the 200 patients with positive cPCR included in this study 145 (72.5%) had positive qPCR.

In the individuals with cardiopathy (group A) qPCR was positive in 73 cases (73%) nevertheless XD was positive only in 13 cases (13%). In group B qPCR was positive in 72 cases (72%) and XD was positive in 15 cases (15%). The Ct (threshold cycle) average and its relation with parasite burden equivalent was described by our group previously (Apt et al., 2015). In group A the parasite burden fluctuated between 7.9–0.009 parasite equivalents per milliliter (par.eq./mL) with an average of 0.39 and a median of 0.025. In group B the parasite burden fluctuated between 4.56–0.006 par. eq./mL, average 0.25 with a median of 0.03. The average levels of parasites did not differ significantly between patients of groups A and B ($p = 0.8175$). In group A no differences were observed in parasite burden between cardiopaths of sub groups II and I of NYHA classification ($p = 0.3601$).

The 100 cardiopaths had 133 electrocardiographic alterations (Table 3). The group A was divided in two subgroups according to NYHA classification. In sub group I, the 44 patients showed electrocardiographic alterations of grade I NYHA. They were 39 cases, 17 men and 22 women aged, 27–81 years who showed since bradycardia from 41 to 58 pm in the ECG tracing. In five other women aged 36–59 right bundle branch block incompletely blocked was observed. In sub group II 14 men and 42 women had more than one electrocardiographic alteration. In 12 cases, nine women and three men aged 38–79 years had sinus bradycardia ranged. Three of these women had first degree A-V block and prolonged QTc interval associated with bradycardia in two and left ventricle hypertrophy in the other. One women had left anterior hemiblock, another had bradycardia in association with prolonged QTc interval. One women had repolarization alteration associated to bradycardia another left ventricular hypertrophy associated to bradycardia and the last two showed ischemia in association with bradycardia. Two men had first degree A-V block associated to bradycardia another one prolonged QTc interval associated to bradycardia. Prolonged QTc time appeared in 19 cases (15 women aged 30–79 years) and 4 men aged 38–49 years). This was and isolated finding in four cases in the other 14 it was associated.

Seventeen cases 13 women and 4 men aged 49–77 years showed left anterior hemiblock, alone in six cases, three in association to prolonged QTc interval, two with left ventricle hypertrophy, one with ventricular extrasystole incomplete right bundle branch block in association with prolonged QTc interval, one with sinus bradycardia, one with auricular extrasystole and myocardial ischemia, two cases with complete right bundle branch block one of them associated to prolonged QTc interval, and the last one was associated to first degree A-V block.

First A-V block was present in 9 patients, isolated in four and the other five were associated, two with sinus bradycar-

Table 1
Clinical, epidemiological, serological, parasitological, comorbidity, electrocardiographical, and Echo-Doppler characteristics of 100 chronic chagasic cardiopaths. Group A.

No.	Age	Sex	Locality	Origin		Control date	Serology		qPCR	Xenodiagnosis	ECG	NYHA group	Comorbidity
				Urban	Rural		IIF-IgG	ELISA D.O.	par. eq./ml				
1	55	M	Combarbalá		x	7/6/2010	(+) 1/1280	2507	0.54	(+)	SB	I by ECG	Cholecystectomy
2	56	F	Combarbalá		x	7/6/2010	(+) 1/640	1568	0.4	(+)	IRBBB	I by ECG	
3	59	M	Salamanca		x	1/13/2011	(+) 1/640	1413	0	(−)	SB	I by ECG	
4	52	F	Combarbalá		x	7/1/2012	(+) 1/1280	1831	0	(−)	SB	I by ECG	Histerectomy
5	68	M	Combarbalá		x	7/1/2012	(+) 1/1280	1734	0.49	(+)	SB	I by ECG o III by EF *	Hypertension
6	53	M	Salamanca		x	4/1/2013	(+) 1/160	0.485	0.26	(+)	SB	I by ECG	
7	57	M	Combarbalá	x		7/1/2012	(+) 1/320	1641	0.3	(−)	SB	I by ECG	
8	53	F	Combarbalá		x	7/1/2012	(+) 1/640	1986	0	(−)	SB	I by ECG	Bronchial asthma
9	81	F	Illapel		x	7/1/2012	(+) 1/320	1121	0	(−)	SB	I by ECG	Hypertension, prediabetes, hypercholesterolemia, compensated congestive heart failure
10	60	F	Illapel		x	4/1/2013	(+) 1/1208	2327	0.51	(−)	SB	I by ECG	
11	56	M	Illapel	x		8/1/2013	(+) 1/160	0.784	0.06	(−)	SB	I by ECG	Hypercholesterolemia
12	55	F	Combarbalá		x	6/6/2013	(+) 1/640	1541	0.009	(−)	SB	I by ECG	Hypertension, hypothyroidism, hypercholesterolemia
13	59	M	Combarbalá		x	8/1/2013	(+) 1/640	1871	0.13	(+)	SB	I by ECG	
14	71	M	Combarbalá		x	8/1/2013	(+) 1/320	1315	0.04	(+)	SB	I by ECG	Hypertension
15	72	F	Combarbalá		x	8/1/2013	(+) 1/640	1754	0.02	(−)	SB	I by ECG	Hypertension, hypercholesterolemia
16	38	M	Combarbalá		x	6/6/2013	(+) 1/320	1.06	0.02	(+)	SB	I by ECG	Prediabetes
17	46	M	Salamanca	x		11/1/2013	(+) 1/1280	1483	0.02	(+)	IRBBB	I by ECG	Diabetes mellitus, lumbar hernia
18	60	M	Illapel	x		7/1/2012	(+) 1/1280	1.51	0.02	(−)	IRBBB	I by ECG	
19	27	F	Salamanca		x	11/1/2013	(+) 1/160	0.328	0.02	(−)	SB	I by ECG	
20	65	M	Illapel	x		7/1/2014	(+) 1/640	1116	0.07	(−)	SB	I by ECG	
21	25	F	Illapel		x	7/1/2014	(+) 1/640	0.734	0.04	(−)	SB	I by ECG	
22	62	F	Illapel		x	7/1/2014	(+)1/160	0.417	0.02	(−)	SB	I by ECG	Hypertension, pancreatitis, cholecystectomy, hypercholesterolemia
23	41	M	Illapel		x	7/1/2014	(+) 1/160	0.405	0	(−)	SB	I by ECG	
24	36	F	Salamanca		x	7/1/2014	(+)1/640	1122	0.03	(−)	SB	I by ECG	
25	35	F	Other	x		8/21/2013	(+) 1/80	0.447	0	(−)	SB	I by ECG	
26	57	F	Salamanca	x		7/1/2014	(+)1/1280	1651	7.9	(−)	SB	I by ECG	
27	36	F	Combarbalá		x	10/1/2014	(+)1/640	1357	0.02	(−)	IRBBB	I by ECG	
28	53	F	Illapel	x		5/1/2014	(+)1/80	1716	0	(−)	SB	I by ECG	Hypothyroidism, hypercholesterolemia

29	70	M	Illapel		x	12/1/2014	(+)1/640	1533	0	(−)	SB	I by ECG	
30	49	F	Illapel	x		12/1/2014	(+)1/640	1677	0.1	(−)	SB	I by ECG	
31	59	M	Illapel	x		12/1/2014	(+) 1/1280	1475	0.14	(+)	SB	I by ECG	Gastritis
32	67	F	Illapel	x		10/1/2014	(+)1/1280	1613	0	(−)	SB	I by ECG	Hypertension, cholecystectomy
33	78	F	Illapel	x		10/1/2014	(+)1/1280	1554	0.01	(−)	SB	I by ECG	Hypertension
34	62	F	Salamanca	x		10/1/2014	(+)1/160	0.384	0.01	(−)	SB	I by ECG	
35	59	M	Salamanca		x	12/1/2014	(+)1/640	1406	0	(−)	SB	I by ECG	Hypertension
36	45	F	Salamanca	x		7/1/2014	(+)1/1280	1280	0.01	(−)	SB	I by ECG	Hypertension, prediabetes, hypercholesterolemia
37	45	F	Salamanca		x	10/1/2014	(+)1/1280	1671	0.01	(−)	SB	I by ECG	Hypertension
38	69	F	Salamanca		x	6/1/2015	(+) 1/1280	1548	0	(−)	IRBBB	I by ECG	Hypertension
39	46	F	Salamanca	x		12/1/2014	(+)1/1280	1352	0	(−)	SB	I by ECG	
40	61	F	Illapel		x	6/1/2015	(+) 1/1280	1752	0	(−)	SB	I by ECG	
41	46	M	Illapel		x	6/1/2015	(+) 1/1280	2004	0	(−)	SB	I by ECG	
42	29	F	Combarbalá	x		10/1/2015	(+)1/1280	1589	0	(−)	SB	I by ECG o III by EF *	
43	71	M	Salamanca		x	10/1/2015	(+)1/1280	1628	0	(−)	SB	I by ECG	Hypertension
44	43	F	Salamanca		x	10/1/2015	(+)1/640	1243	0	(−)	SB	I by ECG	Cholecystectomy, prediabetes
45	73	F	Salamanca		x	6/1/2009	(+) 1/640	1628	0.37	(−)	LVH, LAHB	II by ECG	Hypertension, depression, diabetes mellitus, hypercholesterolemia, hypothyroidism
46	49	M	Illapel		x	7/6/2010	(+) 1/640	1905	0.17	(−)	QTc↑, VE, LAHB, IRBBB	II by ECG	Hypertriglyceridemia
47	60	M	Salamanca	x		1/10/2011	(+) 1/640	1974	0	(−)	LAHB	II by ECG	Hypercholesterolemia
48	54	F	Combarbalá	x		8/1/2011	(+) 1/40	0.544	0.4	(−)	QTc↑, LAHB	II by ECG	Hypertension, hypercholesterolemia, prediabetes
49	47	F	Combarbalá		x	8/1/2011	(+) 1/640	1313	2.38	(−)	QTc↑	II by ECG	Hypertension, cholecystectomy, depression, gastritis
50	60	F	Combarbalá		x	8/1/2011	(+) 1/640	1356	0.34	(−)	QTc↑, LAHB	II by ECG	Gastritis, depression
51	70	M	Combarbalá	x		8/1/2011	(+) 1/1280	2059	0.39	(−)	CRBBB	II by ECG	Hypertension, artrosis
52	43	F	Combarbalá		x	8/27/2011	(+) 1/1280	2176	0.38	(−)	LPHB, CRBBB	II by ECG	
53	48	M	Combarbalá		x	1/19/2011	(+) 1/1280	2022	0.53	(−)	SB, LVH	II by ECG	Esophagus achalasia
54	69	F	Combarbalá		x	8/1/2011	(+) 1/640	1403	0.49	(−)	LAHB	II by ECG	Hypertension

Table 1 (*Continued*)

No.	Age	Sex	Locality	Origin		Control date	Serology		qPCR	Xenodiagnosis	ECG	NYHA group	Comorbidity
				Urban	Rural		IIF-IgG	ELISA D.O.	par. eq./ml				
55	71	F	Illapel	x		9/1/2011	(+) 1/640	1225	0.8	(−)	LAHB, SB	II by ECG	Hypertension, hypercholesterolemia
56	77	F	Illapel	x		9/1/2011	(+) 1/640	2099	0.44	(−)	AE, LAHB, Lateral ischemia	II by ECG	Hypertension, prediabetes, cholecystectomy
57	55	M	Combarbalá		x	7/1/2012	(+) 1/1280	1987	0.05	(+)	I°st AV block	II by ECG	
58	50	F	Combarbalá		x	7/1/2012	(+) 1/1280	1719	0.57	(−)	QTc↑, SB, I°st AV block	II by ECG	
59	79	F	Combarbalá		x	7/1/2012	(+) 1/640	1837	0.1	(−)	SB, QTc↑, I°st AV block	II by ECG	Cholecystectomy
60	77	F	Combarbalá		x	7/1/2012	(+) 1/640	1589	0	(−)	QTc↑, SB, I°st AV block, LVH	II by ECG	Hypertension, cholecystectomy, histerectomy
61	60	F	Illapel	x		6/23/2011	(+) 1/1280	1974	0.38	(−)	SB, Anterior ischemia	II by ECG	Scoliosis, depression
62	53	F	Illapel	x		7/1/2012	(+) 1/1280	2037	0	(−)	QTc↑, Repolarization alteration	II by ECG	Hypertension
63	30	F	Illapel	x		9/1/2011	(+) 1/640	1179	0.58	(−)	QTc↑, VE	II by ECG	Hypothyroidism
64	75	F	Illapel		x	7/1/2012	(+) 1/1280	2068	0.01	(−)	QTc↑, VE	II by ECG	Hypertension, cholecystectomy
65	72	F	Combarbalá		x	7/1/2012	(+) 1/80	1331	0.07	(−)	Repolarization alteration	II by ECG	Cholecystectomy
66	64	M	Combarbalá	x		7/1/2012	(+) 1/640	1603	0.01	(−)	LVH	II by ECG	Hypertension
67	41	F	Illapel		x	7/1/2012	(+) 1/80	1415	0.01	(−)	QTc↑, SB	II by ECG	Hypercholesterolemia
68	47	M	Illapel		x	7/1/2012	(+) 1/640	1776	0.01	(−)	QTc↑, LAHB, CRBBB	II by ECG	Hypercholesterolemia
69	31	F	Illapel		x	5/20/2008	(+) 1/1280	1552	0.36	(−)	QTc↑	II by ECG	
70	76	M	Illapel	x		7/1/2012	(+) 1/1280	2379	2.1	(+)	Repolarization alteration	II by ECG	Hypertension, gastritis, severe hearing loss
71	70	F	Illapel		x	4/1/2013	(+) 1/640	1371	0.19	(−)	Anterior ischemia	II by ECG	Hypertension, bronchial asthma
72	67	F	Combarbalá		x	10/1/2012	(+) 1/320	1393	0.32	(+)	AE	II by ECG	Hypertension, hypercholesterolemia, hypothyroidism, depression
73	38	M	Illapel		x	1/1/2010	(+) 1/1280	2506	0.18	(−)	QTc↑, SB	II by ECG	
74	51	F	Combarbalá		x	7/1/2012	(+) 1/160	0.362	0.15	(−)	QTc↑	II by ECG	
75	49	F	Combarbalá	x		6/1/2013	(+) 1/80	1319	0.05	(−)	LAHB	II by ECG	
76	70	F	Combarbalá	x		11/1/2013	(+) 1/320	1358	0.03	(−)	QTc↑, CRBBB	II by ECG	Arthritis
77	48	M	Combarbalá		x	11/1/2013	(+) 1/640	1651	0.03	(−)	QTc↑, CRBBB	II by ECG	
78	62	F	Illapel	x		7/1/2012	(+) 1/1280	1224	0.06	(−)	SB, Anterior ischemia	II by ECG	Compensated congestive heart failure
79	52	M	Illapel	x		7/1/2014	(+) 1/1280	1389	0	(−)	SB, I°st AV block	II by ECG	
80	56	F	Salamanca		x	7/1/2014	(+)1/160	0.447	0	(−)	CRBBB	II by ECG	
81	53	F	Salamanca		x	4/1/2013	(+) 1/640	1016	1.15	(+)	SB, Repolarization alteration	II by ECG	Histerectomy, depression

82	53	F	Salamanca	x		1/1/2011	(+) 1/1280	1407	0.07	(−)	LAHB, CRBBB	II by ECG	Depression
83	49	F	Combarbalá		x	5/1/2014	(+) 1/1280	1629	0.01	(−)	Anterior ischemia	II by ECG	
84	67	F	Salamanca	x		8/1/2013	(+)1/160	0.324	0.04	(−)	CLBBB	II by ECG	Hypertension, hypercholesterolemia
85	70	F	Combarbalá		x	8/1/2011	(+) 1/640	1038	0.13	(−)	QTc↑, LAHB	II by ECG	
86	51	F	Illapel		x	7/1/2011	(+) 1/640	0.976	0.03	(−)	I°st AV block, LAHB	II by ECG	Gastritis, artrosis
87	54	F	Combarbalá		x	5/1/2014	(+) 1/1280	1709	0.02	(−)	LAHB	II by ECG	
88	58	M	Salamanca	x		7/1/2014	(+) 1/640	0.821	8.5	(−)	I°st AV block	II by ECG	
89	56	F	Combarbalá	x		10/1/2014	(+)1/80	1869	0.04	(−)	QTc↑	II by ECG	Cholecystectomy, diabetes mellitus, hypercholesterolemia
90	55	F	Combarbalá	x		10/1/2014	(+)1/160	0.496	0.19	(−)	CLBBB, QTc↑	II by ECG	Hypertension, cholecystectomy
91	53	F	Combarbalá	x		7/1/2014	(+) 1/160	0.558	5.6	(−)	LVH	II by ECG	Hypertension, histerectomy
92	62	M	Illapel		x	12/1/2014	(+)1/1280	1471	0	(−)	I°st AV block, SB	II by ECG	
93	53	F	Salamanca		x	10/1/2014	(+)1/160	0.41	0	(−)	LAHB	II by ECG	
94	70	F	Combarbalá	x		10/1/2014	(+)1/80	1776	0.01	(−)	CLBBB	II by ECG	Hypercholesterolemia, gastritis, cholecystectomy, depression
95	66	M	Combarbalá	x		12/1/2014	(+) 1/1280	1761	0.009	(−)	LAHB, LVH	II by ECG	Hypertension, cholecystectomy, prostate adenoma
96	53	F	Salamanca	x		6/1/2015	(+) 1/1280	1910	0	(−)	CRBBB	II by ECG	
97	64	F	Illapel		x	6/1/2015	(+)1/640	1541	0	(−)	Repolarization alteration	II by ECG	Hip osteoarthritis
98	60	F	Combarbalá		x	10/1/2015	(+)1/1280	1499	0	(−)	I°st AV block	II by ECG	Hypercholesterolemia
99	62	F	Salamanca		x	6/1/2015	(+)1/1280	1099	0.009	(−)	I°st AV block	II by ECG	
100	55	F	Salamanca	x		10/1/2015	(+)1/1280	1554	0.009	(−)	LAHB	II by ECG	

LVH left ventricle hypertrophy, LAHB left anterior hemiblock, SB sinus bradycardia, IRBBB incomplete right bundle branch block, QTc↑ prolongued QTc interval, VE ventricular extrasystoles, CRBBB complete right bundle branch block, CLBBB complete left bundle branch block, LPHB left posterior hemiblock, AE auricular extrasystoles, EF ejection fraction. * These cases present low ejection fraction by Echo-Doppler.

Table 2
Clinical, epidemiological, serological, parasitological, and comorbidity of 100 chronic chagasic patients without cardiopathy. Group B.

No.	Age	Sex	Locality	Origin		Control date	Serology		qPCR	Xenodiagnosis	Comorbidity
				Urban	Rural		IIF-IgG	ELISA D.O.	par. eq./ml		
1	43	M	Combarbalá	x		7/1/2012	(+) 1/640	2106	0.03	(−)	
2	32	F	Illapel		x	9/1/2011	(+) 1/1280	1952	0.37	(−)	
3	76	F	Combarbalá		x	7/1/2012	(+) 1/320	1375	0.4	(+)	Diabetes mellitus, hypercholesterolemia, cataract
4	38	F	Illapel	x		9/1/2008	(+) 1/80	1692	1.13	(−)	
5	66	F	Salamanca		x	7/1/2012	(+) 1/320	0.685	0.02	(−)	Esophagus achalasia, hypothyroidism,gastritis
6	30	F	Illapel	x		7/1/2012	(+) 1/640	1785	0	(−)	
7	32	M	Illapel		x	1/1/2010	(+) 1/1280	1784	0.03	(+)	
8	56	F	Illapel	x		7/1/2012	(+) 1/1280	2114	0.29	(+)	Hypertension, hypercholesterolemia, cholecystectomy
9	21	M	Salamanca		x	8/29/2010	(+) 1/40	1302	0	(−)	
10	57	F	Illapel	x		7/1/2012	(+) 1/320	1085	0	(−)	Hypertension, cholecystectomy, hypercholesterolemia
11	56	F	Salamanca		x	1/13/2011	(+) 1/640	1804	0	(−)	Hypertension, cholecystectomy, artrosis
12	61	F	Illapel	x		1/1/2011	(+) 1/1280	2196	0.01	(−)	
13	34	F	Illapel	x		7/1/2012	(+) 1/320	1.09	0.06	(−)	Bronchial asthma
14	44	F	Salamanca		x	7/1/2012	(+) 1/1280	2248	0	(−)	Hypertension
15	53	F	Salamanca		x	7/1/2012	(+) 1/640	1726	0	(−)	Hypertension
16	20	F	Illapel		x	7/1/2012	(+) 1/1280	2288	0.13	(−)	
17	56	F	Illapel	x		7/1/2012	(+) 1/1280	2.24	0.01	(−)	Hypertension, gastric ulcer
18	61	M	Salamanca	x		7/1/2012	(+) 1/320	1.37	0.01	(−)	Colon cancer
19	62	F	Illapel	x		9/1/2011	(+) 1/1280	1654	0.5	(−)	Hypertension, artrosis, hypothyroidism
20	55	F	Salamanca		x	1/13/2011	(+) 1/1280	2276	1.04	(+)	
21	65	M	Salamanca		x	7/1/2010	(+) 1/40	1495	1.27	(−)	Hypertension
22	60	F	Combarbalá		x	7/1/2012	(+) 1/1280	2575	0	(−)	Cholecystectomy, hydatidosis
23	63	F	Combarbalá		x	8/1/2011	(+) 1/1280	2.15	1.04	(+)	
24	47	F	Combarbalá		x	7/1/2012	(+) 1/160	1.47	0.02	(−)	
25	61	F	Combarbalá		x	8/1/2011	(+) 1/640	1673	0.33	(−)	Cholecystectomy
26	42	F	Combarbalá		x	7/1/2012	(+) 1/640	1645	0.06	(−)	
27	58	F	Illapel		x	11/1/2013	(+) 1/1280	1633	0.39	(−)	Hypertension, hypothyroidism
28	56	M	Illapel	x		11/1/2013	(+) 1/640	1817	0.02	(+)	
29	55	M	Illapel	x		11/1/2013	(+) 1/640	1796	0	(−)	Hypertension, bronchial asthma
30	43	F	Illapel	x		11/1/2013	(+) 1/320	1717	0.34	(−)	
31	49	F	Salamanca		x	11/1/2013	(+) 1/640	1503	0.03	(−)	Hypertension, cholecystectomy
32	72	F	Salamanca		x	11/1/2013	(+) 1/1280	1629	0	(−)	Hypertension, diabetes mellitus, cerebellar ataxia
33	58	F	Combarbalá		x	11/1/2013	(+) 1/640	1772	0.04	(−)	Hypertension, hypercholesterolemia
34	51	F	Combarbalá	x		11/1/2013	(+) 1/160	0.743	0	(−)	
35	42	F	Illapel		x	8/30/2010	(+) 1/160	0.634	0	(−)	
36	33	F	Combarbalá		x	8/1/2010	(+) 1/320	1478	0.1	(+)	
37	53	F	Salamanca		x	7/1/2010	(+) 1/1280	1635	0.5	(+)	
38	38	F	Salamanca		x	7/1/2010	(+) 1/1280	1689	0	(−)	
39	60	F	Illapel		x	7/1/2012	(+) 1/640	1441	0.02	(−)	Hypertension
40	65	F	Combarbalá	x		7/1/2012	(+) 1/1280	1944	0.07	(−)	Cholecystectomy
41	41	F	Illapel		x	7/1/2012	(+) 1/640	1602	0	(−)	Fibroids, nephrolithiasis
42	52	M	Combarbalá		x	7/6/2010	(+) 1/1280	2337	0.59	(+)	Esophagus achalasia
43	57	F	Illapel		x	01−14-2013	(+) 1/40	1094	0.04	(−)	Cholecystectomy, hypercholesterolemia
44	48	M	Combarbalá		x	7/1/2012	(+) 1/320	1347	0.04	(−)	Chagasic megacolon
45	46	F	Combarbalá		x	6/1/2013	(+) 1/160	0.411	0.37	(−)	Cholecystectomy
46	20	F	Salamanca		x	10/1/2012	(+) 1/160	0.549	0.67	(+)	
47	50	F	Salamanca	x		4/1/2013	(+) 1/640	1539	0	(−)	
48	62	F	Illapel		x	7/1/2012	(+) 1/320	0.862	0.02	(−)	Osteoporosis
49	39	M	Combarbalá	x		8/1/2013	(+) 1/640	1185	0.09	(+)	
50	57	F	Illapel	x		5/1/2013	(+) 1/1280	1256	1.57	(−)	Cholecystectomy, diabetes mellitus

Table 2 (*Continued*)

No.	Age	Sex	Locality	Origin		Control date	Serology		qPCR	Xenodiagnosis	Comorbidity
				Urban	Rural		IIF-IgG	ELISA D.O.	par. eq./ml		
51	30	F	Illapel	x		8/30/2010	(+)1/1280	1275	0.03	(−)	
52	66	F	Salamanca	x		9/1/2011	(+) 1/1280	1395	0.15	(−)	
53	58	F	Salamanca	x		9/1/2011	(+) 1/640	1025	0.77	(−)	
54	49	M	Other	x		4/10/2013	(+) 1/640	1026	0.02	(−)	
55	36	F	Other	x		5/17/2013	(+) 1/1280	1145	3.44	(−)	
56	28	F	Other	x		5/17/2013	(+) 1/1280	1163	0	(−)	
57	40	M	Other	x		7/12/2013	(+) 1/1280	1358	0.38	(−)	
58	43	F	Other	x		3/1/2014	(+) 1/320	0.872	0.03	(−)	
59	57	M	Other	x		9/1/2014	(+) 1/160	0.417	0	(−)	
60	45	F	Salamanca	x		5/1/2014	(+)1/80	0.947	0.02	(−)	Cholecystectomy, pancreatitis
61	30	F	Salamanca		x	5/1/2014	(+)1/160	0.688	0.03	(−)	
62	36	F	Illapel		x	1/14/2011	(+) 1/1280	1281	0.09	(−)	
63	63	F	Combarbalá	x		11/1/2013	(+)1/160	0.43	0.16	(−)	Hypertension
64	56	F	Other	x		1/16/2013	(+) 1/320	0.625	0.23	(−)	
65	57	F	Other	x		8/20/2014	(+) 1/1280	1894	0.018	(−)	
66	28	F	Other	x		8/21/2013	(+) 1/320	0.819	0.07	(−)	
67	57	F	Other	x		10/16/2013	(+) 1/1280	1521	0.28	(−)	
68	63	F	Illapel		x	7/1/2014	(+) 1/1280	1.94	0	(−)	Hypertension, thrombosis saphenous vein, hip osteoarthritis
69	51	F	Salamanca	x		7/1/2014	(+) 1/1280	1643	4.56	(−)	
70	62	M	Salamanca	x		7/1/2014	(+)1/1280	1826	0.06	(−)	Prostate adenoma
71	59	F	Salamanca	x		7/1/2014	(+)1/1280	1447	0.07	(−)	Breast cancer surgery
72	60	M	Combarbalá	x		5/1/2014	(+) 1/1280	1203	0.01	(−)	
73	26	F	Combarbalá		x	10/1/2014	(+)1/1280	1553	0.01	(−)	
74	33	F	Combarbalá		x	10/1/2014	(+) 1/640	1335	0	(−)	
75	49	F	Illapel	x		10/1/2014	(+) 1/1280	2059	0.01	(−)	
76	58	F	Illapel		x	10/1/2014	(+)1/640	0.76	0.03	(−)	Hypothyroidism, hypercholesterolemia, gastritis
77	58	M	Illapel	x		10/1/2014	(+)1/160	0.373	0.55	(+)	
78	53	F	Salamanca		x	10/1/2014	(+)1/160	0.507	0.54	(+)	Pancreatitis
79	53	M	Salamanca		x	10/1/2014	(+)1/1280	1705	0	(−)	
80	63	M	Salamanca	x		10/1/2014	(+)1/640	1481	0.01	(−)	Hypertension, hypercholesterolemia, onychomcosis, prediabetes
81	69	F	Salamanca	x		10/1/2014	(+)1/128	1761	0.3	(−)	Hypertension, hypercholesterolemia, vasculitis
82	68	F	Salamanca		x	10/1/2014	(+) 1/640	1446	0.03	(−)	
83	56	F	Salamanca	x		10/1/2014	(+)1/160	0.394	0	(−)	Hypertension, cholecystectomy, histerectomy, lumbar disc disease
84	57	F	Combarbalá		x	12/1/2014	(+) 1/320	0.931	0.33	(−)	Gastric ulcer
85	63	F	Combarbalá		x	1/1/2011	(+) 1/80	0.586	0	(−)	
86	41	F	Combarbalá		x	5/1/2014	(+)1/1280	1677	0	(−)	
87	50	F	Combarbalá		x	12/1/2014	(+) 1/1280	1838	0.006	(−)	Hypertension, diabetes mellitus, nephrolithiasis
88	57	F	Combarbalá	x		10/1/2014	(+)1/1280	1668	0	(−)	Fibroids
89	36	F	Salamanca	x		12/1/2014	(+) 1/1280	1572	0.11	(−)	Hypothyroidism
90	59	F	Salamanca		x	12/1/2014	(+) 1/80	0.919	0	(−)	Hypertension, diabetes mellitus, hypothyroidism
91	39	M	Other	x		10/1/2014	(+) 1/1280	1447	0.11	(−)	
92	77	M	Salamanca	x		12/1/2014	(+)1/1280	1537	0.18	(+)	
93	58	F	Salamanca		x	12/1/2014	(+) 1/640	1.11	0	(−)	Hypertension, cholecystectomy,
94	57	F	Salamanca		x	12/1/2014	(+)1/160	0.837	0.004	(−)	Cholecystectomy, hypothyroidism
95	30	F	Illapel	x		5/1/2014	(+)1/80	0.537	0	(−)	
96	54	F	Salamanca	x		12/1/2014	(+) 1/160	0.566	0.007	(−)	Hypothyroidism, hypercholesterolemia
97	69	M	Illapel		x	12/1/2014	(+)1/80	1003	0.28	(+)	Hypertension, polyneuropathy
98	71	F	Illapel	x		6/1/2015	(+) 1/640	1139	0.009	(−)	
99	47	F	Illapel		x	6/1/2015	(+) 1/1280	1589	0	(−)	
100	34	F	Combarbalá	x		6/1/2015	(+)1/160	1003	0	(−)	

Table 3
Electrocardiographic alterations of 100 chagasic cardiopaths by NYHA classification.

	Grade I		Grade II	
	n	%	n	%
Arrhythmias				
Auricular	39	88.7	12	13.4
Ventricular			3	3.3
A-V Block first degree			9	10.1
Intraventricular block				
Unifascicular	5	11.3	26	29.2
Bifascicular			4	4.5
Ischemia image			5	5.7
Repolarization alteration			5	5.7
Hypertrophic pattern			6	6.8
Prolonged QTc interval			19	21.3
Total alterations	44	100	89	100
Total tracings	44		56	
Proportion of alterations per tracing	1.0		1.59	

dia and prolonged QTc interval, two with sinus bradycardia, one with prolonged Qtc interval, sinus bradycardia and left ventricular hypertrophy. Associated pathology did have 59% of patients of group A and 47% of group B. This difference is statistically significant ($p = 0.008$). The most frequent concomitant pathology in groups A and B was hypertension (31 and 23% respectively) followed by hipercholesterinemia in group A (16%) and cholecystectomy in group B (13%) (Tables 1 and 2).

4. Discussion

At present it is not known which person with chronic Chagas disease in indeterminate period will develop cardiomyopathy and who will be maintained asymptomatic with normal ECG all his life. There are some promising studies of biological markers such as brain natriuretic peptide (BNP) that could determine the progress of the cardiopathy (Cardoso et al., 2016). Others such as metallproteinases (Bautista-López et al., 2013) and micro RNAs of myocardium (Ferreira et al., 2014) could determine the passage of chronic infection to cardiopathy. These last investigations need to be confirmed with a larger number of patients.

Some factors of the interrelation of *T. cruzi* with the host and the environment could explain why some persons with chronic Chagas disease in latent or indeterminate period develop a cardiomyopathy and others remain all his life in this period. In relation to human host some studies have demonstrated that haplotype B40Cw3 confers some protection against the development of Chagas cardiopathy (Rothhammer et al., 1986; Llop et al., 1988). No correlation diversity frequencing between age, sex or symptomatology of the host in chronic chagasic patients according to variance analysis have been obtained (Llewellyn et al., 2015).

Zhang and Tarleton in 1999 in a neuron model confirmed by PCR *T. cruzi* its presence in heart muscle tissue, and demonstrate a correlation between the presence of the parasite and the presence of disease. Today it is accepted that the heart damage that occurs in 30–40% of chronic chagasic patients is originated by the persistence of *Trypanosoma cruzi* in heart tissue (Schijman et al., 2004; Benvenuti et al., 2008).

In this survey the majority of the patients of groups A and group B live in rural zones, whose dwellings are appropriate for the development of the vectors and where they have more contact with infected bugs (Gomes et al., 2013). This zone is actually free of *Triatoma infestans*, the most important vector of the domestic cycle (OPS/OMS, 2000).

The higher average age of group A 56.4 with statistically significant differences with group B 50.5 confirm the fact that Chagas heart disease, requires a long period for development (Rassi et al., 2009; Morillo, 2013).

In both groups females predominated due to the greater attendance of women in clinical control for Chagas disease while their husbands were working. No association between sex and cardiopathy was observed, this is consistent with results obtained by other investigators (Pereira et al., 1990; Silva Sde et al., 2007; Llewellyn et al., 2015), but do not agree with Basquiera et al., 2003 who reported more males with Chagas heart disease.

The percentages of detection *T. cruzi* by XD and qPCR in the group A were 13 and 73% respectively. In group B the percentages were 15 and 72% respectively. The percentage of positive XD in both groups was concordant with the literature where the sensitivity in chronic Chagas patients fluctuated between 5.3 and 50% (Pereira et al., 1992; Siqueira-Batista et al., 1994). No discordance was observed between the results of XD and qPCR, however, that there are 4 cases of group A (4%), and 5 of group B (5%), who had more than 1 par.eq./mL with negative XD. This results could be due to the presence of *T. cruzi* genotypes that are not developed in the biological vector (Coronado et al., 2006a,b). The concordance between cPCR and qPCR in group A and B was 73 and 72% respectively ($Z = 0.1584$; P value = 0.874). The quantification limit of qPCR for *T. cruzi* in our laboratory is 0.01 par. eq./mL.

The parasite burdens obtained in this study in groups A and B are lower than those observed by Melo et al., 2015 in Brazil who detected a median of 1.39 and 1.8 par. eq. /mL in cardiopaths and patients without cardiopathy. Our results are also lower than that found in México, Argentina and Spain (Bolivian cases) with indeterminate chronic Chagas disease. The median par. eq./mL in these cases were 0.24; 0.18; and 0.16 respectively (Ramírez et al., 2015). No significant differences were found between average parasite burden in patients of groups A and B nevertheless in group A 5 cases were found with more than 2 parasites/ml. In this study no significative differences in average parasites were found in cardiopaths of grade II and I of NYHA. This last two results differ from Mosca et al., 1985 who demonstrated higher parasitemia by XD in cardiopaths than chronic chagasic patients without cardiopathy and with Sabino et al., 2015 who found that the severity of the cardiopathy is associated to higher DNA in blood by PCR.

The diagnosis of Chagas cardiopathy was made based on clinical examination, results of the ECG and cardio Echo-Doppler which allowed ruling out the most important cardiomyopathies of other etiologies.

In relation to co-morbidity, group A had a greater number of patients with associated pathology than group B (59 versus 47%), this statistically significant difference could be due to the older age of the group with cardiopathy. Hypertension was the most frequent pathology observed in both groups (Tables 1–2). Patients of group A with hypertension and/or hypercholesterinemia, the cardiopathy was due to Chagas disease and not to hypertension and or hypercholesterinemia as the ECG showed no left ventricular hypertrophy, no signs of ischemia and the cardiac Echo-Doppler was normal. Atherosclerosis was discounted as a confounding variable of the clinical date of heart pathology in the cohort and no patients had a history of angina. The ECG analysis of the cohort showed no signs of ischemia and the cardiac Echo-Doppler was normal.

In groups A and B 8 and 13 women respectively had been cholecystectomized, this figure is consistent with the high frequency of biliary pathology in Chile where 55% of women over age 50 have gallbladder pathology (Braghetto et al., 2011). Two men of group A were also cholescystectomized, confirming that this pathology is also common in men. Seventeen patients had gastrointestinal involvement: Three has esophagus achalasia 1 of group A and 2 group B, one patient of group B had megacolon, another an operated colon cancer, two had chronic gastritis, two chronic pancreatitis and the last two a gastritis ulcer. Five patients of group A had chronic gastritis and one a chronic pancreatitis.

The electrocardiographic tracing was the basic clue for diagnosis of Chagas cardiomyopathy; 133 abnormalities were observed in the 100 cardiopaths. Sinus bradycardia was the most frequent alterations (51 cases, 38.3%). In 39 patients it was the only alterations of the serial ECG performed (at least three), none of this cases corresponded to athletes or people who do heavy work or received bradycardic vagotonic drugs that could causes this pathology. The twelve associated cases correspond to the following: First degree A-V block and prolonged QTc interval (3) one with LAHB, another two with prolonged QTc interval, one with repolarization alteration, two with ischemia, another left ventricule hypertrophy and the last two first degree A-V block.

Prolonged QTc interval is one of the first elements altered in the ECG of chagasic cardiopaths according to our experience, this was present in 19 cases (21.3%). In four cases it was the only alteration present, in the remaining 15 it was associated. LAHB was present in 17 cases, alone in six cases, three in associated to prolonged QTc interval, two with left ventricle hypertrophy, one with ventricular extrasystole incomplete right bundle branch block in association with prolonged QTc interval, one with sinus bradycardia, one with auricular extrasystole and myocardial ischemia, two cases with complete right bundle branch block, one of them associated to prolonged QTc interval, and the last one associated to first degree A-V block.

According to the electrocardiographic alterations 56 cases (56%) correspond to grade II and 44 (44%) to grade I of the NYHA Association Classification, similar percentages have been observed in Brazil (Rassi et al., 2010). The most frequent alterations of the ECG in group A were sinus bradycardia and prolonged QTc interval.

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