Clinical, laboratory and epidemiological data of chronic chagasic patients residing in the northwestern region of the State of Paraná, Brazil

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ABSTRACT. Clinical, laboratory and epidemiological data of 48 chagasic patients living in the northwestern region of the State of Paraná were analyzed. The clinical study revealed abnormalities in 59.5% of electrocardiograms and 27% of chest x-rays. Prior to etiologic treatment, all patients had positive IIF and ELISA, except for one case with a IIF titer of 20. Hemoculture, CoML and PCR were positive in 41.7%, 89.6% and 83.3% of subjects respectively. The subjects included women (52.1%) and men (47.9%) with average age of 46.9 ± 10.1 years mainly from the States of Minas Gerais and São Paulo (70.9%) and Paraná (18.8%). The majority acquired the infection through vector contact (95.8%) and 79.2% discovered the disease because they felt ill and looked for medical care, while 8.3% discovered their illness through blood donation. Understanding the clinical, laboratory and epidemiological data of chagasic patients is of considerable importance in order to formulate etiologic treatment proposals for these patients. The majority of the subjects in this study are in the productive phase of their lives and do not present clinical abnormalities, thus raising questions related to treatment which will be evaluated in future studies.

Key words: Chagas disease, hemoculture, PCR, lytic antibodies, epidemiology data, State of Paraná.

RESUMO. Dados clínicos, laboratoriais e epidemiológicos de pacientes chagásicos residentes no noroeste do Paraná, Brazil. Neste trabalho, foram avaliados os dados clínicos, laboratoriais e epidemiológicos de 48 pacientes chagásicos residentes na região noroeste do Paraná. No estudo clínico, o eletrocardiograma e o raio-X de tórax estavam alterados em 59,5% e 27% dos pacientes, respectivamente. Dos testes laboratoriais realizados antes de se propor o tratamento etiológico, a IFI e ELISA apresentaram resultados positivos em todos os pacientes, exceto um caso com título da IFI 20. A hemocultura, LMCo e PCR foram positivas em 41,7%, 89,6% e 83,3%, respectivamente. Observamos que 52,1% da população era feminina e 47,1% masculina com idade média de 46,9 ± 10,1 anos. Quanto à procedência, 70,9% eram procedentes de Minas Gerais e São Paulo e 18,8% relataram ser paranaenses. A grande maioria (95,8%) adquiriu a infecção por via vetorial, 79,2% descobriu a doença de Chagas ao procurar o médico e 8,3% quando foi doar sangue. O estudo dos dados clínicos, laboratoriais e epidemiológicos de pacientes chagásicos torna-se hoje de maior interesse porque, diante desse quadro devemos propor o tratamento etiológico desses pacientes. A grande maioria dos pacientes desse estudo está na fase produtiva de suas vidas e não apresenta alterações clínicas, fatos que levaram a proposição do tratamento etiológico desses pacientes, etapa esta que será avaliada em trabalhos futuros.

Palavras-chave: doença de Chagas, hemocultura, PCR, anticorpos líticos, dados epidemiológicos, noroeste do Paraná.

Chagas disease is a major medical-social problem in Brazil and it currently affects some four million people (Dias and Schofield, 1998). It has been calculated that from 5,074,000 deaths that occurred in Brazil between 1977 and 1983, 8.2% were caused by this disease (Silveira, 1986). Chagas disease is a chronic illness that may be incapacitating and one that has a profound socioeconomic and cultural impact, in fact, it is the main reason for early retirement in the country (Schmunis, 1994).

In Brazil, roughly 80% of the Chagas disease endemic area is under epidemiological surveillance and household infestation rates are reported to be less than 5% (Dias, 1994). There has also been a strong reduction in chagasic infection among blood donors (Dias and Schofield, 1998). Data provided by the executive council of the WHO show that progress has been made in reaching the goal of eliminating Chagas disease in Latin America by the year 2010 (Who, 1998). These reports may provoke optimism with regard to the reduction or elimination of new infections, but they do not alter the fact that nowadays there is a large number of people living with the disease. If assisting these patients becomes a basic public health issue for authorities, these patients will have a better perspective for improved quality of life.

In the State of Paraná, a national survey conducted by Camargo *et al.* estimated chagasic infection prevalence at 4% (Camargo *et al.*, 1984), which represents a total expected case of 166,511 infections for the rural population of 4,164,943 inhabitants (Silveira and Resende, 1994). According to this data, the State of Paraná has the fourth-highest number of estimated chagasic cases, after the State of Minas Gerais, Bahia and Rio Grande do Sul (Silveira and Resende, 1994). This way the group of chagasic patients in Paraná can be considered similar to those of the rest of the country.

In 1992, the Chagas Disease Laboratory at Maringá State University found that 6.3% of individuals from five municipalities in northwest Paraná had positive serology for *Trypanosoma cruzi* (Gomes *et al.*, 1992). Recently this laboratory has established a scheme to evaluate clinically, diagnose, treat and follow chagasic patients living in this region of the state. In the present paper we showed the clinical, laboratory and epidemiological data of chagasic patients before specific treatment.

Material and methods

Study population. The study was conducted July 1994 through July 1998 among 48 chagasic patients residing in different municipalities of northwest Paraná (for map, see Figure). Patients were referred to the Chagas Disease Laboratory of the Maringá State University from either blood banks or the public health system (SUS, cardiology department) or from surveys of rural areas conducted in partnership with the National Health Foundation (FNS/MS).
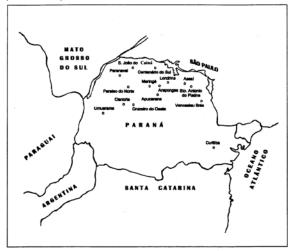

Figure 1. Northwestern Paraná municipalities; location of residences of study population

Clinical study. Patients were clinically evaluated in the Regional Specialty Center, which is part of Health Region 15 from the State of Paraná (CRE/SUS/Maringá/PR). In addition to clinical evaluation, subjects received electrocardiograms and chest x-rays.

Serologic tests. Serologic tests were performed in non-treated patients. Five milliliters of blood were collected from subjects and the following tests were performed on each sample: indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA) and complement-mediated lysis (CoML).

The immunofluorescence test was carried out according to the technique described by Camargo (1966) using *T. cruzi* antigen (Biolab) and anti-IgG fluorescein conjugates (Biolab), in accordance with Camargo (1973). Titers equal to or greater than 40 were considered significant. Anti-*T. cruzi* antibodies of the IgG type were analyzed.

ELISA was carried out with Abbott's Chagas enzyme-immunoassay reagents according to the manufacturer's instructions. Sera with absorption equal to or greater than the cut-off value plus 10% of its value were considered positive.

CoML was performed following the method described by Galvão *et al*. (1993). Briefly, cell culture trypomastigote (CCT) suspensions were diluted to 6 x 10⁶/ml in 10% M199 fetal bovine sera media (FBS). Only suspensions resistant to human complement (HC) direct lysis were used in the CoML test. The determination of specific lytic activity in the human sera was performed by *in vitro* incubation for 30 min of 50µl of the CCT suspensions with an equal volume of sera diluted 2 and 4 times in M199 containing 10% FBS. Aliquots of 50µl of this mixture were incubated with 50µl of HC in an ice bath and parasites were counted immediately (zero time) and again after incubation at 37°C for 45 min. Each reaction was monitored using positive and negative control sera and the CoML reference value was >20%.

Hemoculture. Thirty milliliters (30 mL) of blood were collected from each subject and hemoculture was performed in LIT media according to Chiari *et al*. (1989). Hemocultures were incubated in 50-mL plastic Falcon tubes at 28°C, homogenized once a week and examined 30, 60, 90 and 120 days after incubation.

Polymerase chain reaction (PCR). At the same time that subjects' blood was collected for hemoculture, 15 ml were drawn into 50-ml plastic Falcon tubes containing an equal volume of Guanidina-HCl 6M/EDTA 0.2 M (Sigma Chemical Company, USA) pH 8.0 (Avila *et al*., 1991). The blood samples were boiled at 100°C for 15 min (Britto *et al*., 1993). After boiling, the samples were stored at 4°C until use. A small aliquot (200µl) was removed, the DNA extracted sequentially with phenol-chloroform and chloroform, and precipitated with two volumes of ethanol in the presence of 100mM sodium acetate and 40µg of glycogen (Boehringer Mannheim). The pellet was resuspended in 20µl of sterile distilled water and stored at 4°C until use (Gomes *et al*. 1998).

The PCR conditions were as follow: mixture contained 2µl of the DNA solution, 10mM Tris-HCl (pH 9.0), 75mM KCl, 3.5mM MgCl₂ , 0.1% Triton X-100, 0.2mM each of dATP, dCTP, dGTP, dTTP (Sigma Company Ltda), 20 pmol of 121 (5'AAATAATGTACGGG(T/G)-GAGATGCATGA -3') and 122 (5'GGTTCGATTGGGGTTGGTGTA ATATA3') primers (Wincker *et al*., 1994), 1.0 unit of Taq DNA Polymerase (Promega) and water up to 20µl. These primers anneal in the constant regions of the minicircles of kDNA (kinetoplast DNA) of *T. cruzi* for amplification of a 330 bp fragment. The reaction mixtures were overlaid with 30µl of mineral oil and subjected to 35 cycles of amplification in a MJ Research Programmable Thermal Controller (PTC-150). The temperature profile was 95°C for 1min for denaturation (with a longer initial time of 5 min at 95°C), 65°C for 1 min for primer annealing and 72°C for 1min for extension (with a final incubation at 72°C for 10 min to extend the annealed primers) as described by Gomes *et al*. (1998). Five microliters of the PCR product were analyzed by electrophoresis on a 6.0% polyacrylamide gel and visualized by staining with silver salts (Santos *et al*., 1993).

Epidemiological study. The epidemiological data from subjects were obtained, with their consent, through a questionnaire that included queries regarding sex, age, origin, the way the subject learned of his/her illness and the way the subject believed he/she became infected.

Statistical analysis. Hemoculture, PCR, and CoML results were compared by the chi-square test (Mantel-Haenszel - Statistica Program version 6.0).

Results

Clinical data. Tables 1 and 2 show that of 48 patients studied 37 were evaluated by electrocardiogram and chest x-ray. Abnormal results were observed for 22 (59.5%) and 10 (27%) of subjects, respectively. From 22 individuals with abnormal electrocardiogram 12 (54,5%) presented multiple abnormalities. Chest x-rays revealed that of 37 subjects the cardiomegaly affected 18.9% (Table 2).

Table 1. Chagasic patients electrocardiogram results Chagas Disease Laboratory/Maringá State University - July 1994/July 1998

Electrocardiogram results	Number	Percent (%)
LAH[a]	1	2.7
IRBBB[b]	2	5.4
RBBB[c]	4	10.8
Ventricular extrasystole	2	5.4
Abnormal ventricular repolarization	1	2.7
RBBB + LAH	2	5.4
RBBB + Ventricular extrasystole	2	5.4
Abnormal ventricular repolarization + Ventricular extrasystole	4	10.8
RBBB + LAH + Ventricular extrasystole	2	5.4
RBBB + LAH + Ventricular extrasystole + sinus bradycardia	2	5.4
Normal	15	40.5
Total	37	100

[a]LAH: Left anterior hemiblock; [b]IRBBB: Incomplete right bundle branch block; [c]RBBB: Right bundle branch block

Table 2. Chagasic patients Chest x-rays results Chagas Disease Laboratory/Maringá State University - July 1994/July 1998

Chest x-rays results	Number	Percentage (%)
Cardiomegaly	7	18.9
Abnormalities of the aorta	3	8.1
Normal	27	73
Total	37	100

Analysis of laboratory tests. Results of laboratory tests in chagasic patients seen at the Chagas Disease Laboratory at State University of Maringá from July 1994 to July 1998 may be seen in Table 3. Studied subjects presented IIF titers that varied from 40 to 1,280, except for one case with a titer of 20. This patient had positive CoML, hemoculture and PCR, thus dispelling the possibility that she was not chagasic. ELISA test was positive for all individuals. Hemoculture, CoML and PCR were positive in 20 (41.7%), 43 (89.6%) and 40 (83.3%) subjects respectively. Comparative analysis of these laboratory tests showed that PCR displayed positive results significantly higher than hemoculture (p= 0.027). On the other hand, there was no significant difference between PCR and CoML positive results (p= 0.927) (Table 4 a, b).

Table 3. Chagasic patients laboratory tests results Chagas Disease Laboratory/Maringá State University - July 1994/July 1998

Result	IIF	ELISA	CoML	Hemoculture	PCR
+	47	48	43	20	40
-	1	0	5	28	8
Total	48	48	48	48	48

IIF: indirect immunofluorescence; ELISA: enzyme-linked immunosorbent assay; CoML: complement-mediated lysis test; PCR: polymerase chain reaction

Table 4. Comparative analysis between PCR versus hemoculture and PCR versus CoML tests from chagasic patients Chagas Disease Laboratory/Maringá State University - July 1994/July 1998

a

Test results		PCR +	PCR -	Total nº. (%)
Hemoculture	+	20	0	20 (41.7)
	-	20	8	28 (58.3)
	Total nº (%)	40 (83.3)[c]	8 (16.7)	48 (100)

PCR: polymerase chain reaction; [c]: p=0.027

b

Test results		PCR +	PCR -	Total nº (%)
CoML	+	36	7	43 (89.6)
	-	4	1	5 (10.4)
	Total nº (%)	40 (83.3)[d]	8 (16.7)	48 (100)

CoML: complement-mediated lysis test; [d]: p= 0.927

Epidemiological data of the study population. Of studied population 52.1% were women and 47.9% were men between the ages of 27 and 64 years. With regard to origin, 39.6% of subjects were born in Minas Gerais, 31.3% in São Paulo, 18.8% in Paraná and 10.4% in northeastern states.

The majority of subjects acquired the infection through vector transmission (95.8%). They discovered the disease because they felt ill and looked for medical attention (79.2%) or through blood donation (8.3%), routine examinations (10.4%) and epidemiological surveys (2.1%).

Discussion

The study of the clinical, laboratory and epidemiological data of chagasic patients has become increasingly more important because through understanding these data we can and must propose etiologic treatment for these patients. As reported by Ribeiro and Rocha (1998), health care for chagasic patients, particularly for those that present the indeterminate chronic phase, can not continue to be passive observation, waiting for cardiopathy to set in. Defining groups at risk, identifying groups for treatment and/or retraining professionals, using clinical, epidemiological and cardiac evaluation data should be a primary concern for professionals who care for chagasic patients. In addition, there is significant evidence that etiologic treatment can prevent the progression of Chagas disease since patients treated with benzimidazole have presented fewer changes in electrocardiograms and are more likely to remain in the same clinical stage in which treatment had started (Viotti *et al.*, 1994) .

The majority of the clinical and laboratory data observed in this study does not differ markedly from reports by authors working in other regions of Brazil (Coura *et al.*, 1996; Gontijo *et al.*, 1996; Galvão *et al.*, 1993; Avila et., 1993; Wincker *et al.*, 1994a; Wincker *et al.*, 1994b; Britto *et al.*, 1995a; Britto *et al.*, 1995b; Junqueira *et al.*, 1996). However, bigger differences are seen regarding data from the backcountry of the State of Paraíba.

We found that 59.5% of our subjects had abnormal electrocardiograms while Pereira and Coura (1987) observed alterations in electrocardiograms of 26.5% of individuals studied in the backcountry of Paraíba. The most frequent abnormalities in the electrocardiograms of the patients studied here were right bundle branch blockage, followed by altered ventricular repolarization and ventricular extrasystole. However, multiple abnormalities were observed in 54,5% of subjects (Table 1). Chest x-rays showed irregularities in 27% of patients. The most common was cardiomegaly (18.9%) (Table 2). No case of cardiomegaly was found in the radiological study of patients in the back-country of Paraíba. Pereira and Coura (1987) speculated that this finding was the result of the less malignant nature of Chagas disease in this region.

It was not possible to conduct a digestive tract analysis as part of the clinical study for two reasons. First, there was no professional available within the SUS network to follow the patients, as there was in the cardiology sector. Second, since subjects were seen through SUS, the cost/benefit of radiological study versus digestive tract analysis had to be considered and it is recommended when there is clinical indication of compromised function of the esophagus or colon. As reported by Ribeiro and Rocha (1998), digestive tract analysis is justified if asymptomatic digestive tract damage alters the risk for the patient or permits curative or preventative treatment of asymptomatic alterations. If not, then the analysis increases the cost and complexity of chagasic care without any immediate, tangible benefit. However, we would like to point out that although it has not been possible to evaluate the digestive tract of these patients until now, we have done and continue to do everything in our power to better assist chagasic patients in the northwestern region of Paraná. It should also be mentioned that health care services for these patients in this region only began in the 1990s. Today chagasic patients have at their disposal the Chagas Disease Laboratory that has a multiprofessional team including a medical doctor, professors, a psychologist and a social worker as well as students, assistants and professionals of the National Health Foundation, our partner in certain studies.

The laboratory test analysis demonstrated that all subjects had positive IIF and ELISA, except for one case with a titer of 20 to IIF. The hemoculture confirmed infection in only 41.7% of cases. This data agree with other authors (Chiari *et al.*, 1989; Fernandes *et al.*, 1995; Galvão *et al.*, 1993; Mora, 1996; Silva *et al.*, 1996), who in general observed positive results until 50% of patients when a single hemoculture was performed. This result differs from that found by Coura *et al.* (1996) who reported that 25.7% of patients from Caatinga, Piauí, had positive hemocultures. Since one of our objectives was to propose etiologic treatment for patients, CoML was carried out because this test has been used as a control of cure. PCR was also done since we were interested in knowing if it could also be used to evaluate the efficacy of specific treatment. CoML was positive in 89.6%. Galvão *et al.* (1993) observed that CoML was positive in 100% of 101 non-treated chagasic patients. PCR was able to detect *T. cruzi* DNA in 83.3% of the 48 analyzed patients. Comparative analysis between hemoculture and PCR showed that this test was much more sensitive than hemoculture in confirming chagasic infection (Table 4a). This finding is in agreement with various authors who compared PCR with hemoculture or xenodiagnosis (Avila *et al.*,

1993; Britto *et al.*, 1995a; Britto *et al.*, 1995b; Coura *et al.*, 1996; Junqueira *et al.*, 1996; Wincker *et al.*, 1994a e b). When PCR was compared with CoML the majority of subjects had positive results for both tests (Table 4b).

The epidemiological data of subjects revealed that vector transmission was the main via of infection, which has also been reported by Gontijo *et al.* (1996). But other characteristics of patients in the present study differed from other Brazilian populations. The average age of subjects analyzed here was 46.9 ± 10.1 years. However, patients seen at the Chagas Disease Laboratory in Belo Horizonte, MG, had an average age of 37.78 years (Gontijo *et al.*, 1996) and the majority of patients from the backcountry of Paraíba and Piauí were over 50 years old. Regarding sex, these authors reported that patients seen in Belo Horizonte were predominantly male. Coura *et al.* (1996) observed a predominance of women in Paraíba and men in Piauí, while our study had an almost equal percentage of men and women. The majority of subjects (79.2%) discovered they had Chagas disease because they felt ill and sought medical care at health posts and 8.3% were referred by blood banks, while Gontijo *et al.* (1996) reported that the majority of chagasic patients (78%) were referred by blood banks. Regarding origin, 18.8% of subjects were from Paraná but the majority were from Minas Gerais and São Paulo (70,9%), a breakdown that makes sense considering that northwest Paraná is an area with a high rate of migration from those two states.

The clinical, laboratory and epidemiological data of the study population illustrate that the majority of subjects are in the prime of their lives and a percentage of them did not present significant clinical alterations, which is the current indication for etiologic treatment (Coura *et al.*, 1997; Viotti *et al.*, 1994). Specific treatment for *T. cruzi* was proposed for these patients. The clinical and laboratory evaluation of this treatment will be the object of future studies.

Acknowledgments

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