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Early Evaluation of Etilological Treatmetn in Young Adults, Chronically Infected *Trypanosama Cruzi* Detected Using Polymerase Chain Reaction

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ABSTRACT: Fourteen patients (age limits: 18-30) chronically infected with Trypanosoma cruzi and presenting positive blood PCR reactions, were submitted to etiological treatment with benznidazole, in Nagapattinam district rural an area. A control group of five patients of the same age and positive by PCR did not receive treatment. Post-treatment follow-up was performed with PCR and conventional serology. PCR became negative in 12/14 of the treated patients after six months follow up, compared to 1/5 in the control group ($p = 0.01$). All patients remained serologically positive after treatment. The reduction of PCR signals of infection after treatment may become an early evidence of cure in chronically infected young adult patients.

I. INTRODUCTION

Chagas disease, caused by the parasite *Trypanosama cruzi*, is transmitted to humans and animal by insects (vectors) located in America, mainly in rural areas of Latin America. Humans can be infected by blood transfusions or tissue and organ transplants from infected donors, from a mother to her child (congenital transmission), orally by consuming contaminated food with faeces from infected insects, or by accidental exposure in the laboratory. Additionally, this disease has extended, due to migrations, to countries such as Spain (6) and the USA (7). The etiological treatment of chronically infected patients has been recommended by the World Health Organization (10), Brazil (8) and Argentina (9) treatment guidelines also recommend treatment for such patients, stating that the decision should be taken by each patient and his/her physician. Conventional serologic (Enzyme Linked Immunosorbent Assay - ELISA, Indirect Hemagglutination - IHA) and parasitologic (Hemoculture, Xenodiagnosis) methods are recommended for the control of therapeutic efficacy. However, in the chronic phase, conventional reactions only become negative years after treatment (1, 10), and conventional parasitologic methods have low sensitivity (4). The Polymerase Chain Reaction (PCR), detecting Trypanosoma cruzi DNA (13), showed higher diagnostic sensitivity than conventional methods in chronic Chagas disease patients (14). In this work, we explored the usefulness of PCR as a tool for early evaluation of the efficacy of etiologic treatment in young adults infected by T. cruzi. Treatment was also controlled with conventional serology (IHA, ELISA)

II. MATERIALS AND METHODS

Subjects

Ninety-one patients were interviewed, age ranged from 18 to 30 years old, and they had two positive serological tests (ELISA and IHA). The PCR test was applied to all of them. The reaction was positive in 36/91 (65.5%). Treatment was initiated in 18 patients and 14 completed the whole treatment course. The control group was composed of five patients who rejected treatment or received it after this study. Out of 19 patients thirteen were women. All lived inNagapattinam rural , an area with no Victorial transmission, and signed an informed consent. Exclusion criteria were: previous treatment for T.

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cruzi, pregnancy, liver or kidney disease, low leukocyte or platelet counts, alcoholism, severe pathology associated to Chagas disease and breast-feeding. Treatment Two groups were studied the treated group (TG), included 14 patients (age range 23.6 ± 3.5 years), which received benznidazole at 5 mg/kg/day for 60 days, by oral route. The control group (CG), included 5 patients (age range 26.6 ± 4.9 years), which did not receive treatment. The follow-up was of 6 months post-treatment for TG and from 2 weeks to 6 months for CG. Serology After separating the serum, IHA and ELISA tests were done, using Wiener Laboratory kits (government research area) and following the recommended procedures and cut-off points (1/16 dilution for IHA and 0.22 absorbance for ELISA)

Polymerase Chain Reaction (PCR)

The procedure used by Britto et al. (3) with minor modifications was used. Blood (5 mL) was diluted 1:1 in 6 M-Guanidine EDTA 0.2 M buffer. The sample was heated at 100º C 10 minutes. DDNA was extracted in minipreps using 100 µsample aliquots, using phenol-chlorophorm-isoamilic alcohol and sodium acetateethanol precipitation. Amplification mixtures included primers 121 (5'-AAA TAA TGT ACG GGT GAG ATG CAT GA-3') and 122 (5'-GGT TCG ATT GGG GTTGGT GTA ATA TA-3'). Amplification was carried out in a thermal cycler (M. J. Research, Watertown, Masssachusetts, USA) with the following cycle sequence: cycle 1(x2) 1 min at 98º C and 2 min at 64º C; cycle 2 (x33): 1 min at 94 ºC and 2 min at 64º C; cycle 3 (x1): 1 min at 72º C and 2 min at 25º C. Amplified productswere electrophoresed in 0.2 % agarose minigels, stained with ethidium bromide and revealed with ultraviolet light. Positive samples displayed a 330 bp band. Because cross-contamination or PCR artifacts are a constant risk, a maximum of 5 samples was processed, together with a negative and a positive control, per extraction session. PCR mixtures and DNA extraction were performed in separate chambers. All reagents were prepared in aliquots, using exclusive pipettes and filter tips. Amplicons were electrophoresed in a separate room.

Statistical analysis

The significance of differences between proportions was calculated with the Fisher's exact test.

III. RESULTS

The main outcomes of ELISA, IHA, and PCR for each patient are shown in Tables 1 and 2. PCR became negative in 12/14 (85.7%) TG patients at month 6 post-treatment, and in 1/5 (20%) CG patients (p = 0.01). All patients remained serologically positive after treatment (TG), or in the follow-up of the CG. Seven of 14 (50%) patients in the TG presented adverse effects of benznidazole. These were: allergic dermatitis (4/7; 57.2%), peripheral polyneuritis (2/7; 28.6%), and asthenia (1/7; 14.3%). Nevertheless, treatment could be completed in all patients, by either diminishing temporarily the dose or by symptomatic medication. In two cases, adverse effects disappeared spontaneously after the first days of treatment.

Table 1. Result of serological tests and PCR before/ after treatment with benznidazole of young adults, infected with Trypanosoma cruzi .

Patients	Before treatment			After treatment		
	IHA	ELISA	PCR	IHA	ELISA	PCR
1	1/1024	+ve	+ve	1/1024	+ve	-ve
2	1/32	+ve	+ve	1/16	+ve	-ve
3	1/256	+ve	+ve	ND	ND	-ve
4	1/512	+ve	+ve	1/128	+ve	-ve
5	1/64	+ve	+ve	1/256	+ve	+ve
6	1/256	+ve	+ve	1/32	+ve	-ve
7	1/512	+ve	+ve	1/256	+ve	-ve

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8	1/1024	+ve	+ve	1/512	+ve	-ve
9	1/1024	+ve	+ve	1/512	+ve	-ve
10	1/1024	+ve	+ve	1/1024	+ve	+ve
11	1/1024	+ve	+ve	1/512	+ve	-ve
12	1/64	+ve	+ve	1/512	+ve	-ve
13	1/1024	+ve	+ve	1/512	+ve	-ve
14	1/32	+ve	+ve	1/64	+ve	-ve

IHA: indirect Hemagglutination ELISA: Ezyme linked immunesorbent assay ND: not done
+ve: positive, -ve: Negative.
Table 2. Results of serological tests and PCR of a group of non treated Trypanosoma cruzi infected, young adults .

Patients	Beginning			Follow up		
	IHA	ELISA	PCR	IHA	ELISA	PCR
A	1/64	+ve	+ve	ND	ND	+ve
B	1/128	+ve	+ve	1/256	+ve	+ve
C	1/64	+ve	+ve	ND	ND	+ve
D	1/16	+ve	+ve	ND	ND	+ve
E	1/128	+ve	+ve	ND	ND	+ve

IV. DISCUSSION

The criterion of cure for Chagas disease is still the negativization of conventional serologic and parasitological tests (5), even though there is not much experience using this criterion in chronically infected adults. During this stage of the disease, conventional parasitologic tests display low sensitivity and serological reactions become negative very late, even in cases of successful treatment (1, 10). PCR could overcome both difficulties: it could provide results on the efficacy of treatment with higher sensitivity than conventional parasitological tests, and earlier in time than serological tests. In this work, a significant negativization of PCR was demonstrated in the TG. Although these results are promising for monitoring treatment, the relatively low sensitivity of PCR should be considered. 65.5% of chronically infected patients were positive by PCR. Similar results were observed by other authors (11). A negative result after treatment therefore does not indicate cure, although it could provide a supporting evidence of cure. Only two patients presented a positive PCR after treatment. The possibilities of contamination and lack of treatment compliance were reasonably discarded. Therefore, these results could indicate failure to therapy. This fact has been shown in other studies (2), and could be due to infection with resistant parasite strains (12). An important proportion of adverse effects of benznidazole was observed. However, most of them were mild or could be resolved with simple clinical indications. To evaluate the effectiveness of PCR as method for assessing therapy in chronically infected adults, its sensitivity should be increased and patients should be followed-up for longer periods.

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