SYSTEMATIC MONITORING OF PATIENTS WITH CHAGAS DISEASE REVEALS BENEFITS OF ETIOLOGICAL TREATMENT

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ABSTRACT

The objective of this work was to monitor before, 4.5 and 10 years after the etiological treatment 17 patients with chronic Chagas disease using serological, parasitological and molecular methods. The indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) results before and after 4.5 years of treatment showed no variation. The IIF reduced 2 to 3 titles in six (66.7%) of nine patients 10 years after of treatment, with ELISA remaining unchanged. Hemoculture was positive for 7 patients before treatment and for none after 4.5 years of treatment, remaining these results for nine patients in follow-up of 10 years. PCR was positive for 82.4% (14/17) patients before treatment, for 35.3% (6/17) and 11.1% (1/9) after follow-up therapeutic by 4.5 and 10 years, respectively. The results indicates the reduction of circulating parasites, justifying the specific therapy implemented and revealing beneficial action of the drug, improvement in the patient's prognosis and the importance of systematically monitor patients with Chagas' disease treated etiologically using several techniques simultaneously.

KEYWORDS: Chagas disease, etiologic treatment, systematic monitoring, several laboratories techniques.

1. INTRODUCTION

In terms of public health and economic impact, Chagas' disease is the most important parasitic infection in Latin America. Five at six millions of people are infected by *Trypanosoma cruzi* and 30% of chronic chagasic patients may develop severe abnormalities in the electrocardiogram and chagasic cardiomyopathy[1]. In endemic areas, cardiovascular problems are the main cause of death in patients with 30 to 50 years old[2]. This fact justifies the proposal of etiologic treatment for infected individuals.

Current knowledge seems to indicate that parasite persistence, coupled with an unbalanced immune response, plays a pivotal role in the development of the characteristic pathology present in both acute and chronic human Chagas' disease[3,4]. A high frequency of parasites and/or antigens associated with myocardial inflammation is an important guide to the therapeutic procedures in the chronic phase[5]. Once *T. cruzi* infection is confirmed and when clinical conditions allow, etiological treatment can benefit even patients in the chronic phase of the disease[6,7].

For evaluation of the efficacy of the etiological treatment, the serological methods (indirect immunofluorescence, IIF; and enzyme-linked immunosorbent assay, ELISA) have limitations, with the results remaining positive years after the treatment[8,9]. However, a significant decrease in titers of IIF antibodies detected in long-term follow-up of patients treated etiologically suggests that eventually the titers will be negative, which is a sign of cure[9,10].

Parasitological methods (hemoculture and xenodiagnosis) are less sensitive to monitor the etiological treatment and on the other hand polymerase chain reaction (PCR) is highly sensitive for detecting *T. cruzi* DNA[11,12] and has been proposed as a confirmative proof in the post-therapeutic monitoring of Chagas' disease[6,13,14].

In this study, we showed that systematic monitoring of chronic chagasic patients before, after 4,5 and 10 years of etiologic treatment using several techniques simultaneously is important to confirm the benefits of treatment.

2. MATERIAL AND MÉTHODS

Patients. Seventeen patients with Chagas' disease living in southern Brazil were referred by public health authorities to the Chagas' Disease Laboratory of the State University of Maringá (Laboratório de Doença de

Chagas da Universidade Estadual de Maringá, LDC/UEM). The patients gave their informed consent to terms approved by the Institutional Ethics Committee under protocol number 375/2007, accepting to participate in this study. Eleven were women and 6 men, between the ages of 27 and 59 years, with a mean of 44.5 ± 10.1 years.

Laboratory tests. Serological, parasitological and molecular tests were performed before, 4.5 years after etiologic treatment for all patients and 10 years after for nine patients.

From patients, five milliliters of blood were collected and indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) tests were carried out. The IIF test was performed using *T. cruzi* antigen and anti-IgG fluorescein conjugates (Biolab, Rio de Janeiro, Brazil), in accordance with the manufacturer's recommendations. A titer of 40 or higher was considered positive. ELISA was performed with Abbott's Chagas enzyme-immunoassay reagents, ELISA the Chagatest-ELISA recombination v.3.0 diagnosis kits (Wiener, Argentina) and Chagas Test Elisa III (Bioschile Ingenieria Genética S.A, Chile), 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 reagent. Positive and negative controls for Chagas' disease were included for the tests.

Hemoculture was performed with 30 ml of venous blood collected in 50 ml heparinized tubes and centrifuged at 4°C to harvest the plasma. The packed cells were washed by centrifugation in 15 ml of liver infusion tryptose medium-LIT at 4°C, resuspended in LIT, homogenized and divided into five 15 ml plastic Falcon tubes, and incubated at 28°C[15]. All tubes were mixed gently once a week and examined monthly for 120 days.

At the same time that patients' blood was drawn for hemoculture, 10 ml was drawn into 50 ml plastic Falcon tubes containing an equal volume of Guanidine-HCl 6M/EDTA 0.2 M (Sigma Chemical Company, USA), pH 8.0 to make polymerase chain reaction (PCR). The blood samples were boiled at 100°C for 15 min, and were then stored at 4°C until use. DNA extraction, the conditions of the PCR reaction and revelation of the amplified products were as previously described[11]. PCR controls were added to each series of samples to establish that carryover DNA contamination did not occur. For each blood sample, extraction and amplification of DNA were performed in duplicate. In order to exclude the possibility that negative results of the PCR were due to the presence of reaction inhibitors, 10 picograms (pg) of previously extracted *T. cruzi* DNA were added to the negative samples that were amplified again.

Treatment: Patients were treated with benznidazole (Rochagan-Roche) at doses of 5 to 7 mg/Kg/day for at least 30 days. Treatment was indicated for patients up to 60 years of age, who generally had good health and wish to receive the treatment.

3. RESULTS

As seen in Table 1 the IIF and ELISA results before and after 4.5 years of treatment showed no variation. The IIF had reduced 2 to 3 titles in six (66.7% - 423; 057; 167; 283; 036; 427) of nine patients 10 years after of treatment, with ELISA remaining unchanged.

Hemoculture was positive for 7 patients before treatment and for none after 4.5 years of treatment, remaining these results for the nine patients in follow-up of 10 years (Table 1).

PCR was positive for 82.4% (14/17) chronic chagasic patients before treatment, for 35.3% (6/17) and 11.1% (1/9) after follow-up therapeutic by 4.5 and 10 years, respectively (Table 1).

Table 1. Laboratory test results from chronic chagasic patients before, 4.5 and 10 years after of etiologic treatment with benznidazol at a dose of 5-7 mg/Kg for at least 30 days

Patient	IIF		ELISA		Hemoculture		PCR	
	Before	4.5/10*	Before	4.5/10*	Before	4.5/10*	Before	4.5/10*
423	320	160/40	2,000	2.000/2,460	-	-/-	-	-/-
057	160	80/40	2,000	2.000/2,182	+	-/-	+	-/+
167	160	320/40	2,000	2.000/2,861	-	-/-	+	+/-
283	320	320/80	1,782	2.000/3,157	-	-/-	+	-/-
036	320	160/80	1,589	2.000/2,904	+	-/-	+	-/-
427	320	160/40	1,636	2.000/2,862	+	-/-	+	-/-
150	160	80/160	0.996	0,607/0012	+	-/-	+	-/-
218	160	160/80	1,664	2.000/2,256	-	-/-	+	-/-
200	160	320/160	1,808	2.000/2,334	-	-/-	+	-/-
323	40	80/ND	1.611	2.000/ND	-	-/ND	-/ND	-/ND
401	160	160/ND	2.000	2.000/ND	+	-/ND	+/ND	-/ND
411	320	320/ND	1.611	2.000/ND	-	-/ND	+/ND	+/ND
2784	160	160/ND	1.030	1.098/ND	+	-/ND	+/ND	+/ND
367	20	40/ND	1.298	1.327/ND	+	-/ND	+/ND	+/ND
224	320	320/ND	ND	2.000/ND	-	-/ND	+/ND	-/ND
304	1280	640/ND	1.940	2.000/ND	-	-/ND	+/ND	+/ND
139	160	80/ND	1.812	2.000/ND	-	-/ND	-/ND	+/ND

ND= Not done.

4. DISCUSSION

In human Chagas' disease, the problem of adequate parasitologic evaluation has always generated much study and controversy, because negative results do not necessarily indicate a lack of parasitemia or parasitologic cure post-treatment. The effectiveness of drug, the tests used for diagnosis, the characteristics of parasite and of host and the parasite-host relationship are some factors that complicates the evaluation of etiological treatment. To ensure the benefit and build consistent theoretical foundation in applied research is important to systematically monitor, by different and longer periods of time, patients treated, despite of the difficulties inherent in the extended follow-up of subjects in research. The Brazilian Ministry of Health (1997)[16] recommends that to follow up treated chagasic patients, serological tests should be assessed both before and after treatment. In our study, a group of patients was systematically monitored by laboratory analyses before, 4.5 and 10 years after of etiologic treatment.

The hemoculture and PCR results observed with 4.5 years after treatment has not changed after 10 years, consolidating the drug effect with relation to the presence of circulating parasite. As PCR is efficient in detecting low levels of blood parasitism, i.e., this method can reveal the presence of one parasite per 20 ml of blood[17] or as few as 0.1 fg of *T. cruzi* k-DNA[11], a negative PCR result after therapeutic evaluation indicates a reduction in circulating parasites, since the parasitemia is influenced by benznidazole[13,18,19,20]. As the presence of parasite has a pivotal role in the development of the characteristic pathology, the reduction of parasitemia suggests a beneficial action of the drug and an improvement in the patient's prognosis. This was observed for the majority of patients who had positive PCR results before and negative results after treatment, justifying the specific therapy implemented. The failure of all blood samples to yield a positive PCR can be explained by the intermittent presence and variable quantity of circulating parasites at the time of blood collection; i.e., it was possible that parasites were present in one sample but not in another[21,22]; alternatively, changes in the host immune response may have modified the level of parasitemia.

Other results that confirm treatment' benefits is that the number of patients that showed IIF antibody titers of 160 or less ten years after etiological treatment was higher in relation to the number of patients who showed these values before the treatment. Luquetti *et al.* (2008)[23] reported that titers of 160 or lower in treated patients can indicate a tendency toward cure, since these titer levels occur infrequently in patients with untreated Chagas' disease. Fabbro *et al.* (2001)[24] and Streiger *et al.* (2004)[25] have also observed a lower mean of antibody titers detected by IIF for groups of treated patients in relation to untreated patients, and the decline of titers is accepted and recommended by other authors as a sign of cure[23,26]. Reduction in antibody titers and decrease in parasitemia leading to negative parasitological and serological tests was also observed for 100% of the patients at the end of seven years of follow-up[27].

5. CONCLUSION

We concluded that systematic monitoring of patients with Chagas disease performing three methods with different principles and in three different periods of time allows to check benefits of etiological treatment, especially with negative results for high sensitivity method as PCR. Complete evaluation associating these results with clinical data allows accurate assessment of the current condition of patients undergoing chemotherapy. Based on this experience, we recommend therapy with benznidazole, the only drug currently available in Brazil for the etiological treatment of Chagas disease[28], including chronic chagasic patients who show changes in their electrocardiograms and chest X-rays.

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