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Persistence of *Trypanosoma cruzi* in the chronic phase of Chagas disease

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One of the most important determinants of congestive heart failure and sudden death in Latin America is Chagas disease, provoked by infection with the intracellular protozoan parasiteTrypanosoma cruzi. It affects approximately 20 million people [1] and represents a serious public health problem in Central and South America [2]. Chagasic cardiopathy appears to carry the worst prognosis and has become the most frequent cause of heart failure and sudden death, as well as the most common cause of cardio-embolic stroke in Latin America. Chagas disease also represents an increasing challenge for clinicians in the United States [3]and some European countries [4] due to the continuous immigration of people from disease-endemic countries .

The acute phase, characterized by high intracellular parasite growth, usually affects children or young adults in endemic areas . Between 10 to 30 years after this acute stage, about 30% of the patients develop a myocarditis with electrocardiographic abnormalities increase in heart size and observed histological alterations, such as necrosis of the cardiac fibers, focal or diffuse inflammatory infiltrates and interstitial fibrosis of varying intensity [6]. The mechanisms involved in the pathophysiology and progression of the chronic chagasic cardiomyopathy are still unclear. The difficulty of finding trypanosomes in patients undergoing the chronic phase, suggested that persistent antigenic stimulation could be implicated in the pathogenesis of Chagas disease, with CD4 + lymphocytes generated in response to the parasite's
proteins also reacting against the myocardial tissue [7]. However, later PCR-based studies have confirmed the presence of the parasite during the chronic phase of Chagas disease [8]. These studies would therefore suggest that the persistence of the parasite in all the stages of the infection and its replication are responsible for the cell destruction [9,7]. Nevertheless, it is uncommon to find trypanosomes in the cardiac tissue; this has been attributed to the ability of the cardiac muscle cells to control the intracellular multiplication of *T. cruzi* when they are stimulated with certain cytokines[6].

For all these the indication for treatment in patients infected with *T. cruzi* beyond the acute phase is a subject of debate because of the knowledge that 30% of infected people progress to heart disease and 70% remain asymptomatic, and the drugs currently used are not easily tolerated .

The purpose of the present was to study 20 patients with long term positive serology for Chagas disease to make a possible association between clinical symptoms and *T. cruzi* presence.

Material and Methods

Patients

Twenty four patients with positive serology for Chagas disease since long time ago, with and without hypertension and diabetes associated, with an age between 65 and 79 years were studied.

A group of ten patients with hypertension and diabetes and negative serology for Chagas disease were also analyzed.

Written and oral consent was obtained in all patients and the study was approved by the Ethics Research Committee.

Cardiac function was evaluated by electrocardiographic and echocardiographic studies . The presence or absence of parasites was determined by the Polymerase Chain Reaction (PCR).
in blood obtained in a single extraction.

DNA extraction from blood

Blood samples from each patients were mixed with equal volume of guanidine hydrochloride 6M / EDTA 0.2 M .DNA was extracted using conventional phenol:chlorophorm:isoamylic techniques precipitated with ethanol and re-suspended in sterile nuclease free water. The samples were conserved at - 20ºC until used for the amplification of the parasite DNA by PCR.

PCR

The amplification of a 188 bp nuclear fragment of the parasite DNA was performed using two specific primers: **TCZ-1** (5'-CGAGCTCTTGCCCACACGGGTGCT-3') and **TCZ-2** (5'-CCTCCAAGCAGCGGATAGTTCAGG-3'). PCR was performed in 25 µL reaction mixtures containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of each dNTP, 2.5 µL DNA (~ 2.5 ηg), 0.5 U of Taq polymerase (Promega) and 25 ρmols of each oligonucleotide. After an initial 5 min denaturation step at 95ºC, 40 cycles of amplification were performed on DNA Thermal Cycler (Ivema T-18) with a step program consisting of 30 sec denaturation at 95ºC, 30 sec annealing at 60ºC and 30 sec extension at 72ºC. Additional 5 min extension step at 72ºC was performed at the end of reaction. PCR products of the predicted size (188 bp) were separated by electrophoresis in a 1.2 % agarose gel stained with ethidium bromide and examined under UV light. Each reaction was performed in triplicate .

Data analysis

Percentages were analyzed using Pearson Chi-square test. The PCR results for a given sample were considered negative after three repetitive negative reactions; one positive reaction was considered enough for a positive result.

Results

Table 1 shows the clinical characteristics of the patients under the present study. It can be observed that patients with hypertension and diabetes did not present any cardiac abnormalities even though their ages were similar to the chagasic patients.On the other hand chagasic patients and chagasic patient with hypertension and diabetes presented important cardiac alterations and 25% of the chagasic group had positive PCR and 20% of chagasic and hypertension and diabetic group has positive PCR.

Figure 1 shows *T. cruzi* specific PCRs that were performed in order to verify the presence of the parasite in the blood from the serological positive patients . As observed in figure 1 specific PCRs were positive in two of the the blood samples analyzed.

Discussion

Chagas disease is a major cause of heart failure across much of Latin America. About 30% of the infected individuals develop chronic Chagas heart disease, the most severe form of the disease [10]. However, the pathogenesis progression of the infection is still unknown [5]. The lack of a correlation between the microscopic visualization of the parasite and the intense inflammatory mononuclear cell infiltrates,rich in T cells, in tissues affected during the chronic phase of Chagas disease, has led to the formulation of the autoimmune hypothesis to explain the characteristics of this later stage of the infection [11]. On the other hand, the persistence of the parasite in infected individuals, as evidenced by the reactivation of parasitemia in immunocompromised patients[12], the association of cardiac lesions with the presence of specific parasitic antigens [6].

The direct pathogenic role of the parasite has obtained strong support in later years, after the eapplication of new molecular and immunohistochemical techniques, which have shown a close correlation between the presence of the parasite and the tissue damage [13]. Most researchers in the field now agree that chronic low grade parasite persistence in tissue drives the tissue damage and the autoimmune component of the disease.

In the present work we detected parasites in one blood sample in 25% of chagasic patients and in 20 % of chagasic patients with other associated pathologies.

Conclusion

Present work clearly demonstrates that in chronic chagasic patients with or without m associated diseases one simple blood samples can detect circulating parasites. These clearly

demonstrates that the pathology is active, that cardiac damages are going to increase and the the treatment of this patients are clearly indispensable to stop the evolution of the chagasic cardiopathy.

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Table 1: Clinical characteristics of the patients studied

	Chagasic patients	Chagasic patients+hyprtension +diabetes	Hypertensive and diabetic patients
Number of patients	4	20	10
Age	62 ± 6	70 ± 4	66.5 ± 5
Different Blockades	25%	40%	0%
Reduced left ventricular ejection fraction	25%	30%	0%
Left ventricular dilatation	25%	40%	0%
Palpitations	25%	60%	0%
Disnea	25%	60%	0%
PCR +	25%	20%	0%

Legends to Figures

Figure 1 . *T. cruzi* specific PCRs were performed in order to verify the presence of the parasite in the blood serological positive patients.

Figure 1