High similarity of *Trypanosoma cruzi* kDNA genetic profiles detected by LSSP-PCR within family groups in an endemic area of Chagas disease in Brazil

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**ABSTRACT**

**Introduction**: Determining the genetic similarities among *Trypanosoma cruzi* populations isolated from different hosts and vectors is very important to clarify the epidemiology of Chagas disease. **Methods**: An epidemiological study was conducted in a Brazilian endemic area for Chagas disease, including 76 chronic chagasic individuals (96.1% with an indeterminate form; 46.1% with positive hemoculture). **Results**: *T. cruzi* I (TcI) was isolated from one child and TcII was found in the remaining (97.1%) subjects. Low-stringency single-specific-primer-polymerase chain reaction (LSSP-PCR) showed high heterogeneity among TcII populations (46% of shared bands); however, high similarities (80-100%) among pairs of mothers/children, siblings, or cousins were detected. **Conclusions**: LSSP-PCR showed potential for identifying similar parasite populations among individuals with close kinship in epidemiological studies of Chagas disease.

**Keywords**: *Trypanosoma cruzi*. Endemic area. LSSP-PCR.

*Trypanosoma cruzi* has a multiclonal structure with wide biological diversity and high genetic polymorphism. This heterogeneity is associated with the presence of different parasite populations in the same host, a broad geographic distribution, infection stage, and susceptibility or resistance to specific treatments.

*Trypanosoma cruzi* populations can be divided into six distinct discrete typing units (DTUs) named TcI-VI, which show highly heterogeneity within and between types. TcII, TcV, and TcVI are associated with the domestic transmission cycle and are the main causes of Chagas disease in the southern and central regions of South America. TcI isolates are associated with wild and domestic cycles, and are frequently detected in the northern parts of the Amazon region and in endemic areas of Venezuela, Colombia, and Mexico. TcIV and TcIII circulate in the wild and are relatively poorly studied.

The prevalence and geographic distributions of clinical forms of Chagas disease show differences among and within countries. These differences appear to be related to the genetic characteristics of hosts and strains of the parasite within a specific region. However, the data reported to date are inconclusive, making assessment of epidemiological studies difficult.

Currently, the application of molecular techniques for epidemiological studies on Chagas disease has been proposed, which has stimulated research aiming to evaluate the relationship between the transmission mechanisms, DTUs of *T. cruzi*, and clinical forms of the disease.

The low-stringency single-specific-primer-polymerase chain reaction (LSSP-PCR) technique has been successfully applied for *T. cruzi* characterization. This method has shown excellent potential for evaluating intraspecific differences or similarities of *T. cruzi* populations and shows stability and reproducibility in experimental and human studies.

In this study, the epidemiological application of LSSP-PCR in the characterization of TcII samples demonstrated similar genetic profiles circulating among individuals with very close kinship. Seventy-six chronic Chagas disease individuals from an endemic post-control vector program area in the State of Bahia (Brazil) were evaluated; the age of subjects ranged from 2 to 56 years (mean, 31.62 ± 12.25 years). *Trypanosoma cruzi* infection was detected by positive serology of anti-*T. cruzi* using indirect immunofluorescence and an enzyme-linked immunosorbent assay. This research project was approved by the Ethics Committee (nº 388) of the Universidade Federal do Triângulo Mineiro (UFTM) and all procedures were carried out with the informed consent of patients.

The clinical records of the patients were classified according to their symptoms and electrocardiographic and radiological abnormalities (esophagogram, opaque enema, and chest X-ray);
96.1% of patients presented the indeterminate form and 4% presented the cardiac form of Chagas disease.

Parasitemia and parasite isolation were evaluated by hemoculture and demonstrated the presence of *Trypanosoma cruzi* in 46.1% (35/76) of the individuals, without significant differences with respect to gender (40% male and 60% female) and age group (range, 4-56 years; mean, 31.43 ± 11.95 years) (p > 0.05). The degree of kinship in the study population was also determined, and 13 pairs of the 35 individuals with positive hemoculture showed kinship.

Deoxyribonucleic acid (DNA) extraction of the positive hemoculture samples preserved in guanidine-ethylenediaminetetraacetic acid was performed in duplicate by the phenol-chloroform method. *Trypanosoma cruzi* DNA controls represented by positive and negative samples were included in all DNA extractions and PCR procedures.

Genetic characterization of *Trypanosoma cruzi* minicircles, using the primers 121 (5’-AAATAATGTACGGGGGAGATGCATGA-3’) and 122 (5’-GGTTCCAGTGCGGTTGTTGTTTAATAA-3’). The PCR was carried out in a final volume of 20µL containing 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 3.5mM MgCl2, 75mM KCl, 0.2mM each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), 0.4 unit of Taq DNA polymerase (Promega; Madison, WI, USA), 20pmol of each primer, 2µL (1.5ng/µL) of DNA, and 30µL of mineral oil. The amplification consisted of initial denaturing at 95°C (5min) and 35 cycles: 95°C (1min), 65°C (1min), and 72°C (1min), followed by final extension at 72°C (10min). The PCR-amplified products were submitted to electrophoresis on a 1.5% agarose gel containing 0.5% low-melting-point agarose and stained with ethidium bromide. The 330-bp fragments of *Trypanosoma cruzi* PCR-amplification products were submitted to electrophoresis on a 7% polyacrylamide gel and the products of multiplex PCR were viewed on a 7.5% polyacrylamide gel stained with silver nitrate.

For the statistical analysis, the chi-squared test was used to determine the association between positive hemoculture with the patients’ age group and gender. The Shapiro-Wilk test was used to investigate whether the age distributions of the groups was normal. If so, the Student’s *t*-test for independent samples was performed. Associations between patient age groups and the complexity level of the genetic profiles for *T. cruzi* obtained from LSSP-PCR were investigated using regression analysis. The significance level used was 5% (p < 0.05). The analyses were performed using the Statistica for Windows program, version 8.0 (StatSoft, Inc.; USA).

TcII populations were predominant and were detected in 97.1% (34/35) of the hemoculture-positive individuals; these data are concordant with previous reports, which confirm the predominance of TcII in Brazil. TcI was found in a single six-year-old patient who did not receive blood transfusion and whose mother did not present positive serology for *T. cruzi*. This finding suggests the existence of vector transmission in the studied region. *Trypanosoma rangeli* was not detected in any of the analyzed samples.

Intense kDNA polymorphism with only 46% of the bands shared among the TcII populations (mean number of bands, 9.26 ± 2.68) was demonstrated by LSSP-PCR analysis (Figure 1), which agrees with previous reports. High genetic variability has previously been detected by LSSP-PCR among TcI strains.

It has been demonstrated that the percentage of polyclonal *T. cruzi* populations progressively decreases during the chronic phase of Chagas disease. Therefore, for a single endemic area, the *T. cruzi* populations isolated from younger patients should present complex genetic profiles. In the present study, a weak but not significant (p > 0.05) inverse correlation (r = −0.3) was observed between patient age and the complexity level of the genetic profiles of kDNA obtained by LSSP-PCR. This result may be related to the small number of patients in the youngest age group, from zero to 20 years (n = 5).

Despite the high polymorphism of TcII isolates, very similar genetic profiles were observed between specific pairs of *T. cruzi* samples, particularly for eight pairs of patients with very close degrees of kinship. The isolate pairs 34/35, 3/4, and 14/15 corresponded to mother-child pairs and shared 92%, 100%, and 100% of their bands, respectively. The pairs 16/22, 1/2, and 10/11 corresponded to siblings and presented band similarities of 80%, 90%, and 96%, respectively. The pairs 8/9 and 16/17 were cousins and shared 92% and 94% of their bands, respectively (Figure 1). It is important to emphasize that the electrophoretic kDNA profiles were reproducible for all samples when a second LSSP-PCR was performed.
FIGURE 1 - Polyacrylamide gel (7.5%) stained with silver, representing the genetic profiles of *Trypanosoma cruzi* isolates obtained using LSSP-PCR. The percentages of bands shared between each pair of patients (highlighted) and their level of kinship are as follows: pairs 8/9 (92%) and 16/17 (94%) were cousins; pairs 1/2 (90%), 10/11 (96%), and 16/22 (94%) were siblings; pairs 3/4 (100%), 14/15 (100%), and 34/35 (92%) were mothers and children. MW: molecular weight (100 bp); LSSP-PCR: low-stringency single-specific-primer-polymerase chain reaction.

Most of the data reported in the literature have demonstrated unique and exclusive LSSP-PCR profiles for individual patients, although no report has described the differential distribution of *T. cruzi* populations in tissue and peripheral blood samples obtained from the same human host. Here, the high similarity of *T. cruzi* kDNA minicircles associated with kinship suggests the possibility of congenital transmission and/or the presence of similar or identical *T. cruzi* blood populations circulating within the same home or family group. This hypothesis can be supported by evidence of nearly identical patterns of kDNA minicircles between each mother and infant in congenital transmission described in a study using other molecular techniques.

Similarities among the LSSP-PCR *T. cruzi* genetic profiles were also observed among different individuals infected with Tcl from the same geographical region, although it was not possible to associate the kDNA genetic profile with the geographical or biological origin of the studied samples.

These data reinforce the potential of the LSSP-PCR technique for field studies of human Chagas disease and show the importance of using different genetic markers of *T. cruzi* to monitor the transmission of human Chagas disease, particularly for endemic areas that have been certified as free from vector transmission by *Triatoma infestans*. These findings also suggest the existence of vector transmission in the study area and may represent an important warning sign for Brazilian epidemiological surveillance programs.

The detection of *T. cruzi* populations that are genetically related and associated with a high degree of kinship, i.e., children and their mothers, is a new approach in the molecular epidemiology of Chagas disease and may provide new strategies for future studies of *T. cruzi* congenital transmission.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**FINANCIAL SUPPORT**

This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): case n° 307153/2009-6; Universal Announcement - Ministério da Ciência, Tecnologia e Inovação (MCTI): MCT/CNPq 14/2008 (case n° 481231/2008-0); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES): 14/2013 (case n° 481068/2013-9).

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