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**Genotyping of *Trypanosoma cruzi* by RFLP-PCR in chronic patients
attended on Study group in Chagas disease – GedoCh – UNICAMP**

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Abstract

Trypanosoma cruzi (*T. cruzi*) is the agent of Chagas disease (CD), which affect 6 to 7 million of people around the world. For a better comprehension of aspects of disease, as a pathogenesis, epidemiology and genetic profile, the protozoan is subdivided into six discrete typing units (DTUs): TcI, TcIIa, TcIIb, TcIIc, TcII d and TcIIe, based on biological and biochemical (e.g. biodemes and zymodemes) features. Different strains could be parasites in different host or vector. Besides this, the high variation on the treatment success of Chagas disease could be associated with strains variety found in the ambient. Some research, unsuccessfully, tries to relate the *T. cruzi* strains with the clinical forms of CD, once the indeterminate form is more prevalent (20-30%) in active vectorial transmission areas, while the cardiac and digestive forms presented major morbidity and mortality. The aim is to determinate the genotype of the *T. cruzi* in non-treated chronic chagasic patients. Were collected blood of fifteen non-treated patients attended on the study group in Chagas disease (GedoCh - Campinas State University/SP/Brazil) for the realization of blood culture technique with liver infusion tryptose (LIT) medium added fetal bovine serum 10% and penicillin/streptomycin. Epimastigotes forms of *T. cruzi* were maintained on the LIT medium and DNA was extracted by phenol-chloroform method. The genotyping assay was realized by polymerase chain reaction restriction fragment length polymorphisms (RFLP-PCR) based on single nucleotide polymorphisms (SNPs) in the *HSP60*, Histone *H1* and *GPI* loci, and PCR product size polymorphism of the LSU rDNA and mini-exon loci. Those patients were diagnosed with CD for serological methods, except one,

which diagnosed by PCR method. Due low parasitaemia found in chronic Chagas disease patients, only four patients presented parasitaemia by blood culture techniques and could be genotyping. All four samples were classified by RFLP-PCR in TC II strain. Of these patients, two are natural from state of Minas Gerais, being that one presents indeterminate form of CD and one presents digestive form of CD; and two are natural from state of São Paulo, being that one presents indeterminate form of CD and one presents digestive form of CD. These results demonstrate homogeneity in the strains and clinical forms found on the southeast of Brazil, but it is necessary greatest studies to prove this hypothesis.

Keywords: *Trypanoma cruzi*, Genotyping, RFLP-PCR.