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Short Communication

Polymerase Chain Reaction: Molecular Tool Employed in the Diagnostic of Chagas Disease

María Elizabeth Márquez Contreras*

Laboratory of Parasite Enzyme, Science Faculty, University of Los Andes, Mérida, Venezuela

***Corresponding Author**: María Elizabeth Márquez Contreras, Laboratory of Parasite Enzyme, Science Faculty, University of Los Andes, Mérida, Venezuela.

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Chagas disease is caused by the parasite *Trypanosoma cruzi*, during its evolution it is produced an acute phase, followed by a chronic phase, with a low parasitemia and a clinic that goes from no symptoms to severe heart disease. This infection it constitutes the third most common parasite disease after malaria and schistosomiasis. It has been estimated a prevalence of 6 a 7 million people in 21 countries where the disease is endemic [1,2]. In view of its medical, social and economic importance is indispensable to have very effective diagnostic methods to detect the presence of *T. cruzi*. The conventional diagnosis tests are based on detecting the presence of tripomastigotes in blood, amastigotes in tissues, serological testing, or cultures [3]. But these methods have several disadvantages: xenodiagnoses and hemoculture are laborious, time consuming, are not commercially available, requiring special laboratory biosecurity conditions and have poor sensitivities, since more than half of untreated infected individuals show a negative result [4,5], limiting their usefulness in diagnosis and monitoring of drug efficacy [6]. The circulating trypomastigotes are usually absent in chronic stages of the disease and because most of the available serological tests use epimastigotes as antigens, these methods usually exhibit a high degree of cross-reactivity [7]. The recent application of the PCR have increased the specificity and sensitivity of detection of *T. cruzi*, compared to the traditional methods [8]. This method utilizes gene specific oligonucleotide primers that target a distinct gene sequence within a sample, and amplifies that gene sequence hundreds of thousands of time. These copies of the DNA sequence (known as amplicons or PCR products) can then be visualized following agarose gel electrophoresis to determine the presence/absence of the target sequence within the sample [9]. This technique has been used to detect *T. cruzi* in the blood of chronic chagasic patients [10], may be a useful tool for evaluating the efficacy of trypanocidal chemotherapy in different clinical and epidemiological settings [11,12] and may also be used as a complement to serological tests in blood banks [13,14]. PCR can distinguish between infection with other hemoflagellates, does not depend on the immunocompetence of the patient, is sensitive enough for use in chronic patients, and can distinguish a current infection from a previous one or a newborn infection from a maternal one [15]. Other studies showed that PCR is a sensitive method that may detect parasite DNA in up to 95% of blood samples from chronic chagasic patients [16]. Furthermore, PCR is a useful auxiliary technique for confirmation of diagnosis in patients with inconclusive serology [13,14]. An important factor that is affecting the sensitivity and specificity of the diagnosis by PCR is the DNA sequence targeted by the primers. Therefore, the most attractive sequences as templates in PCR diagnosis are conserved, unique to the target group and produced as multiple copies in the parasite's genome, which increases the sensitivity of the detection. DNA targets that have been most widely used in diagnostic PCR´s are the *T. cruzi* kinetoplast minicircle DNA (kDNA) sequence of 330-base pair (bp) (kDNA) and the nuclear mini satellite DNA (satDNA) sequence of 195-base pair (bp), which contain many repetitive sequences that are highly suitable for PCR detection. The kDNA-PCR which amplify repetitive sequences of trypanosome kinetoplast DNA (kDNA) has provided excellent results in T. cruzi detection in human blood [15]. The kDNA specific primers 121 [17] and 122 [8,9], have been designed to amplify the 330-base pair (bp) fragment of the kinetoplast minicircles [18]. This approach has proven to be highly specific as it allows the successful detection of different *T. cruzi* strains, and also sensitive because it does not recognize other kinetoplastids [19,20]. Studies using PCR assays targeting multicopy kDNA minicircles reported 100% sensitivity in chronic Chagas disease patients, highlighting that an excess of human DNA does not interfere with the selective parasite DNA amplification process. The sensitivity of the kDNA-PCR protocol

is about 5 fg of total *T. cruzi* DNA in the reaction tube, equivalent to one parasite in 10 ml of blood [21]. In a study conducted in ChD patients receiving etiological treatment, using qualitative PCR (primers 121 and 122, protocol more currently used to detect *T. cruzi*), demonstrated a progressive decrease in the thicknesses of the 330 base pair (bp) kDNA fragments, probably indicating a decrease in the number of circulating parasites [22]. Therefore, the PCR technique may be tested to assess the evolution of parasite load (before, during, and after etiological treatment). This molecular method as well is more sensitive and allows an earlier diagnosis of congenital infection than conventional techniques [23]. *T. cruzi* satellite DNA (Sat-DNA) is present in 120,000 copies in the parasite genome, is a 195-bp repeated sequence, and it represents 10% of the parasites' total DNA, which makes it a highly sensitive target [24]. To establish the sensitivity of sat-DNA amplification primed by TCZ1 and TCZ2 primers, genomic DNA from various sources and templates were used (Y, Tulahuén, Corpus Christi, and Sylvio X-10/4 isolates) and obtained from patients in widely separated geographical areas (Brazil, Chile, and Texas) yielding a 188 bp amplification band; thus suggesting that a fragment size is universally present in these parasites [25]. Moreover, TCZ1 and TCZ2 are highly specific, because they do not amplify DNA of closely related species, as *Leishmania spp*. (*L. mexicana*, *L. major*, *L. braziliensis*, *L. donovani*) which overlap with *T. cruzi* in some endemic areas, as well as the African trypanosomes (*T. brucei brucei*, *T. brucei rhodesiense*, *T. brucei gambiense,* and *T. congolense*) which are also phylogenetically related to *T. cruzi* [26]. Additionally, mammalian hosts of *T. cruzi* such as mice or humans do not have DNA sequences that are amplified to any significant degree with these primers [24]. Sat-DNA PCR tests showed high specificity and sensitivity values of 0.05 - 0.5 parasites/ml, whereas specific kDNA tests detected 5×10^{-3} parasites/ml [27]. Studies conducted in infected monkeys and in patients using serum samples to amplify Sat-DNA revealed a high sensitivity and specificity in the detection of DNA from *T. cruzi* at any stage of the disease [28]. Despite all the advantages of PCR the implementation of this technology in disease endemic countries is limited due to need of sophisticated equipment (automated thermocycler and electrophoretic equipment). The electrophoresis used for detection of PCR amplicons is laborious and involves the use of toxic ethidium bromide to stain the gels and subsequent use of a hazardous ultraviolet transilluminator. Moreover, PCR is relatively expensive for mid-level laboratories and not affordable for low income countries. Most PCR protocols use phenol-chloroform for DNA extraction, this compound has biohazards issues and is no longer recommended for use in clinical laboratory routine or hospital settings. Finally, a risk of diagnostic tests based on nucleic acid amplification is contamination leading to false positive results. However, the use of PCR as a diagnostic tool is still debated, as the extreme sensitivity that has been reached is prone to false positive results and, most importantly, parasite DNA may be present without infection with live parasites, as it has been observed during congenital infection in mouse models [29]. Various factors may contribute to the overall performance of PCR assays: the epidemiological characteristics of the study populations, the blood volume collected, the conditions of conservation of the sample, the method used to isolate DNA, parasite target-sequences e.g., nuclear satellite DNA (satDNA) or kinetoplast DNA (kDNA) and primers selected for PCR, reagents used and thermo-cycling conditions. The blood sample volume is an important factor to be considered in chronic patients with low levels of parasitaemia following treatment, and the differences in PCR sensitivity can be explained by the intermittent presence and quantity of circulating parasites at the time of blood collection [30]. The PCR results depends on the amount of circulating parasites in patients' blood stream. *T. cruzi* circulates in very small amounts at the chronic phase and dynamics about its circulation is not predictable. It is possible that, even if a patient is infected, the collected sample does not have an adequate amount of the parasite DNA leading the test to a negative or undetectable result. A possible solution to this limitation is the collection of several serial blood samples at different times or increasing the blood volume per test may overcome this problem [31]. Recently, an international survey evaluated PCR methods for the detection of *T. cruzi*, showing large variations in accuracy and a lack of quality controls worldwide among the 48 reviewed PCR studies. This international collaborative study was launched by expert PCR laboratories from 16 countries from which the four best performance tests were evaluated. The results indicated the limitations of PCR for the diagnosis of patients with chronic disease, and the authors recommended PCR only for posttreatment follow-up, the diagnosis of congenital disease in newborns, post-organ transplantation control, AIDS patients and oral transmission patients [27]. Others authors distinguish what its usefulness is primarily focused in the chronic phase, congenital Chagas diagnosis and post therapeutic follow-up due to its ability to detect low parasitemias [32]. In conclusion the PCR is a very powerful molecular tool due to its high level of sensitivity and specificity for detection of *T. cruzi* in blood, which has many advantages over conventional methods for the diagnosis of Chagas disease. These excellent features makes it a suitable tool for the follow-up a chemotherapeutic treatment of chagasic patients. Another important feature of this test is that it can be used to detect the elimination of parasites in blood or cardiac cells [9].

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