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Case Reports

Molecular diagnosis and treatment monitoring of congenital transmission of *Trypanosoma cruzi* to twins of a triplet delivery☆

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Abstract

Congenital transmission of *Trypanosoma cruzi* was diagnosed in 2 triplets born to a triamniotic bichorionic delivery. Only the sisters sharing the placenta became infected, as diagnosed by microhematocrit and/or polymerase chain reaction of 3 parasite targets. The neonates' parasitologic response to benznidazole was monitored. Molecular strategies allowed genotyping lineage IId and identical minicircle signatures in both triplets, showing they harbored the same maternal parasite populations.

Keywords: Triplets; Congenital Chagas disease; PCR diagnosis; *Trypanosoma cruzi* lineage; Minicircle signatures; Placenta

Trypanosoma cruzi, the causative agent of Chagas disease, infects 10 to 18 million people in 21 endemic countries of America (World Health Organization [WHO], 2002).

The success of the control of vector-transmitted Chagas disease and screening programs in blood banks in the reduction of the incidence of human cases has uncovered the public health relevance of congenital transmission of Chagas disease (CCD), which has been gradually emerging in nonendemic areas, being partially responsible for the urbanization of Chagas disease (Freilij and Altcheh, 1995; WHO, 2002). The transmission rates of CCD vary in different geographic regions, ranging from 0.1% in regions of Brazil and Argentina to 7% or more in some areas of Bolivia, Chile, and Paraguay (for a review, see Schijman, 2006). The vertical transmission of *T. cruzi* cannot be prevented, but early diagnosis enables prompt treatment, achieving cure rates close to 100%, thus, avoiding disease progression (Freilij and Altcheh, 1995; Schijman et al., 2003). Early diagnosis of CCD is assessed by microhematocrit (MH) or microstrout concentration methods (Freilij and Altcheh, 1995). In cases of negative MH findings, polymerase chain reaction (PCR) tests, which depict higher sensitivity, may be applied (Russomando et al., 1998; Schijman et al., 2003; Solari et al., 1998; Virreira et al., 2003). Herein, we describe the molecular diagnosis and follow-up of an extraordinary case of triplets born to a Chagas disease mother from a tri-amniotic bichorionic delivery.

1. Case report

Triplet sisters were born to an 18-year-old woman with indeterminate Chagas disease, who had acquired *T. cruzi* at the endemic province of Chaco, Argentina, and did not receive trypanocidal treatment. The cesarean delivery was performed in Buenos Aires city, which is nonendemic for Chagas disease. The newborn triplets were delivered at gestational week 35, without signs or symptoms of

☆ The study was approved by the Ethics Committee of the Htal. I. Pirovano under written informed consent for PCR analysis.

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hepatomegaly or myocarditis, which may be detected in a proportion of CCD newborns (Freilij and Altcheh, 1995; Schijman, 2006).

The 3 sisters were studied at 16 days of life by MH, being only sister II (Fig. 1A) MH positive. A 2nd sample was withdrawn 30 days after delivery in the remaining sisters, being sister I MH positive and sister III MH negative (Fig. 1A). Sister III was diagnosed as noninfected at 7 months of age on the basis of her negative serologic reactivity (indirect hemagglutination test <1:16 [Polychaco, Argentina] and ELISA (ratio <1.2; Wiener, Argentina), after consensus criteria for CCD diagnosis (Freilij and Altcheh, 1995; WHO, 2002) (Fig. 1A).

For molecular diagnosis, 1 mL of neonatal blood was obtained and mixed with an equal volume of buffer guanidine 6 mol/L–EDTA 0.2 mol/L, pH 8.0 (GEB) (Schijman et al., 2003). Three different PCR procedures were carried out from 100-μL aliquots of GEB samples: the 330-bp variable (vkDNA) and 120-bp conserved (ckDNA) regions of the minicircle genome (Avila et al., 1991) and the 195-bp repetitive nuclear satellite sequence (Sat-DNA) (Diaz et al., 1992) were amplified using a Taq DNA polymerase coupled with proprietary antibody for hot start PCR (Taq Platinum DNA polymerase; Invitrogen, Carlsbad, CA) (Burgos et al., 2007).

A

Patient	weight of birth (gr)	Diagnostic method	Age at diagnosis 16d	30 d	75d	7m
Sister I	1900	MH				ND
		T.cruzi PCR	ND			
		Serology				
Sister II	1970	MH				ND
		T.cruzi PCR				
		Serology				
Sister III	1200	MH				ND
		T.cruzi PCR	ND			
		Serology				

B

Target	Sequence	*T. cruzi* lineage I	IIb	IIe	IId	Sister I	Sister II
Nuclear	SLIS	475	425	425	425	425	425
Genomic	24s α rDNA	124	139	139	124+139	124+139	124+139
Markers	18s rDNA	175	165	165	165	165	165
Minicircle Variable Region	ATGAGGGG	Y	N	N	N	N	N
	GGTAGTAT	N	Y	Y	N	N	N
	TATTAGATT	Y	N	Y	N	N	N
	TAGTGGT	Y	Y	Y	N	N	N
	TGGGTAG	N	N	Y	Y	Y	Y
	TATAGTTT	Y	Y	Y	Y	Y	Y
	TGTTTGA	Y	N	Y	Y	Y	Y
	AGTTGTTA	Y	N	N	Y	Y	Y
	TATGTTT	Y	Y	Y	Y	Y	Y
	ATTATGTT	Y	Y	Y	Y	Y	Y

Fig. 1. (A) Triplets were followed up by MH, PCR, and IgG-based serodiagnosis at 16, 30, and 75 days and at 7 months of life. Black boxes = positive findings; white boxes = negative findings; NA = not available; ND = not done; d = days; m = months; MH = microhematocrit; *T. cruzi*-PCR = amplification of the variable and constant regions of minicircle and the 195-bp satellite DNA sequence. Positive PCR findings mean that the 3 PCR protocols rendered amplification; serology: ELISA and indirect hemagglutination. Sister I received benznidazole treatment at 30 days of life and sister II at 16 days of life (arrows). (B) Repetitive strings in CCD twins' populations were detected in 330-bp variable minicircle amplicons annotated under the GenBank accession nos. DQ835646-66. SL-IS = intergenic spacer of the spliced leader region; amplicon sizes are in base pairs. N = not observed; Y = observed.

Sisters I and II gave positive PCR findings in all pretreatment tested samples (Fig. 1A). Treatment with benznidazole (5 mg/kg per day) for 60 days was given to both infected neonates when their MH-positive result was obtained. No adverse effects were observed; parasitologic response was assessed by negative parasitologic and PCR findings. Sister II showed positive PCR but negative MH findings 15 days after treatment initiation (Fig. 1A). Both patients' cure was confirmed by conversion of serologic tests to negative after their 7th month of age.

The parasite lineage was identified using a PCR-based algorithm targeted to the intergenic regions of spliced leader genes (SL-DNA), the D7 domain of the 24s-alpha ribosomal DNA, and a fragment of the 18s ribosomal DNA (Burgos et al., 2007) and by sequence analysis of repetitive motifs within the vkDNA amplified regions (Telleria et al., 2006) (Fig. 1B). Lineage IId was identified in both neonatal samples. To profile the parasite minicircle signatures in neonatal samples, fluorescent low stringency single primer PCR (LSSP-PCR) was performed from the vkDNA amplicons, using primer S35G as driver (Vago et al., 1996). LSSP-PCR showed peaks of the same molecular weight in both sisters' samples, confirming that the same parasite populations were transmitted to both twins, although minor differences in the areas of some fluorescent peaks were detected. For example, the relative abundance of peaks between 210 and 230 bp with respect to those between 285 and 295 bp differed in both sisters' samples (Fig. 2, see arrows), suggesting variations in the relative loads of the corresponding parasite subpopulations. The molecular characterization of the *T. cruzi* populations infecting the mother was shown in a previous work (Burgos et al., 2007), demonstrating that the whole bloodstream maternal *T. cruzi* populations were transmitted to both triplets.

As mentioned above, sister III was not infected; this could be explained because this triplet did not share the placenta of the other 2 CCD ones. This case points to the protective role of the placenta in preventing parasite invasion. The mechanisms by which the placental trophoblastic layer prevents fetal infection are not well understood; it has been shown that lysosomal enzymes expressed by the trophoblasts might restrict parasite propagation (Frank et al., 2000). Besides, a pathogenetic role for human placental alkaline phosphatase (PLAP) has been proposed; PLAP participates in the process of *T. cruzi* invasion into placental syncitiotrophoblast cells (Sartori et al., 2005). It is tempting to