SHORT REPORT: AMNIOTIC FLUID IS NOT USEFUL FOR DIAGNOSIS OF CONGENITAL TRYpanosoma CRUZI INFECTION

MYRNA VIRREIRA, SABRINA MARTINEZ, CRISTINA ALONSO-VEGA, FAUSTINO TORRICO, MARCO SOLANO, MARY CRUZ TORRICO, RUDY PARRADO, CARINE TRUYENS, YVES CARLIER, AND MICHAL SVOBODA*

Laboratoire de Chimie Biologique, Faculté de Médecine, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium; Facultad de Medicina, Universidad Mayor de San Simon (U.M.S.S.), Cochabamba, Bolivia; Laboratoire de Parasitologie, Faculté de Médecine, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

Abstract. Although Trypanosoma cruzi can be transmitted transplacentally and induce congenital infection, no data are available about the presence of this parasite in human amniotic fluid. We examined 8, 19, and 4 amniotic fluid samples (collected at delivery or by aspiration of gastric content of neonates) from control uninfected mothers (M−B−), infected mothers delivering uninfected newborns (M+−B−), and mothers of confirmed congenital cases (M+−B+), respectively. Polymerase chain reaction (PCR), using nuclear and kinetoplastic parasite DNA primers (Tcz1-Tcz2 and 121-122), was performed for all control M−B− samples, but positive for 5 of 19 M+−B− and 2 of 4 M+−B+ samples. To determine the number of parasites in the positive samples, real-time PCR using S35/S36 kinetoplastic DNA was performed. Only one M+−B+ sample presented a high parasitic DNA amount, whereas the other six PCR-positive samples displayed traces of T. cruzi DNA. In conclusion, the release of parasites in amniotic fluid is probably a rare event that cannot be helpful for the routine diagnosis of congenital Chagas disease.

Trypanosoma cruzi, the causative agent of Chagas disease, is transmitted mainly by insect vectors, but also by alternative routes such as blood transfusion and congenital transmission. The maternal–fetal transmission rate of T. cruzi infection in the Southern cone countries varies widely from 1% in Brazil to 4–12% in Argentina, Bolivia, Chile, and Paraguay. As recently shown in studies of placenta from Bolivian mothers infected with T. cruzi, the trans-placental transfer of maternal blood parasites mainly occurs through the placental membranes rather than by crossing villous trophoblast. During the parasite multiplication in the chorial plate, amniotic cells can be infected, making possible the release of T. cruzi parasites in amniotic fluid (AF). If this occurs, AF could be considered as a possible biologic sample for the diagnosis of congenital infection, as is the case for the diagnosis of congenital toxoplasmosis and viral diseases. However, as far as we know, study of T. cruzi in human amniotic fluid has not been reported, and its predictive or diagnostic value remains unknown. This study aims to investigate the presence of parasitic DNA in amniotic fluids of T. cruzi-infected mothers, using polymerase chain reaction (PCR) methods.

Mothers were admitted to the Bolivian maternity German Urquidi (Universitary Hospital Viedma, Universidad Mayor de San Simon) in Cochabamba. Samples of AF were collected either at the time of membrane rupture before delivery, with precautions to avoid maternal blood contamination, or by aspiration of the gastric fluid content (GAF) in newborns, immediately after birth. A total of 31 AF/GAFs have been studied: 4 (all GAF) from congenital cases of T. cruzi infection (mothers and newborns are infected, M+−B+); cases 1–0311 and 1–0480 were asymptomatic; cases 1–0098 and 1–0899 displayed splenomegaly and hepatomegaly, respectively. 19 (16 AF and 3 GAF) from infected mothers having delivered uninfected babies (M+−B−), and 8 (all AF) from control cases (mothers and newborns are uninfected, M−−B−). Infection in mothers was assessed using standard serological tests, and congenital infection with T. cruzi was sought for by direct microscopic examination of blood buffy coat collected in microhematocrit heparinized tubes, or hemoculture, as previously described. The absence of T. cruzi infection in parasitologically negative newborns was confirmed by PCR performed on umbilical cord blood. Clinical data of newborns were collected as previously described. This study was approved by the scientific/ethic committees of the “Universidad Mayor de San Simon” and the “Université Libre de Bruxelles,” and written consent of the informed mothers was obtained before sample collection.

Parasites were not detected by direct microscopic examination of centrifugation pellet of eight AF samples of 5 mL from M+−B− mothers, and the other samples were not examined using this parasitological technique. All AF/GAF samples (0.5–5 mL) were mixed with the same volume of the buffer guanidine-HCl, 6 mol/L, and EDTA 0.1 mol/L and boiled for 15-minute DNA extraction was performed on 200 μL of guanidine-mixed samples, using the “QuiAmp DNA blood” kit (Qiagen) according to the manufacturer’s instructions. PCR amplifications were made using primers targeting either nuclear (primers TCZ1/TCZ2) or kinetoplastic parasite DNA (primers 121/122), as previously described. In all PCR amplifications, DNA extracted from a negative sample (AF from uninfected mother) was included. All PCR were performed at least twice in duplicates. A PCR amplification of a fragment of the human β-globin gene was systematically performed to assess the integrity of extracted DNA.

To get information on the parasite amount detected in PCR-positive samples, the relative intensity of their kDNA amplicons (obtained with primers 121/122) was compared with those of amplicons obtained, in the same PCR assay, from DNA prepared from known amounts of T. cruzi parasites. Parasite amounts equivalent to 0.0002, 0.02, and 2 parasites/assay corresponded to 0.4, 0.8, and 10,000 parasites/mL of extracted material, taking in account the dilution with guanidine and that performed during extraction of DNA (Figure 1). To obtain more accurate quantitative information on PCR-positive samples, real-time PCR (qRT-PCR) using kinetoplastic DNA primers (modified S35/S36) was performed as previously described, increasing the hybridization tempera-
ture to 63°C, to reduce amplification of unspecific bands originating from human DNA. The SyberGreen system (Roche Diagnostic, Vilvoorde, Belgium) in LightCycler ver.2 apparatus (Roche Diagnostic, Vilvoorde, Belgium) was used according to the manufacturer’s instructions. The same DNA amount of negative AF sample was added to all reference standard samples. As shown in the Figure 2A, a reproducible correlation could be obtained between the number of cycles and the number of parasites ranged from 0.0002 to 2 parasites/assay (corresponding to 0.4–4,000 parasites/mL).

All the eight control AF samples were negative in PCR for both TCZ1/TCZ2 and 121/122 primers. Five of the 19 (26.3%) M+B− and 2/4 (50%) of M+B+ AF/GAF samples were PCR positive for both kinds of primers. Comparisons of amplicon intensities of all the positive PCR samples with amplicons from known amounts of parasites showed only one M+B+ sample (GAF from the asymptomatic case 1-0311) displaying a strong intensity corresponding to several thousands of parasites per milliliter. All the other positive samples (one of the M+B+ group 1-0899 and five of the M+B− group 1-0343, 868, 962, 1025, 1057) showed lower amplicon intensities corresponding roughly to 1 parasite/mL. Complementary studies by qRT-PCR confirmed these observations. The M+B+ GAF sample 1-0311 showed a high concentration of parasites (18,400 parasites/mL), whereas all the other samples that were weakly positive in PCR displayed very low T. cruzi DNA concentrations, around the limit of reliable qRT-PCR detection, corresponding to < 0.4 parasites/mL (Figure 2B).

However, the electrophoretic analysis of these qRT-PCR products showed the presence of the expected amplicon, showing the actual presence of T. cruzi DNA traces in these samples.

Altogether our results show T. cruzi parasites being hardly detectable in AF (collected from amniotic sac or aspirated from newborn stomach), because 1) only one of the four samples from mothers of congenital cases (M+B+) displayed a significant and PCR-detectable parasite DNA amount and 2) the other M+B+ PCR-positive sample contained only traces of parasitic DNA. Moreover, 26% of AF/GAF from M+B− mothers delivering uninfected babies also displayed such DNA traces. This might suggest that placenta of M+B− mothers might also be infected without parasite transmission to the fetus, in agreement with previous reported data. However, detection of such traces of parasitic DNA raises the question of the actual presence of living parasites in samples. Indeed, sample contamination by maternal blood cannot be formally excluded. Although serious precautions have been taken for AF collection from amniotic sac, GAL might have been contaminated by swallowing maternal blood during vaginal delivery of neonates (all PCR-positive samples came from vaginally delivered babies). The GAF containing significant parasite amount was from an asymptomatic congenital case, suggesting that parasite detection in AF probably does not relate to Chagas disease severity. Moreover, parasites can be more easily detected in umbilical cord blood. Therefore, if T. cruzi can be released in AF after placental invasion, this is probably a rare event that cannot be helpful for the routine diagnosis of congenital Chagas disease.

Received June 21, 2006. Accepted for publication July 24, 2006.

Acknowledgments: The authors thank Marisol Cordova and the staff of the maternity German Urquidi (Cochabamba, Bolivia) for the management of patients; Miguel Guzman, Myrian Huanca, Rudy Parrado, and Marco Antonio Solano (CUMETROP/LABIMED, U.M.S.S., Cochabamba, Bolivia) for the serological and parasitological diagnosis of patients.

Financial support: M. Virreira is fellow of the “Xénophilie” grant (ULB, Belgium). C. Alonso-Vega is fellow of the “Association pour la Promotion de l’Education et la Formation à l’Etranger” (APEFE, APEFE,
“Communauté Française de Belgique”). This study was supported by the Conseil Interuniversitaire de la Communauté Française de Belgique (CIUF), the Fonds National de la Recherche Scientifique Médicale (Belgium, conventions 3.4.595.99/3.4.615.05), and the Community and Child Health (CCH) Chagas control program (USAID).

Authors’ addresses: Myrna Virreira, Sabrina Martinez, and Michal Svoboda, Laboratoire de Chimie Biologique, Faculté de Médecine, Université Libre de Bruxelles (U.L.B.), Route de Lennik 808, CP 611, B-1070 Brussels, Belgium. Cristina Alonso-Vega, Faustino Torrico, Marco Solano, Mary Cruz Torrico, and Rudy Parrado, Facultad de Medicina, Universidad Mayor de San Simon (U.M.S.S.), Avenida Aniceto Arce 371, casilla 922, Cochabamba, Bolivia. Carine Truyens and Yves Carlier, Laboratoire de Parasitologie, Faculté de Médecine, Université Libre de Bruxelles (U.L.B.), Route de Lennik 808, CP 611, B-1070 Brussels, Belgium.

REFERENCES