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

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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 kinetoplastid DNA primers (Tcz1-Tcz2 and 121-122), were negative 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 kinetoplastid 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.

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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 placentas 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 parasite-specific 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/ethnic 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 (Quiagen) according to the manufacturer’s instructions. PCR amplifications were made using primers targeting either nuclear (primers TCZ1/TCZ2) or kinetoplastid 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, 40, and 4,000 parasites/mL of extracted fluid, 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 kinetoplastid DNA primers (modified S35/S36) was performed as previously described, increasing the hybridization tempera-
mothers delivering uninfected babies also displayed T. cruzi parasites. 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.

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