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ORIGINAL ARTICLE

MATERNAL RISK FACTORS FOR THE TRANSMISSION OF CONGENITAL CHAGAS DISEASE

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ABSTRACT

The present article looks at the association between the epidemiological history of women infected with *Trypanosoma cruzi* and the risk of vertical transmission. Eighty-three chronically infected mothers and their 237 children were studied, using a cohort design. All patients reside in Santa Fe city, Argentina. Twenty-five women transmitted the infection to 38 children. The potential risk factors evaluated in the mothers were exposure to vector transmission, blood transfusion history, maternal seropositivity, parasitemia and age at birth of the child. 72% (18/25) of the mothers who transmitted the infection to their children, had little or no contact with the vector, while only 28% (7/25) of the mothers presented a history of medium or high risk of vector infection. The differences were significant ($p < 0.05$). Forty-one percent of the women who presented maternal history as the probable route of infection, transmitted the parasite to more than one child (1.86 ± 0.33; CI95% = 1.03-2.68). In addition, the most frequent history, among the women who transmitted the disease to their children, was the absence of exposure to vector transmission and transfusion with unknown maternal serology. The route of infection was probably transplacental. These observations suggest that there are family genetic characteristics involved in vertical transmission. The parasite was found in 71% of the mothers who transmitted the infection to their children and were able to perform xenodiagnoses. After controlling for the other variables, the logistic regression analysis showed that xenodiagnosis (+) is a risk factor for congenital transmission; the relative risk was 12.2 (95% confidence interval: 2.9 - 50.1). No differences were found when analyzing the mother's age and transfusion history. The highest risk of congenital transmission was associated with detectable parasitemia and less maternal exposure to the vector.

KEY WORDS: Chagas Disease; congenital transmission; epidemiological factors; *Trypanosoma cruzi*.

INTRODUCTION

Chagas disease is a parasitic infection caused by *Trypanosoma cruzi*; it is a serious public health problem in Latin America where *T. cruzi* is mainly transmitted to humans through the infected feces of an insect vector (triatomines/vinchucas). In addition, the infection can also occur through blood

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transfusion, organ transplantation, mother-to-child transmission and other less usual forms such as laboratory accidents and oral ingestion. According to estimates by the World Health Organization, between 6 and 7 million people are infected worldwide (WHO, 2018). Moreover, approximately 1,800,000 women of childbearing age are capable of transmitting the infection to their children (OPS, 2006).

Since the 1990s, important advances have been made in the fight against triatomines. These policies, complemented by blood bank control measures, have achieved a significant reduction in the number of infections with *T. cruzi* through vector and transfusion routes (Zaidemberg et al., 2004; Coura & Dias, 2009). In this scenario, transplacental transmission has acquired greater relative importance, as it is now the most frequent route of acute infection.

Migrations from rural to urban centers have led to the urbanization of Chagas disease. Furthermore, large numbers of people have migrated from Latin America to Europe, United States, Canada and Japan, countries with little knowledge of the disease and, consequently, with poor control measures in blood banks and obstetrical services. This situation has led to the globalization of Chagas disease (Klein et al., 2012).

At birth, a child with congenital infection may present clinical symptoms of varying severity, but most (70 to 80%) are asymptomatic. Therefore, it is necessary to follow-up every newborn child of an infected mother, using parasitological studies in the first months of life and serological studies after 10 months (Ministerio de Salud de la Nación, 2012). Children in whom infection is detected are most likely to be cured, given the effectiveness of trypanocidal treatment at this early stage. However, less than 20% of the children born to infected mothers receive timely diagnosis and treatment (Basombrio et al., 1999; Gurtler et al., 2003; Spillmann et al., 2013).

At present, there is still not enough knowledge about the risk factors involved in congenital transmission. Several factors such as the parasite strain, as well as the immunological status and genetic characteristics of the mother could be involved.

Other possible factors involved in the risk of congenital transmission are the epidemiological history of the mothers (age, geographic origin and length of residence in an endemic area and risk of reinfection, length of stay in a zone with low or zero exposure to vector transmission, route of infection, detectable parasitemia and transmission in family groups, among others). At the Centro de Investigaciones sobre Endemias Nacionales (CIEN), patients infected with *T. cruzi* have been followed up for more than three decades. These cohort studies make it possible to take repeated measures in order to know the personal characteristics and/or exposure of the mother and her children. This information is useful for investigating the factors that could be involved in congenital transmission.

This work analyzes whether there is an association between the epidemiological history of mothers chronically infected with *T. cruzi* and the congenital transmission of the parasite.

MATERIAL AND METHODS

This study was carried out at the CIEN, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe city, Argentina.

This Center has performed epidemiological, serological and clinical follow-up of people infected with *T. cruzi* from 1977 to the present. The patients who attend the center arrived spontaneously or were referred there from other services.

Routine of the service

After confirming *T. cruzi* infection in the patients by means of serological studies of venous blood samples, a clinical examination, including ECG and chest X-ray, is performed.

Personal, family, epidemiological, serological and clinical data are recorded in a previously prepared form. The clinical and cardiological controls of adult patients are performed by a cardiologist. Children and newborns of an infected mother are controlled by a pediatrician. Epidemiological, serological and clinical controls of patients (stable residents in Santa Fe city) chronically infected with *T. cruzi* are carried out biannually. The data obtained in each control are recorded in the clinical histories of the respective patients. The information is always collected by the same medical specialists and/or biochemistry professionals, as appropriate.

Design

On the whole, the study presents a retrospective observational cohort design, although the information obtained during the writing of this paper was also included (February 2016 - December 2017).

The cohort was made up of mothers infected with *T. cruzi*, with epidemiological, serological and clinical follow-ups, selected by convenience sampling among the patients who attended CIEN from 1990 to December 2017. The children of these women were studied in order to detect congenital infection.

Children born during the study were controlled until their first year of life. The children born before the study were invited to the Center to confirm or rule out infection and for collection of migratory and transfusion data.

Inclusion criteria

- For the mothers

a) positive serology for *T. cruzi* infection, in at least two out of the three tests performed: Indirect Hemagglutination (IHA); Indirect Immunofluorescence (IIF) and Direct Agglutination with 2-mercaptoethanol (DA) or an Enzyme-linked Immunoassay (ELISA);

b) stable residence in the city of Santa Fe for 5 years or more;

c) without trypanocidal treatment before the birth of the children;

d) epidemiological data (place and date of birth; places of residence and time of permanence; type of previous housing and peridomicile; presence or absence of vinchucas; transfusions before the birth of their children; a history of maternal serology for *T. cruzi* infection; other means of discovering that they were infected).

- For the biological children of these women:

a) confirmed diagnosis of absence or presence of *T. cruzi* infection by serological methods after 10 months of life, or by parasitological methods (xenodiagnosis or Strout) in the first months of life;

b) birth data (place, date, birth weight, type of delivery, gestational age), places of residence prior to diagnosis, previous transfusion history.

Data collection

The infected women were screened using their medical records. Those without children were excluded. The medical records of all mothers were preselected. After verifying the information contained in the medical record of each mother and her offspring, we proceeded to:

a) the selection of mothers and children if they met all the inclusion criteria;

b) make an appointment with the mothers in our Center and, if necessary, with their children, in order to obtain missing data and/or blood samples for laboratory studies.

The information collected from the mothers and their children was subsequently uploaded to a database generated *ad hoc*.

Assessing exposure to vectorial transmission

In order to estimate the risk of exposure to vectorial transmission, a composite quantitative variable "VET" was constructed, based on a score calculated by combining the mother's housing condition (in 2 levels: 0.5-1), endemicity level of place of previous residence (in 4 levels: 0 to 3), and

duration in years of residence in a transmission area (1: less than one year; 2: between 1 and 5 years; 3: from 5 to 10 years, and 4: greater than 10 years).

The VET score was attributed values from 0-12, and then grouped in fourth strata (null, low, moderate and high) for the analysis.

Definition of maternal-fetal transmission of Trypanosoma cruzi

Newborn with positive parasitological test (xenodiagnosis or Strout) in the first months of life and/or with at least two serological tests (IHA, IIF, DA or ELISA) after 10 months of life presenting maternal serology as the only infection route.

Ethics

The working protocol was approved by the Ethics and Security Committee of the Faculty of Biochemistry and Biological Sciences, at the Litoral National University.

All women were informed of the purpose of the study, and those who agreed to participate signed an informed consent form in their name and in the name of their under aged children.

Statistical analysis

The comparison of proportions was performed using Fisher's exact test or Pearson's $\chi 2$; Student's t-test was used to compare the means.

The relationship between the maternal factors and the transmission risk was analyzed by logistic regression. In all cases, the relative risk (RR) and respective confidence intervals (CI 95%) were calculated. Analysis was conducted in Stata 9.0.

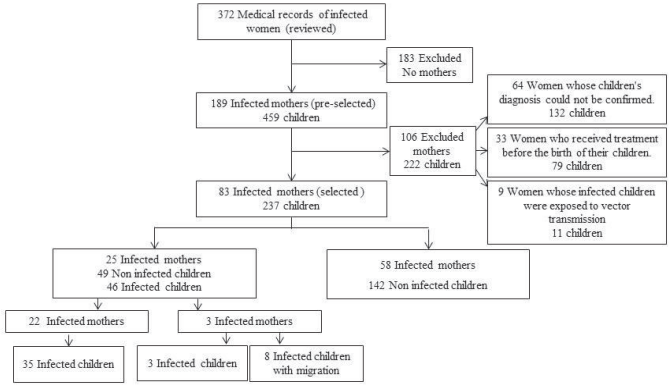
RESULTS

The medical records of 372 women of childbearing age, with chronic *T. cruzi* infection, who attended the CIEN were reviewed. One hundred eighty-nine mothers were pre-selected from which the following were excluded: 64 women whose children's diagnosis could not be confirmed at CIEN; 33 women who received treatment before the birth of their children and 9 mothers whose infected children were exposed to vector transmission.

The inclusion criteria were met by 83 mothers, and 237 biological children (Figure 1). Twenty-five mothers transmitted the parasite to 38 children (38/237 = 16 %), whose only antecedents were positive maternal serology.

All infected children received specific antiparasitic treatment.

Figure 1. Study Flowchart



Of the 237 children, 32 were followed-up from their birth and during the first months of life by parasitological methods (Strout). Infection was detected in 4 cases. In the rest, follow-up was carried out by serological methods after 10 months of age (average 13 months). In the 205 children who entered the study after 24 months of age, a serological diagnosis was confirmed at an average age of 8 years.

From February 2016 to December 2017, 6 newborns and 10 children between 4 and 10 years of age were included.

According to the history of the 83 mothers analyzed, the probable routes of infection were the following: 44.6% (37/83) were born and/or lived in areas presenting risk of vector infection; 39.7% (33/83) received transfusions before the birth of their children. Only 20 women reported a maternal history of infection: 17 were infected and 3 were not (serology was confirmed at CIEN). The history of many of them included more than one factor described in Table 1.

The geographical origin of the women studied was: Santa Fe 75%; Chaco 10%; Entre Ríos 7%; Santiago del Estero 4% and others 4%.

Regarding the risk of VET of the 25 mothers who transmitted the infection to their children, 72% had little or no contact with the vector during their lifetime, while 28% presented history of medium or high risk of vector infection. The differences were significant (Pearson's $\chi 2$, $p < 0.05$).

A transfusion history was reported by 11 out of 25 mothers (44%) who transmitted the infection to their children and by 22 out of 58 mothers (38%) who did not transmit it (Pearson's $\chi 2$, $p > 0.05$).

The average age of the mothers who transmitted the infection to their children was 24.05 ± 6.08 years, and those who did not transmit the infection had an average age of 24.74 ± 5.34 years. There were no differences in the ages of mothers at the birth of children with or without congenital infection (unequal variance student's t-test, $p > 0.05$).

Table 1. Joint distribution of mothers according to probable routes of infection: vector (VET), transfusion and vertical (infected mother).

VET	Infected Mother		Unknown Mother Serology	Transfusion + Infected Mother	Transfusion + Uninfected Mother	Transfusion + Unknown Mother Serology	Total
	Yes	No					
Medium or high	6	0	19	0	1	11	37
Null or low	7	1	17	4	1	16	46
Total	13	1	36	4	2	27	83

Table 2. Potential maternal risk factors associated with congenital *T. cruzi* transmission

Maternal factor	Transmission (+) (N=25)	Transmission (–) (N=58)	Relative Risk (CI 95%)	p value
Mother age	24.05 ± 6.08	24.74 ± 5.34	0.94 (0.89 – 1.00)	0.059
VET				
Null or low	18/25 (72%)	28/58 (48%)		
Medium or high	7/25 (28%)	30/58 (52%)	0.36 (0.14 – 0.97)	0.046
Transfusion				
Yes	11/25 (44%)	22/58 (38%)		
No	14/25 (56%)	36/58 (62%)	1.28 (0.49 - 3.32)	0.605

Xenodiagnosis was performed in 35 out of 83 mothers studied. This parasitological test was positive in 17 women (48.6%). Of the 25 mothers who transmitted the infection, xenodiagnosis was performed on 7 of them and the parasite was found in 5 (71%).

Table 2 shows the results obtained in the logistic regression analysis of each maternal factor studied (mother's age at the birth of the child, VET and transfusion).

When the risk of mother-to-child transmission, based on parasitemia (xenodiagnosis), was subjected to a multivariate analysis through logistic regression, controlling the mother's age at the birth of the child, the transfusion history and VET, xenodiagnosis (+) was a risk factor in the *T. cruzi* mother-child transmission (RR = 12; CI 95% = 2.9 - 50.1).

The history of maternal infection could not be analyzed because most of the mothers were unaware of its existence (63/83). However, when the risk of congenital transmission was analyzed in the women whose maternal serology was confirmed, this antecedent (+) (grandmother +) was present in 17 mothers. Of these, 7 transmitted the infection to 13 children; the average number of children with congenital infection per mother was 1.86 ± 0.33; CI 95% = 1.03 - 2.68. The 3 women with maternal history of infection (-) (grandmother -) had 7 children (-).

In the mothers who transmitted the infection to their children, the most frequent epidemiological profile was: unknown maternal serology (grandmother), no transfusions and null or low VET (7/25); the second most frequent unknown maternal serology (grandmother), transfusions and null or low VET (6/25). In the mothers who did not transmit the infection, the predominant profile was unknown maternal serology, no transfusions and high VET (16/58).

Three out of 83 mothers had twin pregnancies; one of them transmitted the infection to both her 2 twin sons and also to a later child. The other two mothers did not transmit the infection to their twin children nor to their other children.

The moment in which the mothers found out about their infection status was: 33.7% (28/83) pregnancy control; 32.5% (27/83) asymptomatic check up; 15.7% (13/83) pre-employment examination; and 18% (15/83) in other circumstances (mother and/or infected sybling, entry to the university, blood donor and others).

DISCUSSION

In this study, a greater risk of transplacental transmission was observed in women with little or no contact with the vector during their lifetime, compared to those who lived a longer time exposed to vector infection.

These results agree with the observations made by other researchers (Rendell et al., 2015; Kaplinski et al., 2015). They also found that parasitemia was higher in the women who did not live in houses infested by triatomines, suggesting that greater exposure to the vector might control the level of parasites in the blood and reduce the risk of vertical transmission (Rendell et al., 2015).

We evaluated the level of parasitemia through xenodiagnosis. By multivariate logistic regression, the probability of transmission was 12.2 times higher in mothers with xenodiagnosis (+) compared to those with xenodiagnosis (-). Several studies have shown that a high parasite load increases the risk of congenital transmission (Kaplinski et al., 2015; Brutus et al., 2010; Bua et al., 2012; Bern et al., 2009; Murcia et al., 2012).

However, it is necessary to take into account that a possible measurement bias could affect the results of this study. The only parasitological method used in the mothers studied was xenodiagnosis. This presents low sensitivity in the chronic phase and was not performed on all mothers.

Forty-one percent (7/17) of the women who presented maternal history as the probable route of infection transmitted the parasite to more than one child (1.86 ± 0.33; CI 95% = 1.03 - 2.68). In addition, in the case of the twin pregnancy when both children were born infected, the mother also transmitted the infection to further children. These observations suggest that there are family genetic characteristics involved in vertical transmission. Previous research (Zulantay et al., 2013; Sanchez Negrette et al., 2005) found a higher occurrence of congenital transmission in family groups.

The most frequent profile among the women who transmitted the disease to their children proved to be the "absence of exposure to vector transmission and transfusion, with unknown maternal serology". Most women analyzed did not know the serology of their own mothers and thus it was not possible to reliably measure the significance of this antecedent but the absence of vector exposure and transfusions suggests that the route of infection was transplacental.

The presence of a transfusion history was not significant for the risk of congenital transmission. Similarly, no differences were found between the ages of the mothers at the birth of their children with or without congenital infection, coinciding with other investigations (Rendell et al., 2015; Sánchez Negrette et al., 2005) and differing from yet others (Bittencourt et al., 1985; Torrico et al., 2004).

The sample in the present study, made up of patients that attended CIEN spontaneously or were referred there from other services, may be affected by a selection bias as the population sample was selected by convenience sampling, not randomly or probabilistically, therefore we could not estimate the real congenital transmission rate.

Considering this limitation, cases of congenital Chagas disease occurred in 38 of the 237 children analysed (16%). Although this percentage cannot be compared to the values reported for our region which range from 1% to 12% (Carlier & Torrico, 2003), there is, therefore, difficulty in determining the real incidence of congenital Chagas disease. This comes to light when observing that the majority of children with congenital infection were diagnosed after their first year of life, at an average age of 8 years.

Studies performed by this group (Fabbro et al., 2014) and other researchers (Sosa Estani et al., 2009; Moscatelli et al., 2015) showed that the risk of transplacental transmission is significantly reduced if the mother receives trypanocidal treatment prior to pregnancy. It is necessary to extend the coverage of early diagnosis to be able to provide timely treatment. The urgency of this is highlighted by the fact that 33.7% of the mothers analyzed became aware of their infected condition during pregnancy control, when the administration of trypanocidal drugs is contraindicated.

Late diagnosis in mothers and their children are risk factors that perpetuate the chain of parasite transmission. Although this was not the main objective of this study, we presented and discussed it because of its health impact.

The mechanisms underlying mother-child transmission of the parasite are very complex. The factors that intervene in this process have not been elucidated so far. The results of several studies, (Carlier & Truyens, 2015; Vekemans et al., 2000; Cuna et al., 2009; Hermann et al., 2004; García et al., 2008), suggest that the transmission and morbidity of congenital Chagas disease are a product of the complex interaction between one or more infectious strains of the parasite and the immunological status of mother and fetus; both of which are influenced by environmental and genetic factors.

Some recent studies have found differences in congenital transmission according to the type of *T. cruzi* that predominates in each region (Luquetti et al., 2015).

Based on these results, this group is studying the possible influence of specific infectious strains of *T. cruzi* and the genetic components of the mothers on the vertical transmission of the parasite.

Even though further research is required to determine with greater precision the risk of congenital transmission, the results of this study suggest establishing health strategies for the early detection of infected girls and young women, and for the monitoring and diagnosis of their children before the first year of life.

This is even more necessary given that most women live in areas of low or no endemicity, and less maternal exposure to reinfections increases the risk of congenital transmission.

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