

# Etiological treatment of young women infected with *Trypanosoma cruzi*, and prevention of congenital transmission

Tratamiento etiológico de mujeres jóvenes infectadas con *Trypanosoma cruzi* e prevención da transmissão congênita

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## ABSTRACT

The objective was to detect *Trypanosoma cruzi* infection in 32 children in Salta, Argentina, born to 16 chronically infected young women who were treated with benznidazole. Tests were performed to assess the efficacy of treatment after 14 years. At the end of the follow up, 87.5% of the women were non-reactive to EIA tests, 62.5% to IHA and 43.8% to IFA. 62.5% of the women were non-reactive according to two or three serological tests. No infected children were detected among the newborns of mothers treated before their pregnancy.

**Key-words:** *Trypanosoma cruzi*. Chagas disease. Treatment. Congenital transmission. Prevention.

## RESUMO

O objetivo foi detectar a infecção do *Trypanosoma cruzi* em 32 crianças nascidas de 16 jovens mulheres cronicamente infectadas e tratadas com benznidazol, em Salta, Argentina. Testes foram feitos para avaliar a eficácia após 14 anos do tratamento. Ao final do seguimento 87.5% das mulheres foram não reativas ao EIA, 62.5% ao IHA e 43.8% ao IFA. 62.5% das mulheres foram não reativas de acordo a 3 ou 2 testes serológicos. Nenhuma criança infectada foi detectada entre os recém-nascidos de mães tratadas antes da gravidez.

**Palavras-chaves:** *Trypanosoma cruzi*. Doença de Chagas. Tratamento. Transmissão congênita. Prevenção.

Chagas disease, or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi*. It is a major cause of morbidity and mortality in Latin America. Infection is transmitted mainly by vectors but also congenitally and by transfusion of infected blood.

Specific treatment with benznidazole® 5-7 mg/kg/day for 60 days proved effective among children aged 6 to 12 years during the chronic phase of *Trypanosoma cruzi* infection<sup>15,23</sup>. The goals of specific treatment against *Trypanosoma cruzi* infection are to eliminate the parasite from infected individuals, to decrease the chances of developing the illness (Chagas disease) and to

interrupt the chain of *Trypanosoma cruzi* transmission<sup>22</sup>. Focusing on interrupting the chain of transmission, the hypothesis is that etiological treatment of infected women of reproductive age may prevent congenital transmission. However, the most up-to-date etiological treatment with currently available drugs is not recommended for pregnant women<sup>19</sup>. The objective of this study was to assess *Trypanosoma cruzi* infection among children born to women who were treated with benznidazole at young ages (6 to 15 years old). The information acquired will enable further evaluation of etiological treatments for preventing congenital *Trypanosoma cruzi* transmission.

## MATERIAL AND METHODS

In this study, we assessed the presence of specific anti-*Trypanosoma cruzi* antibodies and parasites in blood samples from women who received treatment when they were 6 to 15 years old and who were evaluated 14 years later. We also assessed the presence of specific *Trypanosoma cruzi* antibodies in their children older than nine months of age. We carried out a clinical observational study in eight villages in the Departments of Mosconi, San Martín and Orán in the north of the Province of Salta in Argentina. Women were treated with benznidazole 6mg/kg/day for 60 days in 1991, and were evaluated in 2005. Also in 2005, all

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children born to these women undergoing follow-up were studied by means of serological tests to detect specific *T. cruzi* antibodies. This area has been under continuous triatomine surveillance by sanitary agents since 1982 (i.e. they undertake regular insecticidal actions against infestations).

In 1991 and 2005, 8-10ml of maternal venous blood was obtained, and in 2005, 5ml of blood was obtained from their offspring. Whole blood samples (1ml) were stored with equal volumes of guanidine in order to perform the polymerase chain reaction (PCR), and serum was obtained by centrifugation. The serum was stored in two aliquots; one of them was frozen at -70°C and the other one was stored with an equal volume of buffered glycerin. The samples with glycerin were later used for determining antibodies, when performing serological tests. We carried out an enzymatic immunoassay (EIA)<sup>11</sup>, an indirect hemagglutination assay (IHA)<sup>9</sup>, an indirect immunofluorescence assay (IFA)<sup>1</sup> and an EIA using F29 antigen<sup>20</sup>. The guanidine-EDTA blood (GEB) mixture was stored at room temperature for the first seven days, and then at 4°C until DNA extraction<sup>24</sup>. The PCR was performed following the technique described by Wincker et al<sup>28</sup>. Mothers were tested in 1991 and 2005 and their offspring were tested in 2005 with the same serological tests, under quality control<sup>12</sup>. The women were tested by means of xenodiagnosis in 2005 using four boxes, with 10 *Triatoma infestans* third or fourth-instar nymphs each<sup>10</sup>. All the tests were performed at the National Institute of Parasitology Dr. Mario Fatała Chaben in Buenos Aires, Argentina.

Descriptive data were presented as the prevalence with 95% confidence interval (CI) when appropriate, or as means and standard deviations for continuous data. McNemar's tests of serological and molecular data were used to make comparisons of change of status of infection between before treatment and 14 years after treatment. The means were compared using the ANOVA and Mann-Whitney U-tests.

The results from the serological tests were shown as log<sub>2</sub> of dilution of IHA (cutoff dilution ≥ 1/32) and IFA (cutoff dilution ≥ 1/32) and as absorbance of EIA (cutoff ≥ OD 0.200) and EIA-F29 (cutoff ≥ OD 0.170). The data analyses were done using Epi Info version 3.4 (CDC). This study was approved by CeNDIE committees, under the authority of the Minister of Health of Salta Province and the local hospitals of Pichanal, Embarcación and Gral. Mosconi. Informed consent to take samples was obtained from all women for themselves and their children. Signed informed consent for treatment of women in 1991, and oral informed consent were required for taking samples in 2005, in accordance with the current care guidelines for patients infected with *Trypanosoma cruzi*.

## RESULTS

Sixteen women who had children during the 14 years of follow-up and 32 offspring were included in the study. The median age of the women was 26 years (ranging from 21 to 29 years of age). The median number of children studied per mother was two (ranging from one to four children). Four (25%) mothers and eight (25%) children were living in rural areas. No special antecedents of illness were recorded during physical examinations. The median age of the children was five years (ranging from 1 to 11 years of age) and the frequency of females among the children was 53.1%. In 1991, all the mothers had at least two reactive serological tests, using EIA, HA and IFA, before treatment. **Table 1** shows the results from the serological tests on the mothers in 1991 and 2005 and the results from tests on their children in 2005. Most of the serological techniques performed on the women showed high rates of negativization at the end of follow up, that is, 14 years after treatment. The rate of negativization was mainly in relation to EIA (87.5%), EIA-F29 (77.8%) and IHA (57.1%). IFA shows a significant decrease in titers, although the rate of negativization (43.8%) was not significant 14 years after

**TABLE 1**

Evolution of serological tests on women treated with benznidazole in 1991 and their children born during 144 months of follow-up. Salta, Argentina, 1991-2005.

Follow up	EIA			IHA			IFA			EIA F29			
	n	reactive	%	P value	reactive	%	P value	reactive	%	P value	reactive	%	P value
<b>Mothers</b>													
pre-treatment 1991	16	16	100.0		14	87.5		16	100.0		9	69.2	
end of follow-up 2005	16	2	12.5	0.001	6	37.5	0.04	9	56.3	0.07	2	7.7	0.04*
<b>Children</b>													
controls 2005	32**	0	0.0	NA	0	0.0	NA	0	0.0	NA	0	0.0	NA
		Mean	SD	P value	Mean	SD	P value	Mean	SD	P value	Mean	SD	P value
<b>Mothers</b>													
pre-treatment 1991	16	0.512	0.108		7.4	3.2		6.8	0.9		0.291	0.218	
end of follow-up 2005	16	0.161	0.038	<0.0001†	3.1	2.7	0.0003	3.7	2.7	0.0001†	0.105	0.048	0.02***

EIA: enzymatic immunoassay, IHA: indirect hemagglutination assay, IFA: indirect immunofluorescence assay, NA: not applicable, EIA: enzymatic immunoassay using F29 antigen.

\* McNemar test was done with 13 samples matched in 1991 and 2005

\*\* One child was tested for EIA and IHA; both results were unreactive

\*\*\* Kruskal-Wallis test

† Serological tests on the mothers 1991-2005. Differences in proportions of reactive tests: EIA: 72.2% (95% CI: 30.1-83.0); IHA: 41.2% (95% CI: 1.2-52.6); IFA: 33.3% (95% CI: -2.3-44.2); EIA-F29: 50.0% (95% CI: 2.2-63.9)

treatment (Table 1). Final evidence of cure using serological criteria (three serological tests unreactive) was reached for six (37.5%) women, while four (25%) were discordant, with two tests non-reactive. None of the xenodiagnoses performed at the end of the follow-up were positive, although we found positive PCR results in two (12.5%) mothers (Table 2). No differences were found regarding age or residence in a rural area between the women with two or three unreactive serological tests and the women with two or three reactive serological tests.

Among the 32 children under study, we did not find any evidence of congenital *Trypanosoma cruzi* infection (Table 1). No exposure to vectorial or transfusional transmission could be demonstrated, and other serological tests were unreactive. We used the most sensitive current technique for diagnosing congenital *Trypanosoma cruzi* transmission, which is the serological test in infants older than nine months of age<sup>3 7 17</sup>.

TABLE 2

Parasitological and molecular tests on mothers treated with benznidazole, Salta, Argentina, 1991-2005.

Follow-up	Xeno				PCR			
	n	positive	%	P value	positive	%	P value	
Pre-treatment 1991	12	ND	-	-	11	91.7		
End of follow-up 2005	16*	0	0.0	NA	2	12.5	0.02**	

Xeno: xenodiagnosis, PCR: polymerase chain reaction, ND: not done, NA: not applicable.

\* Total number of mothers with xenodiagnosis test n=15.

\*\* McNemar test was done with 12 samples matched in 1991 and 2005.

PCR on mothers 1991-2005. Differences in proportions of positive results: PCR: 61.5% (95% CI: 8.4).

## DISCUSSION

Our results show the benefit of specific treatment for these women, who in most cases showed absence of parasitemia. The rate of positive xenodiagnosis among untreated patients who were infected during the chronic phase is expected to be between 30 to 50% for both adults and children<sup>10 23</sup>. We and others<sup>15 23 25 27</sup> have demonstrated that when patients are treated during the chronic phase of *Trypanosoma cruzi* infection and are cured, their antibodies disappear gradually and take several years to become definitively negative. Additionally, the time required to become negative differs depending on the test used<sup>23</sup>. On the other hand, consistent results have been observed among patients treated during the chronic phase, with evidence of absent or low parasitemia, even though antibodies were still present in the serum for a long term after treatment<sup>6 8 10 15 23 25</sup>.

Up until now, the benefit of specific treatment to clear or reduce parasitemia in infected populations, in order to prevent congenital transmission (one of the main non-vectorial means of transmission), had been hypothesized but never demonstrated.

The number of cases with congenital infection among this population was expected to be one or two cases, based on the rates of congenital infection recorded close to our study area, which have ranged from 4 to 13%<sup>5 7 16 18 21 29</sup>, but not a single case was detected among the offspring of these mothers treated before pregnancy.

The low number of patients under observation did not allow us to measure the risk of transmission among reactive mothers: only two (12.5%) mothers showed evidence of infection through positive PCR. It is known that reactive serological tests after treatment do not mean infection in all cases, and that discordance among tests after treatment suggests a degree of negativization during follow-up<sup>24</sup>. In the same way, no xenodiagnosis was positive after treatment, whereas at least 30% of xenodiagnoses are expected to be positive among adult patients with untreated chronic infection<sup>24</sup>. We can hypothesize that a positive effect regarding prevention of congenital transmission occurs in the population when at least 60% of the women treated before pregnancy show evidence of cure.

Our results support the concept that specific treatment for young women is useful at the level of secondary prevention because previously infected women were cured. Our results suggest that it is also helpful at the primary prevention level because such treatment would prevent congenital transmission. The main limitation of this study was its sample size and therefore an observation with a larger sample size will be necessary in order to confirm our finding. However, we do not have controls (untreated mothers) against which to measure the relative or absolute effect of the intervention for preventing congenital transmission. These limitations do not allow us to reach a final conclusion, but only to support the hypothesis.

For as long as appropriate drugs for use during pregnancy are not available, the alternative of treating young infected women or women of reproductive age (requiring contraceptive practices during the treatment) could be a highly effective strategy for preventing transmission of congenital *Trypanosoma cruzi*.

It is most important that we find timely methods for interrupting congenital transmission. After eliminating transmission by vectors and by blood transfusions, eliminating congenital *Trypanosoma cruzi* infection will be a critical final step in eradicating Chagas disease from large regions of Latin America.

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