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

Tratamento etiológico de mulheres jovens infectadas com *Trypanosoma cruzi* e prevenção 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.


**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 Martin and Oran in the north of the Province of Salta in Argentina. Women were treated with benzimidazole 6mg/kg/day for 60 days in 1991, and were evaluated in 2005.
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)\(^1\), an indirect hemagglutination assay (IHA)\(^2\), an indirect immunofluorescence assay (IFA)\(^3\) and an EIA using F29 antigen\(^4\). The guanidine-EDTA blood (GEB) mixture was stored at room temperature for the first seven days, and then at 4°C until DNA extraction\(^5\). The PCR was performed following the technique described by Wincker et al\(^6\). Mothers were tested in 1991 and 2005 and their offspring were tested in 2005 with the same serological tests, under quality control\(^7\). The women were tested by means of xenodiagnosis in 2005 using four boxes, with 10 *Triatoma infestans* third or fourth-instar nymphs each\(^8\). All the tests were performed at the National Institute of Parasitology *Dr. Mario Fatala 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 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**

<table>
<thead>
<tr>
<th>Follow up</th>
<th>EIA</th>
<th>IHA</th>
<th>IFA</th>
<th>EIA F29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-treatment 1991</td>
<td>16</td>
<td>16</td>
<td>100.0</td>
<td>14</td>
</tr>
<tr>
<td>end of follow-up 2005</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>0.001</td>
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<tr>
<td><strong>Children</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>controls 2005</td>
<td>32**</td>
<td>0</td>
<td>0.0</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
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</tr>
<tr>
<td>EIA</td>
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<tr>
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<td>0.108</td>
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<tr>
<td>end of follow-up 2005</td>
<td>16</td>
<td>0.161</td>
<td>0.038</td>
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</tr>
</tbody>
</table>

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. 

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. 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. 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.

**REFERENCES**


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