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# IgG Autoantibodies Induced by *T. cruzi* During Pregnancy: Correlation with Gravidity Complications and Early Outcome Assessment of the Newborns

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**Abstract** *Objective* The aim of the present research was to evaluate the correlation of vertically transmitted IgG antibodies induced by *T. cruzi* and newborn early outcome assessment, mainly birth weight and gestational age. *Methods* We performed a cross-sectional study with 183 pregnant women (64 with asymptomatic Chagas disease) and their newborns. Both were subjected to complete clinical examination. Peripheral parasitemia was assessed in mother and neonates by parasite detection through microscopic examination of the buffycoat from mother's peripheral and cord blood. Antibodies induced by *T. cruzi*, such as anti-FRA, anti-B13, anti-p2β and anti-*T. cruzi* were assessed by immunoassay. Birth weight, general condition evaluation by APGAR Score and gestational age by Capurro Score, were determined in newborns. *Results* The rate of stillbirth background and pregnancy-induced hypertension were higher in patients with Chagas disease ($p = 0.01$ and $p = 0.02$, respectively). Parasitemia was detectable in 17 mothers and 4 newborns. The newborns of mothers with detectable parasitemia presented decreased gestational age ($p = 0.006$) and body weight ($p = 0.04$). Mostly all the mothers with Chagas disease and all their newborns have positive values of antibodies induced by *T. cruzi*; however, only anti-p2β showed to be related to the presence of complication during pregnancy (OR 2.35, $p = 0.036$), and to low birth weight (OR 1.55, $p = 0.02$). *Conclusions* Low birth weight and decreased postnatal estimation of maturity were related to detectable parasitemia in the mother. Also, vertical transmission of *T. cruzi*-induced autoantibodies might have clinical implication in newborns given the negative association between anti-p2β values and weight.

**Keywords** Chagas disease · Antibodies · Anti-p2β · Low birth weight

## Significance

Although it has been described previously that *T. cruzi* induces antibodies with pathogenic role named anti-p2β and anti-B13, to our knowledge, they were only explored in regard to heart and digestive involvement in humans but not including pregnant women and newborns. However, it has been demonstrated the presence of antibodies induced by *T. cruzi* in congenital cases of Chagas disease. In this regard, we explored the relation between anti-p2β and anti-B13, and the clinical profile of pregnant women and their newborns finding that anti-p2β might be related to the presence of complications during pregnancy and a marker of low birth-weight.

Miguel Hernán Vicco and Luz Rodeles have contributed equally to this study.

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## Introduction

American Trypanosomiasis (Chagas disease), which is caused by the protozoan *T. cruzi*, nowadays is a worldwide infection and public health problem affecting several million people across America and Europe [1, 2]. *T. cruzi* transmission is caused mainly by the bite of an infected hematophagous triatome insect. However, another important mechanism of infection is transmission of the parasite from mother to newborn in about 5 % of pregnancies [3]. Although congenital Chagas disease was usually described as a symptomatic infection clinically presented with hepatosplenomegaly, cardiomegaly, lymphadenopathy, low birth weights and prematurity, it is well known that most cases of congenital transmission of Chagas disease are asymptomatic [3–7].

Although it has been widely disclosed that inflammatory response plays a major role in the development of symptomatic Chagas disease, it has been described that *T. cruzi* also might induce humoral response pathogenically involved in the progression of the disease [8]. Among the several auto antibodies described, antip2β and anti-B13 were implied in the induction of heart tissue lesions, in humans and in animal models [8–10]. Anti-B13 antibodies were shown to promote immune response to cardiac myosin because of the sequence homology between the B13 antigen epitope (AAAGDK) and the human cardiac myosin heavy chain hexapeptide (AAALDK) [9–12]. On the other hand, anti-p2β antibodies cross-react against the β1 adrenergic receptor (β1-AR) because of the antigenic acidic epitope present on the C-terminal end of the p2β, named R13 (EEEDDDMGFGLFD), which bears similarity to the AESDE acidic motif on the second extracellular loop of the β1-AR. These autoantibodies were linked to increased chronotropic effect and myocyte apoptosis [9, 13–17].

As regards pregnancy, it is well known that during the third trimester the production of antibodies and auto-antibodies is increased likely due to higher serum levels of estriol with estradiol which favor humoral responses [18–21]. Other important immunological process that occurs during pregnancy is the IgG placental transfer. This process, and the IgG concentration in newborn, is mainly related to the length of gestation and level of the motheŕs antibodies [22]. IgG antibodies placental transfer could be protective or harmful for the newborn; in several autoimmune pathologies, placental transmission of antibodies is detrimental to the neonate [23].

Considering the previous demonstration on the presence of anti-p2β in seven congenital cases [24], we aimed to analize whether there is correlation between the level of IgG auto-antibodies induced by *T. cruzi* in mothers and their newborns, regarding their potential contribution to the presence of gravidity complications and mainly, early clinical profile of the offspring.

## Materials and Methods

### General Study Population and Subject Evaluation

We performed a prospective cross-sectional study lasting 1 year in the public maternity hospital Odón Ortega in Yacuiba, in south Bolivia, which is an endemic region of Chagas disease. Informed consent for participation was obtained from subjects before their inclusion in the study. Ethical approval was obtained from ethics committees of the Faculty of Medicine and the Medical College of Bolivia. Sampled mothers were subjected to a complete clinical assessment. Data related to the presence of eclampsia, preeclampsia or hypertension induced by pregnancy was included. Most of the included individuals were from rural areas living in precarious houses.

As regards to the newborns, they were also subjected to a complete clinical assessment, including body weight, valuation of general conditions by the APGAR score (Appearance, Pulse, Grimace, Activity, Respiration) based on five criteria, and the presence of cardiac or gastrointestinal lesions. Gestational age of a newborn was estimated with the Capurro score expressed in days [25].

Serological test for *T. cruzi* in the mothers was performed according to the recommendation of the World Health Organization (with two different tests: ELISA and indirect hemagglutination or indirect immunofluorescence, yielding positive results) [26]. In the present work, both ELISA and indirect hemmagglutination were used for the diagnosis of Chagas disease. Only when there were discrepancies between the serological results, indirect immunofluorescence was employed in order to confirm or rule out the diagnosis of *T. cruzi* infection.

Exclusion criteria included: (a) age <18 years old; (b) symptomatic Chagas disease; (c) history of other heart disease and/or the presence of equivalents of coronary artery disease such as diabetes or peripheral arterial disease; (d) history of pregnancy induced hypertension or history of hypertension; (e) systemic complaints, concomitant infections; (f) high risk pregnancy, and (g) previous treatment with anti-*T. cruzi* compounds or immunosuppressive drugs. Then two groups of mothers were conformed, those with Chagas disease and a control group of healthy women.

Peripheral parasitemia was assessed in mother and neonates by parasite detection through microscopic examination of the buffycoat [27] from mother's peripheral and cord blood. We defined confirmed congenital cases of

Chagas disease in neonates as newborns showing circulating *T. cruzi*. Although some authors suggest the use of polymerase chain reaction (PCR) for earlier diagnosis of congenital *T. cruzi* infection [28, 29], this method is not yet validated [30–34].

### Assessment of Antibodies Induced by *Trypanosoma cruzi*

Protein expression and purification were done according to the technics described elsewhere [35]. Briefly, *E. coli* BL21(DE3) cells bearing the plasmidic constructions pET-32a/p2β, B13 and FRA (flagellar repetitive antigen) were grown overnight in LB medium, supplemented with 0.1 mg ml/1 ampicillin at 37 °C, with agitation. Protein expression was induced in 1 mM isopropyl-β-D-thio-galactopyranoside and purified with a Ni-nitrilotriacetic acid column (GE) [36, 37]. Total homogenates of epi-mastigotes (TH) were obtained as described elsewhere [35].

In order to determine the level of total IgG antibodies induced by *T. cruzi*, peripheral blood samples were obtained by venipuncture from all parturients before delivery, and cord blood was collected from newborns after cutting the umbilical cord. Antibodies were measured by immunoassay (ELISA) as previously described [35]. Microtiter plates were coated with 0.5 µg of specific antigens (p2β, B13, FRA and homogenate parasites) and incubated overnight at 4 °C. Then, after they were washed thrice, and blocked, they were incubated with a 1:100 dilution of human serum in 1 % skimmed milk in PBS at 37 °C for 60 min. The plates were washed and peroxidase-conjugated goat anti-human IgG (KPL) was added. Plates were read at 450 nm in an ELISA reader after incubation with trimethylbenzidine in $H_2O_2$. For each specific antigen sera was processed in duplicate. ELISA negative standard cutoff values were calculated as the mean optical density of the negative serum samples plus 2 standard deviations. The levels of antibodies were expressed as the ratio between the optical density (OD) of the mean of the sample and the OD of the negative standard cut off. This index is referred as IOD (Index of the Optical Density of autoantibodies in relation to the Negative control). An IOD $\leq$1 was considered negative [38, 39].

### Statistical Analysis

The variables of the study were analyzed with MedCalc version 12.2.1. The Kolmogorov–Smirnov method was employed to analyze normal distributions of the continuous variables. The data are expressed as mean ± SD. Groups were compared in relation to age, number of pregnancies, gravidity complication such as eclampsia, pre-eclampsia and pregnancy-induced hypertension, born and stillbirth. Also neonatal age, weight, APGAR and Capurro scores were compared between groups. Chi square test ($X^2$) or Fisher's exact test were used for categorical variables, whereas *T* Student test or one-way ANOVA (Student–Newman–Keuls post hoc test for all pairwise comparisons) were used to compare quantitative data. A logistic regression model was applied to assess the potential predictive values of above described antibodies for gravidity or newborn complications.

## Results

### Sampled Individual Characteristics

At first 429 pregnant women were potentially eligible because of age >18 years old and agreement to participate. However, 79 presented concomitant infections, 44 diabetes, and 57 history of hypertension or pregnancy induced hypertension, and 19 high risk pregnancy, so they were excluded. The remaining individuals were patients with symptomatic Chagas disease, so they were also excluded from the research. In this regard, the sample was composed of 183 women aged 25.16 ± 6.06 years (mean ± SD) and 172 newborns. Clinical characteristics of women are summarized in Table 1.

Sixty-four women had Chagas disease, and were older than the seronegative ones (Independent samples *T* test; $p < 0.001$). Sixty-nine neonates were born from mothers with Chagas disease.

No difference was observed between the chagasic and no chagasic mother groups in relation to the presence of eclampsia or pre-eclampsia, but pregnancy-induced hypertension was associated with Chagas disease (Fisher's exact test; $p = 0.02$). Also, when they were grouped according to "presence of complication or not", Chagas disease was more prevalent among the former ones ($X^2$; $p = 0.007$). In relation to dead newborns (n = 11), there was no difference between groups; however history of stillbirth was associated to Chagas disease ($X^2$; $p = 0.01$). Newborn age, weight and APGAR score were similar in both groups, and they were not related with motheŕs age. From the whole neonates sample, only 11 neonates were premature and 7 newborns presented low birth weight (weight <2.500 g), which was not related to mother infection with *T. cruzi* (n = 5), mothers' age, gravidity complication or presence of circulating parasitemia in the mother (n = 2) or in the newborn (n = 1).

Parasitemia was detectable in 17 mothers and in 4 newborns (whose mothers had detectable parasitemia), all of them being asymptomatic. The newborns of mothers with detectable parasitemia presented decreased gestational

**Table 1** Clinical characteristics of pregnant women with and without positive anti-*T. cruzi* serology and their newborns

| | Mother without Chagas disease (n = 119) | Mothers with Chagas disease (n = 64) | *p* |
|---|---|---|---|
| Age | 23.91 ± 3.48 | 27.48 ± 4.43 | <0.001 |
| Complications (n) | 14 | 20 | 0.007 ($X^2$ 7.21) |
| Eclampsia | 3 | 5 | NS |
| Pre-eclampsia | 4 | 4 | NS |
| Pregnancy-induced hypertension | 7 | 11 | 0.02 |
| Death newborns (n) | 6 | 5 | NS |
| History of Stillbirth | 9 | 13 | 0.01 ($X^2$ 6.39) |
| New born age (days) | 39.4 ± 1.37 | 39.2 ± 1.67 | NS |
| APGAR 1 Score | 7 ± 1 | 7 ± 1 | NS |
| APGAR 2 Score | 9 ± 1 | 9 ± 1 | NS |
| Weight (Kg) | 3451.84 ± 0.5 | 3473.9 ± 0.51 | NS |

*NS* non statistically significant, $X^2$ Chi squared test

age (39.5 ± 1.6 vs. 38.2 ± 1.3; $p = 0.006$) and body weight (3542.6 ± 472.6 vs. 3228.57 ± 581.7; $p = 0.04$) in comparison with the ones delivered by mothers with Chagas disease but undetectable parasitemia.

## Antibodies

In 96.87 % of the pregnant women group with positive Chagas serology, autoantibodies anti-p2β, anti-B13 and anti-FRA were detected. Antibody concentrations were not correlated with age, also their level did not differ in regard to the presence of eclampsia, pre-eclampsia or pregnancy-induced hypertension. However when they were grouped in one variable as "presence of complication or not", we observed that anti-p2β were increased in this group (Independent samples *T* test; $p = 0.02$). Also, mothers with detectable parasitemia, showed increased levels of anti-p2β antibodies (Independent samples *T* test; $p < 0.05$). Considering this result, we applied a logistic regression model step-forward in order to assess the predictive value of anti-p2β antibodies for the presence of complication. The variables included were age, anti-p2β antibodies, number of pregnancies, history of pre-eclampsia/eclampsia, history of stillbirth, and body weight. The analysis revealed that anti-p2β antibodies (OR 2.35, 95 % CI 1.06–3.49; $p = 0.036$), age (OR 2.82, 95 % CI 1.1–4.99; $p = 0.027$), history of stillbirth (OR 1.8, 95 % CI 1.01–2.27; $p = 0.04$) contributed significantly to the presence of complications (Hosmer and Lemeshow test of 11.43; $p = 0.78$).

The rates of anti-*T. cruzi* antibodies measured in newborns were not correlated to those of the corresponding mothers. Similar result of absence of correlation was also observed for the three autoantibodies. On the other hand, concentrations of anti-*T. cruzi* antibodies and autoantibodies were higher (Independent samples *T* test; $p < 0.001$; Fig. 1a–d). Furthermore, children with detectable parasitemia showed increased levels of anti-p2β (4.67 vs. 2.48; Mann–Whitney test, $p = 0.02$), irrespective of their mothers antibody values. Finally, in regard to the clinical profile on the mother and the neonates, neither the rates of anti-B13, anti-FRA nor anti-*T. cruzi* showed any relation. On the other hand, considering that β1 adrenergic receptors stimulation have been linked to body weight reduction [40–45], we explored if anti-p2β values in the newborns correlated negatively with their weight. The test yielded that anti-p2β scarcely was negatively related with birth weight ($r = -0.3$; corrected $R^2 = 0.077$; $p = 0.028$).

In order to analyze the predictive value of detectable parasitemia in mothers and the IOD of anti-p2β in the newborns to predict birth weight, we conducted a logistic regression model step-forward. In this regard, we transformed the continuous variable birth weight to a categorical variable named "presence of low birth weight" using 2.500 g as cutoff. The variables included in the model were mother age, children and mothers IOD of autoantibodies and anti-*T. cruzi* antibodies, number of pregnancies, history of pre-eclampsia/eclampsia, history of stillbirth, presence of complications. The analysis revealed that both, detectable parasitemia in mother and newborn (OR 1.97, 95 % CI 1.11–2.51; $p = 0.04$; and OR 2.03, 95 % CI 1.05–3.42; $p < 0.001$, respectively), and anti-p2β (OR 1.55, 95 % CI 1.23–2.25, $p = 0.02$), contributed significantly to the presence of complications (Hosmer and Lemeshow test of 6.58; $p = 0.51$).

## Discussion

Congenital Chagas disease is mainly asymptomatic, however some cases show severe morbidity with hepatosplenomegaly, anaemia, meningoencephalitis, low birth weights and prematurity [3–7, 46]. Indeed, most of the

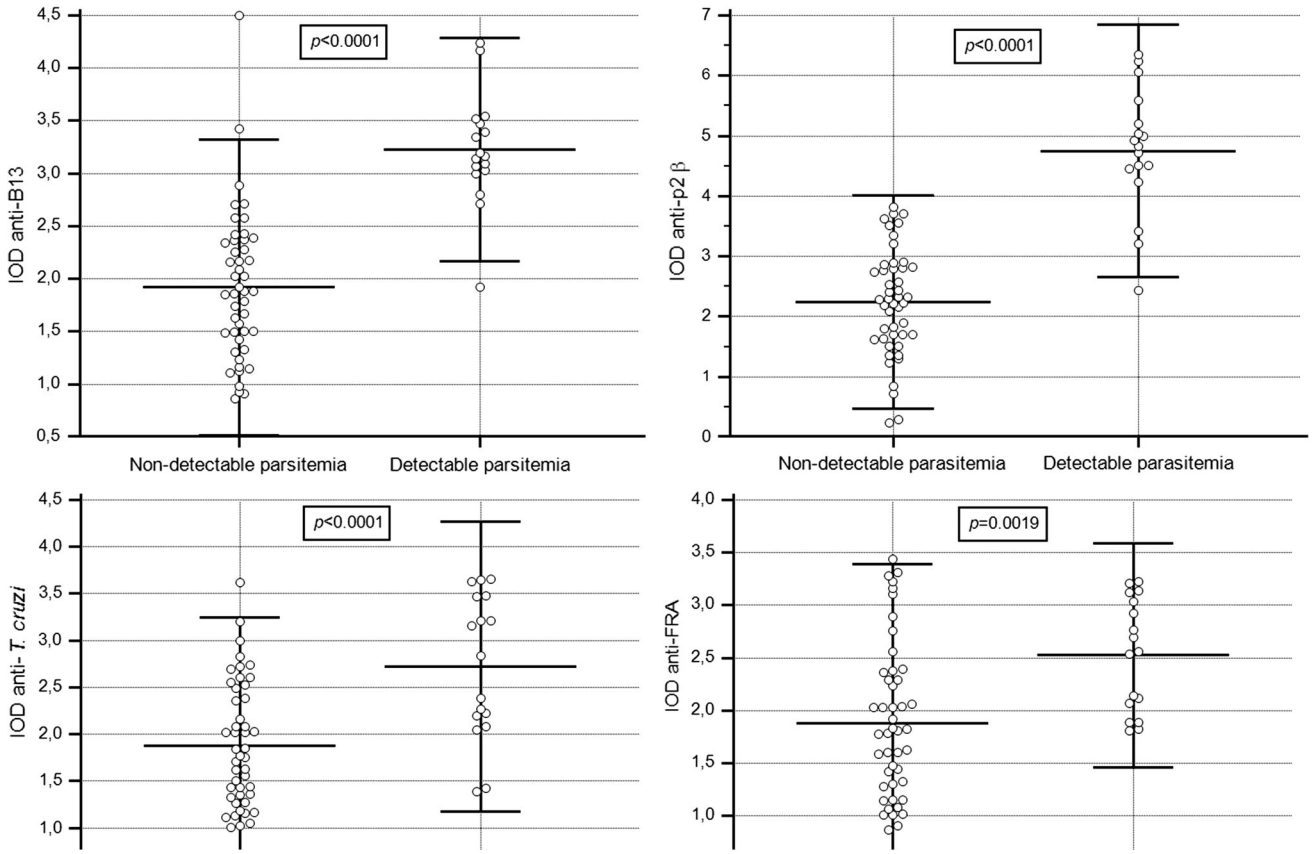


**Fig. 1** Levels of anti-*T. cruzi* antibodies and autoantibodies expressed by their IOD (index of optical density of antibodies in relation with negative control)

neonates born from chagasic mothers in our casuistry were asymptomatic, with only 5 cases being premature, and another one showing low birth weight. In our work, detectable parasitemia in the mothers was related to low birth weight and decreased postnatal estimation of maturity by Capurro score. Considering that the placenta is the principal barrier for vertical transmission, symptomatic congenital Chagas disease might be due to the intrauterine infection [47]. Referred to the presence of complications, we observed that pregnancy-induced hypertension was significantly associated with *T. cruzi* infection of the neonate. Previously it has been observed that intrauterine infection is related to pregnancy complications [48]. On the other hand, to our knowledge, there is no other previous report describing the relation of pregnancy-induced hypertension and *T. cruzi* infection, however we have reported that Chagas disease might be a risk factor for high blood pressure [49].

As regards to the vertical transmission of antibodies induced by *T. cruzi*, Aznar et al. [24] reported the presence of anti-p2β in seven congenital cases. However, it was not explored whether it had some clinical implication for the newborns. It has been described that the newborn IgG antibodieś concentration might be correlated with the maternal ones [43]; however recently evidence suggest that placental transfer depends on the amount of cell surface receptors and it is not determined by the maternal antibodies levels [22]. Similarly, we observed that there was no correlation of the levels of the auto antibodies induced by *T. cruzi* between the groups of mothers and neonates.

Anti-B13, anti-FRA and anti-*T. cruzi* antibodies were not related to the maternal and neonatal clinical profile. As regards to anti-p2β levels, they were higher in pregnant women with presence of complications (eclampsia, preeclampsia and pregnancy-induced hypertension). Furthermore, by multiple regression model we observed that anti-p2β could be a predictor of the complications. This might be related to the β1 adrenergic receptor stimulation by anti-p2β; since the β1 adrenergic receptor is involved in the regulation of blood pressure [50–52] as well as the induction of IL-6 [53], which has been associated with the development of preeclampsia [54]. In the case of newborns, only anti-p2β antibodies were particularly augmented in those with detectable parasitemia. Finally, anti-p2β concentration and birth weight were negatively correlated in all newborns, and also, anti-p2β showed to be a

marker of low birth weight (OR 1.55, $p = 0.02$). The inverse correlation between anti anti-p2β and birth weight might be due to the agonist effect of anti-p2β on the β1 adrenergic receptors which are known to induce serum triglyceride levels and body weight reduction [40–45]. Moreover, the use of β-blocker drugs has been related to weight gain [55].

In conclusion, we observed that the average weight of newborns born from chagasic mother, is lower than that of newborns born from non-chagasic mothers. Also, patent parasitemia in the mothers appeared to be related to decreased postnatal maturity estimation in newborn. In addition, we also appreciated that anti-p2β autoantibodies induced by *T. cruzi* might play a role in the presence of complication (eclampsia, pre-eclampsia and pregnancy-induced hypertension) during the course of pregnancy, and also in the development of low birth weight.

## Limitation of the Study

Our study was performed in a single-center with a relatively reduced sample size. Also, because it is a cross-sectional study, it is difficult to establish if antibodies have pathogenic role in the newborn birth.

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**Compliance with Ethical Standards**

**Conflict of interest** There was no conflicts of interests.

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