J Bras Patol Med Lab. 2019; 55(6): 693-704.

10.5935/1676-2444.20190062

Investigation of Chagas disease within the same family: case study

Investigação da doença de Chagas em um mesmo núcleo familiar: estudo de caso

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ABSTRACT

Chagas disease (CD) is a neglected endemic disease. Its classic form of transmission occurs through hematophagous triatomine insects. Its classic form of transmission occurs through hematophagous triatomine insects. There are cases of the disease in non-endemic regions that occur through alternative transmissions, as this possibility also exists. The aim of this study was to report a case among members of a same family (born and resident in Taquarituba, São Paulo, Brazil) diagnosed with CD. The family matriarch lived in a mud house in the countryside and reported contact with the triatomine during childhood. Two grown-up children are also seroreactive; both reported not having contact with the insect as children. Medical record analyzes and new laboratory tests were performed. Clinical history and recent tests have confirmed positivity for CD in the matriarch and her grown-up children. Parasitological techniques have shown negative results, evidencing that they are in the chronic form of the disease. Congenital transmission may have occurred between them, as well as the possibility of vector transmission by secondary species cannot be ruled out, since the patients come from a municipality considered endemic for CD in the past.

Key words: congenital Chagas disease; *Trypanosoma cruzi*; mechanism of transmission; diagnostic methods.

RESUMO

A doença de Chagas (DC) é uma enfermidade endêmica negligenciada. Sua forma clássica de transmissão ocorre por meio de insetos triatomíneos hematófagos. Há casos da doença em regiões não endêmicas ocorridos por transmissões alternativas, uma vez que também existe essa possibilidade. O objetivo deste estudo foi relatar um caso de membros da mesma família (natural e residente em Taquarituba, São Paulo, Brasil) diagnosticados com DC. A matriarca da família morava em uma casa de barro na zona rural e relatou contato com o triatomíneo na infância. Dois filhos também são sororreagentes; ambos relataram não terem tido contato com o inseto quando crianças. Análises de prontuário e novos testes laboratoriais foram feitos. O histórico clínico e os recentes exames confirmaram a positividade para DC, tanto na mãe quanto nos filhos. As técnicas parasitológicas demonstraram resultados negativos, constatando forma crônica da doença. Transmissão congênita pode ter ocorrido entre eles, assim como também não se pode descartar a possibilidade de ocorrência de transmissão vetorial por espécies secundárias, visto que os pacientes são oriundos de um município considerado endêmico para DC no passado.

Unitermos: *doença de Chagas congênita;* Trypanosoma cruzi*; mecanismos de transmissão; métodos de diagnósticos.*

First submission on 03/18/19; last submission on 03/19/19; accepted for publication on 05/08/19; published on 12/20/19

RESUMEN

La enfermedad de Chagas (EC) es una infección endémica que ha sido descuidada. Su forma clásica de transmisión ocurre mediante insectos triatominos hematófagos. Hay casos de la enfermedad en regiones no endémicas que ocurrieron por vías alternativas de transmisión, puesto que también hay esa posibilidad. El objetivo de este estudio fue reportar un caso de miembros de una misma familia diagnosticados con EC. La matriarca de la familia vivía en una casa hecha de barro en la zona rural y reportó contacto con el triatomino en su infancia. Dos hijos son también sero-reactivos, pero no reportaron contacto con el insecto cuando eran niños. Se hicieron análisis de historial médico y nuevas pruebas de laboratorio. El histórico clínico y las pruebas recientes confirmaran la positividad para EC, tanto en la madre como en los hijos. Las técnicas parasitológicas demostraron resultados negativos, comprobando la forma crónica de la enfermedad. Transmisión congénita puede haber ocurrido entre ellos, así como no se puede descartar la posibilidad de ocurrencia de transmisión vectorial por especies secundarias, ya que los pacientes proceden de un municipio que fue considerado endémico para EC en el pasado.

Palabras clave: *enfermedad de Chagas congénita;* Trypanosoma cruzi*; mecanismos de transmisión; métodos de diagnósticos.*

INTRODUCTION

American trypanosomiasis, or Chagas disease (CD), is caused by a flagellated protozoan, *Trypanosoma cruzi* (*T. cruzi*). There are occurrences of this manifestation in 21 countries in the Americas, with an incidence of 28 thousand cases/year and approximately 12 thousand deaths/year, and it is estimated that there are around 8 million infected individuals[1].

Considered one of the main neglected infectious endemic diseases, mainly due to the low investment of the pharmaceutical industry and the disease occurring exclusively in the American continent; its transmission occurs by the triatomine bugs (vector). The disease is known in non-endemic regions by other means: alternative routes of transmission (oral, congenital, blood transfusion, laboratory accidents, and organ transplants)[2-4].

Classical transmission occurs through the triatomine, which ingests blood repast from a protozoan-infected mammalian host. By performing a new blood meal on a healthy individual, the insect eliminates metacyclic trypomastigotes with feces and urine, which will penetrate the bite wound; inside the vertebrate host cells, they become amastigotes, which multiply. After formation of intracellular nests, another differentiation in trypomastigote occurs; this form overflows the cell and is released into the bloodstream and can reach other cells of any tissue or organ[5].

Alternative mechanisms for CD transmission are very important for public health control. There is concern about these forms of transmission, as they are presenting new epidemiological occurrences, especially the oral and congenital cases. There are reported cases of congenital CD in South American countries. Brazil presents congenital transmission risk estimated at 1%; in other countries such as Argentina, Bolivia, Chile and Paraguay, the percentage reaches 8%[6, 7]. In endemic countries, vertical transmission rate is 5%, compared to other localities (2.7%)[8].

Early diagnosis is important in infants of mothers with Chagas disease, as there are no approaches to prevent *T. cruzi* maternal-fetal transmission to the fetus, since specific treatment of the disease during pregnancy due to side effects and toxicity is not recommended of the drug[9]. When diagnosis is possible, disease control and its evolution to clinical forms are more likely to occur[10]. For the diagnosis of pregnant women with suspected CD, serological tests and direct parasitological methods are performed, usually detectable when in the acute phase, with high parasitemia levels. In the chronic or undetermined phase of infection, in which there is a low parasitemia level, indirect parasitological or serological methods, or molecular biology tools are performed[11].

Treatment for CD is performed by oral administration of benzonidazole, which causes adverse reactions to the patient, or nifurtimox, which is highly toxic drug. However, only the former is usually administrated in Brazil. Treatment is recommended regardless of the mechanism acquired, and is not indicated for pregnant women, lactating women, alcoholics and patients with a history of liver failure[12].

CASE REPORT AND METHODOLOGY

Case report of three members of a same family detected with CD. These patients underwent medical follow-up at the Infectious Disease Outpatient Clinic of the Hospital das Clínicas of the Faculdade de Medicina de Botucatu (HC-FMB). The family

is from and are resident in the city of Taquarituba, in the rural area of the state of São Paulo, a municipality considered in the past as an important endemic area of CD, which requires constant epidemiological surveillance[13].

The matriarch (67 years old) reports that during childhood she lived in a rural area, in a mud house. She remembers having had contact with the triatomine insect and seeing it countless times on the wall of the old house; she currently lives in a brick house in a peri-urban area. This patient was found to be carrying chronic CD in 2013, through a screening performed on her family members by serological diagnoses. She has nine children; two of them were confirmed seroreactive for *T. cruzi* infection; four other children presented negative results; and three of them refused to perform the tests.

The positive son (27 years of age) found to be a carrier for the disease in 2012; he reported that they had never contacted the vector. From this fact, the other family members were called for examination. Among the siblings who agreed to be serologically diagnosed at the HC-FMB, one sister (30 years old) was found to be seroreactive in 2017; she also reported have never having contact with the vector at any stage of her life. Both reported that they showed no clinical manifestation similar to that occurring during the acute phase of the infection, and that during childhood they lived in a brick house in a peri-urban area; now, in the youth/adult phase, they are living in an urban area. This patient has a four-year-old daughter who has not undergone any specific tests so far.

Parasitological diagnoses were performed at the Laboratory of Infectious and Parasitic Diseases of the Department of Tropical Diseases of the FMB. Direct diagnoses, which consist of a small drop of venous blood or digital puncture placed on a slide for reading under a microscope, were applied[14, 15]. The modified Strout concentration method was applied[16], which allows demonstrating the parasite in a blood collection sample. The sample undergoes a double centrifugation for microscope sediment analysis[17, 18]. This method is effective when the patient is in the acute phase, and the protozoan infection occurs, and it is possible to visualize its trypomastigote form in the blood[14].

The *T. cruzi* determination of the chronic phase of the disease is performed by indirect methods using enrichment techniques (blood culture). According to Junqueira *et al*. (2011)[18], this technique presents 30% to 79% sensitivity. Blood components were seeded in a biphasic semisolid media composed by blood agar in association with enriched liquid medium, as proposed by Camargo (1964)[19]. The introduction of modifications makes the technique more sensitive and promotes the proliferation of trypanosomatids in the culture medium[20].

In this phase there are also high levels of antibodies against *T. cruzi* antigens detectable by conventional serological methods, such as indirect immunofluorescence reaction (IIF), indirect hemagglutination (IHA) and immuno-enzymatic assay (ELISA)[21]. Serological tests were performed using indirect chemiluminescence (ICL), IHA, and immunofluorescence, as well as complete blood count (CBC), and C-reactive protein (CRP); all conducted by the HC-FMB Clinical Laboratory.

RESULTS AND DISCUSSION

The parasitological method performed in the patients with Chagas disease indicated that they are not in the acute phase of the disease. Laboratory tests for *T. cruzi* detection are directed according to the phase of infection the patient is in (acute or chronic). The parasitological method presents high specificity in individuals who are in the acute phase, but is low sensitivity during the chronic phase[22]. Sub patent parasitemia at this stage may interfere with the results of this method, which suggests negativity, emphasizing that a negative test does not exclude the possibility of infection[23, 24].

In the chronic phase, the parasite can be detected using serological methods that depend on the binding of immunoglobulin G class (IgG), an anti-*T. cruzi* specific antibody[3]. According to Ostermayer and Castro (1997)[25], serological testing reference values (reagent, non-reactive and inconclusive) are established by each laboratory according to the technique applied, the type/brand of the kit, etc. Based on the patients' clinical history of this case report, it was observed that the applied serology (chemiluminescence and IHA) in the matriarch in 2013 was reactive (14.74s/Co). These tests were not performed in later years due to the loss of clinical follow-up of the patient. She returned to followed-up only in 2017. The son, when diagnosed in 2012, presented chemiluminescence of 8.40s/Co and reagent IHA; he repeated specific post-treatment examinations in 2013, obtaining serological results of 9.61s/Co and reagent IHA. This patient was the only one receiving treatment. Recently, in 2017, chemiluminescence (7.57s/Co) was performed and non-reactive IHA. For his sister, chemiluminescence was 5.07s/Co, positive indirect immunofluorescence (IFI) and non-reactive IHA. The chemiluminescence of the child presented non-reactive result (0.04s/Co) and negative IFI.

All patients presented normal CRP parameters, as well as on blood count. Only the eosinophil rate (23%) in the matriarch was altered; in the daughter, increased cholesterol was observed (223 mg/dl). According to Rassi and Rassi Jr. (2013)[26] (*apud* II Brazilian Consensus on Chagas Disease – Consenso Brasileiro de Doença de Chagas)[24], laboratory tests may present alterations on

blood count with increase or decrease in global leukocytometry levels, intense lymphocytosis, plasmacytosis and neutropenia. It is noted that from the second week of the disease there is a high occurrence of atypical lymphocytes and leukocytoids, however, it cannot be stated that CD is related to the matriarch's eosinophilia, as there are other factors and other triggering conditions that alter this rate[27].

The clinical record of CD positive children indicated that they are in the undetermined chronic phase. The patient in this phase presents positive serology on serological test, echocardiogram (ECG), and esophageal and chest x-Ray within normal parameters, which may persist for years[3, 28].

Unlike the matriarch, who in 2013 was diagnosed with the undetermined chronic form because she presented no development of the disease at that time, in 2017 there was an alteration in her electrocardiogram on imaging exams, including right bundle branch block in the heart, that evolved to the chronic cardiac form. Chronic Chagas heart disease is the most common clinical manifestation and presents a certain degree of severity; it develops in about 20%-30% of patients with abnormal conduction system dispositions, arrhythmia, heart failure, arterial thromboembolism and atrioventricular block[3, 29].

Regarding the child, it was observed that she received negative results for the applied tests, ruling out the contact with the protozoan.

According to Lima and Silva (1952)[30], and Coda *et al.* (1958)[31], the municipality of Taquarituba, central west of the state of São Paulo, was formerly considered an important region for CD, since a distribution of the *Triatoma infestans* vector infected by the *T. cruzi* occurred. In addition, some studies mention that the area could be an important disseminator of infection in the 1950s, since most samples for such studies indicated positivity for infection in both, insects and patients[32, 33]. At first, the matriarch may have contracted the infection during this period, as she reported frequent contact with the insect during childhood.

With regard to her grown-up children with Chagas chronic disease, the possibility of congenital transmission cannot be ruled out, nor can the hypothesis that other transmission mechanisms are still involved, such as oral and mainly vectoral, because the patients come from an endemic municipality. At this point, there is no way to determine which mechanism of transmission occurred, and at what time of life the children contracted the infection. Based on other studies, although the implementation of the Chagas Disease Control Program in the State of São Paulo was efficient in the control of *T. infestans*, there are still secondary triatomine species (*Panstrongylus megistus*, *T. sordida* and *Rhodnius* genus) capable of carrying the infection[34-38].

It should be noted that this neglected disease should attract more attention from public health services, especially by the pharmaceutical segment, due to the development of new treatments, especially in endemic regions where there are still remnants of infection in young patients, as evidenced in the study by Navarro *et al.* (2013)[39]. In this context, it would be necessary to direct attention to the entire population of these areas, such as young people of childbearing age, especially pregnant women, performing the identification and clinical monitoring of this group. Furthermore, the work carried out by entomological surveillance must be intensified and continuous in the fight against still residual vectors, and secondary species.

ACKNOWLEDGEMENTS

To the Universidade Estadual Paulista Julio de Mesquita Filho (UNESP)/FMB for offering the Professional Improvement Program; to the HC-FMB and also to the Experimental Research Unit of the same institution, specifically the Infectious Diseases Laboratory (Laboratório de Moléstias Infecciosas) of the Department of Tropical Diseases for granting its structure for the development of this study.

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