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FIRST REPORT OF CHAGAS DISEASE TRANSMITTED BY BLOOD TRANSFUSION IN-PATIENT OF PUEBLA, MEXICO.

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ABSTRACT

A case of transmission of *Trypanosoma cruzi* by transfusional route is reported in a 38-year-old woman who underwent serological and PCR diagnosis. The inappropriate use of the norm causes that the patient is currently positive for antibody to T. cruzi and has a possible Chagas cardiomyopathy.

INTRODUCTION

American trypanosomiasis is a disease caused by Trypanosoma cruzi (KinetoplastidaTripanosomatidae), which is endemic in the American continent and is spread throughout several regions of the world owing to human migrations. In America the vector transmission is the most important route documented. Vector control and surveillance strategies are not efficient in several regions of Mexico, thus increasing species populations and jeopardizing disease control initiatives [1]

Transfusion route has reached some relevance in the last years. In USA and Canada seven Chagas's disease patients contaminated by transfusion have been reported; in Mexico four cases have been identified; none of these patients had clinical symptoms[2] .

A program for the control of blood products with blood screening for the detection of antibodies against *Trypanosoma cruzi* was established [3]

The use of different antigens and different commercial kits for sero-diagnosis is one of the main problems since the prevalence figures are not comparable and reliable. Guzman et al.[4] reported the problem of discordance between commercial and non-commercial kits (made in own "home" or "labs"), recognizing the latter as having greater sensitivity and specificity in prevalence studies. The state of Puebla is located in the south central part of the Mexican republic and is adjacent to important transmission areas such as Veracruz and Morelos [5] and anti-T. cruzi antibodies are detected in a number of blood donors (1.5%) [6,7].

CASE REPORT

This study reports a case of a 38-year-old female patient who underwent a cholecystectomy surgery in an institution of the Instituto Mexicano del Seguro Social (IMSS), where she was transfused with 450 ml. of whole blood after a postsurgical bleeding. After the remission of sepsis and the anemia which justified the transfusion she was released from doctor care. During the following 6 months, nonspecific symptoms and signs appeared, such as: cramps, general malaise, arthralgia and frequent headaches, being symptomatically treated until the symptoms completely remitted. However, intermittent febrile symptoms were associated with other infections.

After a year of the surgical intervention she made a blood donation and she was notified as anti-Trypanosoma cruzi antibodies seropositive, so she went to the Parasites and Vectors Laboratory of the Faculty of Biological Sciences of the Benemérita Universidad Autónoma de Puebla (BUAP) where anti-T.cruzi antibodies were detected by means of the same commercial kit (Chagatest Wiener Lab, Rosario, Argentina) which is the only test used in blood banks for antibody screening in Puebla. The optical density value determined by our laboratory in the patient's serum was 2.10 units, cut off value of the reagent set 0.230 positive control 1.84 units. Two samples of 10 ml. of her blood were taken, and then an indirect xeno-diagnosis with five Meccus pallidipenis Stall, third stage nymphs was performed, of one colony established and fed with hen's blood previous to supplying the patient's blood. The specimens were kept in a plastic container at 27° C±2 temperature and 75% relative humidity, being fed every 15 days with rabbit blood. In view of the positivity of both tests, a new blood sample was taken to perform a real-time polymerase chain reaction (PCR), which was positive and confirmed the case. Currently the patient's PCR is negative (Figure1). The PCR assay used to detect T cruzi was the amplification of a fragment of 235 pb as describe previously [8]. The PCR products were subjected to electrophoresis on 1.8 % agarose gel stained with ethidium bromide and visualized by using trans illumination.

Figure 1 Title: Patient´s Polimerase Chain Reaction (PCR) after treatment.

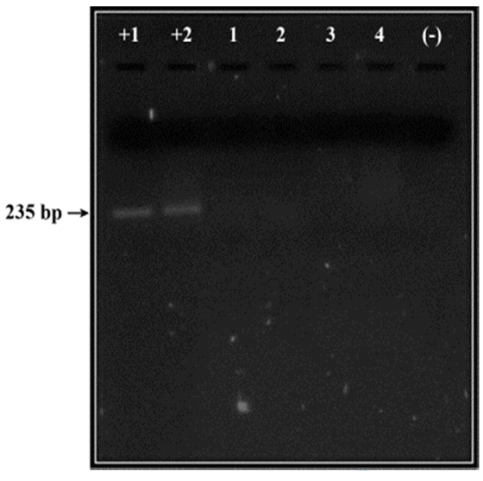

Figure1. 1.8% agarose gel stained with ethidium bromide. +1) Positive control *T. cruzi*strain "Y", +2) Positive control *T. cruzi* strain "LJ01", 1) DNA of the patient using 5 µL oftemplate, 2) DNA of the patient using 10 µL of template, 3) DNA mouse infected with patient's blood using 5 µL of template, 4) DNA mouse infected with patient's blood, using10 µL of template, (-) negative

control using vector DNA from *M.pallidipennis* not contaminated by *T. cruzi*.

Due to failures of haemovigilance service and the time elapsed it was impossible to locate the donor. The detection of antibodies led to an entomological survey in the area of residence of the patient, study which reported never knowing about the presence of or having seen triatomines in that area. The intensive search of the vector in the patient residence area by specialized personnel yielded negative results. Furthermore, the patient referred that she had not found or seen triatomines ever, as well as that she had never visited rural area during the last 5 years. All these facts together make vector transmission unlikely.

Before treatment the patient was referred to a cardiology clinic, where an electrocardiogram (EKG) was indicated showing some ventricular changes suggestive of lesions of the chronic phase of the disease.

The patient's electrocardiogram (EKG) was remarkable for sinus bradycardia, with conserved axis and segment intervals and no other conduction abnormalities (Figure 2).Her cardiac ultrasound (ECHO) showed an ejection fraction of 60%; the patient was referred to a medical institution for treatment.

Figura 2 Title: Patient´s Electrocardiogram with alterations in the shunts.

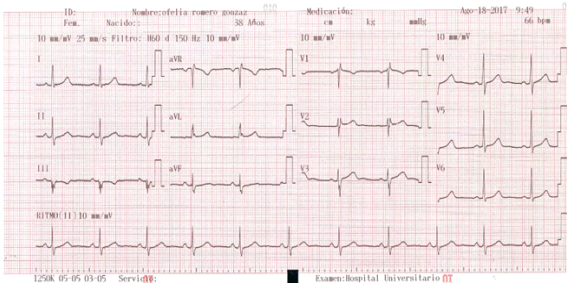

Figure 2: EKG of the patient on admission. This EKG shows marked sinus bradycardia with borderline low voltage QRS (QRS amplitude max: 6 mm in limb leads and 7 mm inprecordial leads; HR: 41 beats/min (R-R interval: 1380 ms); PR: 154 ms; QRS 84 ms;QT/QTc 400/414 ms).F.C 66 bp

DISCUSSION.

In the case reported here, the levels of IgG detected through the serology was elevated which is probably related to the result presented in EKG and ECHO. Georg *et al.*[9], found correlation between IgG1 levels and the severity of Chagas´ cardiomyopathy. Chronic Chagas cardiomyopathy is the most important chronic form of Chagas' disease due to its high morbidity and mortality and its significant medical and social impact [10].

Apparently, the patient is in the indeterminate phase, considering the alterations referred.

The Chagas disease presents two phases, acute and chronic, each with distinct clinicalfeatures. In the chronic phase, two forms are typical, the indeterminate form, whichrepresents about 60–70% of the cases, is diagnosed when there are positive serologic testresults, but no specific organic injury of the esophagus, bowels, and/or heart isdetected [11]. Among patients with the indeterminate form, nearly 2–5% per year evolve todeterminate forms, which are generally mild. In the determinate forms, cardiac anddigestive involvement are the main features and cardiac lesion is the worse prognostic.

The WHO and the Official Mexican Standard NOM - 253-SSA1-2012 intended for the useof human blood and its components with therapeutic purposes lays down, in the case of *T.cruzi,* two mandatory tests to confirm their safety. Reda Gilber*et al*[12] comparing two serological methods, one IIF test with the use of anti-human IgG and IgM fluorescein conjugates and one ELISA test (Chagatek, bio Merieux, Buenos Aires, Argentina) to a PCR test, for the diagnosis of Chagas' disease, showed that this ELISA test was not able to detect acute cases.

To explain the case and considering that the blood analyses of the donor are carry out in all cases through a single commercial trial we postulate two hypotheses. The Chagatek of bio Mérieux and the Chagatest of Rosario Argentina are used for the detection of human IgG immunoglobulin, which owing to the high specificity of the conjugate (both test use a monoclonal antibody against human IgG,conjugated to horseradish peroxidase) could make these tests unable to detect acute cases,where there is present only anti-*T cruzi* specific IgM.. A first hypothesis would be that the donor was in the acute phase of the infection. Studies conducted on risk factors and seroprevalence from areas near the city of Puebla show one of the highest values recorded (10 %) for Mexico. This result shows the necessity, in addition to the Chagatest, of a second test able to demonstrate the presence of anti-*T. cruzi* specific human antibodies of the IgM class in blood banks to avoid disease false negatives donors.Another hypothesis is the sensitivity, due of the little sensitivity of commercial kits in relation of own "home or labs" designed kits.

It is necessary to establish, beyond the universal donor screening, a centralized system for reagent quality control and serological testing in all laboratories participating in blood collection in all countries were this disease is emerging because human migrations, in accordance with the standards and requirements established, given the high incidence of this parasitic disease in Latin America.

The authors declare that there is no conflict of interest.

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