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RELATO DE CASO

SEVERE DIGESTIVE PATHOLOGY ASSOCIATED WITH CHRONIC CHAGAS' DISEASE IN ECUADOR: REPORT OF TWO CASES

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DNA extracted from peripheral blood of two Ecuadorian patients showing severe digestive pathology was amplified by the polymerase chain reaction using a Trypanosoma cruzi *specific oligonucleotide primers derived from the primary sequence of a cDNA encoding for a 24 kDa excretory/secretory protein. The positive PCR results together with the clinical findings confirmed that both patients had a digestive pathology due to Chagas' disease. This pathology could be more frequent than previously described in the chagasic endemic regions of Andean countries.*

Key-words: Chagas' disease. T. cruzi. *PCR. Megaesophagus. Megacolon. Ecuador.*

The acute phase of Chagas' disease is characterized by high parasitemia levels in which the blood parasites may easily be detected by microscopic observation. However, most acute infections remain undiagnosed and clinical disease may only become apparent many years later during the chronic phase of disease in which some patients may develop cardiac, digestive and/or neurological pathology[3]. During the chronic phase, blood parasites are not detectable by direct parasitological methods, but evidence for infection may be obtained by serological means. More recently, the presence of parasite DNA in peripheral blood in patients with chronic infection has been demonstrated by PCR[2 14].

The mechanisms underlying the development of pathological disorders in chagasic patients are poorly understood. The infecting strain as well as autoimmune factors have been shown to be involved in the pathological process[10 11]. In Ecuador, the existence of chagasic endemic regions is well documented[4 5] with a new foci with evidence of transmission recently reported[1]. Although in Ecuador cardiomyopathy has been associated with Chagas' disease[8], little is known in relation to digestive pathology. We herein report two cases with severe digestive pathology suggestive of Chagas' disease, in which circulating *T. cruzi* DNA was demonstrated by PCR.

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CASE REPORTS

Case 1. A 65-year-old man living in the province of Esmeraldas (northwestern area of Ecuador) but born in an area highly endemic for Chagas (province of Loja, southwestern region), sought medical assistance due to chronic constipation. His chief complaint was abdominal pain and no bowel movements for the last two weeks. Physical examination revealed abdominal distension and tenderness. Rectal examination revealed the presence of a fecaloma. Hematological and biochemical blood tests were all within normal limits. Radiological studies of the abdomen confirmed the presence of a large amount of fecal material in the rectosigmoidal region along with pronounced achalasia of esophagus. (Figure 1A). Oesophago-gastroduodenoscopy revealed the presence of esophagectasis, while a colonoscopy showed dolichomegacolon. Histopathological studies on biopsies taken from the esophagus and colon showed neuronal lesions in the esophageal and intestinal mesenteric plexuses. A diagnosis of dolichomegaesophagus and megacolon was made.

Case 2. A 63-year-old woman born and permanent resident of the province of Morona Santiago (southeastern Ecuador), sought medical

help due to chronic constipation and abdominal pain. Physical examination revealed a painful distended abdomen. Radiological studies showed the presence of a megacolon associated with dolichomegacolon (Figure 1B). On hospital admission, the patient was dyspneic due to abdominal distension. An emergency laparotomy was done which revealed an extremely dilated colon, in particular the sigmoid, occupying the whole abdominal cavity. A fecaloma was found in the rectum. A sigmoidectomy and colostomy was performed. Histopathological studies on the tissues showed ganglionar cells with degenerative changes. A diagnosis of dolichomegacolon was made.

Diagnosis of T. cruzi *Infection*. Serology. Since the histopathological studies showed nonspecific changes, and since megaviscera can be associated with Chagas' disease, sera from both patients were tested for anti-*T. cruzi* antibodies. ELISA tests were performed using as antigens an epimastigote crude extract and a recombinant *T. cruzi* specific antigen (rTc24)[8]. A strong positive reaction against both antigens was seen with sera from both patients.

DNA preparation and Tc24-based polymerase chain reaction. Antibodies against rTc24 reflect the presence of an active *T. cruzi* infection or an unsuccessful treatment[9], while those directed against crude lysate indicate exposure

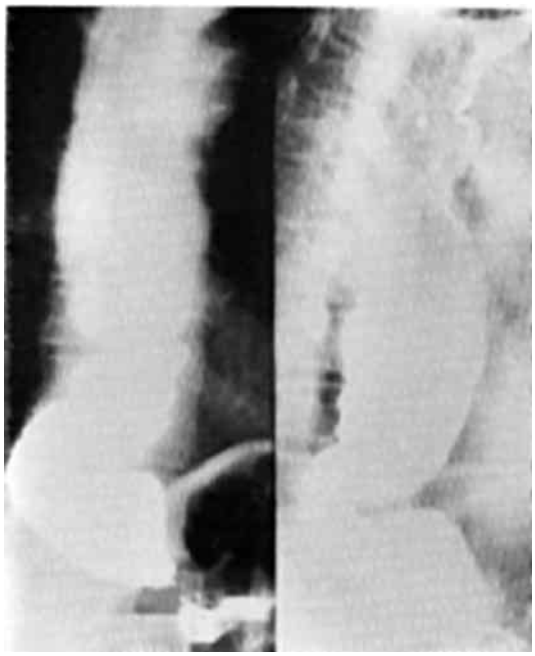

Figure 1A - Chagasic megaesophagus showing stenosis typical of achalasia (case 1).

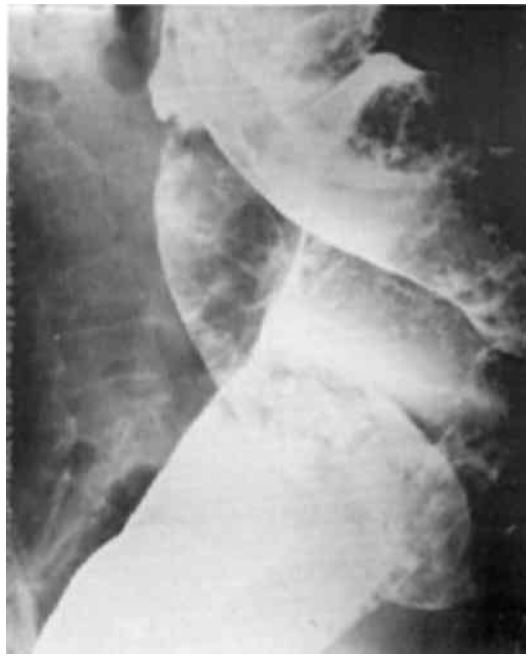

Figura 1B - Chagasic megacolon with enlargement of the sigmoid.

to the parasite. To confirm the possible existence of a *T. cruzi* infection, a PCR assay[6] was performed as follows: 5ml of venous blood from both patients were collected with EDTA. 200µl of each sample were brought up to 400µl with PCR grade water and mixed with the same volume of 2X lysis buffer (20mM EDTA, 20mM Tris-HCl, 300mM NaCl, 0.4% SDS). Proteinase K (final concentration 100µg/ml) was added and the tubes were incubated for one hour at 56°C. Samples were then extracted once with phenol/chloroform/isoamyl alcohol (25:21:4) and once with pure chloroform followed by ethanol DNA precipitation. As a negative control

blood sample serologically negative for *T. cruzi* from a endemic area was used, while a pGEX-2T plasmid containing the Tc24 cDNA was used as the positive control. For PCR assay, 5µl of purified DNA templates were added in a final volume of 50-µl reaction containing 100 pmol of Tc24-specific primers: T1 5´ GACGGCAAGAACGCCAAGGAC 3´ and T2 5´ TCACGCGCTCTCCGGCACGTTGTC 3´, and 2.5 units of Taq DNA polymerase (Promega, Madison, WI, USA). After 35 cycles at 94°C (1 min, denaturation), 60°C (1 min, annealing) and 72°C (2 min, elongation), the amplified products were analyzed by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. Both samples were positive for *T. cruzi* (Figure 2) as shown by the presence of amplified DNA.

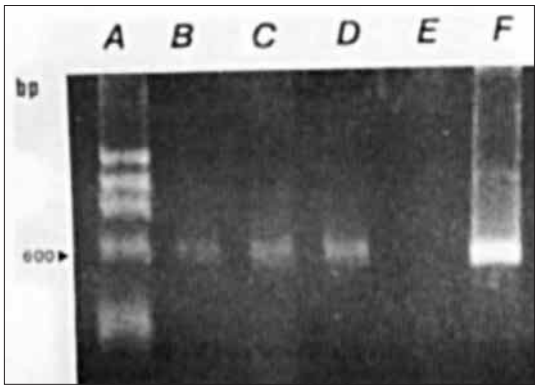

Figure 2 - Agarose gel after electrophoresis and ethidium bromide-staining showing PCR products corresponding to the following templates: B) Ten times diluted DNA from blood of case 1. C) Total DNA from blood of case 1. D) Total DNA from blood of case 2. E) DNA from a non-chagasic individual. F) DNA from a pGEX-2T plasmid containing the Tc24 cDNA which makes 636 base pairs (bp). DNA molecular weight markers are shown in lane A and corresponds to ØX174 Hae III digested plasmid. The fragment of 600 bp denotes positivity of the PCR assay as shown in the positive control, lane F.

DISCUSSION

This report definitively demonstrated the presence of digestive pathology associated with Chagas' disease in Ecuador. The positive PCR results confirmed that the parasite *T. cruzi* was still present in both patients. In Latin America, there is evidence suggesting geographic differences in the prevalence of megaviscera in Chagas' disease[7]. In chagasic endemic regions in Brazil, Argentina and Chile, the prevalence of megaviscera is high, while in the Andean South and Central American countries megaviscera are absent or very rare. Digestive pathology with probable chagasic etiology has been reported in Mexico while in Venezuela only changes in esophageal motility has been shown in *T. cruzi* infected patients[12]. The cases we report here emphazise that digestive pathology associated with *T. cruzi* infection may not be as rare as previously thought.

It is of interest that the patients in this study came from different geographical endemic regions in Ecuador; Morona Santiago province located in the eastern side of the Andes and Loja province located on the western side. Recently, additional cases from other provinces, Pastaza and El Oro have been documented (R. Guderian, unpublished observations) supporting the notion that digestive pathology due to American trypanosomiasis may be underestimated in Ecuador.

Antiparasitic treatment of chronic cases with severe pathology is still controversial. Surgical intervention is recommended in chagasic patients with complicated megaviscera[10]. In the two cases described in this report, both patients showed complications such as fecaloma. Surgery was done in case 2 with a satisfactory result. In case 1, clinical treatment was sufficient to improve esophageal function.

Control strategies for Chagas' disease currently rely on reduction of transmission by insecticide spraying. However, asymptomatic chronic cases constitute a potential risk of transfusion-associated transmission of *T. cruzi* and reactivation of the disease in immunodepressed *T. cruzi* infected individuals. Thus, in co-infections with HIV, the classical clinical manifestations of the disease may become altered, as neurological disturbances such as meningoencephalitis have been observed[13]. More detailed surveys to determine seropositivity, quality of blood products and their derivatives used for transfusion, and consequent pathology due to Chagas' disease are still required in Ecuador to define the epidemiology of this disease.

RESUMO

DNA obtido do sangue periférico de dois pacientes equatorianos, que apresentavam severa patologia digestiva, foi amplificado pela "polymerase chain reaction" (PCR) utilizando os oligonucleotídoes

específicos do Trypanosoma cruzi, *derivados de uma seqüência primária de cDNA codificado de 24 kDa proteína excretória/secretória. Os resultados positivos da PCR junto com os achados clínicos confirmam que os dois pacientes tinham uma patologia digestiva de origem chagásica. Esta patologia poderia ser mais freqüente que a descrita previamente nas regiões endêmicas chagásicas das cidades dos Andes.*

Palavras-chaves: Doença de Chagas. T. cruzi. *PCR. Megaesôfago. Megacólon. Ecuador.*

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