Parasitology International 61 (2012) 735–737

Contents lists available at SciVerse ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Case report

Chronic Chagas disease with cardiodigestive involvement and the TcVI infective form of *Trypanosoma cruzi*. A case report

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ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 19 March 2012
Accepted 26 April 2012
Available online 4 May 2012

Keywords:
Trypanosoma cruzi
Chagas
Cardiovascular involvement
DTU TcVI
Megacolon

ABSTRACT

We report a patient with megacolon associated with TcVI infective lineage form of *Trypanosoma cruzi*. Although this megacolon was considered idiopathic, Chagas disease was suspected and diagnosed because of the concomitant cardiovascular involvement. Based on this case, we discuss the suitability of Chagas diagnosis in patients with tract motility involvement.

Case Report. A 63-year-old woman born in an area endemic for Chagas disease (Jujuy province), who emigrated to Santa Fe province when aged 22 years, was admitted to Hospital J. B. Iturraspe of Santa Fe City with abdominal pain and distension, together with 6-day constipation. Antecedents recorded on admission included appendectomy 38 years before and arterial hypertension (treated with beta blockers and angiotensin-converting enzyme inhibitors). Neither toxic habits nor antecedents of cardiovascular, metabolic, pulmonary or neurological diseases were recorded. Vital signs were within the normal range. The patient presented abdominal bloating accompanied by increased borborygmus and pain on deep palpation. A hard but not painful elastic mass with net borders (diameter 5–7 cm) was detected in the left flank, and the rectal ampoule was occupied by feces.

Routine laboratory showed red cells 4.35×10^6 µl, hemoglobin 13.3 g/dl, hematocrit 39.4%, leukocytes 14.9×10^6µl, neutrophils 90%, blood urea 0.78 g/l, creatinin 1.98 g/l, serum albumin 3.9 g/dl, total proteins 7.14 g/dl, serum Na + 139 mEq, serum K + 3.9 mEq. Hepatic function was within the normal range. The abdominal X-ray revealed a marked dilation of descendant colon and sigmoid (Fig. 1), which was considered an idiopathic case of megacolon, before practicing a colonic fibroscopy. During the preoperative cardiac risk assessment the chest X-ray examination showed a cardiothoracic index > 0.5, whereas the 12-lead electrocardiogram revealed alterations compatible with atrial fibrillation. Also the 2D and in M mode echocardiogram indicated mild enlarged cardiac cavities with normal ejection fraction and walls of normal thickness and uniformly reduced motion. Because of the epidemiologic antecedent for Chagas disease, presence of specific antibodies for *Trypanosoma cruzi* was investigated. ELISA and indirect hemagglutination were performed using a commercial kit (Wiener Lab, Rosario, Argentina), which yielded positive results in both cases. After the colonic fibroscopy, revealing abundant feces and normal mucosa, and colonic lavage, the patient experimented favorable clinical evolution and was released from hospital, remaining under periodic controls.

Upon obtaining the informed written consent approved by the ethical committee of the Faculty of Biochemistry, National University of Litoral, 10 ml of venous blood was obtained for *T. cruzi* genotyping, which was kept and lysed in polypropylene tubes containing guanidine 6 M EDTA 0.2 M pH 8.0. Genotyping was performed by means of hybridization tests with the minicircle hypervariable region of the *T. cruzi* kinetoplastid and genotype-specific DNA probes, as previously described [1,2].

DNA extraction: Blood sample was processed using a 200-µl aliquot with the phenol–chloroform extraction method, ethanol precipitation, and DNA resuspension in 200 µl sterile distilled water. Each set of purifications was performed with the respective positive and negative controls. All PCR-amplified DNA from blood samples were analyzed with Southern blot assay with a panel of four genotype-specific probes from *T. cruzi*

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doi:10.1016/j.parint.2012.04.007

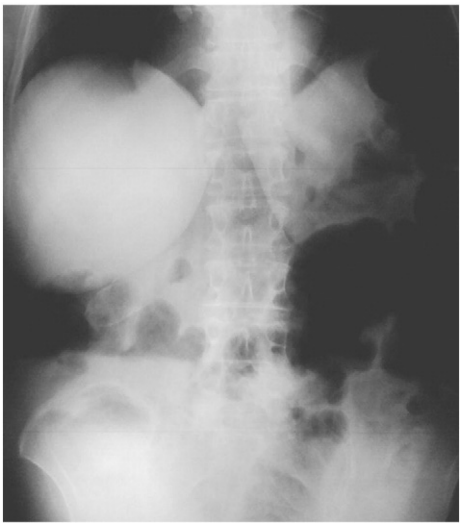

Fig. 1. Abdominal X-ray showing megacolon.

clones: sp104 cl1 (TcI), CBB cl3 (*TcII*), NR cl3 (DTU *TcV*), and v195 cl1 (DTU *TcVI*). These probes derive from the whole kinetoplast so they can hybridize to all minicircle sequences present in a parasite clone. Clone sp104 was first isolated from *Mepraia spinolai* (silvatic cycle of Chile), the CBB and NR clones were isolated from chronically infected individuals, and clone v195 from *Triatoma infestans* (domestic cycle of Chile). The probes were radiolabelled with ^{32}P, and membranes were exposed and analyzed in a Personal Molecular Imager-FX (Bio-Rad, USA). Moreover, probes were hybridized against a panel containing minicircle amplicons of reference *T. cruzi* clones. Those procedures revealed that the four genotype DNA probes were specific, and the patient presented a DTU *TcVI* because the PCR-amplified DNA only hybridized with the TcVI probe, as shown in Fig. 2.

The chronic stage of Chagas disease may be categorized into three major clinical forms: Chagasic cardiomyopathy, digestive tract involvement and the indeterminate type. Although cardiac cases have

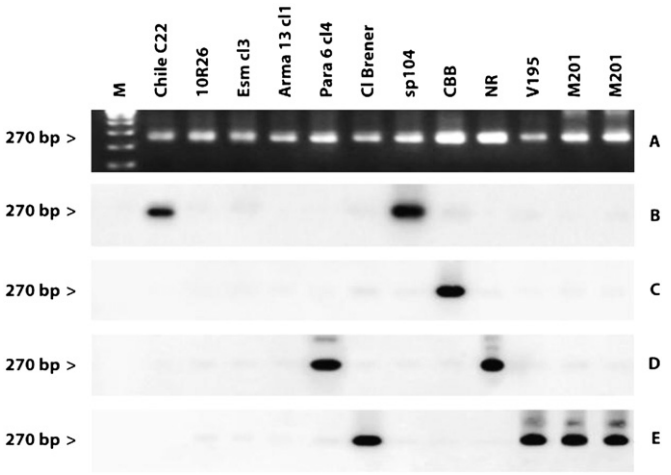

Fig. 2. Genotyping of the *T. cruzi* infecting strain: Agarose gel electrophoresis and hybridization with specific DNA probes. (A) Ethidium bromide staining of the 270-bp minicircle region amplified by PCR with primers CV1 and CV2 from kDNA of different reference strains of all *Trypanosoma cruzi* DTUs; M is a 100-bp ladder used as a molecular size marker. Strips Chile C22 , 10R26, Esm cl3 , Arma 13 cl1 , Para 6 cl4 , CL Brener, sp 104 cl1, CBB cl3, NR cl3, V195 cl1 correspond to reference strains TcI, TcIV, TcII,TcIII TcV, TcVI, TcI, TcII, TcII, TcV and TcVI, respectively. M201 corresponds to a duplicated running of the human subject infecting strain. B, C, D and E shows amplified DNA blots transferred to a nylon membrane hybridized with 32P-labeled with probe: (B) Probe sp104 cl1(TcI); (C) Probe CBB cl3(TcII); (D) Probe NR cl3(TcV); (E) Probe V195 cl1 (TcVI).

been reported across the American continent, megaesophagus and megacolon are common in the Southern Cone countries of Latin America, where about 15% of chronic patients suffer from these conditions [3]. Not only do complications vary among geographical regions but also the distribution of the six discrete typing units (DTU) or lineages TcI, TcII, TcIII, TcIV, TcV and TcVI of *T. cruzi* vary among regions where the infection is endemic. TcI is prevalent in northern Brazil, Central and North America and the other DTU infecting humans (TcII to TcVI) are found in the southern region of Latin America [4–8]. It has been repeatedly reported that in Argentina, where the infection presents heart and digestive tract involvement, TcV is the predominant DTU while TcVI is uncommon [9–12]. In a recently published epidemiological study carried out in Argentina [12], from 239 patients, the circulating TcVI infective form was identified in a minority of cases.

Our case deals with a patient with TcVI circulating *T. cruzi* parasites, who was admitted to hospital with digestive complaint and whose diagnosis was suspected based on her epidemiological antecedents and visceral involvement. Although Chagasic cardiomyopathy is systematically informed by cardiologists and reported by government statistical departments, there is scarce official information about Chagasic megaesophagus and/or megacolon. Since in medical practice, patients with digestive disorders are not routinely assessed for the presence of Chagas disease, even in those with positive epidemiologic data, digestive Chagasic involvement may be underestimated. However, some information can be obtained from nonofficial surveys. For example, Bozelli et al. [13] reported the presence of both conditions in 14.7% of 95 patients diagnosed with chronic Chagas disease in Paraná, Brazil. Of 34 patients with megacolon or/and paralytic ileus without a possible mechanical cause who attended our hospital in the last 2 years, 22 were investigated for *T. cruzi* infection, with 17 cases being seropositives. Our casuistic suggests a high incidence of megacolon or/and paralytic ileus due to *T. cruzi* infection in our region. As illustrated in this case, despite the epidemiological data and the visceral involvement, Chagas disease is not always suspected. In line with the view raised by other authors [14,15], the assessment for Chagas disease etiology when megaesophagus, megacolon or paralytic ileums are present needs to be taken into consideration.

The pathogenic mechanism of digestive Chagas disease has not yet been fully elucidated but it was determined that parasite persistence plays an important role in the progression of the disease, leading to organ damage [16]. This was also verified by Batista et al. [17], who reported positive nested polymerase chain reaction for *T. cruzi* DNA in tissues even in patients with negative or inconclusive serologic finding but with a clinically compatible megacolon. In another study, DNA from the *T. cruzi* was found to be present in the gastrointestinal tissues in individuals with cardiac and/or digestive involvement [18], and the pathological alterations in target organs were correlated with the load of parasite DNA in the tissue. Besides the relevance of parasite presence and its burden in the pathogenesis of the organ damage, the potential association between the genetic features of *T. cruzi* and tissue pathology cannot be disregarded. In the present case, the bloodstream parasites corresponded to a single TcVI lineage, which, to our knowledge, was not previously reported in patients with cardiodigestive form of Chagas disease. Indeed, TcVI DTU was frequently associated with Chagasic miocardiopathy [12,19,20], but not with digestive form of the illness. In a study performed in Bolivia, the circulating parasite strains were genotyped in 117 patients with megacolon (100 megacolon and 17 cardiodigestive involvement). None of them had TcVI strains [21]. The tropism of different strains to particular tissues was previously described in human cases, for which it cannot be excluded that different *T. cruzi* lineages were present in blood and GI tissue in this or other cases [18]. Particular tropism towards colon has been analyzed only in an epidemiological study where 18 Bolivian patients with megacolon were assessed

[22]. These authors did not find tropism of one particular lineage of *T. cruzi* by comparing infecting lineages in the tissues of patients. In contrast to our finding, these authors described the presence of TcV and TcII in the colon tissue of the patients, but no TcVI.

Data from the present case suggest that, in addition to the known involvement in cardiac damage, TcVI DTU may be also associated with Chagasic colon lessions.

Acknowledgments

This work was funded by the CONICET (Argentinean Research Council) and Universidad Nacional del Litoral, Argentina. M.H.V. and I.B. thank CONICET for their fellowship. I.S.M. and O.B. are members of the CONICET career.

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