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Evaluation of different diagnostic methods of American Cutaneous Leishmaniasis in the Brazilian Amazon

Thais Tibery Espir [a, b, *], Thayanne Sá Guerreiro [a], Maricleide de Farias Naiff [a], Luanda de Paula Figueira [a], Fabiane Veloso Soares [a], Susi Simas da Silva [a], Antonia Maria Ramos Franco [a]

[a] Coordenação de Pesquisas em Sociedade, Ambiente e Saúde, CSAS, Laboratório de Leishmaniose e Doença de Chagas, Instituto Nacional de Pesquisas da Amazônia (INPA), 69060-001BR, Avenida André Araújo 2936, 69060-001 Manaus, AM, Brazil
[b] Faculdade de Medicina, Departamento de Saúde Coletiva (FM-DSC), Universidade Federal do Amazonas (UFAM), 60077000 Manaus, AM, Brazil

HIGHLIGHTS

- Diagnostic methods were evaluated.
- Clinical forms make difficult diagnosis.
- Parasitological exams is the most indicate and cheaper method.

GRAPHICAL ABSTRACT

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ABSTRACT

Epidemiological studies have been conducted to better understand the dynamics of American Cutaneous Leishmaniasis (ACL) in the Amazon region where distinct species of *Leishmania* circulate. In endemic areas, the optimal diagnosis must be made in the earlier clinical presentation to avoid the complications of chronic disease. The scarcity of financial support, laboratory infrastructure and trained persons are the major obstacles in this reality. This paper describes the result of performing different diagnostic methods for ACL in Amazonas State between the years 2010 and 2011. The tests used were the intradermal skin test (Montenegro's skin test), ELISA (Enzyme-Linked Immunosorbent Assay), direct examination, culture isolation and identification of *Leishmania* species. A total of 38 suspected human cases of ACL were diagnosed by different methods, of which 71.0% (n = 27) were positive by direct examination, 75.6% (n = 28) had positivity in the culture isolates and, of these, 54.0% (n = 19) had infection with *Leishmania* (*Viannia*) *guyanensis*. The positivity of the intradermal skin test with the leishmanin solution was observed in 77.0% of cases analyzed and the serology with detection of IgG and IgM showed the presence of antibodies in 100% of exams realized results, showing variation in the titles of antibodies. The success of Leishmaniasis treatment depends on an effective and early diagnosis. Parasitological diagnosis is highly specific, but sensitivity is subject to variation because the tissue distribution of parasites generally is not homogeneous and depends on the specie of parasite. Moreover, parasitological tests require invasive procedures and depend on restrictive conditions for the collection of biological sample, which limit their use in large-scale for epidemiological studies. ELISA has been the most widely used serological

* Corresponding author. Instituto Nacional de Pesquisas da Amazônia, INPA, Manaus, Amazonas, Brazil.
E-mail address: thaisinpa@gmail.com (T.T. Espir).

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method for the diagnosis of Visceral Leishmaniasis (VL) as it is easy to perform and has a low cost. However, flaws in specificity are observed in the diagnosis of cutaneous leishmaniasis. Actually the diagnosis needs to be done as an associated methods depending on the question to be solved.

1. Introduction

Neglected Diseases consist in a group of diseases that affect mainly the under developed countries, and may be endemic in developing countries and may have a serious threat to industrialized countries (Alvar et al., 2012). The World Health Organization (WHO) estimates that a billion of people in 149 countries suffer from one or more neglected tropical diseases (NTD). Leishmaniasis is among the seventeen diseases considered as an NTD by WHO, occupies the category of emerging and uncontrolled diseases (Lindoso and Lindoso, 2009; Alvar et al., 2012) and is present in 98 countries and territories (WHO, 2010; Alvar et al., 2012). Over 350 million people live in risk areas and, each year, 500 000 develop Visceral Leishmaniasis (VL) and 1.5 million with Cutaneous form of the illness (Desjeux, 2004; Alvar et al., 2012). Factors such as environmental changes, uncontrolled migration, ecotourism, working in dangerous areas, difficulties in treatment, immunocompromised hosts and migration of non-immune people to endemic areas, contribute to the increase in the number of cases (Desjeux, 2001; Shaw, 2007; Gomes et al., 2014).

In Brazil, the American Cutaneous Leishmaniasis (ACL) is distinguished for its wide distribution, occurring in all states of the country. Amazonas State notified 2230 new cases of the disease only in the year 2011 and the transmission occurred mainly in the cities of Manaus (752 cases), Presidente Figueiredo (213) and Rio Preto da Eva (203) (Sinan, 2012). This importance is not only in their high incidence and wide geographical distribution, but also the possibility to assume chronic clinical forms that might determine disfiguring and disabling injuries, with major repercussions in the psychosocial field of the individual (Gontijo and Carvalho, 2003). A total of seven species of *Leishmania* (ATL) are found in Brazil (and all in the north region): *Leishmania* (*Viannia*) *braziliensis*, responsible for localized mucocutaneous and disseminated cutaneous leishmaniasis; *L.* (*V.*) *guyanensis*, *L.* (*V.*) *lainsoni*, *L.* (*V.*) *shawi*, *L.* (*V.*) *lindenbergi* and *L.* (*V.*) *naiffi* by localized cutaneous leishmaniasis; *L.* (*Leishmania*) *amazonensis*, by localized cutaneous and diffuse cutaneous leishmaniasis (Lainson et al., 1987; Silveira et al., 2002; Gramiccia and Gradoni, 2005). The Amazon region is responsible for 37% of new cases and the most prevalent species are *L.* (*V.*) *guyanensis* and *L.* (*V.*) *braziliensis* (Arias and Naiff, 1981). A comparison of clinical and etiologic agents revealed that 83% of reported cases in the region are caused by *L.* (*V.*) *guyanensis* (Naiff et al., 1999; Figueira et al., 2008; Espir et al., 2014). The endemic disease associated with this pathogen affects on rural and urban outbreaks (75.9% and 24.1% of caoty to ulcerated lesions of other diseases such as Virchowian leprosy, paracoccidioidomycosis, tropical ulcer, syphilis, cutaneous tuberculosis, cancer, among others (Gontijo and Carvalho, 2003). Furthermore, there are limitations of conventional diagnostic methods therefore the diagnosis must be made by the association of clinical, epidemiological and laboratory testing aspects. These include the identification of amastigotes in the tissue by immunocytochemical techniques and in imprints (printing affixing biopsy) of aspirate lesion and histopathological evaluation; Isolation of promastigotes *in vitro*; Serological methods such as indirect immunofluorescence assay (IFA), direct agglutination test (DAT), ELISA (Enzyme-linked immunosorbent assay) and Western blot analysis, based on the presence of specific antibodies against parasite antigens; cell mediated immunity as the Montenegro skin test (MST) detection of kDNA by PCR and others (Kar, 1995). One of the most common diagnostic tests, direct observation of amastigotes in stained slides of imprints by Giemsa method under an optical microscope, shows a sensitivity of 50–70%. This technique depends on the number of parasites in the stained smear. The test positivity is inversely proportional to the time of the skin lesion evolution, and is rare after one year (Vega-Lopez, 2003; Bensoussan et al., 2006). Immunological tests employed in clinical practice are indirect indicators of infection by *Leishmania*. The results of MST indicates previous infection and of a cellular immune reaction against the parasite, but does not distinguish whether infection is present or past, so its diagnostic importance is hampered in patients from endemic areas, because asymptomatic individuals may have a positive test (Goto and Lindoso, 2010). In treated patients, the MST still remains positive indefinitely and in cases of infection with *L.* (*L.*) *major* was observed positivity for more than 19 months after treatment (Sassi et al., 1999). MST is positive in more than 80% of patients with cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (ML), while the diffuse cutaneous leishmaniasis (DCL) is persistently negative (Costa et al., 1986; Reis et al., 2009). In this study were evaluated different diagnostic methods of CL in the Amazonas State, Brazil.

2. Materials and methods

2.1. Patients

The study was designed as cross-sectional descriptive and a convenience sample. The results of laboratory tests of 38 patients with cutaneous lesions clinically suspected of ACL, treated between 2010 and 2011 in the Basic Health Unit Manoel Rumão Km 135, from Manaus-Itacoatiara highway and in the Hospital Thomé de Medeiros Raposo located in the municipality of Rio Preto da Eva – Amazonas State, were evaluated. All of the patients came from endemic areas in the north of the Amazon River, Brazil, where predominance of human cases of *L.* (*V.*) *guyanensis* has been reported. Thirty-eight CL patients (23 males and 15 females; mean age 31.22 ± 2.518 (SD) and 37.93 ± 4.113 (SD) years, respectively) with active skin lesions coming from endemic areas located at the Amazonas State-Brazil, were studied. The diagnostic criteria were based on parasitological and immunological parameters as described below. A total of 19 strains isolated from 37 patients were characterized as *L.* (*V.*) *guyanensis* and one as *L.* (*L.*) *amazonensis* by multilocus enzyme electrophoresis (isoenzyme analysis) (Cupolillo et al., 1994).

2.2. Inclusion criteria and diagnostic methods

All patients included in this study were selected from a population of individuals attending at the ambulatory of the Basic Health Unit Manoel Rumão, and in the Hospital Thomé de Medeiros Raposo, Rio Preto da Eva, Amazonas State, that presented a positive clinical picture (skin lesion) and suggestive epidemiology for ATL.