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Visceral leishmaniasis: an update of laboratory diagnosis

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ABSTRACT

Visceral leishmaniasis, is an infection due to obligate intracellular protozoa of the genus *Leishmania*. There exist two varieties of visceral leishmaniasis, that vary in their transmission aspects: zoonotic visceral leishmaniasis and anthroponotic visceral leishmaniasis. Their clinical features are comparable with sevral differences. Laboratory diagnosis of visceral leishmaniasis consists of microscopic observation of parasite, culture from appropriate samples, detection of antigen, serological tests, and identification of parasite DNA. In this review, we will discuss the different techniques of diagnosis and the interet of the recent methods such as rapid diagnostic test and direct agglutination test.

1. Introduction

Visceral leishmaniasis (VL), is an infection due to obligate intracellular protozoa of the genus *Leishmania*[1]. Natural transmission of the parasite happens primarilyby way ofthe bite of contaminated female of phlebotome. In the Old World it is represented by the genus Phlebotomous but in the New World it is called Lutzomyia. Another way of transmission may occur among the intravenous drug abusers by sharing syringes[2]. That parasitic disease is caused by infection by *Leishmania donovani* (*L. donovani*) in the Indian subcontinent as well as Eastern Africa but in the Mediterranean basin, Central Asia and South America it is caused by *Leishmania infantum*[3].

2. Epidemiology and clinical features

It is a health problem of poor and neglected populations. It is affecting 79 countries worldwide and accounts 58 000 new cases every year[4]. There exist two varieties of VL, that vary in their transmission aspects: zoonotic VL is transferred from animal to vector to human while anthroponotic VL is transferred from human to vector to human. In the past, humans are occasional hosts and animals, basically dogs, remain the reservoir of the parasite. Zoonotic VL is located in areas of *Leishmania infantum* transmission as opposed to anthroponotic VL which is located in areas of *L. donovani* transmission[5].

After an incubation interval that usually takes between 2 and 6 months, VL subjects present signs and symptoms of persistent systemic infection such as fever, fatigue, weakness, lack of appetite and losing weight as well as parasitic invasion of the blood and reticulo-endothelial system including enlarged lymph nodes, spleen and liver. Generally fever is coupled with rigor and chills and could be intermittent. Fatigue and weakness are compounded by anaemia, which is a result of the persistent inflammatory state, hypersplenism and occasionally by bleeding. The clinical expression of VL is comparable in various endemic areas but there exist some differences. For instance, enlarged lymph nodes are not often found in Indian VL patients while are frequent in Sudanese

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VL patients[6,7]. Hyperpigmentation, that probably brought the name kala-azar (black fever in Hindi), only has been mentioned in VL patients from the Indian subcontinent, nowadays this sign is rare and was probably an element of extended infections in the time while efficient treatment was inaccessible. Since the disease developments, splenomegaly could augment, producing abdominal distension and also pain, which can be amplified by a concomitant hepatomegaly. Signs and symptoms of bacterial co-infections that include pneumonia, diarrhea or tuberculosis may confound the clinical representation while initial diagnosis. VL signs generally remain for a few weeks to months before patients find treatment or die of bacterial co-infections, major bleeding or intense anaemia[8].

3. Laboratory diagnosis

Diagnosis of VL in laboratory consists of microscopic direct observation of the parasite, culture from appropriate samples, detection of antigen, different serological tests, and identification of parasite DNA. Until day parasitological method stays the gold standard for the diagnosis VL. Microscopic demonstration of amastigotes in splenic aspirates, buffy coat and also peripheral blood mononuclear cells have been proven to have elevated sensitivities and ideal specificity[9].

3.1. Demonstration and isolation of parasite

The frequently used technique for diagnosing VL was the demonstration of parasites in splenic or bone marrow aspirate. The existence of the parasite in liver biopsy, lymph nodes, or aspirate specimens or the buffy coat of peripheral blood may also be revealed. Amastigotes look like round or oval bodies measuring 2 to 3 m in length and are identified intracellularly in monocytes and macrophages. In preparations colored with Giemsa or Leishman stain, the cytoplasm looks pale blue, with a quite large nucleus that stains red. At the same plane as the nucleus, but at a right position, it is a deep red or violet rod-like body known as a kinetoplast[9]. To improve the sensitivity of parasitological diagnosis, antibodies conjugated with fluorescent against surface antigens of the parasite were performed in endemic settings in Spain, Brazil, Italy, Tunisia, and Iran[10].

Culture of the parasite may increase diagnostic sensitivity, however it is laborious, prolonged, and expensive, therefore infrequently employed for clinical diagnosis. Nowdays there exist new techniques of culture increasing sensitivity, like the microculture method. Current improvements concerning this procedure require employing the buffy coat and peripheral blood mononuclear cells[11,12].

3.2. Immunological diagnosis

3.2.1. Non-invasive and rapid methods

Immunological diagnoses are founded on the finding of both leishmania antigens and antileishmanial antibodies in the blood samples. Various serological tests are already produced for VL to substitute parasitological techniques that have been checked out in several endemic regions[13]. Recent serological tests are dependent on four kinds of techniques: ELISA, indirect fluorescent antibody, direct agglutination test and western blot. The sensitivity relies on the assay and its techniques, however the specificity will depend on the antigen instead of the serological test applied[14].

3.2.2. Fluorescent antibody test

The indirect fluorescent antibody test is among the frequently used tests for anti-leishmanial antibody detection employing fixed promastigotes. The test is founded on detecting antibodies, that can be proven in the very initial phases of infection as they are undetectable six to nine months following cure. Titres over 1:20 are significant and higher than 1:128 are diagnostic. In spite of this, there exist a possibility of a cross reaction with trypanosomal sera[15,16]. The indirect fluorescent antibody test demonstrates accepted sensitivity (87% to 100%) and also specificity (77% to 100%)[17,18].

3.2.3. ELISA

Detection of antileishmanial antibodies by means of ELISA is highly frequent, and its sensitivity/specificity largely relies on the antigen employed. Before, ELISA with crude or soluble antigens of promastigotes or amastigotes was applied, however cross reactivity was prevalent resulting in providing it the lowest consideration in diagnosis. With the advancement in technology, numerous recombinant antigens are integrated VL diagnosis with rK39 located on the top of every recombinant antigens (sensitivity: 67%–100%; specificity: 93%–100%)[19].

3.2.4. Direct agglutination test

The direct agglutination test is a semi-quantitative test that employs microtitre plates wherein increasing dilutions of patients serum or blood are combined with stained killed *L. donovani* promastigotes[20,21]. Once specific antibodies are existing, agglutination will be visible over 18 h with the naked eye. This method was already extensively validated in many endemic areas that gave sensitivity and specificity rates of 94.8% and 97.1%[22], respectively.

3.2.5. Rapid diagnostic test

The recombinant K39 protein antigen used in the rapid diagnostic test is accessible, reproducible and low-priced, which is not difficult

to execute, and could give the results during 10 min[23]. This rapid test represented by nitrocellulose strips is immobilized by recombinant antigen *Leishmania* K39. We smear a drop of serum or blood on the pad of the strips that dipped in a small amount of buffer. We can have a result after a few minutes. In the strip of infected patients, appear two pinkish lines in the middle of the nitrocellulose membrane[14,9].

The sensitivity and specifity of the rK39 immunochromatographic test are variable inside different population presenting VL in different areas. In India the test proved 100% of sensitivity and 93%–98% of specificity[24,25]. In Brazil the sensitivity is estimated as 90% and specificity as 100%[26]and finally in the Mediterranean area both sensitivity and specificity found about 100%[27].

3.3. PCR

Molecular approaches for VL diagnosis are actually developed and approved to reduce the limitations of parasitological (elevated invasiveness as well as non applicability to subject conditions) and serological techniques (reduced prognostic value). These kinds of methods are based mostly on qualitative PCR and require amplifying parasite DNA in VL patient's blood sample. Numerous regions of leishmania genome are already selected (elevated copy number of kDNA, small subunit of rRNA, internal transcribed spacer-1) to provide very specific and sensitive primers[28]. PCR assay sensitivity relies on the sample applied. The greatest sensitivity is near 100% in spleen and bone marrow samples[29]. The perfect sample is peripheral blood because of its non-invasive character. Employing peripheral blood, the sensitivity amounts documented range between 70% and 100%[30,31].

4. Diagnosis of VL-HIV co-infection

As reported by the World Health Organization, approximately 35 million people around the world live with HIV. Immunosuppression can reactivate latent *Leishmania* contamination in non-symptomatic subjects and HIV/AIDS patients[32]. VL in HIV subjects will have atypical clinical expression. Just 3/4 of HIV-infected persons, compared to non-HIV-infected patients, show the typical clinical pattern such as fever, splenomegaly, hepatomegaly, and also gastrointestinal involvement[33-36].

Serological tests seem to be not accurate, given that the most of these patients often will not present detectable quantities of antibodies[37]. Negative serology must not conscientiously eliminate a diagnosis of VL among HIV-infected patients. Parasitemia is elevated in HIV coinfection therefore direct identification of parasite or its component in blood by PCR or quantitative PCR is significantly employed not just for diagnosis but additionally for the follow-up of the patients in the course of and after treatment, however tests are usually not easily accessible in poor medical care areas[38].

5. Conclusion

VL is suspected clinicaly but should be confirmed by laboratory tests. The demonstration of parasite in splenic or bone marron still the gold standard of the diagnosis but recently the rapid tests seem to be the best choice of all the technics non-invasive, low-priced with increase sensibility and specificity.

Conflict of interest statement

We declare that we have no conflict of interest.

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