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Review article

Diagnostic modalities in cutaneous leishmaniasis

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Abstract Cutaneous leishmaniasis is a protozoan disease caused by various species of an obligate intracellular parasite *Leishmania.* It is endemic in more than 80 countries on five continents; Africa, Asia, Europe, North America and South America, with a total of 350 million people at risk. In view of the large number of clinical presentations of cutaneous leishmaniasis (CL), a correct diagnosis of the disease becomes important. Clinical appearance along with history of visit to an endemic area may be sufficient at times. These days the diagnosis is generally aided by slit skin smear and impression smears examination, histopathology of the tissue sections and by culture of parasite. However, unusual clinical presentation, superadded infection or persistence of the disease for a prolonged time, has provoked scientists to develop more sensitive methods to detect even a trace of the parasite. The present article is to review the various diagnostic techniques and their relevance in different clinical settings.

Key words
Cutaneous leishmaniasis, parasitological diagnosis, immunodiagnosis, serological tests.

Introduction

Leishmaniasis is a general name given to the diseases caused by infection with any member of the genus *Leishmania.* There is a wide range of clinical forms of leishmaniasis in both the Old and the New World. These are classified into one of the three general types; visceral, mucocutaneous and the cutaneous disease. Each type seems to be related to certain species of the parasite. The tropism of the different parasites to the different tissues is still unexplained. Leishmanial parasite fixed in alcohol and stained with gentian violet was first observed by Cunningham in 1885 and although noted by others, was eventually described definitively by Leishman in 1900 and independently by Donovan in 1903.[1-3] The parasites are transmitted by the female sandflies of the genera *Phlebotomos* and *Leutromyzon*. The promastigotes, in the midgut of the sandfly exists in a motile flagellated form. When these parasites enter the mammalian host via the bite of the sandfly they are taken up by the cells of mononuclear phagocytic system. Inside these cells they transform into non-motile rounded forms called the amastigotes. The intracellular parasitism of these culminates in the symptoms and pathology associated with the disease. Once the parasites have been introduced and taken up by the phagocytic system they are easily disseminated throughout the reticulo-endothelial system. The parasite survives and multiplies as an obligate intracellular parasite of the macrophages.[1-3] The untreated symptomatic disease follows a chronic course that may end up in death of the patient in the visceral form. Most of the times, in an endemic areas, it is adequately

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diagnosed by its clinical appearance. Diagnostic challenge arises when the lesions appear in non-endemic area, when superadded infection or home made remedies alter its clinical appearance, or any atypical variant is seen even in endemic areas.[4,5] In such cases other diagnostic techniques like skin slit smears, impression smears, culture of the parasites, animal inoculation and histopathological study of the biopsied specimens of the skin lesions are required to confirm the diagnosis.[5-8] Modern methods like immunofluorescence, immuno-histochemistry, the use of monoclonal antibodies, DNA probes, polymerase chain reaction (PCR) and electron microscopic studies are sophisticated and sensitive methods and some of these are quite capable of picking up even a trace of antigen in tissue specimen.[9-11] These are used to aid the diagnosis in doubtful cases. Various serological techniques can also be used to support the diagnosis and for screening purposes in endemic areas. A wide variety of topical as well as systemic treatment modalities have been employed for cutaneous leishmaniasis with variable results. Pentavalent antimonials are the drugs of choice for the treatment of lesions involving cosmetically sensitive sites, and multiple or disseminated lesions of CL. However, for simple lesions which are few in number, and where there is no risk of disfigurement or restriction of joint mobility, local therapy is simple, economic, quick, and safe, appears effective and offers an attractive alternative to systemic therapy. Other drugs, such as pentamidine, amphotericin B and oral ketoconazole, are used for resistant cases of CL.[12-16]

Modes of diagnosis

Diagnosis is mainly clinical or is based on histopathological reports. The diagnostic difficulty arises when lesion either becomes chronic or is altered by super-added infection or self-applied home made medications. The disease can also be missed if the patient appears in a non-endemic area or by un-suspecting clinician. A chronic case may easily be mistaken for tuberculosis, the lymphatic spread or the sporotrichoid form can easily be confused with deep mycosis, and similarly a chronic ulcer may be confused with a malignant lesion. In such circumstances advanced laboratory support is needed to confirm the diagnosis. The diagnosis of cutaneous leishmaniasis can be considered under the following headings. A schematic diagram is given as **Figure 1** and different diagnostic modalities along with relevant clinical settings are given in **Table 1**.

A. Clinical diagnosis

After taking a detailed history the diagnosis of cutaneous leishmaniasis should be suspected in the following circumstances.[1,3,4,17]

a) Travel/residence in an endemic area
b) Lesions on exposed parts of the body
c) Few number of lesion (usually 1-3)
d) Duration of several weeks/months
e) Resistance to all types of attempted treatments
f) Usually no pain or itching
g) Morphological patterns; (satellite papules, subcutaneous nodules

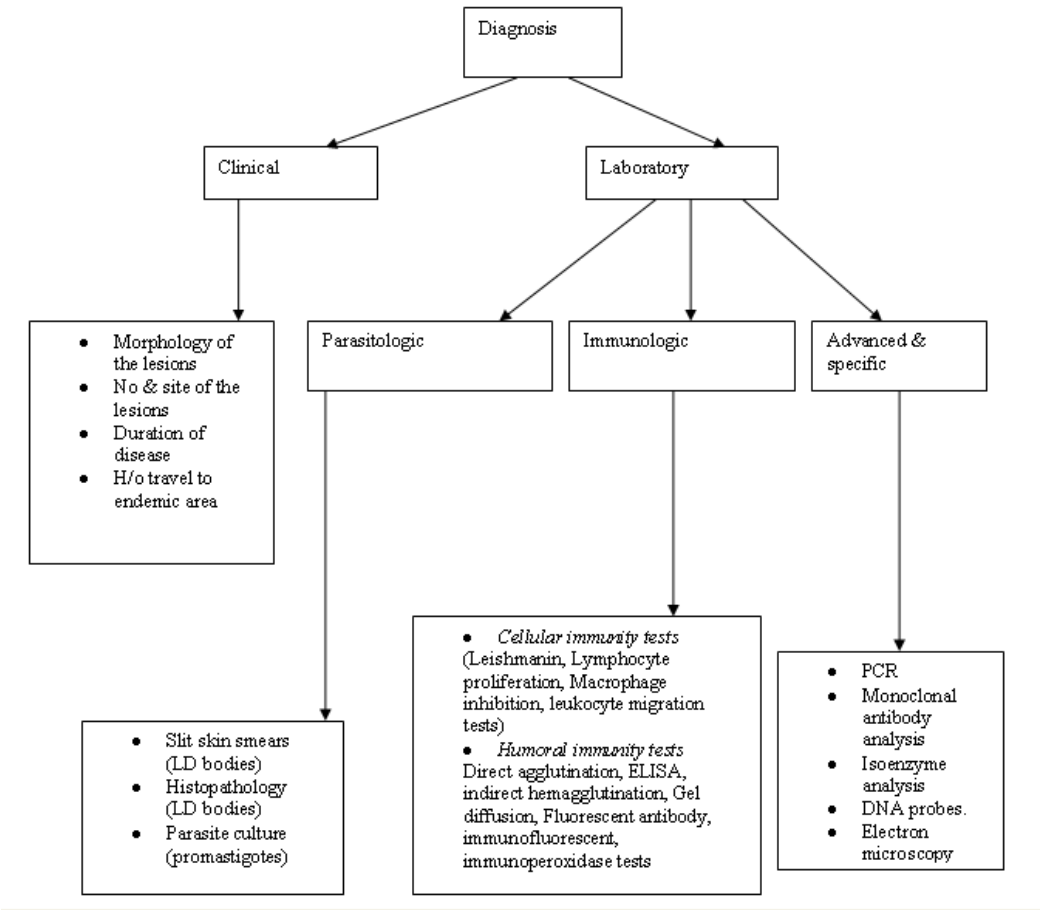

Figure 1 Schematic diagram of diagnostic approach in cutaneous leishmaniasis

No	*Diagnostic modalities*	*Relevant settings*
1.	Clinical diagnosis	• In endemic areas where no laboratory facilities exist • Most rapid diagnosis
2.	Parasitological diagnosis	• To confirm clinical diagnosis with laboratory aid • Delayed but accurate diagnosis
3.	Immunological diagnosis • Cellular immunity tests • Serodiagnosis	• For academic purposes • No diagnostic significance • For screening purposes • To aid clinical & lab diagnoses
4.	Advanced diagnostic tools	• For species characterization • To make absolute diagnosis • For academic & research purposes

Table 1 Various diagnostic facilities along with clinical relevance

with lymphatic spread, skin crease orientation, paired or clustered lesions, volcanic nodules and iceberg nodules)

B. Laboratory diagnosis

1. Parasitological diagnosis

It means the modes of diagnosis in which the parasites are seen in the form of non-motile amastigotes (smear preparations and histopathology) or motile promastigotes (culture).

(a) Stained smears for microscopy

Smears obtained from the lesions and examined under the microscope, after

staining with Giemsa or Leishman stain, is a rapid means of diagnosing cutaneous leishmaniasis. Different methods can be used for making a smear, these include, the impression or touch preparation, a slit smear, scalpel scraping, by using a dental broach or by fine needle aspiration. It is recommended that the sample should be taken from the edge of the active lesion, as far away from crusting, ulceration and secondary infection as possible. Smears and touch preparations should be stained with Leishman or Giemsa's stain. The touch preparations if performed correctly can give a positive yield in over 70%.[5-7,18]

(b) Histopathological examination

The histological picture in cutaneous leishmaniasis differs according to the stage of infection and the clinical type. Both epidermal and dermal changes are seen. The characteristic histological features are usually present in the dermis. A consistent finding is a moderate to heavy dermal infiltrate of lymphocytes, plasma cells and macrophages. In about 80% of cases, epithelioid cell granulomas with giant cells and a rim of lymphocytes are present. In all histopathological examinations it is important to search for amastigotes, which are diagnostic. In general, there is inverse correlation between the ease of detection of amastigote and the age of lesion. Ridley[19] has classified cutaneous leishmaniasis into five histopathological patterns, types A-E. The type A pattern is characterised by infiltration of dermal layers with numerous macrophages laden with numerous amastigotes. Type B is characterised by diffuse necrosis of macrophages. In type C there is focalised necrosis surrounded by disorganised epithelioid cells. Type D pattern is characterised by well-organised and extensive epithelioid granuloma (lupoid leishmaniasis). Type E response is similar to D but with absence of plasma cells.[5,7,19]

(c) Culture

Several different cultures have been used to isolate leishmania, including Novy-McNeal-Nicolle medium (NNN), Modified Evan's medium, Tobie's modified NNN medium and Schneider's insect medium. These are specialised media containing several nutrients necessary for growth of the fastidious Leishmania organisms. The incubation temperature is usually around 25°C. The cultures may be positive in 3-8 days, but may take as long as 4 weeks. The promastigotes, as examined under microscope, are highly pleomorphic with variation in the length of the body and flagellum. There are many cases of cutaneous leishmaniasis on record in which leishmania parasites have been grown successfully from blood of the patients. These cases were due to both L. major and L. tropica, which are classically considered to be only dermotropic.[5,7,8,20]

2. Non-parasitological diagnosis (immunodiagnosis)

Leishmanial parasite contains some antigenic components that evoke both humoral as well as cell-mediated immune responses. Immunodiagnosis may be divided into those tests, which detect and measure antibodies in the serum and those that detect specifically- activated T lymphocytes. The cell-mediated immune response can be demonstrated by delayed hypersensitivity reactions and histopathological picture. To detect humoral response various serological tests have been developed which help in the diagnosis of CL.[5,9,10, 21-24]

(a) Tests for cellular immunity

i. Leishmanin test (Montenegro)

ii. Lymphocyte proliferation assay
iii. Macrophage inhibition test
iv. Leukocyte migration test

(b) Serological tests for diagnosis (to detect anti-leishmania antibodies)

Various serological techniques can also be used to support the diagnosis and for screening purposes in endemic areas. These include:

i. Direct agglutination test
ii. Enzyme-linked immunosorbent assay test (ELISA)
iii. Indirect hemaggulination test
iv. Gel diffusion and counter current electrophoresis
v. Fluorescent antibody test
vi. Immunoperoxidase test
vii. Immunofluorescent test

(c) Tests for diagnosis and species characterisation

Modern methods like immuno-fluorescence, immunohistochemistry, the use of monoclonal antibodies, DNA probes, polymerase chain reaction (PCR) and electron microscopic studies are sophisticated and sensitive methods and some of these are quite capable of picking up even a trace of antigen in tissue specimen. These are used to aid the diagnosis in doubtful cases.[11, 25-29]

Advanced modalities available these days for diagnosis are:

i. Monoclonal antibody analysis
ii. Isoenzyme analysis
iii. Polymerase chain reaction

All these tests are specific and are capable of species determination. PCR is used to amplify the amount of kinetoplast DNA (kDNA) which is species-specific. This when attached to a specific probe can be detected with various methods.

Conclusion

Accurate clinical diagnosis made by an experienced dermatologist can be good enough in settings where laboratory facilities do not exist. Otherwise, light microscopy with routine H&E staining is probably the best diagnostic method in terms of economy and accuracy. More attractive, advanced modalities may become the diagnostic tool of future but being expensive procedure these cannot be used in routine practice.

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