Diagnostic Microscopic Features of Cutaneous Leishmaniasis other than Leishmania Tropica Bodies

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Objective: Delineate the histological features which can help in diagnosis of cutaneous leishmaniasis other than the presence of Leishmania Tropica Bodies

Study Design: Prospective descriptive study.

Place and Duration of Study: Departments of Dermatology and Pathology, Pakistan Institute of Medical Sciences, Islamabad from January 2008 to July 2008.

Material and Methods: 35 patients presenting to Dermatology OPD with initial clinical diagnosis of CL were included in this study. Patients with all ages and both sexes were included. Patients already on treatment were excluded. Skin biopsies were taken, stained with hematoxyline & eosin stain (H & E stain) and studied in collaboration with dermatopathologist. Different histopathological findings were recorded and results analyzed.

Results: Leishmania Tropica Bodies were found in (43 %) of patients. Other histological features helping in diagnosis of CL even in the absence of LT bodies were Plasma cell infiltrate (77 %), Giant cells (34 %), Granuloma (34 %), Lymphocytic infiltrate (28.5 %), Ulcer/scab (17 %), Epithelioid cells (14 %). Most common epidermal features were hyperkeratosis (77%), acanthosis (44%), ulcer/scab (17%), atrophy (8.5%). pseudocarcinomatous hyperplasia and mixed patterns were also seen.

Conclusion: LT bodies considered to be diagnostic for CL are found in 43% of patients other than history and clinical examination; histological features which can add to the diagnosis include plasma cells infiltrates, giant cells, epithelioid cells, granuloma formation, ulcer/scab and mixed dermal infiltrate.

Key words: Cutaneous Leishmaniasis, Leishmania, Skin biopsy.

Introduction

Cutaneous leishmaniasis (CL) is an important health problem in various parts of the world. In endemic areas (Afghanistan, Pakistan, Iran and Iraq) incidence of CL is approximately 2-3%. CL is transmitted by sand flies. In human host they present as intracellular parasites infecting mononuclear macrophages referred as Leishmania Tropica (LT bodies). Cutaneous Leishmaniasis presents a spectrum of manifestations both clinically and histologically. Lesions can present as nodule, plaque or ulcer mostly present on exposed sites.

Various investigations are done for diagnosis of CL. Histopathological findings in acute CL include dermal infiltrate predominantly consisting of macrophages containing large number of leishmania organism called LT bodies. In addition plasma cells and dense mixed inflammatory cell infiltrate are also present in dermis. When ulceration occurs secondary infiltration with neutrophils occur. After several months, in chronic forms number of LT bodies is reduced and granulomatous infiltrate containing epithelioid cells and giant cells appear. The objective of study is to delineate histopathological features that can help in diagnosis of CL other then the presence of LT bodies. As LT bodies cannot be detected in all biopsies especially in chronic lesions and other investigations are costly and difficult to perform.

Materials and Methods
This prospective descriptive study was carried out at Dermatology and Pathology departments of Pakistan Institute of Medical Sciences, Islamabad over a period of 8 months (Jan 2008 – April 2008). 35 Patients of all age groups and both genders were included in the study. Among all 35 patients age range was 16–55 years. Diagnosis was made on the basis of history (patients coming from endemic areas, persistence of lesions), clinical presentation of lesions (nodules, plaques, ulcers) and response to specific antileishmania treatment.

Duration of lesions varied from 4 weeks to 12 months. All cases had localized cutaneous Leishmaniasis. The lesions were clinically grouped into early (duration less than 2 months) and late lesions (duration more than 2 months).

Different clinical lesions were noted as plaque, nodule, crusted lesions and ulcers. Skin biopsy was done in all the patients. Biopsy specimens were routinely processed and stained in the department of pathology using formalin fixation and hematoxyline and eosin stain (H & E Stain). Skin biopsies were studied by dermatopathologist and histopathologist.

In patients where histopathological feature was presence of granuloma/ tuberculoid granuloma ESR, chest X-ray, Mantoux test was done and was asked about family history of tuberculosis to rule out important differential diagnosis of lupus vulgaris.

After history, clinical examination, and skin biopsy patients were given intraleisional or systemic antimonials depending upon site and number of lesions. Patients response to antimonials was noted which further confirmed the diagnosis of cutaneous Leishmaniasis.

All the histopathological changes were noted and data was analysed using Microsoft Excel.

**Results**

A total of 35 patients were included in the study in 8 months period. Among 35 patients 70% were male and 30% were female. (Figure 1) Among all the patients included in the study age range was 16 – 55 years with lesions most commonly seen in patients with age group of 25 – 35 years (Figure 2).

Out of 35 patients LT bodies were seen in 43% of patients. In most of these patients duration of lesion was less than two months. Next commonest presentation was presence of Plasma cells with or without necrosis (77%), Giant cells (34%) & granuloma formation (34%) (Figure 5, 6 and 7). Other histopathological features included lymphocytes, ulcer formation/scab, neutrophils, epithelioid cells, hyperkeratosis, acanthosis, atrophy and pseudocarcinomatous hyperplasia. (Figure 3, 4)

Different histopathological features were grouped as dermal reactions and epidermal changes. In dermal reactions features other than presence of LT bodies included presence of plasma cell infiltrate with or without necrosis, granuloma formation, giant cells, lymphocytes, epithelioid cells and polymorphonuclear leukocytes. Most common epidermal changes were hyperkeratosis and acanthosis. In some cases atrophy and pseudocarcinomatous hyperplasia was seen. Intraepidermal organisms were not seen in any case.

Out of 35 patients 20 patients presented with early lesions (duration less than 2 months). In these patients, most common histopathological finding was the presence of LT bodies in 14 patients (70%); other histopathological findings were plasma cell infiltrate, polymorphs, lymphocytes and early granuloma formation with epithelioid cells, few plasma cells and lymphocytes.

15 patients presented with chronic lesions (duration from 2 – 8 months). LT bodies were seen just in 1 patient with most common histopathological finding in all other patients was well formed epithelioid granuloma. Granulomas were formed of epithelioid cells, lymphocytes and giant cells with no caseation.
Figure 3: Frequency and percentage of different histopathological features

Figure 4: Frequency and percentage of different histopathological features

Figure 5. Skin biopsy: Macrophages containing LT bodies (H & E x 200)

Figure 6. Skin biopsy: Plasma cell infiltrate (H & E x 200)

Figure 7. Skin biopsy: Multinucleated giant cells (H & E x 200)

Discussion
Cutaneous Leishmaniasis has emerged as significant health problem of our country and a top priority for tropical disease programme of World Health Organization. Diagnosis of CL is although clinically obvious to an experienced practitioner in an endemic area, confirmation can be done using various investigations. In our setup slit skin smear and light microscopy are most accurate and cost effective diagnostic methods. So histopathology should be attempted first. Other investigations used to diagnose CL include , culture on NNN medium it yields positive result in 1- 3 weeks. Montenegro test is performed injecting killed promestigotes intradermally and examining the lesion.
after 48 hours. An induration of 5mm or more indicates positive result. However two main drawbacks are that acute infection cannot be identified and positive result may not be obtained in immunosuppressed. Serological tests are not well established and are costly. PCR is more accurate in determining in new onset CL but is costly.

In our study diagnosis was based on history, clinical examination and histopathological features which was further confirmed by response to treatment. On histopathology although the most diagnostic feature is the presence of LT bodies, they cannot be seen in all specimens.

In various studies frequency of LT bodies varies from 38-75%. In our study LT bodies were seen in 43% of patients but they are more numerous in early lesions and gradually tend to decrease in chronic lesions. So we need to establish diagnosis in remaining 57% of patients. We have seen in our study that during evolution of lesions various intermediate features are also seen including presence of plasma cells, giant cells and epithelioid cells that can lead to the diagnosis of CL.

Diagnosis on histological basis in early lesions is not difficult. Difficulties arise when organisms are scanty and histological features other then LT bodies are seen. A frequent finding was the presence of granulomas. Important differential diagnosis at this stage includes other granulomatous disorders including lupus vulgaris, leprosy, rosacea and sarcoidosis. In lupus vulgaris there are tuberculoid granulomas, in leprosy nerve thickening and perineural infiltrate is seen, in sarcoidosis naked granulomas are seen. In rosacea clinical presentation of lesions (Persistent papules/ pustules with history of flushing) are helpful diagnostic features and rosacea does not respond to antimonial therapy. Another feature was the presence of plasma cell infiltrate with or without necrosis, an important differential includes syphilis. In patients with syphilis on histopathological examination along plasma cell infiltrate endarteritis obliterans is seen. Diagnosis of cutaneous Leishmaniasis could not be made on epidermal changes alone, patient must have typical clinical presentation and history of coming from an endemic area, and other causes of atrophy and ulceration need to be ruled out.

Studies has been done in past for diagnosis of CL based on various investigations, histopathological features in detail are not studied very well so far. In our setup most of the patients presenting to us belong to low socioeconomic status. On the basis of history and clinical presentation clinical diagnosis was made and skin biopsy was done. Presence of LT bodies was diagnostic for CL. Difficulties arise when LT bodies are not seen. Other investigations to confirm the diagnosis are available but are costly and time consuming. Most of the patients coming to our OPD cannot afford these investigations so we have to rely on other histopathological findings. Patients were also given meglumine antimoniate and all the patients responded to them with clearance of lesions.

Skin biopsy is not only diagnostic in cases of cutaneous leishmaniasis but the variety of features specially the atypical ones should not be ignored and are also considered important in diagnosis of cutaneous leishmaniasis and simply absence of LT bodies cannot specifically rule out CL and the features shown in our study can also lead to the diagnosis of CL supported with history, clinical examination and response to antimony compounds.

**Conclusion**

In the absence of LT bodies on skin biopsy other histopathological features can lead to the diagnosis of Cutaneous Leishmaniasis, without using other investigations for diagnosis which are costly, time consuming and cumbersome. LT bodies although diagnostic but not easily found in many cases. Features other than it aid to diagnosis of CL and these must not be ignored as they may clinch the diagnosis in appropriate clinical background.

**References**