ORIGINAL ARTICLE

The predictive value of slit skin smears and skin biopsy in the diagnosis of cutaneous leishmaniasis: Comparative study

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ABSTRACT

Background: Slit skin smears and skin biopsy are the most common techniques used for the diagnosis of cutaneous leishmaniasis (CL) in endemic areas.

Aim of work: This study was carried out to compare the predictive value of slit skin smears and skin biopsy in the diagnosis of CL.

Patients and Methods: 30 patient were included in the present study with clinically suspected lesions of CL. Their age ranged from 20 to 55 years. The duration of lesions was from one month to six months. Slit skin smears and skin biopsy were done for every patient, and then the results were compared.

Results: Among 30 cases, 14 cases (46.6%) showed positive slit skin smears for *Leishmania donovani* (LD) bodies and 16 cases (53.4 %) showed negative slit skin smears for LD bodies. Histopathological examination of sections obtained from skin biopsies revealed that 7 cases (23.3%) were negative for LD bodies and no granuloma was seen, 23 cases (76.6%) showed features favoring the diagnosis of CL (21 cases had LD bodies and 2 cases had granulomas formed of epitheliod histiocytes, giant cells, lymphocytes and plasma cells). The sensitivity of skin biopsy was significantly higher than slit skin smears (76.6% versus 46.6% P <o.oo1). All lesions with positive result on slit skin smears also showed positive result on skin biopsies.

Conclusion: Histological examination of sections obtained by a skin biopsy is the most sensitive and conclusive technique for the diagnosis of CL. Slit skin smears can be used as a first step technique for the diagnosis of CL.

KEYWORDS: Cutaneous leishmaniasis(CL), Leishmania donovani(LD)bodies, slit smears, skin biopsy

INTRODUCTION

Leishmaniasis is a parasitic disease caused by a heterogeneous group of protozoan parasites that belong to the genus Leishmania. It is transmitted by the bite of certain species of sand fly (subfamily Phlebotominae). Two genres transmit Leishmania to humans: Phlebotomus and Lutzomyia.[1] Cutaneous leishmaniasis (CL) is endemic in 88 countries, particularly localized in areas of the tropics and subtropics of Africa, western and central Asia.[2]

Human infection is caused by more than 20 different species that infect mammals. A single species can produce more than one clinical form of the disease, and each form can be caused by multiple species. The different clinical presentations of the disease depend on species causing the infection and on host-related factors. The skin, mucosa, and mononuclear phagocytic system may be affected giving three forms of leishmaniasis: Cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis. Cutaneous Leishmaniasis

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presents a spectrum of manifestations both clinically and histologically. Lesions can present as nodule, plaque or ulcer.[3]

Several techniques are used for the diagnosis of CL. Slit skin smears and histopathological examination of skin biopsy are sensitive methods for diagnosis of suggested cases of CL. Direct microscopic examination of slit smear using Geimsa stain reveals leishmania amastigotes in macrophages or extracellular areas. Histopathological findings in acute CL include dermal infiltrate predominantly consisting of macrophages containing large number of leishmania organism called *Leishmania Donovani* (LD) bodies.[4,5] In addition plasma cells and dense mixed inflammatory cell infiltrate are also present in dermis. When ulceration occurs secondary infiltration with neutrophils occur.[6,7] In chronic stage number of LD bodies is reduced and granulomatous infiltrate containing epithelioid cells and giant cells appear.[8,9] Other investigations as culture and PCR are specific but very costly.[10]

There is a need for identifying a sensitive technique to confirm diagnosis of CL. The present study was carried out to compare the predictive value of slit skin smears and skin biopsy in the diagnosis of CL.

PATIENTS AND METHODS

This study was carried out at the department of Dermatology, Al-Azhar university hospitals, Cairo, Egypt over a period of 3.5 years (June 2010-December 2013). Thirty Patients clinically suspected of CL were included in the study. Their ages ranged from 20 to 55 years. An informed consent was taken from all patients. The diagnosis was made on the basis of history (patients coming from endemic areas, persistence of lesions), clinical presentation of lesions (nodules, plaques, ulcers). The duration of lesions varied from 1 to 6 months. Slit skin smears followed by Skin biopsy were done for all the patients. The smears & specimens were processed and stained for cytological and histopathological evaluation.

Slit skin smears

The lesion was cleansed with alcohol pads and allowed to dry. The lesion sites/ ulcers were anaesthetized with 1% lidocaine. A small incision was made in the active margin of lesions/ulcers with the point of the blade. The blade was turned 90 degrees and scraped along the cut edge of the incision to remove and pick up the skin tissue, which was smeared on clean glass microscope slides. After the smears dried completely, they were fixed with 100% methanol, allowed to dry again, and stained with Geimsa stain for microscopic examination at 100 x magnification for the presence of amastigotes.[11]

Skin biopsy

Skin biopsy was taken from the active edge of the lesion with a 4-mm disposable punch. The material was fixed in neutral formalin, routinely processed and embedded in paraffin. Sections were stained with hematoxylin, eosin and Geimsa. The sections were examined under microscope at 40x &100x magnification. Special stains as PAS, Zeihl -Neelsen and Fite Faraco stains for other organisms were also examined.[10,12] The number of LD bodies were graded on a scale (modification of Ridley's parasitic index 1983) as; - (no organism), + (1-9 organisms per standard section); ++ (10-100 organisms per standard section); + + + (>100 organisms per standard section).[13]

After history, clinical examination, slit smears

and skin biopsy, confirmed patients were given intralesional antimonials. Patients response to antimonials was noted which further provided indirect evidence for the diagnosis of cutaneous Leishmaniasis.

RESULTS

Among 30 patients, 19 (63.3%) were males, 11 (36.7%) females. Their ages ranged from 20 to 55 and the mean (± SD) age was 35.3 ± 4.6 years. We observed that the lesions were located on face in 14 (46.6%) cases, including the forehead in 5 cases (16.6%), the nose in 2 cases (6.6%) (Fig. 1), the chin in 7 cases (23.3%). While in 16 cases (53.3) they were found on the parts of extremities, such as the forearms in 4 cases (13.3%), the elbows in 2 (6.6%) cases, the right arm in 1 cases (3.3%) (Fig. 2), the legs in 6 cases (20%) and the knee in 3 cases (10%) as shown in (Table 1). In 4 cases (13.3%), multiple lesions were observed. The size of the lesions ranged from 1 cm to 6 cm. The duration of the lesions ranged from 1 month to 6 months and the mean duration was 2.9 months.

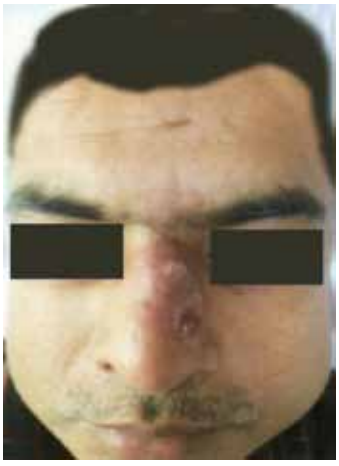

Fig. 1 Reddish brown, crusted, ulcerated, indurated, plaque over the left side of nose since 3 months.

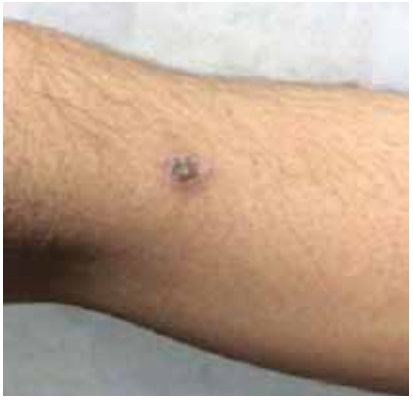

Fig. 2 Reddish brown, indurated nodule in the right arm since two months.

Table 1 Distribution of lesions in all patients

Site of lesion	Number of patient
Forehead	5 (16.6%)
Nose	2 (6.6%)
Chin	7 (23.3)
Forearm	4 (13.3%)
Elbow	2 (5%)
Arm	1 (3.3%)
Knee	3 (10%)
Leg	6 (15%)

Out of 30 cases, 14 cases (46.6%) were positive with skin smears method for LD bodies (Fig. 3A) and 16 cases (53.3%) were negative for LD bodies. Histopathological examination of biopsy in 7 cases (23.3%) were negative for CL, as characterized by absence of granuloma and LD bodies

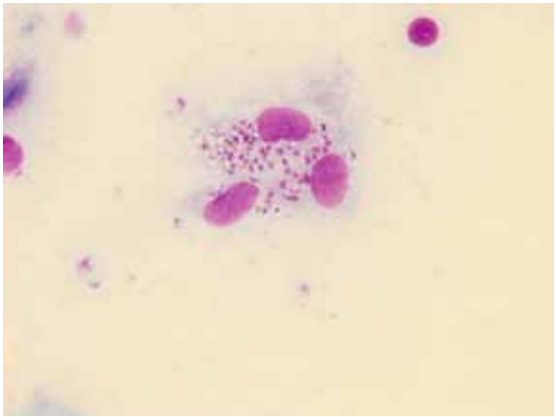

Fig. 3A Positive slit skin smear (Geimsa x 100).

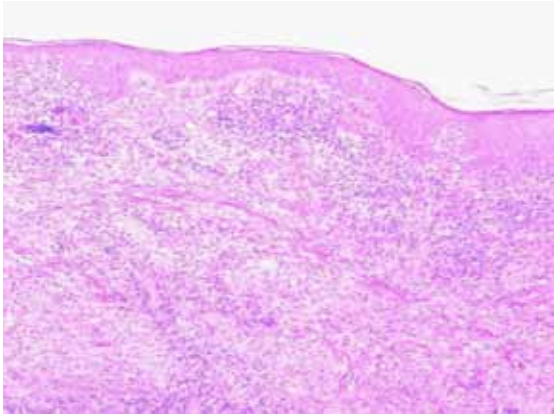

Fig. 3B Dermal infiltrate formed of lymphocytes with numerous histiocytes (H & E x 10).

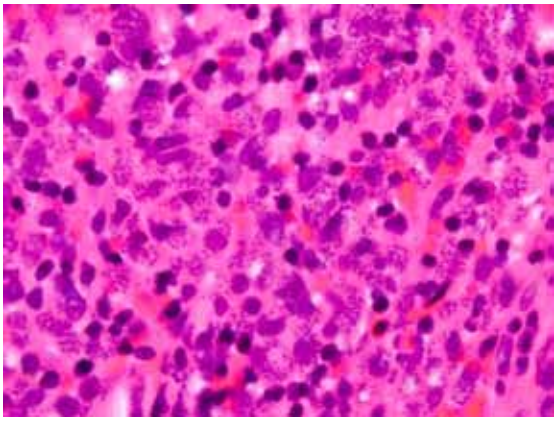

Fig. 3C Leishman-Donovan bodies within the histiocytes and extracellulary (H & E x 100).

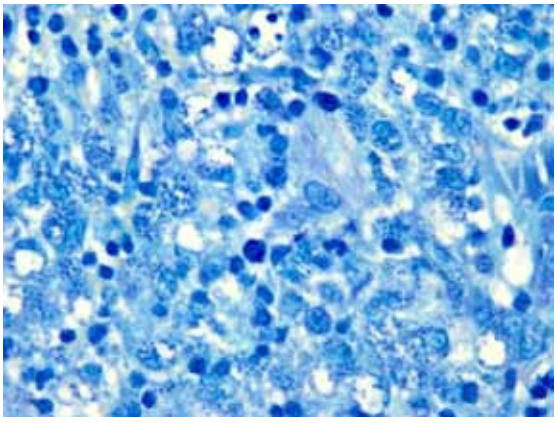

Fig. 3D Leishman-Donovan bodies within the histiocytes and extracellulary (Geimsa x 100).

except with evidence of acute and chronic inflammation. While 23 cases (76.6%) showed evidence for CL (21 cases had LD bodies) (Fig. 3 B, C, D) and 2 cases had granulomas without LD bodies formed of epitheloid histiocytes, giant cells, lymphocytes and plasma cells (Fig. 4). The LD bodies, of size 2-4μ, consisted of a nucleus and a smaller kinetoplast, were present within macrophages and occasionally extracellularly (Fig. 3 A, C, D).

There was no correlation between the patients' age, sex and lesions' duration, size with the results of the diagnostic techniques.

Histopathologic examination in 23 cases of CL revealed epidermal and dermal findings. Epidermal findings were hyperkeratosis in 12 cases (52.1%), parakeratosis in 9 cases (39.1%), liquefaction degeneration of the basal cell layer in 5 cases (21.7%) and follicular plugging in the epidermis in 8 cases (34.8%). While, An ulcer was present in 7 cases (30.4%), epidermal atrophy in 10 cases (43.4%), acanthosis in 5 cases (21.7%), pseudoepitheliomatous hyperplasia in 6 cases (26.2%) and crust in 5 cases (21.7%). Epidermal findings are shown in Table 2.

Dermal histological findings were categorized

Table 2 Histopathological epidermal findings of the CL cases

Epidermal findings	**Number of cases(%)**
Hyperkeratosis	12 (52.1%)
Parakeratosis	9 (39.1%)
Acanthosis	5 (21.7%)
Pseudoepitheliomatous hyperplasia	6 (26.2%)
Follicular plugging	8 (34.8%)
Epidermal atrophy	10 (45.4%)
Liquefaction degeneration of the basal cell layer	5 (21.7%)
Ulceration	7 (30.4%)
Crust	5 (21.7%)

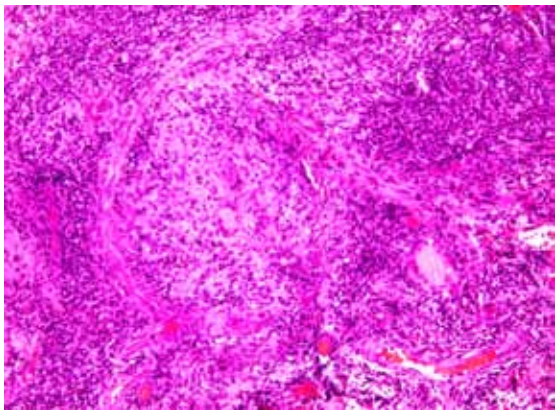

Fig. 4 Granuloma formed of epitheloid histiocytes, giant cells, lymphocytes and plasma cells (H & E x 20).

into 3 patterns (Fig. 3 B, C, D) & (Fig. 4):

Type 1 pattern: Consisted of a diffuse dermal infiltrate composed mainly of macrophages admixed with few neutrophils and lymphocytes. Eosinophils and plasma cells were seen occasionally. The sections showed plenty (+ + +) of LD bodies, which were apparent with both H& E as well as Giemsa stains. This pattern was seen in 11 cases (47.8%).

Type 2 pattern: Consisted of early granuloma formation with focal collection of epitheloid cells, lymphocytes, and few plasma cells. There were no giant cells. There were only a few (+) LD bodies seen. This pattern was seen in 10 cases (43.4%).

Type 3 pattern: Epitheloid granulomas were observed in this pattern. The granulomas consisted of epitheloid cells, histiocytes, lymphocytes, Langhan's giant cells and foreign body giant cells, LD bodies were absent. This pattern was seen in 2 cases (8.6%). Other dermal changes included areas of granulation tissue formation, vasodilatation and edema.

Table 3 Histological dermal findings of the CL cases

Pattern	Dermal infiltration	Number of cases
Type 1	Numerous: histiocytes & LD bodies. Few: neutrophils & lymphocytes. Occasionally: Eosinophils & plasma cells	11(47.8%)
Type 2	Early granuloma with focal collection of epitheloid cells & lymphocytes and few plasma cells. No giant cell. few LD bodeis	10(43.4%)
Type 3	Epitheloid granuloma formed of epitheloid cells, histiocytes lymphocytes, Langhan's giant cells and foreign body giant cells. No LD bodies.	2 (8.6%)

DISCUSSION

The routine diagnosis of CL in endemic areas depends on direct microscopic examination of slit skin smears and histopathological examination of sections obtained from a skin biopsy.[14,15] In current study, the diagnosis was based on history, clinical examination, slit skin smears and histopathological features which was further strengthened by response to treatment.

In this study, the sensitivity of direct smear (46%) was compatible with similar studies performed by Javidi et al,[15] Gazozi et al,[4] and Nazodi et al,[16] (55.4%, 54.33% and 50.4%, respectively) but it is much higher than that reported by Dar et al[17] (25%). An explanation of this difference is that patients in this study were in acute stage of the disease (duration of lesions from 1 month to 6 months) and we prepared and examined from 2 to 4 slides for each patient instead of one. Also, the results of skin smears were consistent with the previous studies which have shown that the sensitivity of this test is affected by the site of the scrapings, the staining quality and the technician proficiency.[18,19]

In current study, sensitivity of histological examination of skin biopsies (76.6%) was comparable with studies of Dar et al,[17] Gazozi et al[4] and Kubba et al[20] (89.74%, 72.34%, 70%, respectively), but it was not comparable with study of Rawlin et al[21] (43.8%). This discrepancy may be explained

by the fact that cases of this study were in acute form (from 1 to 6 months) and also, many serial sections were routinely obtained for histopathological examination. As reported in previous studies,[14,17] and in this study the sensitivity of skin biopsy (76.6%) was higher than the sensitivity of slit skin smear (46.6%). The difference was significant ($P > 0.001$). This indicates that skin biopsy is the most sensitive method for the diagnosis of acute CL.

The dermal findings in CL has been classified into different types. Scrimgeour et al and Bryceson[22,23] reported three histological classification of CL, tuberculoid, macrophage (leptomonad) and intermediate forms. Kurban[24] reported two histological patterns, early lesions (less than 1 year) showing a diffuse macrophage infiltrate, with plenty of organisms and late lesions (more than 1 year) with a granulomatous infiltrate. Mansour et al[25] reported similar pattern. Ridley[13] suggested a three-group classification for CL after a comparison with classification of Brazilian leishmaniasis. Gaffar et al[26] reported four different patterns depending on the various cell populations, granuloma, necrosis and parasite count. In this study and as reported by Gazozi et al[14] three different patterns were observed according to the various cell population, LD bodies and presence of epitheloid cell granuloma. The histological features in CL differs according to the stage of infection, clinical type of disease and host immunity.[27] In a study of comparison of pathological patterns of cutaneous leishmaniasis in different geographical regions, El Hassan et al[28] demonstrated that type 2 pattern was more prominent in Nicaragua and Guyane, while type 3 pattern was more common in Sudan and Saudi Arabia, and hence concluded that the histological types varied from one region to another.

Diagnosis of CL based on histological basis in early lesions is not difficult. Difficulties arise when organisms are absent. During evolution of lesions, various intermediate features are also seen including presence of epitheloid cells, giant cells and plasma cells that can be considered as indicator for diagnosis of CL.[4] In this study, 2 cases showed granulomtous infiltrate formed of epithelioid cells, giant cells, lymphocytes and plasma cells with absence of LD bodies, it was diagnosed as case of CL based on clinicopathological correlation and response to intralesional antimonial therapy that confirmed the diagnosis.

As reported in previous study, diagnosis of cutaneous Leishmaniasis could not be based on epidermal changes alone.[10] Features such as basal cell degeneration, intraepidermal abscesses, hyperplastic rete ridges though nonspecific, may be helpful in differentiating CL from other lesions.[12] Histopathological findings in CL should be differentiated from other granulomatous disorder such as lupus vulgaris, leprosy, sarcoidosis. In lupus vulgaris, there are tuberculiod granulomas with caseation necrosis. In leprosy nerve thickening, perineural and periadenxal infiltrate are seen. Naked granulomas are characteristic for sarcoidosis. Plasma cells and endarteritis obliterans are seen in syphilis.[10]

CONCLUSION

Appropriate diagnosis of clinically suggestive cases of CL is essential for specific treatment and control of the disease. Histopathological examination of sections obtained from a skin biopsy is the most sensitive and conclusive method for diagnosis of CL. Presence of granulomatous infiltrate formed of epitheloid histiocytes, gaint cells and plasma cell with absence of LD bodies can

be considered as a guide for the diagnosis of CL in clinically suspected cases. Slit skin smears can be used as a primary investigation for the diagnosis of CL (a good positive test); however, patients where slit skin smears technique is negative should be subjected to the histological examination if the clinical suspicion of CL is high.

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