

Evaluation of diagnosis of cutaneous leishmaniasis by direct smear, culture and histopathology

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ABSTRACT

Objective: Definite diagnosis of cutaneous leishmaniasis is based on the isolation of the causative organism by smear and culture or its identification in tissue section. This study was conducted to evaluate the efficacy of different sampling techniques. These techniques are tissue sampling with dental broach, slit scrape method, aspiration of the lesion edge and biopsies.

Methods: This study was carried out in the Department of Dermatology, Baghdad Teaching Hospital, Baghdad, Iraq. Sixty patients with cutaneous leishmaniasis were seen, 33 females and 27 males with a mean age of 15.7 years and the mean duration of the lesions was 8-weeks. The total number of lesions was 167, 60% ulcerative and 40% nodular, 40% of patients had single lesion, while 60% had multiple lesions, and the highest number being 11 lesions. Smears and cultures were carried out in all patients using different sampling techniques. Biopsies were taken from 20 patients.

Results: It was found that tissue sampling using dental broach was better than the other smearing techniques. It was positive in 71.5% while culture on Nicolle-Novy-MacNeal media was positive in 80% of cases and these

figures were much higher in comparison to other published studies. The morphology of LD bodies (amastigotes) in smears was mainly spindle shape, other morphological forms like barrel, safety pin and umbrella like were noticed, while the morphology in histopathological sections were rounded with a nucleus and kinetoplast. However, in some sections spindle shape form similar to smear morphology were detected. LD bodies were seen in histopathological sections in 30% of patients. Other histopathological features were mainly abundance of lymphocytes and plasma cells in the wet ulcerative lesions while in dry nodular types there was a tendency to form granuloma with less lymphocytes and scanty plasma cells.

Conclusion: Tissue sampling using dental broach appeared to be more efficient than other sampling techniques used in this study. Histopathological sections have identified the focal presence of the parasites. Thus, multiple site samples from the edge of the lesions are needed.

Keywords: Cutaneous leishmaniasis, dental broach, diagnosis.

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Diagnosis of cutaneous leishmaniasis is mainly clinical by observing characteristic lesions especially in endemic areas, however definite diagnosis should be reached in cases of research purposes and atypical lesions that might be confused with mycobacterial, mycotic or bacterial infections. Therefore definite diagnosis must depend on the

isolation of causative organism by smear, culture, and its identification in tissue section.¹ The present study is a trial to compare these different diagnostic aids and to find the easiest suitable technique.

Methods. A total of 60 cases of cutaneous leishmaniasis were included in this study in the

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Dermatology Department, Baghdad Teaching Hospital, Baghdad, Iraq. There were 33 females and 27 males. Their ages ranged from 2 years to 50 years with a mean age of 15.7 years. Many patients have multiple skin lesions, and the number varied between one and 11 with a mean of 2.75. The total number of the lesions in all patients was 167 of both ulcerative (102) and nodular (65) lesions. Duration of the lesions was from one-20 weeks with a mean duration of 8 weeks. For each patient a full history was taken especially regarding the residence, duration of the lesions and family history. Careful physical examination was performed to identify the number, the site, the size and the type of the lesions whether nodular or ulcerative, scar of previous infection of cutaneous leishmaniasis was also recorded. The diagnosis was based on history, clinical examination and confirmed by stained smears for the presence of leishman bodies (amastigotes), using leishman stain. Cultures were carried out using Nicolle-Novy-MacNeal (NNN) media for the presence of parasites.

Method of sampling.² a. Tissue sampling with dental broach: Smears and cultures were performed in all patients by using dental broach. The dental broach was inserted into the edge of the lesion. Firm pressure was applied into the lesion and then rotation of the broach was performed. Rapid pull of the broach was then carried out. Smearing of the aspirated tissue was carried out on a drop of saline already present on a glass slide. Culture on NNN media was performed from the same lesion by inserting the dental broach containing tissue material into the culture media. As a comparative study to the dental broach, smearing by slit-scrape and aspiration of the lesion was carried out. b. Slit scrape method: The edge of the lesion was incised, the wound edges scraped with a scalpel and the material smeared on a drop of saline already present on a glass slide and material obtained by scraping also inoculated for culture. c. Aspiration of the lesion edge: After injection of sterile saline into the edge of the lesion as much fluid as possible was aspirated then used to inoculate culture and to prepare smear. Smears were stained by leishman stain to detect the presence of Leishman bodies as follows: 1. Allow the smear to dry. 2. Fix in methanol 95% for 30 seconds then stain with Leishman stain for 20 minutes. 3. Rinse in tap water and examine under the oil immersion objective of the light microscope. Cultures were performed on NNN media, these media consisted of nutrient agar and human blood overlaid with a small volume of physiological saline to which an antibiotic like streptomycin was added. Culture media was checked for the appearance of parasite for 4 weeks before they were discarded as negative.

Skin biopsy. Biopsies were taken from 20 patients and stained with hematoxylin and eosin stain as well as Giemsa stain.

Results. A total of 60 patients with cutaneous leishmaniasis were studied, 33 females (55%) and 27 males (45%), their ages ranged from 2 to 50 years with a mean age 15.7 years. Children and young adults below the age of 20 comprised 44 (73.3%) of the patients. Most of the lesions were on the exposed parts of the body. The upper limbs and hands were involved in 48.3%. The lower limbs and feet in 46.7%, and the face was involved in 35%, the ears in 1.7%, while the covered areas of the body were involved less frequently, buttocks in 5% and the trunk in 1.7%. Twenty-four (40%) patients had a single lesion, while 36 (60%) patients had multiple lesions, the highest number being 11 lesions. The total number of skin lesions in all of them was 167 of both nodular (65) and ulcerative (102) lesions. Different morphological pictures were seen such as: Nodulo-ulcerative lesion (the classical type of cutaneous leishmaniasis) ulcerated lesion, granulomatous nodule, impetigo like lesion, rhinophyma like picture and tinea circinata like lesion. Subcutaneous nodules along the lymphatic drainage of hand and forearm were seen in 3 patients together with enlargement of epitrochlear lymph gland. The primary lesions in these patients were mainly located on the hands.

Smear and culture results. Dental broach smear was positive in 71.6% and culture was positive in a high percentage (80%). Slit skin scrape smear was positive in 60% and culture in 60%. While aspiration of the lesion, smear was positive in 40% and culture in 60%. The morphology of LD bodies (amastigotes) in smears was mainly spindle shape (**Figure 1**), other morphological forms such as barrel, round safety pin and umbrella-like were noticed in some smears together with spindle shape. The morphology of leptomands (promastigotes) in cultures was spindle shape with tail (**Figure 2**). The amastigotes were mainly seen extracellular but in a number of patients intracellular forms were also observed (**Figure 3**). In the smears of 4 patients monilia hyphae were seen in addition to LD bodies.

Histopathological findings. The results of biopsies from 20 patients with cutaneous leishmaniasis were as follows:

Epidermal changes. Epidermal hyperplasia with orthoparakeratosis was noticed in 18 patients (**Figure 4**), while in some cases the epidermis showed necrosis and atrophy. Still, some sections showed pseudoepitheliomatous hyperplasia. Follicular plugging and liquefaction degeneration of the basal layer was observed in 5 patients, lymphocytic exocytosis was also recorded in many patients.

Dermal changes. The histopathological changes were characteristic and depended on the duration of lesions and whether they are ulcerative or not. The dermal changes were mainly observed as a spectrum, at one end of the spectrum, especially in the early ulcerative lesion, there were lymphocytic invasions

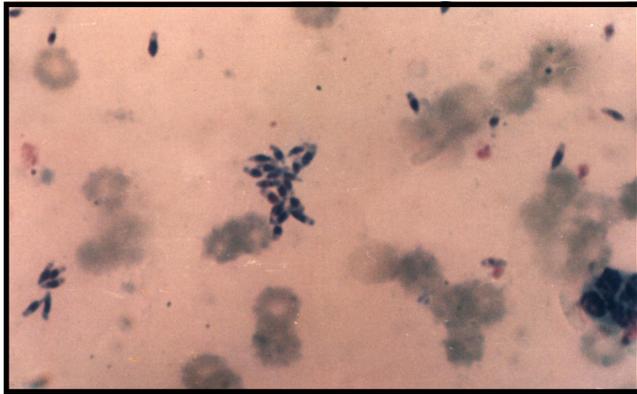


Figure 1 - Smear from cutaneous leishmaniasis showing numerous spindle shapes, extracellular LD bodies. (Leishman stain, oil immersion).

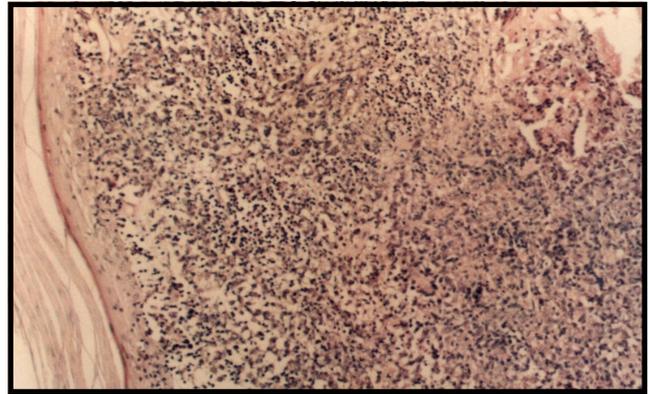


Figure 4 - Low power view of histopathological section of cutaneous leishmaniasis (hematoxylin and eosin).



Figure 2 - Culture from cutaneous leishmaniasis showing leptomand (promastigote) stage. (Leishman stain, oil immersion).

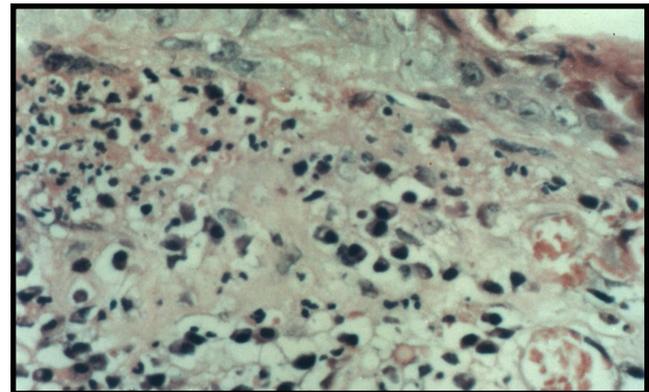


Figure 5 - Massive diffuse infiltration of lymphocytes and plasma cells in the early cases of cutaneous leishmaniasis (hematoxylin and eosin).

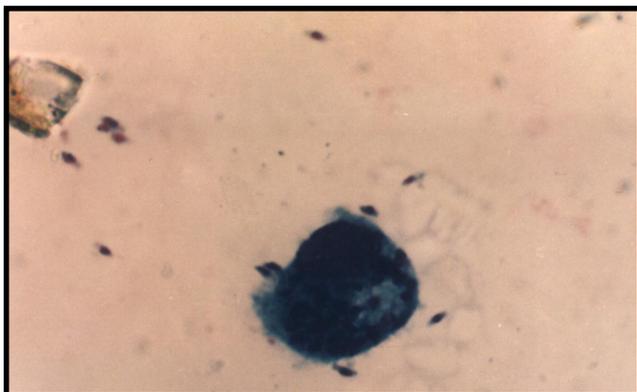


Figure 3 - Smear from cutaneous leishmaniasis showing intracellular and extracellular LD bodies. (Leishman stain, oil immersion).

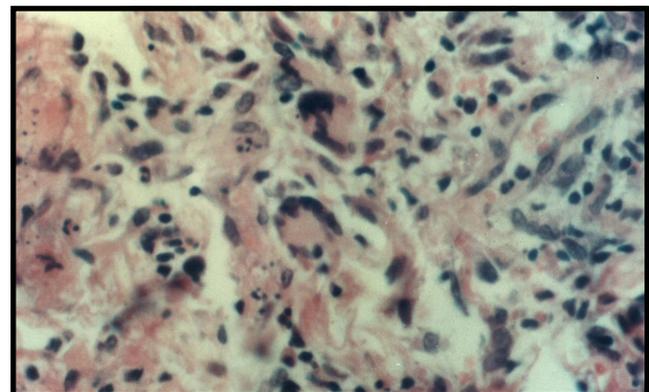


Figure 6 - Tubercles made up of epithelioid and giant cells in the dermis in late cases of cutaneous leishmaniasis (hematoxylin and eosin).

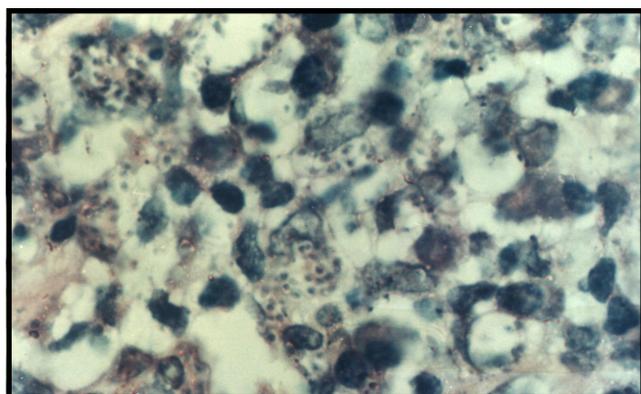


Figure 7 - Leishman bodies in histocyte in the dermis of cutaneous leishmaniasis (hematoxylin and eosin).

of the dermis together with multiple foci of plasma cells (**Figure 5**). At the other end of the spectrum, in the dry and in lesions with longer duration, the dermal changes were mainly granuloma formation with few lymphocytic cells and plasma cells (**Figure 6**). Neutrophils were detected especially when there was secondary infection. LD bodies were detected in 6 biopsies (30%). These bodies were noticed to be present in foci inside the dermis rather than in diffuse form, the parasite was seen intracellularly with extracellular bodies in the surrounding area (**Figure 7**). These LD bodies had the tendency to occur at the lymphocytic spectrum rather than in the granulomatous phase. The morphology of LD bodies in histopathological sections were rounded with a nucleus and kinetoplast, in some sections spindle shape form similar to smear morphology were detected.

Discussion. Cutaneous leishmaniasis is an endemic disease in many countries including Iraq. For the last 20 years, the disease has become epidemic and can be seen in many cities from north to south while previously it was mainly located in Baghdad, Iraq.² The diagnosis of cutaneous leishmaniasis depends on many criteria such as, history and clinical picture. There is a rule in this country: Any boil that stays for a few weeks and does not respond to ordinary therapy should be considered to be cutaneous leishmaniasis unless proved otherwise. The most helpful diagnostic aids are smears and cultures. Histopathological findings may be suggestive and occasionally diagnostics when the LD bodies are identified. Although a smear from the edge of the lesion is an easy and rapid technique to detect the parasite, it should be carried out carefully to eliminate high false negative results. Multiple sites from the edge of the lesion should be examined rather than one site, to increase the positivity results mainly due to the fact that parasites are present in a foci rather than in a uniform pattern in a lesion as had

been demonstrated in this study. In addition to the duration of the lesion, careful examination of the smears by an experienced eye increases the positivity rate of detecting the parasite in the smear. For the above reasons the present work has confirmed a high positivity rate using the dental broach method as the positive smears and cultures were 71.6% and 80%. These findings were much higher than the previous studies.³ In this study the dental broach method of sampling was found to be superior to slit-skin scraping (positivity rate of smear 60% and culture 60%), and aspiration method (positivity rate of smear 40% and culture 60%), this finding has not been reported before. The LD bodies in the smears usually take the round or spindle shape.^{4,5} This was confirmed by the present study, other morphological variants not previously reported were observed in this study like safety pin and umbrella like, these might cause confusion in diagnosis when present alone in the smear. Histopathological findings have confirmed the spectrum pattern and confirmed a previous observation.^{3,6,7} Plasma cells were found in abundance in the early ulcerative wet lesions and were present in typical foci; this observation was not properly documented in the literature. So on any histopathological examination whenever we see plenty of plasma cells in sections, the examiner should check for cutaneous leishmaniasis, in addition to other causes of increase in plasma cells. The presence of LD bodies in tissues was seen in 30% of cases and this was observed by both the hematoxylin and eosin as well as by Giemsa stain. These bodies were present in foci rather than in uniform pattern; this explains the negativity in some sections, smears and cultures especially if these foci are missed by these different techniques. This interesting observation has not been mentioned in previous studies.

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