Fine-needle aspiration cytology in the diagnosis of cutaneous leishmaniasis

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Background: In areas of endemicity without sufficient laboratory infrastructure, cutaneous leishmaniasis (CL) is often diagnosed on the basis of clinical characteristics, but parasitologic confirmation is essential to exclude erroneous diagnoses. We compared fine-needle aspiration cytology (FNAC) with the conventional method of excisional biopsy to assess the efficacy, utility and accuracy of FNAC for the diagnosis of CL.

Materials and Methods: In a consecutive series of 100 patients referred for a suspected CL lesion during June 2001 to May 2002, FNAC and 'excisional biopsy followed by histopathology' were done using hematoxylin and eosin (H&E) stain for both procedures.

Results: The study group included 40 males and 60 females, ranging in age from 1 to 70 with a mean age of 28.4 years. In more than 60% of cases, the lesions were on the face. By histopathological examination, 86 of 100 patients were positive for CL; while FNAC showed 77 cases as positive for CL. Taking histopathology as a standard diagnostic procedure, FNAC showed a remarkably high sensitivity (89%) and specificity (100%). The positive and negative predictive values were 100% and 60%, respectively.

Conclusion: FNAC is easier, less painful and more cost effective than the conventional 'scraping method/biopsy followed by histopathology'. The high sensitivity and specificity eliminate the need for other time consuming and invasive procedures. Limitations include poor sampling and poor yield.

Key words: Cutaneous leishmaniasis, fine-needle aspiration biopsy, sensitivity and specificity, Pakistan

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Leishmaniasis, a disfiguring skin disease, has reached epidemic proportions in the province of Balochistan, Pakistan. The World Health Organization (WHO) estimates that 200,000 people are infected in Kabul alone, the capital of nearby Aghanistan.[1] The magnitude of the problem has increased in the last twenty years because of the influx of refugees into Quetta Valley.[2, 3] Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*, which is transmitted by the bite of the female phlebotomine sandfly. Of the more than 15 species known to cause disease in humans, *Leishmania tropica, L. aethiopica, L. infantum,* and *L. major* are relevant to our part of the world.

In areas of endemicity without sufficient laboratory infrastructure, cutaneous leishmaniasis is often diagnosed on the basis of the clinical characteristics of the lesions. However, parasitologic confirmation is absolutely critical to exclude erroneous diagnoses, which may easily occur because of the wide spectrum of cutaneous presentations caused by *Leishmania spp.* and confusion with other dermal lesions.[4-6] Other lesions such as sporotrichosis and bacterial ulcer, both of which are frequent in regions where leishmaniasis is endemic, may mimic the presentation of cutaneous leishmaniasis.[7] Confirmation of infection is also important because treatment is expensive, toxic, and difficult to administer.[8] Lesions may re-activate and produce the progressive and disfiguring mucosal form if not adequately treated.[9]

Definitive diagnosis in the laboratory requires demonstration of the parasite in smears and/or biopsies, or by isolation of the organism in culture media or in experimental animals. Many methods for demonstration of parasites (histochemical and immunohistochemical) or detection of antibodies have been described, but remain outside the reach of the standard clinical diagnostic laboratory in less developed countries like Pakistan. The newer serologic techniques, such as ELISA (enzyme-linked immunosorbent assay), IFAT (indirect immunofluorescent antibody test), and others, are largely research tools with greatest use in seroepidemiological surveys.[10] We rely on demonstration of parasites in smears stained by hematoxylin and eosin (H&E), and on biopsy/scraping

specimens processed and stained with H&E. Conventional scraping is widely employed, but fine-needle aspiration cytology (FNAC) has been recommended over newer serologic techniques.[4] FNAC is relatively simple and economical, and is suitable for our part of the world. We compared FNAC with the conventional method of excisional biopsy/scraping followed by histopathology to determine the efficacy of FNAC.

Materials and Methods

During the period from June 2001 to May 2002, we performed FNAC and excisional biopsy followed by histopathology on 100 patients referred by the Dermatology Department of Bolan Medical College, Quetta, for suspected cutaneous leishmaniasis. The dermatologist selected the patients with lesions characteristic of the disease in Pakistan.

Both old world and new world cutaneous lieshmaniasis ancompass a broad range of severity and manifestations of infection. Stereotypically, lesions evolve from papules, to nodules, to ulcerative lesions, with a central depression and raised, indurated border, and ultimately, over months to years, to atrophic scars. Some lesions do not ulcerate but persist as nodules or plaques.[11]

Cutaneous leishmaniasis is endemic in certain areas of Pakistan, with the wet form of the disease being the most prevalent.[12] Bhutto et al in a study conducted on CL in the country classified the disease clinically as dry papular, dry ulcerative and wet ulcerative type.[13] These clinical presentations of the lesion along with the area of residence of the patient (e.g. refugee camps where the disease is endemic) were considered when including the patients for the study. The procedure was explained to each patient and informed consent was obtained before the fine needle aspiration was performed.

After the lesion was prepared for aspiration, a standard disposable 24-gauge needle was inserted into the lesion, and the aspirated cytology material in the bore was spread on the slide with a 20-cc syringe filled with air. No local anesthesia was required. For the excisional biopsy, a microtome and an automatic tissue processor were used for preparing a formalin-fixed biopsy material. The procedure took about 18 to 24 hours. The stain used on both the aspiration and biopsy smears was H&E, which produces results similar to Giemsa and Leishman stains. Periodic Acid Schiff stain failed to stain the parasite.[14] A Diff-Quick stain resulted in poorer morphology of the parasite and macrophages because of the drying effect of the stain.

The cytology slides were interpreted without knowing the results of the histology report. The aspirates were considered adequate or satisfactory if more than 4 to 6 well-visualized cells were present. The aspiration was repeated, if necessary, to obtain a satisfactory aspirate. Diagnosis was based on the presence of epitheloid cells tending to form granulomas, lymphocytes, neutrophils, eosinophils, macrophages containing Leishman-Donovan bodies, and extracellular Leishman-Donovan bodies. The final diagnosis was based on a histopathology examination. The criteria for diagnosis were dermal atrophy, hyperkeratosis, keratin plugging, granulomas, histiocytes containing parasites, extracellular parasites, and plasma cell infiltration.

Results

The 100 patients included 60 females and 40 males, ranging in age from 1 to 70 years with a mean age of 28.4 years. The lesions were located on the face in 60% of cases, with the remainder on other exposed areas such as the hands and feet. Eighty-six patients were positive for cutaneous leishmaniasis by excision biopsy/scraping followed by histopathology, while 77 patients were positive by FNAC. Taking histopathology as the standard, the sensitivity of FNAC was 89% and the specificity was 100%. The positive and negative predictive values were 100% and 60%, respectively.

In the aspirates taken by fine needle, the yield was adequate in all specimens. Extracellular Leishman-Donovan bodies and macrophages containing Leishman-Donovan bodies (Figures 1, 2) were found in all 77 cases testing positive for leishmaniasis, while only 24 cases revealed the presence of plasma cells. Neutrophils and eosinophils were also present in varying numbers. Lymphocytes were present in 85% of the skin lesions. Since leishmaniasis is a granulomatous disease, epitheloidcells tending to form granulomas were also present in most of the patients (78%) (Table 1).

In the histopathological exam, dermal atrophy was present in more than 80% of patients, while hyperkeratosis was seen in only 40% (Table 2). Keratin plugging was seen in 32%. Eosinophilic infiltration was negligible, but plasma cells were apparent in 90% of cases. Extracellular parasites and histiocytes containing parasites (Figure 3), the main diagnostic criteria, were found in 70% and 86% of cases, respectively.

Discussion

Cutaneous leishmaniasis is rarely fatal, but the lesions may cause tissue destruction, scarring, serious impairment of vision, and social stigmatization. The disease manifests in a variety of forms, including simple cutaneous leishmaniasis, mucocutaneous leishmaniasis, diffuse cutaneous leishmaniasis, and leishmaniasis recidivans.[4, 15]

FNAC proved to be a suitable alternative procedure for the diagnosis of cutaneous leishmaniasis. It is easier, less painful and more cost effective than the previous conventional scraping method/biopsy followed by histopathology. The diagnostic accuracy of skin aspiration, however, is dependent on the skill and experience of the personnel who perform the aspiration, prepare the slides

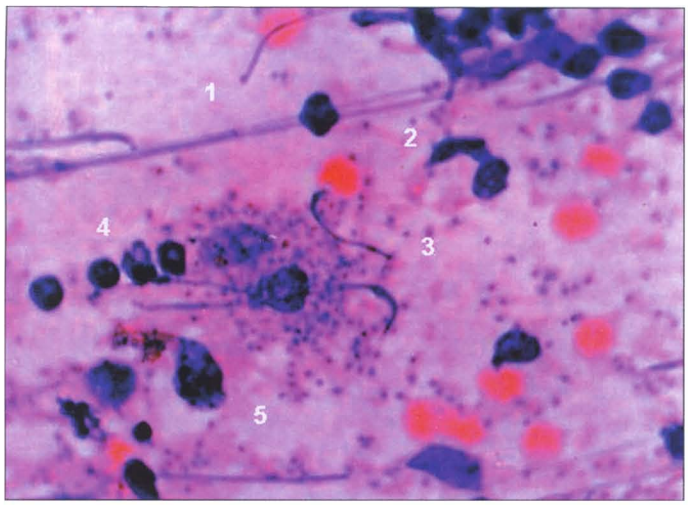

Figure 1. Fine-needle aspiration smear showing 1) red blood cells, 2) lymphocytes, 3) neutrophils, 4) extracellular Leishman-Donovan bodies, 5) a macrophage filled with parasites in amastigote form (H&E stain).

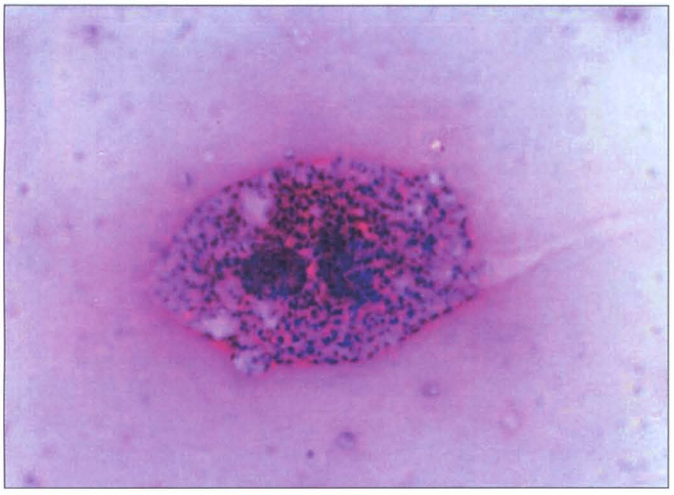

Figure 2. Fine-needle aspiration smear of cutaneous leishmaniasis showing a single macrophage filled with many parasites and some extracellular parasites.

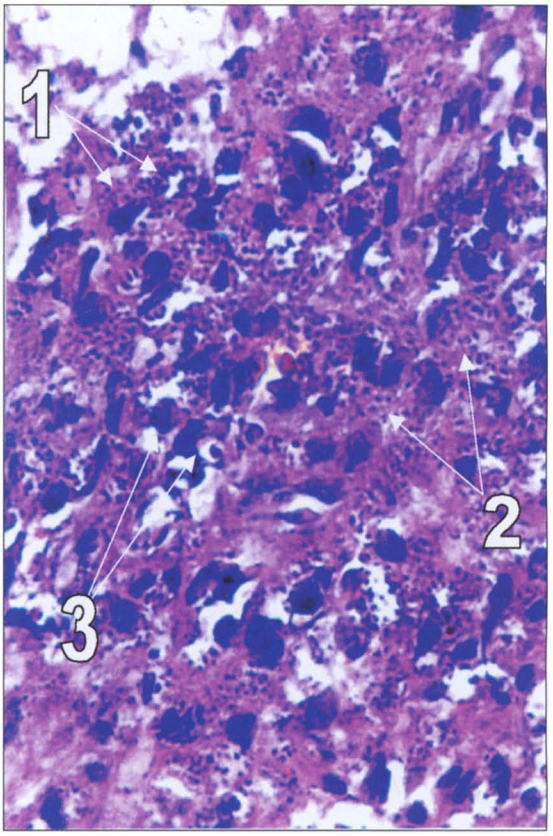

Figure 3. Histopathological section of cutaneous leishmaniasis lesion showing 1) macrophages filled with parasites, 2) some extracellular Leishman-Donovan bodies, and 3) plasma cells (H&E stain).

Table 1. Fine-needle aspiration cytology results for 100 patients with suspected cutaneous leishmaniasis.

	Yield	Granulomas/ epitheloidcells	Lymphocytes	Plasma cells	Other inflammatory cells	Macrophages containing LD bodies	Extracellular LD bodies
Nil	0	22	15	76	6	24	23
Mild*	30	26	25	24	36	36	38
Moderate**	68	34	58	0	52	28	31
Severe†	2	18	2	0	6	12	8

LD = Leishman-Donovan bodies; * Mild showed the presence of up to 6-8 cells; ** Moderate showed up to 9-20 cells; †Severe cases depicted more than 20 cells.

Table 2. Histopathology results for 100 patients with suspected cutaneous leishmaniasis.

	Dermal atrophy	Hyperkeratosis	Keratin plugging	Granulomas	Histiocyte containing parasites	Extracellular parasites	Plasma cell infiltration	Eosinophil infiltration
Nil	0	0	0	13	14	30	10	92
Mild*	18	60	68	30	26	58	10	8
Moderate**	76	40	28	32	28	12	32	0
Severe†	6	0	4	25	32	0	48	0

*Mild showed the presence of up to 6-8 cells; **Moderate showed the presence of 9-20 cells; †Severe cases depicted more than 20 cells.

and interpret the results. Since no local anesthesia was required, the process can be done in an outpatient setting as well.

Fine needle aspiration, though a blind procedure, has been well established for the diagnosis of skin lesions. The skin lesion aspirates contain most of the information necessary not only for the diagnosis of cutaneous leishmaniasis but also for other inflammatory and neoplastic processes.[16] Compared to the standard method, FNAC showed remarkably high specificity (100%) and sensitivity (89%). It could, therefore, be a good alternative for diagnosis of the disease. Due to the criteria formulated, the process is reliable and can be easily performed in a variety of clinical settings. To avoid false negative and false positive results the cytological findings should be considered in a clinicopathologic context. The high positive and negative predictive values (100 and 60% respectively) might have been related to the endemicity of the disease in the province of Balochistan. The free influx of refugees from the neighboring country may further contribute to the problem.

In a typical case of cutaneous leishmaniasis, the diagnosis is not difficult, but highly reactive macrophages, especially when distorted in morphology, may pose a problem in diagnosis. A sample lacking the histiocytes filled with parasites or extracellular Leishman-Donovan bodies should automatically be deemed inadequate. In some cases, it was advisable to repeat the FNAC on the same day, or later. Artifacts from the staining method that mimicked L.D. bodies included air bubbles, nuclear debris and stain particles. All these artifacts were given special attention and were therefore neglected. All these artifacts were ignored while following the criteria stated previously. Extracellular Leishman-Donovan bodies were considered diagnostic only when the full morphology was seen on slides and were accompanied by other defense cells. When extracellular Leishman-Donovan bodies alone were present, especially in a degenerated form, the aspirate was discarded. A high yield was obtained in most of the cases (68%) and was much higher in acute cases than the chronic ones.

The results of this study prove that FNAC is a valuable diagnostic tool for cutaneous leishmaniasis. The greatest advantage of FNAC in our setting is the savings in time and low cost. Limitations of the procedure are related to poor sampling, poor cellular yield in the recidivans type of cutaneous leishmaniasis, poor preservation and the difficulty

faced in differentiating degenerated Leishman-Donovan bodies from the real ones. Complications of FNAC are rare and seldom serious. We observed neither bleeding nor hematoma formation.

FNAC proved to be a reliable, economical and a relatively non-invasive procedure for the diagnosis of cutaneous leishmaniasis. The procedure can be carried out safely in an outpatient clinical setting. The risk of complications in an expert hand is negligible. FNAC could effectively differentiate between acute and chronic inflammatory processes. Fine needle aspirates from the skin contain most of the information processed not only for the diagnosis of cutaneous leishmaniasis, but also for grading in case of malignancies of the skin. Due to its high sensitivity and specificity, it obviates the need for time-consuming and other invasive procedures, especially in areas where the disease is endemic. Precise sampling and a pathologist's expertise could increase the sensitivity and positive predictive value.

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