COMPARISON OF DIAGNOSTIC METHODS IN CUTANEOUS *LEISHMANIASIS* (HISTOPATHOLOGY COMPARED TO SKIN SMEARS)

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ABSTRACT

Present study is carried out to compare laboratory diagnostic methods of Cutaneous leishmaniasis (CL) for the outdoor patients of Bolan Medical College Complex Hospital, Quetta, Balochistan. From November 2005 to December 2007, three hundred cases of CL patients were selected without restriction of age and sex. The lesions were divided into two groups. Early with duration less than 2 months and late duration between 2 to 4 months and were noted as nodules, plaques, ulcers and scarring (in case of relapses). Skin smears were taken on first visit of the patients, followed by skin biopsy for histopathological examination. Result showed that out of 300 cases 163 (54.33%) were positive smears for *Leishmania donovani* (LD) bodies and 137 (45.67%) were negative smears for LD bodies.. While histological examination of all 300 cases showed that only 83 (27.66%) cases were negative for (LD) bodies and no granuloma seen, except with evidence of acute and chronic inflammation. Further analysis of histological observations of positive cases (72.34%) revealed that 91(30.33%) cases had LD bodies,, 78 (26%) cases had only necrotic sloughs showing polymorph neutrophilic infiltration, and 48(16%) cases were having granulomas composed of, epithelioid cells Langhan's type of giant cells and lymphocytes. It is therefore concluded that histopathological examination as compared to skin smears method is more sensitive method for diagnosis of CL.

Keywords: *Leishmaniasis, diagnostic methods, histopathology.*

INTRODUCTION

Leishmaniasis is an infectious disease caused by trypanosomatid protozoan of the genus Leishmania comprising various species that are obligate intracellular parasites of the mononuclear phagocytic cells of vertebrates. About 20 species and subspecies of the genus Leishmania are known to infect man and cause spectrum of disease states. Although *cutaneous leishmaniasis* (CL) can be traced back many years, one of the first and most important clinical descriptions was made in 1956 by Alexander Russell following an examination of a Turkish patient. The disease, then commonly known as "Aleppo boil", which remains through life. It affects the natives when they are children, and generally appears on the face, and have some lesions on the extremities.

Leishmaniasis has been discovered more than 100 years back but has not been eradicated since then (Zubair *et al*., 2005). The leishmaniasis is one of the six major diseases which the tropical disease research (TDR) program of the World Health Organization (WHO) has targeted (Magill *et al*., 2005). They are important public health problem world wide. The endemicity of disease has been confirmed or suspected in 97 countries of the world and affect an estimated 12 million people with more than 400,000 new cases per year and approximately 367 million people are at risk of infection and disease (Reithinger *et al*., 2007). The disease is also endemic in Kuwait, Iraq, some parts of Iran and around 10 Mediterranean coasts The incidence of this disease is on a rise in many parts of the world, without urgent control measures it might become a major health problem worldwide.

CL is common in those areas of Pakistan which are near to Afghanistan border and also tropical and subtropical regions of the Balochistan province, and some case also reported from Northern area of Pakistan, Kurram and Momand agencies of NWFP, North Waziristan and Peshawar. Diagnosis of CL is usually suspected in the endemic regions of Pakistan for typical lesions. Confirmation of CL is achieved by laboratory examination of either skin scrapings or a biopsy from the lesions edge. A touch preparation using Giemsa stain reveals the *leishmania* amastigotes in macrophages or extra cellular areas. Skin scrapings are obtained from edges of lesions/ulcers. There are limitations regarding skin scrapings, especially in older lesions and ulcers, which are showing areas of fibrosis and healing. Other than these microscopy based test, there are serological and bacterial culture based tests available. Due to certain limitations those have less practical significance for diagnostic purpose. Since it is important for the clinically diagnosed patient to confirm by laboratory, we compared routinely performed tests (skin smear and histology) for sensitivity.

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MATERIALS AND METHODS

Patients

The study was carried out in Pathology, Dermatology Department of Bolan Medical College Complex Hospital Quetta and Pharmacy Department, University of Balochistan, Quetta, Pakistan from October 2005 to December 2007; Bolan Medical Complex Hospital is the biggest teaching Hospital of Balochistan providing health care to the patients of Balochistan and Afghanistan including the patients coming from far-flung area for diagnosis and further treatment of CL. From out patients department (OPD), cases of all ages, both sexes that were clinically diagnosed of CL and cases of chronic ulcer were also included in the study. A total of 300 patients reported to the OPD. However, cases with fibroses and skin lesions with secondary bacterial infections were excluded , who had received systemic treatment of CL and also patients using antibacterial drugs that have some potential against CL e.g., amoxicillin and rifampacin. Therefore a total of 300 cases are included in the study.

Study design

Patients were classified into three groups as follow:

1. Lesions, more than two months old.
2. Lesions, more than fourteen months old.
3. Relapse cases (after partial treatment) (table 3)

Clinical impression noted

a) Nodular lesions 94 cases i.e., 31%
b) Atypical erythemic lesions 78 cases i.e., 26%
c) Ulcerative and crusted lesions 128 cases i.e., 42%

1. **Skin Smears**. After cleaning the area surrounding the ulcer base with alcohol pads and allowed to dry. Most of the lesion sites/ ulcers were anesthetized with 1% lidocaine. Smears were made on glass slide, fixed in 95% Ethanol for 3 minutes, stained with hematoxylin and eosin (H&E), and examined for the presence of amastigotes, under microscope at 100 x magnification.

2. **Incisional skin biopsy**. A full thickness biopsy was taken from an inflamed margin of the lesion. Most of the lesions selected were of secondarily infected, cleaned with 0.9% saline or with 70% ethyl alcohol. In infected cases after cleaning with 6% hydrogen peroxide, 2% lidocaine with epinephrine was injected around the lesion and biopsy was taken with scalpel from the infected patients. Biopsy specimen was taken from selected lesions in the Department of Skin BMCH, and was referred to histopathology sections in 10% Formalin coded bottles. After processing with different steps with ethanol, the sections were taken and stained by hemotoxylin and eosin (H & E stains), under microscope at 40x magnification.

- The patterns were graded according to the following histological criteria

1. The pattern of infiltrate, whether diffuse or patchy.
2. Type of cellular infiltrate.
3. Presence of necrosis, and granuloma formation.

RESULTS

Out of 300 patients with CL, 197 (65.66%) were male and 103 (34.33%) were females. Among these patients 268 (89.33%) were adults. The median age of 32 (10.67%) child patients were 9 years (range 6-12) years. A total of 112 (37.35%) were having only a single lesion. The 188 patients (62.65%) with multiple lesions had a median of 3 lesions (range 2-6). Higher proportion of the lesions were located in the facial area, 86 patients (28.66%), on the other hand 42 patients (14%) have developed on lower extremities presented as shown in table 1 and plotted in fig. 1. The lesions were painless, enlarged, slowly approaching towards the central ulceration.

Table 1: Distribution of patients according to lesion site

Lesion Site	No of patients
Elbow	14
Face	86
Forearm	12
Forehead	99
Leg	42
Nose	72
Thigh	4
Wrist	9
Total	300

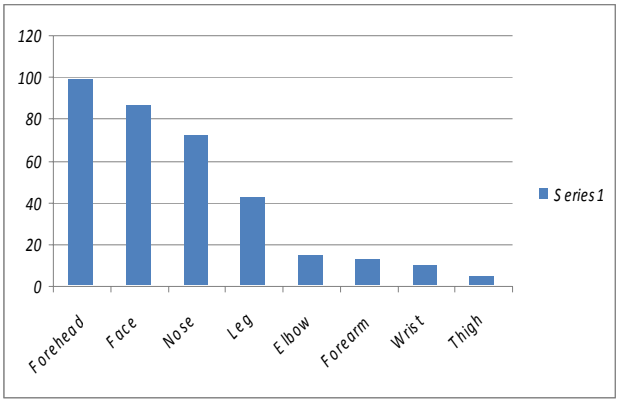

Fig. 1: Distribution of patients according to lesion site, in the figure the number shows the X-Axis and Y-Axis show the sites of the lesions.

Skin smears

Out of 300 cases 163 were positive (54.33%) with skin smears technique for (LD) bodies and 137 (45.67%) cases were negative for LD bodies. All 300 cases were also explored for histopathological examination.

Table 2: comparison of skin smear and histological examination

Skin smears	Hisotogical Exam
Positive cases: 163 (54.33 %)	Positive cases: 217 cases (72%) Cat A: 91 cases (30.33%) Cat B: 48 cases (16%) Cat C: 78 cases (26 %)
	Negative case: 83 cases (28%)
Negative cases: 137 (45.67 %)	Positive cases: Cat A: 73 cases (74%) Cat B: 08 cases (10%) Cat C: 50 cases (24 %)
	Negative case: 6 cases (8 %)

Histopathological sections (biopsy)

Histopathological examination of biopsy taken revealed only 83 cases (27.66%) negative for CL, as characterised by absence of granuloma and LD bodies except with evidence of acute and chronic inflammation. A total of 217 (72.34%) showed evidence for positive cases, which were classified into following three categories:

- 91 cases (30.33%) (Biopsy sections were positive for (LD) bodies).
- 48 cases (16%) cases were having granuloma, composed of epithelioid cells, Langhan's type of giant cells with no caseation necrosis.
- 78 cases (26 %) where LD body have-not seen, only areas with necrotic slough.

A comprehensive comparison of positive and negative cases by skin smears and histopathology is shown in table 3.

Histological grading

- **Type 1**: In this type, there was diffuse dense infiltrate consisting of predominantly macrophages. This pattern was seen in 148 cases.
- **Type 2**: In this pattern, there was diffuse dense infiltrate with a few early granuloma formations. LD bodies were sparse (+), seen within macrophages consisting of 110 cases
- **Type 3**: In this pattern, well formed epithelioid granulomas were seen in the dermis. The granuloma consisted of epithelioid cells, histiocytes and lymphocytes. Langhan's giant cells and foreign body giant cells were seen in 42 cases. LD bodies were absent (table 3). Other dermal changes included areas of granulation tissue formation, vasodilatation and oedema. Late lesions (3-12 months) were clinically seen as crusted plaques, lupoid plaques, dry crusted ulcers, nodules and plaques with areas of scarring, a total of 147 (49%) cases were in this category.

DISCUSSION

Results of study show a significant rise in Leishmania positive cases. In an other study the results of histopathology proved that biopsy specimen shows the percentage of Leishmania found in skin biopsies were 10.1 % (Anwer *et al*., 2007), where as the percentage of biopsy specimen Leishmania positive cases as compared to this study is about 30.33%. The Histopathology proven biopsy specimen Leishmania positive cases have a wide range of variation in morphology, different histological grading patterns have been recognized by various authors. Classification of CL as tuberculoid, (leptomonad) and intermediate forms (Bryceson *et al*., 1969). Where as other scientists has identified histological grading or classification in four categories (Vankataram, *et al*., 2001). In this study we have categorized the histological grading patterns in three grades according to the various cell population and presence of epithelioid cell granulomas. cutaneous leishmaniasis appears in variable patterns clinico-pathologically, and sometimes a simple erythemic lesion, shows (LD) bodies and in few cases has been diagnosed clinically malignant and remained untreated for years. The CL clinically presents according to the stage of infection and the clinical type of disease basically an ulcer appearing at the site which heals slowly, takes months to years depends upon the host immunity. *Cutaneous leishmaniasis*, a wide clinical and histological spectrum. The histological picture in CL differs according to the stage of infection and the clinical type and host immunity. There is evolution of the lesion as it progresses from a papule and nodule into a soft boggy, crusted plaque or nodule (Khawer *et al.,* 2007). The lesion breaks up after 3-4 months into a well circumscribed ulcer, which heals slowly over a period of 3 to 12 months. The histological spectrum in CL has wide range with a great variation in morphology. The appearances may range from predominance of leishmanial granuloma with macrophages showing epithelioid

Table 3: Pathological type with clinical correlation in *Cutaneous leishmaniasis* (CL)

Histological	No of Case	More then 2 Months	More then 14 Months	Relapse Cases
Type 1	148	142	9	0
Type 2	110	22	12	76
Type3	42	Nil	Nil	42
Total	300	164	13	123

differentiation Langhan's type of giant, foreign body giant cells and lymphocytes, to complete absence of epithelioid granuloma but with subacute or chronic mixed cell reaction provoked by secondary infection. In this study, we have categorized the histological patterns of CL into 3 types.Granuloma formation results from macrophages activation into epithelioid cells, considering clinico-pathological relationship, in current study Type 1 pattern was seen mostly in acute lesions. Type 2 and 3 pattern were seen mostly in late lesions but have also been found in some early lesions, and mixed patterns were also seen. As compared to the study done by Simeen *et al*. (2002), it has shown that clinic pathological correlation has been done according to the clinical type of CL as wet and dry type only (Simeen *et al*., 2002) whereas in this study, we have done it according to the basis of duration of the lesions, such as early and late.

CONCLUSION

The yield of positive cases is much higher by histopathology as compared to skin smears, by this technique we can confirm the pathology of Leishmania parasites by LD bodies, and in absence of LD bodies in tissue biopsies, we can even get it confirmed by the presence of granulomas which is another indicator for the diagnosis of Leishmaniasis.. Although histopathology is time consuming, it would be beneficial for the Pathologist and researchers who are working with Leishmania prevailance in the region.

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