

## Short Communication

# Cutaneous leishmaniasis in Karak, Pakistan: Report of an outbreak and comparison of diagnostic techniques

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A total of 339 patients with clinically suspected cutaneous leishmaniasis (CL) were studied from March to April, 2010 in three villages of Karak, Khyber Pakhtunkhwa, Pakistan where an epidemic of the disease was in question. Using polymerase chain reaction (PCR), 78.17% (265/339) were observed having CL. Microscopically, however, only 43.06% (146/339) were diagnosed with the disease. This study reports and confirms epidemic of CL in both gender of all ages in the area. Females (70.94%) were noted to be predominantly affected as compared to males (29%). Clinically, 12.38% of patients had more than three lesions, 29.20% had two lesions, while 58.40% had only single lesion. Most lesions were found on exposed surfaces of the body (predominantly hands, face and feet). The present study confirms that PCR was more sensitive than microscopic examination.

**Key words:** Epidemic, cutaneous leishmaniasis, polymerase chain reaction (PCR), microscopy, Pakistan.

## INTRODUCTION

Cutaneous leishmaniasis (CL) infects about 1.5 million people each year, worldwide, with the bulk of the disease been reported from Afghanistan, Iran, Iraq, Algeria, Saudi Arabia, Peru and Pakistan (WHO, 1990).

CL is prevalent in Pakistan and has been reported from all the provinces (Kassi et al., 2008), where the causative agent is *Leishmania tropica*, a protozoan parasite (Amtul and Shaheen, 2001). The disease it cause is called 'Oriental Sore', 'Aeppo button', 'Jericho boil', 'Dehli boil', 'Bouton de biskra', Ulcer de los chicleore or forest "yaws" of Yucutan leishmaniasis in old world and new world CL (Philip et al., 1975).

CL pathogens are carried by sand flies (*Phlebotomus*) vector and in Pakistan, some 29 species of *Phlebotomus* are present (Lewis, 1967). *Phlebotomus papatasi* was found in Kashmore, Bannu, Dera Ismail Khan, Idak, Tank (Sinton, 1927), Kohat, Lahore Miramshah, Nowshera, Quetta, Rawalpindi (Santon, 1927), while *Sergentomyia (Grassomyia) squamipleuris indica* is distributed in

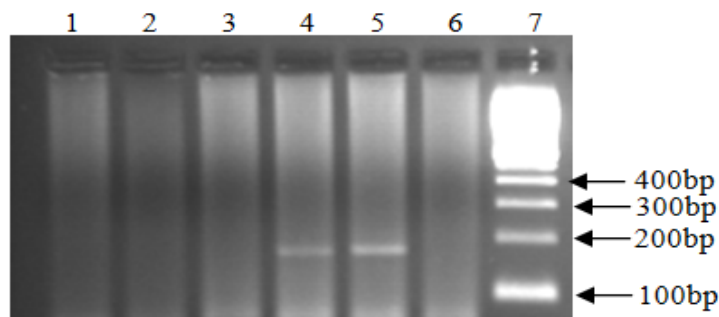
Peshawar, Saharanpur, Lahore, Dera Ismail Khan, Jhelum, Khanki, Tank, Cherat, Gujrat, Rawalpindi, Said Pur, Saidu Sharif and Taxila (Lewis, 1967). Sand flies become infected through feeding on infected animals. Once a sand fly is infected, it can transmit the parasite to both humans and animals for the rest of its life (Rab et al., 1986). Man is usually an incidental host (Rajpar et al., 1983).

CL is prevalent in most parts of Pakistan (Rahman and Bari, 2003; Ayub et al., 2001; Jaffernay and Nighat, 2001). During the past decade, CL has emerged as a challenging infectious disease in the form of new outbreaks in some areas of Pakistan, where it has never be noted before. It has affected people of all ages including children (Mujtaba and Khalid, 1998; Bhutto et al., 2005).

## Geography of Karak

The topography of Karak is composed of mainly a series of high and low mountains of salt range Kohi Hundokash. Karak is situated on south of district Kohat and on North of districts Bannu and Lakki Marwat on main Indus highway from Peshawar to Karachi. It is approximately

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**Figure 1.** PCR amplified product of *L. tropica*. Lanes 1 to 3 and 6 = negative control; lanes 4 and 5 = positive sample; lane 7 = 100 bp ladder marker

**Table 1.** Lesions wise prevalence of CL.

S/N	Number of patient	Number of lesion	Percentage (%)
1	198	1	58.40
2	99	2	29.20
3	42	>3	12.38
Total	339	-	100

123 km from Peshawar, the capital of Khyber Pakhtunkhwa. The total population of the district is 536,000: male 281244 (52%) and female are 254756 (48%), where 86% population inhabits the rural areas.

Presently, there was an outbreak of CL in the area. The present study was thus carried out to document the outbreak; and also to compare two diagnostic techniques for the disease (microscopy and polymerase chain reaction).

## MATERIALS AND METHODS

This study was conducted from March to April, 2010 in three villages (Khurram, Surdog and Shawa) of Karak, Khyber Pakhtunkhwa, Pakistan. A questionnaire was designed to document patient's demographics, including place of origin, any visit to endemic area or contact with animals, medication and awareness of the disease. These patients were then examined for site and nature of lesions.

A total of 339 skin scraping/exudates were taken from the patients with lesions suspected clinically to be CL. Slides were prepared, stained with Giemsa stain and examined under the microscope at 10, 40 and 100x magnification for identification of *L. tropica*. The same samples were then subjected to DNA extraction and PCR. Blood samples were also collected in vacutainer from the patients and a complete blood analysis was also performed.

## DNA extraction and PCR amplification

DNA was extracted from exudate/skin scrapings using DNAzol kit (Trizole USA). DNA was amplified in a thermal cycler (Nyxtech USA) by using Oligonucleotide, LSa (5' -

TCTTGCGGGGAGGGGGTG-3') and LSb, (5' - TTGACCCCAACCACATTTTA-3') specific to kDNA of *L. tropica*. PCR was conducted in a 25 µl of reaction mixture containing MgCl<sub>2</sub>, 10 mM dNTPs and 2 U of *Taq* DNA polymerase (Fermentas USA).

The amplified product (Figure 1) was run on 2% agarose gel visualized under UV light using ethidium bromide (0.5 mg/ml). The CL specific band 185 bp was compared with 100 bp DNA ladder marker (Fermentas USA).

## RESULTS AND DISCUSSION

A total of 339 patients were used in the study, 71% were females and 29% were males. The age range was 1 to 70 years. Patients were noted to have a single lesion (58.4%) of the cases, two lesions (29.2%) and three or more than 3 lesions (12.38%) (Table 1). Most lesions were found on exposed surfaces of the body (predominantly hands/forearms, face and feet/legs.)

Using PCR, 78.17% (265 out of 339 patients) were noted to have CL; microscopically however, only 43.06% (146/339) were diagnosed with the disease; thus showing PCR to be more sensitive as compared to microscopic examination with Giemsa staining in diagnosing CL (Table 2).

The present study here, firstly, reports and confirms another epidemic of CL affecting people of all ages and gender in the area. Similar outbreaks have been reported in other parts of the country as well (Sharma et al., 2005; Mujtaba and Khalid, 1998; Kharfi et al., 2004; Talari et al., 2006). As noted in our study, Talari et al. (2006) also found females to be predominantly affected.

Secondly, even though the disease is usually

**Table 2.** Gender wise CL prevalence of microscopy and PCR.

Parameter	Number of patient	Male (%)	Female (%)	Total (%)
Microscopy	339	60 (17.69)	86 (25.36)	146 (43.06)
PCR	339	77 (29)	188 (70.94)	265 (78.17)

diagnosed with microscopic examination of a dermal scraping or biopsy (Kassi et al., 2004), PCR was found to be a more sensitive method for diagnosis and confirmation of the infection (Chargui et al., 2005; Romero et al., 2001). Cost of doing a PCR and availability of the equipment (as compared to microscopic examination) would be limiting factors to address, given the widespread nature of the disease in the country.

## Conclusions

Our study confirms an outbreak of the disease in the country; showing the rising widespread nature of the disease in Pakistan.

Currently, available methods for the diagnosis of CL have low sensitivity. This constitutes a major obstacle for the diagnosis of the disease and for the study of the effectiveness of treatment schedules. In our study, PCR was noted to be more sensitive in detecting the disease as compared to traditional microscopic examination. Cost of doing the PCR may be a limiting factor for its widespread use in the country.

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