

Original Article

Leishmaniasis in Sindh, Pakistan: outbreak and review of the literature

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Abstract *Background* Cutaneous leishmaniasis (CL) is endemic in Pakistan and is widely spreading day by day. Earlier, we proposed that leishmaniasis is endemic in Jacobabad, Dadu and Larkana districts of Sindh Province. It was pointed out that the disease is dramatically spreading in the country and warned that if the preventive measures were not taken at right time there would be a serious public health problem in the country. Since then, we have regular watch on the disease.

Objective Fresh clinical observations of CL patients who visited our department from 2004 to 2005.

Patients and methods This study was conducted in the Department of Dermatology, Chandka Medical College Larkana. A total of 1640 cases of CL were seen during this period.

Results Among the 1640 patients, 470 were residents of different cities of Balochistan Province; and 1170 were the residents of different cities of Sindh Province like Jacobabad, Ratodero, Shahdadt, Qambar, Warah, Mehar, Dadu, Dokri and Larkana. All the patients were aged between 3 months and 60 years. Seven hundred eighty were males and 860 were females. Duration of the disease ranged from 1 to 12 months. Most of the patients had single lesions but multiple lesions were also seen on the exposed (mainly) and unexposed parts of the body. Clinically, the lesions were classified as dry ulcerative, wet ulcerative, dry papular, nodular, and crusted lesions. Diagnosis was made on the basis of smear test and clinical presentation of the skin lesions. All the cases were treated with the meglumine antimoniate 600 mg/day (adults) and 15 mg/kg/day (children) either intramuscularly for 20 consecutive days or intralesionally for 10 alternate days.

Conclusion Cutaneous leishmaniasis is endemic in the Sindh Province. The molecular characterization and other studies are needed for further evaluation of this disease.

Key words

Leishmaniasis, outbreak, epidemiology, Pakistan.

Introduction

Leishmaniasis are a group of diseases caused by several species of the genus *Leishmania*. Each species tends to occupy a

particular zoo-geographical zone. These parasites can affect several mammalian species including humans. Human leishmaniasis is caused by at least 20 different species and subspecies of the genus *Leishmania*. In general, leishmaniasis is a zoonotic disease and the parasite is transmitted to man from a reservoir mammalian host by a sandfly vector during a bite. The type of infection of leishmaniasis gives wide range of clinical changes that divide the disease into following

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subcategories: A) cutaneous leishmaniasis (CL); B) diffuse cutaneous leishmaniasis (DCL); C) mucocutaneous leishmaniasis (MCL); and D) visceral leishmaniasis (VL). CL can be classified largely into two forms: an Old World form mainly caused by *L. tropica* complex¹; and a New World form caused by *L. (Viannia) braziliensis* and *L. mexicana* complexes.²⁻⁵

Leishmaniasis is endemic in 88 countries in 5 continents with a total of 350 million population at risk. The estimated annual number of new cases of VL and CL are about 5 lacks and 15 lacks, respectively.^{6,7} About one hundred thousand deaths due to VL were estimated among 2.8 lacks people in the endemic area of southern Sudan between 1984 and 1994⁸ and an epidemic of CL is ongoing in Afghanistan and other surrounding states with hundreds of thousands of cases over there.⁹

Leishmaniasis was reported from Pakistan in 1960 for the first time. Initially it was limited to the northern mountainous region, but now it is widely spreading all over the country. In this study, we present our recent observations and the current status and up-to-date information of leishmaniasis and its epidemiology in Pakistan, by surveying the literatures reported to date in Pakistan. The vector sandflies and factors suspected for the spread of disease in country will also be discussed.

Patients and methods

These data were collected from the outpatient clinic of the Department of Dermatology, Chandka Medical College (CMC) Hospital, Larkana, Sindh, Pakistan. A total of 1640 cases were seen in our department from September, 2004 to December, 2005. All the patients examined at our department were either referred by local doctors working in rural health centers

in villages and/or visited on their own. These patients were divided into two groups: (1) those that had a positive history of travel to the Balochistan Province before the appearance of lesion(s) and/or to have come from Balochistan Province; and (2) those that belonged to the newly reported endemic areas of CL in the Sindh Province. All the cases were diagnosed on the basis of clinical findings and smear test for Leishman-Donovan bodies. All the cases were treated with the meglumine antimoniate 600 mg/day (adults) and 15 mg/kg/day (children) as intramuscular for 20 consecutive days or intralesionally for 10 alternate days.

Results

A total of 1640 cases were seen during the 15 months period, which is a quite high number in comparison to previously reported cases from these areas. Among them 470 either had a positive history of travel to the Balochistan Province or were residents of that province. They were likely to have been infected from the previously recognized endemic areas. 1170 were the residents of different cities of Sindh Province like Jacobabad, Ratodero, Shahdadkot, Qambar, Warah, Mehar, Dadu, Dokri and Larkana. Seven hundred and eighty patients were males and 860 were females. Age of the patients ranged from 3 months to 60 years. There was no significant difference between the male and female ratio. Duration of the disease ranged from 1 to 12 months. Most of the cases were seen during winter season; however, rare cases were seen in summer. All the patients had lesions mostly on the exposed parts of the body such as face, neck, ears, hands, arms, feet and legs (**Figures 1-3**); and rarely the lesions were also seen on the other unexposed parts of the body including the back and abdomen. No cases of MCL or VL were seen in this study.



Figure 1 A 16-year-old girl showing the dry ulcerative lesion on cheek.



Figure 2 A 25-year-old man has wet ulcerative lesion on the nose since 2 months.



Figure 3 Papulo-nodular lesions are visible on the leg of male patient.

The two types of sandflies from the villages of parasitologically positive cases were identified as *Phlebotomus sergenti* and *P. papatasi*.

Discussion

Epidemiology of leishmaniasis in Pakistan

Pakistan is a tropical country situated in the

north-west of South Asia, sharing borders with China, Afghanistan, Iran and India. Incidentally, all these neighboring states are also endemic for the leishmaniasis.^{9,10,11}

As regards epidemiology of the disease, Ahmed *et al.*¹² were the first who reported 30 cases of VL in the Baltistan, northern areas from 1957 to 1960. In another study, Ahmad and Burney¹³ observed the increasing incidence of leishmaniasis in northern areas of Pakistan. Baltistan is a part of the northern areas of Pakistan, lying between latitude 35° to 35.5°N and longitude 75.5° to 76.5°E, and comprises of many valleys having altitude of 6000 to 10000 feet above sea level with majority of the population is rural. Later, Ahmad and Burney¹³ presented their studies in detail and all their reported 30 cases of kala azar (VL) were admitted to the combined military hospital, Skardu, during the period of April 1957 to March 1960. Among 30 patients 13 were children below 10 years, 10 were 10-15 years, and 7 were 15-35 years old. They also conducted a survey from June 26 to July 3, 1960 in nine villages of the Baltistan and discovered 60 cases of VL.¹⁴

In 1974, Burney *et al.*¹⁵ discovered new foci in Kharmang Valley and 25 cases of VL from the villages were recorded. In 1975, they recorded only two cases from Parkuta village in Kharmang valley. In 1979, they reported high seropositivity of antileishmania antibodies using the complement fixation test (CFT) and immunofluorescence assay (IFA), in the children age groups 6-10 and 11-15. They also concluded that there was no animal reservoir of infection, and that disease transmission was from man to man via sandflies.

In the eighth decade, the cases of VL were reported from the district of Chilas in the northern areas. Later, cases were also

reported from the sub-Himalayan region of Azad Jammu and Kashmir (AJK), and the neighbouring areas of North-West Frontier Province (NWFP) and Punjab Province.¹⁶ Rab and Evans¹⁷ reported the existence of *L. infantum* in the Himalayas region. They reviewed the record of 10 years and revealed the 239 cases of VL from the Rawalpindi, Islamabad, Gilgit (northern areas) and Muzaffarabad (AJK) hospitals. They observed that 52% were under the age of 2 years, while 86% of all cases were below 5 years old. They also randomly skin tested with leishmanin in 1938 individuals, tested for anti-leishmania antibodies in 580 samples by enzyme-linked immunosorbent assay (ELISA) and direct agglutination test (DAT), and another 1403 as blood samples on filter paper, were tested by DAT alone. Parasites were isolated from 15 patients, 11 from bone marrow, 3 from normal skin and one from spleen. The parasites were typed as *L. infantum* zymodeme LON-49 (=MON-1).

In order to know the role of dogs in the epidemiology of human visceral leishmaniasis, a serological study was conducted in the domestic dogs in the rural communities in the districts of Chilas, Abbotabad, Bagh, Poonch and Muzaffarabad (AJK).¹⁸ A total number of 244 dogs were examined for the evidence of anti-leishmania antibodies. Deoxyribonucleic acid (DNA) probing by ³²P-labelled Lmet 2 cDNA probe showed high sensitivity with aspirates obtained from the popliteal lymph nodes of dogs but not with skin snips. Parasites isolated from dogs in these foci were identified as *L. infantum* by isoenzyme characterization. Hence, they confirmed the role of dogs as the reservoir of visceral leishmaniasis in these endemic foci in northern areas of Pakistan.

In Pakistan, both VL and CL are endemic; and two types of CL, zoonotic CL and anthroponotic CL are reported endemic in

different parts of the country.^{19,20,21} In one study, the isolated parasites from the cutaneous lesions of 13 patients were typed as *L. tropica*; and it was concluded that anthroponotic cutaneous leishmaniasis is caused by *L. tropica* in Pakistan.²² Later, the VL was reported in the 10 children between 2 to 10 years at DHQ Hospital, Timergara district Dir, NWFP.²³

Slowly and gradually the disease has been spreading to the other parts of the country like Balochistan Province and the affected cases have had the both type of leishmaniasis i.e. the CL as well as VL. Nagi and Nasimullah²⁴ reported the presence of several cases of VL in Balochistan Province. Further more, Yasinzai *et al.*²⁵ studied on various aspects of CL and VL in the Balochistan Province. It was observed that the disease was affecting equally the adults and children. However in another study, the VL was more frequently seen in the children.²⁶ The mean age of these children was 2.9 years. They concluded that Pakistani children with VL tended to be younger than the affected children from Africa and were less likely to have lymphadenopathy. Hassan *et al.*²⁷ reported 38 cases of VL from the Rawalpindi Medical College Hospital. The majority of these patients (84.2%) came from Azad Kashmir, especially from areas around Poonch, and the others were from the areas around Muzaffarabad and 2 patients belonged to Gilgit agency, 4 cases came from villages around Murree, Rawalpindi and Abbotabad. They proposed that VL was endemic in these areas.

In the last decade of last century, the disease rapidly spread to the central and south parts of the country, the Central Punjab and Sindh provinces. CL is also reported as endemic in the central part of Punjab. Mujtaba and Khalid²⁸ reported 305 cases of cutaneous leishmaniasis from the Nishtar Medical

College, Multan during 1995 to 1997. They observed only dry type of lesions in their patients and suspected for the presence of *L. tropica* in the region. Later, 173 cases of CL were reported from the central part of same city.²⁹ Although, the dry and wet type of ulcerative lesions are the common features of the CL, unusual clinical variants like acute paronychia, chancriform, annular, palmoplantar, zosteriform and erysipeloid type lesions are also reported in the country.³⁰ No case of visceral leishmaniasis is reported yet from the central part of the Punjab and Sindh provinces.

In 1996, we observed the frequent number of cases in the interior part of Sindh Province. We noticed that most of the CL patients visiting our department had never visited any previously reported endemic areas of leishmaniasis before the appearance of lesion on the body. This prompted us to conduct the careful study regarding the disease. The aim was to investigate whether this disease is really becoming endemic in this interior region of Sindh Province. For this purpose we divided our patients in two groups; 1) those who had traveled or belonged to the endemic areas of leishmaniasis before the appearance of first lesion; 2) those who had neither traveled nor belonged to the endemic areas of leishmaniasis. A total of 1210 cases were seen in our department from 1996 to 2001. We observed that 450 patients had the positive history of travel to the endemic areas of CL in the country. They were likely to have been infected from the previously recognized endemic areas. 750 patients never traveled to the endemic areas before or after the appearance of the lesion, and they were residing in the central part of the Sindh Province i.e. Larkana, Dadu and Jacobabad districts.³¹ On the basis of these findings we proposed that three districts of Sindh province i.e. Jacobabad, Dadu and Larkana were endemic for cutaneous

leishmaniasis. Since then, hundreds of patients are visiting our department every year. The present study was conducted on the similar pattern. A total of 1640 cases were seen during the 15 months period, which is quite high as compared to previously reported cases. Among them 470 were residents of different cities of Balochistan Province; and 1170 were the residents of different cities of Sindh province like Jacobabad, Ratodero, Shahdadkot, Qambar, Warah, Mehar, Dadu, Dokri and Larkana. Every year the number of cases is increasing dramatically.

In order to see the nature of the disease in these new endemic areas, we took a survey of many CL related villages. It appears that leishmaniasis entered in the Sindh Province from the Balochistan Province through the routes of mountainous belt continuing from the borders of Balochistan and Sindh province, under specific conditions. Further studies are required in this regard. The number of patients residing in Sono Khan Chandio Village was much higher than the number of patients from other cities of Sindh Province.^{32,33,34} The possible factors responsible for the outbreak and spread of disease from north to south of the country may be considered as: I) flow of canals and rivers from north to south, II) increased population, III) refugees influx, IV) heavy vehicles (trucks) used for the domestic transportation of fruits, luggage and other purposes, V) military activities, VI) stoppage of anti-insecticidal spray, once a regular practice for agricultural purposes, VII) environmental modifications like construction of water dams can change the temperature and humidity of the soil and vegetation, which may result in changes of the composition and density of sandfly species as well as changes in rodent species.

The incidence of CL in other cities of Sindh Province like Nawabshah, Khairpur,

Shikarpur and Sukkur districts is also on rise (personal communication with Dr. Aziz Memon). Similarly, Malir and Landhi areas of the coastal city of Karachi are being suspected as endemic for cutaneous leishmaniasis (personal communication, Dr. S. Sharaf Ali Shah).

Vector sandflies

Burney *et al.*¹⁴ captured the sandflies from the houses of the VL and CL patients in Baltistan which were identified as *Phlebotomus chinensis*, *P. major*, *P. kandelakii* and *P. burneyi*. They considered *P. burneyi* as a new species of sandfly. *P. papatasi* is the commonest vector in the Gilgit area. At that time, authors could not incriminate the species of *Phlebotomus* responsible for the transmission of disease in the area. In order to know the reservoir host in the Baltistan area, a number of studies were carried out. Although the number of dogs were rare in the houses of the kala azar patients, the bone marrow smear from dogs were checked for the *Leishmania* that was negative. Squirrels, gerbils and lizards were not available in the region. Even not a single monkey or donkey was present in the area. Forty-eight sera from goats, sheeps, cows, horses and yaks collected from affected houses did not reveal complement-fixing antibodies against *Leishmania*. Rodents were captured from the kala azar houses, they were dissected and their smears were examined for L.D. bodies and cultured on Novy-MacNeal-Nicolle (NNN) medium but the results were negative. On the basis of their findings they concluded that the disease might be transmitted from man to man through any of the species of *Phlebotomus* present in the area.

Anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* is a major epidemic in Kabul city of our neighboring state of Afghanistan. Hewitt *et al.*⁹

conducted a study among children in 2 high-rise apartment blocks in the city in Kabul and suggested that most transmission of ACL took place in homes. ACL is also common and endemic in the southern city of Kandhar, the western city of Herat and central provinces of Kabul and Parwan.^{35,36,37} Rowland *et al.*³⁸ conducted the study in the Afghan refugee camp at Timargara, in the district Dir, North West Frontier Province of Pakistan (NWFP). They examined the lesions parasitologically and the amastigotes were detected by microscope in only 36% of lesions, and, 48% of slide negative cases showed positive cultures; however the same cases negative to both microscopy and culture were positive by PCR. They concluded that *L. tropica* was existing type of *Leishmania* and *P. sergenti* was a known vector in the area.

In order to see the behavior, characteristics and various aspects of the disease different studies like molecular characterization of *Leishmania* isolates from patients, reservoir host/s (animals) and vector sandflies, and the molecular type of *Leishmania* from tissues are needed.

Regarding the reservoir host and the existing type of sandflies in the new endemic areas of Sindh Province, studies are under way. At present, we have found two types of sandflies in the villages of parasitologically positive cases that are *P. sergenti* and *P. papatasi*.

References

1. Mebrahtu YB, Lawyer PG, Ngumbi PM, Kirigi G, Mbugua J, Gachihi G, Wasunna K, Pamba H, Sherwood JA, Koech DK, Roberts CR. A new rural focus of cutaneous leishmaniasis caused by *leishmania tropica* in Kenya. *Trans Royal Soc Trop Med Hyg* 1992; **86**: 381-7.
2. Lainson R, Shaw JJ. Evolution, classification and geographical distribution. Peters W, Killick-Kendrick

- R (eds.) *The Leishmaniasis in Biology and Medicine, Biology and Epidemiology*. London: Academic Press 1987; **1**: 1-20.
3. Pearson RD, Sousa ADQ. *Leishmania* species: visceral (kala-azar), cutaneous and mucosal leishmaniasis. In: Mandell GL *et al*, eds. *Principles and practice of infectious diseases, (3rd edition) Ed*. New York: Churchill Livingstone; 1985. p. 2066-2077.
 4. Hashiguchi Y, Gomez EAL, Coronel VV *et al*. Andean leishmaniasis in Ecuador caused by infection with *Leishmania mexicana* and *L. major*-like parasites. *Am J Trop Med Hyg* 1991; **44**: 205-17.
 5. Jones TC, Johnson WD, Barretto AC Jr *et al*. Epidemiology of American cutaneous leishmaniasis due to *Leishmania braziliensis braziliensis*. *J Infec Dis* 1987; **156**: 73-83.
 6. Desjeux P. Leishmaniasis public health aspects and control. *Clin Dermatol* 1996; **14**: 417-23.
 7. Herwaldt BL. Leishmaniasis. *Lancet* 1999; **354**: 1191-9.
 8. Seaman J, Mercer A J, Sondrop E. The epidemic of visceral leishmaniasis in western Upper Nile southern Sudan: Course and impact from 1984 to 1994. *Int J Epidemiol* 1996; **25**: 862-71.
 9. Hewitt S, Reyburn H, Ashford R, Rowland M. Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: vertical distribution of cases in apartment blocks. *Trans Roy Soc Trop Med Hyg* 1998; **92**: 273-4.
 10. Dogra J. Cutaneous leishmaniasis in India: Evaluation of oral drugs (dapsone vs itraconazole). *Eur J Dermatol* 1992; **2**: 568-9.
 11. Momeni AZ. Amin Javaheri M. Clinical picture of cutaneous leishmaniasis in Isphahan, Iran. *Int J Dermatol* 1994; **33**: 260-5.
 12. Ahmad N, Burney MI, Wazir Y. A preliminary report on the study of kala-azar in Baltistan (West Pakistan). *Pak Arm Force Med J* 1960; **10**: 1-10.
 13. Ahmad N, Burney MI. Leishmaniasis in northern areas of Pakistan. *Pak Arm Force Med J* 1962; **12**: 1-11.
 14. Burney MI, Wazir Y, Lari FA. A longitudinal study of visceral leishmaniasis in northern areas of Pakistan. *Trop Doctor* 1979; **9**: 110-6.
 15. Burney MI, Lari FA, Khan MA. Status of visceral leishmaniasis in northern Pakistan. *Trop Doctor* 1981; **11**: 146-8.
 16. Saleem M, Anwar CM, Malik IA. Visceral Leishmaniasis in children: A new focus in Azad Kashmir. *J Pak Med Assoc* 1986; **36**: 230-3.
 17. Rab MA, Evans DA. *Leishmania Infantum* in Himalayas. *Trans Roy Soc Trop Med Hyg* 1995; **89**: 27-32.
 18. Rab MA, Frame IA, Evans DA. The role of dogs in the epidemiology of human visceral leishmaniasis in northern Pakistan. *Trans Roy Soc Trop Med Hyg* 1995; **89**: 612-5.
 19. Burney MI, Lari FA. Status of cutaneous leishmaniasis in Pakistan. *J Pak Med Assoc* 1986; **25**: 101-8.
 20. Jan SN. Cutaneous leishmaniasis in Baluchistan. *Pak J Med Res* 1984; **23**: 64-9.
 21. Rajpar GM, Khan MA, Hafiz A. Laboratory investigation of cutaneous leishmaniasis in Karachi. *J Pak Med Assoc* 1983; **33**: 248-50.
 22. Rab MA, Rustamani L, Bhutta RA *et al*. Cutaneous leishmaniasis: Iso-enzyme characterization of *Leishmania tropica*. *J Pak Med Assoc* 1997; **47**: 270-3.
 23. Rahim F, Rahman F, Ahmad S, Zada B. Visceral leishmaniasis in District Dir, NWFP. *J P Med Assoc* 1998; **48**: 161-2.
 24. Nagi AG, Naseemullah M. Visceral leishmaniasis in Balochistan. *Pak Med J* 1993; **17**: 7-10.
 25. Yasinzai MM, Iqbal J, Kakar JK *et al*. Leishmaniasis in Pakistan: Re-visited. *J Coll Physicians Surg Pak* 1996; **6**: 70-5.
 26. Rathore MH, Buksh D, Hassan M. Visceral leishmaniasis in Pakistani children. *South Med J* 1996; **89**: 491-3.
 27. Hassan K, Ikram N, Bukhari KP *et al*. Visceral leishmaniasis: a study of 38 cases on the basis of geographical distribution. *J Pak Med Assoc* 1995; **45**: 125-7.
 28. Mujtaba G, Khalid M. Cutaneous leishmaniasis in Multan, Pakistan. *Int J Dermatol* 1998; **37**: 843-5.
 29. Ayub S, Khalid M, Mujtaba G, Bhutta RA. Profile of patients of cutaneous leishmaniasis from Multan. *J Pak Med Assoc* 2001; **51**: 279-81.
 30. Raja KM, Khan AA, Hameed A, Rahman SB. Unusual clinical variants of cutaneous leishmaniasis in Pakistan. *Br J Dermatol* 1998; **139**: 111-3.
 31. Bhutto AM, Soomro RA, Nonaka S, Hashiguchi Y. Detection of new endemic areas of cutaneous leishmaniasis in Pakistan: a six year study. *Int J Dermatol*, 2003; **42**: 543-8.
 32. Pathan GM, Soomro FR. Cutaneous leishmaniasis in village of mountainous belt of Larkana district. *J Pak Assoc Dermatol*, **11**: 16-19, 2001.

33. Soomro FR, Pathan GM, Soomro RA. The patterns of cutaneous leishmaniasis and differential diagnosis of misdiagnosed cases in Larkana region, *J Pak Assoc Dermatol* 2002; **12**: 77-82.
34. Soomro FR, Pathan GM, Bhutto AM *et al.* A preliminary survey of Cutaneous Leishmaniasis at village Ghaibi Dero in Larkana District, Sindh, Pakistan. Hashiguchi Y. ed. *Studies on New World leishmaniasis and its transmission, with particular reference to Ecuador. Kochi, Japan. Kyowa Printing, 2004; Co. Research Report Series No.7*: 110-4.
35. Omar A, Saboor A, Saidi S, Sery V. Cutaneous leishmaniasis in Afghanistan. *Afghan J Pub H* 1968; **1**: 1-6.
36. Nadim A, Rostami GS. Epidemiology of cutaneous leishmaniasis in Kabul, Afghanistan. *Bulletin of the World Health Organization* 1974; **51**: 45-49.
37. Nadim A, Javadian E, Noushin MK, Nayh AK. Epidemiology of cutaneous leishmaniasis in Afghanistan. II. Anthroponotic cutaneous leishmaniasis, *Bulletin De La Societe De Pathologie Exotique* 1979; **5**: 461-466.
38. Rowland M, Munir A, Durrani N *et al.* An outbreak of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. *Trans Royal Soc Trop Med Hyg* 1999; **93**: 133-136.

Authors Declaration

Authors are requested to send a letter of undertaking signed by all authors along with the submitted manuscript that:

The material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Journal of Pakistan Association of Dermatologists*.