Research Article

Identification of Cutaneous Leishmaniasis in Humans Through Polymerase Chain Reaction (PCR)

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Abstract | To establish the baseline and the data on samples of Cutaneous Leishmaniasis were collected from three different districts of Sindh-Province, Pakistan to diagnose Leishmania species (spp), parasites through Polymerase Chain Reaction (PCR). Total, 70 human Leishmaniasis tissues from various villages of district, Dadu, Jamshoro and Hyderabad were symptomatically identified. For further confirmation, all identified tissue samples were subjected to Polymerase Chain Reaction (PCR) using, Applied Bio-system thermal cycler. DNA from samples was extracted, quantified, through, Nano drop spectrophotometer and, Polymerase Chain Reaction (PCR) product was run on, Gel electrophoresis and bands were analyzed using, Gel documentation system out of, 70 suspected, Cutaneous Leishmaniasis cases, 61 were diagnosed clinically cases and confirmed by Polymerase Chain Reaction (PCR) for, Cutaneous Leishmaniasis. During this study, both dry and wet types of lesions were observed that, indicated the presence of two strain of parasite. Children and adults between 08-60 years of ages were found more susceptible to infection when compared with above 60 years of ages. The strains were found to be *Leishmania tropica* and *Leishmania major* as was indicated by the nature of lesions and confirmed by Polymerase Chain Reaction (PCR). Cutaneous Leishmaniasis is endemic and increasing in the rural areas of Sindh province, Pakistan. This requires attention of health authorities to take appropriate measures for its effective control, failing which it may create more severe public health problems.

Keywords | Cutaneous Leishmaniasis, Sindh, Leishmania tropica, Leishmania major, PCR, Pakistan.

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INTRODUCTION

Cutaneous Leishmaniasis (CL) is caused by haemo-flagellate genus *Leishmania*, which infect domestic and wild mammals, including human being (Gradoni et al.,1999) Cutaneous Leishmaniasis in Pakistan has been a burning seasonal problem in the Canine and Human being. Present study was therefore conducted to generate baseline data on Leishmaniasis in three districts of Sindh-Province, Pakistan (Al-Samarai et al., 2009; Aneela et al., 2011; Bari et al., 2011). Leishmaniasis is an endemic disease in certain areas of Pakistan and infection transmitted via the bite of phlebotomine sandfly and presence of its vector is associated with occurrence of cases in endemic areas, (Al-Zahrani et al., 2004). Cutaneous Leishmaniasis has emerged as a challenging infectious disease in the form of new outbreaks in different areas of Sindh-Province, Pakistan and Balouchistan, (Cortes et al., 2004; Kakar, and Suleman. 2004). This disease is wide spread and may cause serious health problems in communities through Mediterranean regions and the Middle East, including Pakistan, (Brooker et al., 2004). There are estimated 12 million cases world wide and 1.5 million new cases of Cutaneous Leishmaniasis (CL) are added each year suspected from tissue samples, (Parviz et al., 2008; Moradi., 2009). The disease affects all ages groups of human beings including children. This study was conducted to detect Leishmaniasis through clinical way and confirmed by Polymer-

ase Chain Reaction (PCR) in suspected tissue samples.

MATERIALS AND METHODS

Study Area

Tissue samples were collected from Hyderabad were (18), Dadu (41), and Jamshoro (10) total 70 samples were collected of patients from different health centers and clinics of challenged districts in Sindh-Province, Pakistan for detection of Cutaneous Leishmaniasis (CL) via clinical diagnosis followed by Polymerase Chain Reaction (PCR).

Sample Size

Tissue samples of human beings between 08-60 years of ages were collected from ulcerative areas washed thoroughly with 70 percent alcohol and size were 0.3-0.5 cm. The tissue samples were brought at Molecular & Parasitology Laboratory, Sindh Agriculture University, Tandojam and kept them into bijou bottles that containing 3-5 ml of 70 percent alcohol to keep tissue for further processing.

DNA Extraction

DNA was extracted through Qiagen column kit. Protocol was followed according to manufactures guide.

DNA Purification Protocol

Name of kit: DNeasy Blood & Tissue Kits.

DNA Quantification

DNA was quantified on Nano drop Spectrophotometer, (ND-1000, Thermo, and Scientific, USA) at 260/280 nm wave length.

PCR Conditions

The following thermal conditions as initial denaturation at 94^{0}C for 5 minutes followed by 30 cycles including denaturation at 94^{0}C for 35 seconds annealing at 60^{0}C for 35 seconds, extension at 72^{0}C for 45 seconds and final extension at 72^{0}C for 5 minutes were used. At the end 5µl of the reaction mixture was analyzed by 2% Agarose gel electrophoresis. These primers were evaluated with Leishmania standard species including Leishmania, (MCAN/IR/97/LON 490), *Leishmania major*, (MHOM/IR/75/ER) and *Leishmania tropica*, (MHOM/IR/04/Mash 10).

Primers

Specific oligonucleotide primer as forward (5'-CAA-CACGCCGCCTCCTC TC T-3) and Reverse primer (5'-AAA CAA AGG TTG TCN GGG-3') were used.

RESULTS

Detection of *Leishmania tropica* and *Leishmania major* in human beings via polymerase chain reaction (PCR) in districts Dadu, Hyderabad and Jamshoro.

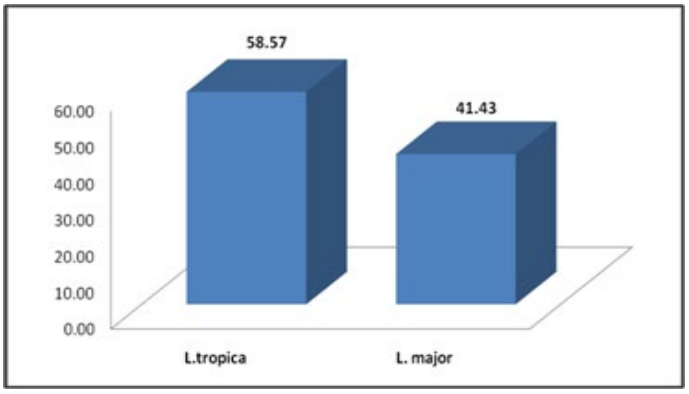

Figure 1: Reveals that, out of 61 Leishmania positive tissues 58.57% tissues were found positive for *Leishmania tropica* and 41.43% tissues were found positive for *Leishmania major*

Prevalence of cutaneous leishmaniasis on different body parts in districts Dadu, Hyderabad and Jamshoro.

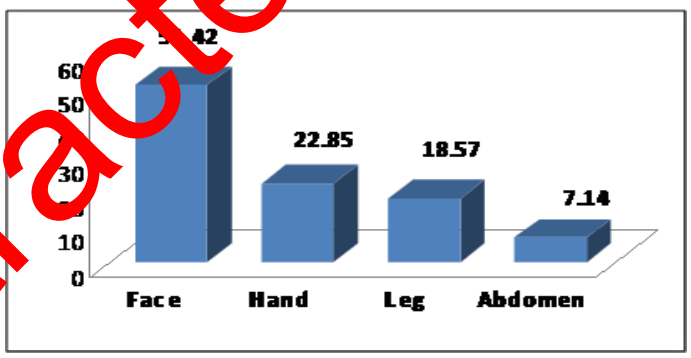

Figure 2: Indicates that, the distribution of infection according to the body parts the 51.42% victims had lesions on the faces where as 22.85% had lesions on hands 18.57% had lesions on legs and 7.14% lesions were observed on the abdomen.

Gel documentation showing positive bands for *Leishmania tropica* at 620 base pairs.

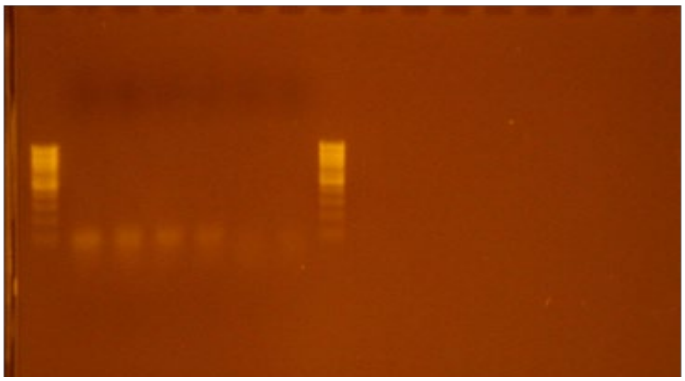

Figure 3: Showing positive bands for *Leishmania tropica* at 620 base pairs. Plate.1 is a Gel documentation which reveals that, *Leishmania tropica* bands appeared at 620 base pairs.

Gel documentation showing positive bands for *Leishmania major* at 670 base pairs.

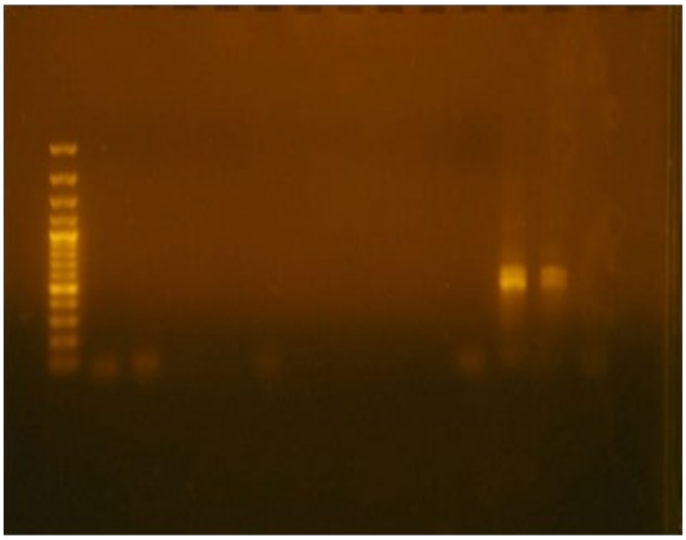

Figure 4: Showing positive bands for the *Leishmania major* appeared at 670 base pairs also reported similar bands of Polymerase Chain Reaction (PCR) product with sizes of 620 base pairs for *Leishmania tropica* and *Leishmania major* in Iran. Establishment of Leishmaniasis is associated with that, of the establishment of sandfly population in Sindh-Province of Pakistan. Sandfly is distributed in rural areas of Sindh-Province which are located near Indus River.

Table 1: District wise infection percentage in humans detected through Polymerase Chain Reaction (PCR).

District	Humans		Prevalence
	Observed	Infected	
Hyderabad	18	15	83.33
Dadu	41	39	95.12
Jamshoro	10	08	80.00
Total	70	61	87.14

Total 70 samples were collected from three different districts of Sindh-Province where, 61 (87.14%) cases were suspected for Cutaneous Leishmaniasis 41% cases belong to district Dadu where as 18 % cases belong to Hyderabad and 10% cases belong to district Jamshoro.

DISCUSSION

Correct diagnosis of Leishmania species is essential to determine the clinical prognosis and a species specific therapeutic approach. The Polymerase Chain Reaction (PCR) technique has opened new windows in the diagnosis of Cutaneous Leishmaniasis, and several approaches have been developed during the last two decades, (Ronger et al., 2009; Shaheen et al., 2007). Accurate identification of the Leishmania species seems to be necessary for a variety of clinical and epidemiological reasons for deciding distinct treatment regimens and also designing appreciate control program, (Guerra et al., 2001). DNA-based techniques have commonly been used as potential tools. During this study, Cutaneous Leishmaniasis was detected in 61 (87.14 %) tissues samples out of 70 samples in three different districts of Sindh province. Our findings are not in agreement with those of which may be due to different study area (Ronger et al., 2009).

During present study we found that, *Leishmania tropica* and *Leishmania major* were predominant for Leishmania species (Soraya et al., 2010), this difference may be attributed due to geographical location which plays an important role strain zoogeography of species and strains (Rassi et al., 2013). During present study, it was seen that, exposure to various body parts also affects rate of infestation and number of infection (Daniela et al., 2012; Seray et al., 2013). Present study shows that, the infection rate for Cutaneous Leishmaniasis through different body parts was 51.42% on face, 22.85% on hand, 18.57 % on leg and 7.14% on abdomen reported 54%, 33% and 13% lesions of Leishmaniasis on face, hands and legs respectively (Aneela et al., 2011; Alfan et al., 2011). Polymerase Chain Reaction (PCR) has provided the ability to diagnose and identify *Leishmania* species. Polymerase Chain Reaction (PCR) based assays have been found significantly sensitive then the histopathological methods also used for further diagnosis (Grazielley et al., 2012; Mormano et al., 2013), Establishment of Leishmaniasis is associated with that, of the establishment of sand fly population. In Sindh, Province of Pakistan, sand fly is distributed in those districts which are located on right bank of river Indus (Fallah et al., 2011, Ershadi et al., 2012, Seray et al., 2013).

Present study covered cases from three districts viz. Dadu, Jamshoro and Hyderabad. As matter of fact that, sand flies population is much distributed in districts, Dadu and Jamshoro as they share their borders with, Baluchistan-Province where population of sand flies is well established. In fact, data on Leishmaniasis in district, Hyderabad does not actually mean that, Hyderabad has high intensity of infection. More cases were seen in district, Hyderabad for the reason that, for interior Sindh, Hyderabad is hub of, Medical facilities, where people from other districts come for medical treatment.

CONCLUSION

Leishmaniasis was discovered 100 years ago but disease has not been eradicated largely organized and collaborated efforts are requires attention of health authorities to take appropriate measures for its effective control, failing which; may create more than 80% prevalence to public health problems. Cutaneous Leishmaniasis is endemic and

spreading mostly found in rural areas of Sindh-Province and their surrounded areas. The disease caused by the intracellular protozoan parasite *Leishmania* represent a major global health problem and World Health Organization (WHO) classified neglected tropical disease. Nearly 10% of the world's population is at risk of acquiring a form of Leishmaniasis. Worldwide it is estimated that there are 12 million active cases of Leishmaniasis, with 2 million new cases occurring each year. Among parasitic infections, this disease is responsible for the highest number of disability adjusted life years a measure of health burden after malaria.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Muhammad Ismail Qureshi was co[illegible]ct[illegible] research work whereas, Abdul Ahad Soomro wa[illegible] the advisor. Muhammad Ismail Qureshi wrote the manus[illegible]pt.

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