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PREVALENCE OF CUTANEOUS LEISHMANIASIS AMONG SUSPECTED PATIENTS IN KHYBER PAKHTUNKHWA PAKISTAN

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Abstract
Cutaneous leishmaniasis, a protozoal disease caused by Leishmania, is endemic in developing countries, including Pakistan. The objective of the present study was to investigate the incidence and risk factors associated with cutaneous leishmaniasis in Khyber Pakhtunkhwa, Pakistan. Exudates of skin lesions were collected from suspected cutaneous leishmaniasis patients from January 2013 to December 2019. All samples were checked for the presence of leishmania using Giemsa-stained microcopy. Out of a total 3917 exudate samples, the rate of positivity for leishmaniasis was 11.99%. The highest frequency of leishmaniasis was recorded in February (18.028%), followed by March (17.128%). On the other hand, less incidence was observed in the months of July (4%) and August (4.5%). The incidence of leishmaniasis in Afghan refugees (15.2%) was high as compared to the Pakistani local population (11.48%). Age-wise distribution showed that leishmaniasis was more frequent in the age group 16–30 years (18.21%), while it was least frequent in the age group 60 years (5.86%). The frequency of leishmaniasis was recorded at 16.24%, 16.73%, and 5.28% in 2016, 2017, and 2019, respectively.
In conclusion, the present study revealed a high incidence of cutaneous leishmaniasis in Khyber Pakhtunkhwa. So proper strategies should be developed by the health care authorities to control the cases of cutaneous leishmaniasis in Khyber Pakhtunkhwa.

Keywords: *Cutaneous leishmaniasis, Microcopy, Prevalence, Khyber Pakhtunkhwa.*

Introduction

Leishmaniasis is considered as one of the leading diseases in tropical and sub-tropical regions, caused by an intracellular protozoan parasite of the genus Leishmania (Babuadze *et al*, 2016). World health Organization (WHO) categorized leishmaniasis it among the 17 neglected tropical disease of poor conturies (Oliveira *et al*, 2004). Leishmaniasis is endemic in 98 countries and results in estimated 1.3 million new cases and 20,000 to 40,000 deaths each year (Salam *et al*, 2014). The important categories of leishmaniasis are cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis, caused by different species of leishmania. Cutaneous leishmaniasis is the most prevalent clinical form of leishmaniasis worldwide and accounts for about 90 % of all leishmaniasis cases occur in countries such as Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria (de Vries *et al*, 2015). In Pakistan, it is one of the major and rapidly increasing public health issues,

especially alongside regions bordering the neighboring Afghanistan and cities that have had the maximum influx of refugees from Afghanistan. About 5000 cases of cutaneous leishmaniasis had been reported during 2002, in the Khyber Pakhtunkhwa province of Pakistan (Kevric *et al*, 2015). This disease is highly endemic in different parts of the country, including the Punjab province, but recently it seems to become an epidemic (Karimkhani *et al*, 2016) and hence disease alerts have been generated in the country through print media (Ullah *et al*, 2009).

CL reported from different parts of Pakistan i.e. Khyber Pakhtunkhwa, Punjab, Baluchistan, and Sindh along with Azad Jammu and Kashmir (Noor and Hussain, 2017; Kakar sulemankhel, 2004; Talat *et al*., 2014). In Pakistan, about 21,000 to 35,000 cases of both anthroponotic cutaneous leishmaniasis (ACL) and zoonotic cutaneous leishmaniasis (ZCL) forms of CL were reported (Shaheen *et al*, 2020).

Cutaneous leishmaniasis this was not common before the arrival of afghan refugees in Pakistan. In Aghan refugee camps leishmaniasis is spreading rapidly due to anthropogenic factors such as human as reservoir for leishmanial (Rahman *et al*., 2009). The potential risk factors associated with increased incidence and transmission of leishmaniasis are rapid growing human population thus creating opportunity for rapid leishmaniasis transmission while some reports showed that it is associated with gender, age and domestic animal (Desjeux, 2001; Richard Reithinger *et al*., 2010). The migration of infected refugees and the local inhabitants are the main source for spreading the infection in non-endemic areas (Alvar *et al*., 2012).

As per literature review, no detailed study has been conducted on the cutaneous leishmaniasis in Khyber Pakhtunkhwa, Pakistan. So, the findings of the study will be helpful to report the current status of leishmaniasis and their risk factors in the study area. Furthermore, the findings will be used by the health authorities to design strategies to reduce cases and spread of leishmaniasis.

Material and Methods

Study area

This study was conducted on leishmaniasis suspect patients from January 2013 to December 2019 in Khyber Pakhtunkhwa, Pakistan. Khyber Pakhtunkhwa is a province in the north-west of Pakistan, previously known as the North-West Frontier Province of Pakistan. According to the study, the total population is over 30 million. A total of 3917 patients were screened for cutaneous leishmaniasis from 2013 to 2019. The prevalence of cutaneous leishmaniasis in each year was determined according to age, gender, month, and ethnicity. The overall period from 2013 to 2019 was also assessed. Furthermore, the demographic information of all patients was also recorded. Ethical approval of the study was obtained from the institutional bioethical committee. In this study, gender-wise prevalence, age-wise prevalence, month-wise prevalence, and ethnic group prevalence was calculated.

Sample collection

The ulcerating lesions of skin was cleaned with alcohol and exudate from was collected with the help of sterile needle, from the raised margin. From the non-ulcerating lesions, the skin scraping was taken after removing of the outer lesion crust. In addition, blood was also collected from the patient.

Microscopy

Small quantity of blood and exudate were smeared on a clean glass microscope slide. Smear was air dried and then fixed with methanol. Geimsa's stain was poured on the smear and left for 15 minutes. Following staining, slide was washed with water and air dried. Smear was examined for the presence of LD bodies (amastigote) under the compound microscope with 40 X lens and 100 X oil.

Statistical analysis

Data was organized and Chi square, OR, 95% confidence interval, and P value were calculated. P-value with less than 0.05 was considered as statistically significant. The overall chi square values were also noted.

Results

Year-wise prevalence of CL

The data shows that the maximum month wise prevalence was observed in February and March were 18.02 % and 17.12%. The minimum disease was less prevalence in the month of July and August during these the overall month wise prevalence was 4 and 4.5% respectively. The results confirm that this disease is more prevalent in the winter as compared to summer because more tests were positive the month of January, February, March, April and December. The results pertaining to month wise prevalence of Cutaneous Leishmaniasis show significant results. The P-value is 0.0000 at 0.05 level of significance. Which means that there is a significant difference among different months regarding prevalence of Cutaneous Leishmaniasis, data given in table 1.

Gender-wise prevalence of CL

Results regarding gender wise prevalence shows that the difference between infected male and female was statistically significant. In male the prevalence was 14.02% while in female it was 9.06%. The prevalence of Cutaneous Leishmaniasis was significantly greater in male as compared to female. The P-value (0.0000) shows the difference between the male and female population is significantly different at 0.05 level of significance, data given in table 1.

Ag-wise prevalence of CL

Overall age wise prevalence clearly shows that the disease was most prevalent in the age group (16-30). The prevalence in this age group was 18.21%. In age group 1-15 the prevalence was 17.54%. The minimum prevalence was observed in age group above 60 years. In this age group prevalence was 5.86%. The P-value for age wise prevalence of Cutaneous Leishmaniasis is 0.0000.

Ethnicity-wise prevalence (Pakistan-Afghanistan)

Results show that Cutaneous Leishmaniasis was more prevalence in afghan refugees than local people. The prevalence in afghan refugees was 15.2% while in local population it was 11.48%. The chi-square analysis shows that prevalence is significantly high in afghan refugees as compared to the local population. The P-value was 0.0131 which is significant at 0.05 level of significance, data given in table.

Year wise Prevalence (2013-2019)

The data pertaining to the Prevalence of Cutaneous Leishmaniasis in different years is given in table. It shows the pattern prevalence in different years. The maximum prevalence of Cutaneous Leishmaniasis was recorded during 2016 and 2017. The prevalence during these years 16.24% and 16.73 respectively. The minimum prevalence was noticed in 2019 which was 5%. The prevalence among different years was significantly different. The P-value is 0.0000 at 0.05 level of significance which highly significant, data given in table 1.

Table 1: Gender-wise, Age-wise, Month-wise, and Population-wise Prevalence

Variables	**Positive n(%)**	**p-value**
Gender		
Male	325 (14.02)	
Female	145 (9.07)	0.0000
Age group (yr)		
1-15	167 (17.54)	
16-30	104 (18.21)	
31-45	93 (11.41)	
46-60	55 (7.746)	
60 <	51 (5.869)	0.0000
Month wise		
January	46(13.85542)	0.0000

February	64(18.02817)	
March	68(17.12846)	
April	64(16.97613)	
May	49(12.34257)	
June	28(8.695652)	
July	11(4)	
August	10(4.545455)	
September	22(6.285714)	
October	26(8.783784)	
November	34(12.09964)	
December	48(15.2381)	
Population		
Local Pakistani	387(11.48)	
Afghan Refugees	83(15.20)	0.0131

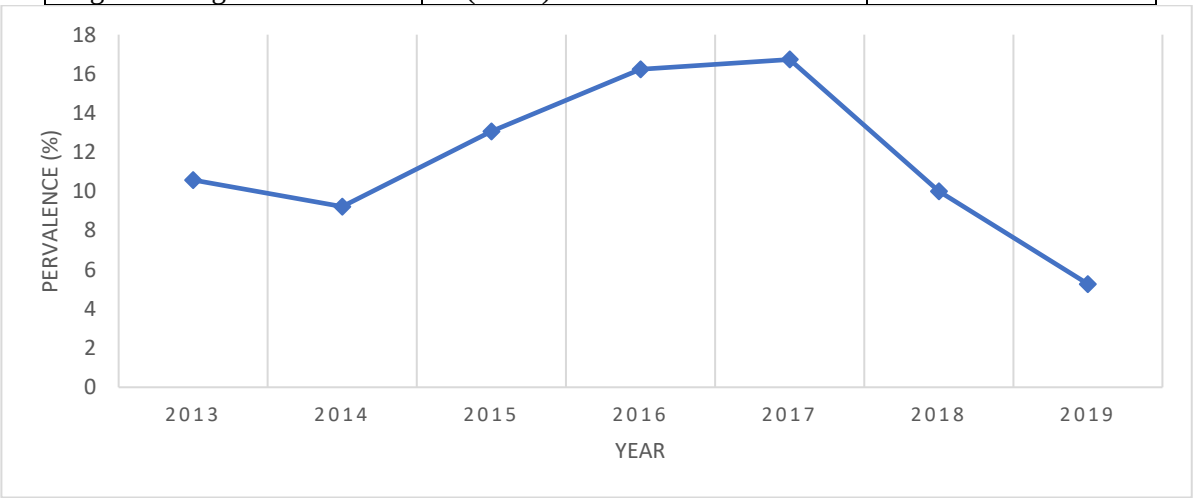

Figure 2: Showing year-wise prevalence

Fig.1: Year wise prevalence of Cutaneous leishmaniasis in Khyber Pakhtunkhwa

Discussion

The prevalence rate of Cutaneous Leishmaniasis was greater in male as compared to female. In male the prevalence was 14.02% while in female it was 9.07%. Alkulaibi *et al* 2019 reported higher prevalence rate in male than female. Shaheen *et al*, 2020 reported the prevalence rate was 58.12% in male and 41.88% in female. Prevalence of Cutaneous leishmaniasis was higher in male (63.6%) than female (36.4%) in District Peshawar (Iqbal *et al*, 2022). In the present study lower prevalence rate in female may due to their cultural clothing in which most of the female body parts, like face, neck, feet and arms are covered while male have more exposed body parts to sand fly bite. Another reason of lower prevalence in female is that they stay indoors while male work mostly outdoor and more prone to transmission of Leishmaniasis.

Cutaneous leishmaniasis prevalence was recorded 18.21% in age group 16-30. It was 17.54% in age group 1-15. The prevalence of Cutaneous Leishmaniasis was higher in lower age groups (Alkulaibi *et al*, 2019). Incidence of Cutaneous leishmaniasis was 33% in age group 10-20 years and 6% in age group 51-60 years (Noor and Hussain, 2004). Rashid *et al* reported 60.62% prevalence in age group up to 19 years, 24.11% prevalence in age group 20 to 39 years and 15.27% prevalence of Cutaneous leishmaniasis in age group 40 years and above. But Nighat *et al* (2022) reported that prevalence of Cutaneous leishmaniasis was 23% in age group ≥5 years while it was 45% in age group ≥16. In the present study the prevalence recorded high in adults is may be due to because they spend lot of time outdoor doing work and other activities.

Results showed that in month wise prevalence of Cutaneous Leishmaniasis the maximum prevalence with 18.03% was recorded in the month of February. The lowest prevalence rate with 4% observed

in August. The prevalence rate was higher in winter than summer. Almost similar results were also reported Ullah *et al* (2021). In our study maximum prevalence rate, 9.38%, in the month of February and lower prevalence rates were reported in the month of May, June and July.

The prevalence of Cutaneous Leishmaniasis was higher in Afghan refugees. The prevalence in Afghan refugees was 15.2% while in local population the prevalence, 11.5%, was less than Afghan refugees. Ullah *et al* (2009) reported higher prevalence rate of Cutaneous Leishmaniasis in Local population than Afghan refugees during a study on prevalence of Cutaneous Leishmaniasis in Lower Dir. In our study the higher rates of prevalence of Cutaneous Leishmaniasis in Afghan refugees than local may be due to the housing conditions. Immunity to the disease can be another reason. That is why the disease was more prevalent in Afghan refugees than locals.

A total of 239 samples were collected, of which 22.17% were positive and 77.82% were negative. In rural areas, the prevalence of cutaneous leishmaniasis was 23.72%, and in urban areas, the prevalence was 20.66%. The prevalence of cutaneous leishmaniasis is high in females, with a percentage of 88.67% and in males, 30.88%. The cutaneous leishmaniasis prevalence was high in the age group 1–15 years, with a percentage of 35.71%, while 9.8% were in the age group 45–60 years. This study shows that in rural areas as a whole, prevalence was recorded as high as compared to urban areas due to a lack of awareness, an open housing system, and a low literacy rate. This study also shows that there has been a tremendous increase in cases of CL, and the disease has become endemic in many regions of Peshawar (Fawad *et al* 2023).

The results regarding prevalence pattern of Cutaneous Leishmaniasis from 2013 to 2019 show that the prevalence rate was highest during 2016 (16.24%) and 2017 (16.73%). The minimum prevalence was observed in 2019 which was 5.28%. The overall prevalence of Cutaneous Leishmaniasis 11.99%. The total number of patients were 3917 and positive case 470. Male positives were 325 and female were 145.

Authors Contributions

MZ presented and supervised the idea, while BA conducted the Field work/lab work and wrote the manuscript.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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Data Availability:

The data presented in this study can be accessed upon a fair request to the corresponding authors.

Ethical Approval:

The present study has been approved from the ethical committee of Islamia College University Peshawar.

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