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Original Research Article

A clinico-epidemiological study on cutaneous leishmaniasis in Erbil, Iraq (2015-2017)

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ABSTRACT

Background: The objective of the study was to determine the incidence of cutaneous leishmaniasis (CL) in Makhmur District (Erbil Province).
Methods: A cross-sectional, observational, descriptive study was performed in the outpatient clinic of Makhmur General Health Center. All the patients who presented at the dermatology clinic during the period from January 2015 to January 2017 were included in the study. The provisional diagnosis was dependent mainly on clinical examination in addition to Giemsa stain.
Results: A total of 1264 cutaneous leishmaniasis cases were diagnosed during the study period with males representing 54.6% of the cases. The study participants ranged from 10 months to 61 years. Age group <15 years were 53.5%. Clinically, 49.3% of patients had one lesion, 51.5% of patients had wet type. Most lesions were found on both limbs (48.8%). The highest number of cases was recorded during February (29.1%) and November (21.8%), while the lowest rate of cases was recorded in July (0.2%). According to stain results, 70.6% of the cases were positive to giemsa stain.
Conclusions: CL is endemic in Makhmur district. Males were infected in higher percent than females and this may be due to cultural, occupational and social factors.

Keywords: Epidemiology, Cutaneous leishmaniasis, Makhmur, Children, Endemic

INTRODUCTION

Leishmaniasis is a group of diseases caused by the blood flagellate Leishmania. These diseases are considered as an important cause of serious health problems in the Middle East including Iraq.[1] It is well known that in 88 countries, more than 12 million persons are infected with leishmaniasis. It is estimated that near 2 million new cases of CL discovered annually in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru.[2,3] Leishmania has two stages in their life cycle: the intracellular amstigote form within macrophages of vertebrates and promastigote stage in the gut of invertebrate host.[4] In endemic and developing countries such as Iraq in which such laboratory facilities like ELISA kits or PCR technique is not routinely available in such district health centers so the diagnosis is mainly dependent on the clinical features of the lesion in addition to the staining a smear from the lesion by Giemsa stain.[5-7] Although CL cases have been reported in Iraq, the

epidemiological and clinical characteristics regarding Makhmur district in Erbil Governerate have not been well documented. Therefore the present study was carried out to describe the epidemiologic conditions of CL in this district.

METHODS

Study population

This observational, descriptive study was carried out in Makhmur town (center of Makhmur district). Makhmur town is situated 67 kms South- west of Erbil city in Erbil governorate. The district had a population of 203801. The city is surrounded by an open land that is used by farmers for agriculture.[8]

The study was conducted during the period from January 2015 to January 2017. The site of the study was the outpatient Dermatology Clinic at Makhmur General Health Center. The research protocol was approved by the ethical committee of Hawler Medical University College of Medicine, Erbil Polytechnic University, and Erbil Health directorate. Informed consent was taken from each patient or the patient's parents if the patient was less than 15 years of age. In this study, the patients with cutaneous leishmaniasis were collected from out patients who were attending the dermatology clinic. Patients were including the inhabitants or resident of the district. Patients with a suspected clinical lesion, or who refuse to be shared in the study or who had received some treatment for CL were excluded from this study.

Sample and diagnostic procedures

Diagnoses of the disease are based on:

1- *Clinical picture*: this is the main step and was achieved by an experienced dermatologist. Examination of the patients was done to assess these points about the lesion: site, number, size, duration, and type as wet or dry CL. A special questionnaire regarding the patients was filled which include age, gender and residence.

2- *Parasitological examination*: after cleaning of the lesion, a sample was obtained from the indurated margin of the lesion and examined. The sample from the cutaneous lesion was taken by fine needle aspiration as the following steps:

   1- The skin around the lesion was disinfected.
   2- The sterile syringe of 1 ml contained 0.2 ml of sterile normal saline was injected intradermal through intact skin in to the active red border of the lesion.
   3- Aspirate the injected fluid as the needle draw back till the bloody stained fluid aspirate.
   4- Small amount of aspirated fluid was taken and smeared on a clean glass microscope slide then left it to dry, then fixed using 100% absolute methanol for 30 seconds and left it to dry again.
   5- Stained with Geimsa stain for 20 minutes, then rinse with tap water and dry the slide, and then examined it under oil immersion lens of the light microscope (Olympus CH2, Japan).
   6- Amastigote was diagnosed as round or spherical shape with distinctive kinetoplast. In this case was declared positive. When no amastigote was seen after 15 minute of inspection, the smears was declared negative.[5,9]

Statistical analysis

The data analysis was performed using descriptive statistics, including median, frequency, and frequency percentage. Comparisons were made using Chi-square test using standard equations. The results were reported with $p \leq 0.05$ as the accepted level of significance.

RESULTS

In the present study, a total of 1264 patients were confirmed to have CL from January 2015 to January 2017. The youngest patient was 10 months of age and the oldest was 61 years.

Table 1: Distribution of CL cases according to different demographic characteristics.

Characteristics	N	(%)
Gender		
Male	690	54.6
Female	574	45.4
Age in years		
<5	269	21.3
5-14	407	32.2
<15	676	53.5
15-24	210	16.6
25-34	191	15.1
35-44	79	6.3
45-54	67	5.3
>55	41	3.2
≥15	588	46.5
Residence		
Urban	471	37.3
Rural	793	62.7

Tables 1 illustrate the distribution of CL cases according to the demographic characteristics. It is noted from this table, CL cases distributed in a higher percent among: males (54.6%), age range between 5-14 years (32.2%), rural resident (62.7%). Regarding the age, the median age of the patients was 28 years (age range: 10 months to 61 years). The higher prevalence of CL was in age group <15 years 53.5%.

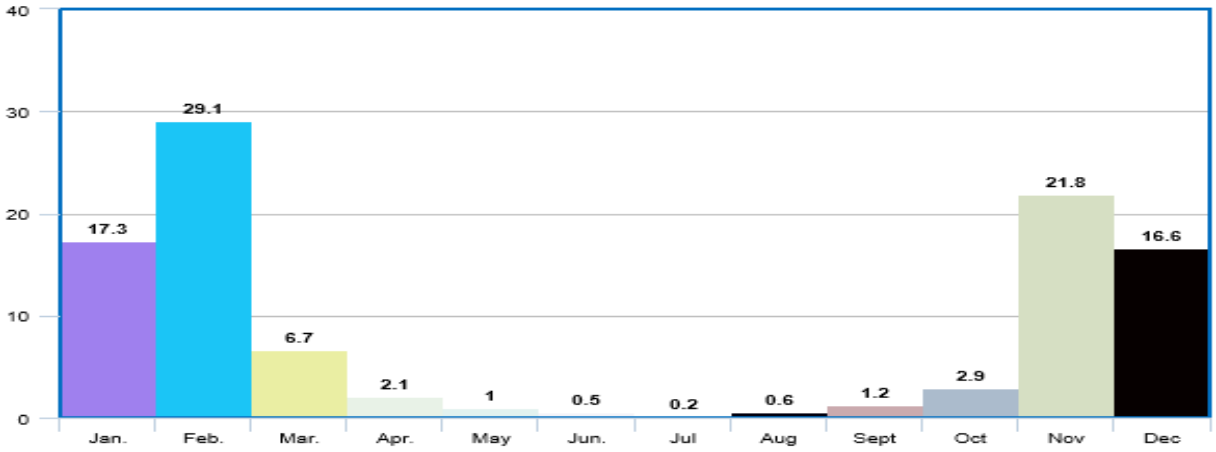

Figure 1: Percentages of monthly distribution of CL patients.

Figure 2 (A-L): Distribution of different lesions regarding: site, size, number, gender, age, type of lesion.

Table 2 shows that in both age groups <15 &and ≥15 years the percentage of male patients distribution were 54.4% and 57.1% respectively, and those both percentages were higher than those of females. The differences in the distribution of both males and females patients in both age groups <15 and ≥15 years was significant (p≤0.05).

Figure 1 shows that the highest rates of infection were recorded during February (29.1%) and November (21.8%) while the lowest rate was recorded during July (0.2%).

Table 2: Distribution of gender according to the age groups (above and below 15).

Age (yrs)	Male N (%)	Female N (%)	Total N (%)
<15	354 (52.4)	322 (47.6)	676 (100)
≥15	336 (57.1)	252 (42.9)	588 (100)
Total	690	574	1264

Chi-square value= 5.98; df= 1; Significant ($p \leq 0.05$).

Table 3 illustrates the distribution of CL cases according to the clinical features of the lesions. The highest percentage of distribution were of lesion of: duration of 2-4 months (56.6%), limbs involvement (48.8%), one lesion (49.3%), wet type lesion (51.5%), and of 1.5×2 cm to 2.5×3 cm (53.3%). The highest number of skin lesions per case which was recorded in this study was 6.

Table 3: Distribution of CL cases in relation to the clinical features.

Characteristics	N	(%)
Duration (months)		
<2	180	14.2
2-4	715	56.6
>4	369	29.2
Site of the lesion		
Limbs	617	48.8
Face	452	35.8
Abdomen and trunk	195	15.4
Number of lesion		
1	623	49.3
2	509	40.3
≥3	132	10.4
Type of lesion		
Wet	651	51.5
Dry	613	48.5
Diameter of the lesion (cm×cm)		
0.5×1 cm - 1.5×2 cm	332	26.3
1.5×2 cm - 2.5×3 cm	674	53.3
≥2.5×3 cm	258	20.4

Table 4: Results of parasitological examination by Giemsa stain.

Result	N	%
Positive	893	70.6
Negative	371	29.4

Regarding the results of parasitological examination, positive Giemsa stain was positive in 893 cases (70.6%) (Table 4).

Figure 2 shows the distribution of different lesions regarding: site, size number, gender, age.

Figure 3 illustrate the amastigote stage obtained from cutaneous lesion stained by Giemsa stain.

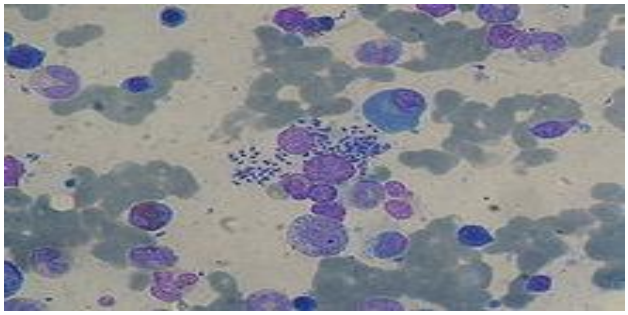

Figure 3: Amastigote from cutaneous lesion, Giemsa stain 100 X.

DISCUSSION

To our knowledge, this is the first study on cutaneous leishmaniasis in Erbil.

In the present study, CL had occurred more in males (54.6%). This result is in agreement with a studies done by in Iraq in which males were representing 56% and 57% of the cases respectively.[1,6]

Studies elsewhere, particularly in endemic areas in Iran, claimed no association between the gender and the rate of infections.[10,11]

Additionally a survey in Iraq from the period (2008-2015 years) in which the total reported cases for this period were 17001 had detected that the CL male infection (50.8%) was more than female infection (49.2%).[12]

In contrast, a study by In Turkey reported a higher rates in females (53.84%).[13] Also another study in Al-Yarmouk hospital Baghdad, total case was 50 and the female percent was 56%.[14]

The differences in the above studies may be related to the variations in factors such as size, design of the study population, in addition to climatic variations. The higher rate of CL among males in the present study was probably due to many factors. For example, the cultural habits of using covered dresses by women, which minimize the chances of the bites and being at home before evening, the onset of the period of sand fly activity as observed by.[15] In addition, men are probably traveling more for work while women were restricted to the house, which is another likely factor for the higher rate in males.[16]

Although all age groups are affected by CL, the highest percent was in the 5-14 years old (31.1%). This result is in accordance with the study achieved by which showed a high infections rate range in these ages.[13] In addition, the present study clarified that the incidence rate of CL infection was 53.5% in patients less than 15 years old. This high result is in agreement with results from Pakistan, Turkey and Greece.[17-19] In endemic regions, a

useful indicator of endemicity of the disease is the incidence of CL in children.[16]

This high rate in the present study may be due to children of this age play outdoors and are, thus, more be exposed to the source of infection than older children or adults.[12] In addition, younger age may probably have poorly developed immune system while the low rate infection of elderly patients may be related to their infection during childhood so consequently they had acquired long term immunity.[13]

In this study the rate of infection in 15 years age and above was 46.5% and this result is differed from that obtained by in which the incidence rate of CL infection was 57%, while it is lower than that reported for Colombia (86%), but in accordance with those findings reported from Turkey (45%).[13,16,20]

In this study, the number of infected cases was higher in rural inhabitants compared to urban areas. The result of this study is in accordance with that of both studies in which the percentages of the rural patients were 56.9% and 51.61% respectively.[15,20]

Factors that may play an important role in presence and distribution of CL in this district area like presence of reservoirs animals like rodents, dogs, living in houses with cracked mud, sleeping on floor or outside, and vegetation near house can enhance sand fly survival & abundance.[14,16,17].

Monthly distribution showed that winter months were reported higher infection cases of CL. This result is in harmony with that obtained by in which the highest percent of CL cases (29.4%) for the period (2008-2015) were observed in February, whereas the lowest percent were observed in July (0.7%).[12] The result of the recent study was nearest to a study in Tikrit/Iraq which had reported that the incidence rate was maximized in October, January, and February.[6]

It is clear that the incidence of CL cases in Iraq is due to highly distribution of sand flies. Distribution of sand flies is depending on local environmental factors (as humidity and temperature), physical factors (as geographical barriers and habitat availability), and biotic factors (as an abundance of vertebrate hosts). Climatic factors as rainfall, winds and temperature may be the most important factors affecting the distribution of sand fly species.[6,12] Additionally, the emerging peaks of cases are probably related to the incubation period of the disease and seasonal activity of the sand fly vectors that extend from August and September for this region after which a peak of infections was recorded until next year's February.[1,6,12]

The higher proportion of the lesions was located on the limbs (48.8%), face (35.8%), abdomen and trunk (15.4%). These observations are in contradict with studies in Iraq and Turkey in which face was the highest site involved, as the results were (43.5%) and (58.52%) respectively.[1,13] In comparison, other studies by, CL lesions occurred mainly on both limbs in a rate of (75%) and (69.3%) respectively.[12,16]

Phlebotomous attack exposed areas of the body, in addition, sandflies prefer to feed from appropriate selected sites with specific chemical attractions such as concentration of carbon dioxide which apparently was felt by the insects more from the feet and hands.[2,13,14]

Considering lesions number, the prevalence were as such: single lesion (49.3%), two lesions (40.3%) and three or more lesions (10.3%). These findings were differ from a study by in which the results were (42% one lesion, 58% multiple lesions).[6] Other study by showed that 36% of patients had single lesion and the remaining patients had double or more lesions.[14] The present findings differ from that of Akcali in which (71.0%) had one lesion and the remaining had multiple lesions.[13] The multiplicity of lesions can be due to the feeding behaviors of sandflies which they are doing multiple bites, presence of high numbers of infected sandflies or insemination following rubbing.[10,11,17]

Classification of CL based on the clinical presentation and theoretical variations in *Leishmania* spp. has been made in several studies.[16,20] Most authors have divided CL into 2 major forms ("wet" and "dry"), without considering the pathogenesis.[6] In this study, the wet type was more frequently observed (51.5% versus 48.5%). Al-Mafraji showed that (36.5%) of the lesions were of dry type while 63.5% were of wet type.[14] A study by demonstrate that wet lesions were observed more frequently (81.3%) than dry type (18.7%).[18] The wet form of the disease was not detected previously in Iraq.[12] Studies suggested that during the Iraq- Iran war battle (1988-1988) there was much crossing and re-crossing of armed forces over the international borders with Iran and because this form of the disease was common in western Iran, it may be speculated that the disease started to cross the borders with the soldiers or the gerbils (the reservoir of the disease) of the area.[12,21]

The highest duration of the disease obtained in this study was duration of 2-4 months with a percent of (56.5%). A study by stated that half of the CL cases (50%) were had duration of lesions under 2 months.[1] Other studies claimed that the duration of lesions was a minimum of 1 month and a maximum of 19 months.[6,10-13,16]

These differences in the durations may be related to two factors. Firstly it may be related to the different species or strains of leishmania causing infection. Secondly it may be related to the differences in the immune status of the patients involved in these studies.[12,14,22] Studies had shown the important role played by cytokines which are produced by CD4 T cells in protection against human leishmaniasis as they activate the macrophages that to kill

the parasites. More recently, the role displayed by CD8 T cells has been better illustrated. These cells participate in the differentiation of Th1 responses in the early events of parasite infection, where as they take part in lesion development after establishment of the infection. Their presence and their cytotoxic activity are directly correlated to the lesion size.[19,22,23] Studies in populations living in endemic areas suggest that some gene loci seem specific to particular clinical manifestations. Genetics of the host could provide critical information for the discovery of key steps in the pathogenesis of leishmania infections.[23]

The present study delineate that Giemsa stain has a detection rate of 70.6% in clinically diagnosed cases. This result is near to that obtained by in which the rates were 73% and 69.5% respectively.[6,21] The success of microscopic detection of amastigotes varies depending on the number of parasites present and duration of lesions. Therefore, failure to observe amastigotes does not exclude a diagnosis of CL and such infection in endemic areas may be diagnosed on the basis of their clinical features as leishmaniasis.[4,9,20] Mohammed had claimed that amastigotes at certain time of the disease are impossible to be detected.[24] The disappearance of such cells infected with the amastigote form in spite of the disease process is still continuous delineate that these phagocytes, giant cells, macrophages and monocytes, at specific point of the disease process become resistant to be infected with the amastigotes.

Recent studies had concluded that amastigotes which are replicated in the macrophages and then released into the extracellular fluid, become unable any more, to invade and consequently to replicate in another macrophages again. This inability is explained by an immune interaction between the host immune system and the leishmania parasite determinants which causes a development of resistance in the phagocytes against the parasites. This occlusion may explain the disappearance of the amastigotes at certain stages of the disease.[23-25] Hepburn clarified that over the next months of the disease process, there is a gradual decrease in the number of amastigotes and macrophages, which consequently will lead to the formation of granulomatous infiltrate consisting of lymphocytes, epithelioid cells and multinucleate giant cells.[25] Therefore Giemsa stain will be negative. In conclusion, this study indicates that cutaneous leishmaniasis is considered a main health problem in this district and its prevention is one of the health directorate priorities.

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