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Epidemiological study of Cutaneous Leishmaniasis in Babylon province/ Iraq

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Abstract

The study included identification of Leishmania parasite from cases of cutaneous leishmaniasis, by examination of smears from patients,Out of 215 cases suspected for cutaneous leishmaniasis included (61) pus and (178) tissue these pus sample were aspirated from the edges of the skin lesions examined in the advanced parasitology laboratory and DNA laboratory in the Department of Biology/ College of Science - University of Babylon at first by direct smear method to see the Amastigote phase. The cases included in this study belonged to some hospitals in the Babylon as Morjan Medical City Hospital (24), Musayyib General Hospital(17), Thu Al-Kifl General Hospital (29)and Hashimyah General Hospital(145), during the period from (October 2019 to March 2020). The distribution of infection in different age groups indicated that majority of cases belonged to (6-15) (39. 5%) age group and the infection was detected in both sexes with a predominance in males (57. 6%). The clinical picture of cutaneous lesions was suggestive to higher percentage of dry type (79. 6%) compared with wet type of lesions. Clinically, (64. 6%) of patients had multiple lesions. Most lesions were found on both face and trunk (45. 6%%). The highest number of cases was recorded during January (45. 2%). According to stain results (60. 6%) from the (61) total pus sample cases were positive to giemsa stain.

Keywords: Epidemiology; Cutaneous leishmaniasis; Leishmania; Direct smear method; Babylon; Province; Iraq

1. Introduction

Leishmaniasis is vector borne disease transmitted by biting of the female Sand fly caused by different species of *Leishmania* its manifested by three major clinical forms: cutaneous, mucocutaneous and visceral leishmaniasis [2,8,5]. cutaneous leishmaniasis exists at least in two forms [6]: 1. Amastigote form was ovoid and non flagellated form of Leishmania, measuring 3-5 µm in length. 2. Promastigote form was Flagellated form found in the sandfly host. Worldwide,vector-borne transmission is the most common mode of transmission. Other modes of transmission such as parenteral, congenital, sexual,occupational exposures, and person to-person transmission could also theoretically occur [10]. identification of Leishmania species is essential to decide the clinical prognosis and a species-specific therapeutic approach [27].

2. Materials and Methods

2.1. Sample and diagnostic procedures

- 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, duration, and type as wet or dry CL. A special questionnaire regarding the patients was filled which include age, gender and residence .
- Parasitological examination: samples from the cutaneous lesion were taken by the chancre fluid was aspirated by disposable syringe, through the steps:
  - The skin around the lesion was disinfected.

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- The sterile syringe (Insulin syringe) of 1 ml/cc contained 0. 2 ml of sterile normal saline was inject intra dermally by intact skin in to the active red border of the lesion
- Aspirate the injected fluid as the needle draw back till the bloody stained fluid aspirate
- Small amount of aspirated fluid was taken, and scarab around lesion taken to keep in ependorf tubes in deep freezing at -20 and smeared on a clean microscope glass slide to staining by Giemsa according to procedure that provide by company of Giemsa stain (SYRBIO / INDIA).
- All staining slides examined under oil immersion lens of the light microscope.

2.2. Statistical analysis

The data analysis was performed using descriptive statistics, including frequency, and frequency percentage. Comparisons were made using Chi-square test using standard equations. The results were reported with p≤0. 05 as the accepted level of significance.

3. Results and discussion

Table 1 The relationship between suspected cutaneous leishmaniasis patients and different gender

Gander	NO.	%	χ 2	P value
Male	124	57. 6%	5. 065	0. 024
Female	91	42. 4%		
Total	215	100%		
X2: represent chi-square value S : significant differences (P <0. 05)				

Table 2 The relationship between suspected cutaneous leishmaniasis patients and different age

Age group (year)	No.	%	Male (124)		Female (91)		Pearson Chi-square	P Value
			No.	%	No.	%		
≤ 5 years	62	28. 8%	37	59. 6%	25	40. 3%	3. 594	0. 464
6-15	85	39. 5%	51	60. 0%	34	40. 0%		
16-25	42	19. 5%	19	45. 3%	23	54. 7%		
26-35	17	7. 9%	11	64. 7%	6	35. 3%		
36-45	9	4. 2%	6	66. 7%	3	33. 3%		
Total	215	100%	124	57. 6%	91	42. 4%		
X2 represent chi-square value S : significant differences (P <0. 05)								

Table 3 Distribution of suspected Cutaneous leishmaniasis suspected patients according to the type of lesion

Type of lesion	No.	%	χ 2	P value
Dry	154	71. 6%	40. 228	0. 000
Wet	61	28. 4%		
Total	215	100%		
X2 represent chi-square value S : significant differences (P <0. 05)				

Table 4 Distribution of suspected cutaneous leishmaniasis patients according to residence

Residence	NO.	%	χ 2	P value
Urban	45	20. 9%	72. 674	0. 000
Rural	170	79. 1%		
Total	215	100%		
X2 represent chi-square value S : significant differences (P <0. 05)				

Table 5 Distribution of suspected cutaneous leishmaniasis patients according to location of lesion

Location	No.	%	χ 2	P value
Face and trunk	98	45. 6%	15. 358	0. 000
Upper limbs	64	29. 8%		
Lower limbs	53	24. 6%		
Total	215	100%		
X2:represent chi-square value S : significant differences (P <0. 05)				

Table 6 Distribution of suspected cutaneous leishmaniasis patients according to number of lesion

Number of lesion	No.	%	χ 2	P value
Single	76	35. 4%	18. 460	0. 000
Multiple	139	64. 6%		
Total	215	100%		
X2: represent chi-square value S : significant differences (P <0. 05)				

The results for this current study illustrate the distribution of CL cases according to the demographic characteristics. It is noted from Table(1), CL cases distributed in a higher percent among: males (57. 6%) comparison with female (42. 4%),this possibly because males more exposure to infected vectors compared with the females, due to the high percent of males were working or sleeping in open areas surfaces of houses with less coverage of body [3],the present study agree with [7]that she revealed that 54 (90%) of suspected patients were males and 6(10%) were females but incompatible with the results of [9] in Syria, who recorded that 527 cases (51%) for females and 505 cases (48. 9%) for males. Table (2) show the age range between ≥5 years to 45 years,the higher prevalence of CL was in age group (6-15) years in percentage (39. 5%) that's agree with [13] who found that most of the injured in Baghdad between the ages of 10-15 years, Table(3) appear the percentage of dry type was (71. 6%) and wet type was (28. 4%),the research result disagree with the outcomes of [15,23,18,25] who are mentioned the wet type as more prevalent that the dry type. Table (4) also appeared in the present study higher percentage in rural resident (79. 1%) comparison with urban areas (20. 9%),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 and abundance. 14,16,17. ,this result identical with [11] they pointed the most of cutaneous leishmaniasis case were observed in the rural area.

Table(5) show these lesions located in higher percent on the face and trunk (45. 6%), these results similar to what was obtained in study carried out in study of [1],which were proven that face was the highest site involved, as the results were (43. 5%). [16]study he was found that the incidence rates of CL lesions were on upper limbs, lower limbs and less frequent in on the face and much less on the trunk respectively. In addition the study appeared in table (6)that the Multiple lesions in high percent (64. 6%) comparison with single lesions,This results agree with another study which

that show (37. 35%) were having only a single lesion and (62. 65%) with multiple lesions [29],but this was not in agreement with those reported by [21,28,26], who mentioned that, most of the patients were with single lesion.

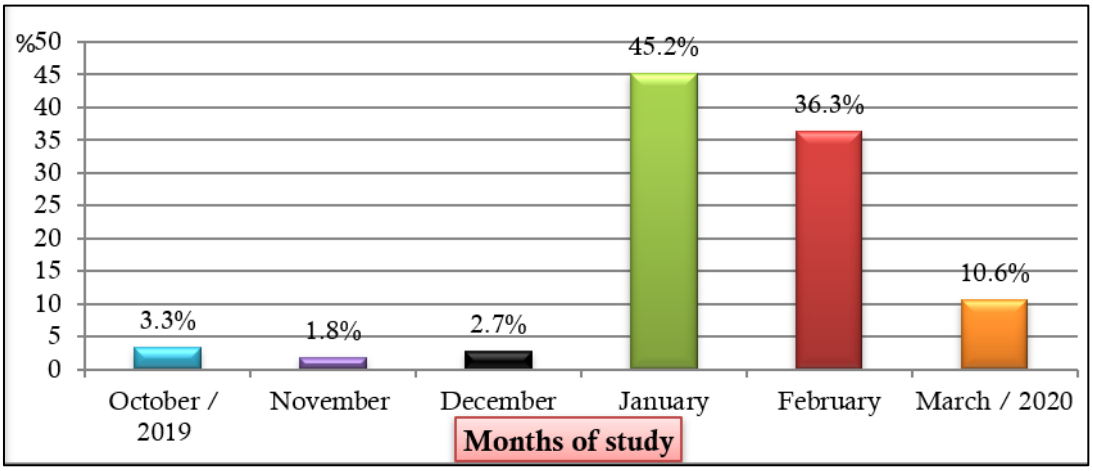

Figure 1 Percentages of monthly distribution of CL patients in Babylon province.

This figure show the higher percentage of CL in January (45. 2%) then the infections disappear in April, the results compatible with [17] in Al-Qadisiyah province and [13]in Baghdad province who showed that the peak of CL cases was in the month of January, and also consistent with [12,20,14] studies in AL-Najaf province. Variations in the monthly distribution for the cutaneous leishmaniasis may be related to an active sand flies related to climatic conditions in the study period of temperature and humidity, and the evolution of insect female that need blood during the life cycle of the maturation and development of eggs to complete their life cycle, especially in the spring [19] also this variation in seasonal peak could be the result of the existence of various dominant reservoir species in these areas particularly for ZCL, and the activity of the sand flies.

Figure 2 (A-F): Distribution of different lesions regarding: site, number and type of lesion

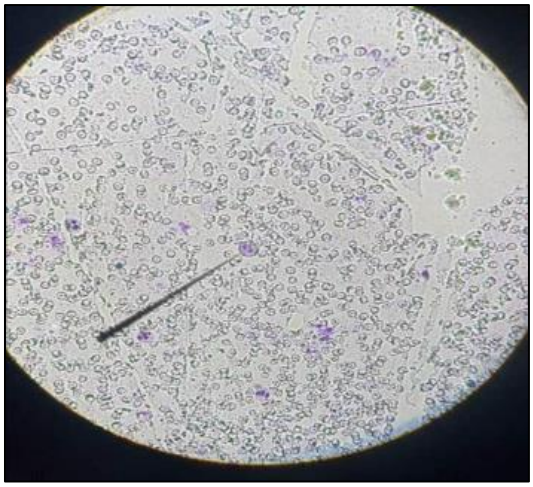

Figure 3 Amastigote phase of Cutaneous leishmaniasis by direct smear (Giemsa stain, X100)

Figure (3) show the microscopical diagnosis of CL is performed by the direct identification of amastigotes in Giemsa-stained lesion smears of biopsies, scrapings, or impression smears. Amastigotes appear as round or oval bodies, about 2–4 lm in diameter, with characteristic nuclei and kinetoplasts [16,24].

Table 7 Results of parasitological examination by Giemsa stain

Result	**No.**	**%**	**χ 2**	**P Value**
Positive	37	60. 6%	2. 770	0. 096
Negative	24	39. 3%		
Total	61	100%		
X2: represent chi-square value S : Non-significant differences (P > 0. 05)				

This table illustrate positive sample in percent (60. 6%) from the total number (61),this result was in agreement with what was reported by [22,29]. These methods have limited sensitivities because they require direct visualization of the parasites and the paucity of parasites within the lesion is a hallmark of lesions with old age. Identification of Leishmania species is essential to determine the clinical prognosis and a species-specific therapeutic approach[27]. The results were agree with the result reported in Iraq by[23], which was 73%. The percent success for microscopic identification of amastigotes in stained preparations varies depending on the number of parasites present, the site of the lesion rendered the sample taken from him and/or the experience of the person examining the slide[24].

4. Conclusion

This study showed that cutaneous leishmaniasis was more common in males, children and young age groups, and rural residents in Babylon province. The disease was mostly presented as dry, multiple lesions and frequently affected the face and trunk, with the highest number of cases recorded in January. Direct Giemsa-stained smear examination confirmed a good proportion of suspected cases, but it still had limited sensitivity in some samples, indicating the need for more sensitive diagnostic methods such as molecular techniques for accurate diagnosis and species identification.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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