

## Cutaneous leishmaniasis in Iraq

Abdulghani Mohamad AlSamarai<sup>1,2</sup>, Hussein Saher AlObaidi<sup>2</sup>

<sup>1</sup>Department of Medicine, Tikrit University College of Medicine, Tikrit, Iraq

<sup>2</sup>Microbiology Department, Tikrit University College of Medicine, Tikrit, Iraq

### Abstract

**Objective:** To determine the incidence of cutaneous leishmaniasis (CL) in Alhaweja District and to clarify the secondary bacterial infection in CL.

**Methodology:** A prospective survey was performed in the outpatient clinic of Alhaweja General Hospital. All the patients who presented at the dermatology clinic during the period from 1 October 2004 to the end of April 2005 were included in the study. The provisional diagnosis was dependent on clinical examination; however, biopsies and confirmatory tests were performed for questionable cases. Venous blood was drawn from patients with cutaneous leishmaniasis for determination of IgM. Swabs from the ulcer were taken for culture to determine secondary bacterial infections.

**Results:** A total of 107 cutaneous leishmaniasis cases were diagnosed during the study period with males representing 57% of the cases. The study participants ranged from 1-60 years. The incidence rate was 45 cases per 10,000. Clinically, 58% of patients had multiple lesions, while 42% had a single lesion. It was found that 36.5% of patients had dry type while 63.5% had wet type lesions. Most lesions were found on upper limbs (57%), while the fewest were found in the ear 1%. The highest number of cases was recorded during February (32.1%), while the lowest rate of cases was recorded in April (3.37%). According to cultures and smears, 73% of the cases were positive to giemsa stain and 43% were positive in cultures. Secondary bacterial infection occurred in 42% of lesions and *Staphylococcus epidermidis* was the most common bacteria (55%) isolated from lesions, followed by *Staphylococcus aureus* (33%). The mean of total IgM levels was significantly decreased in patients with CL in comparison with the control group.

**Conclusions:** CL is endemic in Alhaweja district. CL is an important health problem since secondary bacterial infection was reported in 42% of cases and this infection may influence the natural course of the disease causing more destruction for skin. Giemsa stain was the most sensitive parasitic diagnostic test.

**Key Words:** epidemiology, leishmaniasis, secondary infections, Alhaweja, Iraq

*J Infect Developing Countries* 2009; 3(2):123-129.

Received 4 August 2008 Accepted 15 October 2008

Copyright © 2009 Al samarai and AlObaidi. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Introduction

Leishmaniasis is a parasitic disease caused by haemoflagellate *Leishmania*. The disease is widespread and may cause serious health problems in communities throughout the Mediterranean regions and the Middle East, including Iraq [1-3]. There are an estimated 12 million cases worldwide, and there are about 1.5 million new cases of cutaneous leishmaniasis each year, of which over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru [4]. Old World disease primarily is caused by *Leishmania tropica* in urban areas and *Leishmania major* in dry desert areas [3].

In Iraq, two species are present: *L. tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were

reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in suburban areas [5]. The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. The total incidence rate of cutaneous leishmaniasis in Iraq varies from 2.3 / 100000 to 45.5 / 100000 [5].

In an endemic area, CL is largely diagnosed by its clinical appearance. Diagnostic challenges arise when cases appear in non-endemic areas, when the clinical picture is distorted, or when any atypical variants are seen even in endemic regions [6]. In addition, secondary infection or mistreatment can alter the clinical picture of CL and cause difficulty in diagnosis and delay in treatment. In such cases the diagnosis should be confirmed by examination of smears from lesions, culture, and histopathological

examination [7]. In developing countries such as Iraq, laboratory equipment and materials such as ELISA test kits or PCR technique materials are not available and dermatologists mostly have to rely on the clinical characteristics of the lesion. Giemsa- or leishman-stained smears obtained from the lesions are a rapid means of diagnosis [8]. Although CL cases have been reported in Iraq, the epidemiological and clinical characteristics regarding Alhaweja district have not been well documented.

The objective of this study was threefold: to determine the incidence of cutaneous leishmaniasis in Alhaweja District, to clarify how frequently secondary bacterial infection occurred in CL, and to determine the effect of secondary bacterial infection on the natural course of the disease.

## **Materials and Methods**

### *Study population*

A cross-sectional, observational, descriptive survey was performed in Alhaweja District, a city located 40 miles north of Kirkuk City in Altameem. The district had 236,992 inhabitants for the year 2005 and the city is surrounded by open land that was used by farmers for agriculture. The study was conducted during the period from October 2004 to April 2005. The site of the study was the outpatient Dermatology Clinic at Alhaweja General Hospital, which is the only hospital in the district for patient health care delivery. Out of a total of 23,778 of patients, 107 were infected with cutaneous leishmaniasis and were included in this study. Although the parasite infects all age groups, the patients were divided into two groups according to the development of their immune systems and to the different dietary zinc requirements for maturation and growth for all age groups. The first group included patients 1 to 15 years of age, and the second group patients aged 16 years and older. A control group of healthy individuals (50 subjects) was included in the study for comparison of serum IgM.

The research protocol was approved by the ethical committee of Tikrit University College of Medicine. Informed consent was taken from each patient or the patient's parents if the patient was less than 12 years of age.

### *Sample and diagnostic procedures*

Generally, samples were obtained only from those sites which showed the most indurated margin. For patients exhibiting more than one lesion, a detailed examination of each lesion was performed in order to choose the site of sample extraction. The

lesion was cleaned of debris with saline solution. Purulent or necrotic ulcers were treated with particular care, and debris was removed. None of the patients had received any anti-leishmanial chemotherapy treatment prior to diagnostic examination. Samples for parasitologic diagnosis included dermal scraping of the active indurated margins of the lesions, dermal scraping of the bottoms of the ulcers, and fine needle aspirate for culture [8]. All samples were taken by the same person in order to avoid individual variation. The slides of scraping materials were air dried, fixed with methanol, and stained with Giemsa. The slides were analyzed with a 100X immersion objective and examined by the same person. The aspirate culture was taken from the active indurated margin of the lesions as previously described [9]. Briefly, a 26-gauge needle was used on disposable tuberculin syringes with 0.4 ml of 0.1 M phosphate buffer saline at pH 7.2. The needle was inserted intradermally into the outer border of the lesion and rotated several times, and the tissue fluid was gently aspirated. A 0.2 ml portion of the aspirated materials was used to inoculate 2 tubes with the biphasic culture medium NNN, and the tubes were incubated at 27°C. Every two to three days, the liquid phase of the cultures was examined in order to observe motile promastigotes.

### *Serum IgM Measurement*

Total serum IgM concentration was determined using a single radial immunodiffusion technique.

## **Results**

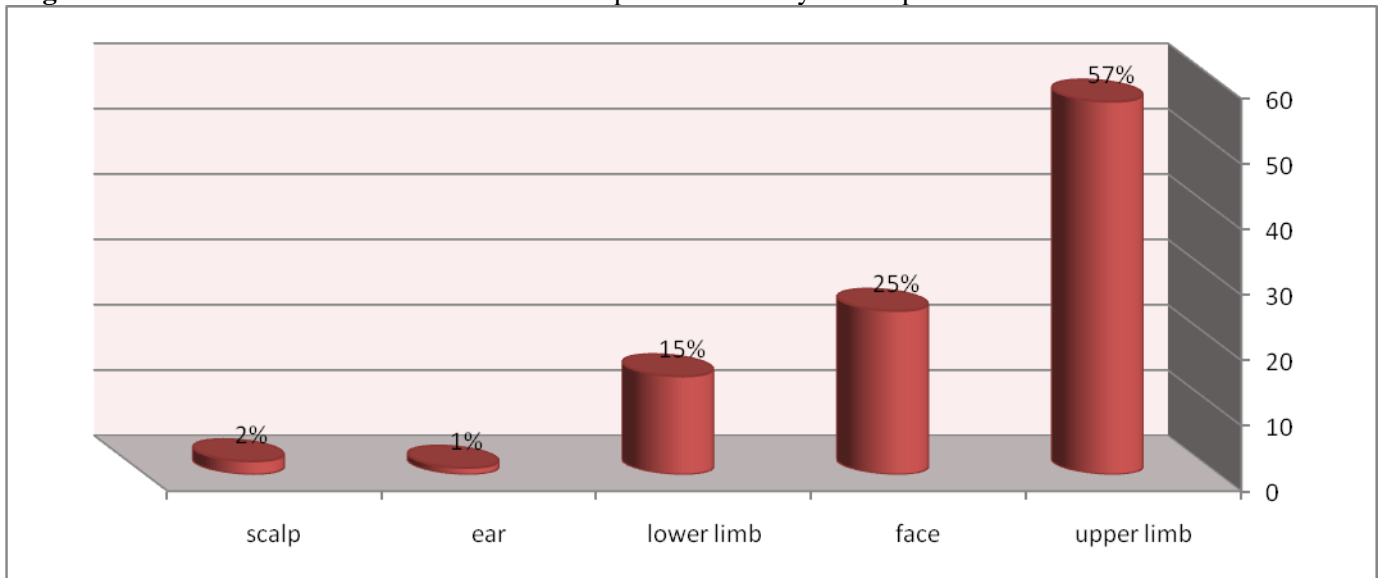
A total of 107 patients with CL were diagnosed, of whom 57% were male. The incidence was 45 cases per 10,000. The age range was from 1 to 60 years, and 43% were between 1 and 15 years. The male patients over age 15 represented 27 cases (25.23%) out of 107, with their ages ranging between 16 and 52 years. Out of the 107 patients, there were 34 (31.7 %) females over 15 years of age ranging between 16 and 60 years (Table1).

It was found that 58% (62 cases) had multiple lesions, while 42% had a single lesion (Table1). The highest number of skin lesions per case was 10. The total number of skin lesions in all CL patients was 187; of these, 68 lesions (36.5%) were nodular (Dry type) while 63.5% were ulcerative (Wet type) (Table1). Most lesions were present on an exposed area of the body. Distribution of the sites of the lesions was as follows: 57% on upper limbs; 25% on face; 15% on lower limbs; 2% on the scalp; and 1%

**Table 1.** Frequency distribution of cutaneous leishmaniasis cases.

	Variable	Percent	Number
Sex	Male	57	61
	Female	43	46
Age in Years	15 and below	43	46
	16 and above	57	61
Male age in years	15 and below	31.77	19
	16 and above	25.23	42
Female age in years	15 and below	11.21	5
	16 and above	31.77	41
Lesion number	Single	42	45
	Multiple	58	62
Lesion type	Dry	36.5	39
	Wet	63.5	68

**Figure 1.** Distribution of skin lesions on different parts of the body of 107 patients infected with CL.



on ears (Figure 1). Figure 2 shows that the highest rate of infection was recorded during February (32.1%), while the lowest rate was recorded during April (7.49%).

Table 2 shows that 73% (78 samples) of the samples were positive for Giemsa stain, while the percentage of positive culture of *Leishmania NNN* media was only 43% (46 samples). Thus Giemsa stain had high sensitivity (93%) but low specificity (43%), whereas the culture method had 55% sensitivity and 90% specificity.

As shown in figure 3, the most common bacteria isolated from CL lesions was *Staphylococcus epidermidis* (55%), followed by *Staphylococcus aureus* (33%), *Streptococcus pyogen* (7%) and *Escherichia coli* (5%). The mean total serum IgM concentration in CL male patients over the age of 15

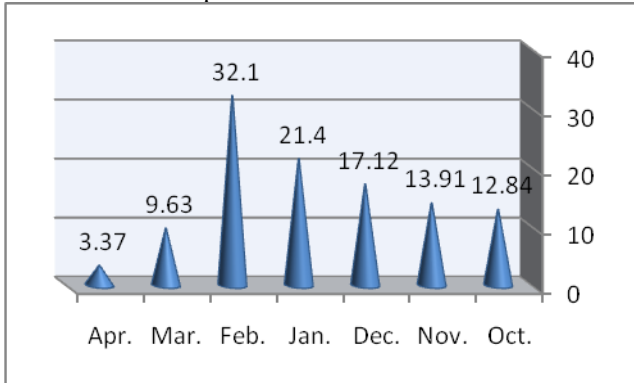
years was  $0.51 \pm 0.003$  g/l, while it was  $1.10 \pm 0.01$  g/l in the control group (Table 3). The difference was statistically significant ( $P < 0.05$ ). However, the mean total serum IgM concentration of CL female patients was lower ( $0.43 \pm 0.002$  g/l) than that in the control group ( $0.56 \pm 0.05$  g/l,  $P < 0.05$ ). In addition, it was found that the mean IgM concentration was  $0.16 \pm 0.001$  g/l in the patient group, while it was  $0.39 \pm 0.003$  g/l in the control group. There was a significant difference ( $P < 0.05$ ) between these two groups (Table 3).

**Discussion**

Alhaweja district is regarded as a rural area. There are many factors that play an important role in the presence and distribution of CL in this district, including the presence of animal reservoirs such as

rodents, dogs, etcetera; the presence of marshes; and the use of clay to build some of the houses in villages

**Figure 2.** The monthly distribution of CL patients in 107 patients who attended Al-Haweja General Hospital from October 2004 to April 2005.



**Table 2.** Detection rate of methods for parasitologic diagnosis of cutaneous leishmaniasis.

Test	Detection rate Positive Negative	Sensitivity	Specificity
Giemsa stain	73 % 27%	93 %	43 %
Culture	43 % 57%	55 %	90 %

that belong to this district area. Furthermore, as an agricultural area, Alhaweja district attracts and harbors many kinds of insects; therefore, its population works long hours in the farms where they are more exposed to insects bites.

In this study the incidence rate was 45 cases per 10,000. This rate was higher than those reported for other geographical areas in Iraq. In two community-based studies, the incidence was 2.5 case / 10,000 for Tikrit [10] in 2000 and 15 cases / 10,000 for Kirkuk city [11] in 2000. Additionally, in a hospital-based study performed in Samara [12], the incidence rate was 5.5 cases / 10,000 for the year 1994. However, the incidence rate was similar to that reported for Afghanistan [13] (46 per 10,000) and Eastern Venezuela [14] (50.1 per 10,000) but higher than that reported for Turkey (4.6 per 10,000) [15].

CL cases occurred more in males (57%) than in females (43%). This result was in agreement with those reported by AL-Obaidi for Tikrit [16], Sarhan for Baghdad [17], Sharifi *et al.* for Iran [18], Talari [19] for Iran, and Arfan for Pakistan. In contrast, AL-Zaidawi [20] in Tikrit and Akcali [15] in Turkey reported higher rates in females. These differences may be explained on the basis of variations between

studies with regard to factors such as the size of the study population, the study design, climatic variations, and culture.

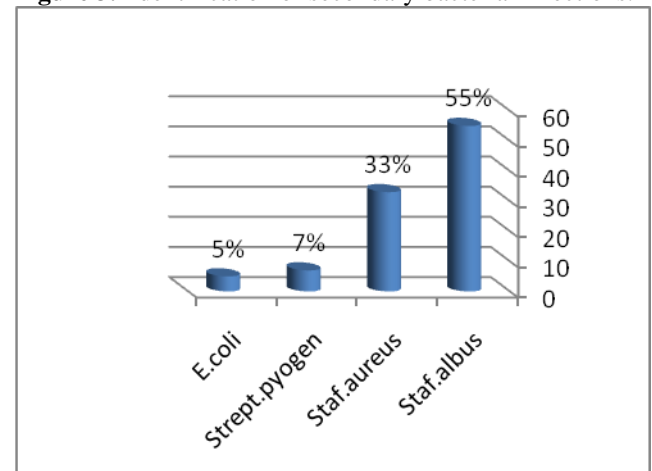
The incidence rate of CL infection was 57% in patients over 15 years old. This finding is lower than that reported for Colombia (86%) [8], but higher than findings reported for Iran (38%) [19] and Turkey (45%) [15]. In the two latter studies, the investigators postulated that the decrease in incidence with age was due to development of immunity by previous infections.

Incidence of CL was 25.23% in male patients over 15 years of age, while in females the incidence of CL was 31.7% in women over 15 years of age. The difference between these two groups of patients (male and female) might be explained by the fact that females in this age group are more exposed to insect bites than male patients in the same age group because most farm workers were females.

**Table 3.** Serum IgM levels in patients with cutaneous leishmaniasis.

Variable	Patients Mean g/l	Control Mean [SD] g/l	P value <
Male Mean SD 95% CI	0.51 0.003 0.5094-0.5106	1.10 0.01 1.097-1.103	0.0001
Female Mean SD 95% CI	0.43 0.002 0.4296— 0.4304	0.56 0.05 0.546-0.574	0.02
Total Mean SD 95% CI	0.16 0.001 0.1598-0.1602	0.39 0.003 0.389-0.391	0.0001

**Figure 3.** Identification of secondary bacterial infections.



The incidence rate of CL in patients aged 15 years and younger was higher in males as compared to females. This difference in the incidence rate of infection could be due to males in this age group playing outdoors without clothes and swimming in the rivers or lakes. This finding was in agreement with those reported for Iraq [16,21,22]. Ulcerative wet type lesions (ZCL) were present in 63.5%, while the nodule dry type lesions (ACL) were present in 36.5%. These observations are in agreement with those reported for Iran [19], Colombia [8], Pakistan [6], and Afghanistan [13], but not those reported for India [7]. The high incidence of ZCL may be due to the presence of reservoir animals in large numbers in this area, especially rodents and dogs. However, today, Afghanistan, Algeria, Brazil, Iran, Iraq, Peru, Saudi Arabia and Syria account for over of the 90% of the world's estimated 1.5 million cutaneous leishmaniasis cases [23,24]. Obviously, dense populations of natural ZCL hosts, together with abundant vector sand flies, are the key elements responsible for the high rate of human infection in the Alhaweja area. ZCL in our country may primarily affect farmers and nomads, who are chiefly exposed to night biting sand flies. In addition, the presence of high gerbil population densities in the area may be blamed as reservoirs of infection that are supported by the crops for which the irrigation canals had been constructed. Furthermore, the canal embankments serve as densely populated and favored rodent/sand fly infestation areas.

This study indicates that the incidence rate of multiple lesions in CL patients is 58%. This result could be due to long periods of exposure to Plebotomine sand flies and the high population density of sand flies in this area.

Regarding to distribution of CL lesions in this study, we found that the higher proportion of the lesions were located on the upper limbs (57%), face (47%) and lower limbs (15%) and less frequently on the scalp (2%) and ears (1%). These observations are consistent with reports concerning Turkey [15,25,26], Colombia [8], and Iran [19].

In comparison, the study by AL-Obaidi [16] found that CL lesions occurred mainly on upper limbs and lower limbs, less frequently on the face, and much less frequently on the trunk. These observations contrast those of Al-Zaidawi [20], who reported that the face was most affected.

Culture, customs and geographic location of Alhawija, along with the presence of contaminated centers and diverse susceptible hosts, are the most

important factors in the spread of the disease. *Phlebotomous* attack exposed areas of the body to suck blood; thus the lesions most frequently appear on the hands, face and legs. The differences in distribution of lesions noted in the studies mentioned above may be explained by the living conditions and habits of the people concerned. For example, some people prefer to sleep outdoors, thus exposing their upper and lower limbs as well as their faces to sand fly bites at night when the insects become more active. In general, the presence and distribution of lesions depend on which parts of the body are exposed and on the susceptibility of the host.

Our findings also indicate that new cases of CL tended to increase in October and reach a maximum in January and February. The incidence rate of infection then starts to decline from March and reaches its lowest point in April. It was observed that the majority of CL patients attended Alhaweja hospital between the months of October and March. This concurs with that reported by AL-Obaidi [16] in Tikrit, but not with those reported for Iran [19] or Afghanistan [13]. This variation in seasonal peak could be due to the existence of various dominant reservoir species in each study area as well as to the activity of the sand flies. The differences in monthly distribution of CL patients might also be related to the development of female insects and their requirement of blood during their life cycle for the maturation and development of eggs, especially in spring season. The lapse of time between when the patient was bitten and the appearance of skin lesions might be related to the long incubation period of leishmaniasis (two to four months).

Ulcerated skin lesions account for the majority of clinical manifestations of cutaneous leishmaniasis. However, the relatively wide range of morphological variations of the skin lesions, which are particularly frequent in New World leishmaniasis, as well as the prevalence of other microbial infections in areas where leishmaniasis is endemic that may mimic the symptoms of a *Leishmania* infection, often complicate the diagnosis of leishmaniasis [8]. Parasitic diagnosis of CL is therefore necessary before the relatively toxic chemotherapy should be applied<sup>9</sup>. Among the diagnostic methods available at the time of study in Iraq, fine-needle aspirate culture has been reported to be the most sensitive method [27-29]. However, direct microscopic examination of lesion scraping continues to be the diagnostic method most widely applied because of the ease of performance, low cost, and speed of this technique

[7]. In this report we provide evidence that Giemsa stain has a detection rate of 73% in clinically diagnosed cases, while culture has a detection rate of 43%. The negative results of both tests may be due to the presence of lesions of long duration, since most of the methods to test for parasites (Giemsa stain, culture, and PCR) analyzed by Ramirez *et al.* [8] are significantly less sensitive in lesions that have been present for longer than 3 months. In our study, the better detection rate by Giemsa stain as compared to culture was in agreement with the results reported by some investigators [8] and in contrast with the findings of others who reported higher sensitivity for culture as compared with Giemsa stain [28,29].

Although the present study indicated that Giemsa stain has higher sensitivity than culture, Giemsa staining showed lower specificity when compared to culture. Taken together, our results show that the combined use of microscopic examination and culture may significantly enhance the sensitivity and specificity of diagnosis. The diagnostic sensitivity of the microscopic examination observed in our study is the second highest reported so far. Furthermore, the sensitivity of microscopic examination can be increased by increasing the number of samples taken per single lesion [27]. Negative results may not always mean absence of *Leishmania* infection because the result of the test may be influenced by secondary bacterial or fungal infection. Furthermore, the *Leishmania* species is regarded as a fastidious microorganism and therefore, the duration of lesion evolution may not be fully elucidated upon testing. In contrast to our study, the culture method was previously reported to be the most sensitive for parasitologic diagnosis [27,28]. In this study we found a significant number of patients (35 of 78) who were diagnosed by Giemsa stain but had a negative result by culture. The low sensitivity of the microscopic method observed previously could significantly underestimate the total number of patients suffering from CL and therefore overestimate the sensitivity of other methods, such as culture [8].

Secondary bacterial infection occurred in 42% of CL lesions. Most of the bacteria were regarded as opportunistic microorganisms. Different kinds of bacteria associated with CL lesions occurred with different proportions: *Staphylococcus epidermidis* 55%, *Staphylococcus aureus* 33%, *Streptococcus pyogenes* 7% and *Escherichia coli* 5%. These observations are in agreement with those found by AL-Obiadi . [16] in Tikrit, but not with those recently

reported for India [30]. Most of these microorganisms are regarded as normal flora in a healthy person; therefore, the presence of *Leishmania* allows these opportunist microorganisms to invade either unprotected injured areas or at times when the immunity of a person was decreased.

In this study, the mean concentration of total IgM concentration in all CL patients was significantly decreased in comparison to that observed in the control groups ( $P < 0.05$ ). The parasite may be actively decreasing total IgM concentrations by degrading IgM via the activity of gp63, the virulence factor of *Leishmania spp.*<sup>31</sup>

In conclusion, this study indicates that CL is endemic in Alhaweja district. CL is a major health problem since secondary bacterial infection was reported in 42% of cases and this infection may influence the natural course of the disease causing further skin damage. Giemsa stain was the most sensitive parasitic diagnostic test.

## References

1. CDC (2004) Update CL in US. Military personnel. Southwest / Central Asia, 2002-2004. MMWR 53:264-265.
2. Hepburn NC (2003) Cutaneous leishmaniasis: an overview. JPGM 49:50-54.
3. Markle WH and Makhoul K (2004) Cutaneous leishmaniasis Recognition and Treatment. American Family Physician. 69: 1-9.
4. Ashford RW, Desjeux P, de Raadt P (1992) Estimation of population at risk of infection and number of cases of leishmaniasis. Parasitol Today 8:104-105.
5. WHO (2003) Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMRO, WHO OFFICE, Baghdad. WHO Office, Baghdad. Communicable Disease Toolkit, IRAQ CRISIS. WHO 2003:39-44. [www.who.int/diseasecontrol\\_emergencies/toolkits/Iraq\\_profile\\_ok.pdf](http://www.who.int/diseasecontrol_emergencies/toolkits/Iraq_profile_ok.pdf).
6. Arfan B, Rahman S (2006) Correlation of clinical, histopathological, and microbiological finding in 60 cases of cutaneous leishmaniasis. IJDVL 72:28-32.
7. Singh S and Sivakumar R (2003) Recent advances in the diagnosis of leishmaniasis. JPGM 55-60.
8. Ramirez JR, Agudelo S, Muskus C, *et al.* (2000) Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. J Clin Microbiol 38:3768-3773.
9. WHO Expert committee (1984) epidemiological aspects. In: Control of the leishmaniasis. World Health Organization, Technical Report Series 701:2-4.
10. Alaa NH (2002) Epidemiology of skin diseases in Tikrit and vicinity: a community based study. M Sc thesis, Tikrit University College of Medicine.
11. Murtada SJ (2001) Epidemiology of skin diseases in Kirkuk. MSc thesis, Tikrit University College of Medicine,

12. AlSamarai AGM. Prevalence of Skin Diseases in Samara, Iraq. MEJIM. In press.
13. Faulde M, Schrader J, Heyl G, Amirih M (2008) Differences in transmission seasons as an epidemiological tool for characterization of anthroponotic and zoonotic cutaneous leishmaniasis in northern Afghanistan. *Acta Tropica* 105:131-138.
14. Jourquera A, Ledezma E, Sousa L, *et al.* (1998) Epidemiologic characterization of American cutaneous leishmaniasis in an endemic region of Eastern Veezula. *Am J Trop Med Hyg* 58:589-593.
15. Akcali C, Culha G, Inaloz HS, *et al.* (2007) Cutaneous leishmaniasis in Hatay. *J Turk Acad Dermatol* 1:1-5.
16. AL-Obaidi HS (2000) Microbiological & Pharmacological studies with a Trial of vaccination against cutaneous leishmaniasis. PhD Thesis submitted to Collage of Medicine, University of Tikrit .
17. Sarhan ER. (1998) Study on Epidemiology of Cutaneous leishmaniasis in Baghdad. MSc Thesis submitted to College of Medicine, University of Baghdad.
18. Sharifi I, Ferkeri AR and Aflatonian MR (1998) Cutaneous leishmaniasis in primary school children in the South-eastern Iranian city of Bam, 1994-1995. *Bull. WHO* 76: 289-293.
19. Talari SA, Shajari G, Talaei R (2006) Clinical finding of cutaneous leishmaniasis as a new focus of Iran. *Internet J Infec Dis* 1 (2).
20. AL-Zaidawi KA (1997) New approach for treatment of cutaneous leishmaniasis by manitol. Diploma dissertation, College of Medicine, University of Tikrit, Iraq.
21. Hussian AK (1993) Intra-lesional therapy of CL with hypertonic sodium chloride solution. Diploma thesis submitted to Drmatology & Venerology Dep. College of Medicine, University of Baghdad.
22. AL-Gorban HA. (1996) Comparison of the intralesional therapy of cutaneous leishmaniasis. A dissertation submitted to the College of Medicine, Univesity of Tikrit. As a requirement for the degree of Diploma in Primary of Health care.
23. WHO (2000) Leishmaniasis and *Leishmania* / HIV co-infection. In: WHO report on global surveillance of epidemic prone infectious diseases, WHO/CDS/CSR/ISR/2000.1.PP:121-127.
24. Philippon E, Gramond C, Chouc C. (2005) Leishmaniasis. *Med Trop* 65:419-420.
25. Uzun S, Uslular C, Yucel A, Acar MA, Ozpoyraz M, Memisoglu HR (1999) Cutaneous leishmaniasis: evaluation of 3074 cases in the Cukurova region of Turkey. *Br J Dermatol* 140:347-350.
26. Gurel MS, Ulukanligil M, Ozbilge H (2002) Cutaneous Leishmaniasis in Sanliurfa: epidemiologic and clinical features of the last four years (1997-2000) *Int J Dermatol* 41:32-37.
27. Navin T, Arana F, Demerida A, Arana B, Castillo L, Silvers D (1990) Cutaneous Leishmaniasis in Guatemala: comparison of diagnosis methods. *Am J Trop Med Hyg* 42:36-42.
28. Weigle K, Dedavalos M, Heredia P, Molineros R, Saravia N, D'Alessandro A (1978) Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. *Am J Trop Med Hyg* 36:489-496.
29. Sharquie KE, Hassan AS, Hassan SA, Al-Hamami IA (2002) Evaluation of diagnosis of cutaneous leishmaniasis by direct smear, culture and histopathology. *Saudi Med J* 23:925-928.
30. Chaudhuri G, Chang KP (1988) Acid protease activity of a major surface membrane glycoprotein (gp63) from *Leishmania mexicana* promastigotes. *Mol Bioch Parasitol* 27:43-52.
31. Ziaie H, Sadeghian G (2008) Isolation of bacteria causing secondary bacterial infections in the lesions of cutaneous leishmaniasis. *Indian J Dermatology*. 53:129-131.

**Corresponding Author:** Prof AGM AlSamarai,  
Department of Medicine, Tikrit University College of  
Medicine, Tikrit, Iraq  
Email: galsamarrai@yahoo.com

**Conflict of interest:** No conflict of interest is declared.