Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2013; 1(6):1021-1025 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2013.v01i06.0081

Research Article

Cutaneous Leishmaniasis in Iraq: A clinicoepidemiological descriptive study

Abdulsadah A.Rahi
College of Science / Wasit University, Iraq

***Corresponding author**
Abdulsadah A.Rahi
Email: abdulsadah1966@yahoo.com

Abstract: Leishmaniasis is a zoonotic disease caused by the intracellular parasite since long time ago. The most common form is cutaneous leishmaniasis (CL), which causes skin sores. Dermal scrapings were analysed both by examination of Giemsa-stained smears and in vitro cultivation methods on NNN and RPMI 1640 media. The present study was conducted from January to May 2013 in Al-Karamah Teaching Hospital in Wasit Province, Iraq. Skin biopsies of 5 to 10 mm in diameter were taken under sterile conditions from the border of the ulcer and divided into three parts, The first part of the sample was smeared onto a glass slide, fixed with methanol, stained with Giemsa and examined by microscopy. A second and third part was inoculated on Novy-MacNeal-Nicolle (NNN) and Roswell Park Memorial Institute (RPMI 1640) media. The cultures were incubated at 25 °C and observed every week for 1 month. A total of 92 suspected individuals of CL were referred for diagnosis, out of whom 97.8 % proved to be positive by direct Giemsa-stained smear diagnosis, 84.8% by culture on NNN medium and 76.1% by cultivation on RPMI 1640 medium. These were mainly in age group 11-20 years old (48.9%), more in males (56.5%) than females (43.5%). There was no significant differences between gender. The present study recorded that median duration of lesions was under 2 month (50 %). Results of our study showed that high number of ulcers (43.5 %) were in face and fifty patients (54.3 %) presented with single lesion. Also the most cases (67.4 %) were appeared in rural areas.
Keywords: Cutaneous leishmaniasis, Culture, Human

INTRODUCTION

Leishmaniasis is a parasitic disease spread by the bite of infected sand flies. There are several different forms of leishmaniasis. The most common form is cutaneous leishmaniasis (CL), which causes skin sores. Visceral leishmaniasis (VL), which affects some of the body's internal organs, (most commonly the spleen, liver and bone marrow) is the most serious of the infections. Leishmaniasis is endemic to Iraq, Kuwait, Iran, Afghanistan, and other places in the Middle East; and poses a health risk to service members deployed there. The sand fly season in Iraq is from April through November and peaks in September/October. While effective treatment is available, prevention remains the best option. Leishmaniasis is not the same thing as Sandfly Fever which is a different disease spread by sand flies. Both VL and CL have been reported in Iraq caused by *Leishmania donovani*, *Leishmania major* and *Leishmania tropica* respectively **[1]**.

The genus *Leishmania* has two morphlogical forms in its lifecycle: the amastigote within macrophages of mammalian host and promastigote in the gut of invertebrate host **[2]**. More than 12 million people in 88 countries are known to be infected with leishmaniasis, but the true burden remains largely hidden. Two million new cases – 1.5 million of cutaneous leishmaniasis over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru, 500 000 of the visceral form of the disease occur annually, but declaration of the disease is compulsory in only 32 countries and a substantial number of cases are never recorded **[3,4]**.

In all areas of Iraq there had also been cases of cutaneous leishmaniasis. The course of the disease is much more gentle than that of kala-azar (VL). In 2001 there were 625 cases of cutaneous leishmaniasis, 955 cases in 2000 and as many as 8779 cases in the peak year 1992 (45 cases for every 100 thousand citizens). Cases of cutaneous leishmaniasis caused by *L. tropica* mostly occur in the suburbs of big cities (Baghdad, Mosul) among large conglomerations of people where the sanitary conditions are unsatisfactory. Incidences caused by *L. major* are much more common; they appear primarily in rural areas, especially in the northern and southern provinces of the country **[5,6]**. The aims of the present study to identify outbreaks of Cutaneous Leishmaniasis in Iraq.

MATERIALS AND METHODS

Samples collection :

Ninety two patients with clinically diagnosed Cutaneous leishmaniasis from both sexes and different ages were included in this study. They were attended to Al-Karamah Teaching Hospital and Health centers in different areas of Iraq during the period from January to May 2013. Patients with a doubtful clinical lesion, or who did not agree to be included in the trial or who had received some definitive treatment for CL were excluded from the trial. Written informed consent was

obtained from all the participating patients. Name, age, sex, address, duration of disease, number of lesions, and site of lesions of all enrolled patients were recorded.

Traditionally, smear samples are taken from the active edge of the leishmanial lesion, presumably due to the maximum number of parasites in the active edge **[7]**. After the smears dried completely,they were fixed with 100% methanol, allowed to dry again, and stained with Geimsa stain for microscopic examination for presence of amastigotes [**8]**. Staining technique is very important in improving the yield of smears. It has been suggested that visualizing the parasites is easier using Giemsa staining [**9]**.

Culturing :

The lesions and the adjacent normal-looking skin around them were cleaned, sterilized with 70% ethanol, and allowed to dry. Similar to the preparation of the slide smears, a small amount of the scraped tissue was inoculated on the liquid phase of Novy-McNeal-Nicolle (NNN) medium (10% of rabbit blood). The cultures were incubated at 25ºC and examined for parasite growth by light microscope every 4 days until promastigotes were seen or up to one month before being discarded as negative. The cultures were made at least in duplicates for each case.

Culture Media:

1- Biphasic medium (NNN):

-Solid phase **[10]** :Brain Heart infusion , 33.3 gm ; Agar, 16 gm ; D-Glucose, 8 gm ; Defibrinated Rabbit Blood , 100 ml ; Gentamycine, 1.25 ml.

-Liquid phase **[11]** : NaCl, 9 gm; KCL,.0.42 gm; Ca2Cl.H2O, 0.32 gm ; NaHCO3, 0.2 gm; Gentamycin , 80 mg/ml ;Glucose, 2 gm and distilled water 100 ml.

2-RPMI-1640 medium **(11)** : RPMI-1640 powder, 10.4 gm; sodium bicarbonate, 2 gm; double distilled water 1000 ml; Hepes solution, 10 ml; Fetal Calf Serum, 10% ; penicillin, 1 U / 100 ml and streptomycine,1 gm / 100 ml.

Statistical Analysis

All the statistical analysis were done by using programs of Pentium-4 computer through the Statistical Package for the Social Sciences(SPSS) program (version-10)[**12]**.

Note: The comparison of significant (P-value) in any test were:

S= Significant difference (P<0.05).

NS= Non Significant difference (P>0.05).

RESULTS

Table-1. represents the diagnosis of *Leishmania* species by using giemsa-stained and culture on NNN and RPMI media. Our study was revealed that the highest infection (97.8 %) appeared by using giemsa-stained method.

Table 1. Identification of *Leishmania* spp. by Giemsa Stain and Culture

Results	Giemsa-smear Stain		Culture on NNN		Culture on RPMI	
	No.	%	No.	%	No.	%
Positive	**90/92**	**97.8**	**78/92**	**84.8**	**70/92**	**76.1**
Negative	**2/92**	**2.2**	**14/92**	**15.2**	**22/92**	**23.9**

Regarding gender differences, in the study areas, CL have been reported more frequently in males (56.5%) than females (43.5%) and high prevalence (48.9%) in age group (11-20) years old (Table 2).

Table 2. Prevalence of CL According to the Age Groups and Gender

Age groups / Year		Gender		Total
		Male	Female	
1-10	**N**	**9**	**10**	**19**
	%	**9.8 %**	**10.9 %**	**20.7 %**
11-20	**N**	**27**	**18**	**45**
	%	**29.3 %**	**19.6 %**	**48.9 %**
> 20	**N**	**16**	**12**	**28**
	%	**17.4 %**	**13 %**	**30.4 %**
Total	**N**	**52**	**40**	**92**
	%	**56.5 %**	**43.5 %**	**100%**

P-value	C.S
0.01	**Significant (P<0.05)**

The comparison of the clinical features of CL cases was shown in table 3. The present study recorded that median duration of lesions was under 2 month (50 %). Results of this study showed that high number of ulcers (43.5 %) were in face and fifty patients (54.3 %) presented with single lesion. Also the most cases (67.4 %) were appeared in rural areas.

Table 3. Distribution of CL cases in Relation to the Clinical Features

Clinical features	No.	%
Duration (months)		
< 2	46	50.0
2-4	34	37.0
> 4	12	13.0
Total	**92**	**100**
Site of Lesions		
Face	40	43.5
Limbs	32	34.8
Abdomen	20	21.7
Total	**92**	**100**
Number of Lesions		
1	50	54.3
2	31	33.7
≥ 3	11	12.0
Total	**92**	**100**
Residence		
Rural	62	67.4
Urban	30	32.6
Total	**92**	**100**

Figure 1. Promastigote forms of *Leishmania* spp

DISCUSSION

The first step in the diagnosis of leishmaniasis especially in endemic areas is clinical clinical symptoms, microscopic observation of the parasites in stained tissue smears by the experienced medical personnel. Showing the parasites in clinical examples, culture methods are used to produce the promastigotes for definitive diagnosis. This method for diagnosis of leishmaniasis has been accepted as Gold Standard **[13]**. Although NNN medium and other diphasic media are routinely used for maintenance and production of *Leishmania* [**14]**, RPMI-1640 media gave the highest mean of growth rate after (2, 4, 6) days of cultivation, The viability gradually declined in all culture media used in our study and reached the minimum value after 10 days of cultivation. The minimizing of viability may be due to the food insufficiency to all parasites in culture media **[15]**.

Ninety- two patients with 150 skin lesions were enrolled in our study ; 52 males (56.5%) and 40 females(43.5%). Previous reports confirmed the same

results indicating that males are more commonly infected than females, most likely because of their exposure, possibly as a result of occupational contact with the outdoor sand fly vectors **[16,17]**. Although it is believed that sex hormones may influence the establishment and the course of parasitic diseases, behavioral factors, making male individuals more likely to be exposed to vectors in fields and other transmission environments, are probably equally or more important **[18,19]**. On the contrary of other studies that found the higher incidence of infection among females than males **[20-22]**. Moreover, the highest proportion of infection (48.9%) was recorded in 11-20 years age group, and the lowest (20.7 %) was in the 1-10 years age group, which is in agreement with previous reports indicating more exposure as a result of educational and occupational situations **[23-27]**.

Half of the CL cases (50%) were had duration of lesions under 2 months and most affected part of the body was face (43.5 %) with single lesions (54.3 %). This can be due to the fact that some ulcers do not necessarily lead to the appearance of scars for several possible reasons,i.e. immune system interference or early healing of the ulcers, spontaneously or due to treatment. These results with agreements with other studies in Iraq **[28]** and other countries **[29-31]**.

Our finding showed that CL was significantly associated with illiteracy and farmers as an occupation which is usually more common in rural population (67.4 %) . Similar findings were reported in previous studies **[32-34]**.

CONCLUSION

There has been a preference for liquid media for promastigote cultivation, although NNN medium and other biphasic-type media are routinely used for maintenance and producing parasites.

References

1. World Health Organization. WHO communicable disease profile for Iraq. 2003; 39-43.
2. Paniker CKJ; Textbook of Medical Parasitology. 5th edition. Jaypee Brothers Medical Publishers.New Delhi, 2002; 221.
3. WHO..The Leishmanisis report of TDR, WHO/Leish Geneva: CTD/TRY, WHO, 2005; 5.
4. Ashford RW, Desjeux P, de Raadt P; Estimation of population at risk of infection and number of cases of leishmaniasis., Parasitol Today. 2002 ; 8: 104-105.
5. Korzeniewski K; Health hazards in Iraq, Lekarz Wojskowy. 2005; 81(3): 176-180.
6. World Health Organization. Communicable disease profile for Iraq. 2003.
7. Robinson RJ, Agudelo S, Muskus C et al.; The method used to sample ulcers influences the diagnosis of cutaneous leishmaniasis. Trans R Soc Trop Med Hyg., 2002; 96: 169-171.
8. Bensoussan E, Nasereddin A, Jonas F, Schnur LF, Jaffe C; Comparison of PCR Assays for diagnosis of cutaneous leishmaniasis. J Clin Microbiol. 2006; 44(4): 1435–1439.
9. Magill AJ. Leishmaniasis. In: Strickland GT, ed. Hunter's Tropical Medicine and Emerging and Infectious Diseases, 8th edn. Philadelphia: WB Saunders. 2000; 665-87.
10. Kagan I G, Norman I; In "Manual of Clinical Microbiology", Washington. 1970; 479.
11. Dawson RM, Elliot DC, Elliot WH, Jones K.M; Data For Bio-chemical Research" .2 ed edition, Clareden press. Oxford , 1978 ; 508-510.
12. SPSS 15.0 Command Syntax Reference 2006, SPSS Inc., Chicago III.
13. Luz ZM, Silva AR, Silva Fde O, Caligiorne RB, Oliveira E, and Rabello A; Lesion aspirate culture for the diagnosis and isolation of Leishmania spp. from patients with cutaneous leishmaniasis. Mem. Inst. Oswaldo. Cruz., 2009; 104: 62-66.
14. McCartry-Burke C, Bates PA, Dwyer M; L. donovani : use of to different commercially available chemically defind media for continuous in vitro cultivation of promastigotes." Exp. Parasitol., 1991; 73: 385-387.
15. Al-Jeboori SR; Effect of temperatures difference on morphology and infectivity of *Leishmania tropica* in golden hamster. M.Sc. Thesis, University of Baghdad., 2000; 114 .
16. Rahman SF, Ghulam M, Pathan PA et al.; A survey of cutaneous leishmaniasis at village Gaibidero, district Larkana, Sindh, Pakistan .J. Gomal Med. Sci., 2009; (7): 2.
17. Kumar R, Bumb RA, Ansari N, and Metha R; Cutaneous leish-maniasis caused by *L. tropica* in Bikaner, India:Parasite identification and characterization using molecular and immunologic tools. Am J Trop Med Hyg. , 2007; 76(5): 896-901.
18. Rastogi V, Nirwan P;. Cutaneous leishmaniasis: an emerging infection in anon-endemic area and abrief update. Indian J Med Microbiol., 2007; 25:272-275.
19. Stewart CC, Brieger WR; Community views on cutaneous leishmaniasis in Istalif, Afghanistan: implications for treatment and prevention. Int Quart Commun Health Education 2009; 29: 123-142.
20. Fellah H, Rhajoui M, Ouahabi S, Belghiti D, Lyagoubi M; Occurrence of Human Cutaneous Leishmaniasis in Zouagha My Yacoub Province (Morocco). Int. J. Agri. Biol., 2007; 9 (1):197-198.

21. Gurel MS, Ulukanligil M, Ozbilge H. Cutaneous leishmaniasis in Sanliurfa: epidemiologic and clinical features of the last four years (1997-2000). Int J Dermatol., 2002; 41(1): 32-7.
22. Aytekin S, Ertem M, Yağdıran O, Aytekin N; Clinico-epidemiologic study of cutaneous leishmaniasis in Diyarbakir Turkey. Dermatol Online J., 2006; 12(3): 14.
23. Shehab A Lafi , Saleem O AL-Mawala , Ali Abdul-Latef AL-Ani, Abdulla S AL-Dulaymi; Bacterial Infections Associated with Cutaneous Leishmaniasis. Al –kindy Col. Med. J., 2007; 4 (1): 23-26.
24. Abdullah M, Qader, Mushriq K. Abood, Tural Y. Bakir; Identification of Leishmania parasites in clinical samples obtained from Cutaneous Leishmaniasis patients using PCR technique in Iraq. Iraqi Journal of Science, 2009; 50(1):32 – 36.
25. Alimoradi S, Hajjaran H, Mohebali M, Mansouri F; Molecular Identification of Leishmania Species Isolated from Human Cutaneous Leishmaniasis by RAPD-PCR. Iranian J Publ Health, 2009; 38(2): 44-50.
26. Khatami A, Firooz A, Gorouhi F, Dowlati Y; Treatment of acute old world cutaneous leishmaniasis: A systematic review of the randomized controlled trials. J Am ACAD Dermatol 2007; 2:29.
27. Sharma NL, Mahajan VK, Kanga A, Sood A, Katoch VM, Mauricio I; Localized cutaneousleishmaniasis due to *Leishmania donovani* and *Leishmania tropica*: preliminary findings of the study of 161 new cases from a new endemic focusin Himachal Pradesh, India . American J Trop Med Hyg., 2005 ; 72: 819-824.
28. Khalifa E, Rafid S, Najim A; Disseminated cutaneous leishmaniasis. Saudi Med J., 2004; 25 (7):951-954.
29. Talari SA, Talaei R, Shajari G, Vakili Z, Taghaviardakani A; Childhood cutaneous leishmaniasis: report of 117 cases from Iran. Korean J Parasitol., 2006; 44: 355–60.
30. Momeni AZ, Aminjavaheri M; Clinical picture of cutaneous leishmaniasis in Isfahan, Iran. Int J Dermatol., 1994; 33: 260–5.
31. Hojat A.N., Mehdi B., Mojtaba N., Mohamad M; Cutaneous Leishmaniasis in school children in border area at southwest of Iran. Sci Parasitol., 2012; 13 (4): 153-158.
32. Sabra M. Ahmed, Hala H; Abou faddan.Cutaneous Leishmaniasis in Gharyan – Libya – a Case-Control Study. Life Science Journal. 2013;10(1).
33. Abdellatif, M ZM, EL-Mabrouk K, Ewis AA; Cutaneous Leishmaniasis infection in Al-jabal Al-gharbi, Libya; an epidemiological study. Korean Journal of Parasitology. 2012; 50 (4):127 – 144.
34. Ranjan A, Sur D, Singh; Risk factors for Indian Kala-azar. Am J Trop Med Hyg., 2005 ; 73:74 – 8.