Isolation of Bacteria Causing Secondary Bacterial Infection In The Dry Lesions Of Cutaneous Leishmaniasis

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Received 27/9/2009 Accepted 4/1/2011

Abstract

Cutaneous leishmaniasis (CL) is a parasitic disease characterized by single or multiple lesions. Secondary bacterial infection is one of the complications of the disease that can increase the tissue destruction and the resultant scar. To determine effectively the incidence of real secondary bacterial infection in the dry type of cutaneous leishmaniasis ,we designed the current study. This was a cross-sectional study performed in the outpatient clinic of dermatology and veneriology at Tikrit Teaching Hospital. A total of 37 patients with clinical diagnosis of Cutaneous leishmaniasis (dry type) were enrolled in this study. Samples were taken from all patients. The skin areas surrounding the lesions were thoroughly cleaned using cotton wool moistened with alcoholic iodine. After appropriate cleaning, the samples were collected aseptically by scraping or sterile swabs of the lesions. All the samples were transferred to Mac Conkey's agar and blood agar. After 24 hours incubation (aerobically& an aerobically) at 37 °C, the species of the bacteria were determined. Then the samples were transferred to Mueller-Hinton agar or nutrient agar for sensitivity test. After 24 hours incubation at 37 °C, the diameters of the inhibition zones were measured to determined the sensitivity Among the 37 patients with Cutaneous leishmaniasis the results of the culture were positive in 12 patients (32.4%), 11 of them (91.6%) have more than one lesion and 9 patients (75%) of them were less than 5 years old, and 3 patients (25%) were more than 5 years old. Bacteria isolated from the lesions were Staph. aureus in 11 patients (91.6%)(and all were sensitive to Rifampicin) and E.Coli in one patient (8.3%) which was sensitive to Amikacin & Norfloxacin. The incidence of secondary bacterial infection with a dry type of CL, was (32.4%), (91.6%) of them had multiple lesions, and (75%) were less than 5 years old. The most common bacterial isolate was Staphylococcus aureus .

عزل البكتريا التي تسبب عدوى جرثومية ثانوية في الاصابات الجافة للاشمانيا الجلدية

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المستخلص

اللاشمانيا الجلدية مرض طفيلي يتميز بإصابات وحيدة أو متعددة العدوى الجرثومية الثانوية إحدى مضاعفات المرض التي يمكن ان تزيد من دمار النسيج والندبة الناتجة. للتشخيص الفعال لهذه العدوى الجرثومية الثانوية الحقيقية في الإصابات الجافة للاشمانيا الجلدية اجريت دراسة مقطعية في العيادة الخارجية للأمراض الجلدية والتناسلية في مستشفى تكريت التعليمي على مجموعة عددها(٣٧)مريضا تم تشخيصهم سريريا بإصاباتهم باللاشمانيا الجلدية النوع الجاف حيث

أخذت عينات الدراسة من مناطق الجلد التي تحيط بالآفات الجلدية التي نظفت كليا باستعمال القطن الطبي المبلل باليود الكحولي وحولت هذه العينات الى الوسط الزرعي(Mac Conkey s & blood agars) بصورة خالية من التلوث وبعد حضانة (هوائية ولا هوائية لمدة اربعة وعشرون ساعة) في (٣٧) درجة مئوية تم تحديد نوع البكتريا ثم حولت العينات الى الوسط الزرعي Mueller-Hinton agar or nutrient agar لاختبار الحساسية بعد حضانة (٢٤ ساعة في ٣٧ درجة مئوية) ،تم قياس قطر المنطقة التي لم تنمو فيها البكتريا لتحديد الحساسية ،من بين المرضى ال(٣٧) المصابين بالليشمانيا الجلدية النوع الجاف كانت نتيجة المزرع البكتيري ايجابية في ١٢ مريض(٣٢.٤%)،١١منهم (٩١.٦%)لديهم اكثر من اصابة واحدة و ٩ مرضى(٧٥%)منهم كانوا اقل من عمر ٥ سنوات،و ٣ مرضى فقط ٢٥% كانوا بعمر اكثر من ٥ سنوات البكتريا التي تم عزلها كانت Staph. aureus في ١١مريض (٩١.٦%) وكانت كلها حساسة الى Rifampicin وفي مريض واحد (٨.٣%) عزلت E.coli والتي كانت حساسة الى Norfloxacin& Amikacin نسبة العدوى الجرثومية الثانوية في النوع الجاف للليشمانيا الجلدية كانت (٣٢.٤%) وكان (٩١.٦%) منهم لديه اصابات متعددة و ٧٥% منهم اقل من عمر ٥سنوات وكان نوع البكتريا الاكثر شيوعا هيStaph. aureus

Introduction

Leishmaniasis is a parasitic disease transmitted by sand flies. It is characterised by a spectrum of cutaneous, mucocutaneous and visceral clinical manifestations that depend largely on the species of the parasite involved and host immune response. According to the recent estimates, 1.5 million new cases of cutaneous leishmaniasis (CL) occur each year .More than 90% of cases occur in five countries in the old world (Afghanistan, Algeria, Iran, Iraq and Saudia Arabia) and two countries in the new world (Brazil and Peru). [1] CL is endemic disease in Iraq and other countries in the Middle East [2] .Beginning in the 1980s (during the Iran-Iraq war) ,It has now reached epidemic status[1,3] CL is a self healing disease but spontaneous cure may take several months or even years [4,5] CL of the old world eventually heals. The rate of spontaneous healing depend on several factors, including parasite load and virulence, host immune response, location of lesion and the presence or absence of secondary bacterial infection. [6]The healing time for lesions caused by Leishmania major may be 9 months or more and those caused by Leishmania tropica it may be in excess of 1 year [4] .Therapy aims shortening the duration of lesions and prevent scarring. [5,7]An important part of therapy for CL is by local care along with antileishmania therapy. The treatment of secondary bacterial infection is essential for healing. On the other hand, the secondary bacterial infection of CL will increase the tissue destruction and the result of scar. [8]To determine effectively the incidence of real secondary bacterial infection in the dry lesions of CL, the present study were designed .

Patients and Methods

This was a cross-sectional study performed in the outpatient clinic of dermatology and venerology at Tikrit Teaching Hospital.A total of 37 patients with clinical diagnosis of Cutaneous leishmaniasis (dry type) were enrolled in this study during the period from 1st of November 2008 to 1st of April 2009.History was obtained from each patient regarding age, gender, address, duration and family history of Cutaneous leishmaniasis. Patients with history of antibiotic use (topical or systemic)in the recent weeks were excluded from the study. The skin areas surrounding the lesions were thoroughly cleaned using cotton wool moistened with alcoholic iodine.After appropriate cleaning, the samples were collected aseptically by scraping or sterile swabs of the lesions. All the samples were inoculated on Mac Conkey's agar and blood agar. After 24 hours incubation(aerobically& an acrobically) at 37 °C, the species of the

bacteria were determined. Then the samples were transferred to Mueller-Hinton agar or nutrient agar for sensitivity test. After 24 hours incubation at 37 °C, the diameters of the inhibition zones were measured to determined the sensitivity

Results

A total of 37 patients with Cutaneous leishmaniasis (85 Lesions) were enrolled, 19 male and 18 female their ages ranged from (3 months-38 years) with mean 6.4 years.The results of the culture were positive in 12 patients (32.4%) among them 11 patients (91.6%) have more than one lesion and 9 patients (75%) of them less than 5 years old, And 3 patients (25%) more than 5 years old. The isolated bacteria from the lesions were Staph. aureus in 11 patients (91.6%) and all were sensitive to Rifampicin and E.Coli in one patient (8.3%) which was sensitive to Amikacin & Norfloxacin .

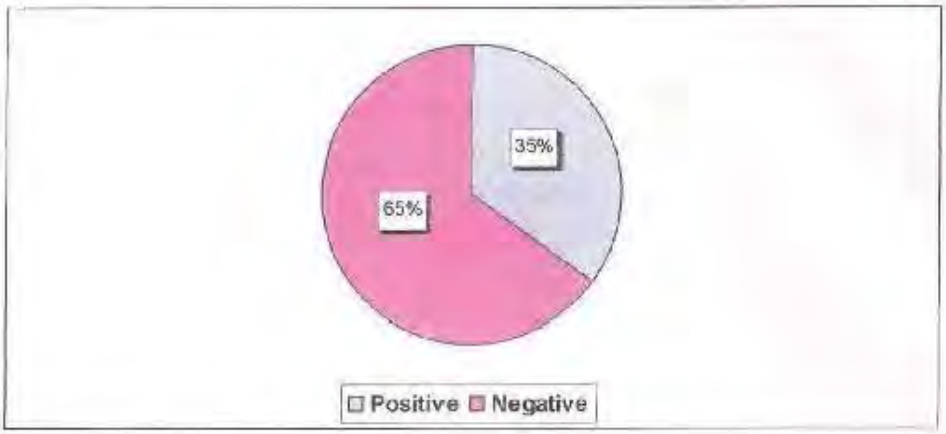

Fig.(1): Sample distribution according to the family history of Cutaneous leishmaniasis

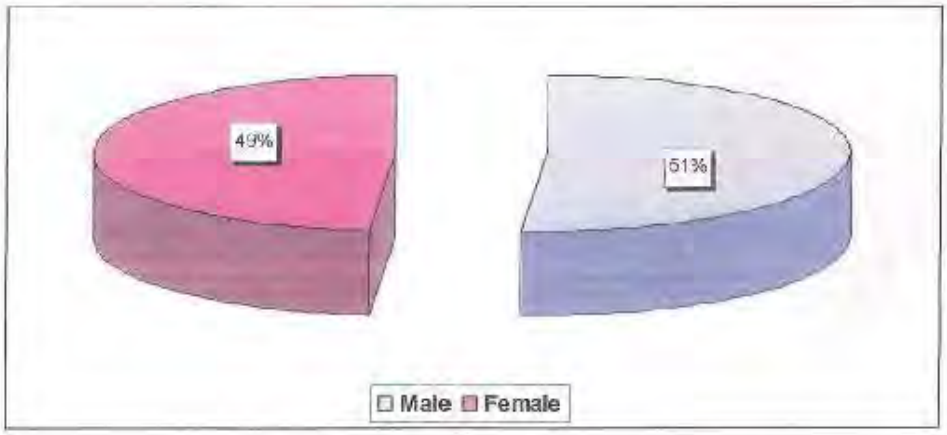

Fig.(2): Sample distribution according to gender.

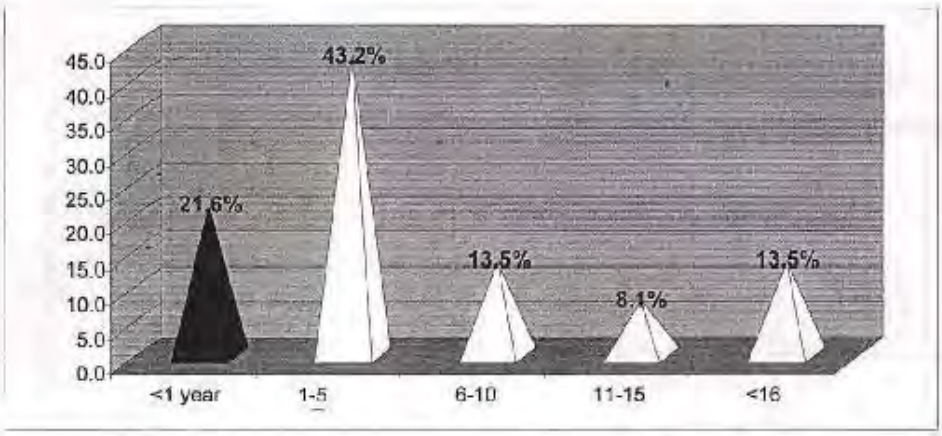

Fig.(3):percentage of infection according to age group (year)

Table(1): Number & percentage of lesions according to age

Age	No. of lesions										Total	
	1		2		3		4		>5			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<1 year	4	23.5					2	50	2	50	8	21.6
1-5 year	7	41.2	4	44.4	2	66.7	2	50	1	25	16	43.2
6-10	3	17.6	2	22.2							5	13.5
11-15	2	11.8	1	11.1							3	8.1
<16	1	5.9	2	22.2	1	33.3			1	25	5	13.5
Total	17	100	9	100	3	100	4	100	4	100	37	100

$X^2 = 12.873$, df=12, p= 0.862**(not significant)**

Table(2): Number & percentage of lesions according to gender.

gender	No. of lesions										Total	
	1		2		3		4		>5			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Male	8	47.1	4	44.4	2	67	2	50	3	75	19	51.4
Female	9	52.9	5	55.6	1	33	2	50	1	25	18	48.6
Total	17	100	9	100	3	100	4	100	4	100	37	100

$X^2 = 1.477$, df =4, p= 0.813**(not significant)**

Table(3): Number & percentage of lesions according to family history.

Family history	No. of lesions										Total	
	1		2		3		4		>5			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Positive	7	41.2	3	33.3	1	33	2	50			13	35.14
Negative	10	58.8	6	66.7	2	67	2	50	4	100	24	64.86
Total	17	100	9	100	3	100	4	100	4	100	37	100

$X^2 = 2.844$, df=4, p= 0.584 **(not significant)**

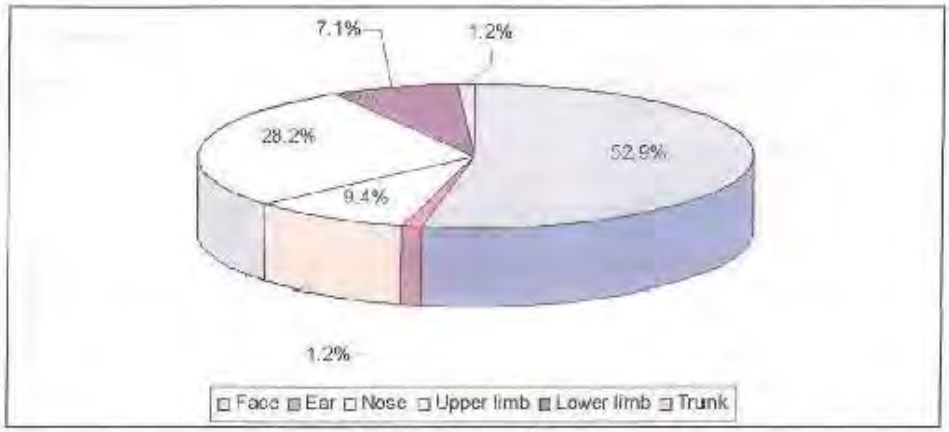

Fig.(4): Percentage of lesions according to Site of the infection.

Table(4): The site & percentage of lesions according to the gender.

Site of lesion	Male		Female		Total	
	No.	%	No.	%	No.	%
Face	32	68.1	13	34.2	45	52.9
Ear	1	2.1	0	0.0	1	1.2
Nose	3	6.4	5	13.2	8	9.4
Upper limb	6	12.8	18	47.4	24	28.2
Lower limb	4	8.5	2	5.3	6	7.1
Trunk	1	2.1	0	0.0	1	1.2
Total	47	100	38	100	85	100

$X^2 = 16.420$, df=5, p= 0.006(significant)

Table(5): The site & percentage of lesions according to the family history.

Site of lesion	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Face	17	70.8	28	45.9	45	52.9
Ear	0	0.0	1	1.6	1	1.2
Nose	4	16.7	4	6.6	8	9.4
Upper limb	3	12.5	21	34.4	24	28.2
Lower limb	0	0.0	6	9.8	6	7.1
Trunk	0	0.0	1	1.6	1	1.2
Total	24	100	61	100	85	100

$X^2 = 9.973$, df=5, p= 0.076(**not significant**)

Table(6): The site& percentage of lesions according to the age

Site of lesion	<1 year		1-5		6-10		11-15		>16		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Face	14	58.3	23	67.6	5	71.4	2	50	1	6.3	45	52.9
Ear	1	4.2									1	1.2
Nose	0	0	3	8.8	2	28.6	1	25	2	12.5	8	9.4
Upper limb	4	16.7	7	20.6	0	0	1	25	12	75.0	24	28.2
Lower limb	4	16.7	1	2.9	0	0	0	0	1	6.3	6	7.1
Trunk	1	4.2	0	0	0	0	0	0			1	1.2
Total	24	100	34	100	7	100	4	100	16	100	85	100

$X^2 = 40.820$, df=20, p= 0.004(significant)

Table(7): The site& percentage of lesions according to the growth of bacteria.

Site of lesion	no growth		Staph epidermidis		Gram –ve E.Coli		Staph. aureus		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Face	20	60.6	1	25	4	100	20	45.5	45	52.9
Ear	0	0.0			0	0	1	2.3	1	1.2
Nose	4	12.1	1	25	0	0	3	6.8	8	9.4
Upper limb	8	24.2	2	50	0	0	14	31.8	24	28.2
Lower limb	1	3.0	0	0	0	0	5	11.4	6	7.1
Trunk	0	0	0	0	0	0	1	2.3	1	1.2
Total	33	100	4	100	4	100	44	100	85	100

$X^2 = 11.605$, df=15, p= 0.709 (not significant)

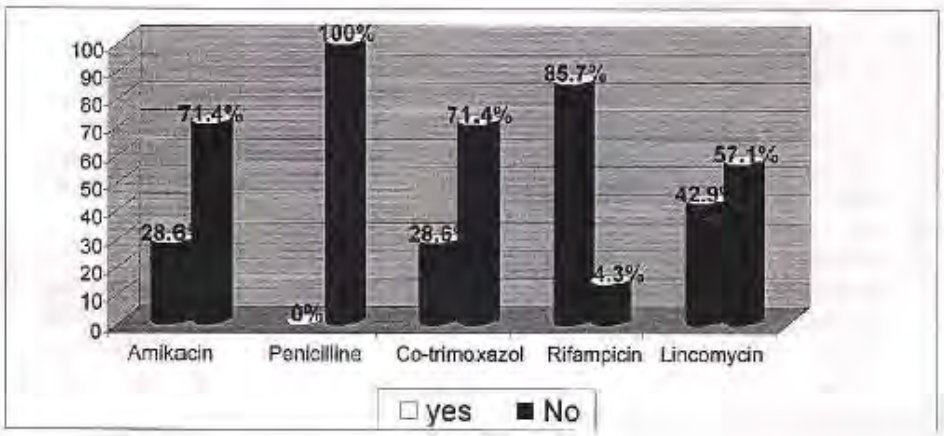

Fig.(5): Sensitivity to antibiotics

Discussion

Secondary bacterial infection is one of the complications of CL . Although some authors emphasize on the rarity of this findings [6], our clinical data are in contrast to this.In our practice we encounter many cases of dry CL, secondary bacterial infection can exacerbate the disease and it will increase the tissue destruction with a resultant scar formation. The appropriate use of antibiotics will decrease the infection in these cases and the resultant scar. One study in Sudan has shown the prevalence of secondary bacterial infection to be 18%. The pathogenic organism was not

identified in this study [8]In another study that was performed in the impetiginized formsof leishmaniasis, Staphylococcus aureus was recognized to be the responsible pathogen. [9] In our study, out of the 37 patients with a dry type of CL, 12 patients (32.4%) had confirmed secondary bacterial infection, and 11 of them (91.6%) had multiple lesions, and 9 of them (75%) were less than 5 years old. The most common bacterial isolate was Staphylococcus aureus in 11 patients (91.6%) and in only one patient (8.3%) gram negative bacteria (E.coli) was isolated. There was no significant association between the no. of lesions according to age, family history and gender ,however there was significant association between the site of lesions according to the gender and age P=0.006 and 0.004 respectively. Regarding to the results of our study we proved for the first time that dry type of CL may be secondarily infected like the ulcerative or wet types on the contrary of the previous studies[10] .Also the increase in the no. of lesions, and the younger age patients (<5 years) especially the males were candidate for this infection. This could be explained that the young boys exposed more to environment which will induce the infection. we suggest that topical antibiotics are needed for dry CL treated with the antileishmania medications to combat the secondary infection and accelerate the tissue healing and to prevent further scar formation as possible. The choice of antibiotics will be optimal if it is directed against Staphylococcus in first line.

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