Detection Of Secondary Infection In Cutaneous Leishmaniasis.

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الخلاصة

لغرض دراسة انتشار الإصابة الثانوية في حالة اللشمانيا الجلدية وعلاقتها في عملية الشفاء.تم تحديد 76 مريض مصاب بداء اللشملنيا بثلاث طرق وهي الحقن بمادة المحلول المائي الملحي ، عينة من الجد و قطرات دم من مكان الإصابة، وقد ظهرت العينات الموجبة بنسبة مئوية وهي على التوالي 39 (68,4%) ،48 (84,2%) و 37 (65%) بينما كانت النسب المئوية للحالات السالبة هي كما يأتي 18 (31,6%) ، 9 (15,8%) و 20(36%) وقد وجدت الإصابات الثانوية فقط في 57 (75%) مريض، كانت الإصابات البكتيرية الثانوية 52 إصابة من مجموع 57 إصابة أي بنسبة (91 %) ، في حين ان الإصابات الفطرية الثانوية كانت 5 من مجموع 57 إصابة أي بنسبة (9%). وجد ان حالات الإصابة البكترية الموجبة لصبغة كرام هي 48 من مجموع 52 وبنسبة (92%) اما حالات الإصابة بالبكتريا السالبة لصبغة كرام هي 4 من 52 وبنسبة (8%) حالة.

ان الإصابات البكترية الموجبة لصبغة كرام أظهرت الأنواع التالية من البكتريا *Staphylococcus aureus* 45 من مجموع 48 (% 93,7) ، *Streptococcus pyogenes* 2 من مجموع 48 (4,2 %) ، *Staphylococcus epidermidis* 1 من مجموع 48 (2,1%) ، بينما أظهرت البكتريا السالبة لصبغة كرام النسب التالية وحسب الأنواع *Escherichia coli* 2 من مجموع 4 (50%) ، *Pseudomonas aeruginosa* 1 من مجموع 4 (25%) و *klebsiella spp* 1 من مجموع 4 (25%).

Abstract

In order to study the prevalence of secondary infection in cutaneous Leishmaniasis and its relationship to the healing process , 76 Leishmaniotic patients were evaluated by three methods . The percentage of positive cases by injection with normal saline, part of skin biopsy and blood drops from lesion was 39 (68.4 %), 48 (84.2%) and 37 (65%) respectively while the percentage of nagative cases was 18 (31.6%), 9 (15.8%) and 20 (36%) respectevely .

Secondary infection only were present in 57 (75%) patients, secondary bacterial infection were found in 52/ 57 (91%) patients , Secondary Fungal infection was found in 5/57 (9%) patients , gram positive bacteria only were present in 48/52 (92%) , gram negetive bacteria were present in 4 /52 (8%) patients.

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The secondary infection gram positive bacteria which appear *Staphylococcus aureus* 45/48 (93.7%) ,*Streptococcus pyogenes* 2/48 (4.2%) ,*Staphylococcus epidermidis* 1/48 (2.1%) , While gram negative bacteria appear *Escherichia coli* 2/4 (50%) ,*Pseudomonas aeruginosa* 1/4 (25%), *klebsiella spp*. 1/4 (25%) .

Introduction

Leishmaniasis is a group of diseases caused by infection with protozoan parasites (single cell microorganisms) called *Leishmania* . The infection is transmitted by bites of sand –flies infected with the microorganisms .A variety of different sand files may act as vector of parasites , and the parasite may be transmitted from person to person or from a range of animals to human [1].

Leishmaniasis has two main clinical presentation forms (cutaneous and visceral Leishmaniasis).

Cutaneous Leishmaniasis is characterized by one or more skin sores (either open or closed) that develop week to months after a person is bitten by infected sand flies [2].

The skin is an intricate habitat for many bacteria , and their type and density are determined by anatomy, humidity , sebum production and host hormonal status. Bacterial skin microbiota are commensal , symbiotic or parasitic relative to the host; the type of relationship established is often inherent to the bacteria but also depends on alterations in the host immune status . In virtually all studies of skin and soft-tissue infections, *Staphylococcus aureus* is the most common pathogen . In most studies , *Streptococcus pyogenes* ranks second in frequency [3].

A raised , red lesion develops at the site of the bite (often weeks or sometimes years afterwards),the lesion becomes susceptible to colonization infection with a number of micro-organisms, such as pathogenic or opportunistic Fungi and bacteria that could cause secondary infections such secondary infection may play an important role in the size and shape of the lesions , as well as in scar development [4].

Materials and Methods

Subjects : A total of 76 patients with cutaneous Leishmaniasis from teaching hospitals in Najaf , Marjan hospital in Hilla , and AL-Hosseine hospital in kerballa between November 2003 and February 2006 .

The study were included 24 healthy individuals. Subjects having secondary bacterial infection in cutaneous lesions and other acute or chronic diseases were not included in the study.

Direct smears examination done by two procedure were evaluated for diagnosis of cutaneous Leishmaniasis. The margin of lesion was aseptically punctured with a hypodermic needle of insulin syringe containing a small amount of saline .The aspirate which is drawn up into the needle was smeared on slides for staining and examined under an oil immersion microscope looking for Leishman - donovan bodies (L.d. bodies) .

Small biopsy silted by the end of the loop , smeared onto a clear glass slide , fixed with methanol , stained with Giemsa stain and examined in the same way as the first method.

Identification of bacterial isoloates was done according to[5].While fungal isolates was done according to [6].

Results

Distribution of cutaneous Leishmaniasis a ccording to direct examination .

The percentage of positive cases by injection with normal saline and part of skin biopsy was 39(68.4%), 48(84.2%) respectively while the percentage of negative cases was 8(31.6 %) , 20 (35%) respectively (table 1) .

Distribution of microorganism that isolated from secondary infection in cutaneous Leishmaniasis .

This study revealed that the bacteria isolated from secondary infection in cutaneous Leishmaniasis were found in 52/57 (91%) patients , (gram positive were present in 48/52 (92%) patients from it, while gram negative were present in 4/52 (8%) and secondary fungal infection was found in 5/52 (9%).

The type of gram positive bacteria in the secondary infection which appear *Staphylococcus aureus* 45/48 (93.7%), *Staphylococcus pyogens* 2/48(4.2%), *Staphylococcus epidemidis* 1/48 (2.1%) , *Escherichia coli* 2/4 (50%) ,*Pseudomonas aeruginosa* 1/4 (25%), *klebsiella spp*. 1/4 (25%) respectively (table 2).

Figure -1: Photograph pf patients with cutaneous leishmaniasis and secondary bacterial infection.

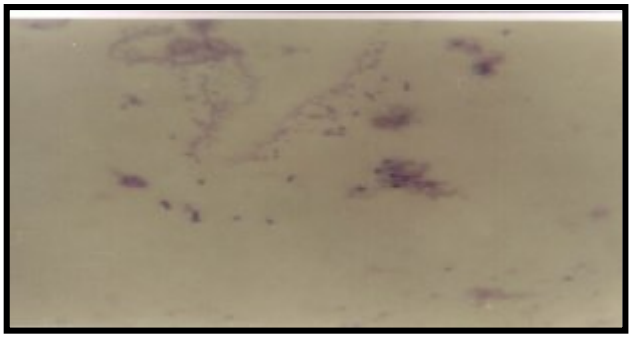

Figure -2: Direct skin smears (Giemsa stain, 100X) showing amastigotes from normal saline.

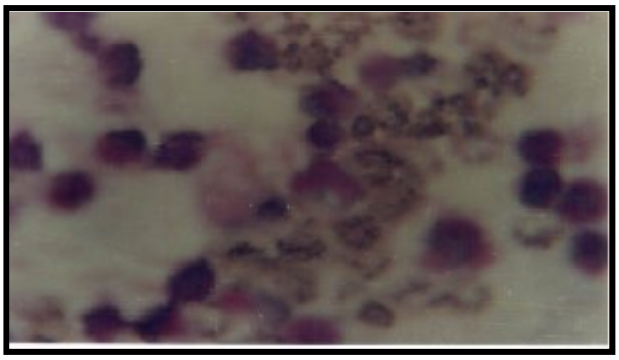

Figure -3: Direct skin smears (Giemsa stain 100X) showing amastigotes from skin biopsy.

Table -1 : The percentage of infection according to direct skin smear examination .

Age(year)	Injection with normal saline		Part of skin biopsy		By the two Methods	
	positive	negetive	positive	negetive	positive	Negetive
10 and Less	6	4	6	4	6	4
11-20	10	4	12	2	9	5
21-30	10	4	13	1	8	6
31-40	7	3	9	1	8	2
41 and more	6	3	8	1	6	3
Total	39	18	48	9	37	20
%	68.4	31.6	84.2	15.8	65	36

Table -2 :The microorganism that isolated from secondary infection in Cutaneous Leishmaniasis .

Isolates	Single		mixed		Total	
	number	%	umber	%	number	%
S. aureus	45	83	9	17	54	87.1
S. pyogenes	2	29	5	71	7	11.3
S. epidermidis	1	100	Zero	Zero	1	1.6
Total of g+ ve	48	77	14	23	62	100
E. coli	2	40	3	60	5	55.6
P.s aeraginosa	1	50	1	50	2	22.2
Klebsiella spp.	1	50	1	50	2	22.2
Total of g- ve	4	44.4	5	55.6	9	100
Isolates of fungi and yeasts	5	71.4	2	28.6	100	100

Discussion

Cutaneous Leishmaniasis is a complex disease with a wide spectrum of clinical manifestations determined by the causative *Leishmania species* [7] .It remains as a major public health problem in Sub-tropical countries [8] .

The disease mechanisms responsible for healing or chronicity of experimental and human Leishmaniasis are essentially confined to the immune system . Bacterial , fungal and parasitic infections sometimes result as illnesses affecting the integrity of skin and immune system .[9, 10]

When nodules due to infection with Leishmania parasite ulcerate ,they become susceptible to colonization with number of microorganisms , such as pathogenic fungus and bacterial that could provoke secondary infection [11 , 12] .

It is most frequently diagnosed by clinical evaluation , this evaluation usually suffer from lack of standardization [13]. And is hampered by the fact that even Fairly typical acute Lesion can be confused with other dermatological problems.

This study showed that 57/75 positive case with secondary infection in skin lesions of patients with cutaneous Leishmaniasis ,some a pproximate results were seen in other studies , like study of[14 , 15 , 16] .

Because the skin is a fairly dry habitat, Gram-negative bacteria are , with one exception , rarely found on the skin in comparison with the Gram-positive bacteria . The exception is the genus Acinetobacter, which is found colonizing the moister areas of skin such axillae , groin and antecubital fossa. Anumber of Enterobacter species can be found on the hands, which become temporarily colonized and constitute a source for cross – infection . However , *Pseudomonas aeruginosa* and *Proteus mirabilis* can be found in the toewebs of normal individuals and can be found as skin invaders in persons with very moist feet [16] .

A recent study of the SENTRY Antimicrobial Surveillance programme on the major pathogens isolated from skin and soft – tissue infections showed that, in Latin America , the most common pathogens, in decreasing order of prevalence, were *staphylococcus aureus* (32.8%) , *Esherichia coil* (13.1%), *Pseudomonas aeruginosa* (11.9%) ,*Enterococcus species* (7.7%) , *Klebsiella pneumoniae* (5.8%) , *Enterobacter species* (5.6%) and *Acinetobacter species* (4.1%) [17] . Anaerobes play an important role in complicating skin infections, and peptostreptococci typically are the most common of the anaerobic isolates [18].

Few studies were found in the literature on the prevalence of bacteria and /or fungi in lesions of cutaneous leishmaniasis [9] described the predominant presence, in Iran, of cogulase- positive staphylococci in ulcers of cutaneous leishmaniasis as well as of streptococcus species Pseudomonas species .

Kelbsiella species and *E.coil.* [19 , 20] . Also observed that *staphylococcus aureus* was prevalent in cutaneous leishmaniasis .

Chiclero s ulcers, a from of cutaneous leishmaniasis due to Leishmania mexcana , have a high prevalence of bacterial contamination (90.9%), but *staphylococcus aureus* is found at similar levels (20%) to other micro-organisms such as *streptococcus Pyogenes* , *pseudomonas aeruginosa*, *Morgaenella morganii* and *Enterococcus species* [17] .

These da. ta suggest that the Leishmania parasites that are present in the cutaneous Leishmaniasis lesions induce a local suppressive effect , allowing better survival of both the parasite and the contaminating bacteria . However , inter-relationships between leishmanial and bacterial infections are not alaweys synergistic . The prevalence of bacterial infections in lesion in which Leishmania parasites were detected was significantly lower (26.5%) than for lesions in which no parasite was found with the bacteria (45%) .This apparently suggests some antagonistic mechanism of the bacterial contaminants and Leishmana parasites were always present simultaneously [9] .

Leishmania parasite and their metabolites might induce a local immunosuppressant in cutaneous Leishmaniasis Lesion and may facilitate concomitant bacterial infection [21] .

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