Diamond Jubilee year

ISSN: 0019-5154

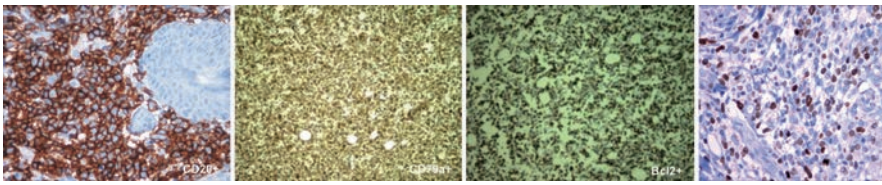
Indian Journal of Dermatology

Volume 60 Issue 2 March-April 2015

Issue highlights

- Male genital lichen sclerosus
- Immunomodulators in warts
- Serendipity in dermatology
- Zinc-responsive acral hyperkeratotic dermatosis
- Quality of life and psychological morbidity in vitiligo patients
- Clinico-mycological study of dermatophyte toenail onychomycosis
- Skin prick test in patients with chronic allergic skin disorders
- Facial angiofibromas treated with topical sirolimus
- Novel uses of skin biopsy punches
- Diffuse cutaneous mastocytosis with bullous lesions and pulmonary involvement
- Multiple synchronous verrucous carcinomas of the scalp
- Secondary syphilid on healed varicella

Cutaneous B cell lymphomas



IJD®
www.e-ijd.org

PubMed

Medknow

Wolters Kluwer

Bacterial Contamination in Cutaneous Leishmaniasis: Its Effect on the Lesions' Healing Course

Pouran Layegh, Kiarash Ghazvini[1], Toktam Moghiman[2], Fatemeh Hadian[2], Naghmeh Zabolinejad, Fakhrozaman Pezeshkpour

Cutaneous Leishmaniasis Research Center, [1]Microbiology and Virology Research Center, Qaem Hospital [2]Research Center for Skin Disease and Cutaneous Leishmaniasis, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Address for correspondence:
Dr. Toktam Moghiman, Cutaneous Leishmaniasis Research Center, Qaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Taghiabad Square, Mashhad, Iran, P.O. Box: 9176699199.
E-mail: t_moghiman@yahoo.co.uk

Abstract

Background: The colonization of aerobic and anaerobic microbial agents on cutaneous leishmaniasis (CL) lesions, especially acute erosive ulcerative ones, has been mentioned in previous studies showing controversial results on the healing course of lesions with the use of antibiotics. **Aims:** The purpose of this study was to evaluate the prevalence of secondary bacterial infections in CL lesions and the effect of its elimination on the lesions' improvement rate. **Materials and Methods:** This cross-sectional clinical trial was performed on 84 acute CL patients. The required skin samples were taken. Cultivation for bacteria was conducted. Patients with positive culture results were divided into two groups. Both groups received standard anti-leishmania treatment, whereas only one group was treated with cephalexin 40-50 mg/kg/day for 10 days. The improvement rate was evaluated in the following visits based on changes in the lesions' induration size. **Results:** Among the 84 studied patients, 22.6% had a negative culture result whereas the result was positive in 77.4%. The most common pathogenic germs were *Staphylococcus aureus* (52.3%) and *Staphylococcus epidermidis* (9.5%); 34/5% of the positive lesions received antibiotic treatment. Finally, among the lesions with a 75-100% improvement rate, no significant difference was observed between the antibiotic-treated and -untreated groups (36.1% vs. 63.9%, respectively, P = 0.403). **Conclusions:** The most common pathogen was *S. aureus* and, as a primary outcome, the simultaneous treatment for microbial agents did not have any considerable effect on the improvement rate of CL lesions.

Key Words: *Acute cutaneous leishmaniasis, aerobe, anaerobe, antibiotic, bacterial colonization*

What was known?
Colonization of aerobic and anaerobic microbial agents on cutaneous leishmaniasis (CL) lesions has shown controversial results on the lesions' healing process with the use of antibiotics.

Introduction

Cutaneous leishmaniasis (CL) is a chronic disease that is endemic in many parts of the world, but 90% of CL cases live in six countries, including Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria.[1] According to the World Health Organization reports, 12 million people are affected with this condition worldwide, and around 350 million are estimated to be under high risk of contamination.[2] CL is usually treated with systemic and intra-lesional antimonial compounds that have many side-effects.[3]

Colonization of microbial agents (aerobic and anaerobic) on CL lesions, especially acute erosive ulcerative ones, has been investigated in previous studies.[4-10] There are also several reports on the positive role of antibiotic therapy of colonized bacterial leishmaniasis lesions leading to higher rates of improvement.[7,8] It is to note that no specific protocol is available for approaching clinically suspicious CL lesions or those with positive laboratory results on smear or culture. On the other hand, most clinicians encounter this situation during clinical practice. Regarding the newly published references on chronic wound management, there is a difference between the clinical diagnosis of infection in acute and chronic wounds.[11]

As the Khorasan state in North East of Iran is a well-known endemic area for CL, especially due to *L. tropica*, and no such study has yet been performed in this area, and by considering CL as a chronic wound, we aimed at assessing the bacterial species (aerobic and anaerobic) colonized on acute CL lesions in this region and the effect of their elimination by antibiotic therapy on the lesions' healing course.

Access this article online	
Quick Response Code:	Website: www.e-ijd.org
	DOI: 10.4103/0019-5154.152560

Materials and Methods

This interventional clinical trial was performed on 84 acute CL patients who were referred to the Dermatology

Clinic of Qaem Hospital in Mashhad, Iran, over a 2-year period from 2009 to 2011. Diagnosis was initially made clinically and then confirmed through parasitological studies by direct skin–slit smear stained with Giemsa. Inclusion criteria consisted of acute CL lesions with less than 1 year duration, with either crust, erosion or ulcer on the surface and having indication for topical treatment. Patients with a history of recent antibiotic therapy or concurrent use of topical medications with antibiotic or antiseptic effects were excluded from the study. The study goals were explained to all patients and an informed consent was obtained from each participant. The study protocol was reviewed and approved by the Ethics Committee of the Research Council of Mashhad University of Medical Sciences. After enrolment, all data were recorded into a well-designed questionnaire and laboratory tests (smear and culture) were performed in the microbiology lab of Qaem Educational Hospital as per the protocol outlined below.

Sampling procedures

After cleaning the surface of the lesion and the surrounding areas with normal saline solution, appropriate samples were taken from the base of the ulcerative lesions.

Bacterial identification

Microscopic evaluations of the direct skin smears were performed. Aerobic and anaerobic cultivation for bacteria were done by inoculation on appropriate culture media. Two plates were inoculated for each specimen and then incubated at 36°C for 48 h and 96 h aerobically and anaerobically, respectively. Primary characterization of isolates was based on the microscopic Gram stain examination and also on the morphological and cultural characteristics of the colonies.

Bacterial genus and species were identified by standard identification testing according to certain guidelines.[12] The antibiotic susceptibility profile of isolates was studied according to the CLSI guidelines.[13]

Treatment

Patients with positive culture results for bacteria were randomly divided into two groups by a computer-based randomization program to compare the effect of antibiotic therapy on the lesion's improvement rate. Both groups received the same anti-leishmania treatment protocol as intralesional glucantime (Glucantime; Aventis, France) was injected into each lesion once a week to the point when the lesion's surface became fully infiltrated and up to a maximum dose of 2 mL, whereas only one group was treated with oral cephalexin 40-50 mg/kg/day for 10 days.

Follow-up and outcome

Clinical evaluations were performed weekly during the treatment course up to the end of the course or the occurrence of complete improvement. The evaluations were performed by a single clinician for all patients. The final evaluation was performed at the end of the treatment course. At each visit, the clinical response was determined on the basis of the following criteria:

1. Significant improvement (decrease in induration size between 75% and 100%)
2. Moderate improvement (decrease in induration size between 50% and 75%)
3. Partial improvement (decrease in induration size between 25% and 50%)
4. No improvement (decrease in induration size < 25%).

Statistical analysis

The collected data were analysed using SPSS software package, version 11.5, and Chi-square test, Mann–Whitney test and *t*-test. In all these statistical tests, a $P < 0.05$ was considered as statistically significant.

Results

Figure 1 shows the flowchart of participants based on the CONSORT 2010 guidelines. In this study, 84 patients with confirmed CL were enrolled. During the study period, seven patients in the intralesional glucantime group and three patients in the intralesional glucantime plus antibiotic group were excluded due to taking another therapeutic method simultaneously or losing access for further follow-up. The patients' mean age was 28.2 years, ranging from 16 months up to 70 years.

The demographic characteristics of patients are summarized in Table 1.

The mean age in the positive-culture and negative-culture groups were 30.51 ± 21 and 20.31 ± 17.2, respectively.

Table 1: Demographic characteristics of the studied population

Characteristic	Frequency (%)
Sex	
Male	45 (53.6)
Female	39 (46.4)
Age/years (mean±SD)	28.20±20.67
Lesions' location	
Head and neck	21 (25)
Upper extremity	38 (45.2)
Lower extremity	17 (20.2)
Upper+lower ext.	4 (4.8)
Head and neck+upper ext.	3 (3.6)
Head and neck+upper+lower ext.	1 (1.2)
Duration of lesions/months (mean±SD)	5.45±3.22
Lesions' duration classification (month)	
<6	58 (69.0)
7-12	26 (31.0)

SD: Standard deviation

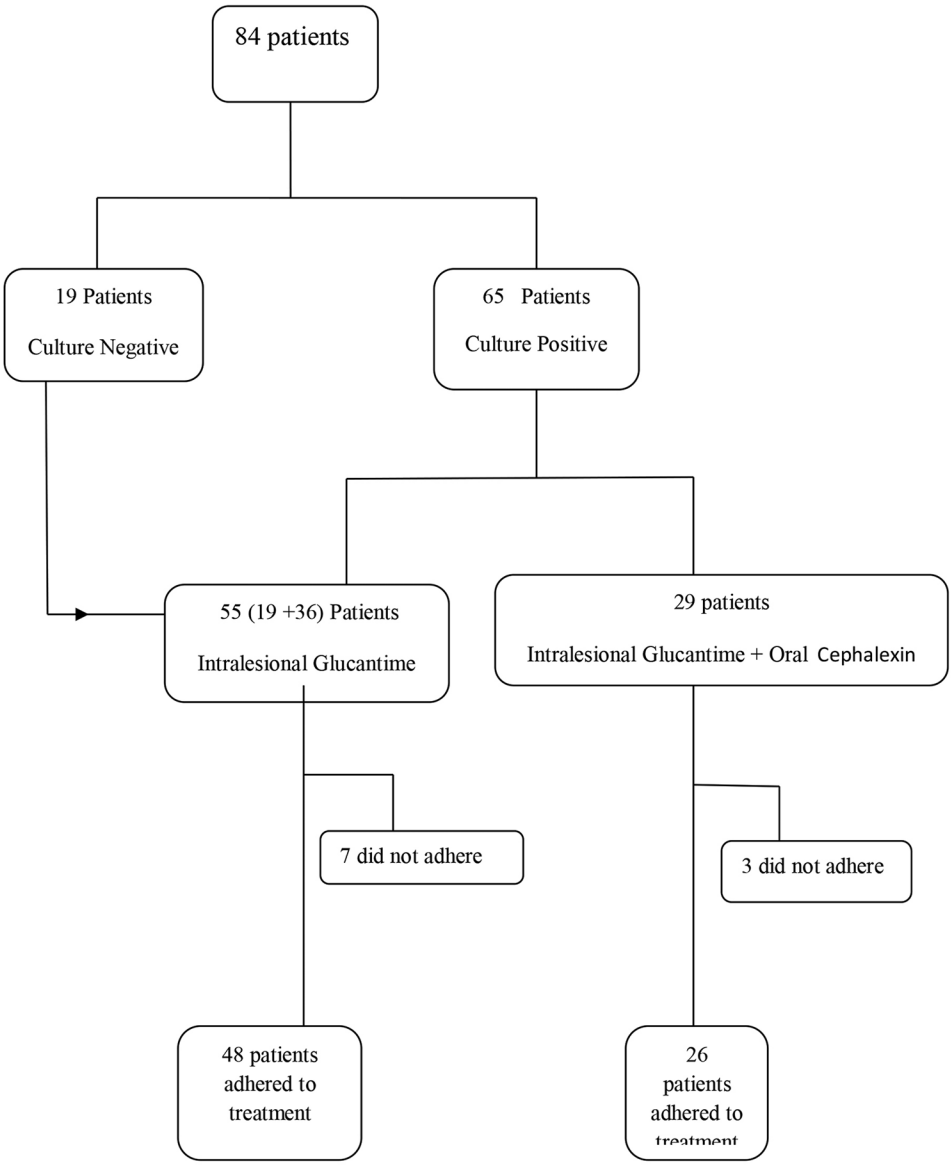


Figure 1: Flow chart of the studied cases

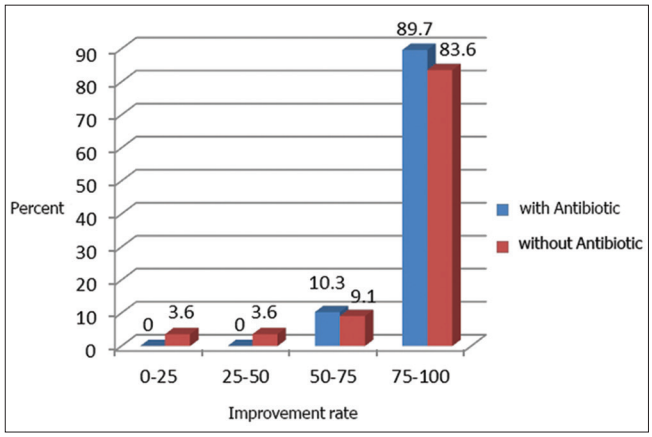


Figure 2: Correlation between lesions' location and culture results

Table 2: Frequency distribution of pathogenic bacteria isolated from cutaneous leishmaniasis lesions of the studied cases

Bacteria	Frequency	Percent
Staphylococcus aureus	44	52.3
Staphylococcus epidermidis	8	9.5
Beta *haemolytic streptococcus* (group A)	3	3.6
Escherichia coli	2	2.4
Coagulase negative *staphylococcus*	2	2.4
S. aureus + *S. epidermidis*	2	2.4
Streptococcus viridans	1	1.2
E. coli + *Enterococcus*	1	1.2
E. coli + *S. epidermidis*	1	1.2
E. coli + *S. aureus*	1	1.2

No significant relationship was found between the age and sex of the patients and the culture results (P = 0.058 and P = 0.437, respectively).

Considering microbial culture results, among the 84 cases, 19 (22.6%) had a negative result while the result was positive in the other 65 (77.4%); Table 2 shows the types of bacteria isolated from the studied lesions.

Figure 2 shows the correlation between lesions' location and culture results; based on the Chi-square test, a significant correlation was seen between them (P = 0.002), showing that lesions located on the upper extremities have a higher rate of culture positivity.

The average time duration of lesions in the positive-culture group was 6.7 ± 8.2 months; this was 4.8 ± 3.2 in the negative-culture group and there was no meaningful correlation between them (P = 0.274).

Eventually, no significant difference was revealed between the groups with or without antibiotic therapy and the lesions' improvement rate (P = 0.403).

Discussion

Secondary bacterial infections are one of the major complications of CL. Although some authors emphasize on the rarity of this finding, our clinical trial showed contrary results. Secondary bacterial infections can prolong the disease duration, increase tissue destruction and the resulting scar.[14] Several studies in Iran and elsewhere have been performed on the rate of secondary bacterial contamination in CL lesions where, in some studies, the improvement rate has been evaluated with the application of antibiotic therapy. The results have been controversial.[7,8,10,14-16]

Glucantime is regarded as the first-line treatment for CL. In a study by Sadeghian *et al*. in 2011, the therapeutic effect of glucantime showed a decrease in CL lesions with secondary bacterial infection (P < 0.01). Therefore, they concluded that in the cases of unresponsiveness to treatment, the lesions should be evaluated for bacterial infection before repeating the treatment.[17]

In a study by Ziaei *et al*. in 2008 among the 1255 confirmed CL patients, 274 (21.8%) had positive cultures for secondary bacterial infection. The bacteria isolated from these lesions were *Staphylococcus aureus* in 190 cases (69.3%), coagulase-negative Staphylococcus in 63 cases (23.0%), *Escherichia coli* in 3.6%, *Proteus* sp. in 2.2% and *Klebsiella* sp. in 1.9%. The results show an overall incidence of 21.8% for secondary bacterial infection. This incidence was significantly higher in ulcerated lesions compared with non-ulcerated ones (P = 0.00001).[7,14] In this study, the use of a local antiseptic solution has been suggested as a prophylactic factor for secondary bacterial infection, and, in those already infected, the administration of antibiotics, mainly anti-*S. aureus*, has been recommended as the best acceptable choice.[7]

Secondary bacterial infection was reported in 42% of the cases studied by Alsamarai in Iraq. He also emphasized that this infection may influence the natural course of the disease causing more destruction for the skin.[4]

In another study by Shirazi *et al*. in Iran, bacteriological experiments showed 47 cases (55.9%) with lesions infected by bacterial infections. The most prevalent bacterial isolates included group D *Streptococcus* (19.1%), *Enterococcus* spp. (19.1%) and *S. aureus* (12.7%).[5]

In the study by Edrissian *et al*., it was revealed that bacterial infections should be considered in the diagnosis and treatment of suspected CL lesions and that erythromycin could be a good choice for treating bacterial infected leishmaniasis lesions.[8]

Regarding the high prevalence of CL in our region and the need for a reliable approach toward such cases, the present study was performed.

In the Vera *et al*. study, 54.2% of the studied cases had a positive culture, it was more frequent in lesions located below the knee. *S. aureus* was the predominant species (89%). In this study, the healing process of the CL lesions, evaluated 1 month after finishing treatment, was not influenced by secondary bacterial infection, which is in consensus with our findings.[10]

Fontes *et al*. also found a 67.7% rate of bacterial infection mostly due to *S. aureus* (95.2%). They suggested to highly consider secondary bacterial infection, especially *S. aureus*, in the diagnosis and treatment of American Tegumentary leishmaniasis.[9]

In an investigation undertaken by Isaaq-Marquezand *et al*. in Mexico, the results indicated the need to eliminate bacterial purulent infections by antibiotic treatment before antimonial administration to CL patients infected with *L. mexicana*.[15]

In a study by Van Der *et al*. on the prevention of complications due to early antibiotic therapy, it was stated that open lesions are more prone to secondary bacterial infection and, due to the probability of tissue destruction and other complications, early treatment is highly recommended. Based on this study, there is no evidence on the best time and route of antibiotic administration. Most physicians suspect secondary bacterial infection based on the clinical manifestations and often add antibiotics (especially anti-gram positive bacteria) to anti-leishmania treatment.[16]

In our study, among the 84 studied cases, 65 (77.4%) had a positive culture result indicative of secondary bacterial infection. The most common germs were *S. aureus* and *Staphylococcus epidermidis*. It was found that

the simultaneous treatment of CL lesions with antibiotics did not have any significant effect on the improvement rate of these lesions (P = 0.403).

The current study, besides several other similar studies, introduced *S. aureus* as the most common germ isolated from CL lesions. This finding is well justified by considering the colonization of *S. aureus* on healthy skin.

The colonization of other microbial germs like gram negative agents on the lesions could be due to secondary contamination because of their location, which has been reported more commonly in previous studies in comparison to ours.

Acknowledgments

The authors wish to thank the Research Council of Mashhad University of Medical Sciences for supporting this study, which was a medical student's thesis (No. 6397) financially. Also, Dr. M.T. Shakeri is highly acknowledged for his kind assistance in data analysis.

What is new?
The simultaneous treatment of cutaneous leishmaniasis (CL) lesions with antibiotics does not have any significant effect on the improvement rate of such lesions.

References

1. Klaus SN, Frankenburg S, Ingber A. Epidemiology of cutaneous leishmaniasis. Clin Dermatol 1999;17:257-60.
2. Layegh P, Yazdanpanah MJ, Vosugh EM, Pezeshkpoor F, Shakeri MT, Moghiman T. Efficacy of azithromycin versus systemic meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. Am J Trop Med Hyg 2007;77:99-101.
3. Layegh P, Rahsepar S, Rahsepar AA. Systemic meglumine antimoniate in acute cutaneous leishmaniasis: Children versus adults. Am J Trop Med Hyg 2011;84:539-42.
4. AlSamarai AM, AlObaidi HS. Cutaneous leishmaniasis in Iraq. J Infect Dev Ctries 2009;3:123-9.
5. Shirazi MH, Ranjbar R, Asgari V, Mohebali M, Hamidian M. Study of bacterial infections among the patients with suspected cutaneous leishmaniasis. Pak J Biol Sci 2007;10:4555-8.
6. Gonçalves Eda G, Reis Filho SA, Oliveira EG, Pareira AL, Silva AR, Costa JM. Bacterial infection in cutaneous leishmaniasis: Bacterial pattern and sensitivity to antibiotics. Rev Soc Bras Med Trop 2009;42:219-21.
7. Ziaei H, Sadeghian G, Hejazi SH. Distribution frequency of pathogenic bacteria isolated from cutaneus leishmaniasis lesions. Korean J Parasitol 2008;46:191-3.
8. Edrissian GH, Mohammadi M, Kanani A, Afshar A, Hafezi R, Ghorbani M, *et al*. Bacterial infections in suspected cutaneous leishmaniasis lesions. Bull World Health Organ 1990;68:473-7.
9. Fontes CO, Carvalho MA, Nicoli JR, Hamdan JS, Mayrink W, Genaro O, *et al*. Identification and antimicrobial susceptibility of micro-organisms recovered from cutaneous lesions of human American tegumentary leishmaniasis in Minas Gerais, Brazil. J Med Microbiol 2005;54:1071-6.
10. Vera LA, Santos JB, Macêdo VO, de Magalhães AV, Ciuffo IA, Santos CG. Evaluation of secondary bacterial infection's influence on the course of cutaneous leishmaniasis in Corte de Pedra, Bahia. Rev Soc Bras Med Trop 2001;34:233-7.
11. Dow G. Infection in chronic wounds. In: Krasner DL, Rodeheaver GT, Sibbald RG, eds. Chronic Wound Care: A Clinical Source Book for Healthcare Professionals, 3rd ed. Wayne: HMP Communications; 2001:343-56.
12. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby: 2007.
13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. Cls document M100-S19. Wayne, Pa: CLSI; 2009.
14. Ziaie H, Sadeghian G. Isolation of bacteria causing secondary bacterial infection in the lesions of cutaneous leishmaniasis. Indian J Dermatol 2008;53:129-31.
15. Isaac-Márquez AP, Lezama-Dávila CM. Detection of pathogenic bacteria in skin lesions of patients with chiclero's ulcer. Reluctant response to antimonial treatment. Mem Inst Oswaldo Cruz 2003;98:1093-5.
16. Van Der Vliet D, Le Guern AS, Freitag S, Gounod N, Therby A, Darie H, *et al*. Pseudomonas aeruginosa otochondritis complicating localized cutaneous leishmaniasis: Prevention of mutilation by early antibiotic therapy. Am J Trop Med Hyg 2006;75:270-2.
17. Sadeghian G, Ziaei H, Bidabadi LS, Baghbaderani AZ. Decreased effect of glucantime in cutaneous leishmaniasis complicated with secondary bacterial infection. Indian J Dermatol 2011;56:37-9.

How to cite this article: Layegh P, Ghazvini K, Moghiman T, Hadian F, Zabolinejad N, Pezeshkpour F. Bacterial contamination in cutaneous leishmaniasis: Its effect on the lesions' healing course. Indian J Dermatol 2015;60:211.

Received: September 2012. **Accepted:** December 2012.
Source of support: Nil, **Conflict of Interest:** Nil.