Original Article

Intralesional Streptomycin: New, Safe, and Effective Therapeutic Option for Cutaneous Leishmaniasis

Wisam Majeed Kattoof

Department of Dermatology, College of Medicine, Mustansiriyah University, Baghdad, Iraq

Abstract

Background: Leishmaniasis encompasses a spectrum of chronic infections in humans in which organisms are found within phagolysosomes of mononuclear phagocytes. Cutaneous leishmaniasis is divided into Old World and New World cutaneous leishmaniasis caused by *Leishmania tropica*. Aminoglycosides as it antibacterial, were also tried as a new therapeutic option for parasitic infestation. Proliferation rate and protein synthesis in the promastigote stage of the parasite are inhibited by aminoglycoside such as streptomycin. **Aim:** The aim of this study is to estimate the effectiveness of intralesional streptomycin as a new antileishmanial agent. **Settings and Design:** This was a therapeutic trial with controls**. Materials and Methods:** Sixty three lesions (57.8%) from a total of 109 lesions were treated with intralesional injection of streptomycin after dilution with distilled water (20%) and 46 lesions (42.2%) were used as a control. **Statistical Analysis:** Statistical Analysis System program and Chi-square test were used. **Results:** In Group A, clinical cure was achieved in 40 lesions (83.3%). Moderate response was noticed in 8 lesions (16.7%). In Group B, none of the remaining 39 control lesions shows moderate, marked or total clearance degree of response. Only 3 lesions (7.7%) showed a slight degree of response and 1 lesion (2.7%) with mild degree. **Conclusion:** Intralesional streptomycin solution 20% can be considered as a new effective therapeutic option for cutaneous leishmaniasis.

Keywords: Cutaneous leishmaniasis, intralesional injection, streptomycin

Introduction

Leishmania is a protozoan parasite that produces the known tropical disease "leishmaniasis" when infecting human macrophages. The approximate incidence is up to 1.2 million cases of the cutaneous form and in visceral form ranges between 0.2 million and 0.4 million. Till the current time, chemotherapy is the only proved option to treat leishmaniasis, but its efficacy is greatly limited because resistance gets larger (especially antimonials) side effects frequently develop and it price is high.[1]

Female Phlebotomus sand fly bite transmits intracellular protozoa which leads to the development of leishmaniasis. As sand fly can egest >1000 parasites per bite, one bite from infected sand fly is enough to bring on the disease. Susceptibility to Leishmanias is increased sharply, especially with high proportion of poverty and malnutrition. In endemic areas, children are put on the line greater than adults.[2]

Within several weeks or months after exposure particularly on the exposed parts of the body (mostly the face, arms, and legs), cutaneous leishmaniasis will present as open or closed sores. Over time, the size and appearance of sores can change. They typically present as small papules which progress to nodular plaques and oftentimes lead to open sores with central ulcer and raised border, with scales or crust on the surface. The lesions can be painful (especially if open sores become infected with bacteria) but usually are painless. Regional lymphadenopathy and satellite lesions can be marked some times. The sores (even without treatment) usually heal in time, but this can last for months or years and scarring is the end result.[3]

Diagnosis was based on the clinical manifestations in endemic areas. The organism in the amastigote form can be visualized by the aspiration of fluid from the ulcer. Molecular techniques (polymerase chain reaction, DNA hybridization, and antigen detection) may be done. A Montenegro test which

Address for correspondence: Dr. Wisam Majeed Kattoof, College of Medicine, Mustansiriyah University, Baghdad, Iraq.
E-mail: wissammajeed200@yahoo.com

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/MJ.MJ_11_18

How to cite this article: Kattoof WM. Intralesional streptomycin: New, safe, and effective therapeutic option for cutaneous leishmaniasis. Mustansiriya Med J 2018;17:42-6.

can done by injecting the dead promastigotes intradermally can signal the infection, while blood tests are not ready to give help.[4]

Lesions of cutaneous Leishmanias were classified as simple or complex (multiple, >4 cm in size, if there is lymphatic spread, lesions located over joints or in cosmetic areas, >6-month duration, and in immunosuppressed cases). Options of treatment differ regarding the lesion being simple or complex, simple one usually resolves spontaneously without need for but this can end with scarring. Topical therapy includes imiquimod and topical paromomycin. Cryotherapy and heat and photodynamic therapy are used with good cure rate. Intralesionally, lesion of cutaneous leishmaniasis can be treated with the antimonials (sodium stibogluconate). Another option is the nonantimonial therapies (system in amphotericin B, miltefosine, pentamidine, antifungal drugs [azoles], zinc sulfate, and allopurinol). Sodium stibogluconate and meglumine antimoniate (intravenous or intramuscular) are the cornerstone of treatment for complex lesions of cutaneous leishmaniasis.[5,6]

Antibacterial agents are commonly added to the culture of Leishmania to reduce the risk of bacterial contamination (often penicillin and aminoglycoside such as streptomycin or gentamicin). Despite the widespread use of antibacterial agents, little is known about the degree to which different concentrations might inhibit the growth of Leishmania. To determine the effect of commonly used antibiotics on the growth of *Leishmania donovani*, T. R. Navin and R. D. Pearson tested different concentrations of penicillin, cefamandole, gentamicin, streptomycin, tobramycin, and amikacin. No growth inhibition was observed when *L. donovani* amastigotes were allowed to convert to promastigotes and grow in the presence of penicillin, cefamandole, and amikacin. In contrast, streptomycin and tobramycin both inhibited growth at concentration above 10 µg/ml. Newly converted promastigotes show a similar pattern of growth inhibition as that of amastigotes, and streptomycin inhibited growth by 63%.[7]

Several aminoglycoside antibiotics which were used mainly to treat bacterial infections have also been tried as antiparasitic agents. "Among these aminoglycosides, paromomycin has shown antileishmanial activity."[8-12] Paromomycin (topically as ointments for cutaneous leishmaniasis or as intramuscular injections for visceral type) has been used successfully.[8,13-15] The selective activities of these antibiotics might be on bacterial ribosomes; this leads to the inhibition of their dissociation into subunits, and this will end with concomitant decrease of protein synthesis[11] and/or mistranslation errors by the specific interaction with the decoding sites of small rRNA.[16,17] The bactericidal effect of aminoglycosides has been proposed to explain through a multistep model (antibiotic uptake, mistranslation by chain-elongating ribosomes, membrane damage, and subsequent ribosomal blockade preventing further protein synthesis).[18] This model might also be related to the paromomycin action on Leishmania as the good targets for it are parasite ribosomes.[11] Proliferation rate and protein synthesis in the promastigote stage of the parasite are inhibited to a large extent by paromomycin while being slightly less affected by other aminoglycosides such as streptomycin.[19]

The aim of this study is to estimate the effectiveness of intralesional streptomycin as new, effective, safe, and costless antileishmanial agent.

Materials and Methods

This therapeutic trial with controls was accomplished in AL-Yarmouk Teaching Hospital in Baghdad Province from the period of November 2016 to May 2017. Forty-six patients with cutaneous leishmaniasis presented to the dermatology departments were involved in this study. The diagnosis of each case depends on the clinical examination and the suspicious cases deal with it by skin smear with Giemsa stain to prove the diagnosis.

Inclusion criteria include any patient presented with cutaneous leishmaniasis regardless of age and sex, lesions <5 cm in diameter and <5 in numbers, and searching for treatment for the first time. Exclusion criteria include history of hypersensitivity to aminoglycosides, pregnancy, patients who received an immunosuppressive therapy in the last month, any possible herbal or home remedies therapy, recent vaccination, patients with liver problems, and if the lesions lie near the eyes or on the ears and nose.

A well-designed and informative questionnaire was prepared for this study, and information about the age, sex, and residence as usual was included. The questionnaire contains details about the site, number, size, duration, and type of lesion (weather dry or ulcerative). Furthermore, there is a field for the follow-up records with date of each visit and for the possible side effects. The drug which tested as a new therapeutic agent for cutaneous leishmaniasis is streptomycin as streptomycin sulfate BP equivalent to one-gram streptomycin base from Troge Medical GMBH, Hamburg, Germany. The powder diluted with 5 ml of distilled water to reach a concentration of 20%, which can be considered as the base for the first trial with such antibiotic. It is injected intradermally using needle (30G) at 45° angle to the surface of lesion and 0.5 ml of the solution injected per each cm^2. The intralesional streptomycin was given once a week for a maximum of four sessions and followed up for another 4 weeks.

Lesions have been divided into two groups: Group A lesions injected with intralesional streptomycin and Group B (control) in which we leave one lesion without intervention from each case. For assessment of the end result after completing the four sessions, we used the following criteria.[20]

- Slight: Decrease in indurations and erythema of the lesion
- Mild: Decrease in size of up to 30%
- Moderate: Reduction in the size (30%–60%)
- Marked: Reduction in the size >60%.

Total clearance of the lesion and both marked + total clearance were considered to be a clinical cure.[20] Photography was done for most of the lesions. The diameter of each lesion was measured by a calibrated tape.

Possible side effects (pain on injection anecrosis, hyperpigmentation) are recorded and the degree of scarring also size up by the adherent dermatologists. This study was ethically approved by Iraqi Council for Medical Specializations. Each patient was fully informed about this new therapeutic agent and a spoken rather than written agreement to be the case was obtained. In the Statistical Analysis for this study, Chi-square test was used for different parameters.

Results

In total, 46 patients with cutaneous leishmaniasis enlist in this therapeutic trial with 109 lesions, of which 63 lesions (57.8%) were treated with intralesional injection of streptomycin in Group A and 46 lesions (42.2%) were used as a control in Group B. Table 1 shows in detail the demographic and clinical facets for the patients.

Regarding the characteristics of the lesions, 26 patients (56.5%) presented with the dry type and 20 patients (43.5%) with the ulcerative type; their size ranges from 1 cm to ≤4 cm with mean 2.7 cm ± 0.97; 32 patients (69.6%) have two lesions (13 patients [40.6%] with the ulcerative type and 19 patients [59.4%] with the dry type), 11 patients (23.9%) have three lesions (four patients [36.4%] with the ulcerative type and seven patients [63.6%] with the dry type), and three patients (6.5%) have four lesions (all with ulcerative type); the presenting site for each lesion varies as the patient may have more than one site of involvement as explained in Table 2.

All the patients in Group A received the intralesional injection of streptomycin in the first visit (63 lesions: 33 lesions [52.4%] of the dry type and 30 lesions [47.6%]), after which 7 patients escape from the regimen with a total number of 22 lesions (7 lesions from the control Group B and 15 lesions from Group A [2 dry lesions and 13 ulcerative lesions]). At the end of the designed regimen, 39 patients with a total of 48 lesions (31 dry lesions [64.6%] and 17 ulcerative lesions [35.4%]) complete the four sessions.

Clinical response was assessed a week after the fourth session, and all lesions in Group A show a degree of response; clinical cure was achieved in 40 lesions (83.3%) (28 dry lesions [58.3%] and 12 ulcerative lesions [25%]) and this is clearly shown in Figures 1 and 2. Moderate response was noticed in eight lesions (16.7%) (five ulcerative lesions [10.4%] and three dry lesions [6.3%]), and Figure 3 shows this response, and all of the above and remaining results appear in detail in Table 3.

In Group B, none of the remaining 39 control lesions shows moderate, marked, or total clearance degree of response. Only three lesions (7.7%) showed a slight degree of response and one lesion (2.7%) with mild degree as showed in Table 4.

Table 1: The demographic plus clinical facets of patients

Demographic and clinical facets	*n* (%)
Sex	
Male	21 (45.7)
Female	25 (54.3)
Residence	
Urban	28 (60.9)
Rural	18 (39.1)
Duration (mean weeks±SD)	5.3±2.3
Mean years of age±SD	21.9±6.39

SD: Standard deviation

Table 2: Frequency of distribution for the lesions

Location of lesions	Number of lesions (%)
Upper limb	43 (39.4)
Lower limb	28 (25.7)
Hands	18 (16.5)
Feet	11 (10.1)
Face	9 (8.3)
Total	109 (100)

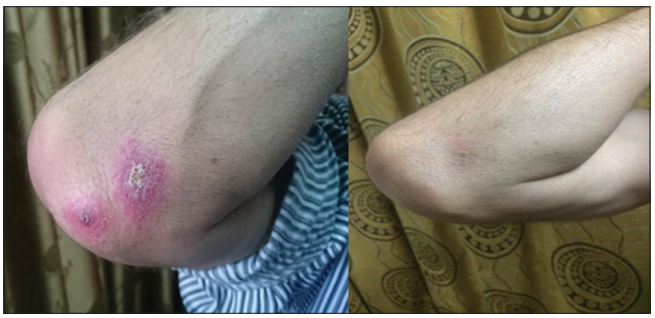

Figure 1: Fifty-two years old male with two lesions (total clearance)

Side effects to intralesional streptomycin that we report: <20% of patients complain of mild degree of pain at the time of injection, this temporary; edema in the first session (4.2%); 34 lesions (70.8%) of the treated lesions expose a noticeable grade of hyperpigmentation, and this usually resolved with time. None of the local side effects (injection pain or edema) interrupt the therapy. Regarding the end result of scarring at the end of the 8th week, the patients were satisfied with the four sessions as minimal or no scarring can be seen at the end of the regimen. No systemic side effects from using streptomycin solution intralesionally were observed through the whole period of study.

Discussion

No universally accepted therapeutic regimen is available for cutaneous leishmaniasis, and it remains a therapeutic and public health challenge.[21,22] Cutaneous leishmaniasis is a self-healing disease, but this may take several months; however, many therapeutic agents were used to prevent or minimize scarring or to shorten the duration of healing.[20]

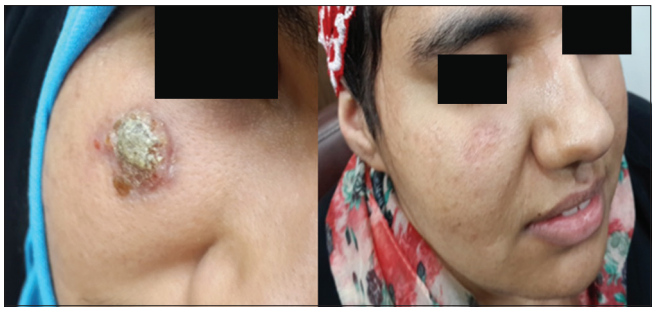

Figure 2: Eighteen years old female with single lesion (total clearance with minimal scarring)

Table 3: The response grade of control lesions of cutaneous leishmaniasis regarding their types and numbers

Grade of response	Ulcerative lesions, *n* (%)	Dry lesions, *n* (%)	Total, *n* (%)	χ^2
No response	13 (33.3)	22 (56.4)	35 (89.7)	8.41**
Slight	1 (2.6)	2 (5.1)	3 (7.7)	1.07 (NS)
Mild	0	1 (2.6)	1 (2.6)	0.89 (NS)
Moderate	0	0	0	0.00 (NS)
Marked	0	0	0	0.00 (NS)
Total clearance	0	0	0	0.00 (NS)
Total	14 (35.9)	25 (64.1)	39 (100)	9.26**
χ^2	9.83**	11.37**	14.05**	-

**P<0.01. NS: Nonsignificant

Table 4: The response grade of treated lesions of cutaneous leishmaniasis regarding their types and numbers

Grade of response	Ulcerative lesions, *n* (%)	Dry lesions, *n* (%)	Total, *n* (%)	χ^2
No response	0	0	0	0.00 (NS)
Slight	0	0	0	0.00 (NS)
Mild	0	0	0	0.00 (NS)
Moderate	5 (10.4)	3 (6.3)	8 (16.7)	1.84 (NS)
Marked	8 (16.7)	11 (22.9)	19 (39.6)	2.52 (NS)
Total clearance	4 (8.3)	17 (35.4)	21 (43.7)	8.61**
Total	17 (35.4)	31 (64.6)	48 (100)	9.17**
χ^2	6.46**	9.74**	9.31**	-

**P<0.01. NS: Nonsignificant

The demographic data of this study reveal that the age group most commonly infested with leishmaniasis are adults with mean age 21.9 years ± 6.39, and this compatible with an epidemiological survey for cutaneous leishmaniasis in the Mediterranean region people younger than 10 years were less affected than older ones.[23] No big difference was observed in the frequency of male-to-female involvement. Patients from urban areas in comparison to rural areas were attending and were registered into this therapeutic trial with a high prevalence.

The species of Leishmania, host immune responses and vector virulence factors determine the clinical outcome of infection, resulting in visceral leishmaniasis, mucocutaneous or cutaneous.[24] Set up on the factors mentioned above, clinically

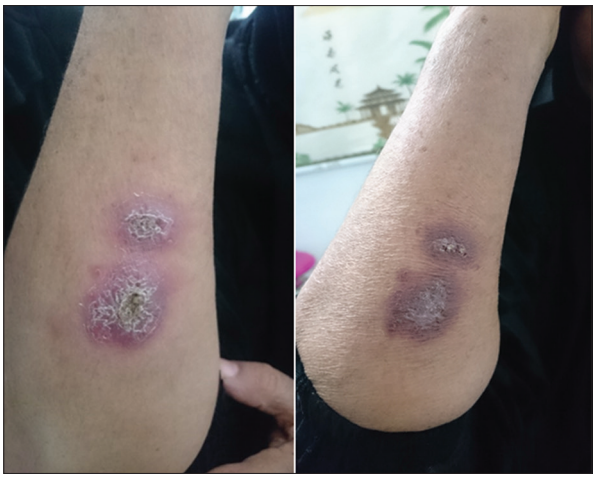

Figure 3: Sixty-eight years female with multiple lesions on the upper and lower extremities (marked response with noticeable degree of hyperpigmentation)

lesions enrolled in this trial appear with higher percentage for the dry type of cutaneous leishmaniasis (60.9%) than ulcerative type (39.1%). Regarding the site of involvement, the frequently involved site was upper limbs (39.4%); this is predictable as it is usually exposed during the summer months; the face being on the bottom (8.3%) attributed to the exclusion criteria for lesions nears the ears, eyes, and on the nose.

Iraq, considered to be one of the endemic areas with cutaneous leishmaniasis and because the antimonial drugs are so expensive, this intended us to search for a new option of antileishmanial agents, better given intralesionally as a first therapeutic trial to avoid the systemic side effects which may result if we had to use it in a high dose. Intralesional streptomycin was selected for this trial in patients with cutaneous leishmaniasis for many reasons; First of all, aminoglycosides such as paromomycin markedly inhibit proliferation rate and protein synthesis in the promastigote stage of the parasite while being slightly less affected by other aminoglycosides such as streptomycin.[19] High level of drug will be supplied at the site of lesion by the intralesional route; from the view of cost, this can be reduced manifold.

The uppermost observation from this study is that clinical cure was accomplished in 83.3% of the lesions injected intralesionally with 20% streptomycin solution weekly for a maximum of 4 weeks; none of the patients in total who involved in this trial showed slight, mild, or no response at all.

Host immune responses to Leishmania infection dictate prognosis, and the clinical outcome is dependent on the immune response (T-helper-1 or T-helper-2 responses). Therefore, immunotherapeutic interventions are potential.[25] Based on this fact, clinical cure rate for the dry lesions was a cut above that for ulcerative lesions (dry lesions [58.3%] and ulcerative lesions [25%]).

It is groundbreaking that our first trial with this drug appears with such high cure rate, and this can be close to other studies

planned to use intralesional route for many chemicals and drugs (100% cure rate in 12 cases treated with chloroquine,[26] rifamycin with a success rate of 92.7% in 25 patients,[27] ciprofloxacin 0.2% [81.5%],[28] and metronidazole 5% [87%]).[29] Side effects in this study are limited such as mild pain at the time of injection, slight edema which soon disappears, and the most eminent hyperpigmentation, and this appears frequently in many intralesional therapeutic trials such as chloroquine[26] and rifamycin.[27] About the end result of healing, minimal or no scar at all in the whole study sample.

Conclusion

Regarding aspects of efficacy, economy, and safety for injecting the drug intralesionally, streptomycin solution 20% is used as new therapeutic option for skin lesions of leishmaniasis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, *et al.* Leishmaniasis worldwide and global estimates of its incidence. PLoS One 2012;7:e35671.
2. World Health Organization. Leishmaniasis: The Disease and its Epidemiology. Nairobi, Kenya: World Health Organization; 2014.
3. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, *et al.* Diagnosis and treatment of leishmaniasis: Clinical practice guidelines by the infectious diseases society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis 2016;63:e202-64.
4. Markell and Voges. "Leishmaniasis." Medical Parasitology. 9th ed. Elsevier Inc.; 2006. p. 246-7.
5. Markle WH, Makhoul K. Cutaneous leishmaniasis: Recognition and treatment. Am Fam Physician 2004;69:1455-60.
6. Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H, *et al.* Diffuse and disseminated cutaneous leishmaniasis: Clinical cases experienced in Ecuador and a brief review. Trop Med Health 2016;44:2.
7. Navin TR, Pearson RD. Inhibition of leishmania donovani growth by streptomycin and tobramycin. Ann Trop Med Parasitol 1987;81:731-3.
8. Bryceson AD, Murphy A, Moody AH. Treatment of 'old world' cutaneous leishmaniasis with aminosidine ointment: Results of an open study in London. Trans R Soc Trop Med Hyg 1994;88:226-8.
9. Ei-On J, Jacobs GP, Weinrauch L. Topical chemotherapy of cutaneous leishmaniasis. Parasitol Today 1988;4:76-81.
10. Maarouf M, Lawrence F, Brown S, Robert-Gero M. Biochemical alterations in paromomycin-treated leishmania donovani promastigotes. Parasitol Res 1997;83:198-202.
11. Maarouf M, Lawrence F, Croft SL, Robert-Gero M. Ribosomes of leishmania are a target for the aminoglycosides. Parasitol Res 1995;81:421-5.
12. Mishra J, Saxena A, Singh S. Chemotherapy of leishmaniasis: Past, present and future. Curr Med Chem 2007;14:1153-69.
13. Ogle JM, Carter AP, Ramakrishnan V. Insights into the decoding mechanism from recent ribosome structures. Trends Biochem Sci 2003;28:259-66.
14. Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. N Engl J Med 2007;356:2571-81.
15. Thakur CP, Olliaro P, Gothoskar S, Bhowmick S, Choudhury BK, Prasad S, *et al.* Treatment of visceral leishmaniasis (Kala-Azar) with aminosidine (= paromomycin)-antimonial combinations, a pilot study in Bihar, India. Trans R Soc Trop Med Hyg 1992;86:615-6.
16. Fourmy D, Recht MI, Blanchard SC, Puglisi JD. Structure of the A site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. Science 1996;274:1367-71.
17. Wimberly BT, Brodersen DE, Clemons WM Jr., Morgan-Warren RJ, Carter AP, Vonrhein C, *et al.* Structure of the 30S ribosomal subunit. Nature 2000;407:327-39.
18. Davis BD, Chen LL, Tai PC. Misread protein creates membrane channels: An essential step in the bactericidal action of aminoglycosides. Proc Natl Acad Sci U S A 1986;83:6164-8.
19. Fernández MM, Malchiodi EL, Algranati ID. Differential effects of paromomycin on ribosomes of leishmania Mexicana and mammalian cells. Antimicrob Agents Chemother 2011;55:86-93.
20. Sharquie KE, Al-Talib KK, Chu AC. Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony. Br J Dermatol 1988;119:53-7.
21. Herwaldt BL. Leishmaniasis. Lancet 1999;354:1191-9.
22. Berman JD. Human leishmaniasis: Clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin Infect Dis 1997;24:684-703.
23. Abdellatif MZ, El-Mabrouk K, Ewis AA. An epidemiological study of cutaneous leishmaniasis in Al-Jabal Al-Gharbi, Libya. Korean J Parasitol 2013;51:75-84.
24. World Health Organization. Leishmaniasis. Eastern Africa: World Health Organization; 16 January, 2010.
25. Ameen M. Cutaneous leishmaniasis: Advances in disease pathogenesis, diagnostics and therapeutics. Clin Exp Dermatol 2010;35:699-705.
26. Noor SM, Khan MM, Hussain D. Intralesional chloroquine in cutaneous leishmaniasis. J Pak Assoc Dermatol 2005;15:18-21.
27. Al-Sudany NK, Ali YJ. Intralesional 8.33% rifamycin infiltration; new treatment for cutaneous leishmaniasis. J Dermatol Dermatol Surg 2016;20:39-45.
28. Al Hamdi K, Awad AH, Moker HM. Evaluation of intralesional 0.2% ciprofloxacin as a treatment for cutaneous leishmaniasis. East Mediterr Health J 2010;16:89-93.
29. Al-Waiz M, Sharquie KE, Al-Assir M. Treatment of cutaneous leishmaniasis by intralesional metronidazole. Saudi Med J 2004;25:1512-3.