



INVESTIGATING PHARMACEUTICAL AND THERAPEUTIC PRESCRIPTIONS OF CUTANEOUS LEISHMANIASIS IN DEHLORAN CITY DURING 2015

SOMAYEH SHAHSAVARI¹, RAZI NASERIFAR², EBRAHIM FATOLLAHI², MAHMOUD RAFIEIAN-KOPAEI^{3*}

¹*Biotechnology and Medicinal Plants Research Center, Ilam University of Medical Sciences, Ilam, Iran*

²*Ilam University of Medical Sciences, Ilam, Iran*

³*Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

ABSTRACT

Leishmania is parasite protozoan of blood- tissue flagellates that causes the leishmaniasis disease. Pharmaceutical and therapeutic methods are used for the treatment of leishmaniasis. The use of pentavalent antimony drugs, cryosurgery, cryotherapy are therapeutic methods of leishmaniasis. In this study, we investigate patients with suspected cutaneous leishmaniasis who referred to the leishmaniasis treatment center of Dehloran city (Ilam province, Iran) during April 2015 to March 2016 and are treated with different medical checks. In this study, 410 patients with suspected leishmaniasis lesion were enrolled to the study which referred to the leishmaniasis treatment center of Dehloran city during April 2015 to March 2016. Suspected people were enrolled to the study after sampling and Giemsa staining and confirmation of leishmaniasis lesion. Patients were investigated after filling the forms and consent for follow-up treatment. The analysis of the results showed that 15.9%, 14.1%, 64.7% and 2.2% of patients are treated with systemic antimony, topical antimony, cryotherapy and topical antimony with cryotherapy, respectively and 0.4% of patients were under blank treatment and 2.7% of patients were treated with other leishmaniasis therapeutic methods. The used treatments for leishmaniasis depend on the severity, location, number and so on. Given that in 64.7% of patients, cryotherapy was performed alone so it is known that this type of treatment was a priority in 2015 because of the lack of Glucantim drug vials. Given that the subjects had leishmaniasis lesions in sensitive and non-sensitive points of body, they were exposed to different treatment regimes. Cooperation of patient with physician and patience until the end of the treatment process was another effective factor affecting the success of treatment in the study.

KEYWORDS: *Parasitic diseases, Leishmaniasis, Treatment, Dehloran, Iran*

INTRODUCTION

Leishmania is parasite protozoan of the blood-tissue flagellates¹. Leishmaniasis disease is one of the six important tropical diseases emphasized by the World Health Organization²⁻⁴. Leishmaniasis is a zoonotic disease and created as three forms of skin lesions, visceral and mucosal-skin⁵. Leishmaniasis causes lesions in the skin and unfortunately scar remains after recovery⁶. Leishmaniasis disease is traditionally known in Iran and in the ancient books of Iran including Avicenna law, a lesion is referred as *Khayronieh* that is long live ulcer and its treatment was difficult

and it was resistant against different drugs. It is thought that this is a leishmania lesion due to the mentioned signs and symptoms⁷. The disease vectors in the urban cutaneous leishmaniasis is a farm sand fly called Sergenti Phlebotomus that transfers parasite from human to human. In the rural cutaneous leishmaniasis, the main vector is semi-wild sand fly called Papatasi Phlebotomus that transfers the parasite of pathogen from some rats to the humans⁸. Although nearly twenty thousand cases of cutaneous leishmaniasis are reported in Iran but probably the actual cases are more than 4 to 5 times of that. Cutaneous leishmaniasis is seen as rural and urban type in

Iran. The rural type is common in many rural areas of 15 provinces of country and urban type exists in many urban areas as endemic including Bam city⁹⁻¹³. It has been reported in provinces like Ilam, Yazd, and Isfahan to a large extent¹⁴. Sudan, Saudi Arabia, Iran, Brazil, Afghanistan, Peru, Bolivia are incidence focus of leishmaniasis¹⁵. The statistics show that the annual deaths due to the leishmaniasis are about 59,000 people¹⁶. Pharmaceutical and Therapeutic methods are used for leishmania treatment. The use of pentavalent antimony drugs, cryosurgery, cryotherapy are therapeutic methods of leishmaniasis¹⁷⁻²³. In this study, we investigate patients with suspected cutaneous leishmaniasis who referred to the leishmaniasis treatment center of Dehloran city (Ilam province, Iran) during April 2015 to March 2016 and are treated with different medical checks.

MATERIALS AND METHODS

People in the study

In this study was done during April to March 2014 on 410 patients who had active lesions suspected to leishmaniasis, referred to the cutaneous leishmaniasis treatment center of Dehloran city (CLTCDC). After filling the form and informed consent of patients for follow-up treatment were studied.

Sampling of suspected cutaneous leishmaniasis

Sampling was performed by Shirzadi (2012) method. For this purpose, suspected cases with completed patient-finding forms were sent to the leishmaniasis laboratory of city. In the lab, specimens were taken from different parts of the cutaneous lesions. Some samples were taken from various wounds from patients who had multiple lesions. From multiple and large wounds (≥ 3 cm) three smears were prepared from each sample. The inflamed and swollen sides of skin lesions are the most important parts with the highest density of amastigote. The important point is that with more samples taken from the tissue, it is more likely to have parasite in the sample. Since skin lesions might have secondary bacterial or fungal infections, it was necessary to clean the location of the lesion where we wanted to take sample. In most cases we changed the alcohol cotton several times²⁴.

Staining samples with Giemsa

Giemsa staining was performed by Shirzadi (2012) method. For this purpose, First smear was prepared from samples and the samples were allowed to be dried without the use of flame at room temperature. Then it was poured on the 70 °C methanol slide for

30 to 60 seconds. Giemsa stain with a PH of 7.2 for 30 to 50 minutes was added to the dried slides. Then slides shortly in water with PH around 7.2 were rinsed and the slides were dried. Finally, slides were observed with the lens 10.40 and 100²⁴.

Treatment type

Intralesional meglumine treatment

Intralesional meglumine treatment was performed by Shirzadi (2012) method. For this purpose, the location of the lesion was disinfected with iodine or alcohol cotton. With a fine needle No. 27 or 30 with an angle of 45 degrees as the tip of the needle was toward the center of the lesion, at the border of healthy skin and induration beginning it was injected into the lesion sideline. 0.1 ml of the drug was injected in the dermis so that the lesion margin became white and this operation was repeated at intervals of 1 cm in total lesion margins in whole environment of the lesion. In large lesions if the center of the lesion was not injured, 0.1 to 0.2 ml of drug was injected in the center of the lesion²⁴.

Cryotherapy method

Cryotherapy was performed by Shirzadi (2012) method. For cryotherapy, a sufficient amount of liquid nitrogen was poured in disposable cups. Cotton swab was kept for several seconds in liquid nitrogen to be completely impregnated with nitrogen, then it was quickly placed on the lesion and for 10 seconds was pressed on lesion, so that the lesion became white. This action was repeated on total surface of lesions until the lesion became white and up to 2 mm margin of healthy skin around the lesion. This method is superior to Cryospray²⁴.

Systemic therapy

Systemic therapy was performed by Shirzadi (2012) method. For systemic therapy, If the lesion was in the face, or the number of lesions was greater than 5, or more than 3 cm in diameter, Aspvrvt Qiu, the joint damage and also in recurrence or treatment failure, systemic therapy was used²⁴. Systemic therapy was carried out with intramuscular injection of meglumine. The recommended amount was administered based on the daily net antimony for the systemic treatment of 20 mg pentavalent antimony per kg body weight, equivalent to 75 mg of meglumine on a daily basis in rural leishmaniasis. Systemic therapy was applied for 2 weeks and systemic therapy was prescribed for 3 weeks in urban leishmaniasis. If after 4 weeks after completion of systemic or topical therapy no signs of recovery were observed

in the lesion, systemic therapy was again administered with the previous dose as treatment failure. If there was no response to the second systemic therapy (clinical resistance) the patient was referred to a dermatologist. Observing the lesion dressing has always been necessary until complete remission²⁴.

Other treatments

Other treatments, if necessary, were combination of the above methods.

RESULTS

The analysis of results showed that 15.9%, 14.1%, 64.7% and 2.2% of patients are treated with systemic antimony, topical antimony, cryotherapy and topical antimony with cryotherapy, respectively and 0.4% of patients were under blank treatment and 2.7% of patients were treated with other leishmaniasis therapeutic methods. Further results of treatment type, number of treatment in each group and the percentage of treatment in each group are shown in table 1.

Table 1
Type of treatment, number of treatment in each group and the percentage of treatment in each group

Type of treatment	Number of treatment in each group.	The percentage of treatment in each group
Systemic antimoan	65	%15.9
Local antimoan	58	%14.1
local antimoan associated with cryotherapy	9	%2.2
Other treatment	11	%2.7
Cryotherapy alone	265	%64.7
Blank	2	%0.4

DISCUSSION

According to the reports of Disease Control Center, the number of patients with different types of Leishmaniasis is annually 20 thousand people in the country that have a high incidence and prevalence²⁵. Many factors such as the type of urban and rural disease, the number of wounds, scars on the body of patients are effective in the selection of treatment methods of Glucantime administration by systemic and local methods, cryotherapy and so on²⁶. The results showed that 15.9%, 14.1%, 64.7% and 2.2% of patients are treated with systemic antimony, topical antimony, cryotherapy and topical antimony with cryotherapy, respectively. The results obtained by Saghabipour et al (2014) showed that the effectiveness of therapeutic method together cryotherapy with glucantime intralesional injection is more than singly glucantime intralesional injection²⁷. In Alkhawajah et al (1997) study, the efficiency of intralesional therapeutic method with pentavalent antimony compounds was reported as 68 to 100 percent²⁸. Depending on the circumstances, there are physical and surgical treatments²⁹. Physical and non-injectable methods are used due to the problems of injectable drugs, limited injection facilities in rural areas and

tendency of patients to use non-injectable treatment methods³⁰. Iran's Ministry of Health put the use of cryotherapy treatment for leishmaniasis on its agenda. In some cases, this method is used once every two weeks in treating patients who have an indication of local glucantime injection³¹. Cryotherapy is a physical method of cutaneous leishmaniasis treatment because leishmania parasite is sensitive to cold³¹. The results of Bassiouny et al (1982) study showed that cryotherapy causes the treatment of cutaneous leishmaniasis during 4 to 5 weeks³². Leishmania parasite is sensitive to cold because it decomposes Leishmania parasite in the dermis connective tissue less than one hour in terms of histology. Also, cryotherapy can cause tissue damage; rupture of the macrophages membrane and the release of their Leishman object within the tissue and probably increases the presentation of antigen to the immune system^{33,34}. The results of Gurei et al (2000) study conducted in Turkey showed that performing the cryotherapy method for three month improved 78 percent of ulcer lesions³⁵. Also, the study conducted by Asiliyan et al in Isfahan showed that 57.3% of patients were treated³⁶ while in Saudi; the efficiency of cryotherapy method was reported 30 percent³⁷. The studies showed that intralesional glucantime injection has fewer side effects than systemic injection^{38,39}. One

of the main reasons for failure of treatment in the local glucantime injection is inadequate infiltration of drug in the lesion^{40,41}. The results of study by Naserifar and colleagues (2017) showed in Dehloran city during 2014 in 38.1% of patients treated with anti-o systemic, 38.3% of patients treated with systemic antimony, 15% of patients with cryotherapy alone, 8.1% of patients treated with anti-local antimony associated with cryotherapy and 1% from other therapies, respectively for treatment of cutaneous leishmaniasis⁴². Probably one of the reasons for using 64.7% of cryotherapy is the emphasis of Iran Health Ministry on its use and its effective treatment mechanism that Leishmania is sensitive to cold and will be disappeared.

REFERENCES

1. Ardehali S, Rzaii R, Nadim A. The leishmania parasites and leishmaniasis. 1st ed. Tehran: Tehran University Press 1994; 1-25.
2. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M; WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. *PloS one* 2012; 7(5): 35671.
3. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comparative immunology, microbiology and infectious diseases* 2004; 27(5): 305-318.
4. Grimaldi Jr, Gabriel, Robert B. Tesh, and Diane McMahon-Pratt. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. *The American journal of tropical medicine and hygiene* 1989; 41(6): 687-725.
5. Berman J. Recent developments in leishmaniasis: epidemiology, diagnosis, and treatment. *Current Infectious disease reports* 2005; 7(1): 33-38.
6. Postigo JA. Leishmaniasis in the World Health Organization Eastern Mediterranean Region, Elsevier B.V., 1:S62-5.
7. Alvajhi A. A. Cutaneous leishmaniasis of the Old World. *Skin Therapy* 2003; 8(2): 1-4.
8. WHO technical report series 949, Control of the leishmaniasis, World Health Organization, 36-104.
9. Mohebali M. Visceral leishmaniasis in Iran: review of the epidemiological and clinical features. *Iranian journal of parasitology* 2013; 8(3): 348.
10. Momeni AZ and Aminjavaheri M. Clinical picture of cutaneous leishmaniasis in Isfahan, Iran. *International journal of dermatology* 1994; 33(4):260-265.
11. Nadim A and Faghih M. The epidemiology of cutaneous leishmaniasis in the Isfahan province of Iran: I. The reservoir II. The human disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1968; 62(4): 534-542.
12. Javadian E, Nadim A, Tahvildare-Bidruni G, Assefi V.. Epidemiology of cutaneous leishmaniasis in Iran: B. Khorassan Part V: Report on a focus of zoonotic cutaneous leishmaniasis in Esferayen. *Bulletin de la Societe de Pathologie Exotique et de ses Filiales* 1976; 69(2): 140.
13. Daneshbod KH. Visceral leishmaniasis (kala-azar) in Iran: a pathologic and electron microscopic study. *American journal of clinical pathology* 1972; 57(2): 156-166.
14. Ministry of Health and Medical Education. Epidemiologic status of Leishmaniasis in 1998. Tehran: Ministry of Health and Medical Education, Department of Health Affairs, Ministry of Health and Disease Control 1378; 1057.
15. Soufizade A. A Study on Vectors and Reservoirs of Cutaneous Leishmaniasis Using Molecular Methods in Kalaleh City Focus in Order to Providing Preventive Programs. Thesis for Master Degree of Health Sciences in Medical Entomology and Vector Controlling, Faculty of Health, Tehran: Tehran University of Medical Sciences; 2007; 99 100.

CONCLUSION

Given that the subjects had leishmaniasis lesions in sensitive and non-sensitive points of body, they were exposed to different treatment regimes. Cooperation of patient with physician and patience until the end of the treatment process was another effective factor affecting the success of treatment in the study.

CONFLICT OF INTEREST

Conflict of interest declared none.

16. Yaghoobi-Ershadi MR, Javadian E. Epidemiological Study on Sandflies in a Hyperendemic Focus of Zoonotic Cutaneous Leishmaniosis in Iran. *Indian J Med Res* 1997; 105:61-66.
17. Davidson, Robert N. Practical guide for the treatment of leishmaniasis. *Drugs* 1998; 56(6): 1009-1018.
18. Amer M. Treatment of leishmaniasis. *Zoonotic and parasitic diseases: proceedings of the Third International and Pan-Arab Seminar held in Amman, Jordan, 17-20 Oct. 1989.* IDRC, Ottawa, ON, CA, 1990.
19. El-On J, et al. Topical treatment of cutaneous leishmaniasis. *Journal of investigative dermatology* 1986; 87(2): 284-288.
20. Arevalo, Iracema, et al. Successful treatment of drug-resistant cutaneous leishmaniasis in humans by use of imiquimod, an immunomodulator. *Clinical Infectious Diseases* 2001; 33(11): 1847-1851.
21. Dorlo, Thomas PC, et al. Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis. *Journal of Antimicrobial Chemotherapy* 2012; 67(11): 2576-2597.
22. Bassiouny A, et al. Cryosurgery in cutaneous leishmaniasis. *British journal of dermatology* 1982; 107(4): 467-474.
23. Leibovici, Veka, and Homayoun Aram. Cryotherapy in acute cutaneous leishmaniasis. *International journal of dermatology* 1986; 25(7): 473-475.
24. Care guide of cutaneous leishmaniasis in Iran. Shirzadi MR: Ministry of Health and Medical Education, Department of Health, Center for Infectious Disease Management, Office of diseases transmitted between humans and animals, 2012; 4-114.
25. Islamic Republic of Iran Ministry of Health & Medical Education. Principles of disease prevention and surveillance. Tehran: Center for disease control 2008: 249-255.
26. Ministry of Health and Medical Education (I.R.Iran) center for Disease Management. Principles of leishmaniasis prevention & surveillance. Tehran 2007: 32-36.
27. Saghafipour A, Rassi Y, Norooze M. Comparison of Efficacy of Intercessional Injection of Glucantime and Cryotherapy with Intralesional Injection in the Treatment of Zoonotic Cutaneous leishmaniasis:a Randomized Clinical Trial. *Knowledge & Health* 2013; 8(2):62-66.
28. Alkhawajah AM, Larbi E, Al-Gindan Y, Abahussein A, Jain S. Treatment of cutaneous leishmaniasis with antimony:intramuscular versus intralesional administration. *Ann Trop Med Parasitol* 1997; 91(8):899-905.
29. Herbert MG. *Protozoal Diseases.* London:Oxford University Press; 1999:1-281.
30. Morsy TA, Abdel Rahman EG, Ahmed MM. Treatment of cutaneous leishmaniasis with Pentostam or cryosurgery. *J Egypt Soc Parasitol.* 1989; 19(2):533-43.
31. Al-Gindan Y, Kubba R, Omer AH, el-Hassan AM. Cryosurgery in old world cutaneous leishmaniasis. *Br J Dermatol* 1988; 118(6):851-4.
32. Bassiouny A, El Meshad M, Talaat M, Kutty K, Metawaa B. Cryosurgery in cutaneous leishmaniasis. *Br J Dermatol* 1982; 107(4):467-474.
33. Bassiouny A, El Meshad M, Talaat M, Kutty K, Metawaa B. Cryosurgery in cutaneous leishmaniasis. *Br J Dermatol* 1982; 107(4):467-74.
34. Leibovici V, Aram H. Cryotherapy in acute cutaneous leishmaniasis. *Int J Dermatol* 1986; 25(7):473-5.
35. Gurei MS, Tatli N, Ozbilge H, Erel O, Seyrek A, Kocigit A, et al. Efficacy of cryotherapy and intralesional pentostam in treatment of cutaneous leishmaniasis. *J Egypt Soc Parasitol* 2000; 30(1):169-76.
36. Asilian A, Momeni A, Faghihi G, Sadeghi V, Sadeghi M, Sadeghi H. A comparison between cryotherapy alone and combined with intralesional Glucantime injection in the treatment of papulonodular Leishmaniasis. *J Dermatol* 2002;6(2):30-33.
37. Moskowitz PF, Kurban AK. Treatment of cutaneous leishmaniasis:retrospectives and advances for the 21st century. *Clin Dermatol* 1999; 17(3):305-315.
38. Faris RM, Jarallah JS, Khoja TA, al-Yamani MJ. Intralesional treatment of cutaneous leishmaniasis with sodium stibogluconate antimony. *Int J Dermatol* 1993; 32(8): 610-2.
39. Kellum RE. Treatment of cutaneous leishmaniasis with an intralesional antimonial drug (Pentostam). *J Am Acad Dermatol* 1986; 15(4 Pt 1): 620-2.
40. Minodier P, Parola P. Cutaneous leishmaniasis treatment. *Travel Med Infect Dis* 2007; 5(3): 150-8.
41. Flraig MJ, Rupec J, Ruzickat, Rupec RA. Topical treatment of persistent cutaneous

leishmaniasis with paromomycin. *Hautarzt* 2007; 58(8): 689-90, 692.

42. Naserifar R, Bahmani M, Abdi J, Adineh SH, Nourmohammadi GHA, Sahraei E, Kaidkhordeh M, Mirshekari M and Rafieian-
Kopaei M. The process of effectiveness and the treatment of cutaneous leishmaniasis in Dehloran city, Iran during 2014. *International Journal of Advanced Biotechnology and Research* 2017; 8(2): 1344-1348.