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The Evaluation of Intralesional Glucantime and Cryotherapy plus Intralesional Glucantime Therapeutic Efficacy on Zoonotic Cutaneous Leishmaniasis: A Randomized Clinical Trial

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Abstract

Background: Currently, Glucantime is considered the first-line treatment of cutaneous Leishmaniasis. This study aimed to evaluation of intralesional Glucantime (IG) and Cryotherapy (Cryo) plus intralesional Glucantime (Cryo and IG) therapeutic efficacy on Zoonotic Cutaneous Leishmaniasis in Qom province, Iran.

Materials and Methods: In this randomized controlled trial of 197 CL patients reported from Qom health centers, Iran during (2014-2017), 112 cases were entered to this study. According to the Iranian Ministry of Health therapeutic guide line of CL, 54 cases with 116 lesions received IG weekly, and 58 patients with 115 lesions received the IG once a week coupled with Cryo using liquid nitrogen once every two weeks. The average volume of injected solution per lesion was 0.2 ml to 0.5 ml into the border of the healthy skin and the onset of erythema in the lesions using insulin syringe needles. In second therapeutic method; cryotherapy with liquid nitrogen was performed by dipstick technique. At first, the lesion size and induration of lesions were recorded. After 7 and 12 weeks of treatment lesions healing rate was monitored. Healing was defined as complete re-epithelialization and disappearance of induration.

Results: Out of 112 CL patients, 54 cases with 116 lesions received IG, and 58 patients with 115 lesions received Cryo and IG. At 7th week after the initiation of treatment, complete healing was observed in 56 out of 116 lesions (48.1%) in the group which received weekly IG and 83 out of 115 lesions (72.2%) in the group which received biweekly Cryo plus IG. At 12th week after the treatment, the cure rate was 91% and 100% in group which received weekly IG and group which received IG once a week coupled with cryotherapy (p=0.001).

Conclusion: Based on the findings, IG once a week coupled with cryotherapy was more effective compared with weekly IG and reduced the period of treatment.

Key Words: Cutaneous Leishmaniasis, Cryotherapy, Intralesional Glucantime.

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1- INTRODUCTION

Leishmaniasis is a parasitic disease transmitted by some species of sand flies (1). It is estimated that in 98 countries, mostly developing ones, approximately 350 million people are at risk of acquiring leishmaniasis, 12 million are infected and the new cases are estimated to be 2 million annually. Two most common clinical forms of the disease Cutaneous Leishmaniasis (CL), and Visceral Leishmaniasis (VL) are mainly observed in 14 of the 22 countries of Eastern Mediterranean Regional Office (EMRO) region including Iran (2). Self-healing Zoonotic CL (ZCL) due to *L. major* and Anthroponotic CL (ACL) due to *L. tropica* are two known types of CL which spread out in the most parts of the country (3). Totally, about 17 out of 30 provinces of the country are endemic foci of ZCL. According to the official reports of Ministry of Health and Education of Iran, the average incidence rate of the CL is usually between 20 to 40 cases per 1,000,000; however, the disease prevalence is increasing and new foci of CL are emerging in Iran (4).

Currently, antimony compounds; Glucantime (Meglumine antimoniate) and Pentostam (Sodium stibogluconate) is used in many countries for the treatment of CL patients (5). In Iran, the Glucantime drug as intralesional and systemic is using for treatment of leishmaniosis. According to the guideline of Centers for Disease Control and Prevention (CDC) office, Ministry of Health and Education of Iran, identified indicators for the systemic treatment with Glucantime are included: cutaneous lesions on the face of patients, having ≥ 5 skin lesions, the diameter of skin lesions > 3 cm, sporotrichoid forms of disease, having lesions on the joints and relapse or failure to the treatment. Otherwise, the local treatment with intralesional Glucantime (IG) is used for treatment of CL patients. Local treatment using IG to complete recovery of the patient or the maximum for 12 weeks is prescribed (6). Due to problems of injectable anti-Leishmania drugs, being limitations in facilities to Glucantime injection in rural areas and the willingness of patients to receive the non-injection treatment strategies, some scientific studies are continuing to achieve effective, therapeutic and inexpensive methods with minimal side effects to this disease (7). Scientists have been placed under investigation therapeutic efficacy of various drugs such as Azithromycin, Paromomycin, Amphotericin B, and Trichloroacetic acid (TCA) on CL, and have reached different findings (8). Alavi et al. indicate that therapeutic effects of azithromycin on cutaneous leishmaniasis were 46.4% (9). Mostaghim et al. reported the therapeutic efficacy of Paromomycin plus Gentamicin on CL patients was 86% (10). Also, Haftbaradaran et al. studied the therapeutic effects of 5% TCA cream in treatment of CL, found that recovery rate of 6.3%, 86.7% and 100 % in the second, seven and eight weeks of treatment period was obtained respectively; they indicate at the end of eight weeks after treatment all patients had recovered (11).

Up to now, some studies about the efficacy of cryotherapy in the treatment of CL have been performed in Old World foci of CL (7, 12-14). Recently use of cryotherapy also was on the agenda of the Ministry of Health. In the cases that have indication for receiving IG once a week, they should receive cryotherapy using liquid nitrogen once every two weeks and for at most three months additionally (15). There are some foci of ZCL in rural areas of the Qom province. Every year a number of patients referred to regional Health Centers for treatment (16, 17). This randomized controlled trial (TCTR20171120004), was done to evaluate therapeutic efficacy of intralesional Glucantime and Cryotherapy plus intralesional Glucantime on Zoonotic

Cutaneous leishmaniasis in Qom province, central Iran. This study can monitor the impact of each of the two therapeutic methods on the field and persuade the policy makers of the Ministry of Health to continue to prescribe or eliminate the method that does not effect on treatment of leishmaniasis.

2- MATERIALS AND METHODS

2-1. Study design and population

The Qom province (Iran) is bounded by Tehran province in the North, Esfahan province in the South, Semnan province in the East, and Markazi province in the West with an area of approximately 11,240 square kilometers (0.68% of total area of Iran) (18). This study was performed in all endemic foci of CL in Qom province during March 2014 to March 2017. All suspected patients with skin lesions (212 cases), who referred to regional health centers of Qom province, they were examined for CL using clinical and parasitological tests. Any skin lesions consistent with the clinical symptoms of CL (one or more painless papule that change to nodule with a central crust.

The papules and nodules get bigger, can develop central ulceration, and take approximately one year to heal without therapeutic intervention) which lasts more than 14 days should be classified as suspected cases. After screening, samples of suspected cases of CL were taken from the borders of the suspected lesion (s), fixed with methanol and stained with Geimsa and examined under the microscope. The disease was diagnosed based on clinical examination and microscopic observation of the intracellular amastigotes of the parasite in the Geimsa stained smear (19). In skin lesions of 197 cases leishman body were observed. Out of 197 cases of CL referring to health centers, 112 cases that had inclusion criteria for entering this study were selected and entered to the present study.

2-2. Methods

This study was randomized controlled trial. According to the Iranian Ministry of Health therapeutic guide line of CL, 112 patients were entered to study as two groups and the intervention was as follows: 54 cases with 116 lesions received IG weekly and 58 patients with 115 lesions received the IG once a week coupled with Cryo using liquid nitrogen once every two weeks and for at about three months (12 weeks) (20). Glucantime® is prepared as a 5ml injection solution; 300mg/ml injectable solution that contains 425mg of pentavalent antimony in 1.5gr of N-methylglucamine antimoniate (15). Glucantime intralesional injection method is included: for each patient, after wearing a gloves and disinfecting the lesion with alcohol or betadine, the following procedure was performed: the solution was injected using insulin syringe needles in adjacent intact skin of the lesions with 45-degree angle so that the lesions were indurated and white. The average volume of injected solution per lesion was 0.2ml to 0.5ml that injected into the border of the healthy skin and the onset of erythema in the lesions. This was repeated at 1cm intervals on the entire margin of the lesion throughout the lesion environment.

In this study, Glucantime injection was weekly done in the lesions by the expert general physicians in the health centers, and the probable side effects were evaluated by the expert medical entomologists. In second therapeutic method; cryotherapy with liquid nitrogen was performed by dipstick technique. It consists of application of saturated, cotton – tipped applicator on the lesion until 2-3mm halo forms around it. The freeze time ranges between 10 - 25 seconds. This procedure was repeated and followed once every two weeks and for at about three

months (12 weeks) (20). The dosage and type of drug for the treatment of patients is provided based on the national control guidelines for cutaneous leishmaniasis by Department of Zoonotic Diseases Control of Iran's Ministry of Health, and is ordered by a general physician and injected by a public health staff. Healing was defined as complete re-epithelialization and disappearance of induration.

2.3-Ethical consideration

Firstly, coordination and permission letter from department of prevention and diseases control of Qom provincial health center was taken. It should be mentioned that before the start of the study, the aim of the study was demonstrated and the target group were ensured about the confidentiality of their data. Meanwhile, 112 people were all those who their leishmania infection confirmed in Qom health centers during three years of study and voluntarily entered the study after completing the consent form.

2-4. Inclusion criteria

The inclusion criteria for the study were as follows: diagnosis of leishmaniasis based on the observation Leishman bodies in smears from skin lesions, the onset of the disease should be less than 4 weeks ago, completion of epidemiologic survey forms and having age 10 years or older (20).

2-5. Exclusion criteria

The exclusion criteria were included; taking any drugs; antibiotics or anti-Leishmania during the last 4 weeks, lactating and pregnant mothers, having large skin lesions with a diameter of 5 cm and ultimately the patients with lesions of more than three on their bodies.

2-6. Data Analyses

In two groups, the lesion size and induration of lesions were evaluated and recorded after 7 and 12 weeks, and treatment of lesions was monitored. Rehabilitated lesions were assessed using clinical examination and determination of the induration size and erythema in the lesions. The data was analyzed with Chi-square and Fisher's exact test. $P > 0.05$ was considered significant level. Analysis was conducted using SPSS software17.0 software.

3-RESULTS

Out of patients participating in this present study, 53.7% and 58.62% were males in two groups; one group: received IG weekly and other group: received the IG once a week coupled with Cryo using liquid nitrogen once every two weeks respectively. The mean age in this two groups were 21.6 ± 2.0 and 20.9 ± 4.0 years old, respectively. Chi-square test showed that there were no significant differences among the demographic variables such as gender and age groups in patients who received IG weekly in comparison of received the IG once a week coupled with Cryo group ($P>0.05$).

Some demographic and epidemiologic characteristics of patients in two groups were showed in **Table.1**. Out of a total of 197 CL patients 112 cases with *L. major* were studied. 54 cases with 116 lesions received IG and 58 patients with 115 lesions received Cryo and IG (**Figure.1**). Of the total number of people participate in this study, 49 cases (43.75%) were under the age of 18 years old. It means, 42.59% of cases who received IG and 44.83% of patients who 115 received Cryo and IG had under the age of 18 years old **(Table.1).** At 7th week after the initiation of treatment, complete healing was observed in 56 out of 116 lesions (48.1%) in the group which received weekly IG and 83 out of 115 lesions (72.2%) in the group which received biweekly Cryo and IG. At 12th week after the initiation of treatment, complete healing was observed in 105 out of 116 lesions (91%) in the group which

received weekly IG and all of 115 lesions (%100) in the group which IG once a week coupled with cryotherapy. By the end of 7th week, RR (0.95CI) amounted to 1.86 (1.1 – 3.15) and at the end of 12th week they tapped to 3.4 (2.23- 2.27) which were statistically significant (P<0.02). The significant difference was observed between the two groups in terms of complete healing rate (P<0.001) (**Table.2**).

Table-1: The demographic characteristics of patients participating in study of evaluation of Intralesional Glucantime and Cryotherapy plus Intralesional Glucantime Therapeutic Efficacy on Zoonotic Cutaneous leishmaniasis, Qom province, Iran (2014-2017)**.**

Characteristics	Categories	Type of treatment	
		Number (%) of patients who received Intralesional Glucantime	Number (%) of patients who received Intralesional Glucantime + Cryotherapy
Gender	Male	29 (53.7%)	34 (58.62%)
	Female	25 (46.3%)	24 (41.38%)
Age	<18 years old	23 (42.59%)	26 (44.83%)
	≥18 years old	31 (57.41)	32 (55.17%)
Mean age of patients	(Year)	20.9±19.47	20.1±19.42
Location of lesions	Hand	27 (50%)	30 (51.72%)
	Foot	22 (40.75%)	21 (36.24%)
	Face	1 (1.85%)	1 (1.72%)
	Head and neck	1 (1.85%)	2 (3.44%)
	Trunk	3 (5.55%)	4 (6.88%)
Mean number of lesions	(Number)	2.14±0.85	2.12±0.94
Type of lesions	Nodule	18 (33.34%)	17 (29.31%)
	Populous	31 (57.5%)	32 (55.17%)
	Ulcer	5 (9.25%)	9 (15.52%)
Mean size of lesions	(cm)	2.38±2.85	1.98±1.62

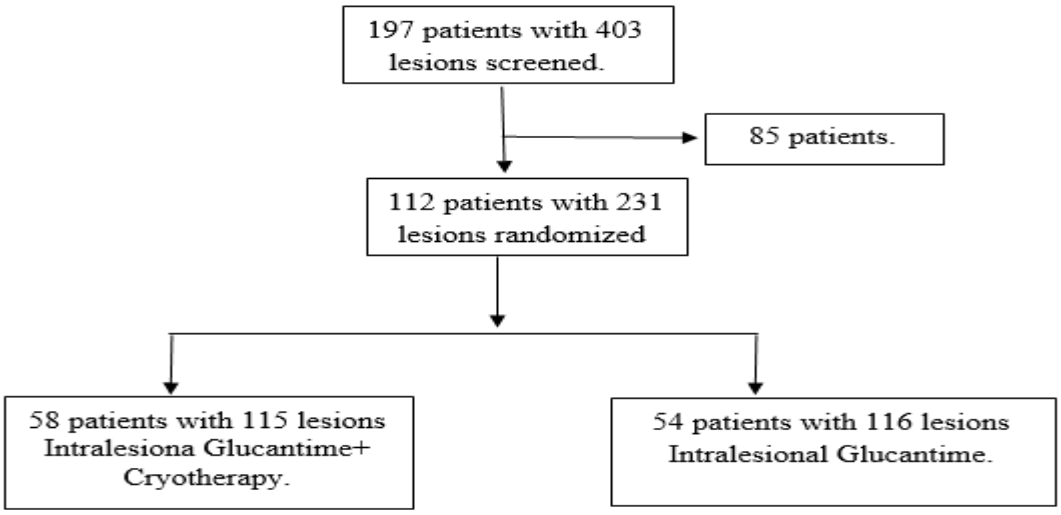

Fig.1: A total of 112 patients completed follow – up after two types of treatments; Intralesional Glucantime and Cryotherapy plus Intralesional Glucantime Therapeutic Efficacy on Zoonotic Cutaneous leishmaniasis, Qom province, Iran (2014-2017).

Table-2: Analysis of cure rate between two groups; Intralesional Glucantime and Cryotherapy plus Intralesional Glucantime Therapeutic Efficacy on Zoonotic Cutaneous Leishmaniasis, based on weeks of follow-up, Qom province, Iran (2014-2017).

Weeks of follow-up	After 7 weeks		After 12 weeks	
	Intralesional Glucantime	Intralesional Glucantime + Cryotherapy	Intralesional Glucantime	Intralesional Glucantime + Cryotherapy
Cure rate	Number (%)	Number (%)	Number (%)	Number (%)
Cured	56 (48.2%)	83 (72.2%)	105 (91%)	115 (100%)
Not cured	60 (51.8%)	32 (27.8%)	11 (9%)	0 (0%)
P-value	0.02		0.001	
Chi- square	5.41		11.45	

4- DISCUSSION

In this clinical trial study, Cryo and IG were compared with IG in the treatment of CL patients. In several studies (21, 22), the efficacy and effectiveness of many therapeutic methods for treatment of CL have been investigated in many foci of the disease in the world. According to the findings of present study, Cryo and IG was more effective in comparison of IG alone (more than 72% in versus 48% cure rate after 7 weeks of follow-up and 100% in versus 91% cure rate after 12 weeks of follow-up). This finding is concurrent with the results of studies on the therapeutic effect of IG by Alkhawajah et al. and Sharquie et al. (21, 22). Alkhawajah et al. and Sharquie et al. in the studies on the therapeutic effect of Glucantime observed that the cure rate of IG on CL patients was 68- 100% (21, 22). But in a similar study, Yazdanpanah et al. found that 37.1% of patients were treated with IG and 22.2% of patients were treated with cryotherapy clinically and they evaluated that cryotherapy and intralesional Glucantime have a same effect in terms of therapeutic effects (23). Also, Salmanpour et al. found that the cure rate of IG on CL was 72% (24). Other previous studies demonstrated that the therapeutic effect of IG was less than the findings of present study; for instance, the findings of a study in Isfahan city; central Iran have showed that cure rate of IG has been 41.7% (25). It is well known that cryotherapy is also a physical method for treating CL patients and it can be useful in the treatment of CL; because of leishmania parasite is susceptible to cold (15). According to the results of this study, cryotherapy was used with intralesional Glucantime injection simultaneously (Cryo and IG), and its cure rate was 100% after 12 weeks follow up. Up to now, this combination method has not been studied. Based on the results of Bassiouny et al. cryotherapy is one of the best strategies for treating of CL, they observed that 30 patients with CL were treated completely using cryotherapy after 4 - 5 weeks of treatment follow up (26), which this is in agreement with our findings of the present study that all of CL patients were cured. As demonstrated in this study, IG once a week coupled with cryotherapy once every two weeks and for at most three months were most effective strategies for curing of CL. Also, it was previously proven that cryotherapy alone was less effective in treatment of CL; the cure rate was 78 %, 57.3% and 30% in Turkey, Iran, and Saudi Arabia respectively (27-29). Regarding the cryotherapy, previous studies have shown

that this stimulus produces decreases in the local tissue temperature and metabolism (30), which results in cryonecrosis that destroys the amastigotes and activates an immune response produced by the liberation of antigenic substances (31).

4-1. Limitations of the study

This study was designed on number of CL patients referring to health center of Qom province. This makes the sample size was limited. In our knowledge this is can be one of the limitations of this the study.

5- CONCLUSION

According to the finding, to the best of our knowledge, Cryotherapy and intralesional Glucantime (the intralesional Glucantime once a week coupled with cryotherapy using liquid nitrogen once every two weeks and for at most three months) is the effective therapeutic strategy for treating Cutaneous Leishmaniasis patients.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

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