Comparison of Three Different Therapies for Cutaneous Leishmaniasis and Identification of the Etiologic Isolates in Isfahan, Iran

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Abstract

Background: In Iran, zoonotic and anthroponotic cutaneous leishmaniasis (CL) are caused by Leishmania major and L. tropica respectively. Despite extensive studies, no effective therapies have ever been reported for CL. The main objective of this research was to determine and compare the three different protocols for treatment of CL patients referring to Skin Diseases and Leishmaniasis Research Center (SDLRC), affiliated to Isfahan University of Medical Sciences, Isfahan, Iran from September 2017 to October 2018.

Methods: In a randomized controlled parallel groups clinical trial, 150 selected CL patients who met our inclusion criteria were randomly assigned to one of the three therapy groups: A, intra-lesional glucantime plus 50% trichloroacetic acid (TCA), B, intra-lesional glucantime and C, systemic glucantime. All patients in the three groups received the complete course of treatment and were followed for 6 months. To identify the etiologic agents, smears and PCR-RFLP were used after parasite culture. Also, clinical characteristics, history of previous involvement, endemic emigration and demographic data were collected.

Results: The results showed that the mean value of healing period was 53.12 ± 25.88 (median: 45, IQR: Q1 = 30-Q3 = 77) days in group A, 57.22 ± 44.02 (median: 42.5, IQR: Q1 = 30-Q3 = 60) days in group B, and 73.56 ± 41.08 (median: 71, IQR: Q1 = 45-Q3 = 90) days in group C; the observed differences were statistically significant (P = 0.024). There was a significant difference between group A and group C (P = 0.049), and between group B and group C (P = 0.047) in terms of mean healing period. Finally, complete recovery rates of 80%, 62% and 42% were shown in the three medicinal groups of A, B and C, respectively (P = 0.022).

Conclusion: In this study, the average duration of lesion healing among the three groups was the shortest in patients with IL glucantime plus 50% TCA treatment regimen. Also, the use of 50% TCA in patients suffering from CL was associated with a significant improvement in the depth of scars, the time and the percentage of recovery, and the low cost of this agent in the treatment of CL.

Keywords: Cutaneous leishmaniasis, Etiologic isolates, Identification, Isfahan, Therapy

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Introduction

Leishmaniasis, a sandfly-borne disease, is caused by different species of intracellular parasites of the genus Leishmania, which are transmitted to mammalian hosts after completion of their metacyclogenesis cycle in the gut of sandfly vectors. They next into reticuloendothelial cells of vertebrate hosts where they are transformed into amastigotes. Involvement with leishmaniasis has been reported around the world with the exception of the islands of Oceania. The disease is estimated to affect about 12 million people in about 100 countries, and more than 350 million people are at risk. The annual rate of new occurrence in the world is about 1.5 to 2 million. Cutaneous leishmaniasis (CL) is one of the most neglected diseases and many cases of the disease are not reported annually. The predominant clinical form of the disease is CL with a prevalence of about 70%–75%. This form is very common in Central Asia, the Americas south of the United States, Africa and the Indian subcontinent. L. major and L. tropica cause zoonotic CL and anthroponotic CL in Iran, respectively. Occasionally, L. infantum has been reported as an etiologic agent of CL. People with zoonotic CL in Iran are about 20,000 cases annually; some believe that the number of involved cases is higher and it is the most important parasitic disease in this country.

Leishmaniasis treatment requires a multi-factorial approach. Clinical and epidemiological types of the disease, overlapping infections, parasite types, and geographical environments are important variables that must be addressed before protocols of effective therapy are implemented. While leishmaniasis is a treatable disease, failure of treatment in some patients with CL is inevitable

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because of multiple factors. The drug of choice for the treatment of all types of leishmaniasis is pentavalent antimonials (SbV), especially glucantime. Impairing the synthesis of ATP and GTP from ADP and GDP in Leishmania amastigotes reduces the parasite’s viability. This action takes place through inhibition of glycolysis and citric acid cycle and inhibition of glycolytic activity and fatty acid oxidation by antimonials compounds. Trichloroacetic acid (TCA) is an appropriate and usual topical treatment for many skin lesions. TCA can penetrate into the midreticular dermis and destroy the epidermis and dermis lesions up to the top surface of the epidermis. After treating ulcers with TCA, accelerated cellular generation appears within two weeks, proving that its application in ulcers due to CL can revolutionize the treatment process. Dowlati reported that some cases of CL with skin lesions who did not respond to SbV compounds required a variety of treatments. One accompanying treatment for CL is using 50% TCA. Due to complications of high doses of meglumine antimoniate, using topical complementary compounds such as TCA 50% can be effective in prevention of scar formation. However, there are have been reports of complications due to using this agent. One of the most important factors associated with disease treatment is the species of the etiologic agent. Polymerase chain reaction (PCR) is an efficient tool to identify the etiologic agents of CL in patient-derived samples. Speciation of isolated organisms up to the species level is possible through some developed molecular methods. Three genes, including the internal transcribed spacer (ITS1) of the ribosomal DNA (rDNA), RNA 7SL, and heat shock protein70 genes (Hsp70) have been fully described in the scientific literature for identification of Leishmania isolates from endemic regions. Gene sequencing is globally validated as a method of choice for use in molecular, clinical, and epidemiological studies. The main objective of this study is comparison of three different therapies for CL and identification of the etiologic isolates in Isfahan, Iran. In this study, the nucleotide variations of ITS1 sequences were used to characterize the agent parasite.

Materials and Methods
Study Design and Participants
This randomized clinical parallel groups trial was carried out in the Isfahan province, Iran, from September 2017 to October 2018 on CL-positive patients who referred to Skin Diseases and Leishmaniasis Research Center (SDLRC). In central Iran, the province of Isfahan lies between the central Zagros mountain range and the great desert. It occupies an area of around 107 000 km² between latitudes 30° 42’ and 34° 30’N and longitudes 49° 36’ and 55° 32’ E.

The inclusion criteria were positive direct slide microscopy of CL with no history of CL treatment. The exclusion criteria were pregnancy, and age under one year or above 90 years. In this study, the positive cases of CL were referred to the specialized laboratory of leishmaniasis to characterize the etiologic agents. The sample size of the study was determined by considering type one error rate α = 0.05, statistical power, 1-β = 0.8, and mean duration of treatment as one of the main outcomes to be 6.8 ± 1.7 and 5.2 ± 1.0 weeks in glucantime and glucantime plus 50% TCA and complete cure rate 40% and 90%, respectively in the mentioned groups, resulting in 45 patients for each group; finally, 50 patients were recruited. Among 389 patients suspected of CL, 150 positive patients identified by smear preparations were recruited. The total sample size was randomly allocated by using permuted block randomization method of size 6 to intervention groups equally.

Demographic Data
Some demographic data (gender, age and habitat), clinical status contained location (body organ, history of previous CL, endemic emigration) of and the onset of lesions, duration, traveling to endemic regions and history of previous involvement were collected.

Smear Preparation
Smears were taken from the margins of the suspected leishmanial ulcers using No. 15 bistoury blade scalpels. For each patient, several smears were prepared. After drying at room temperature and fixing with methanol, they were stained with Giemsa dye solution, washed and examined with light microscopy to find parasitic amastigote forms.

Culture
For isolation and mass production of the leishmanial agents, skin samples were taken from the patients. To do this, the skin ulcers was washed with a local antiseptic solution, then the samples were removed with a sterile surgical razor and transferred to the culture medium, Novy-MacNeal-Nicolle (NNN); the cultures were incubated at 24 ± 1°C. They were then sub-cultured in a complemented RPMI 1640 cell culture medium (Gibco-BRL) containing 10% fetal calf serum (Sigma-Aldrich). Promastigotes were grown in the culture medium until late logarithmic phase, then centrifuged at 3000 rpm for 10 minutes and the resulting pellets were kept at −20°C for molecular studies.

Molecular Identification
DNA Extraction
DNA was extracted from the washed promastigotes as follows: Promastigotes (5 × 10⁶) were suspended in 200 µL of sterile PBS, 200 µL of binding buffer, and 40 µL of proteinase K and then incubated at 70°C for 10 minutes. DNA was dissolved in 200 µL of distilled water or 200 µL of elution buffer, and then extracted. DNA was run on a 0.8% agarose gel at 80 V in tris/borate/EDTA (TBE) 1× solution, then the samples were removed with a sterile surgical razor and transferred to the culture medium, Novy-MacNeal-Nicolle (NNN); the cultures were incubated at 24 ± 1°C. They were then sub-cultured in a complemented RPMI 1640 cell culture medium (Gibco-BRL) containing 10% fetal calf serum (Sigma-Aldrich). Promastigotes were grown in the culture medium until late logarithmic phase, then centrifuged at 3000 rpm for 10 minutes and the resulting pellets were kept at −20°C for molecular studies.

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buffer for 20 to 40 minutes and then visualized under UV light after staining with GelRed.

**Amplification of ITS1 Gene by PCR**

Ribosomal ITS1 containing small subunit of (SSU) ribosomal RNA (rRNA) and 5.8 S rRNA regions were amplified using the following primers:

1. LITSr (5'-CTGGATCATTTTCCGATG-3')
2. L5.8s (5'-TGTACCATATCCGCACTT-3')

DNA amplification was carried out in a 50 µL reaction mix of 200 µM deoxyribonucleotide triphosphates (dNTPs), 2.0 mM MgCl2, 2 U Taq polymerase (Roche Biotech, Germany), 10 pmol of each primer, and 100 ng of extracted DNA. The amplification stages were as follows: initial 5-minute denaturation at 95°C accompanied by 30 cycles of 95°C for 20 seconds, 50°C for 30 seconds, and 72°C for 1 minute, plus 72°C for 6 minutes after extension. Ten microliters of the PCR product was used alongside a 50-base pair (bp) ladder on a 1% agarose gel containing GelRed at 80 V for 25 to 45 minutes. The gel was placed on a UV trans illuminator and compared with Leishmania reference strains.

**RFLP Analysis of Amplified ITS1**

The PCR products along with DNA of reference strains of *L. major* (MRHO/IR/75/ER), *L. tropica* (MHOM/IR/99/YAZ1) and *L. infantum* (MCAN/IR/97/OLON49) were digested with HaeIII (Fermentas, Leon-Rot, Germany) at 37°C for 2 hours. Restriction fragments were placed onto 2% agarose gels in 1×TBE buffer and visualized on a UV trans illuminator after staining with GelRed.

**Patient Treatment by Different Protocols**

**A) IL Glucantime Plus 50% TCA**

Periodic treatment of ulcer location with 50% TCA every 2 weeks, with and topical glucantime injections twice a week in between were performed until complete wound healing or complete epithelization of the lesion for a maximum duration of 8 weeks. In topical treatment with 50% TCA, the site of the lesion was defatted with warm soap water and then 50% TCA was applied to the lesion using a cotton-head applicator embedded in TCA solution. After whitening, the position was neutralized with water and rubbed on with vaseline. Afterwards, if the ulcer did not improve, direct smear was performed and then systemic glucantime injections were repeated up to three times. All patients in the three groups who received the complete course of treatment were followed for 6 months.

**B) IL Glucantime**

IL glucantime injection was performed twice a week before the lesions were completely healed up to eight weeks later. IL injection was performed from the safe margin of the ulcer in quantities sufficient to blanch the lesion and 1 mm surface of normal skin surrounding it. Afterwards, if the ulcer did not improve, direct smear was performed and then systemic glucantime injections were repeated up to three times. All patients in the three groups who received the complete course of treatment were followed for 6 months.

**C) Systemic Glucantime**

The first course of treatment was 20 mg/kg of glucantime for 21 days followed by a 10–14-day rest period.

**Outcome Measures**

Monthly follow-up for the next 6 months was performed for all patients in the three medicinal groups. The clinical response was graded as total improvement (full lesion re-epithelization), partial improvement (50%–75% lesion size improvement), and no difference in the lesion appearance. Scar improvement was also measured based on the patient’s satisfaction, the lesion morphology, and the induration level.

**Statistical Analysis**

The SPSS statistical software (Ver16, Chicago, IL, USA) was used for data entry. Continuous data were reported as mean ± standard deviation (SD) along with median (interquartile range (IQR): first quartile(Q1)-third quartile (Q3) for non-normal continuous data). Normality of data was evaluated using Kolmogorov-Smirnov test and Q-Q plot, and homogeneity of variance was evaluated using Leven test. Non-normally distributed continuous data were subjected to logarithmic transformation. Chi-square test was performed to determine significant differences between the three study groups for categorical data and Fisher exact test was used when more than 20% of crosstab cells were expected to have a frequency less than 5. The effect of treatment approach on complete recovery was evaluated using logistic regression, and odds ratios (OR) and 95% confidence intervals (95% CI) for OR were reported. Both crude and adjusted ORs, after adjusting for the confounders, were reported. One-way analysis of variance (ANOVA) was used for comparing continuous data between the groups, followed by Tukey’s post-hoc test for pairwise group comparisons. Analysis of covariance was used for comparing the mean duration period when adjustment was made for confounders. Statistical analysis for main outcomes was conducted based on an intention-to-treat approach. Statistical significance level was set at *P* < 0.05.

**Results**

**Sampling**

In this study, all of the CL suspicious patients referred to SDLRC were investigated. Out of 389 patients suspected of CL, 180 did not fulfill the inclusion criteria and 54 did not agree to participate in our study. Finally, 150 patients confirmed through direct sampling and microscopic investigations were recruited and completed the study. Figure 1 presents the information on distribution,
randomization, exclusion, and final number of patients evaluated in the three groups.

Basic Demographic & Clinical Characteristics
The number of patients in group A was 20 females (40%) and 30 males (60%). Group B included 20 females (40%) and 30 males (60%), while group C included 19 females (38%) and 31 males (62%). The chi-square test illustrated that groups were not significantly different in terms of sex distribution ($P = 0.972$).

As shown in Table 1, the mean age values of the subjects in the three groups were 33.64, 33.62 and 31.2 years, respectively. In all three studied groups, the majority of participants were male. The majority of participants in treatment groups had low educational levels. Legs and arms were the two most affected organs among the studied participants in the three groups. History of previous CL was positive only in 4% and 8% of patients treated with IL glucantime plus 50% TCA (A) and IL glucantime (B), respectively, and none of the participants in the systemic glucantime group (C). Many of participants (more than 70%) in all three studied groups had history of emigration to endemic areas.

Smear Preparation
Among 389 CL suspected patients, 150 CL positive cases were selected via direct microscopy after Giemsa staining of slides containing impression smears from their lesions.

Parasite Culture
For parasite isolation, samples were taken from the protruded margin of the ulcers under sterile conditions and cultured in screw capped tubes of N.N.N medium. The tubes were then incubated at 24°C from 1 to 4

Table 1. Basic Demographic and Clinical Characteristics of Patients with CL in the Three Groups

| Variables                  | IL Glucantime Plus 50% TCA | IL Glucantime | Systemic Glucantime |
|----------------------------|----------------------------|---------------|---------------------|
| Age                        | 31.16 ± 19.76              | 33.62 ± 18.58 | 33.64 ± 19.37       |
| Gender                     |                            |               |                     |
| Female                     | 20 (40)                    | 20 (40)       | 19 (38)             |
| Male                       | 30 (60)                    | 30 (60)       | 31 (62)             |
| Education                  |                            |               |                     |
| Illiterate                 | 13 (26)                    | 20 (40)       | 13 (26)             |
| Below diploma              | 19 (38)                    | 9 (18)        | 17 (34)             |
| High school diploma        | 15 (30)                    | 16 (32)       | 17 (34)             |
| University education       | 3 (6)                      | 5 (10)        | 3 (6)               |
| Body organ                 |                            |               |                     |
| Arm                        | 21 (42)                    | 29 (58)       | 17 (34)             |
| Leg                        | 22 (44)                    | 13 (26)       | 18 (36)             |
| Face                       | 6 (12)                     | 4 (8)         | 10 (20)             |
| Neck                       | 0 (0)                      | 0 (0)         | 1 (2)               |
| Trunk                      | 1 (2)                      | 4 (8)         | 4 (8)               |
| History of previous CL     | 2 (4)                      | 4 (8)         | 0 (0)               |
| Endemic emigration         | 37 (74)                    | 37 (74)       | 46 (92)             |

Continuous and categorical data are presented as mean ± SD and frequency (percentage), respectively.
weeks until movable promastigotes appeared. For mass production of promastigotes, they were passaged into the complete RPMI 1640 medium.

Molecular Identification

The PCR Results of ITS1-rRNA and PCR-RFLP

The appearance of 350 bp bands related to isolates in conventional PCR were in agreement with the band extracted from the reference *L. major* (Figure 2). When these bands were digested with *Hae* III restricted enzyme, two bands of 220 and 140 bp appeared with complete homology to the reference strain, *L. major* (MRHO/IR/75/ER). Other references strains were *L. tropica* (MHOM/IR/99/YAZ1, lane 2) and *L. infantum* (MCAN/IR/97/LON49, lane 3). Therefore, it was concluded that all the isolates obtained from patients were *L. major* (Figure 3).

CL Healing Period and Percent Recovery in the Three Medicinal Groups

Figure 4 shows the mean healing period of CL lesion in the three groups. The mean duration of healing period was 53.12 ± 25.88 (median: 45, IQR: Q1 = 30–Q3 = 77) days in group A, 57.22 ± 44.02 (median: 42.5, IQR: Q1 = 30–Q3 = 60) days in group B, and 73.56 ± 41.08 (median: 71, IQR: Q1 = 45–Q3 = 90) days in group C; the observed differences were statistically significant (*P* = 0.024). After adjustment for the confounding effect of endemic emigration, the observed differences remained significant (*P* = 0.036).

The results of Tukey’s HSD post hoc test showed that there were no significant differences between group A and group B (*P* = 0.99); however, there was a significant difference between group A and group C (*P* = 0.049), and between group B and group C in terms of mean healing period (*P* = 0.047). However, after adjusting for the confounding variable (emigration to endemic area), the difference between IL glucantime and IL glucantime plus 50% TCA was statistically significant (*P* = 0.022), the difference between Systemic glucantime and IL glucantime plus 50% TCA was not statistically significant (*P* = 0.057) and no significant difference was observed between Systemic glucantime and IL glucantime (*P* = 0.99).

Figure 5 shows the recovery status of lesions in the three studied groups. Chi-square test showed there were significant differences between the three groups in terms of healing of lesions (*P* = 0.002). In group A, 40 (80%) of patients showed complete recovery, 8 (16%), partially recovery and 2 (4%) no recovery. In group B, 31 (62%) had complete recovery, 12 (24%) partial recovery and 7 (14%) no recovery. In group C, 21 (42%) had complete recovery, 22 (44%) partial recovery and 7 (14%) no recovery.

The odds (95% CI) of complete recovery in group A were significantly higher OR = 6.67 (1.27–34.98) (adjusted OR = 5.64, 95% CI: 2.25–14.13) than group C (*P* = 0.025). Furthermore, although the crude odds of complete recovery were higher in group B than group C, OR = 1.48 (0.45–4.85) the difference was not statistically significant; however, after adjusting for the effect of the confounding variable (emigration to endemic area), it was statistically significant (adjusted OR = 2.30,
95% CI: 1.02–5.20). The odds of complete recovery in patients of group A were higher than group B OR = 4.52 (0.88–23.28); however, the difference was not statistically significant in either crude or adjusted models (adjusted OR = 2.45, 95% CI: 0.98–6.03) \((P = 0.079)\).

**Discussion**

CL is reported in more than 90 countries, and Iran is an important focus of the disease. So far, no medication has been reported to induce complete recovery against leishmaniasis. Therefore, intensive research should be performed in pharmacological fields to achieve desired results. Antimony compounds have been introduced as the drug of choice for treatment of CL. Due to the complications of systemic administration of high doses of antimony compounds, application of local compounds such as 50% TCA has been considered. The healing effect of 50% TCA plus glucantime has been reported as an effective combination in reducing the scars and increasing the efficacy of glucantime. Following the evolution of numerous studies for better understanding the compounds or drug combinations to treat leishmaniasis, in this unique design, the comparison of the therapeutic effect of the three methods used for the treatment of CL patients was designed and implemented.

Parasite taxonomy appears to be necessary to properly understand the epidemiological changes based on population structure, genetic variation of the parasite and to determine the exact treatment regimen. For assessment of prognosis and effective care, early diagnosis and speciation of the etiologic parasite are critical. Species identification is very useful in deciding the correct care scheme and disease prevention plans. In recent years, molecular methods such as PCR have been used for detection of the disease agents. It has been shown that molecular methods have higher specificity than the traditional culture methods. In the present study, the frequency distribution of species in the three treatment groups was identified as *L. major* by PCR method. Afkar et al in Bam city of Iran showed that 91.1% of the species were *L. tropica* and 8.9% were *L. major*. Pagheh et al in Golestan province of Iran showed that all of the specimens were *L. major*. Vaeznia et al in Mashhad province of Iran, used PCR-RFLP and detected 34% *L. major* and 66% *L. tropica* as agents of CL in a sample of 50 patients. Eslami et al in Yazd, Iran, identified 49.01% of samples to be *L. major* and 50.98% to be *L. tropica*.

People traveling and moving over the time have caused epidemiological changes and created new foci in some parts of Iran. For example, a focus of *L. major* was created in Pakdasht, Tehran due to people traveling to Sabzevar city (a focus of *L. major*). Significant differences were found in terms of traveling to endemic areas among the three treatment groups in this study.

Glucantime (meglumine antimoniate) is a pentavalent antimony component and is the most commonly used drug for treatment of CL. We showed that the therapeutic effect of IL glucantime plus 50% TCA solution was significantly better than IL and systemic glucantime. However, the healing effect was better in the IL glucantime group compared to the systemic glucantime group. Previously, the efficacy of IL glucantime has been shown for treatment of CL and it was shown that IL injection of glucantime does not reduce the therapeutic effect of the drug as compared to its systemic injection, while the side effects of IL injection were reduced. In our study, decreased induration, clinical healing of the lesions, and the reappearance of epidermis lines were more than 80%. Tallab et al investigated the effects of three IL glucantime injections daily, every other day, and weekly. The results showed that daily IL glucantime injection was more effective than treatment on every other day or on a weekly basis. Hejazi et al showed that IL glucantime injection that was performed weekly resulted in the resolution of CL ulcers. In the first treatment course, the patients received 20 mg/kg of glucantime for 21 days, and then, a rest period of 10–14 days, and then IL injections were performed for 5–7 weeks.

Some discrepancy observed across similar studies is possibly due to the parasite strain. According to Sadeghian...
et al, there is a significant relationship between glaucantime activity in the lesions of non-infected CL and secondary bacterial infection. In other words, in bacterial infected lesions, the treatment response was reduced.\textsuperscript{43} Alkhavajah et al showed that the presence of secondary bacterial infection can lead to lack of response to treatment. Therefore, in this situation, before repetition of antimony treatment, secondary bacterial infection should be treated with appropriate antibiotic therapy.\textsuperscript{42}

It has been shown that the efficacy of IL therapy with five-compound antimony is 68–100%,\textsuperscript{22,44} which is approximately in agreement with our results (62% complete recovery). However, Faghihi et al reported that the rate of complete recovery of the lesions with IL glucantime injection was 41.7%.\textsuperscript{45}

If there is no glucantime available or there is susceptibility to glucantime or if treatment with this drug fails, an alternative local supplemental drug will be 50% TCA solution.\textsuperscript{8} Nilforoshzadeh et al showed that the use of 50% TCA resulted in significant healing in scars, time, and recovery percentages.\textsuperscript{8} The findings of a study by Cho et al indicate that treatment of atrophic scars through chemical reconstruction of skin scars (CROSS) is more successful than the simple application of TCA in the activation of fibroblasts in the dermis and in the increase of collagen.\textsuperscript{46}

Considering that the best therapeutic effect was observed in group A in our study, it may be concluded that TCA, due to its acidic properties, reduces the tissue barrier in the proportional glaucantime penetration and thus facilitates the delivery of glucantime to target cells. In addition, TCA alone can degrade parasite-infected macrophages in CL lesions. Other properties to be mentioned about this agent are suitable penetration of the dermis and destruction of scar lesions, stimulating and increasing the rate of cell proliferation, including fibroblast repair cells and skin collagen within two weeks after use and removing the scars caused by the disease.

The results of this study regarding the effect of treatment approaches reflected in odds ratios should be interpreted with caution due to the small sample size and sparsity of data and possibly sparse data bias.\textsuperscript{47} Also, although the current study is a randomized clinical trial and it is expected that through randomization, the potential confounders to be balanced between study groups, due to the apparent sparsity in our data, we could not adjust for the confounding effects of some limited unbalanced confounders across the intervention groups. Accordingly, more studies with large sample sizes are suggested to confirm our results. Another limitation of our study is the lack of previous data about the effect of systemic glucantime on CL; accordingly, the sample size was determined based on available data about the effects of glucantime and glucantime plus 50% TCA.

In conclusion, in this study, the average duration of lesion healing among the three groups was the shortest in patients with IL glucantime plus 50% TCA treatment regimen. Also, treatment with IL glucantime plus 50% TCA, IL glucantime and systemic glucantime caused lesion healing in 80%, 62%, and 42% of cases, respectively. In the present study, use of 50% TCA in patients suffering from CL was associated with a significant improvement in the depth of scars, the time and the percentage of recovery and the low cost of this agent in treatment of CL.

**Authors’ Contribution**

LA contributed in the conception of the work, conducting the study, drafting and revising the draft and approval of the final version of the manuscript. SHH supervised the research work, contributed in the conception of the work, conducting the study, drafting and revising the draft and approval of the final version of the manuscript. NA contributed in the conducting the study and revising the draft and approval of the final version of the manuscript. FS contributed in the conception of the work, conducting the study, drafting and revising the draft and approval of the final version of the manuscript.

**Conflict of Interest Disclosures**

None.

**Ethical Statement**

This study was ethically reviewed and approved by the Ethics Committee of Islamic Azad University, Science and Research Branch with study project number: IR.IAU.SRB.REC.1398.085. Voluntary participation of CL-positive patients was one of the main conditions for the study and therefore, consent forms were obtained from them. In the case of children’s participation, the consent form was signed by their parents. The principle of confidentiality of patients’ information was observed and they were used only to achieve the objectives of the study.

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