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Short Communication

The topical treatment of old world cutaneous leishmaniasis with gentian violet along with cryotherapy: a pilot single-blind randomized controlled clinical trial

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Abstract

Introduction: The promising non-clinical antileishmanial effects of gentian violet (GV) encouraged us to evaluate the additive effect of GV on cryotherapy. **Methods:** For 8 weeks, 59/161 cutaneous leishmaniasis patients/lesions underwent cryotherapy alone (group 1) or cryotherapy accompanied by 1% GV application (group 2). The primary endpoint was clinical response. **Results:** Ultimately, 54.7% and 45.3% of the significantly cured lesions belonged to groups 1 and 2, respectively, which was not statistically significant. The clinical response was significantly different between the two groups at the end of the fourth week. **Conclusions:** Although the clinical response of the two groups was significantly different at the end of the fourth week, application of GV did not increase the efficacy of cryotherapy.

Keywords: Leishmaniasis. Treatment. Topical. Gentian violet. Cryotherapy.

Leishmaniasis is a neglected tropical disease caused by the vector-borne protozoan parasite *Leishmania*. Cutaneous leishmaniasis (CL) is one of the three main clinical presentations of *Leishmania* infection[1].

Several treatment modalities have been applied for management of old-world CL with variable efficacies[2]. Although pentavalent antimonies are generally considered the mainstay of CL treatment, the safety of treatment has been challenged[3].

Some guidelines recommend local therapy for the treatment of limited-size CL lesions[4].

Several topical therapeutic options have been studied for the treatment of CL. Among them, topical paromomycin with or without combinations, photodynamic therapy, carbon dioxide laser, and thermotherapy resulted in high cure rate, while cryotherapy showed moderate cure rate in a systematic review[5].

Gentian violet (GV) is a triphenylmethane (TPM) dye discovered in 1861 and has been used as an antibacterial agent since 19th century. In addition to its antibacterial activity, GV has antimycotic, antiviral, antihelminthic, and antitrypanosomal effects[6]. Although the results of the study that evaluated *in vitro* and *in vivo* antileishmanial activity of GV and 10 other TPMs were promising[7], to the best of our knowledge, no clinical trial has been conducted to assess the efficacy of GV.

Thus, this pilot single-blind randomized controlled clinical trial was designed to appraise the antileishmanial effect of GV in humans.

Study design and site: This study was a pilot parallel investigator-blind 1:1 randomized controlled clinical trial and was conducted in a teaching hospital at the Shiraz University of Medical Sciences. The study protocol was registered in the Iranian registry of clinical trials (IRCT2017071316557N2).

Patients with clinical diagnosis of leishmaniasis confirmed by direct smear and/or polymerase chain reaction were included in the study. However, if the lesions were absolutely typical for leishmaniasis, the patient was included without laboratory work up. The exclusion criteria were: patients with lesions lasting more than 4 months, receiving systemic or topical antileishmanial treatment or cryotherapy in the recent one month before study, pregnancy, lactation, patients with more than 10 lesions, and lesions located in cartilaginous sites (auricle and nose).

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Patients with clinically infected lesions were recruited after a complete course of oral antibiotic therapy.

Sample size: The sample size calculation was based on the confidence interval approach of Cocks and Torgerson[8] for pilot randomized trials. Considering the proportion of patients with significant cure approximately equal to 30% among those treated with cryotherapy combined with gentian violet, a power of 80% and a significance level of 5%, we would require a pilot sample of 60 participants (30 in each group) in order to detect a minimum difference of 10% between the treatment groups.

Randomization: The patients were randomly allocated to two groups [MEDCALC software version 8 (Ostend, Belgium)] by permuted block randomization (in blocks of size 4). Thirty-three (33) patients were allocated to each group, but some patients did not complete the treatment after allocation. Thus, during follow-up, there were 30 patients in GV and cryotherapy combination group, whereas 29 patients completed the study in cryotherapy group. The details are shown in **Figure 1**.

Interventions: Patients in both groups underwent weekly liquid nitrogen cryotherapy using cryospray (Sarmadarman, Tehran, Iran) for 8 weeks. Liquid nitrogen was sprayed approximately 10 cm away from the lesion for 15 s with a double freeze-thaw cycle. In addition to cryotherapy, patients in one of the groups applied 1% gentian violet (GV) ointment twice daily over the lesions for 8 weeks at their home without supervision. The ointment was prepared by dissolving 1 g of GV (Merck, Darmstadt, Germany) in 100 g of Eucerin (Abidaryaco, Isfahan, Iran). The patients were assessed at the beginning of

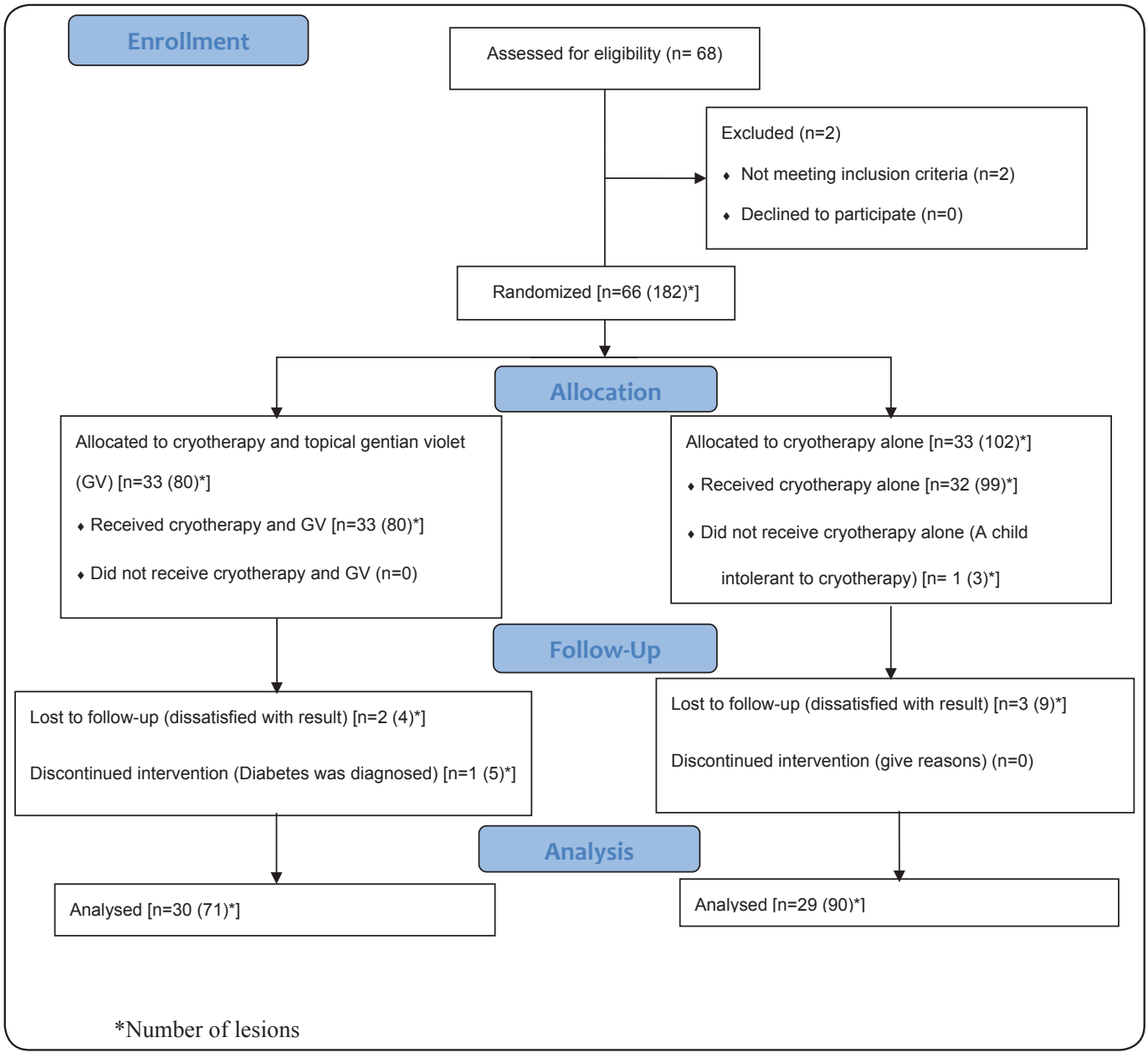

FIGURE 1: CONSORT flow diagram of participants through enrollment, allocation, and follow-up stages of the study

allocation, and also at the end of 4th and 8th weeks of treatment by an investigator who was unaware of the treatment. The patients in the GV group were recommended to wash the lesion(s) with water and soap or cleanse with alcohol to wash out the purple color of GV before the 4th and 8th week visits.

Outcome measures: The primary endpoint of this study was defined as clinical cure[9] as shown below.

Significant cure: more than 75% reduction in the size of lesion (largest indurated diameter multiplied by the shortest indurated diameter of the lesion, measured by a ruler).

Partial cure: marked by 50–75% reduction in lesion size.

Failure to respond: less than 50% reduction in the size of lesion or increase in lesion size.

The clinical cure was reported at the end of the 4th and 8th weeks of treatment.

Ethical considerations: The protocol was approved by the ethical committee of the Shiraz University of Medical Sciences (Ethical code: IR.SUMS.med.REC.1394.29). The ethical principles of the 1975 Declaration of Helsinki were followed. The patients (and parents or legal guardian for patients younger than 18 years) were informed about the study and asked to complete the written consent form.

Data analysis: The data were analyzed using SPSS software version 18 (Chicago, IL, United States). Data of the groups were compared using Chi-square test. The significance level was set at 0.05.

This study lasted from October 2015 to February 2016 and a total of 68 cases were screened. Sixty-six patients with 182 lesions were recruited into the study. After allocation and during follow- up, the cases declined to 59 with 161 lesions (**Figure 1**).

The baseline characteristics of the patients and lesions recruited into the study groups are shown in **Table 1**.

Table 2 presents a comparison of the treatment with cryotherapy combined with GV and the treatment with cryotherapy alone.

At the end of the study, the rate of significant clinical cure was not different between the two groups ($P = 0.549$).

In the 4th week follow-up, 14 (70.0%) and 6 (30.0%) partially cured lesions were treated with cryotherapy combined with GV and cryotherapy alone, respectively. In the same time, 18 (33.3%) of GV-administered group and 34 (66.7%) of the patients treated with cryotherapy alone healed significantly. The therapeutic responses of the two groups were significantly different in the 4th week follow-up ($P = 0.02$).

No side effect was reported in either group except the transient purple staining of the skin in GV-treated patients.

Despite the variable clinical responses in the 4th week of follow-up, adding gentian violet did not increase the efficacy of cryotherapy in the treatment of cutaneous leishmaniasis.

Investigating topical regimens is an expanding field in pharmacological studies due to their convenience and fewer side effects. Various topical medications have been studied for CL treatment. Few of these topical treatments could be strongly recommended based on qualified studies[2].

Gentian violet, also known as crystal violet, is a triphenylmethane dye used for the Gram staining of bacteria.

TABLE 1: Baseline characteristics of patients and lesions in cryotherapy combined with gentian violet (GV) and cryotherapy alone groups

Variant	Cryotherapy / GV group	Cryotherapy group
Number of patients	30	29
Number of lesions	71	90
Methods of diagnosis		
Direct smear	24	25
PCR*	1	0
Clinical	5	4
Mean age ± SD (years)**	29.4 ± 2.8	27.3 ± 3.2
Sex		
Male	18 (60%)	14 (48%)
Female	12 (40%)	15 (52%)
Site of involvement		
Lower extremities	42 (60%)	39 (44%)
Upper extremities	18 (25%)	45 (50%)
Trunk	11 (15%)	3 (3%)
Head and neck	0 (0%)	3 (3%)
Mean duration of disease ± SD (months)**	2.08 ± 0.16	2.10 ± 0.19

*Polymerase chain reaction; **Standard deviation.

TABLE 2: Comparing effects of cryotherapy combined with gentian violet (GV) and cryotherapy alone on clinical cure of cutaneous leishmaniasis lesions

Clinical efficacy	Cryotherapy / GV group Number of patients (%)	Cryotherapy group Number of patients (%)	*P* value
Week 4			
Significant cure	18 (33.3)	34 (66.7)	
Partial cure	14 (70.0)	6 (30.0)	**0.02**
Failure	39 (43.8)	50 (56.2)	
Week 8			
Significant cure	48 (45.3)	58 (54.7)	
Partial cure	5 (31.2)	11 (68.8)	0.549
Failure	18 (46.2)	21 (53.8)	

It has also been used clinically for treatment of various infections caused by various Gram-positive and Gram-negative bacteria including methicillin-resistant *Staphylococcus aureus*, fungi such as *Candida*, parasitic protozoa such as *Trypanosoma Cruzi*, parasitic roundworms such as *Strongyloides* and *Enterobius*. In addition, anti-angiogenic and antitumor activity of GV has also been mentioned[6].

de Souza Pietra et al.[7] tested 9 synthetic triphenylmethane derivatives along with GV on *Leishmania (L.) amazonensis*, *Leishmania (V.) braziliensis*, and *Leishmania major in vitro*. GV was the most effective agent in this study. In BALB/c mice infected with *Leishmania (L.) amazonensis* and subsequently treated with 1% GV gel twice daily, no parasite was detected after 20 days of treatment[7].

However, these promising results were not reproduced in our clinical trial, which may, at least in part, be explained by the difference in the preparation of the GV (ointment versus gel). To the best of our knowledge, our study is the first clinical trial to evaluate the clinical efficacy of topical GV in the treatment of cutaneous leishmaniasis.

The mechanism of action of GV is not clear exactly. Different hypotheses have been proposed to explain the effects of GV, especially the antimicrobial effects[10]. Among these, two hypotheses are mostly emphasized: 1) inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and 2) formation of a covalent complex between GV and thioredoxin reductase 2 (TrxR2) in mitochondria. The latter mechanism was considered to be more admissible explanation for the role of GV in the treatment of leishmaniasis[6]. TrxR2 is also considered as the target for GV in treatment of cancer and another parasitic infection, malaria[11].

Although gastrointestinal and hematological side effects as well as carcinogenicity have been reported in rodents following the systemic use of GV, there is no evidence of significant systemic toxicity following external topical application of GV[12].

Limitations on the parameters essential for ideal efficacy of topical formulations may explain the discrepancy between the outcomes of *in vitro* and animal model studies on antileishmanial effect of GV and clinical outcome in our study. Designing more efficient formulations by emerging delivery systems like liposomes, microsponges, lipid nanoparticles, polymeric particles, dendrimers, dendritic-core multishell nanotransporters or even appropriately designing conventional formulations may improve clinical efficacy of topical GV in treating CL[13,14].

Besides this limitation in topical medication formulation, our study results may be limited by the small number of patients and lack of follow-up after cessation of treatment. Additionally, we did not determine the parasite species in our study; however, the most common species in our province causing leishmaniasis is *Leishmania major*[15].

In conclusion, despite the variable therapeutic effects of GV-added cryotherapy and cryotherapy alone in the early stages of treatment, topical gentian violet ointment did not increase the efficacy of cryotherapy in the treatment of CL.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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