Topical terbinafine in the treatment of cutaneous leishmaniasis: triple blind randomized clinical trial

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Abstract Leishmaniasis is a spectrum of disease condition with considerable health impacts, caused by different species of *Leishmania*. This disease is currently endemic in 98 countries and territories in the world. There are many treatment modalities for cutaneous leishmaniasis. The use of topical terbinafine in the treatment of cutaneous leishmaniasis has recently been considered. Eighty-eight participants more than two years old with proven acute CL by a positive direct smear were randomly allocated to one of the two study arms: first group received meglumine antimoniate (Glucantime) 20 mg/kg/day intramuscular injection (IM) plus a placebo ointment (Mahan Vaseline) for 20 days. The second group received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus topical terbinafine, for 20 days and were monitored closely by

dermatologist during the course of the study. Crude regression analysis showed that there was no significant difference between placebo and intervention group regarding partial or complete treatment (partial treatment: $HR_{crude} = 1.1$, CI 95 % = 0.7–1.7; complete treatment: $HR_{crude} = 1.1$, CI 95 % = 0.8–1.7). Although, there was no statistically significant difference between the two treatment groups, but clinically it seems that the treatment rate in those who receive glucantime plus terbinafine was more effective than the other group. However this rate depended on the type of lesions. As data indicated ulcerated nodules, papules and plaque in experimental group have been completely improved two times faster than placebo group. Ulcerated nodules, nodules and plaque were partially improved faster in those used terbinafine than placebo ointment.

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Introduction

Leishmaniasis is one of the neglected diseases which cause disfiguring. The disease is prevalent in Euroasia, Africa and Latin America which is more severe and common in low socioeconomic. Iran is one of the Eastern Mediterranean (EMRO) countries where is entangled in this health problem especially in the southeast.

Leishmania is a protozoan parasite, which is transmitted by female sandfly bite to mammalian hosts. At least twenty species of *Leishmania* cause different types of leishmaniasis. *Leishmania major* and *Leishmania tropica* have been involved as the cause of cutaneous leishmaniasis (CL) in Iran. However, *L. tropica* the cause of anthroponotic

cutaneous leishmaniasis (ACL) is particularly prevalent from CL patients in Kerman province, south-eastern Iran (Sharifi et al. 2012, 2011; Aflatoonian et al. 2014).

Lesions of CL are self-cured during 6–12 months but the important point is that the progress of lesions makes disfiguring especially it is more important in cosmetic zones (face) and also CL patients potentially are reservoirs of *L. tropica*. Therefore treatment of CL patients can be an effective preventive strategy for the disease.

There are many modalities which have been recommended. One of the most common anti-leishmanial therapies during last decades are pentavalent antimonials (Glucantime). But their usefulness has limited by cardiotoxicity and hepatotoxicity (Najim et al. 1998).

An alternative method is antifungal therapy which has recently been used in the treatment of CL patients. One of the fungicidal drug is terbinafine, a member of allylamine group, which destroys the wall of parasite by inhibiting the synthesis of ergosterol via squalene epoxidase. In the early 21st century, researchers designed experimental studies to evaluate the efficacy of terbinafine on different species parasitic (Zakai and Zimoo 2002). They have suggested that the use of terbinafine could be effective against *Trypanosoma cruzi* and *Leishmania major*.

Although, there are some occasional reports of low side effects of terbinafine, it is safer and relatively free of side effects than the other anti-leishmanial drugs especially for children (Gubta et al. 2005).

The present clinical trial was conducted to evaluate the efficacy of topical terbinafine in the treatment of cutaneous leishmaniasis due to *L. tropica* in Kerman province in the Southeast of Iran.

Materials and methods

Participants

A sample size of 44 participants per treatment group was planned with a probability of a type I error at $\alpha = 0.05$ and $\beta = 0.1$ to determine a 20 % difference between topical terbinafine and placebo in outcome.

Eighty-eight participants more than two years old with proven acute CL by a positive direct smear in all lesions were recruited. The planned treatment was explained to each patient or parents of those less than 18 years and they provided written informed consent prior to randomization. A careful history of CL lesions was taken by masked dermatologist.

Before randomization, patients who fulfilled the following criteria were enrolled: males and females ≥ 2 years of age; parasitological diagnosis of cutaneous leishmaniasis from all lesions with direct smear; ulcerative lesions

with duration of less than one months which were not on exposed areas or joints; the size of lesions has to be < 3 cm; the number of lesions should be 3 or less; they must not receive antileishmanial therapy during last two weeks, and with no history of hypersensitivity to Glucantime. Cases of pregnant or nursing women, patients with hepatic, renal, or heart diseases were excluded.

Study design and location

This triple-blind, randomized clinical trial was performed in Shahid Dadbin Clinic of Leishmaniasis Research Center, at Kerman University of Medical Sciences, between 2008 and 2010. This clinic is a referral center for CL patient throughout the province (Fig. 1).

Interventions

Eighty eight of the 90 participants meeting inclusion criteria were randomly allocated to one of the two treatment arms: First group (placebo group) received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus a placebo ointment (Mahan Vaseline) 32.25–75.5 mg/day (1/16–1/8 of fingertip unit according to the size of the lesion), for 20 days. Second group (intervention group) received meglumine antimoniate (Glucantime) 20 mg/kg/day IM plus topical terbinafine 32.25–75.5 mg/day (1/16–1/8 of fingertip unit according to the size of the lesion), for 20 days and were monitored closely by dermatologist during the course of the follow-up examinations. Participants with clinical failure at 2 months were offered crossover treatment.

The size and induration were measured with a standard scale ruler in 2 perpendicular directions by an independent observer who was not aware of the treatment options at the baseline, 14, 30, 44 and 110 day (three month) after completing the treatment of the course.

End points

Clinical cured was defined as: Complete improvement: full re-epithelialization for ulcerative lesions and decrease in induration size > 75 % (with or without a negative direct smear result); partially improved: decrease in the indurations size between 25 and 75 %; no change: decrease in the indurations size < 25 %.

Ethics

The clinical trial was reviewed and approved by the Medical Ethical Committee of Kerman University of Medical Sciences (project number K-85/33) and ACTR number: ACTRN12609000113257.

Fig. 1 The profile of study

Randomization and blinding

The randomization sequence was generated by the use of a randomization table. A simple block randomization list with a block size of 4 was prepared by a team member (F.G.) who was not involved in the recruitment and follow-up of the patients. The randomization allocation concealment was performed by sending the randomization numbers in envelopes to a pharmacist who was responsible for giving the assigned treatment after each eligible patient was enrolled. The recruited patients were referred to the pharmacist to receive their assigned drugs. The pharmacist evaluated the compliance of the patients by counting the remaining sachets. This study was a triple blind trial in which the outcome assessors (A.F., A.K., and M.H.G.) were unaware of the drugs used by the patients.

Statistical analysis

We tested the effect of the two treatments of measurements on the mean of changing in lesions size and indurations. The effect of other factors on the response to the treatment like age, type of lesions and location of lesions were matched. Statistical significance was defined by a P value <0.05 for all calculations.

The size of each lesion was computed based on its dimensions. We considered the lesion as completely treated if its size had been decreased completely ($>75\%$) comparing to baseline size. Partial treatment would be defined if the size decreased between 25 and 75 %, and no treatment would be ascertained if it decreased less than 25 %.

Cox proportional hazards model was used in order to compare treatment progression between intervention and placebo groups. In this regard, two types of models were

fitted; one for complete treatment as the event of interest and the other for partial treatment. Since participants sex and morphology of their lesion(s) showed significant difference in two groups, adjusted hazard ratios (HR) were estimated in Cox regression models after controlling for their effect. Other potential confounders were identified with backward selection method. Stata software (version 11) was used in order to analyze the data.

Results

Participants' mean age was 19.8 ± 16.9 years. In intervention group most participants were female ($n = 32$, 71.1 %), however, those in placebo group were mostly males ($n = 25$, 55.6 %); this difference was statistically significant (P value = 0.043; Table 1).

Table 1 Baseline characteristics of study participants in placebo and terbinafine groups

	Placebo	Intervention	P value
Age (year)			
Mean (SD)	19.2 (17)	20.4 (17.1)	0.7010*
Number of lesions			
Mean (SD)	1.6 (0.8)	1.6 (0.7)	0.8883**
Size (cm)†			
Mean (SD)	2.6 (3.2)	2.4 (2.2)	0.6522*
Duration of follow-up‡			
Mean (SD)	4.4 (2.6)	4.1 (2.5)	0.0722*
Sex n (%)			
Male	25 (55.6)	13 (28.9)	0.043§
Female	20 (44.4)	32 (71.1)	
Ulcerous lesion ^a (%)			
Yes	15 (25)	23 (38.3)	0.116§
No	45 (75)	37 (61.7)	
Lesion location ^a (%)			
Face	18 (30)	20 (33.3)	0.332§
Hand	40 (66.7)	35 (58.3)	
Foot	2 (3.3)	5 (8.4)	
Body	0 (0)	0 (0)	
Lesion morphology ^a (%)			
Dry nodule	7 (11.7)	24 (40)	<0.0001⌘
Ulcerated nodule	17 (28.3)	3 (5)	
Nodule	20 (33.3)	20 (33.3)	
Papule	5 (8.3)	2 (3.3)	
Ulcer	10 (16.7)	4 (6.7)	
Plaque	1 (1.7)	7 (11.7)	

† Minimum dimension; * maximum dimension; ‡ in week; * independent t-test; ** Mann–Whitney U; § Pearson Chi square; ⌘ fishers exact test

^a The total could be larger than 90 since each patient could have more than one lesion

Our follow up was designed for a period of 3 months after completing of the treatment phase, unfortunately we lost most of the patients during the phase of the follow up and we managed only to follow up 24 participants (9 participants of intervention group, 15 participants of placebo group) for three months. In average, each patient was followed for 4.3 ± 2.5 weeks. Most participants referred with one lesion ($n = 67$, 74.4 %). Lesions were most frequently located on participants' hands ($n = 75$, 61.5 %). Dry nodules were the most frequent morphology of the lesions ($n = 31$, 26.6 %); there was a significant difference in both groups regarding the morphology of the lesions ($P < 0.0001$). Table 1 shows baseline characteristics of study participants in both groups.

In placebo group, treatment response (partial and complete treatment) was observed after more cream administrations comparing to intervention group (more follow-ups), however, this difference was not statistically significant (placebo: 4.4 ± 2.6 , intervention: 4.1 ± 2.5 weeks; $P = 0.0722$). Before showing partial treatment, in average each lesion received 4.9 ± 2.2 cream administrations (placebo: 5.1 ± 2.1 vs. intervention group: 4.6 ± 2.3 ; $P = 0.266$). Moreover, before showing complete treatment, in average each lesion received 6.4 ± 2.2 cream administrations (placebo: 6.8 ± 2.1 vs. intervention group: 5.9 ± 2.1 ; $P = 0.0539$).

Crude regression analysis showed that there was no significant difference between placebo and intervention group regarding partial or complete treatment (partial treatment: $HR_{\text{crude}} = 1.1$, CI 95 % = 0.7–1.7; complete treatment: $HR_{\text{crude}} = 1.1$, CI 95 % = 0.8–1.7). After controlling for ulcerous lesions, sex, and ulcerated nodules, nodules, and plaque, the trend of partial treatment became faster in terbinafine group, however this difference was not statistically significant but it was comparable. After controlling for ulcerous lesions, sex, and ulcerated nodules, papules, and plaque, the trend of complete treatment became relatively two times faster in terbinafine group and there was a statistically significant difference [P -value 0.018, HR 95 % CI 1.9 (0.5–2.4)]. Table 2 shows crude and adjusted HRs for partial and complete treatments.

Discussion

Our findings confirmed that the treatment rate was more effective in terbinafine group than the placebo group. However this rate depended on the type of lesions. As data indicated ulcerated nodules, papules, and plaque in experimental group have been completely improved two times faster than placebo group. Ulcerated nodules, nodules, and plaque were partially improved faster in those used terbinafine than placebo ointment.

The pharmacokinetic profile of terbinafine in the dermatological diseases has been well demonstrated specially

Table 2 Comparison of treatment response in placebo and terbinafine groups

Treatment response	Crude	
	HR (95 % CI)	P-value
Partial treatment (/no treatment)		
Crude	1.1 (0.7–1.7)	0.574
Adjusted for		
Ulcerous lesion	1.1 (0.8–1.7)	0.562
Sex	1.2 (0.8–1.7)	0.457
Shape ^a	1.2 (0.8–2)	0.390
Ulcerous lesion, shape, sex	1.3 (0.8–2.2)	0.233
Complete treatment (/no treatment)		
Crude	1.1 (0.8–1.7)	0.610
Adjusted for		
Ulcerous lesion	1.2 (0.8–1.7)	0.477
Sex	1.3 (0.8–1.9)	0.246
Shape ^a	1.6 (1.0–2.8)	0.069
Ulcerous lesion, shape, sex	1.9 (0.5–2.4)	0.018

^a Ulcerated nodules, nodules, and plaques

in fungal diseases. This drug is a lipophilic allylamine compound. terbinafine penetrates keratinized tissues, and enters the stratum corneum and sebum by direct diffusion through the dermis and living epidermis (Faergemann 1997, Faergemann et al. 1990).

Murine models of Chagas disease has been used to show the effectiveness of terbinafine against *Trypanosome cruzi* and the results suggested that terbinafine is effective on the parasite and it inhibits the ergosterol synthesis via the inhibition of squalene epoxidase (Ryder and Mieth 1992; Ryder 1992). Ergosterol is an essential component of parasite and fungal cell membranes. Furthermore, excess squalene damages cellular membranes and may cause the release of lytic enzymes from vacuoles (Ryder 1992). The ergosterol is found in large quantities in *Leishmania* cell membrane (Beach et al. 1979). Hence, it could be used in the treatment of other infectious diseases like leishmaniasis.

A combination therapy of terbinafine and ketoconazole has had effectiveness on leishmaniasis due to *Leishmania amazonensis* (Andrade-Neto et al. 2011). Our findings also supported this efficacy on *L. tropica*.

A pilot study has been done on 27 participants, showed that using terbinafine in the dose of 250–300 mg/day for 4 weeks could make 0–70 % improving rate. In which, complete treatment was 28.5 %, partial treatment was 43 %, and absence of treatment was 28.5 % (Bahadman and Tallab 1997). In Iran, our colleagues in Isfahan, has conducted a trial to compare the efficacy of Glucantime plus oral terbinafine with systemic Glucantime in the treatment of CL. They reported 88 % of improving rate that it might be due to longer follow up strategy (three months) and because of

using Glucantime which is effective in previous trials (Ebrahimian et al. 2011). In terbinafine group, we estimated the rate of complete, partial and no response to treatment to be 0.01, 0.01, and 0.10 per person every fortnightly, respectively. In comparison with mentioned studies we followed up the participants for 4.1–4.4 weeks.

The effect of terbinafine on *Leishmania* parasite has been experimentally reported from Saudi Arabia. The efficacy of terbinafine on *L. major* was compared between case and control groups and they reported that case group in which Balb/c mice treated with terbinafine had significantly smaller leishmanial lesions (Bahashwan 2011). This trial supported the findings.

The most strong point of terbinafine is relatively free of serious side-effects except for occasional reports of loss of taste, hepatitis, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, neutropenia, and pancytopenia (Ottervanger and Stroker 1992; Lowe et al. 1993; McGregor and Rustin 1994; Carstens et al. 1994; Kovacs et al. 1994). We confirmed this advantage because none of our participants complained about terbinafine side-effects.

The strengths of our study include a randomization, large sample size, and parasitological laboratory confirmation. However, larger sample size makes more generalization about topical terbinafine.

Further studies with at least 3 months follow up are recommended to evaluate the side effects and response. In addition, investigation in different endemic regions could help to estimate the efficacy of terbinafine in various species.

Conclusion

Our findings confirmed that clinically the treatment rate was more effective in patients received terbinafine than the placebo group. However this rate depended on the type of lesions. As data indicated ulcerated nodules, papules, and plaque in experimental group have been completely improved two times faster than placebo group. Ulcerated nodules, nodules, and plaque were partially improved faster in those used terbinafine than placebo ointment.

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