Efficacy of topical 5% imiquimod with cryotherapy versus intralesional meglumine antimoniate in the treatment of anthroponotic cutaneous leishmaniasis

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Conflict of interest: None to declare

Register code: K/88/58

Background: Cutaneous leishmaniasis (CL) is a major world problem. Several types of treatment regimens have been suggested. Imiquimod demonstrated a leishmanicidal activity by increasing local cytokine production. The aim of this study was to determine the efficacy of topical 5% imiquimod with cryotherapy vs. intralesional meglumine antimoniate (MA) in treatment of anthroponotic (dry type) CL.

Method: This is a prospective, randomized, open trial study (from Iran) from September 2008 to September 2010, including 50 patients (25 patients in the combined imiquimod and cryotherapy group and 25 patients in the intralesional MA group). Patients were randomly assigned to receive combined cryotherapy biweekly with imiquimod three times per week or intralesional MA weekly until complete cure or up to 12 weeks, whichever earlier. The primary end point was clinical cure, defined as complete re-epitelialization of 100%, complete flattening of induration compared with baseline at weeks 2, 6, 12 and follow up were done 1, 2 and 3 months after complete cure.

Results: 50 participants divided into 25 patients in group A and 25 patients in group B completed the study. Complete cure was 65.5% (16/24 patients) in group A and 83.3% (19/23 patients) in group B. No complication was detected in patients treated with MA. Pain and eczematous reaction were detected by 4 patients and local infection in 1 patient treated with imiquimod.

Conclusion: Although Meguimine antimoniate seems to be a more effective therapy for cutaneous leishmaniasis, this study revealed no significant difference in clinical response between combination of imiquimod and cryotherapy with intralesional MA in patients with cutaneous leishmaniasis in an endemic area of L. tropica.

Keywords: cutaneous leishmaniasis, imiquimod, cryotherapy, meglimine antimoniate

Received: February 22, 2011
Accepted: June 15, 2011

Iran J Dermatol 2011; 14: 42-47

INTRODUCTION

Cutaneous leishmaniasis (CL) is a major health problem, which is increasing in incidence [1]. Leishmaniasis is endemic in more than 60 countries worldwide [2]. More than 90 percent of CL occurs in Iran, Afghanistan, Syria, Saudi Arabia, Brazil and Peru [3].

Leishmaniasis is a disease caused by the protozoa of the heterogeneous Leishmania species, transmitted by the bite of a female sandfly and from the sub–family of phlebotominae [4].

CL caused by Leishmania tropica (anthroponotic, ACL in urban areas) or by Leishmania major

(zoonotic, ZCL in rural areas) is endemic in Iran [5,6].

CL initially starts as a papule at the site of a sandfly bite which then increases in size and eventually ulcerates. It may take 3-18 months to heal in over 90% of cases [7]. The incubation period lasts from 2 weeks to several months and cases up to 3 years have been reported [8,9]. CL is a self healing disease, but this can take months or even years [3]. Treating of CL will accelerate cure and reduce scarring and risk of transmission. This is especially important at cosmetically important sites [10].

To date, there is no vaccine against leishmaniasis and the available drugs are toxic, expensive and difficult to administer. Moreover, there are evidences of emerging resistance of the parasite to the commonly used drugs. Treatment of CL should be directed towards the eradication of amastigotes and reduction of the size of lesion with minimal scarring and possible toxicity. Several types of treatment regimens have been suggested for CL but until todays, there is no single treatment modality has been indisputably shown to be superior to others [11]. Options in the treatment of CL include intralesional injection as pentavalent antimony, hypertonic sodium chloride solution and zinc sulphate; topical treatments as paromomycine ointment, 5% imiquimod cream, topical amphotericin B; physical therapy as cryotherapy, localized controlled heat, Co_2 laser, photodynamic therapy; oral treatments as azoles, azithromycin, miltefosine, zinc sulphate and intramuscular or intravenous drugs such as systemic antimonials, pentamidine and amphotericin B [12].

Imiquimod is an imidazoquinoline amine that has been approved by Food and Drug Administration (FDA) as a 5% cream for external genital and perianal warts. Imiquimod is an immune response modifier that stimulates innate and adaptive immune pathways, resulting in antiviral, antitumor and immunoregulatory properties. Imiquimod induces cytokine production, most likely via activation of Toll-like receptor 7 (TLR7). Imiquimod is a stimulator of the innate immune response via the induction, synthesis of cytokines, such as IFN, IL6 and TNF [13].

Imiquimod demonstrated a leishmanicidal activity by inducing the expression of the inducible nitric oxide synthase (iNOS) gene and the release of nitric oxide [14]. Imiquimod also stimulates of the Th1 cytokine IFN. Imiquimod is generally well tolerated with the most frequent adverse reactions being mild to moderate inflammation with erythema, erosion, excoriation, flaking and edema [15].

In an open study of 12 patients with CL resistant to MA, Imiquimod in combination with MA cured 90% of the patients [16].

In a randomized double-blind clinical trial with use of Imiquimod, a 72 percent cure rate was observed when the cream was used in conjunction with MA in patients with CL who had failed to respond to antimony alone [17].

In this study, the efficacy of combination treatment with topical imiquimod cream and cryotherapy was compared with intralesional MA in a randomized, open trial clinical study.

PATIENTS AND METHOD

Participants

The study was done on patients aged between 5 to 65 years who had CL. The exclusion criteria were: chronic systemic disease such as renal failure, myocarditis, hepatitis and pancreatitis, immune suppression, breast feeding, pregnancy, sporotrichoid and lupoid forms, diameter of lesion >3 cm, disease duration >9 months, number of lesions >2, the past history of sensitization to MA or imiquimod, mucosal lesions and history of receiving other treatment in a recent month.

All the patients with positive smear or skin biopsy with positive Leishman body were enrolled in this study.

Participants, his or her guardian (patients younger than 18 years) were informed about the study and sign of consent form were taken.

Study setting and location

The study was carried out in the Kerman province of Iran which is an endemic area for ACL caused by L. tropica [18]. The eligible patients were recruited among patients with CL who were referred to the Dermatology Clinic and Leishmaniasis research center of Afzalipour Hospital of Kerman, Iran.

Intervention

Of 105 patients screened, 75 were entered the

treatment study. Patients were randomly allocated to one of two treatment groups. 39 Patients (24 female and 15 male) were enrolled in group A and 36 patients (21 female and 15 male) in group B (Figure 1).

Group A were treated with combined cryotherapy (biweekly) and 5% imiquimod (Aldara, 3M pharmaceuticals) cream 3 times per week.

Cryotherapy with liquid nitrogen was performed by dipstick technique. It consists of application of a saturated, cotton – tipped applicator on the lesion until 2-3 mm halo forms around it. The freeze time ranges between 10 and 25 seconds. This procedure was repeated every other week.

The patients were also treated with 5% imiquimod cream 3 times per week (Mondays, Wednesdays and Fridays). Imiquimod was provided as 250 mg sachet. A box of sachets was given to each patient, asking them to apply a thin layer of cream on lesions at bedtime, to massage it into the skin thoroughly and wash the lesion 6 to 10 hours after application with soap and water.

Group B patients were treated with intralesional MA weekly (Glucantime, 1.5 gram in 5 cc solution as ampule; Rhodia laboratories, Rhonepoulenc, France). First the lesions were cleaned by povidon iodine. Thirty or 27 gauge needle was used for injection. The solution was injected intradermally in each lesion from all directions until the lesion had completely blanched (0.5 -2cc per lesion per week, depending on the size).

In both groups, the procedure was continued

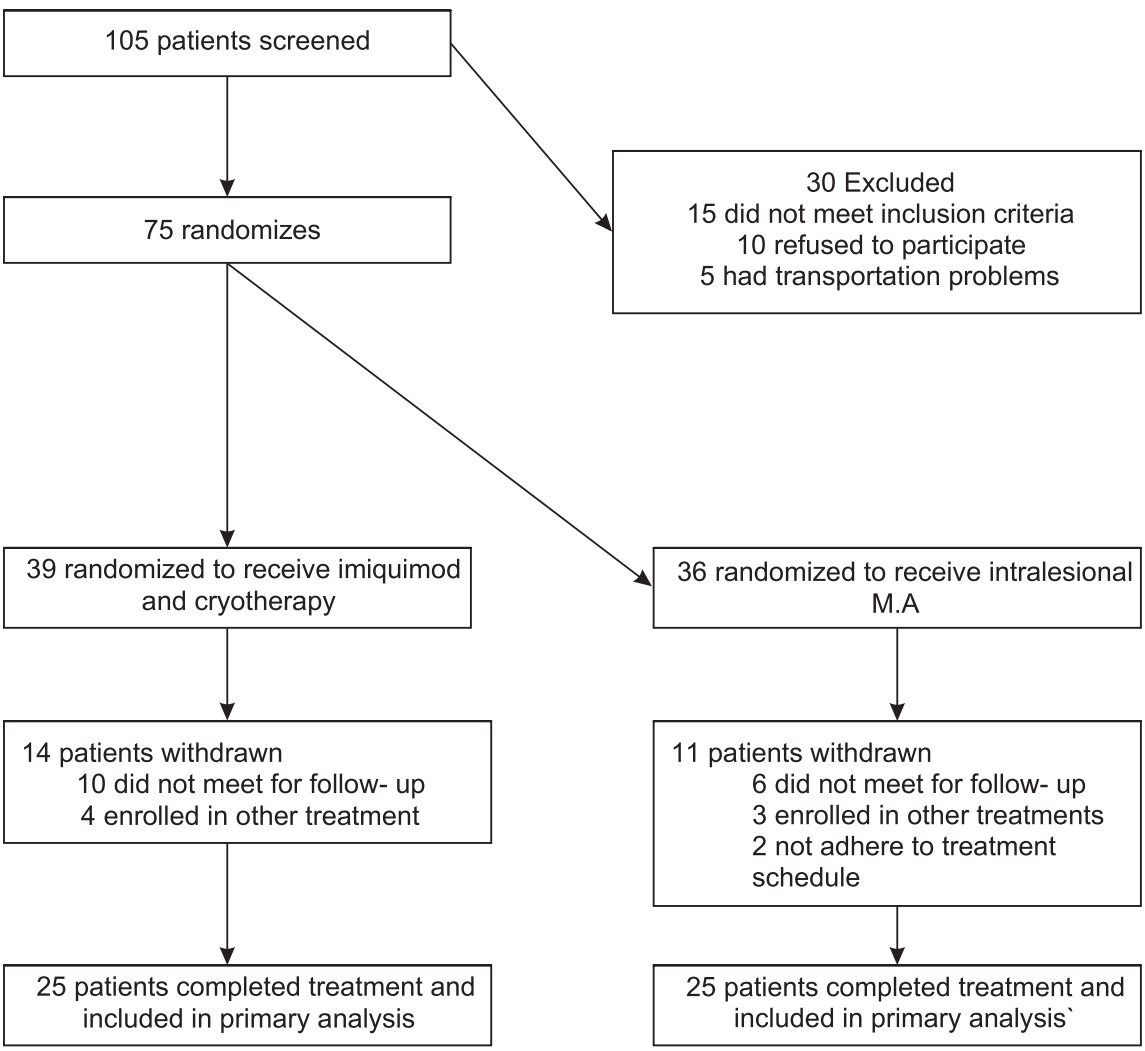

Figure 1. A total of 50 patients completed follow – up after two types of treatments.

until complete cure or up to 12 consecutive weeks, whichever was earlier. The patients were visited every week after initiation of treatment. Follow up evaluation was done by clinical assessment of treated lesions every month until 3 months.

Demographic information of the patients, characteristics of the skin lesions (such as induration, ulceration and edema) were registered by special designed observation recording form.

The induration size was defined as the greatest diameter of induration of the skin lesion in centimeter that was measured with the collis.

End points

The primary end point of this study was the clinical and laboratory cure of the lesions, defined as complete re-epitelialization of 100% (+/-scar), complete flattening of induration and negative smear of the lesions compared with baseline in each visit and also the time of completed cure.

The secondary end points included adverse-effects of two types of treatments and the relapse rate (defined as reappearance of lesions at the site or periphery of previously healed lesions or an increase in the size of lesions after initial improvement) that were assessed at months 1,2 and 3 after the end of treatment.

The proposal and consent form were reviewed and approved by the ethics committee of the center for research and training of Kerman University of medical sciences.

Statistical analysis

Data, expressed as Mean ± Standard deviation, were analyzed by SPSS software package (version11.5). Chi-square-test and t-test were used for determining the significance of difference between the two groups of treatment. Repeated measure model of ANOVA was used to determine the temporal variation of size of lesion during 12 weeks and the effect of treatment. The difference was considered significant when $p<0.05$.

RESULTS

A total number of 75 patients with mean age of 15.6±12.2 were entered in the study and 50 (19 male and 31 female) of them (responding rate 88%) completed the follow- up treatment.

The statistic analysis revealed no significant difference in respect to gender, age, location and type of the lesions between two randomized groups (Table 1).

Twenty four patients in group A and 23 patients in group B completed the treatment and had follow-up for twelve weeks. In group A, 16 of 24 patients (65.5%) responded to treatment while in group B, 19 of 23 patients (83.3%) responded to treatment and had complete cure. No difference was observed between two groups (P=0.16) (Table 2).

Repeated measure model of ANOVA showed that temporal variation for size of lesions was significant for both groups (p= 0.000) and no difference was observed in regard to type of treatment (p=0.57) (Figure 2).

Cure rate in week 6 and 12 seemed to be greater in group B; 13.3% (9/25) in group A, 35% (3/25) in

Table 1. Baseline Demographic and disease characteristics

Group Variable	M.A (N= 25)		Imiquimod + Cryotherapy (N=25)		P. Value
	%	No	%	No	
Sex					
Female	61.9%	15	62.5%	16	0.59
Male	38.1%	10	37.5%	9	
Site					
Upper limb	71.4%	19	28.2%	7	
Lower limb	4.8%	1	9.4%	2	0.04
Face	23.8%	5	62.6%	16	
Type of lesions					
Plaque or ulcerated	57.1%	14	31.3%	7	0.056
Nodule	42.9%	11	68.8%	18	

Table 2. Analysis of cure rate between 2 groups based on weeks of follow-up

Week	Group M.A	Imiquimod+ Cryotherapy	P. value
Two			
Cure rate	0%	0%	
Mean size of lesion	1.39 ± 0.38	1.76 ± 0.39	0.52
N	0	0	
Six			
Cure rate	35%	13.3	
Mean size of lesion	0.60 ± 0.29	0.94 ± 0.24	0.09
N	9	3	
Twelve			
Cure rate	83.3%	65.5%	
Mean size of lesion	0.05 ± 0.04	0.33 ± 0.11	0.16
N	19	16	

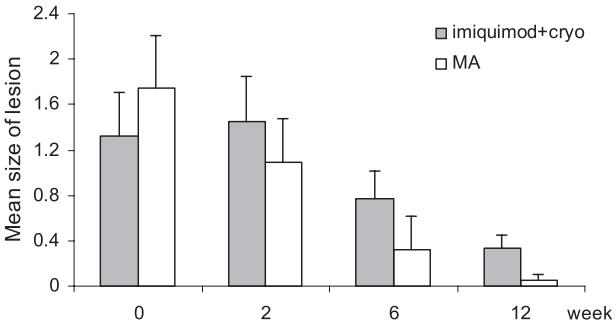

Figure 2. Variation of the size of lesion in 12 weeks of treatment

group B in week 6 and in week 12, 65.5% (16/24) in group A vs. 83.3% (19/23) in group B. But by using fisher exact test there was no significant difference between two groups in weeks 6 (P=0.09) and 12 (P=0.16) of treatment (Table 2). Three months after the end of treatment, relapse was observed in 2 of 25 patients treated with imiquimod and in 3 of 25 patients treated with MA.

The only adverse effects related to topical treatment were pain and eczematous reaction in 4 patients and local infection in 1 patient treated with imiquimod and they were minimal and most of them were treated by non–steroidal anti-inflammatory drugs (NSAID), topical steroid and topical antibiotic.

DISCUSSION

Although CL is a self-healing disease, it is recommended for patients with ACL to receive treatment because of the prolonged course, potential scar formation and role of infected humans as reservoir [19].

Unfortunately, no ideal therapy for CL is available, and its treatment has been remained a challenge. Pentavalent antimonials remain the mainstay of treatment [5]. However, a high rate of adverse events, length of treatment, and relapses in up to 25 percent of cases highlight the limitations of these drugs [3].

In this clinical trial, combined imiquimod and cryotherapy was compared with intralesional MA in the treatment of dry type CL.

Imiquimod is an immune response modifier that increases local cytokine production, with a subsequent activation of both the innate (rapid, nonspecific) and adaptive (specific, cellular and humoral) immune systems [20].

In this study, we did not observe significant difference in clinical response between two therapeutic methods (65/5% in group A vs. 83.3% in group B).

In previous study in Iran (Kerman), ninety-nine patients with biopsy-confirmed CL were enrolled in an open label study. After 40 days of treatment, there was a response rate in 23%, 35% and 37% in weekly intralesional MA (n=35), imiquimod (n=29), and combination treatment group of imiquimod 5% cream and intralesional meglumine antimoniate (n= 35), respectively, indicating a better response in patients with combination of intralesional MA plus imiquimod cream compared with patients treated with MA [21]. In contrast to this study, clinical response in our imiquimod group was higher, 65.5% versus 35%, which this difference may be due to cryotherapy combined with imiquimod.

Miranda et al, recruited 40 patients with clinical resistance to antimony in Peru. All patients received MA (20mg/kg/d intramuscular or intravenous) and were randomized to receive either topical 5% imiquimod cream or placebo as control every other day for 20 days. Lesions resolved more rapidly in the imiquimod group. The cure rate in the imiquimod- treated group was 50% at one month (vs. 15% in the placebo group), 61% at 2 months (vs. 25%), and 72% at 3 months (vs. 35%) (P<0/05 at all time points) [17]. This study was performed in some parts of Peru, that were endemic for the new world CL, but our study was conducted in an endemic area of old world CL caused by L. tropica. All patients in the Miranda study, previously has been treated with MA (intramuscular or intravenous), but none of the patients in our study were treated previously with MA.

In a study in Mashhad (Iran), Firooz et al treated 59 patients with Imiquimod and intramuscular MA (20 mg/kg of pentavalent antimony daily for 2 weeks) and the control group was treated with placebo and intramuscular MA. This study revealed no beneficial effect of combining a 4 week course of treatment with 5% imiquimod cream and a standard course of treatment with MA in patients with CL in an endemic area of L. tropica [19].

In Firooz et al study, patients were treated with combined MA (intramuscular) and imiquimod, but in our study patients were treated with imiquimod and cryotherapy and in control group patients

received MA intralesionally. This may be explained the diversity of responses between two studies.

Our results thus demonstrated that topical application of imiquimod with cryotherapy has the less significant effect in comparison with MA alone for the treatment of Old World CL. This therapy may have particular advantages for cases with facial lesions and for children, because intralesional injection with pentavalent antimony is a relatively painful procedure which needs to be performed regularly every 1-2 weeks, but imiquimod is well tolerated.

Clinical trials for CL are usually confronted due to differences in the design, duration of treatment, sample size, end point definition, causative organisms, etc. It seems that combination therapy has important place for treatment of CL. Imiquimod can be one of this combination because of immunomodifying effect in regard to pathogenesis of CL. Further studies are needed to evaluate this effect.

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