Brief Report

Chitosan-based biocompatible dressing for treatment of recalcitrant lesions of cutaneous leishmaniasis: A pilot clinical study

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Abstract

Background: Chitosan has a biocompatible, biodegradable and nontoxic nature. The effectiveness of nano-chitosan films in the treatment of cutaneous leishmaniasis has been confirmed previously in susceptible laboratory animals.

Aims: The aim of this study is to evaluate the safety and efficacy of a chitosan-based biocompatible dressing in patients with cutaneous leishmaniasis who were either nonresponsive to or had medical contraindications for conventional treatments.

Materials and Methods: A total of 10 eligible patients were included in this single arm, single center study. The sterile chitosan film was immersed in saline serum and was cautiously extended over the wound to avoid air occlusion. Sterile Vaseline gauze was then applied and the film was kept on the wound site for 7 days and was repeated every week until the healing was completed. Complete clinical response was defined as complete re-epithelialization of the skin lesion as well as microscopic negative results for amastigote forms of *Leishmania* sp.

Results: All patients showed either significant (30%) or complete (70%) improvement after 8 weeks of therapy and at 16 weeks post treatment all cases were completely cured. It was well tolerated and there were no product-related adverse events such as allergic reaction or infection. Moreover, no recurrences were observed in any patients after 6 months follow-up.

Limitations: The lack of a control group, relatively small sample size and failure to evaluate the histological and molecular effects of chitosan were the limitations of this study.

Conclusion: Our findings confirmed that chitosan can be safely and effectively used for the treatment of cutaneous leishmaniasis. We were unable to find any previous clinical study in evaluating the efficacy of chitosan for cutaneous leishmaniasis on human subjects. Further studies are recommended to design a randomized, double-blinded clinical trial with more volunteers who infected with different species of *Leishmania* and various clinical forms of cutaneous leishmaniasis.

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Key words: Biocompatible materials, chitosan, cutaneous leishmaniasis, dressing, humans, topical treatment

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Introduction

Cutaneous leishmaniasis is one of the most important health problems worldwide particularly in tropical regions.[1-3] It is estimated that more than 90% of patients with cutaneous leishmaniasis are living in Iran, Syria, Afghanistan, Saudi Arabia, Brazil and Peru.[4] *Leishmania major* and *Leishmania tropica* are the most common pathogens in Iran with variable clinical presentations.[5-7] Zoonotic cutaneous leishmaniasis caused by *L. major* is endemic in many rural foci in the north-east, center and south parts of Iran.[8] Anthroponotic cutaneous leishmaniasis caused by *L. tropica* is the most frequently found in large and medium sizes of Iranian cities such as Kerman.[3,9,10]

Owing to disfiguring nature of lesions and scar formation that may lead to lifelong social stigma, treatment is considered imperative.[11]

Pentavalent antimonial derivatives are the first-line treatment in many cases of cutaneous leishmaniasis,[12] and other medications such as fluconazole have been used with variable success.[13] Unfortunately, in recent years antimonial treatment failure has been reported in 10–12% and 16% of anthroponotic and zoonotic cutaneous leishmaniasis cases in Iran, respectively, because the prevalence of parasite resistance to this treatment is increasing.[14-16] It signifies the crucial need to develop new, safe and accessible drugs, especially in particular patient's populations.[14,17]

Topical treatments are favorable options in some patients because of a better compliance and lower systemic toxicity and costs.[18] Topical agents should be easy to apply, improve parasite elimination and prevent relapse or scar formation. These modalities comprise intralesional antimony, cryosurgery and others; however, there are limited evidence about the safety and effectiveness of some modalities.[18,19] Chitosan, a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine, is derived from chitin with a biocompatible, biodegradable and nontoxic nature.[20] Various studies have demonstrated its effectiveness in reducing pain and inhibiting the growth of microorganisms.[21,22] The effectiveness of nano-chitosan films in the treatment of cutaneous leishmaniasis caused by *L. major* has been demonstrated in an animal study.[17]

The current study was designated for evaluation of a chitosan-based dressing (IPPISKIN) in patients with cutaneous leishmaniasis who are either nonresponsive to or have medical contraindications for conventional treatments.

Materials and Methods

Study design

This study was a clinical study for evaluation of the safety and efficacy of a chitosan-based biocompatible dressing in 10 patients with cutaneous leishmaniasis who are either nonresponsive to or have medical contraindications for conventional treatments. This study took place at the dermatology clinic, Shohada-e-Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran from January 2009 to February 2013.

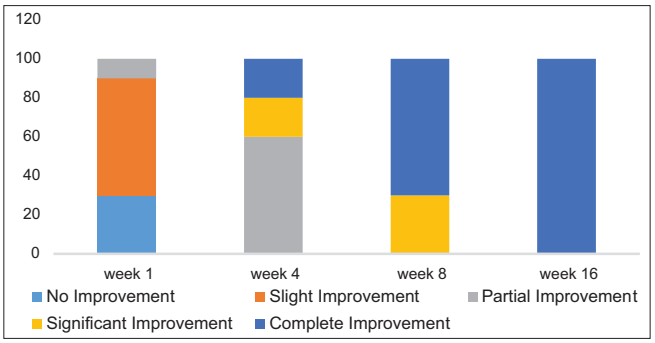

Figure 1: The status improvement of the lesions 1, 4, 8 and 16 weeks after treatment

Patients

A total of 10 eligible patients (5 females and 5 males, mean number of lesions ± SD: 5.3 ± 5.1) were included. The diagnosis of cutaneous leishmaniasis was confirmed both clinically and parasitologically (by direct smear and/or skin biopsy). Samples were prepared from the skin lesions of suspected patients, fixed with absolute methanol and stained with Giemsa 10%. For confirmation of Giemsa-stained smears prepared from the lesion (s) and, if required, culture in RPMI 1640 and NNN media were done. Infection was confirmed by demonstration of *Leishmania* amastigotes in the stained smears by light microscope with a high magnification (1000×).

A questionnaire was completed for each patient.

Drug administration

The material used in our study was poly (vinyl alcohol)/chitosan/clay nanocomposite film that was first introduced by Mahdavi *et al*.[23]

The sterile chitosan films were kept in aluminum-sealed bags. After opening, they were immersed in saline serum in order to hydrate the film and make it pliable. The film was cautiously extended over the wound to avoid air occlusion. Sterile Vaseline gauze was then applied and the films were kept on the wound site for 7 days. No further treatment was performed. This dressing was repeated every week until the healing was completed.

Evaluation of tolerability

The tolerability (occurrence of local adverse events such as infection, irritation, edema, odor and excessive granulation tissue) and acceptability of the dressing were evaluated qualitatively and documented during each dressing change. Acceptability parameters comprised ease of application and removal (very easy, easy, difficult and very difficult), adherence of the dressing to the wound bed (none, minimal, moderate, high and very high) and

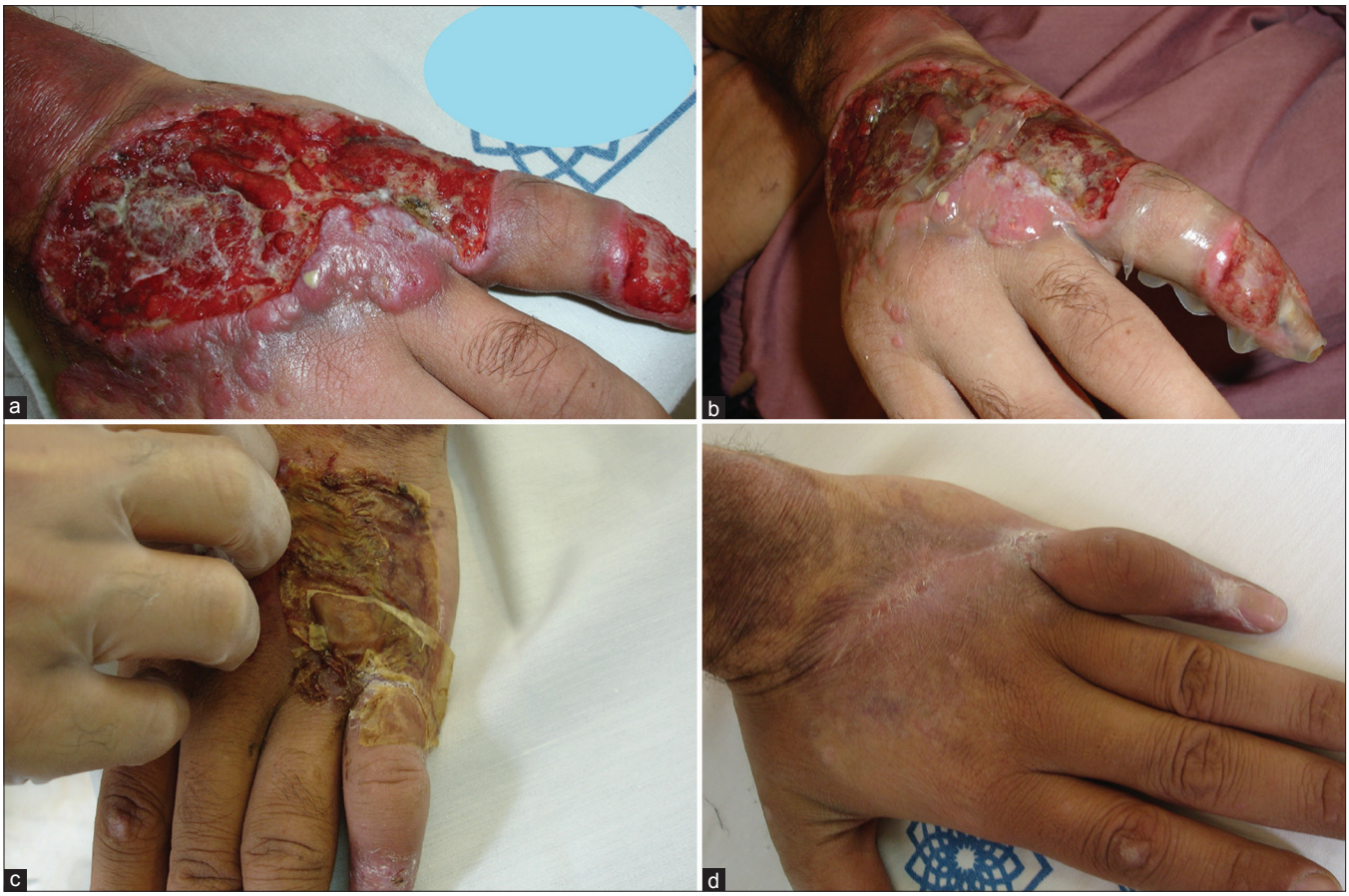

Figure 2: A chronic wound on the dorsal hand of a renal transplant patient with cutaneous leishmaniasis. (a) The baseline image. (b) Treatment with IPPISKIN dressing. (c) 8 weeks after treatment. (d) Complete wound healing at the 16th week

bleeding or pain at removal (none, minimal, moderate, high and very high).

Evaluation of effectiveness

The lesions were evaluated and photographed every week during the treatment course by a dermatologist who did not participate in performing the procedure or analyzing of the data. All of the lesions were evaluated at the 1st, 4th, 8th and 16th weeks during treatment.

Clinical outcome was defined as follows: (1) Complete improvement (full re-epithelialization of the lesion as well as microscopic negative results for amastigote forms of *Leishmania* sp.); (2) significant improvement (>75% decrease in size); (3) partial improvement (50–75% decrease in size); (4) slight improvement (25–50% decrease in size) and (5) no improvement (<25% decrease in size). The study endpoint—the time needed for complete improvement—was also documented for each patient and a 6-month follow-up was performed.

Ethical approval

This study was reviewed and approved by the Ethics Committee of our institute and informed consent was sought from the patients or their parents according to legal requirements.

Data analysis

Descriptive statistical analyses were performed with SPSS 21 software (Chicago, IL, USA). The numeral data were represented as a mean ± standard deviation and categorical data as frequency and percentage with a probability (*P*) value of <0.05 as statistically significant.

Results

A total of 10 eligible patients were included in this single arm, single center study. All of the subjects had cutaneous leishmaniasis for more than 12 months. After 4 months of follow-up, all of them showed complete improvement with minimal or no scar formation. The demographic features and other clinical data are demonstrated in Table 1.

The most common sites of the lesions were the extremities. One patient had disseminated disease with the total number of 16 lesions. The most persistent lesion was observed in a 16-year-old adolescent who had facial leishmaniasis for 13 years. Seven patients had received conventional treatments including intralesional (±cryotherapy) or intramuscular antimoniates, oral azoles and miltefosine before their presentation in our clinic but their disease was persistent. The other three were included due to the comorbidities that made other treatments contradictory (renal transplantation in one and renal dialysis in two cases).

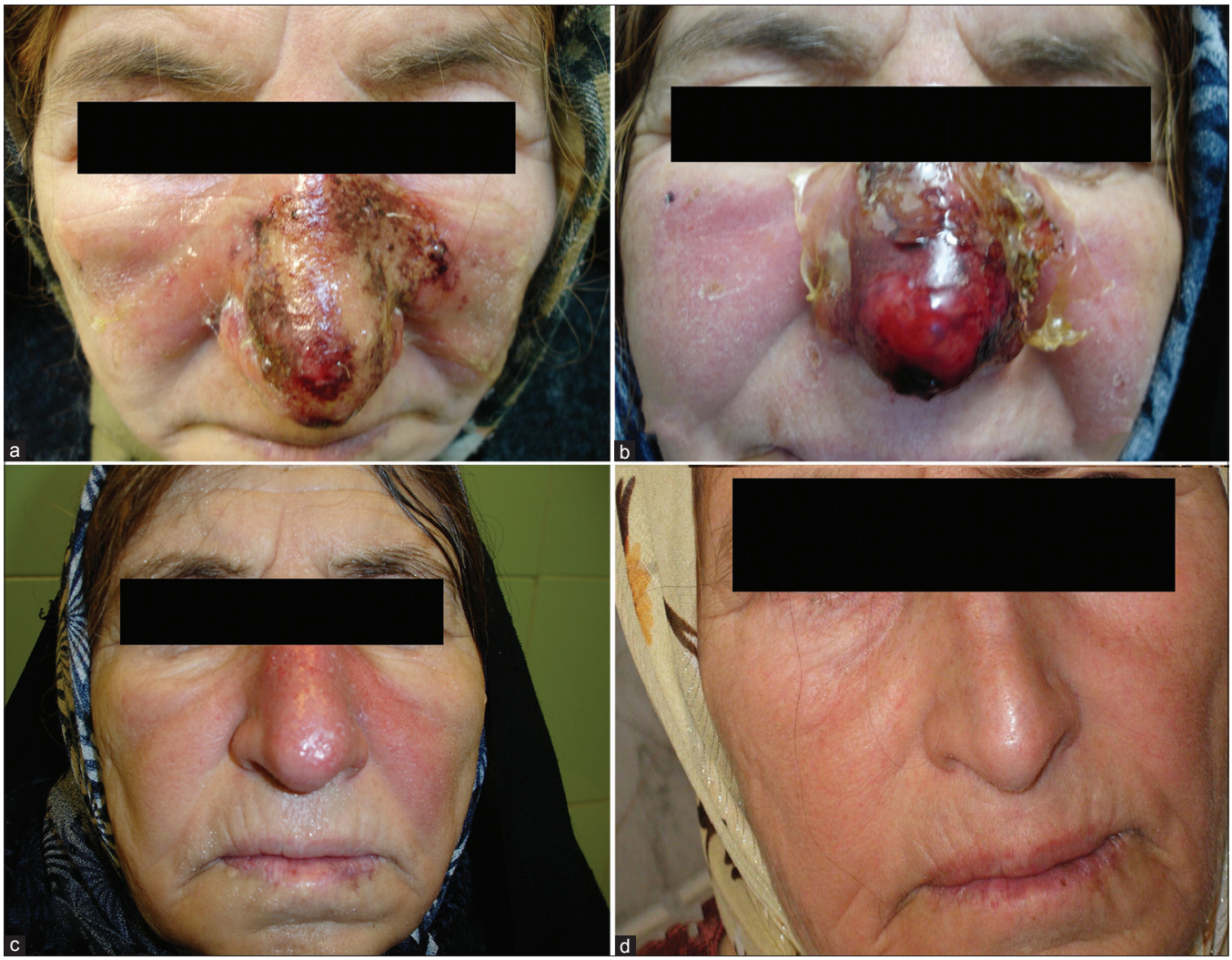

Figure 3: Cutaneous leishmaniasis on the nose. (a) The patient's wound at baseline. (b) Treatment with IPPISKIN dressing. (c) Two weeks after treatment with a significant improvement. (d) The subject's wound was fully improved at 4 weeks

All of the patients showed either complete or significant improvement after 8 weeks of therapy [Figures 1-4]. The mean day before complete improvement was 57.5 ± 33.5 (21–120 days). The subject with disseminated disease had the longest duration of treatment before complete improvement of all lesions. There were no product-related adverse events. Dressing application and removal was reported "very easy" and "easy" in 90 and 10% of cases, respectively. There was no pain or bleeding at dressing removal, owing to minimal–moderate adherence to the wound bed.

None of the patients had recurrences after 6 months follow-up.

Discussion

Our findings confirmed that chitosan can be safely and effectively used for the treatment of cutaneous leishmaniasis in patients who are not good candidates for systemic drugs or nonresponding cases.

Current treatments for leishmaniasis have been considered to have low approval, not only due to the high toxicity of the products but also due to the increasing resistance to present drugs. Topical therapy such as topical liposomal amphotericin B, is a promising treatment due to minimal toxicity and satisfactory efficacy.[18,19]

There is compelling evidence for the beneficial effect of chitosan in several tissue injuries both in human and animal studies because of its attractive properties, including easy film formation, biocompatibility, tending to retain moisture and being biodegradable.[24] Recently, there have been many studies on the antifungal and antimicrobial properties of chitosan,[25,26] and special attention has been paid to chitosan nanoparticles containing drugs that have great potential for the treatment of various diseases, including leishmaniasis, malaria and cryptosporidiosis.[27] Besides, various chitosan derivatives have been evaluated for wound healing.[28]

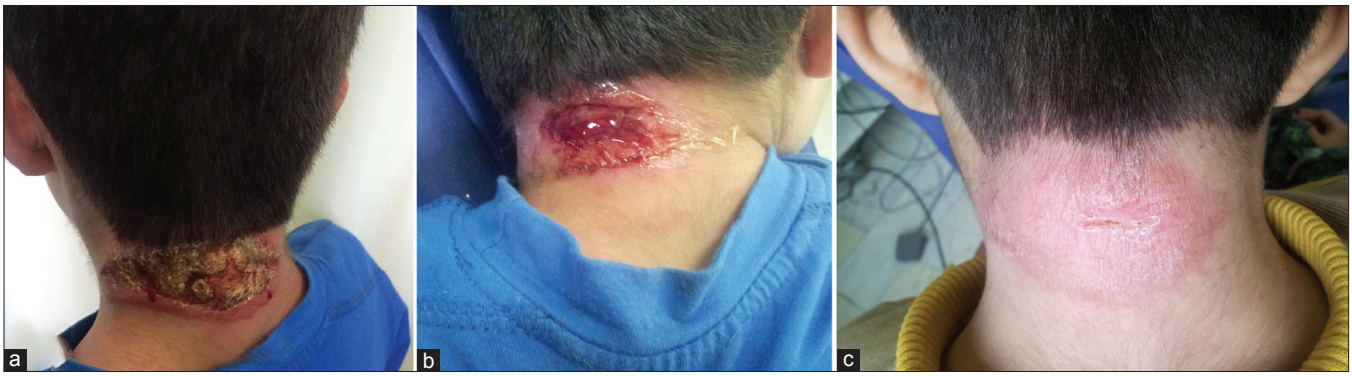

Figure 4: A chronic wound on the neck of a child with cutaneous leishmaniasis. (a) The baseline image. (b) Partial improvement 4 weeks after treatment with IPPISKIN. (c) Complete improvement after 8 weeks

Table 1: The demographic features and clinical data of the patients

Characteristics	
Age (year)	
Mean±SD	48.6±23.8
Range	11-75
Sex (*n*)	
Male	5
Female	5
Comorbidities, *n* (%)	
CKD	3 (30)
Diabetes	3 (30)
Hypertension	3 (30)
None	3 (30)
Number of lesions	
Mean±SD	5.3±5.1
Range (*n*)	1-16
Distribution, *n* (%)	
Face and neck	2 (20)
Extremities	5 (50)
Trunk and extremities	2 (20)
Disseminated	1 (10)
Previous treatments, *n* (%)	
Antimonates IM	2 (20)
Miltefosine*	2 (20)
Cryotherapy	1 (10)
Antimoniates IL plus oral azole	1 (10)
Antimoniates plus cryotherapy	3 (30)
None	3 (30)

*Also these two patients had history of treatment with IM antimoniates.
CKD: Chronic kidney disease, IL: Intralesional, IM: Intramuscular

Mechanisms of chitosan's wound healing acceleration have been investigated by some *in vitro* and *in vivo* studies, but it needs to be further elucidated.[29] Nevertheless, several mechanisms have been proposed, such as enhancing the function of polymorphonuclear leukocytes, macrophages and fibroblasts, and therefore, promoting granulation and organization.[30] Interestingly, recent studies have shown that chitosan and chitin have antileishmanial activities in addition to promote re-epithelialization. In a study conducted on animals, Hoseini *et al*. found that chitinous microparticles have a significant activity against *L. major* with induction of cell proliferation and tumor necrosis factor-α and interleukin-10 production and could be considered as a new therapeutic modality in leishmaniasis.[29]

A study by Bahrami *et al*. on the effect of nano-chitosan films on the healing of skin wounds caused by *L. major* in mice revealed that nano-chitosan film enhanced the wound contraction rate, re-epithelialization and reduced scar formation. In addition, combination of meglumine antimoniate with chitosan film significantly reduced lesion size and parasite load.[17] In another study, Danesh-Bahreini *et al.* prepared chitosan nanoparticles containing L*eishmania* superoxide dismutase to develop a new nanovaccine for leishmaniasis. They demonstrated that formulation of superoxide dismutase in biodegradable and stable chitosan nanoparticles increases the immunogenicity toward cell-mediated immunity. Therefore, it was proposed that it might be effective in leishmaniasis prevention and control.[31]

As it has been mentioned before, recent studies have focused on the use of chitosan as a drug carrier. Several studies have demonstrated the effectiveness of various drugs loaded on chitosan for the treatment of leishmaniasis.[32-35]

Conclusion

None of the studies mentioned above were performed on human subjects. Our study is novel from this aspect. However, there are some limitations to our study such as the lack of a control group, relatively small sample size and failure to evaluate the histological effects of chitosan. Further studies are recommended to design a randomized, double-blinded clinical study with more volunteers infected with known *Leishmania* species and various clinical forms of cutaneous leishmaniasis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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