

Interleukin-10 and Interferon- γ Levels in Patients with Cutaneous Leishmaniasis Treated with Cryotherapy

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Abstract

Pentavalent antimonials, as the 1st choice for the treatment of cutaneous leishmaniasis (CL), have various side effects. Also, there are some reports of drug resistance. Due to its safety, cryotherapy can be a good alternative or complementary treatment in CL. The aim of this study was to explore the possible systemic immunological mechanisms of cryotherapy besides its local effects in the treatment of CL. Twenty patients with CL were selected. The disease was confirmed via a direct smear. A venous blood sample was collected to determine IL-10 and IFN- γ levels before starting cryotherapy. Then, 1 week after 8 sessions of cryotherapy were completed (i.e., 63 d), a 2nd venous sample was taken in order to compare the results with the pretreatment levels of these cytokines using the ELISA method. Cryotherapy resulted in no change in the levels of IL-10 and significantly increased the IFN- γ levels in our patients with CL. Given these *inconclusive* or even *mixed* results, a larger sample size is needed in order to better assess the systemic immunological effects of cryotherapy.

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What's Known

- In general, it is known that the clinical cure for cutaneous leishmaniasis by systemic drugs is associated with a Th1 response, characterized by increased INF- γ and a Th2 response by decreased IL-10.
- Killing the parasite with local freezing is a conventional mechanism of cryotherapy.

What's New

- Cryotherapy caused no change in the levels of IL-10 and significantly increased IFN- γ in cutaneous leishmaniasis patients.
- Larger sample-sized studies are required to assess the systemic effects of cryotherapy on IFN- γ and other key cytokines

Introduction

Leishmaniasis has a worldwide distribution. Old World cutaneous leishmaniasis (CL) is usually due to *Leishmania tropica* (*L. tropica*), *L. major*, *L. infantum*, and *L. aethiopica*. Both susceptibility to infection and delayed resolution of leishmaniasis have been related to an inadequate Th1 response,^{1,3} the latter playing an important role in cell-mediated immunity. The secretion of interferon- γ (IFN- γ) and interleukin-2 (IL-2) are associated with a resolving infection.¹

IFN- γ is the most potent cytokine involved in the induction of cidal activity against phagocytized organisms. It induces the production of oxygen species and activates the Th1 system. This process is also activated by IL-12.^{1,3,4} Tumor necrosis factor- α (TNF- α) is also important for the control of *leishmania* infections.⁵ It is produced by activated macrophages and natural killer cells and it amplifies the macrophage activation triggered by IFN- γ .

Old World CL is often a self-limiting disease. Pentavalent antimonials are the standard therapy for this disease. Common associated side effects of this group of drugs are cardiotoxicity,

pancreatitis, thrombocytopenia or hepatitis, and rarely, acute renal failure. Meglumine antimoniate and sodium stibogluconate are the 2 most commonly used antimonials. Other treatments include cryotherapy, heat therapy, pentamidine, azoles, amphotericin B, allopurinol, and paromomycin.

Cryosurgery is a modality that is used for the treatment of many cutaneous lesions. A study in Jordan showed that cryotherapy at weekly intervals for 1 to 4 sessions was effective in treating CL due to *L. major*, especially for smaller lesions.⁵ Another study in Sri Lanka showed that the compliance rate for cryotherapy was 40%. Frequent hospital visits, long course of treatment, limited availability, and side effects were reasons for this poor compliance.⁶ A study compared the efficacy of cryotherapy with that of intralesional antimonials for the treatment of CL in children and reported that cryotherapy was an effective treatment without any important post-treatment side effects.⁷ Another investigation demonstrated that combined cryotherapy and intralesional meglumine antimoniate was more effective than either cryotherapy or intralesional meglumine antimoniate alone for the treatment of CL.⁸ Another study showed that the combination of cryotherapy with intralesional injections of antimonials was much more effective than the use of intralesional meglumine antimoniate alone.⁹ Elsewhere, investigators revealed that cryotherapy could be an alternative to intralesional pentostam injections.¹⁰

In general, the clinical cure for CL has been associated with a Th1 response characterized by increased IFN- γ and decreased IL-4 and IL-10. Increased expressions of IL-10 and transforming growth factor- β in chronic lesions of human CL were reported in another study.¹¹ Heat therapy for CL has been shown to elicit a systemic cytokine response similar to that of antimonial therapy.¹² The role of IL-10 in the persistence of *L. major* was investigated in a study by Belkaid et al.¹³ Anti-IL-10 antibody protection against visceral leishmaniasis was shown in another study.¹⁴ Murray et al.¹⁵ demonstrated that the deactivating effects of IL-10 regulated the outcome in experimental visceral leishmaniasis and that IL-10 receptor blockade represented a potential immunochemotherapeutic approach in this infection.¹⁵

The aim of the present study was to explore the possible systemic immunological mechanisms of cryotherapy besides its local effects by measuring IFN- γ and IL-10 levels (Th1 and Th2 responses, respectively) in the serum of patients with CL.

Patients and Methods

The present cross-sectional study was conducted at the Molecular Dermatology Research Center, Shiraz University of Medical Sciences, from September 2012 to May 2013. Twenty Old World CL patients who were diagnosed via a *leishmania* skin smear and who met all the inclusion and exclusion criteria were selected consecutively from a pool of suspected CL patients who had referred to the clinic.

The criteria for inclusion were comprised of age ≥ 14 years, having ≤ 6 cutaneous lesions and none > 10 cm, having no signs and/or symptoms of mucous membrane involvement, having no previous history of leishmaniasis, and receiving no specific *leishmania* treatment. Patients with impaired sensation, hypersensitivity to cold, and impaired circulation, as well as patients with angina pectoris, all of which are considered contraindications for cryotherapy, were excluded from the study.

All the patients signed an informed consent form, and the study was approved by the Ethics Committee of Shiraz University of Medical Sciences.

Physical examination of the patients included visualization of the nasal and oropharyngeal mucosa and a complete lymph node examination.

The skin lesions were documented in detail, including their number, location, and size (expressed in mm, great axis+small axis/2). The duration and clinical evidence of bacterial superinfection was noted, and a photograph of the lesion was taken. The study was designed to measure IL-10 and IFN- γ levels in the CL patients before and after treatment with cryotherapy. A venous blood sample (10 mL) was collected to determine baseline IL-10 and IFN- γ levels before the commencement of cryotherapy. Subsequently, at 1 week after the completion of 8 sessions of cryotherapy (day 63), a 2nd venous blood sample (10 mL) was drawn in order to compare the results with the pre-treatment levels. The design of the study took into consideration that it is unethical to treat the patients just for 8 weeks in the event that there was no complete cure. Hence, the treatment and follow-up of the patients was continued until the complete improvement of the lesions, even if it took several months.

Cryotherapy was performed for 8 sessions on a weekly basis. The lesion was 1st washed with saline. Then, the open-spray method was used, which consisted of a cryosurgical unit, liquid nitrogen, and spray-tip attachments. A fine spray of liquid nitrogen was directed at the lesion from a distance of approximately 1 to 2 cm. An

intermittent spray of liquid nitrogen was used for deeper lesions in order to achieve a deeper freeze. The lesion was thereafter covered with a gauze bandage. No additional treatment was administered.

Human IFN- γ and human IL-10 were measured in the patients' sera using the Platinum ELISA (eBioscience, USA).

The current study was a before-and-after study, and descriptive statistics were applied for the analysis of its results. The data analyses were done using SPSS (19 student version; SPSS Inc., Chicago, IL, USA). The Wilcoxon signed-rank test was used to verify differences between the cytokine levels before and after cryotherapy. Effect size was utilized for quantifying the size of the difference between the 2 groups with and without the deletion of some data.

Five (25%) male and 15 (75%) female patients participated in this study. Overall, 13 (65%) CL patients had the rural (wet) form and 7 (35%) had the urban (dry) form of the disease. The mean level of IL-10 was 3.63 pg/mL before cryotherapy (SD=6.16) and 0.36 pg/mL after cryotherapy (SD=0.54). The mean level of IFN- γ was 1.033 pg/mL before cryotherapy (SD=1.08) and 2.566 pg/mL after cryotherapy (SD=1.94). The decrease in the levels of IL-10 ($P=0.031$) and the increase in the levels of IFN- γ ($P<0.001$) in the CL patients treated with cryotherapy were statistically significant.

Since the amounts of IL-10 from 3 patients were very high, we calculated the P value and effect size in 2 different situations, with and without the extreme values of IL-10 in the 3 mentioned patients (tables 1 and 2). By calculating the effect size, we intended to seek clinical correlations from our analysis in addition to statistical associations. Cohen has classified effect sizes as small ($d\geq 0.2$), medium ($d\geq 0.5$), and large ($d\geq 0.8$).

Calculation using the extreme values of IL-10 in the 3 patients showed a significant P value of 0.023 ($P<0.05$). The calculated effect size was 4.854 with an SD of 5.8 for this group and was analyzed as large and significant (effect size >0.8). However, with the deletion of the extreme values of IL-10, the P value was calculated as 0.75 with an SD of 2.8, which was insignificant ($P>0.05$). The calculated effect size was 0.378 for this group, which was considered small and trivial.

From what has been discussed above, after the removal of the 3 patients' data, the P value and effect size for the IL-10 levels were insignificant statistically and clinically.

Table 1: Demographic data and IL-10 serum levels of cutaneous leishmaniasis patients before and after cryotherapy with the extreme values of IL-10 in 3 patients (n=20)

Patient	IL-10 Before (pg/mL)	IL-10 After (pg/mL)	Gender	Age
1	0.18	0.00	Female	29
2	23.00	0.65	Female	28
3	1.50	0.77	Female	27
4	0.00	0.25	Female	18
5	0.02	0.00	Male	37
6	0.00	0.37	Female	15
7	0.00	0.40	Female	30
8	12.37	1.79	Female	20
9	0.10	0.00	Male	44
10	7.05	0.00	Male	50
11	3.04	0.00	Female	35
12	0.00	0.03	Female	16
13	0.00	0.00	Female	27
14	1.00	0.25	Female	18
15	2.85	0.00	Female	75
16	0.00	0.09	Female	31
17	0.00	0.00	Male	24
18	11.83	1.72	Female	24
19	9.64	0.16	Male	45
20	0.00	0.74	Female	38

Table 2: Demographic data and IL-10 serum levels of patients with cutaneous leishmaniasis before and after cryotherapy without the extreme values of IL-10 in 3 patients (n=7)

Patient	IL-10 Before (pg/mL)	IL-10 After (pg/mL)	Gender	Age
1	0.18	0.00	Female	29
3	1.50	0.77	Female	27
4	0.00	0.25	Female	18
5	0.02	0.00	Male	37
6	0.00	0.37	Female	15
7	0.00	0.40	Female	30
9	0.10	0.00	Male	44
10	7.05	0.00	Male	50
11	3.04	0.00	Female	35
12	0.00	0.03	Female	16
13	0.00	0.00	Female	27
14	1.00	0.25	Female	18
15	2.85	0.00	Female	75
16	0.00	0.09	Female	31
17	0.00	0.00	Male	24
19	9.64	0.16	Male	45
20	0.00	0.74	Female	38

Discussion

Cryotherapy appears to be a reasonable alternative in the treatment of CL insofar as it is effective and usually requires a limited number of applications. In addition, cryotherapy can be

easily performed in the field. Killing the parasite with local freezing is a conventional mechanism of cryotherapy. However, to our knowledge, there is no study on the possible systemic effects of cryotherapy to date.

Belkaid et al.¹³ investigated the persistence of *L. major* in genetically resistant C57BL/6 mice after the healing of their dermal lesions. IL-10 was shown to play an important role in parasite persistence. There are a few studies on the importance of decreased IL-10 levels in the treatment of leishmaniasis. Nonetheless, in a study on heat therapy, Lobo et al.¹² found a decrease in IL-5, IFN- γ , and TNF- α levels after heat therapy and concluded that heat therapy for CL elicited a systemic cytokine response similar to that of antimonial therapy as the 1st line therapy in the treatment of CL. Bhattacharjee et al.¹⁴ demonstrated that the in vivo neutralization of IL-10 by anti-IL-10 antibodies (mAb) was able to confer protection against leishmaniasis. Murray et al.¹⁵ demonstrated that the deactivating effects of IL-10 regulated the outcome in experimental visceral leishmaniasis and that IL-10 receptor blockade represented a potential immunochemotherapeutic approach in this infection. These studies prompted the authors of the present study to seek possible systemic cytokine alterations or effects after cryotherapy. Whether the alteration in the cytokine response is a direct result of cryotherapy is not known.

The results from the current study showed that after removing the extreme values of IL-10 in 3 patients, the effect size was reduced considerably. In other words, cryotherapy had no effect on the IL-10 levels, while the INF- γ levels increased significantly after cryotherapy.

Financial constraints precluded us from selecting more than 20 patients for this research. More studies with larger sample sizes and with measurement of these 2 and, indeed, more key cytokines are recommended to confirm our findings.

Conclusion

Cryotherapy caused no change in the levels of IL-10 but significantly increased the IFN- γ levels in our patients with CL. In order to have a better and more accurate assessment of the possible systemic immunological effects of cryotherapy and changes in the levels of key cytokines, studies with larger sample sizes are required.

Conflict of Interest: None declared.

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