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*Original article*

# The Effect of Sun Radiation on the Course of Cutaneous Leishmaniasis in BALB/c Mice

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## Abstracts

**Objective(s)**
Studies have described immunomedulatory effects of sun exposure and ultraviolet radiation on infectious and neoplastic diseases. Here the effect of exposure to low potency radiation of sun on the course of leishmaniasis in mice was studied.
**Materials and Methods**
Fifteen BALB/c mice were exposed to suberythemogenic doses of sun (mean 180 mJ/cm$^2$/day of UVB) 2 months before and 4 months after *Leishmania major* inoculation to food pad. Control group was kept in the sun protected environment. From 2nd to 17th week after inoculation, size of the lesion was recorded in each group weekly and at last week the parasite burden in spleen was detected. Results were compared between two groups.
**Results**
Seven mice from case group and 9 mice from control group survived up to last week. The mean lesion size was 0.90±0.59 cm in exposed and 4.01±3.59 cm in unexposed mice ($P$= 0.037). Parasite burden in spleen of case and control groups were 5.5±4.61 and 106.94±279.76 respectively ($P$= 0.006).
**Conclusion**
Chronic exposure of BALB/c mice to suberythemogenic doses of sun suppressed skin lesion and decreased the extension of *L. major* to spleen.

**Keywords:** Immunity, Leishmaniasis, Sunlight

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## Introduction

Cutaneous leishmaniasis (CL) is a common infectious disease, caused by different species of leishmania genus. The prevalence of the disease is approximately 12 million, with a population at risk of approximately 350 million. Lesions occur mainly in the exposed parts of the body and usually heal spontaneously within months, but in some patients the disease persists for more than 1 year (1, 2). In previous studies the role of the host susceptibility and leishmania species in clinical course of skin lesions have been described (3). However the role of environmental factors including UV exposure was not considered enough.

Chronic and lupoid forms of old world CL (which persists for years and is usually resistant to formal treatments of CL) almost always are seen on the face or exposed sites of the body (1, 4, 5). This form of CL usually aggravates in warm seasons (3). Pathogenesis of this chronic form of CL is not completely understood. Immunomodolatory effects of sun radiation has been described in pathogenesis of many infectious and neoplastic diseases of skin and some clinicians attempted to explain this phenomenon by abnormal immune response in the sun exposed skin. The role of ultraviolet radiation and its immunologic impact on clinical features of post Kala-azar dermal leishmaniasis (PKDL) were discussed by some authors (6,7), but there is not sufficient studies to show the effects of chronic exposure to radiation of natural sun on clinical courses of CL which has different immunopathogenesis than PKDL and viceral leishmaniasis (VL).

The aim of this study was to evaluate the effects of chronic exposure to sun radiation on the clinical course of CL in BALB/c mice.

## Materials and Methods

Present interventional prospective study was performed on 30 female BALB/c mice with 4 to 8 week of age (obtained from Razi Institute, Mashhad, Iran). The mice were divided equally into case and control groups. The case group was exposed to sun two months before and four months after parasite inoculation and the control group was kept in sun protected environment. The two groups were compared for the clinical course of the skin lesion and the burden of parasite in spleen.

### *Sun irradiation*

The mice in case group were daily exposed to suberythemogenic doses of sun from June 10, 2007 to Dec 11, 2007. Because the UVB is most important erythemogenic component of sun radiation and usually is used to determine sun burn threshold, we used UVB dosimetry to estimate suberythemogenic sun exposure. This dose was determined as 180 mJ/cm$^2$ according to the study of Khaskhely and coworkers (8). Irradiation time was calculated as following:

Irradiation time (sec)= irradiation dose (1000× (mJ/cm$^2$))/irradiation intensity (mw/cm$^2$)

The intensity of sun UVB was measured with UV meter (Waldmann factory). The mice were exposed to the natural sun in order to reach this dose of UVB. In the first experiment, exposure begun with an amount of 180 mJ/cm$^2$, but 15 mice did not tolerate this dosage and died with symptoms of sunburn during first week. Another 30 mice were tested and in order to reach a daily mean dose of 180 mJ/cm$^2$, the exposure was started with amount of 100 mJ/cm$^2$ and increased gradually to 330 mJ/cm$^2$ during last month so that mean daily dose was 180 mJ/cm$^2$ for 6 months. The mice were exposed from 4-6 to 25-34 min per day due to sun UVB dosimetry as mentioned above.

### *Leishmania major inoculation*

Suspension of $1\times10^8$/ml *L. major* promastigotes (MRHO/IR/75/ER) in PBS at stationary phase was used and $2\times10^6$ promastigotes in 50 µl of PBS were selected for each inoculation. *L. major* inoculation was performed subcutaneously in both groups in the sole of left feet and 50 µl of PBS simultaneously was injected in the right feet.

### *Assessment of lesion size*

Two weeks after inoculation, swelling, nodule and lesion gradually appeared in the site of *L. major* inoculation. Only mild swelling appeared in the site of buffer inoculation. From the second week on, the sole of both feet

was measured by caliper weekly up to 15 weeks. The mean size of lesions (difference of the measured volume between two feet was estimated as the leishmania induced lesion size) in each group was determined and then used in a statistical analysis.

### *Assessment of parasite burden in spleen*

Assessment of parasite burden in spleen was performed 18th week after *L. major* inoculation for 6 mice of each group. The mice spleens were aseptically removed and homogenized in 1 ml RPMI-FCS, then diluted with the same medium in eight serial 10-fold dilutions in each well of flat-bottom 96-well microtiter plate and incubated at 25±1 °C for 7 days. The motile parasites were identified with an inverted microscope in wells and the mean of the last positive well multiplied by the dilution factor was recorded as quantitative parasite burden in spleen (9, 10).

### *Statistical analysis*

We used one- way ANOVA of Instant statistical software for statistical analysis, and Tukey-kramer test as the *post hoc* test. Significant level was set at 0.05.

## Results

During this study 8 mice of the case group and 6 mice of the control group died and following results are based on observations of survived mice.

### *Change of the footpad thickness (lesion size)*

Table 1 illustrates size of the lesion in both groups from 3rd to 17th week after inoculation. These values show the lesion mean size for the mice in each group. The lesion size in non-exposed mice was more than exposed mice. The difference was statistically significant from 7th week onward. Figure 1 shows the lesion changes in both groups. The difference is apparent and statistically significant between case and control groups. Examples of the lesion in case (UV exposed) and control mice are presented in Figure 2.

### *Parasite burden in spleen of the studied groups*

At the end of the study, 6 mice were randomly selected from each group and parasite burden in spleen of two groups were evaluated. In case group mean leishmania parasite in spleen was lower than the control group (5.50±4.617 vs 106.94±279.760). This difference was statistically significant between two groups ($P$= 0.006).

Table 1. Comparison of lesion size in case and control group during 15 weeks.

| Parameters | Mean of sole feet thickness in unexposed group | Mean of sole feet thickness in exposed group | *P*-value |
|---|---|---|---|
| First week | 1.3883±1.11079 | 0.5567±0.33551 | 0.001 |
| 2th week | 1.8775±1.05612 | 0.6375±0.42186 | 0.009 |
| 3th week | 2.0425±1.08234 | 0.7933±0.53950 | 0.052 |
| 4th week | 1.8075±1.02495 | 0.7283±0.56642 | 0.018 |
| 5th week | 1.9364±1.09055 | 0.8100±0.63647 | 0.202 |
| 6th week | 1.8291±1.22736 | 0.7200±0.61759 | 0.091 |
| 7th week | 2.2627±1.55978 | 0.7542±0.53009 | 0.013 |
| 8th week | 2.4636±1.75241 | 0.9445±0.44630 | 0.010 |
| 9th week | 2.4027±1.84409 | 0.9033±0.44834 | 0.011 |
| 10th week | 2.3918±2.02253 | 0.8808±0.55737 | 0.018 |
| 11th week | 2.3664±2.16785 | 0.9033±0.49286 | 0.017 |
| 12th week | 2.8543±2.57601 | 0.7143±0.55009 | 0.016 |
| 13th week | 3.6586±3.28820 | 0.7120±0.46832 | 0.031 |
| 14th week | 3.8500±3.33032 | 0.9500±0.60568 | 0.043 |
| 15th week | 4.0171±3.59948 | 0.9040±0.59714 | 0.037 |

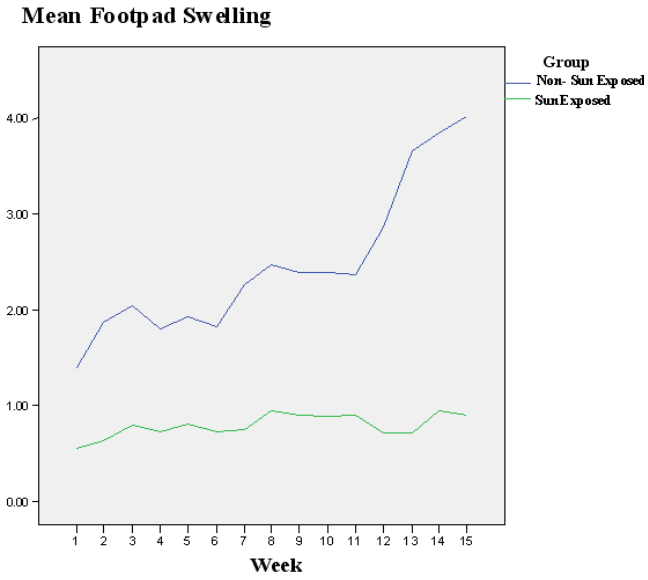


Figure 1. Comparison of lesion size changes in case and control groups.

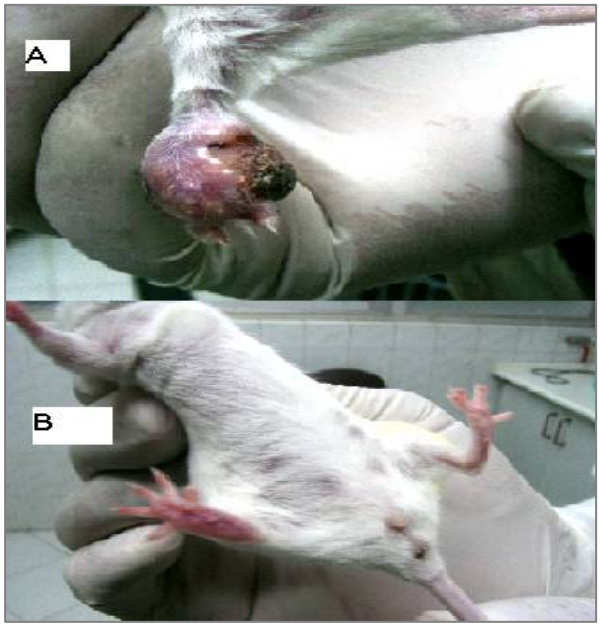


Figure 2. *Leishmania major* induced lesionBALB/c Sun-protected (A) and sun-exposed (B) mice

## Discussion

Different studies have evaluated the suppressive effect of UVB on the host immunity against some infections such as Borrelia (11), Candida (12), Herpes (13), Mycobacterium (14, 15) and Schistosoma (16). UVB irradiation causes decreasing of T cells, natural killer cells and Langerhans cells in human and mice tissues and leads to local and even systemic immunosuppression. Moreover, high doses of UVB cause disturbance in the cellular immune response and induces excretion of Th2 cytokines and decreases the level of Th1cytokines (17). In experimental models the association of Th1 response with control of leishmania infection, and of Th2 cell development with progressive disease, has been well established (18). IFN-γ (the most powerful macrophage activating cytokine) excretion by Th1 cell activates macrophages to a parasiticidal state and leads to leishmania resistance, while IL-4 and other Th2 cytokines exacerbate the development of lesions because of their down-regulatory effects on macrophages (19, 20). Although this Th1 and Th2 pattern of response in mice models is clearly related with self healing and progressive types of CL, induction of Th2 response by natural sun radiation is not established as it is shown for experimental exposure to UVB waves.

In our study, the effect of chronic exposure to low dose sun radiation was evaluated on clinical course of *L. major* lesions in BALB/c mice which have been under daily irradiation of suberythemogenic doses of sun two months before and four months after inoculation. We calculated mean daily doses of UVB to estimate suberythemogenic exposure.

Our results were unpredictable and changed our idea about immunosuppressive effects of sun radiation in clinical course of CL. The size of lesion from the first weeks was considerably lower in the sun exposed group and the parasite burden in spleen was significantly lower in this group.

It is worth noting that these results are against the present hypothesis of local and systemic immunosuppressive effects of UV. Many earlier studies indicated an immunosuppressive effect of sun radiation have been done using the high and episodic doses of irradiation and also the UVB was used alone and/ or with higher doses than what is obtained in natural sun irradiation (21-23).

These paradoxical results enforced us to further search in previous studies: Narbutt and Coworkers studied 30 healthy candidates for immunologic effects of exposure to suberythemogenic doses of UV during 10 successive days. They reported that frequent and low dose irradiation of UVB can protect against the effects of an erythemal UVB dose

on immunity (24). McLoone and Norval showed the adaptation with UV suppressive effects on mice peritoneal macrophages' activity. They evaluated the phagocytic activity of mice peritoneal macrophages after repeated exposures to solar simulated radiation (SSR) plus an additional higher dose at the end. The macrophage activity in mice exposed longer to low dose SSR was less affected by final immunosuppressive dose. (25) These studies demonstrate the opposite effect of UV irradiation on host immunity regarding its frequency and doses. The effect of low dose and suberythemogenic UVB on clinical course of leishmaniasis was studied for the first time by Giannini in 1986. He evaluated the local effect of UVB irradiation after inoculation and concluded that low dose UVB (similar to what humans receive at their usual life in rural areas) can suppress the appearance of cutaneous leishmaniasis (26). Khaskhely *et al* have supported these findings. They exposed BALB/c mice to low dose of UVB for 4 successive days and *L. amazonesis* inoculation was performed 12 hr after the last dose. The cutaneous lesion was considerably suppressed in the irradiated group compared to the non-irradiated group. Serum and cutaneous levels of Th1 cytokines (TNF-α, IFN-γ) were increased in irradiated mice. Moreover, histopathologic analysis of the lesion showed a considerable decrease of cellular infiltrate and tissue's parasite in irradiated mice (8). But none of the above-mentioned studies has used natural sun and the possible effects of other sun waves have not been evaluated. Our results demonstrated that repeated doses of low potency sun radiation before and after *L. major* inoculation in BALB/c mice can considerably suppress the appearance of cutaneous lesions and in addition decrease parasite extension to and its proliferation in spleen. Possible mechanism that may explain this phenomenon is Th1 response induction and amplification by exposure to the repeated and low dose of UV that may lead to excretion of IL-2, TNF-α and IFN-γ as it has been shown by Khaskhely and coworkers (8, 27). This paradox in biological effects between repeated-low doses and episodic-high doses of UV irradiation also can be seen in carcinogenic effects of UV, while a high and episode dose of UV is more predisposing for basal cell carcinoma (28, 29) and melanoma (30, 31), on the other hand squamous cell carcinomas develops in chronically sun exposed skin and are less aggressive.

Although lupoid leishmaniasis is due to *L. tropica* infection and all experimental studies bear the limitation of using *L. major* for induction of CL lesion in mice, they could at least partly evaluate CL immunopathogenesis and effects of environmental factors on that.

## Conclusion

We conclude that chronic exposure to the low doses of UV has protective effects against development of skin lesion in leishmaniasis and also suppresses the parasite extension to spleen and the non healing feature of lupoid leishmaniasis can not be explained by immunosuppressive effect of sun radiation.

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