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Gender differences in the severity and features of lesions among cutaneous leishmaniasis patients

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Objective To determine if there is any differences in the severity and features of lesions among patients complaining of cutaneous leishmaniasis in an endemic region.
Methods A cross-sectional, observational, descriptive study was performed (January 2014 to June 2019) in the dermatology clinics of: Rizgary Hospital, Hawler Teaching Center for Skin Diseases, Shadi Health Center, Hawler Institute of Health Prevention. All the patients were referred from rural districts of Makhmur and Kalack. The provisional diagnosis was dependent mainly on clinical examination in addition to Giemsa stain. Parasite (amastigote) grading and distribution of number, site, type, and size of lesions according to the gender of patients were studied.
Results A total of 1264 cutaneous leishmaniasis cases were diagnosed during the study period. According to stain results, 70.6% of the cases were positive to Giemsa stain. Parasite grading and parasite number/field were higher significantly in males. Features of case severity according to the characters of the lesions (number, size, site, type) were more noted in males than females.
Conclusion Male patients are more prone to more severe infections than females.
Key Words cutaneous leishmaniasis, Makhmur, Kalack, gender, lesion

Introduction

Leishmaniasis is a parasitic ailment brought about by the infection with blood flagellate *Leishmania*. The disease is widespread and it is believed as a cause for a grave health dilemma in countries throughout the Mediterranean regions and the Middle East, including Iraq.[1,2] Cutaneous leishmaniasis exhibit different clinical appearances depending on parasite and the host immune system.[3,4,5] Several studies have declared the differences between males and females regarding total mortality, vulnerability to allergic and autoimmune diseases, or particular infectious disease danger.[6,7,8] Possibly, males are more likely to be engaged in actions such as attacks, campaigns, traveling, and crowding, which increases the likelihood of contact with parasites.[2,3] Regardless of the variation in the probability of encountering different risks, immunological divergences present between both genders that may lead males to be more prone to parasitism.[8,9]

The current research was carried out to delineate any gender differences in the severity of cutaneous leishmaniasis lesions in regarding to parasites grading and features of the lesions (number, size, site, type) amongst patients in endemic zone.

Materials and Methods

Study Protocol

This is a cross-sectional, descriptive study conducted during the period of January 2014 to June 2019 performed in the dermatology clinics of: Rizgary Hospital, Hawler Teaching Center for Skin Diseases, Shadi Health Center, Hawler Institute of Health Prevention. This study was carried out with the collaboration of prevention Health Department, Erbil Medical Technical Institute, Erbil Polytechnic University, with Departments of: Microbiology , Anatomy & Histology, College of Medicine, Hawler Medical University, Erbil, Iraq.

Ethical Considerations

This study was approved by: the Ethics Committee of Hawler Medical University, Erbil; the Committee of Erbil Medical Technical Institute, Erbil Polytechnic University, Iraq; Health Directorate of Erbil; Education Directorate of Erbil. Informed consent was taken from each patient. The patients were informed about study's objectives and they could withdraw thereof if they wished so to do.

Study Population

In the present study, the patients of age ≥18 years with cutaneous leishmaniasis were selected from outpatients attending the dermatology clinics mentioned above. Patients who were inhabitants or resident of the rural districts surrounding Erbil city of Makhmur and Kalack were involved in this study.

Makhmur district is situated 67 km South-west of Erbil City in Erbil governorate while Kalack district is situated 32 km west of Erbil. Both districts are open land used for agriculture with large number of villages.[10] Exclusion criteria include patients with a suspected clinical lesion, patients who rejected to share in the study or who had received partial treatment for CL. In addition, prepubertal, children, and pregnant patients with lesions were excluded from the study to bypass any bias or misconception results which may appear due to sexual immaturity or hormonal changes during pregnancy.

Sample and Diagnostic Procedures

Diagnoses of the disease are based on:

1. Clinical feature: This was achieved by an experienced dermatologist. Examination of the patients was done to estimate the following points about the lesion: site, number, size, duration, and type as wet or dry CL.

2. Parasitological examination (Giemsa stain & Parasite grading): after cleaning of the lesion, a sample was obtained from the indurated margin of the lesion and examined. The sample from the cutaneous lesion was taken by fine needle aspiration as the following steps:
   1. The skin around the lesion was disinfected by 70% ethanol.
   2. The sterile syringe of 1 ml containing 0.2 ml of sterile normal saline was injected intradermal through intact skin in to the active red border of the lesion.
   3. Aspirate the injected fluid as the needle draw back till the bloody stained fluid aspirate.
   4. Small amount of aspirated fluid was taken and smeared on a clean glass microscope slide then left it to dry, then fixed using 100% absolute methanol for 30 s and left it to dry again.
   5. Stained with Geimsa stain for 20 min, then rinse with tap water and dry the slide, and then examined it under oil immersion lens of the light microscope (Olympus CH2, Japan).
   6. Amastigote was diagnosed as round or spherical shape with distinctive kinetoplast. In this case was declared positive. When no amastigote was seen after 15 min of inspection, the smears was declared negative.[2,4,11]
   7. The average parasite number on each slide was graded based on the numbers of *Leishmania* amastigotes in high power field (HPF) using 10 × eyepiece and 100 × objective lenses on the following criteria:
      –, amastigotes could not be observed in the whole slide.
      +, 1 amastigote in the whole slide up to one amastigote per field in a total of at least 100 fields.
      ++, 2 to 10 amastigotes per field in a total of at least 50 fields.
      +++, 11–20 amastigotes per field in a total of at least 50 fields.
      ++++, 21 or more amastigotes per field in a total of at least 10 fields.[12]

Statistical Analysis

The data analysis was performed using descriptive statistics, including mean ± standard deviation, frequency, and frequency percentage. Comparisons were made using Chi^2 test or Student's *t*-test as appropriate by using standard equations. The results were reported with $p \leq 0.05$ or $p \leq 0.01$ or as the accepted level of significance accordingly.

Results

In the present study, a total of 1264 patients were confirmed to have CL from January 2014 to June 2019.

Regarding the results of parasitological examination, Table 1 denotes that Giemsa stain was positive in 893 cases (70.6%). Table 2 illustrates that from the total 1264 patients, males represent 54.6% of the cases. The details of the distribution of positive and negative results in addition to results of parasite grading in both genders are also clarified. From the total 893 positive cases, 506 cases (56.7%) were males and the remainder 387 (43.3%) were females. The difference in the distribution of positive and negative Giemsa stain results in both gender was significant ($p \leq 0.05$). Table 2 also delineates the distribution of various parasite grading in both males and females in which the difference is significant ($p \leq 0.05$). Table 3 shows the comparison between both genders with regard to the parasite number/field in these grades (+2, +3, +4). These grades were taken into consideration because according to the instructions by Ramírez et al[12] which is used in this study, the mentioned grades harboring more than one parasite per field. Table 3 manifests clearly by using Student's *t*-test that the means of the parasites number per field in above-mentioned grades varies significantly between males and females ($p \leq 0.05$).

Table 4 demonstrates in detail the differences in the distribution of the number, site, type, and size of the lesions according to the gender of the patients. It is noted clearly from Table 4 that the differences regarding the number, site were highly significant ($p \leq 0.01$) while the differences regarding type and size of the lesion were significant ($p \leq 0.05$).

Table 1. **Results of parasitological examination by Giemsa stain**

Result	N	%
Positive	893	70.6
Negative	371	29.4
Total	1264	100

Table 2. **Distribution of results of parasitological examination (Giemsa stain) and grading according to the gender**

Giemsa stain	Male	Female	Total
	N (%)	N (%)	N (%)
Positive	506 (56.7)	387 (43.3)	893 (100)
Negative	184 (49.6)	187 (50.4)	371 (100)
Total	690 (54.6)	574 (45.4)	1264 (100)
Chi-square value = 5.33	**df = 1**	**Significant ($p \leq 0.05$)**	
Grading (Positive cases only)			
+1	72	84	156
+2	137	92	229
+3	170	109	279
+4	127	102	229
Total	506	387	893
Chi-square value = 10.688	**df = 3**	**Significant ($p \leq 0.05$)**	

Table 3. **Comparison between both genders regarding parasites number/ Field in grades +2, +3, +4**

Grades	Male	Female	Mean ± SD	Mean ± SD
+2	7.4 ± 1.1	6.84 ± 2.6	t = 2.241 df = 227	**Significant ($p \leq 0.05$)**
+3	14.83 ± 4.16	13.34 ± 5.82	t = 2.49 df = 277	**Significant ($p \leq 0.05$)**
+4	26.54 ± 5.8	24.92 ± 6.14	t = 2.047 df = 227	**Significant ($p \leq 0.05$)**

Table 4. **Distribution of number, site, type and size of lesion according to the gender of patients**

Number of lesion	Male	Female	Total
	N (%)	N (%)	
1	313(50.2)	310(49.8)	623(100)
2	286(56.2)	223(43.8)	509(100)
≥3	91(68.9)	41(31.1)	132 (100)
Total	690	574	1264
Chi-square value = 14.2	**df = 2**	**Highly significant (p ≤ 0.01)**	
Site of lesion			
Limbs	366(59.3)	251(40.7)	617(100)
Face	219 (48.5)	233 (51.5)	452(100)
Abdomen & trunk	105 (53.8)	90 (46.2)	195(100)
Total	690	574	1264
Chi-square value = 14.2	**df = 2**	**Highly significant (p ≤ 0.01)**	
Type of lesion			
Chi-square value = 14.2	**df = 2**	**Highly significant (p ≤ 0.01)**	
Site of lesion			
Limbs	366(59.3)	251(40.7)	617(100)
Face	219 (48.5)	233 (51.5)	452(100)
Abdomen & trunk	105 (53.8)	90 (46.2)	195(100)
Total	690	574	1264
Wet	375 (57.6)	276(42.4)	651(100)
Dry	315 (51.4)	298 (48.6)	613 (100)
Total	690	574	1264
Chi-square value = 4.82	**df = 1**	**Significant (p ≤ 0.05)**	
Diameter of lesion			
0.5 × 1cm–1.5 × 2cm	171 (51.5)	161(48.5)	332 (100)
1.5 × 2cm–2.5 × 3cm	357(53)	317(47)	674(100)
≥2.5 × 3 cm	162(62.8)	96(37.2)	258 (100)
Total	690	574	1264

Fig. 1 shows the distribution of different lesions regarding site, size, number, gender.

Fig. 2 illustrates the amastigote stage obtained from cutaneous lesion stained by Giemsa stain.

Discussion

To our knowledge, this is the first study of such quality and aims on cutaneous leishmaniasis in Erbil.

The results which had been obtained from the present study suggest that gender may have a role in severity and pathogenesis of infection. It is clear from Tables 2 and 3 that males had higher rates of infections and this was illustrated by the higher percent of male patients and the higher percent of males who yield a positive Giemsa stain. In addition, significantly higher rate of parasite grading and parasites number/field (parasite burden) in males were also noted. Table 4 obviously clarified that differences in the distribution of number, site, type, and size of lesions were significant and highly significant among both gender.

The present study delineate that Giemsa stain has a detection rate of 70.6% in clinically diagnosed cases. This result is near to that obtained in which the rates were 73% and 69.5% respectively.[2,13] The success of microscopic detection of amastigotes varies depending on the number of parasites present and duration of lesions. Therefore, failure to observe amastigotes does not exclude a diagnosis of CL and such infection in endemic areas may be diagnosed on the basis of their clinical features as leishmaniasis.[4,14,15] It had been claimed that amastigotes at certain time of the disease are impossible to be detected. The disappearance of such cells infected with the amastigote form in spite of the disease process is still continuous delineate that these phagocytes, giant cells, macrophages, and monocytes, at specific point of the disease process become resistant to be infected with the amastigotes.[15,16]

Several studies have illustrated that immunological differences are noted between both sexes that may lead to increased parasitism in males.[6,7] Females typically have higher immune responses than males. This elevation of immunity among females is beneficial against infectious diseases, while from the other side, it may be injurious because of the increased susceptibility of females to develop autoimmune diseases.[8,9]

In pre-pubertal children, sex differences in response to *Leishmania* infection were notified in which boys are more likely to develop visceral leishmaniasis than girls. During the critical period of sex differentiation, the extent to which sex steroids alter the development of the immune system and responses to infection prior to puberty and into adulthood should be considered.[17,18]

Researches from various diverse endemic foci in the New and Old Worlds had concluded that regardless of cultural and occupational factors, men were noted to get cutaneous or visceral leishmaniasis more than women.[17,18,19,20,21]

Mice infected by *Leishmania* in experimental studies also reveal that males are more subjected to infection than females. In mice infected with *Leishmania major*, disease evolution was found to be different in males and females according to the route of inoculation, i.e., the intradermal route was more severe in females and the intravenous route was more severe in males.[22,23]

Other study, comparing pregnant or castrated mice to normal controls, demonstrated that susceptibility to *L. major* or *L. mexicana* strongly depended on hormone levels, which in turn regulated the expression of different cytokines.[24] The relative resistance of female mice to *L. mexicana* infection compared to male mice was related to increased expression of gamma interferon (IFN- γ). Male mice castration reduces, whereas treatment of the females with testosterone increases, vulnerability to *L. major.*[22]

Experimental infection of inbred age-matched male and female hamsters demonstrated that male animals were more susceptible to infection with *Leishmania* (*Viannia*) spp. than female animals. This difference was evident for strains of both

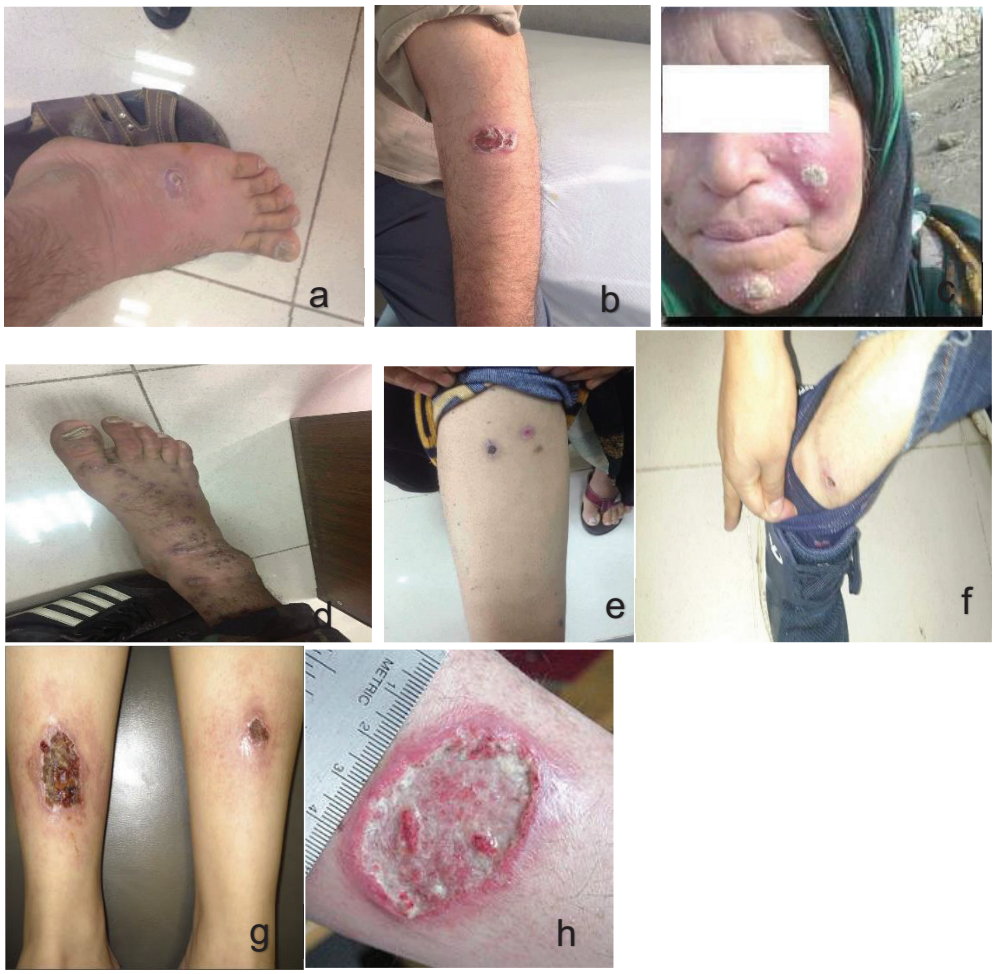

Fig. 1 **(a-h) Distribution of different lesions regarding: site, size, number, gender, type of lesion.**

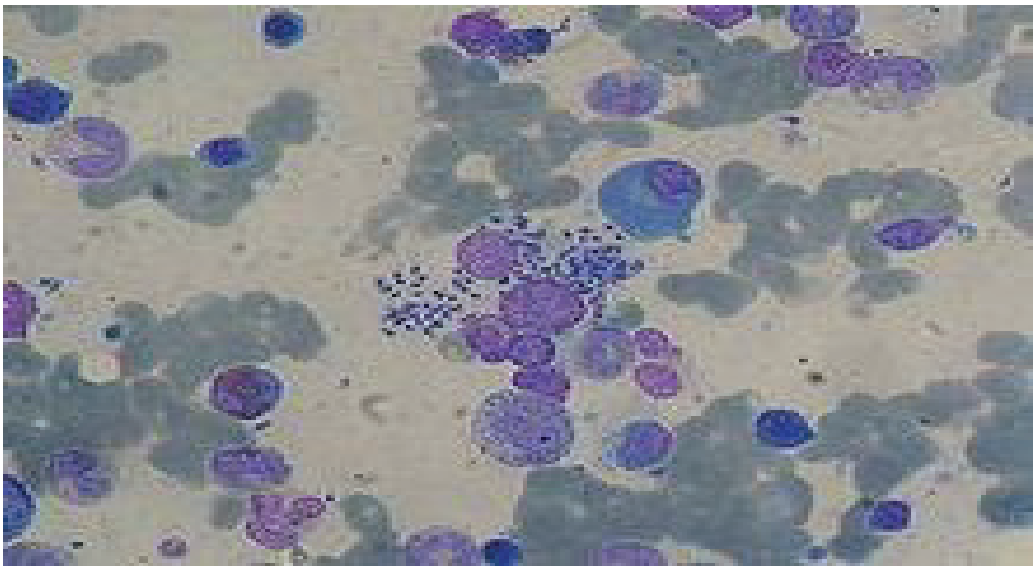

Fig. 2 **Amastigote from cutaneous lesion, Giemsa stain 100 X.**

L. (*V.*) *panamensis* and *L.* (*V.*) *guyanensis* when either primary lesion size or severity or frequency of dissemination (cutaneous metastases) was assessed.[25]

In addition, the exogenous administration of the opposing sex hormone to male and female hamsters demonstrated that testosterone had a disease-promoting effect, possibly through a direct effect on the immune response or by blocking a protective effect of estrogen.[26]

Strikingly, male hamsters had significantly more-severe disease than female animals when lesion size, lesion severity (degree of tissue necrosis), parasite burden in the draining lymph node, and rate of parasite dissemination were

evaluated.[25] Associated with the increased severity of disease in the male animals was a significantly greater intralesional production of IL-4, IL-10, and TGF-β cytokines known from other studies to exacerbate experimental *Leishmania* sp.[26,27]

Study by Wilcoxen et al[28] had demonstrated that there are gender-dependent differences in the secretion of IL-10 and IL-12 by antigen-presenting cells (APCs). APCs from male mice secreted IL-10 but not IL-12 during T-cell activation, and this pattern was reversed in APCs from female mice.

Males and females differ in their innate immune responses suggesting that some sex differences may be germline encoded. The number and activity of cells associated with innate immunity differ between the sexes. Phagocytic cells, including macrophages and neutrophils, can kill parasites by generating reactive oxygen metabolites and nitric oxide, as well as by secreting enzymes.[7] Responses of the innate immune system play a crucial role in the initial recognition and response to parasites and may alter the expression of sex differences in parasite infection.[6] Pattern recognition receptors, such as toll-like receptors (TLR) are closely involved in arranging host–innate responses to infection and serve as a bridge between innate and acquired immunity. Whether the sexes differ in the expression of TLR, mannose receptors, or scavenger receptors that bind to and mediate internalization of parasitic particles has not been reported, but could influence dimorphic responses to infection.[17,18]

Among humans and lizards, the phagocytic activity of neutrophils and macrophages is higher in females than males.[6] Following parasitic or antigenic stimulation, the production and release of prostaglandin E2, thromboxane B2, and nitric oxide is reportedly higher in females than males.[7] Humoral immune responses (i.e., antibody production by B-cells) are typically greater in females than males.[18]

Gonadal hormones exert specific effects on the male and female immunocompetence at both the cellular and the molecular level. Estrogen receptors are expressed in most cells of the innate and adaptive immune system including T cells, B cells, neutrophils, macrophages, dendritic cells (DC), and natural killer (NK) cells.[29] Androgen receptors were identified in T and B lymphocytes. During pregnancy, activated lymphocytes also express progesterone receptors.[7]

Estrogens affect innate immune cells. Estrogens at levels of ovulatory phase or pregnancy suppress cytotoxicity of NK cells.[29] Notably, macrophages treated in vitro with estradiol showed decreased secretion of the proinflammatory cytokines interleukin (IL)-1b, IL-6, and tumor necrosis factor (TNF)-a, whereas long-term in vivo administration led to increased secretion of IL-1b, IL-6, and IL-12p40 after Toll-like receptor (TLR) 4 activation and eventually an enhanced activation status.[30,31,32]

Estrogen receptor signaling also regulates lineage development of DCs. High estradiol levels promote the development of conventional IL-12-producing DCs and the expansion of IFN-γ-producing killer DCs. In addition, production of IL6, IL-8, and chemokine (C-C motif) ligand 2 (CCL2) by immature DCs is increased.

Both estrogens and androgens reduce the numbers of immature T lymphocytes enhancing thymic involution during puberty and pregnancy.[28,29]

Adaptive immunity in men is distinct from women as androgens accelerate the growth and expansion of Th1 responses and trigger CD8+ T cells, while estrogens encourage Th2 responses and animate antibody production.[8] In comparison of women at reproductive age to age-matched men, the CD4+/ CD8+ ratio is significantly increased.[7]

In parallel to the low estrogen levels are the increased manifestation of the transcription factor T-bet (T-box expressed in T cells), which eventually switch the balance toward Th1 immunity and IFN-γ expression.[33] High estrogen levels constrain IRF1 (interferon regulatory factor 1) supporting Th2 immunity and IL-4 expression.[34]

Sex steroid hormones also modified B-cell expansion and function. Estradiol reduces apoptosis of immature B cells and consequently increases the appearance of autoreactive B cells from central and peripheral checkpoints.[8] However, estradiol also increases somatic hypermutation and class-switch recombination leading to high-affinity Ig producing cells.[34] These effects might contribute to an improved humoral response in women and explain the increased susceptibility to autoimmune diseases. In contrast to estrogens, progesterone suppresses somatic hypermutation and class-switch recombination.[33]

Estrogens also exhibit indirect effects on the immune system by modulating the levels of growth hormone, prolactin, or thymosin.[8] Thus, the general paradigm on sex steroid hormones influencing the immune system stipulates that estrogens have immune-enhancing effects. In contrast, progesterone and androgens such as testosterone exert mainly immunosuppressive properties.[17,18,25]

The X chromosome expresses several genes implicated in immunological processes, such as Toll-like receptors, multiple cytokine receptors, genes involved in T-cell and B-cell activity, and transcriptional and translational regulatory factors, while in turn the Y chromosome encodes for a number of inflammatory pathway genes, which are exclusively expressed in men.[37] Most alleles on one X chromosome are randomly silenced during X chromosome inactivation already during embryogenesis.[29] Polymorphism of X-linked genes and cellular mosaicism for X-linked parental alleles may offer additional advantages to women during host responses, in particular by providing a more adaptive and balanced cellular machinery during innate immune responses.[37,38]

This sexual dimorphism commences already during intrauterine development, for example, a male fetus experiencing a chronic inflammatory environment primarily being induced by the maternal immune system in the male placenta via decidual sites yet also likely due to a higher gestational infection rate of male placenta.[38] Later in life very probably due to socioeconomic behavior, such as higher pathogen exposure during agricultural or occupational activities, men are more susceptible to many infections caused by viruses, bacteria, parasites, and fungi. They are significantly more predisposed especially to environmental and vector-borne diseases such as leptospirosis (3.5- to 4-fold increased incidence), schistosomiasis (1.5-fold), brucellosis, or rabies.[6,7,8] The present study concluded that there is a gender differences regarding the severity and features of lesions among patients infected with cutaneous leishmaniasis.

Limitations & Recommendations

The constraints of this study were:

1. It is important to highlight that CL has very different clinical manifestations depending on the condition of the host's immunity and the species of parasite. Proper identification of the species is achieved by PCR which was not applied in this study because such a technique is time consuming and not usually available.
2. Cytokines play a vital role in the host immune response to infection by initiating the healing process and/or accelerating the progression of the disease in cutaneous leishmaniasis (CL). Determination of the cytokine expression pattern of IFN-γ, IL-4, IL-11 and IL-12p40 in CL patients was not done.
3. Proper studies on the effects of environmental, occupation, life style factors on the severity of infection with CL are mandatory.

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Conflict of interest

The authors acknowledge no conflict of interest in this study.

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