Identification of etiologic agents of cutaneous Leishmaniasis in palpebral position followed by effective therapy

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Abstract:
CONTEXT: Cutaneous Leishmaniasis (CL) is a self-healing lesion but prevention of complications and involvement of vital organs such as palpebra requires proper treatment.
AIMS: The main objectives were to detect agents of CL in palpebral region and estimate the proportion of palpebral lesion also possible complications among CL patients in a zoonotic CL endemic area.
SETTINGS AND DESIGN: The study was performed from April 2012 to March 2013 in a total of 1613 CL suspected patients by interview and physically exam.
SUBJECTS AND METHODS: The samples were used for direct smear using Giemsa stain method, or cultured in Novy-McNeal-Nicol medium. For further checked, nested polymerase chain reaction (PCR) was used for negative palpebral samples. Of the 1613 examined samples, 848 (81.4%) by direct smear, 188 (18%) by culture and 6 (0.6%) by nested PCR were positive, respectively. A total of 233 of the patients showed lesions on the face of whom 15 (male = 5, female = 10, 1.43% of all and 6.43% of facial lesions) presented with palpebral CL. The results of nested PCR indicated that all the palpebral cases were due to Leishmania major.
CONCLUSIONS: About 93% of the patients with palpebral lesion were under 5 years old and were infected in the hyper endemic regions, but no ocular complication was seen in any of them. Based on the results, it seems that early referring to the medical center for proper diagnosis and treatment is the main reason for prevention of further complications.
Keywords: Cutaneous Leishmaniasis, facial lesion, Iran, ocular effects, palpebral position

Introduction

Leishmaniasis is a zoonotic disease reported from 98 countries.1,2 Cutaneous Leishmaniasis (CL) is a self-healing skin lesion but due to long duration of healing and disfiguring scar especially when it is close to a vital organ, treatment is strictly necessary and recommended.

There are reports that periocular lesions lead to unpleasant effect on ectropion, which might induce vision disorders.3 Although, the possibility of insect bite is not common around the eye regions4 because of eyelashes and eyelids movements, 2–5% of facial cutaneous lesions are situated on the lids.5,6 Nasolacrimal duct involvement was also reported.7,8 Three cases of ocular involvement were reported, which resulted in blindness possibly due to delay in treatment or ineffective therapy.9 Leishmaniasis of different parts of eye (e.g. cornea) and other parts of eye globe have not been reported yet. However, eyelid scarring and other complications such as entropion, ectropion, trichiasis and lacrimal punctum involvement might also lead to ocular complications.10,11 These complications cause corneal and conjunctival dryness and...
might lead to corneal lesion. To our knowledge through search on internet, no case of Leishmaniasis directly on eye globe is reported.

The main objectives of this study were to detect agents of CL in palpebral region and estimate the proportion of palpebral lesion and possible complications among CL patients in a zoonotic CL endemic area.

Subjects and Methods

This is a cross-sectional descriptive study. The target population was CL suspected patients who were referred to the Skin Disease and Leishmaniasis Research Center (SDLRC), Isfahan, Iran, during April 2012 to March 2013. Patients with suspected CL lesion(s) and a positive direct smear, culture/and nested polymerase chain reaction (PCR) were included in the study. All of the patients were under observation by an ophthalmologist for ocular inspections to diagnose any possible ocular complications.

To collect the samples, the lesion was cleaned by 70% alcohol. The specimen was collected by scraping the inner walls of the slit which was made perpendicularly in the margin of the lesion. The collected sample was smeared on to a glass slide and inoculated into Novy-McNeal-Nicol (NNN) culture media. The smear was air dried and fixed with 100% methanol. In order to stain the samples, the stock Giemsa liquid stain was diluted 1:10 with saline buffer and the slide was immersed in Giemsa stain for 10 min and then was washed and allowed to air dried.[13] The slide was checked under microscope using high power to search for amastigotes. Negative samples were cultured in NNN medium and incubated at 25°C and checked for the growth of promastigotes at 2 days interval for 4 weeks. As mentioned above nested PCR as a sensitive tool carried out to confirm CL suspected patients on the palpebra samples, which were negative in direct smear/and culture and identify the causative parasites to species level.

DNA was extracted with the kit (Genetbio) according to the manufacturer’s instructions.

Two pairs of primers were used with Noyes sequences.[13] The primers sequence was selected based on the conserved regions of the template generate 750 bp bond for *Leishmania tropica* and 560 bp bond for *Leishmania major*. Nested PCR is a two steps stage PCR. The first step primers are as follows:
- **CSB2XF:** 5’CGAGTACGAAACTCCCCGTCCA3’
- **CSB1XR:** 5’ATTTTTCCGATTTTCGCAGACG3’

The PCR product obtained in the first stage was used as a template for the second round. The materials used were as the same as the previous round with the exception that PCR product was used instead of primary DNA. Primers used in the second step were:
- **13Z:** 5’ACTGGGGGTGGTGAAAATAG3’
- **LiR:** 5’TCGCAGAACGCCCCCT3’

After the final stage of PCR, the products were electrophoresed on Agarose gel containing ethidium bromide 1%. Parasite species were identified based on weight index of the PCR products in comparison with *L. major* and *L. tropica* control samples.

The data were analyzed using descriptive statistics and Chi-square test with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). \( P < 0.05 \) was considered as significant.

Results

Overall, 1613 CL suspected patients were physically examined. Demographic information and lesions’ characteristics were recorded. Out of 1613 examined samples, 848 (81.4%) were parasitologically positive by direct smear, 188 (18%) of 765 cultured showed promastigote growth and positive. Of 28 CL suspected patients on the palpebra 7 (25%) and 2 (7%) were positive by direct smear and culture respectively. Out of 19 negative CL suspected palpebral cases which were tested by nested PCR 6 (0.6% of total and 21.4% of CL suspected palpebral cases) were positive and 13 samples were negative with all 3 procedures and the lesions were not recognized as CL. All 15 palpebral samples showed a 560 bp bond in nested PCR Agarose gel electrophoresis [Figure 1].

Out of 1042 confirmed CL patients (female = 509; 48.9%, male = 533; 51.1%) with age ranges between 1 and 78 years mean ± standard deviation 16.70 ± 13.47 years). A total of 233 (22%) showed facial lesions, other affected area included 809 cases (78%) and 15 (male = 5, female = 10) patients showed palpebral involvement, which is about 6.43% and 1.43% of the facial and total positive cases, respectively.

The majority of the patients with palpebral lesion presented with only one lesion (11 patients), 2 patients showed 2 lesions and 2 patients presented 3 lesions on the palpebras [Figures 2 and 3].

Most of the patients with facial lesion showed only 1 lesion [Table 1].

About 93% of the patients with palpebral lesions were infected in the hyper endemic regions of CL. Most of them were resident of north and east north of Isfahan and the remaining (~7%) were infected in the other regions of the Isfahan province [Figure 4].
There was a significant direct correlation between the number of palpebral/face and total body lesions ($P < 0.01$, Figures 5 and 6).

The results also showed that palpebral lesion had a direct correlation with the number of facial lesions it means that in cases with more facial lesions more palpebral lesions is possible (correlation coefficient = 0.319, $P < 0.05$).

All of the parasitologically proven patients received a full course of systemic Glucantime; 20 mg/kg at most 2 ampules/day for 14 days. Complete cure of the lid lesions occurred in all of the patients and followed-up for 1-year showed no relapse. No complications such as dry eye, corneal dryness, corneal lesions, entropion, ektropion, trichiasis and eye tissue involvement was recorded in ophthalmological examinations during the period of 1-year follow-up [Figures 7 and 8].

**Discussion**

Cutaneous Leishmaniasis caused by *L. major* and *L. tropica* is a major public health problem in many parts of Iran.$^{[14]}$ Wide spectrum of clinical manifestation of the disease depends on the Leishmania strains and the immune status of the host.$^{[15]}$

**Table 1: The frequency of facial lesion**

| Lesion counts | Case counts | Frequency | Percentage |
|---------------|-------------|-----------|------------|
| 1             | 151         | 14.5      |            |
| 2             | 39          | 3.7       |            |
| 3             | 27          | 2.6       |            |
| 4             | 7           | 0.7       |            |
| 5             | 3           | 0.3       |            |
| 6             | 3           | 0.3       |            |
| 7             | 1           | 0.1       |            |
| 8             | 1           | 0.1       |            |
| 15            | 1           | 0.1       |            |
| Sum           | 233         | 22.4      |            |

**Figure 1:** Agarose (1.5%) gel electrophoresis of nested polymerase chain reaction products in isolates of patients with palpebral cutaneous Leishmaniasis. Lane 1 – Molecular weight ladder, Lane 2 – *Leishmania tropica* positive control, Lane 3 – *L. major* positive control, Lane 4-10 – Patients’ samples. A band of 560 bp was represented for *L. major*.

**Figure 2:** Facial cutaneous Leishmaniasis in a 1-year-old girl. Note that two lesion are on the eyelid.

**Figure 3:** Facial cutaneous Leishmaniasis with six lesions (the most abundant) in a 40-day-old baby. Note that three lesions are situated on the eyelids.

**Figure 4:** Prevalence of palpebral Leishmaniasis according to residency in endemic and nonendemic areas.
In this group of patients CL was most frequently seen on the facial region, followed by the arms and legs. Nonetheless, ocular involvement is rare. Previous reports of involvement of the eyelid was 2.5% of all CL cases and 2–5% of the facial CL lesion. This might be due to palpebral movement, which could prevent sand fly bite in this region of the skin. Our study showed that 1.43% of all CL lesions and 6.43% of the facial lesions were located on the eyelids, and periocular regions. In a study completed in Turkey, among 987 patients (female = 484, male = 503) with age ranges of 0–7 years old, 33 lesions were in the eye of facial, lids and periocular areas were 1.93% of all CL 3.75% of the facial lesions. Our results indicated that 80% of the patients with palpebral Leishmaniasis were under 10 years old [Figures 2, 3, 5-7 and 9-13]. Distribution of palpebral Leishmaniasis according to age groups presented in Figure 14. Childhood CL is frequent in endemic areas, which might be due to the susceptibility to the infection and the older population are immune due to previous infection.[17]

The results of nested PCR indicated that all of the recognized cases of palpebral CL in the current study were due to *L. major* as a band of 560 bp for *L. major* was observed (reference strain MRHO/IR/75/ER) but no band of 750 bp correspond to *L. tropica* was seen (reference strain MHOM/IR/Mash10) [Figures 1]. These results are similar to previous studies which showed CL in Isfahan, Iran is zoonotic type caused by *L. major*.[18,19]

Clinical feature of ocular Leishmaniasis is similar to the other ocular lesions such as bacterial nodule, chalazion, hordeolum and dacryocystitis, which propound the importance of the on time diagnosis and proper treatment of the infection.[8,11,14,20,21]

Despite of the reports of ocular complications in some eyelid infections, in the present study no ocular complication was seen. An ocular Leishmaniasis was reported from Iran before which was similar to chalazion and complication with trichiasis.[22] Satici

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**Figure 5:** Facial cutaneous Leishmaniasis in an 18-year-old boy. Note that one of the lesions is palpebral Leishmaniasis

**Figure 6:** Facial cutaneous Leishmaniasis in a 9-year-old boy, note the two lesions on the eyelid

**Figure 7:** Palpebral lesion in a 2.5-year-old girl at the first visit

**Figure 8:** The same patient after effective therapy, the lesion disappeared completely without any ocular complication
et al. from Turkey in 2004, reported, out of 33 lesions on eyelids and periorbital parts, 3 developments as blepharocconjunctivitis, and in one mechanical ptosis, lagophthalmos and blepharocconjunctivitis was seen in
CL patients. It was concluded that ocular complications might be missed because of the failure to conduct ophthalmological examination of CL patients with eyelid involvement and, as a result, proper treatment and follow-up might not be carried out. Relatively sufficient knowledge of the residents of endemic areas in regard of CL is necessary to assure presentation of the patients to the health care centers and therefore reduce the risk of ophthalmic complications by CL.

In one study, a female patient with a lesion of 1.1 cm × 1.1 cm size and 6 months duration in lateral one-third of the left palpebral, which was clinically similar to basal cell carcinoma was diagnosed by histopathological examination as CL, proper treatment resulted in healing of the lesion and during 2 years follow-up no ocular complication was seen. In another study, an 11-year-old girl with a history of 6 months redness of the upper eyelids of two eyes was reported and was misdiagnosed as a blepharitis case. Common antibiotic therapy showed no response and had led to a nodular ulcerated lesion. The etiologic agent was Leishmania, the lesion was cured after a full course of systemic Glucantime.

Analyzing of the current data in comparison with the other studies showed a higher percentage of palpebral lesions in the patients facial CL, which might be due to misdiagnosis palpebral CL lesion in nonendemic areas.

Based on the results, it is concluded that one reason for the absence of ocular involvement after palpebral infection might be early diagnosis and treatment of the lesions. However, eyelid lesion should be diagnosed and treated by using standard therapy.

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

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Conflicts of interest
There are no conflicts of interest.

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