



The new situation of cutaneous leishmaniasis after Syrian civil war in Gaziantep city, Southeastern region of Turkey



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ABSTRACT

Cutaneous leishmaniasis (CL) is an important public health problem with around 2.000 autochthonous reported cases each year in Turkey. Due to the civil war in Syria, Turkey received around three million refugees and they are mainly located at either camps or homes in south/southeastern part of Turkey. In the present study, we aimed to collect samples from CL suspected patients admitting to State Hospital in Gaziantep City and perform parasitological and DNA-based techniques for diagnosis as well as species identification of the parasite for better understanding the prevalence of each species among Turkish and Syrian patients in the region.

The collection of samples was carried out between January 2009 and July 2015. The lesion aspiration samples were taken and stained with Giemsa stain followed by microscopical examination for parasitological diagnosis. After the DNA extraction from Giemsa stained slides, real time and semi-nested PCRs both targeting ITS1 region were performed for molecular diagnosis and species identification.

A total of 567 people were admitted to the hospital with the suspicion of CL and 263 (46.4%) of them were found to be positive by parasitological examination. One hundred seventy-four (66.15%), 88 (33.46%) and 1 (0.38%) of them were Turkish, Syrians and Afghan, respectively. Slide samples obtained from 34 CL suspected patients were analyzed by PCR and 20 of them were found positive. Eighteen (13 Turkish and 13 Syrians) of the positive samples were identified as *L. tropica*, while two (1 Turkish and 1 Syrian) of them were *L. infantum*.

In conclusion, the effects of Syrian civil war on the epidemiology of CL in Gaziantep city is demonstrated in the present study. The use of molecular tool in the diagnosis of leishmaniasis is effective, sensitive and time saving which will enable the species typing. Species typing of the causative agent in endemic areas will bring valuable data to epidemiological knowledge.

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1. Introduction

Leishmaniasis is an arthropod borne disease transmitted by female Phlebotominae (Diptera: Psychodidae) sand flies. There are three main clinical forms of leishmaniasis (cutaneous, visceral and mucocutaneous) and both cutaneous and visceral forms are seen in Old World countries (Ready, 2010). According to WHO, leishmaniasis is one of the most neglected diseases in the world and more than 270.000 cases were reported annually with estimation of 0.9–1.6 million cases each year (Alvar et al., 2012). Cutaneous leishmaniasis (CL) causes skin lesion and it is least fatal form of the disease and characterized as self-healing by leaving a scar in the infection site (WHO, 2010).

Turkey is an endemic country for leishmaniasis and CL cases are mostly reported in south/south eastern regions. According to reports of Ministry of Health of Turkey, more than 40.000 CL cases were reported between 1990 and 2010 (MoH, 2015). The causative agents for CL in Turkey were reported as *L. tropica*, *L. infantum*, *L. donovani* and *L. major* (Svobodová et al., 2009; Özbilgin et al., 2016). Cutaneous leishmaniasis has been reported since 1960s in Syria and the disease was mainly restricted to two endemic areas (Aleppo and Damascus). World Health Organization (WHO) was published a report in 2010 about the CL incidence in Syria with more than 25.000 reported cases per year and Syria was declared as one of the most affected countries by CL (WHO, 2010). During the last 3 years, CL incidence has increased and in early 2013, cases were peaked to 41.000 reported CL cases (MoH Syria, 2013). Due to ongoing civil war, more than 7 million people displaced into neighboring countries (Lebanon, Jordan, Iraq and Turkey) and as a consequence, the disease has begun to re-emerge in neighboring countries. Turkey

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received around three million refugees and they are mainly located either in camps or homes in south/southeastern part of Turkey where main sand fly vectors, *Phlebotomus sergenti*, *P. tobbi* and *P. papatasi*, of *L. tropica*, *L. infantum* and *L. major* are present (Alten et al., 2016).

Direct microscopical examination of lesion aspiration samples taken from margins of cutaneous lesions is the most common diagnostic method of CL. Species typing of the causative agent is important especially when four *Leishmania* species are present. However, detection of the causative agent is not possible by microscopical examination, several molecular techniques are available to identify *Leishmania* species. The internal transcribed spacer 1 (ITS1), which is located between the genes coding 18s rRNA and 5.8 rRNA, was reported to be successful for species typing for both Old and New World CL (Van der Auwera and Dujardin, 2015; Al-Jawabreh et al., 2006; Schönian et al., 2003).

In the present study, we aimed to evaluate the epidemiological status of CL among patients admitting to state hospital in Gaziantep, southeast of Turkey on the Syrian border. This hospital receives many Syrian refugee patients. The microscopical examination and DNA-based techniques for diagnosis as well as species identification of *Leishmania* parasite were performed for better understanding the prevalence of each species among Turkish and Syrian patients in this particular region.

2. Material and methods

2.1. Slide preparation and microscopical examination

Sample collection was carried out between January 2009 and July 2015 from patients with suspected CL lesions that admitted to Dr. Ersin Arslan State Hospital in Gaziantep City. Demographic data including (age, gender, type and location of the lesion(s) and nationality) was recorded for each patient. In terms of the ethical statement, all patients signed informed consent form. Smear samples were collected, fixed (methanol), stained (Giemsa) and examined microscopically for the presence of amastigotes, the immotile form of the parasite. Local ethical committee of the Dr. Ersin Arslan State Hospital approved this work with protocol number: 2016/266

2.2. DNA extraction and species typing

DNA was extracted from slides obtained from 34 CL suspected patients in the period between 2014 and 2015. Giemsa stained slides were washed using molecular grade water and gently scraped with sterile surgical blade at room temperature. Suspensions were transferred to 1.5 ml collection tubes and DNA extraction was carried out using Qiagen DNeasy Blood&Tissue isolation kit (Hilden, Germany) by following the manufacturers instructions.

All samples were studied by a real time PCR targeting ITS1 region using primers and probes as published previously (Ozensoy Toz et al., 2013). Reaction mixture was contained 5 µl of QuantiTect® Probe PCR mix (Qiagen, Hilden, Germany), 10 pmol of each primer and probe and 20 ng DNA. Three international reference strains *L. infantum* (MHOM/TN/1980/IPT1), *L. tropica* (MHOM/SU/74/SAF-K27) *L. major* (MHOM/SU/73/5ASKH) were included in the experiments in order to obtain standard melting curves.

To increase the sensitivity, all samples resulted as negative by real time ITS1 PCR was also studied by a semi-nested PCR. First round of semi-nested PCR was performed as classical PCR using LITSR/L5.8S primers. The products of first amplification were used as template in real time PCR using LITSR/ITS1R-TR1 primers for the

second round of the semi nested PCR (el Tai et al., 2000; Ozensoy Toz et al., 2013).

3. Results

3.1. Parasitological examination of the patients

A total of 567 CL suspected patients (all CL suspected patients have at least one cutaneous lesion) were admitted to state hospital. According to parasitological examination of the Giemsa-stained slides, 304 (53.61%) of them were detected as negative, while 263 (46.38%) were diagnosed as *Leishmania* positive. First Syrian patient was admitted in 2012 and the number of Syrian patient peaked in 2013 to 76 patients (Fig. 1A). In following year incidence gradually reduced in both Turkish and Syrian patients. Only 34 out of 567 samples were included in molecular diagnosis due to inadequate laboratory equipment's.

3.2. Detailed information of *Leishmania* positive patients

Out of 263 microscopically positive patients, 134 (51%) and 129 (49%) were male and female, respectively. The majority of the leishmaniasis patients were Syrian (174 patients, 66%), followed by Turkish patients (88 patients, 33%) and one patient from Afghanistan (0.4%) (Fig. 1B). Most of the patients were aged in 0–10 (93 patients, 35%) and more than half of the patients were aged in 0–20 (141 patients, 54%) (Fig. 1C).

The most common site of the lesions was face (55.6%) followed by hand (21%), arm (11%), foot (8%) and leg (3%). Nape (0.4%) and abdomen (1%) was found to be infrequent site of infection among the patients (Fig. 1D). Two hundred-nine (79%) and 54 (21%) of the patients had one lesion and two or more lesions, respectively. The maximum number of lesions was three and observed in two patients.

3.3. Molecular identification of *Leishmania* species

Among slide samples obtained from 34 suspected CL patients, 15 were found to be positive for the presence of *Leishmania* amastigotes while 19 were negative by microscopy. Real time PCR was resulted in 13 positive cases out of 34, while 7 more positive results were detected by semi-nested PCR. Two microscopically positive patients were resulted as negative by both PCR methods. PCR analyses revealed 20 positive patients in total and the causative agents were detected as *L. tropica* (18 patients, 90%) and *L. infantum* (2 patients, 10%).

4. Discussion

Cutaneous leishmaniasis (CL) is one of the notifiable diseases in Turkey since 1980s and 46,003 cases were reported between 1990 and 2010 (Gürel et al., 2012). Turkey has seven different geographical regions and depending on this the climate and endemicity of vector borne diseases have been changed, which vary in climate and thus affecting rate of vector-borne disease. Therefore, the old world *Leishmania* species (*L. tropica*, *L. infantum*, *L. major* and *L. donovani*) as well as the hybrids are seen in the country (Ozensoy Toz et al., 2013; Svobodová et al., 2009; Özbilgin et al., 2016). In order to reveal the epidemiological status of the disease and treatment regimen, species typing of the causative agent is crucial in such endemic areas with four species. Species identification has also great impact in terms of public health since the identification of the causative agent is related with drug resistance and clinical outcomes.

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