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Original Article

Identification of *Leishmania major* and Other Causative Species in Syrian Patients Infected with Cutaneous Leishmaniasis

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Abstract

Background: Leishmaniasis presents in three main clinical forms: Cutaneous (CL), Visceral (VL), and Mucocutaneous (MCL). In Hatay, CL is generally caused by *L. infantum* and *L. tropica*, with *L. major* appearing less frequently. Following the 2011 migration wave from Syria, we aimed to identify the causative *Leishmania* species, particularly L. major, among Syrian patients living in households and tent cities in Hatay, Turkey.

Methods: Smear preparations from 250 Syrian patients diagnosed with CL at Hatay Mustafa Kemal University, Turkey between 2011 and 2019 were retrospectively analyzed. DNA was isolated from these preparations, and molecular typing was performed by Real-Time Polymerase Chain Reaction (RT-PCR) using primers and probes specific to the ITS1 gene region.

Results: Molecular analysis revealed that 182 (72.8%) of 250 Syrian patients were infected with *L. tropica*, 59 (23.6%) with *L. infantum/donovani*, and 9 (3.6%) with *L. major*. While *L. tropica* and *L. major* are known causative species in Syria, the detection of *L. infantum* cases suggests the possibility of local transmission by sand flies, the primary vector in the Hatay region, during the patients' stay in tent cities.

Conclusion: The findings highlight the distribution of different *Leishmania* species among the Syrian population in Hatay. Implementing species-specific treatment protocols and maintaining effective notification and control activities are crucial for preventing the further spread of Cutaneous Leishmaniasis in the region.

Introduction

Leishmaniasis, one of the neglected tropical diseases, is caused by more than 20 species of *Leishmania* (1). Leishmaniasis is found in approximately 102 countries worldwide (2). Approximately 600,000 to 1 million new cases of Cutaneous Leishmaniasis (CL) and 50,000 to 90,000 new cases of Visceral Leishmaniasis (VL) are reported each year. In recent years, climate change, war, migration, and increased travel have facilitated the parasite's access to people in developed countries (1).

Leishmaniasis has remained a public health issue in Turkey for many years. The Ministry of Health reported a total of 46,003 new CL cases between 1990 and 2010 and a total of 21,370 CL cases between 2011 and 2017 (3, 4). Anthroponotic CL is endemic in the Southeast Anatolia, Eastern Mediterranean, and Aegean regions. While Şanlıurfa accounts for 50% of CL cases, Diyarbakır, Çukurova, Hatay, Osmaniye, Maraş, Aydın, Antalya, Niğde, and Muş are among the other endemic provinces (4).

The distribution of CL in a region is closely related to vector prevalence, climate, sociocultural conditions, and the geographical distribution of reservoirs. Migration from endemic regions to other regions is also an important factor in the spread of the disease (5, 6). Large population movements have made the spread of CL inevitable in countries where Syrians have relocated. Due to the turmoil in Syria, approximately 5 million Syrians have fled to neighboring countries such as Turkey, Lebanon, Jordan, and Iraq, causing an increase in cases in these countries (7, 8).

Leishmaniasis has been prevalent in Syria since the mid-1980s, historically concentrated in the rural areas of Damascus and Aleppo. Cases of CL caused by *L. major* and *L. tropica*, as well as cases of VL caused by *L. infantum*, have been reported (9, 10). Following the civil war that began in 2011, there has been an increase in the number of cases in Deir ez-Zor, Hasakeh, and Raqqa in the northeast, and Idlib in the northwest, which are not endemic for leishmaniasis (11).

Refugees from Syria first settled in Hatay and then spread throughout Turkey. This process initially led to an increase in CL cases in certain provinces, and later to cases being reported throughout Turkey. While *L. tropica*-induced anthroponotic CL cases are generally seen in Turkey, cases caused by *L. infantum*, *L. donovani*, and *L. major* have also been reported in recent years (10). *L. tropica* is the causative species in the Southeastern Anatolia region of Turkey, while *L. tropica* and *L. infantum* are found in the eastern part of the Mediterranean region (12).

Hatay is located in the Mediterranean Basin in the southeastern part of Turkey, and CL is considered an endemic disease (12). *L. infantum* was identified as one of the main species causing CL cases in Hatay in studies conducted before migration (13). In addition to *L. infantum, L. major* and *L. tropica* are also among the causative species of CL in Hatay (14, 15).

In this study, the aim was to determine the epidemiological, clinical, and demographic characteristics of Syrian patients living in Hatay after migration who were diagnosed with CL, as well as the causative species, particularly *L. major*.

Materials and Methods

Ethics Committee Approval

Ethical approval for the study was obtained from the Non-Interventional Clinical Research Ethics Committee of Hatay Mustafa Kemal University. (Research Protocol Number: 2023/06/19). This study was supported by Hatay Mustafa Kemal University Scientific Research Projects under the project code No: 22.GAP.040.

Study Area

Hatay, located in southern Turkey, has an area of 5,524 km² excluding lakes. It has a typical Mediterranean climate with mild, rainy winters and hot, dry summers. Annual average temperatures range from 15.1 to 20 °C, and annual average rainfall amounts range from 562.2 to 1,216.3 mm (16, 17).

During the first wave of Syrian migration to Hatay, a total of five tent cities were established in Altınözü, Yayladağı, and Reyhanlı (18).

Sample Collection and Microscopic Examination

Smear preparations were prepared from skin scrapings taken from Syrian patients who applied to the Department of Parasitology at Hatay Mus-

tafa Kemal University Faculty of Medicine after migrating in 2011. stained using the Giemsa staining method, and examined under a microscope (100X magnification). A total of 250 archived smear preparations in which *Leishmania* amastigotes were detected, resulting in a positive diagnosis, were randomly selected from the archive and included in the study. Molecular species identification was performed using real-time PCR with species-specific primers and probes. The age, gender, demographic, epidemiological, and clinical characteristics of the patients were recorded. The information obtained from the patients was analyzed.

DNA Isolation and Genotyping

The smear samples from the selected specimens were processed using a commercial DNA isolation kit (QIAamp DNA Mini Kit, Qiagen, Germany) according to the protocol provided.

To determine the species of *Leishmania* parasites in the samples, primers targeting the genes encoding SSU rRNA and 5.8S rRNA, including the internal transcribed spacer-1 (*ITS-1*) region, were used (Forward primer: 5'-CTGGATCATTTTCCGATG-3' and Reverse primer: 5'-GAAGCCAAGTCATCCATCGC-3') and probe (Probe 1: 5'-CCGTTTATACAAAAAATATAC-GGCGTTTCGGTTTFluo-3' and Probe 2: 5'-LCRed-640-GCGGGGTGGGTGCGTGTGTG-Pho-3') were used to perform Real-Time Polymerase Chain Reaction (RT-PCR). For PCR, a total volume of 25 µL of PCR mixture was prepared using the QuantiTect Probe PCR Kit Master Mix (Qiagen) and performed on a Rotor-Gene device with melting analysis (12).

Results

The data of 250 patients included in the study consisted of 151 males (60.4%) and 99 females (39.6%). The patients were primarily from Aleppo (126, 50.40%), Idlib (77, 30.80%), and Hama (28, 11.20%) (Fig. 1, 2). When examining the age range of the patients, the highest number of cases (152, 60.8%) was found among children and young adults aged 0–20, while the rate was 60 (24%) among those aged 20–40 and 15.2% (38 cases) among those over 40.

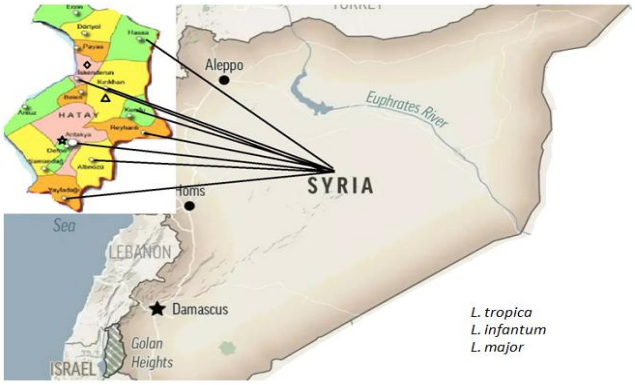

Fig. 1: Cities from which patients came from Syria (This image was generated using OpenAI's ChatGPT (DALL·E image generation tool), based on author-provided input.)

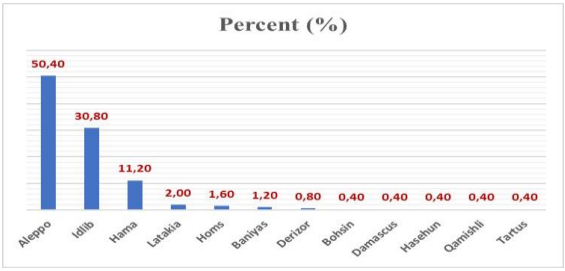

Fig. 2: Percentage of patients according to the regions they came from in Syria (%)

When comparing clinical appearance and lesion durations, it was determined that nodular lesions were most common in lesions that developed between 0-6 months and 7-12 months, papular lesions were most common in lesions that developed between 13-18 months, and

ulcerative lesions were most common in lesions that developed after 19 months. Of the patients, 165 had an acute course and 85 had a chronic course, as shown in Table 1 and Fig. 3.

Table 1: Distribution of lesion clinical forms and status by number and percentage according to lesion duration

Variable	Clinical forms of lesions n (%)				Lesion status n (%)	
Months	Nodule	Papule	Recidivans	Ulcer	Acute	Chronic
0-6	102 (56.0)	64 (35.2)	1 (0.5)	15 (8.2)	165 (90.7)	17 (9.3)
7-12	27 (50.0)	14 (25.9)	1 (1.9)	12 (22.2)	0 (0.0)	54 (100.0)
13-18	3 (30.0)	6 (60.0)	0 (0.0)	1 (10.0)	0 (0.0)	10 (100.0)
≥19	1 (25.0)	0 (0.0)	1 (25.0)	2 (50.0)	0 (0.0)	4 (100.0)
Total	133	84	3	30	165	85
χ^2; p	24.340; .002				197.875; < .001	

Fig. 3: Clinical appearance of lesions found in patients

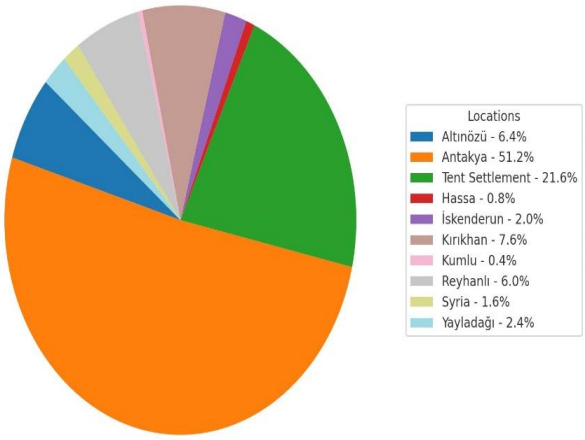

Fig. 4: Percentage distribution of regions where patients live after migration

When examining the distribution of lesion sites on the body, 111 (44.4%) patients had lesions on the upper extremities, 63 (25.2%) on the trunk, and 30 (12.0%) on the lower extremities, while 46 (18.4%) had lesions in multiple areas.

After migrating to Hatay, the majority of patients settled in Antakya city center (128, 51.2%) and tent cities (54, 21.6%) (Fig. 4).

After applying RT-PCR to a total of 250 Syrian patient samples, 182 (72.8%) were *L. tropica*, 59 (23.6%) were *L. infantum/donovani*, and 9 (3.6%) were *L. major*.

Statistical Analysis

IBM SPSS version 29.0 (IBM Corp., Armonk, NY, USA) was used to conduct the statistical analyses of the data. Frequency analyses and chi-square tests of independence were conducted to examine the categorical variables in the data. In all statistical analyses,

the level of alpha for statistical significance was set at $P < .05$.

A chi-square analysis was performed to test the relationship between clinical forms of the lesion and lesion duration. A 4x4 crosstabulation was obtained from the chi-square analysis. As a result of the chi-square test of independence, a statistically significant relationship was found between the clinical form and lesion duration (χ^2 (9, N = 250) = 24.340, P = .002) (Table 1).

As shown on Table 1, the chi-square test post hoc analysis revealed that among people with ulcer, a 7-12 months or 19+ months lesion duration is significantly more prevalent than others, while a 0-6 months lesion duration is significantly less prevalent. Moreover, among people with recidivans, having the lesion for 19+ months is significantly more prevalent. The remaining clinical forms do not have any significant relationship with lesion duration. A chi-square analysis was performed to test the relationship between lesion status (chronic vs acute) and lesion duration. A 2x4 crosstabulation was obtained from the chi-square analysis. As a result of the chi-square test of independence, a statistically significant relationship was found between the clinical form and lesion duration (χ^2 (3, N = 250) = 197.875, $P < .001$). As shown in Table 1, the chi-square test post hoc analysis revealed that among people with acute lesion, a lesion duration of 0-6 months is significantly more prevalent. In contrast, among people with chronic lesion, a lesion duration of 0-6 months is significantly less prevalent, while lesion durations of 7-12 months, 13-18 months, and ≥19 months are significantly more prevalent.

Discussion

The study aimed to type *Leishmania* species from Syrian patient preparations using RT-PCR. The data obtained revealed that *L. infantum, L. tropica*, and *L. major* species were observed in Hatay, Turkey's border province, particularly after migration. According to the data, it is considered important to use molecular methods for typing in the diagnosis of CL in order to contribute to treatment approaches.

Turkey has a favorable environment for the emergence of vector-borne diseases such as CL due to its geographical location in a hot and humid climate (19). In recent years, research on the effects of this disease has been of great importance in clarifying the epidemiological profile of CL in Turkey and better understanding the mechanisms of infection (20). Various studies have conducted detailed investigations into topics such as environmental factors affecting the spread of CL, genetic variations of the pathogen, and immune responses of individuals (21).

In the treatment of leishmaniasis, drugs such as pentavalent antimony compounds, miltefosine, and paromomycin are used. However, in addition to the high cost of these drugs and their toxic effects, factors such as neglect of CL patients, deficiencies in laboratory services, difficulties in vector and reservoir control, and delays in diagnosis and treatment contribute to the spread of the disease. The definitive diagnosis of CL is based on direct microscopic examination to detect the parasite, and the causative species can be identified using molecular methods (22). In the study, 182 of the 250 specimens collected from Syrian patients using RT-PCR, which includes the ITS-1 region, were typed as *L. tropica*, 59 as *L. infantum/donovani*, and 9 as *L. major*.

Between 1990 and 2010, 46,003 cases of CL were reported in Turkey (12). Since 2011, an increase in the number of CL cases has been observed throughout Turkey due to the impact of migration movements, with 2013 being the peak year with 5,362 cases (3). The WHO has classified 12 countries, including Turkey and Syria, as countries with more than 2,500 cases of CL per year and has designated these countries as high-risk (2).

In the old world, CL is characterized by an itchy papule that can develop into a nodule, plaque, or ulcer. The study found that the

nodular form was more prevalent among refugees from Syria. CL lesions generally heal spontaneously within a year but leave scars. When the lesion durations of 250 patients were examined, 66% (165 patients) were acute and 34% (85 patients) were chronic lesions lasting more than a year. In line with other studies, the highest incidence of lesions was observed in children and young adults aged 0-20 years, accounting for 60.8% (152 cases), while the incidence was 24% (60 cases) in the 20-40 age group and 15.2% (38 cases) in those over 40 years of age. When examining the causative agent of the lesions in patients from Syria who came to Hatay, anthroponotic Leishmaniasis caused by *L. tropica* was higher at 72.8%. Zoonotic *L. major* (3.6%) and *L. infantum* were detected at lower rates (23.6%).

With the migration movements that occurred as a result of the civil war in Syria in 2011, it has been reported that other species such as *L. major* have also become active in the regions to which people have migrated (11). After Istanbul and Gaziantep, Hatay is the third province in Turkey with the highest number of Syrians (23). Between 1994 and 2004, covering the period before the migration, 1,079 cases of CL were identified in Hatay (24). Between 2006 and 2011, a total of 535 cases were reported, with the majority of these cases originating from endemic districts such as Altınözü, Hassa, Reyhanlı, Samandağ, Kırıkhan, and İskenderun (25). Prior to migration, the species most commonly responsible for CL was *L. infantum* (13). With the wave of migration from Syria in 2011, CL has become a disease that can be seen in every region of Turkey, and post-migration, *L. tropica* and *L. major* have begun to appear among the causative species (14, 15).

Following the Syrian migration, changes have begun to occur in the *Leishmania* pathogens generally observed in our provinces. In this context, while *L. tropica*, which is anthroponotic, is observed in Şanlıurfa, cases caused by *L. major* and *L. infantum* have been reported post-migration (26, 27). In some studies conducted using molecular methods in different provinces of our country after the migration, *L. major* was detected. In a study conducted by Özbilgin et al. between 2011 and 2014, 18 *L. major* cases were detected by isolation from CL cases in different provinces of Turkey (15). In studies conducted in Hatay before migration, the causative agent of CL was mostly *L. infantum*, while after migration, both Turkish and Syrian cases were found to be caused by *L. tropica* and *L. major* (12). In studies conducted in Hatay before migration, *L. major* was found in CL cases originating from abroad. In five truck drivers transporting goods to Syria, Saudi Arabia, Iran, Georgia, Uzbekistan, and Azerbaijan, overseas-sourced CL cases were identified as *L. infantum, L. major*, and *L. tropica* (25). The strategic location of migration routes following migration movements has led to an increase in the number of CL cases in Turkey and a shift in the causative species to *L. tropica, L. major*, and *L. infantum/donovani*. In the study, *L. tropica* was found in 72.8% of Syrian patients who arrived after migration, *L. major* in 3.6%, and *L. infantum/donovani* in 23.6%.

Study conducted in other provinces of Turkey have shown that *L. major* is more common in Syrian patients (20). In this study, 9 out of a total of 250 patients had *L. major*. People who came from Syria and stayed in camps or homes were mostly from Aleppo, Idlib, Hama, and Damascus. In a study conducted in Urfa, *L. major* was detected in Syrian refugees, and when their places of origin were examined, they were found to be the same as in our study (26).

The distribution of CL is closely related to climate, vector prevalence, geographical distribution of reservoirs, and sociocultural conditions in a region. One of the important factors in the spread of the disease is migration from endemic to non-endemic areas (28). The detection of *L. infantum* species in 23.6% of cases is noteworthy. This rate suggests the possibility that the lesions may have devel-

oped in Hatay, as a large proportion of patients were unable to recall the onset of lesions clearly and provide accurate information. The fact that these individuals have been living in tent cities in Hatay for a long time after migrating from Syria and their potential exposure to the sand fly vector strengthens the possibility that the high rate of *L. infantum* is due to local transmission.

After the large wave of migration in Turkey, CL has not only been seen in limited areas but has become a parasitic infection detected in almost all regions of Turkey. Especially with the start of the first wave of migration, it has gained momentum in the spread of CL in provinces such as Hatay, where CL is endemic. In Hatay, following the migration, *L. tropica* has been observed more frequently in addition to *L. infantum*, and an increase in rare *L. major* cases has also been noted. Studies have reported *L. major* cases in provinces such as Şanlıurfa, Manisa, Gaziantep, Mersin, and Adana (26, 27, 29, 30).

Studies conducted in countries neighbouring Syria reveal that the distribution of CL species and their clinical characteristics may vary regionally. In a large-scale molecular study conducted by Mohammadiha et al. in Iran, 648 CL isolates obtained from 12 different regions were evaluated using PCR-RFLP and sequence analysis targeting the ITS region. *L. major* was detected in 73% of cases and *L. tropica* in 27%. The researchers reported that *L. major* is particularly associated with the zoonotic cycle in rural areas, whereas *L. tropica* is more prevalent in urban areas with anthroponotic transmission (32). When these findings are compared to the detection of *L. tropica* as the predominant species in Syrian patients in our study (72.8%), it suggests that the cases in Hatay may be more related to the anthroponotic transmission cycle.

In Iraq, a study conducted by Mancy et al. in Al-Ramadi did not include molecular species typing; however, the authors provided detailed clinical observations regarding lesion characteristics. They reported that CL lesions were predominantly located on exposed parts of the body, particularly the face and extremities, and associated this distribution with living conditions and increased likelihood of contact with sand fly vectors (33). These findings are consistent with the clinical observations in our study. Similarly, CL lesions in Syrian patients were predominantly located on exposed body areas. This distribution may be related to the prolonged residence of patients in tent cities and temporary settlements in Hatay following migration. Open living conditions, limited protective measures, and increased exposure to sand flies in tent camps may have contributed to the observed lesion localization pattern.

Conclusion

This study examined smear preparations from 250 Syrian patients diagnosed with KL and included in the archive using the RT-PCR method in Hatay. RT-PCR analysis revealed the presence of *L. tropica* in 182 samples (72.8%), *L. infantum/donovani* in 59 samples (23.6%), and *L. major* in nine samples (3.6%). The detection of *L. infantum* suggests that the infection was most likely acquired within Turkey, particularly in tent cities, through contact with vectors (sand flies). The inability of patients to precisely determine the onset time and place of infection strengthens the likelihood that the lesions originated in Turkey. Molecular-level species typing in the diagnosis and treatment of CL is of great importance due to the differences in the clinical course and treatment response of each species. It was concluded that this study could serve as a guide for public health initiatives to be developed for the management and monitoring of increasing CL cases in Turkey.

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Competing interests

The authors declare no competing interests

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