Cutaneous Leishmaniasis in North Lebanon: re-emergence of an important neglected tropical disease

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Abstract

Background: Cutaneous leishmaniasis (CL) is the most prevalent neglected tropical disease among externally displayed people in the Middle East. In recent years, the Lebanese population has increased >30% mainly due to a mass influx of Syrian migrants, thousands of them carrying CL among other infectious diseases. Here, we revisited the current CL prevalence among refugees in Northern Lebanon.

Methods: This cohort study was conducted at the Al-Bachaer Medical Center in North Lebanon between 01/2017 and 06/2017. Randomly selected, forty eight suspected CL patients were clinically diagnosed by dermatologists, and samples were obtained for microscopic examination and molecular identification by PCR-RFLP. The treatment response to antimonials was assessed at each week and was followed for up 6 months.

Results: *Leishmania tropica* was the most predominant species (91.7%) followed by *L. major* (8.3%). Confirmed cases were treated with 1-2 courses of antimonials, and healing was achieved mainly after receiving a second course of treatment. Importantly, we show evidence of possible local CL transmission by indigenous sand flies in three separate patients who had no history of recent travel to Syria.

Conclusions: This highlights the urgent necessity to implement preventive disease strategies to avoid further dispersion of *L. tropica* CL in North Lebanon.

Keywords

Cutaneous leishmaniasis, Glucantime, Lebanon, *Leishmania tropica*, PCR-RFLP, Syrian refugees.

Introduction

Cutaneous leishmaniasis (CL) is the most prevalent neglected tropical disease (NTD) in the conflict zone in the Middle East, which currently affects more than hundred thousands of refugees annually.[1,2] Ongoing civil war in Syria resulted in mass migration of five million Syrians to neighboring countries.[3] Lebanon, situated to the west of Syria, has the highest per-capita of refugees in the world (232 per 1,000 inhabitants) by hosting more than one million Syrian refugees, an enormous stress on a historically fragile country with a population of just 4 million.[4] As reported by Oxfam T5, the area of North Lebanon, including districts of Tripoli, Zgharta, Becharreh, Minieh-Dinieh, El-Koura hosts 283,728 Syrian refugees that counted as one third of the total T5 population with 833,728 inhabitants. While Akkar district hosts 95,403 Syrian refugees counted as 33% of the total population of Akkar numbering around 293,577 persons.[5]

Historically, Lebanon has had a very low annual prevalence of CL. Only 6 CL cases were recorded in Lebanon during 2000-2012,[6] in addition to few cases of visceral leishmaniasis in rural areas located at the Lebanese-Syrian borders.[7] Recently, outbreaks of CL among Syrian refugees started to be reported by hospitals and primary health care centers.[8] Furthermore, mass migration of Syrians fled from Aleppo and Edleb were reported several thousand cases of CL.[9,10] Moreover, the phlebotomine sand fly vectors *Phlebotomus tobbi*, *Ph. syriacus*, *Ph. alexandri* and *Ph. sergenti* have been identified in high-altitude mountains of Lebanon.[11] Thus, the presence of local sand fly vectors and a continual influx of CL-infected refugees into other areas of Lebanon further increase the risk of disease emergence.

The clinical manifestations of CL vary widely from spontaneous self-healing ulcer to debilitating chronic or disfiguring lesions.[12] The drugs used to treat CL are expensive with significant toxicity and side effects. Meglumine antimoniate (Glucantime) remains the first-line treatment for CL in most countries, including Lebanon.[13] In many cases, CL ulcers develop secondary infections, which according to the World Health Organization (WHO) are first treated with a combination of antibiotics and antifugal creams followed by 1-2 courses of intralesional (or intramuscular) meglumine antimonite depending on the number, size and location of the lesions.[13] The WHO has offered more than 10,000 ampoules of Glucantime for the treatment of CL in Lebanon.

In this study, we report on the epidemiology of CL among refugees in North Lebanon between January to June 2017, and showed that most patients were infected with *L. tropica*. Furthermore, we present evidence of potential autochthonous CL transmission in North Lebanon, which highlights the need of establishing a coordinate disease control program to avoid a further CL dispersion in the region.

Methods

Inclusion of patients

This cohort study was conducted at the Al-Bachaer Medical Center in North Lebanon between 01/2017 and 06/2017. Forty-eight CL patients were clinically diagnosed by well-trained dermatologist according to symptoms (presence of characteristic sore or ulcer lesions). Samples were taken from 18 females and 30 males, ranging in age from 1 to 60 years, with a median age of 10 years (interquartile range, 5-17 years). The majority of patients (79%) were younger than

18 years. Case record studies and information sheets were obtained for all patients and re-labelled with the appropriate study code. The recorded patient information included age, sex, region of origin, date of entrance to Lebanon, address and all relevant clinical data (i.e. lesion size, number(s) and location(s) on the body, clinical features and treatment response). All data were kept in secure computer folders.

Sample collection

Needle aspiration was performed in each patient for microscopy analysis after Giemsa staining. Isohelix (SK-2S, UK) swab samples were taken by rubbing the ulcer approximately 20 times in a clockwise direction after removing the covered crust or scabs; the surrounded area was then cleaned with 70% ethanol. All swabs were then processed for molecular diagnosis as indicated below.

DNA extraction and molecular identification

Total genomic DNA was extracted from all lesion swabs using Qiagen DNeasy Blood & Tissue Kit (Qiagen, USA) according to the manufacturer's instructions. *Leishmania* species were identified by PCR-RFLP (Polymerase chain reaction- Restriction fragment length polymorphism) analysis of the ribosomal Internal Transcribed Spacer 1 (*IST1*), as described previously.[14] Primers used were: LITSR (5′- CTGGATCATTTTCCGATG -3′) and L5.8 (5′- TGATACCACTTATCGCACTT -3′). The PCR mixture contained 4.0 mM $MgCl_2$, 200 µM dNTPs, 500 nM primers, 2 U Taq polymerase (KAPA Biosystem) and 1.5 mM $MgCl_2$. After 5 minutes denaturation at 94°C, PCR was performed using 35 cycles of 30 seconds at 94°C, 30 seconds at 53°C, and 60 seconds at 72°C. There was a final extension step of 72°C for 8 mins.[14] For swab samples negative by *ITS1*-PCR, DNA was extracted from smears and amplified using

the same PCR protocol.[14,15] Subsequently, 10 µL of PCR product was digested with 1 unit of *Hae*III digest (New England Biolabs) for 2 hours at 37°C. Restriction fragments were analysed on 2% (w/v) agarose gels using ethidium bromide. The leishmaniasis infections were identified by clinical assessment and confirmed by laboratory analysis.

Drug Treatment

Clinically diagnosed CL-infected patients were treated with 0.5-5ml (85 mg Sb/ml) of intralesional antimonials twice a week, for 3-4 weeks until healing was completed. Systemic administration of antimonials (20 mg Sb/kg/day for 20 consecutive days) was also used in patients presenting multiple (>5), large (>5 cm) or disfiguring lesions. Treatment failure was defined by the lack of re-epithelialization after receiving one course (12 doses) within 45 days of treatment starting.[13]

Data and statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics v22.0 software. Results were presented as the median with interquartile range for quantitative variables (age) and number (percentages) for qualitative variables. The association of the variable swab sample technique along with PCR, in infected patients with suppurative lesions and dry lesions, was determined using the Fisher's exact test and differences were considered to be statistically significant at $p<0.05$.

Results

Origin and distribution of CL patients

Confirmed patients mainly originated from CL endemic and non-endemic areas of North Syria. Most of them migrated from Edleb (44%; 21/48), followed by Hama (25%; 12/48), Aleppo (17%; 8/48), Rakkah (6%; 3/48), Homos (6%; 3/48) and Tartous (2%; 1/48) (Figure 1). By the time this study was conducted, these patients were residing in North Lebanon, especially in Tripoli district (29%; 15/48) followed by Miniyeh-Dannieh (24%; 10/48) and Zgharta districts (24%; 10/48). They are localized primarily in Badawi camps and Tebbeneh region, which are one of the poorest areas of Northern Lebanon. The level of poverty in these regions is quite high, with insufficient hygiene, inadequate sanitation and infrastructure.

Clinical presentations of CL-infected patients

Forty-three patients developed newly active CL lesion while five had chronic lesions. The duration of the lesions prior to sampling ranged from 4 weeks to 48 months. Furthermore, the majority of the ulcers were reported to have appeared between 1 week up to 3 years after their displacement to Lebanon. A higher proportion of the patients have lesions located in the facial area (67%; 32/48), followed by the arms (31%; 15/48), legs (14.5%; 7/48), trunk (4%; 2/48) and shoulder (4%; 2/48) (Figure 2). Moreover, the lesion size varied from 0.5 to 7 cm with an average mean of 1.3 cm. Nodular lesions were the most predominant clinical presentation (67%; 32/48) followed by papular (23%; 11/48) and plaque lesions (10%; 5/48), and suppuration was predominately present in nodular (66%; 21/32) and plaque forms (40%; 2/5).

Interestingly, CL was also diagnosed in three patients that have settled in Lebanon for more than 32 months and with no recent history of traveling or diagnosed with CL. These individuals lived in very poor areas of Tripoli (Abou Samra-chok), Akkar and Kalamoun, which are characterized for accumulation high amount rubbish and organic matter from animals (well-

147 known sand fly breeding sites) (Figures 1 and 3). All three patients were diagnosed by both
148 microscopy examination and PCR, and presented ulcerative nodules bigger than 2 cm in size for
149 at least 4 months.
150
151 **Microscopy and molecular identification**
152 After DNA extraction from Isohelix ulcer swab and smear aspiration, the 48 samples
153 from CL suspect individuals were positive for *Leishmania* spp. by PCR. *L. tropica* was the most
154 predominant species (44/48; 91.7%) followed by *L. major* (4/48; 8.3%). Patients infected by *L.*
155 *tropica* originated mainly from Edleb (21/44; 48%), Hama (12/44; 27%), Aleppo (7/44; 16%),
156 Homes (3/44; 7%), and Tartous (1/44; 2%). Whereas, Al Rakka (3/4; 75%) and Aleppo (1/4;
157 25%) were the original cities for patients infected by *L. major* (Figure 1).
158 By microscopic examination (ME), 39 samples were positive by identifying at least one
159 *Leishmania* amastigote in smears. The sensitivity of ME for *L. tropica* was 73% (32/44) and
160 100% (4/4) for *L. major*. The majority of suppurative lesions (83%; 19/23) were successfully
161 genotyped by PCR after DNA extraction of Isohelix swabs whereas only 48% (12/25) of dry
162 lesion swabs gave positive results. This indicates that swab sampling method along with PCR is
163 significantly more suitable for the molecular diagnosis of suppurative lesions than dry lesions
164 (Fisher-test=0.016).
165
166 **Treatment response**
167 Among the 48 confirmed CL patients, we followed the treatment response for 43 patients
168 who developed new active lesions. Results showed that only 20 patients (46%) healed after one
169 course of intralesional Glucantime (12 doses within 45 days), whereas, 18 (42%) and 3 (7%) of

the patients required a second and third course of Glucantime, respectively. In addition, two patients (5%) receiving systemic drug treatment were cured after one course of treatment over a period of 20 days.

Discussion

Dispersion of CL continues to be one of the most important health consequences of the current Syrian conflict, particularly in externally displaced Syrian migrants. In this work, we present an update of CL epidemiology in the northern districts of Lebanon, which are in geographical proximity to CL-endemic Syrian regions such as Homos, Hama and Edleb and where ca. 75% of patients included in our study originated from. The molecular analysis showed that *L. tropica* is the main parasite species identified in the above cities in addition to Aleppo and Tartous. Interestingly, most of the patients infected with *L. major* originated from Al-Raqqah, which was the capital of the so-called Islamic State terrorist group over 3 years of war. This region is not historically endemic by CL, but it became one of the most affected by this disease during the Syrian conflict.[1] However, *L. major* is also known to be prevalent in Deir Al-Zour and Al-Hasakeh cities,[16] which are located approx. 150 km from Al-Raqqah. These results support a previous report[1] suggesting that a massive internal human displacement combined with ecological disruption of *Ph. papatasi* (*L. major* vector) habitats in neighbor regions may have contributed to the marked increase of CL in Al-Raqqah region. In June 2017, a new outbreak was also recorded in the western countryside of Dara'a governate, mostly in the town tell Shihab. This city had almost no incidence of CL in the past, but the number of confirmed CL cases has currently increased suggesting that sandflies vector might have been carried by families moving from the infected areas.[17] In parallel, the effect of Syrian civil war on the epidemiology of CL in

193 other neighboring countries such as Turkey was also demonstrated in many recent studies.[18,19]
194 Turkey is a CL-endemic country and it currently hosts around ~3 million Syrian refugees in the
195 south/southeaster part of the country, leading to a large increase in the number of CL cases.[18]
196 Most of the CL-infected individuals included in our study were young children (67%)
197 presenting permanent scars, which are likely to blight their social interactions and incorporation
198 into the Lebanese society. This creates a terrible social stigma due to the double impediment to
199 their mental health of being both refugees and carrying CL.[20] In fact, the presence of CL scars
200 alone can cause depression, anxiety and an overall decrease in the quality of life of infected
201 individuals.[21] These psychological and socio-economic impacts will be substantially more
202 noticeable in refugee settings due to the conditions of some of these places. Therefore, future
203 studies aiming to assess the mental health burden of the CL patients in refugee settings are
204 needed in order to formulate recommendations about how simple interventions to address mental
205 health problems can be introduced.
206 During the first four years of the Syrian conflict, Lebanon maintained open borders with
207 Syria. While there are no formal refugee camps for Syrians were established in Lebanon, roughly
208 18% of Syrians live in informal tented settlements around the country. The others are left to
209 dwell in villages and cities without any centralized or regional plan. They are localized in the
210 Northern districts, one of the most deprived regions with severe poverty levels and a high
211 number of refugee migrants. This implies that approximately half of the population of Lebanon's
212 Northern districts (T5) are refugees living below the poverty line.[22] The huge influx of refugees
213 into these regions has put a strain on the scanty local resources. It has increased the depletion of
214 water, sanitation and hygiene, the lack of basic infrastructure and the accumulation of rubbish,
215 which collectively promote the perfect breeding sites for *Ph. sergenti* vector.[5] Thus, the

combination of this suitable environment, the presence of sand fly vectors and refugee migrants presenting either active or inactive (asymptomatic carriers) CL, exhibit a higher threat for the re-emergence of this disease in some areas in Lebanon. Furthermore, in our study, a possible local CL transmission by indigenous sandflies was observed in the three separate cases, although we cannot rule out that these patients were asymptomatic carriers before they migrated to Lebanon. As the last entomological data concerning sandflies in Lebanon is related to a phlebotomine sand fly collection realized in 1995,[11] the characterization of medically important local sand fly species, including bloodmeal preferences and vectorial capacity, is a high priority to update information on the current distribution and dispersion of sand fly fauna in North Lebanon and to prevent future local outbreaks. In addition, xenodiagnosis of wild-caught sand flies will reveal the possible existence of autochthonous transmission and the necessity to implement a local CL preventive control strategy. Although the number of identified local cases is relatively low in our study, other similar cases could be present but either underestimated, misdiagnosed or underreported. For instance, studies showed that CL incidence in Jordan may be underestimated by 40- to 47- fold in national surveillance data.[23] No empirical assessments of CL underreporting are available from the Lebanese official surveillance data.

Patients treated in Lebanon receive 1-2 courses of intralesional (or intramuscular in case of multiple lesions), highly toxic and painful pentavalent antimonials according to current CL treatment regimens. This creates a problem because, as our data show, most of the confirmed CL cases were infected with *L. tropica*, which is naturally more resistant to Glucantime[24] and in fact most of the cases needed a second (and sometimes a third) course of treatment to achieve clinical cure (49%). The four cases infected by *L. major* were healed with a second course of anti-leishmanial treatment. However, we cannot conclude about species treatment response due to the

low number of *L. major* isolates. The variations to antimonial treatment may either be influenced by different factors such as parasite species, the presence of secondary infections within/around the lesion and the spread of drug-resistant parasites.[24-25]

As for the prevalence of visceral leishmaniasis (VL) among Syrian migrants, some cases were recorded at the coast Syrian borders.[7] *Ph. syriacus* and domestic dogs (*Canis lupus familiaris*) were identified as the main implicated VL vector and reservoir, respectively.[26] Unfortunately, the burden of Syrian crisis on VL and their reservoir in Lebanon has not been yet determined. During 2014–2017, five Syrian refugee children died of VL caused by *L. infantum* because of a late diagnosis and lack of awareness of this disease in Lebanon.[27] Recently, four other cases of VL from Syrian refugees were diagnosed in the Lebanese hospitals and were treated by Amphotericin B.[28] The risk of introduction of *L. major* and/or *L. tropica* besides *L. infantum* is highly regarded with the presence of their correspondent reservoirs and vectors. Cats (*Felis catus*) were identified as susceptible reservoir for *L. tropica* and *L. major* in MENA region.[29] On the other hand, humans are considered the main reservoir for the anthroponotic transmission of *L. tropica*,[29] and *Ph. tobbi, Ph. sergenti*, *Ph. pappatasi, Ph. syriacus* and *Ph. alexandri* sand fly species have been already identified in the Lebanese mountains.[11] Collectively, this suggests that Lebanon currently presents all the conditions for the autochthonous transmission of both forms of Leishmaniasis if not preventive disease control measures are put in place by local health authorities.

While the conflict in Syria approaches its ninth year, the military conflict has stopped in some cities that pushed thousands of refugees to return amid fresh hope for last ceasefires. According to the UNHCR, more than 440,000 internally displaced Syrian and about 22,000 of whose fled abroad had come back to cities, which are partly or wholly controlled by the Syrian

262 government, including Damascus, Aleppo, Hama and Homos.[30] This recent situation is highly
263 dangerous because of the risks of returning to a destroyed and unsafe country lacking
264 international support. The deterioration of basic infrastructure, buildings and hospitals, the lack
265 of access to the primary health care and the increasing rate of many infectious diseases such as
266 leishmaniasis will introduce a high risk of morbidity for persons coming back. Sandflies vector
267 will be at high density in these adequate regions for multiplication and without control measures.
268 As a result, people can contract leishmaniasis and then become reservoirs that could contribute
269 with disease spreading on a large scale, especially with the non-sustainability of this return
270 without the social determinants of global health such as employment opportunities, adequate
271 food and water and health care infrastructure.

272 In Summary*, L. tropica* is the most predominant species among refugees in North
273 Lebanon. The majority of patients needed at least two courses of glucantime to achieved cured.
274 The identification of a possible local transmission in Lebanon highlights the urgent necessity to
275 perform entomological studies to characterize the local sand fly vectors. This will help develop
276 targeted vector control strategies in CL-endemic areas to specifically prevent local *L. tropica*
277 transmission.

278

279

280 **Authors' contributions:**

281 DES, MH and AAS designed the study. DS, SM, RR, HM performed the study fieldwork. DS, SM, RR,
282 HM carried out the molecular identification and analysed the data. DS and SM wrote the first draft of the
283 manuscript. DS, AAS, MM, RR and HM critically revised the manuscript for intellectual content. All
284 authors read and approved the final manuscript. MM and AAS are the guarantors of the paper.

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Competing interests:

None declared.

Ethical considerations

Written informed consent was obtained from all the adults who participated in the study. Consent for inclusion of young children was obtained from parents or guardians. The study was reviewed and approved by the Azm Center for Research in Biotechnology and its application Institutional Review Board (Ethic number: CE-EDST-02-2017) and patient data used in this study was anonymized.

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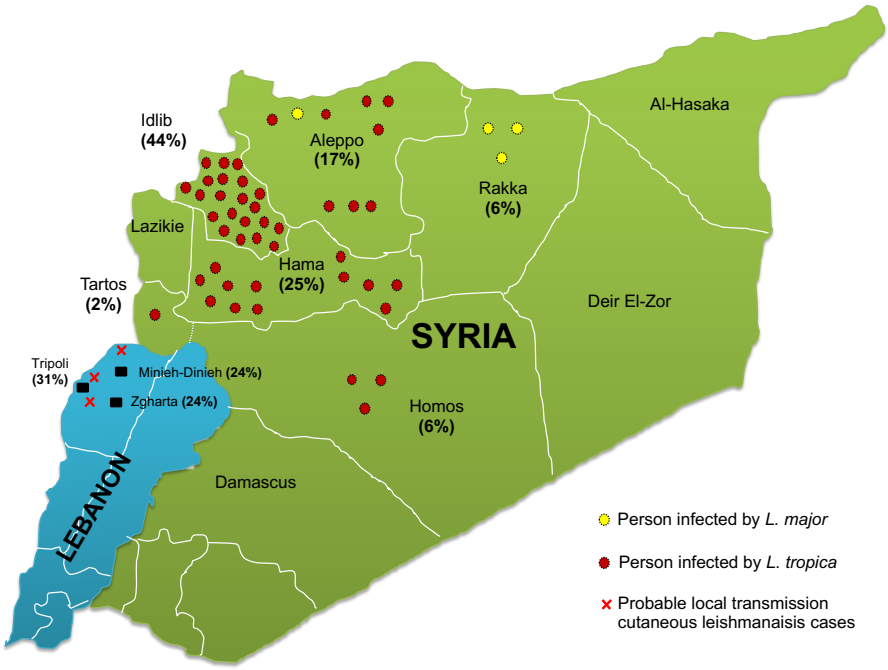

Figure 1. Geographic distribution of Cutaneous Leishmaniasis (CL) cases identified among Syrian refugees in Lebanon (Small box) and their relative region of origin (Round dots). The majority of patients are coming from the northern Syrian regions especially Edleb (44%) and Hama (25%). Infected Syrian refugees are migrated mainly to Tripoli (31%). *L. tropica* (Red dots) is the main genotype identified among CL cases followed by *L. major* (Yellow dots). The green strikes indicated the location of the three probable local transmission CL cases in North Lebanon.

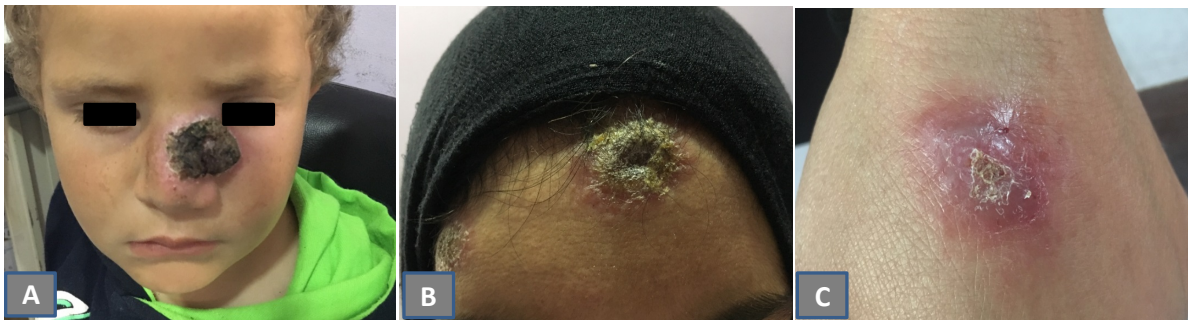

Figure 2. Patterns of cutaneous leishmaniasis among Syrian refugees in Lebanon, 2017. A and B, Disfiguring facial lesions. C, Satellite lesions on the outer edge of CL primary lesion.

Figure 3. Unsanitary living conditions where CL patients live. Accumulated rubbish and presence of animals create perfect sand fly habitats and promote local disease transmission.