DISCLOSURE OF RELEVANT RELATIONSHIPS WITH INDUSTRY

Cutaneous Leishmania

Ibrahim Khalifeh, MD

I do not have any relevant relationships with industry.
Cutaneous Leishmaniania: Is it a Legend?

Ibrahim Khalifeh, M.D.
Assistant Professor
Department of Pathology
American University of Beirut Medical Center

XXIV International Academy of Pathology-Arab Division
History

- Lieutenant-General Sir William Boog Leishman (6 November 1865 – 2 June 1926) was a Scottish pathologist and British Army medical officer.
- He served in India, where he studied enteric fever and kala azar.
- In 1901, while examining pathologic specimens of a spleen from a patient who had died of kala azar he observed oval bodies and published his account of them in 1903.
- Leishman's name was engraved into the history of parasitology by Sir Ronald Ross, who was impressed by Leishman's work and classified the etiologic agent of kala azar into separate genus Leishmania.
Introduction

- Frequent disease: New and Old World
- Global Prevalence: 10 million cases
- Annual incidence: 1.5-2 million
- Annual mortality: 70,000
- Disease burden disability-adjusted life years (DALY): 2.4 million
- Endemic tropical disease
- World health organization (WHO) priority disease
Parasitology
Parasitology

• Leishmaniasis is caused by the vector-borne transmission of the kinetoplastid “leishmania”
• In the vector: Promastigote form.
Parasitology

• In the mammal host: “amastigote form”
  - Exclusively in the macrophages
  - Small, round immobile
Species implicated in human infection

Asymptomatic initial infection

Clinical forms:

**Visceral**
- *L. donovani*
- *L. infantum/chagasi*

**Cutaneous**
- **New World**
  - *L. braziliensis*
- **Old World**
  - *L. major*
  - *L. tropica*
  - *L. aethiopica*

**Mucosal**
- *L. braziliensis*
- *L. guyanensis*
- *L. panamensis*

**Rarely**
- *L. infantum/chagasi*
- *L. donovani*
- *L. colombiensis*
- *L. naiffi*
- *L. lainsoni*
- *L. arabica*
- *L. shawi*
- *L. venezuelensis*
Cutaneous Leishmaniasis
Parasitology

• CL is transmitted by the bite of female sandflies:
  - Phlebotomus in the old world
  - Lutzomyia in the New World

• Leishmaniasis has both zoonotic and anthropoanotic forms
Anthroponotic Cycle

**Amastigotes**
Oval form in cells of mammal host

**Promastigotes**
Flagellated form in the vector

Example of anthroponotic cycle:
*L. tropica L. donovani*

Identification → Culture or direct observation → Smear or biopsy
Example of an animal reservoir cycle in a shared habitat

reservoir – vector – human

*L. infantum*

The epidemiological role of humans is negligible or non-existent as they do not put the parasite back into circulation (or only very rarely); concept of an "epidemiological dead-end".
Parasitology

• The life cycle of the parasite depends on:
  - resist the digestive enzymes of the sandfly
  - adhere to the epithelium of the digestive tube

• Vector independent transmission is very rare: congenitally, transfusion, transplant, lab accidents, etc.
Parasitology

• New world (L. panamensis, guyanensis, amazonensis & mexicana): reservoir is wild animals.
• L. major and infantum: close to human habitats (domestic animals).
• L. tropica and donovani: Human
• Human infection is considered “epidemiologically dead end”

Domestic dog: reservoir for L. infantum
Transepidermal elimination in cutaneous leishmaniasis: a multiregional study

Background: Transepidermal elimination has been documented in a myriad of infectious diseases; however, its occurrence in cutaneous leishmaniasis has not been evaluated.

Methods: Skin biopsies (n = 212) with cutaneous leishmaniasis in Lebanon (n = 46), Syria (n = 53), Saudi Arabia (n = 45) and Pakistan (n = 68) were evaluated. Clinical data collected included age, gender, eruption type (papule, nodule, verrucous or scar), duration and anatomic location. Histopathologically, multiple parameters were recorded including Kellie's parasitic index and transepidermal elimination, interface changes, ulceration and necrosis. Transepidermal elimination was defined as the presence of amastigotes in the epidermis in all layers, limited to the basal layer or present in a perforating plug. All cases were confirmed by polymerase chain reaction (PCR) analysis followed by restriction fragment length polymorphism analysis for molecular subspeciation.

Results: Leishmania tropica was identified in 98.2% and Leishmania major in 11.8% of all cases. Transepidermal elimination was observed in 28.3% of cases (29 perforating plug, 19 all layers and 12 basal layer) with a significant prevalence of L. major in this group (35 vs. 2%, p < 0.001). Cases with transepidermal elimination were associated with interface changes and higher parasitic index (p < 0.001) but not with an increased ulceration rate (p > 0.05). Multivariate analysis showed that transepidermal elimination was independently predicted by L. major [OR (95% confidence interval) = 8.0 (9–712); p < 0.001], parasitic index [OR = 5.4 (2.1–13.3); p < 0.001], interface changes [OR = 6.24 (2.2–17.8); p < 0.001] and necrosis [OR = 0.2 (0.1–0.8); p = 0.026].

Conclusions: We report the largest multiregional cutaneous leishmaniasis series with a 28.3% documented transepidermal elimination incidence of which 48% were perforating plugs; a significant prevalence of L. major was also identified in the transepidermal elimination group. The association of transepidermal elimination with interface changes and a higher parasitic index, without an increased ulceration rate, may reflect a unique biologic alteration in the epidermis, serving to facilitate the extrusion of the parasites through the skin.

Keywords: cutaneous leishmaniasis, molecular subspeciation, transepidermal elimination

Sarah Karam1, Asifa Looy2, Hadil Hamam2, Robert R. Habib3 and Ibrahim Khalifeh1

1Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon.
2Department of Pathology, Shaukat Khanum Memorial Cancer Hospital & Research Center, Lahore, Pakistan
3Department of Dermatology, Hammond Hospital University Medical Center, Saida, Lebanon, and
4Department of Internal Medicine, Outcomes Research Unit, American University of Beirut Medical Center, Beirut, Lebanon

C. transepid. elim. cut. leishm. a multiregional study.

Transepidermal elimination in cutaneous Leishmaniasis: a multiregional study

- Skin biopsies (n=212) with Cutaneous Leishmaniasis
- *L. tropica* (88.2%) and *L. major* (11.8%)
- Transepidermal elimination: 28.3% of cases with a significant prevalence of *L. major* in this group (35% versus 2%, p<0.001).
Transepidermal elimination in cutaneous Leishmaniasis: a multiregional study

• Multivariate analysis:
  1. *L. major* [OR (95% confidence interval) = 80 (9-712); p<0.001]
  2. Parasitic index [OR= 3.4 (2.1-5.3); p<0.001],
  3. Interface [OR= 6.24 (2.2-17.8); p<0.001]
  4. Absence of necrosis [OR= 0.2 (0.1-0.8); p=0.026].
Clinical Presentation
Clinical Presentation

1. Parasitic factors:
   - L. donovani & amazonensis: visceral form
   - L. braziliensis: mucosal form.

2. Host factors:
   - Disease extension
   - Disease evolution
Clinical Presentation

1. Localized Cutaneous Leishmaniasis

2. Multilesional forms:
   - Post kala-azar dermal leishmaniasis
   - Diffuse cutaneous leishmaniasis
   - Disseminated cutaneous leishmaniasis

3. Rare cutaneous forms (still evolving)
1. Localized Cutaneous Leishmaniasis
• Well circumscribed Papule (1w)

• Nodule/plaque (3m)

• Ulcerated/verrucous (5m)

scarring (8m)
Clinical Classification

- Ulcero-crusted type
- Abortive type
- Echtymatous type
- Sporotrichoid type
- Impetigoid type
- Verrucous type
- Tumor-like type
- Hypodermic type
- Eczematous type
- Rhinophyma-like type
- Keloid-like type
- Chalazion-like type
- Hemorragic type
- Pigmented type
- Chanciform type
- Vegetant type
- Recidivan type
- Lupoid type
- Psoriasiform type
- Erysipeloid type
1: Mycobacterium marinum infection
2: Cutaneous leishmaniasis
3: Sporotrichosis
2. Multilesional form

- Post kala-azar dermal leishmaniasis
- Diffuse cutaneous leishmaniasis
- Disseminated cutaneous leishmaniasis
Post kala-azar

- Occurs after Donovani visceral L. In India subcontinent
- Frequency: 5-60%
- DD: Lepromatous Leprosy
- LST: negative 33-100%
- Skin biopsy: negative in 20%
- PCR: positive 83-94%
- Papules: Contains parasite (reservoir)

Post kala-azar dermal leishmaniasis
Abundant hypochromatic papules and nodules on the trunk. Curtsey of Dr. Ali Khamesipour
Diffuse cutaneous leishmaniasis

- Follows localized form
- L. aethiopica & amazonensis
- Clinically resembles Lepromatous leprosy
- LST: negative
- Parasite count: High
- T-cell proliferation in vitro following Leishmania antigen: very weak
- Resistant to treatment
Disseminated cutaneous leishmaniasis

- Multilesional: >10 lesions in > 2 sites
- Brazil and Africa
- HIV patients
- L. amazonensis & major
Tunisia
ZLC (2-8 m)

Curtsey of Dr. Mourad MOKNI

Papule/June

Nodule/Sept

Ulcer/Oct

Scar/jan
Types of CL (Iran)

Anthropoносic CL (ACL)
- Urban type
- Dry lesions
- Chronic
- Longer incubation period (2-8 months)
- Longer duration of self healing (up to 2 years)
- A few lesions usually
- Caused by *L. tropica*

Zoonotic CL (ZCL)
- Rural type
- Wet lesions
- Acute
- Shorter incubation period (2 weeks to 2 months)
- Shorter duration of self healing (up to 1 year)
- Could be numerous lesions
- Caused by *L. major*, *L. aethiopica* & rarely by *L. infantum*

Curtsey of Dr. Ali Khamesipour
Chronic cutaneous leishmaniasis, a great mimicker with various clinical presentations: 12 years experience from Aleppo

Papulonodular form (a-d)

Plaque Form(e-i)

Gyrate From (j)
Tumoral form (e–a)

Verrucous form (b–c)

Ulcerative form (d–f)

Erysipeloid form (g–h)
Histopathology
Ridely’s classification

I. Normal skin/Collagen deg.

II. Pan-necrosis

III. Mixed inflammatory inf.

IV. Scattered giant cells

V. Granulomas

Cutaneous leishmaniasis mimicking inflammatory and neoplastic processes: a clinical, histopathological and molecular study of 57 cases

**Background:** Cutaneous leishmaniasis displays considerable variation in its histopathological and clinical presentation. Clinically, it progresses from a papule into a painless ulcerated and crusted nodule/papule. Microscopically, it progresses from sheets of amastigote-filled histiocytes to granulomatous inflammation.

**Methods:** The study was conducted on 145 skin biopsies from untreated patients with histopathological and/or clinical suspicion of cutaneous leishmaniasis in Lebanon, Syria and Saudi Arabia (1993–2010). The pre-biopsy clinical diagnosis and demographic data were collected. Biopsies were evaluated for the major microscopic pattern, and the parasitic index (PI) was also determined. Diagnosis was confirmed by polymerase chain reaction (PCR) followed by molecular sub-speciation.

**Results:** Of the 145 patients, 125 were confirmed as cutaneous leishmaniasis by PCR. Eighty cases presented with a pre-biopsy clinical diagnosis other than cutaneous leishmaniasis that ranged from dermatitis to neoplasia. Of the 125 cases, 57 showed a major histopathological pattern other than cutaneous leishmaniasis. Identification of amastigotes was equivocal (PI <1) in 36 of the 57 cases. Of interest, all the 18 cases with a pre-biopsy clinical diagnosis other than cutaneous leishmaniasis also showed atypical histopathology for cutaneous leishmaniasis.

**Conclusions:** The manifestations of cutaneous leishmaniasis are broad and may mimic other inflammatory and neoplastic diseases. Pathologists and dermatologists should be aware of such pitfalls and can utilize PCR to confirm the diagnosis of leishmaniasis.

**Keywords:** cutaneous leishmaniasis, mimic, molecular sub-speciation, standard.

145 cases of clinically and/or histologically suspected CL

68 cases
Typical histological features
PCR Positive & PI ≥ 2

68 cases
PCD CL

77 cases
Atypical histological features

20 cases excluded
Negative PCR

39 cases
PCD CL

18 cases
PCD other than CL

45.6% not classical
38/57:
67% PI≤1
Secondary Syphilis

TB

A

B

C
Geography
Geographic distribution of the primary cutaneous leishmaniasis species Old World

Fig 1. Map showing the foci of Anthropomorphic cutaneous leishmanioses in Iran.

Known foci ■
1-Taibad
2-Mashhad
3-Neishabur
4-Sabzvar
5-Tehran
6-Qom
7-Lashan
8-Isfahan
9-Tabas
10-Kerman

Unknown foci ○
11-Shiraz
12-Bushehr
13-Ram
14-Zaheidan & Mirjaveh
15-Mandar-Abbas
16-Yazd
Sidi Saad Dam in KAIROUAN District in 1982

Outbreak ZLC *L. major*

Curtsey of Dr. Mourad MOKNI
Geographical distribution updating of Tunisian leishmaniasis foci: About the isoenzymatic analysis of 694 strains

Diagnosis
Steps in parasitological confirmation in cutaneous leishmaniasis

1. Parasitological confirmation
2. Smear
3. Culture or PCR
4. Species identification
Molecular diagnosis of cutaneous leishmaniasis and species identification: analysis of 122 biopsies with varied parasite index

**Background:** Cutaneous leishmaniasis is endemic in the Middle East and North Africa. Confirming the diagnosis histologically depends on amastigote identification, which varies significantly depending on the infection, strain type, host response and disease stage. Accurate histological diagnosis is mandatory for appropriate therapy.

**Methods:** Skin biopsies from 122 patients from Lebanon, Syria and Saudi Arabia with clinical diagnosis of untreated leishmaniasis were reviewed and clinical data extracted. Cases were classified according to the modified Ridley’s parasitic index. DNA was extracted from formalin-fixed paraffin-embedded blocks. Polymerase chain reaction (PCR) was performed using *Leishmania*-specific ribosomal internal transcribed spacer 1 (ITS1-PCR). Nested ITS1-PCR was performed on cases negative for conventional ITS1-PCR. ITS1-PCR amplicons were digested with HaeIII for subsequent restriction fragment length polymorphism (RFLP) sub-speciation.

**Results:** Of 122 cases, 54 (44.3%) showed a parasitic index of 0–1+ (no unequivocal amastigotes). ITS1-PCR (conventional and nested) was positive for all cases as compared with negative control tissue. RFLP identified *Leishmania tropica* in all cases. Patients with clinically suspected leishmaniasis, whose skin biopsies failed to detect amastigotes represented 44.3% of our cases.

**Conclusions:** In this study, we describe a rapid and optimized protocol from DNA extraction to leishmaniasis sub-speciation. ITS1-PCR showed high sensitivity and specificity in confirming clinically suspected cases.

**Keywords:** cutaneous leishmaniasis, *Leishmania* ribosomal internal transcribed spacer 1 polymerase chain reaction, restriction fragment length polymorphism


---

Lamis Yehia1, Mohammad Adib-Hourieh2, Wasim Fawzi Raslan3, Abdul-Ghani Kibbi4, Asif Loya5, Alireza Fireoz6, Mohamed Satti2, Marwan El-Sabbah1 and Ibrahim Khalifeh6

1Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, Beirut, Lebanon.
2Department of Pathology, Tishreen University, Lattakia, Syria.
3Arabian Health Sciences, Damascus, Damascus, Syria.
4Department of Pathology, Tishreen University, Lattakia, Syria.
5Department of Dermatology, American University of Beirut, Beirut, Lebanon.
6Department of Pathology, Taqwa Hospital, Lebanon.
7Center of Research and Training in Skin Disease and Leprosy, Tehran University of Medical Sciences, Tehran, Iran.
8Department of Pathology, King Abdulaziz Medical City, Jeddah, Saudi Arabia, and
9Department of Pathology and Laboratory Medicine, American University of Beirut, Beirut, Lebanon.

4069 Magenta St, Beirut, Lebanon.

---

Accepted for publication December 14, 2011.
<table>
<thead>
<tr>
<th>PI</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+</td>
<td>1 or more amastigotes per standard section</td>
</tr>
<tr>
<td>2+</td>
<td>10 or more amastigotes per standard section</td>
</tr>
<tr>
<td>3+</td>
<td>100 or more amastigotes per standard section</td>
</tr>
<tr>
<td>4+</td>
<td>1 000 or more amastigotes per standard section</td>
</tr>
<tr>
<td>5+</td>
<td>10 000 or more amastigotes per standard section</td>
</tr>
<tr>
<td>6+</td>
<td>100 000 or more amastigotes per standard section</td>
</tr>
</tbody>
</table>

**Modified Ridley’s Parasitic Index**
ITS1 Polymerase Chain Reaction (ITS1-PCR) Agarose gel electrophoresis of the amplicons of ITS1-PCR for representative samples. The gel reveals 4 specimen that are positive and show a DNA band between 300 and 350 basepairs. The gel also identifies 4 specimens that failed to show the leishmania specific band.

All ITS1-PCR negative samples were subjected to nested ITS1-PCR (100 bpL represents a 100 basepair ladder used as a molecular marker).
• Restriction Fragment Length Polymorphism (RFLP). RFLP pattern typical for Leishmania tropica showing two restriction fragments of 200 and 60 bp.
• RFLP patterns obtained after digestion of representative ITS1-PCR and nested ITS1-PCR amplicons with the restriction enzyme HaeIII. All 125 specimen show an RFLP pattern typical for Leishmania tropica showing two restriction fragments of 200 bp and 60 bp, respectively (20 bpL represents a 20 basepair ladder used as a molecular marker).
Treatment
Major Issues

1. Dermatologic impact
2. Oozing
3. Atrophic scarring/disfiguring
4. Concomitant visceral involvement (10% of New World L.)
5. Disease transition (*L. Tropica*)
6. *L. Major*: spontaneous resolution in 50-75% of cases in 6 months
CL Treatment

• CL is often self-limiting, but treatment is given to accelerate healing, reduce scarring, and prevent the risk of progression and complications

1. Physical treatment including surgery
2. Topical therapy
3. Systemic therapy
Pentavalent antimononate

- **Glucantime**
  Meglumine antimononate
  85 mg/mL (8.5%)

- **Pentostam**
  Sodium Stibogluconate
  100 mg/mL

Both are on the WHO essential drug list. Glucantime is more readily available and less expensive than Pentostam.
Cutaneous Leishmaniasis

- Local and physical can be used for limited CL without the risk of dissemination.
- Systemic treatment is required in cases of *Leishmania* species with the potential to disseminate, immunocompromised host, mucocutaneous or diffuse disease, and severe cutaneous disease.
- Parenteral antimonials are usually first-line agents but are associated with considerable toxicity.
Lesion(s) Requires Treatment

1. Ulcer(s) which are located close to a vital organ or is cosmetically important, such as on the face.
2. Lesion(s) with no evidence of healing for several months after the onset.
3. Special forms of CL, such as sporotrichoid or lymphangietic with satellite lesions, which indicates spreading.
4. CL due to *L. tropica*, which could subsequent systemic involvement or be the reservoir of infection.
5. Requested by the patient
Lesion(s) Recommended not to Treat

- Lesion(s) caused by *L. major*
  - Single or a few lesion
  - Not close to a vital organ
  - Not in the face
  - Lesion size small
  - Requested by the patient
Treatment Strategies

- Single or a few lesions
  - Intralional Glucantime
  - Freeze might be added

- Systemic therapy
  - Multiple lesions
  - Lesion size larger than 3 cm in diameter
  - Lesion close to a vital organ
  - Lesion(s) on face
Collaboration
1. Pathology report
2. H&E and molecular sample
OR
2. Paraffin Block
Collaboration
Kashan, Iran
Spring at American University of Beirut, Lebanon
Acknowledgment

**AUBMC Team**
- Ghazi Zaatar, M.D.
- Robert Habib, PhD
- Lamis Yehia, M.S.
- Jad Saab, M.D.
- Faysal Fedda, M.D.
- Ruba Khattab, M.D.
- Sarah Karram, M.D.

**Collaborators**
- Asif Loya, M.D. (Pakistan)
- Mourad Mokni, M.D. (Tunisia)
- Suad Taraif, M.D. (KSA)
- Wasim Raslan, M.D. (KSA)
- Mohammad Satti, M.D. (KSA)
- Mohammad Houreih, M.D. (Syria)
- Ali Khamesipour, M.D. (Iran)
- Ali Firooz, M.D. (Iran)
- Hadi Hammam, M.D. (Lebanon)