Cutaneous Leishmaniasis in Al-Ahsa Oasis in Saudi Arabia and in Sudan: A Comparative Study

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ABSTRACT

This is a comparative study of cutaneous leishmaniasis (CL) in the Eastern Province of the Kingdom of Saudi Arabia and Sudan. The disease in both countries is caused by *Leishmania major* of different syndromes and the vector is *Phlebotomus papatasi*. The animal reservoir host in Saudi Arabia is *Psammomys obesus* and the Nile rat in Sudan. The clinical manifestations are similar, but some forms encountered in Sudan were not seen in Saudi Arabia. The pathology, immunology, diagnosis and treatment of CL are discussed.

Key words: Al-Ahsa, cutaneous leishmaniasis, Nile rat, *Psammomys obesus*, Sudan

INTRODUCTION AND HISTORICAL BACKGROUND

Cutaneous leishmaniasis (CL) of the Old World is caused by different parasites that include *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica*. The clinical manifestations, the vector, the pathology and response to the treatment vary widely depending on the types of parasite and the clinical manifestations.

This article compares various aspects of CL caused by *L. major* in the Al-Ahsa Region of the Eastern Province of the Kingdom of Saudi Arabia and Sudan. It will be shown that the parasites in Sudan and Saudi Arabia are *L. major* of different zymodemes. The vector in both countries is *Phlebotomus papatasi* and the reservoir of the parasite is *Psammomys obesus* in Al-Ahsa and the Nile rat in Sudan. The clinical manifestations and the pathology are similar in the two countries, but some differences have been documented. This article is based on the author’s experience of work undertaken in both Saudi Arabia and Sudan.

EPIDEMIOLOGY OF CL IN AL-AHSA AND SUDAN

The Al-Ahsa Oasis is the largest natural oasis in Saudi Arabia and all of Asia. It is located just over 40 miles (65 km) west of the Arabian Gulf and measures 30,000 acres (12,000 hectares). Over 60 artesian springs, as well as a large underground aquifer (Encyclopedia Britannica) feed the Oasis. Collectively, these provide water for the million residents and irrigation for over 3 million date palms in the area. In addition to palm trees, the Oasis has abundant wild vegetation, including Chenopod which is the only plant that *P. obesus* feeds on. Besides, indigenous population, there is a sizable number of people from Asia and the Arab World.

In Al-Ahsa, CL attained epidemic proportions in 1973 declining subsequently to reach a plateau in the mid-1980s.[1]
L. major is widely distributed in the area and has been identified as the species causing zoonotic CL with *P. papatasi* as its vector and *P. obesus* as its reservoir host. In the 1980s, the College of Medicine and Medical Sciences of King Faisal University, Dammam, established a field station at Aljisha Village in Al-Ahsa to study CL in the area. A clinic and a laboratory were established in the village. It was attended by Saudi and non-Saudi patients. Most of the latter were Egyptians and Asians working on the farms. No surveys were done and all the data were on patients attending the clinic. Other members of the team studied the vector and the reservoir host. In a clinical study, in which 56 Saudi and 66 non-Saudi patients were examined, it was found that the Saudi patients were considerably younger and had significantly milder disease than the non-Saudi patients.

The endemic areas for CL in Sudan are Kordofan, Darfur in the west and an area in the north along the main Nile. Cases of CL have been described by several authors in Sudan since 1911. These were usually single cases and the parasite was not typed. In the 1970s and 1980s, two major epidemics of CL have occurred in Sudan including Khartoum. The first epidemic on record started in 1976 in the Shendi-Atbara area consistent with a newly-introduced disease in a non-immune population. All the groups were affected. During the epidemic, the incubation period of CL varied from 2 to 4 weeks in the majority of cases. Another outbreak started in Tutti island of Khartoum district in 1985. The disease spread across north and south of Khartoum district and is now endemic in almost all the rural and urban areas of Sudan with the exception of South Sudan. Children are the most affected in the prevailing endemic areas.

**Clinical features**

In a report on the clinical manifestations of CL due to *L. major* in Aljisha village in the Al-Ahsa Oasis, the disease was described as slowly evolving inflammatory lesion(s) that are nodular, noduloulcerative or ulcerative that heals spontaneously with scar(s) in 3-12 months. Features of diagnostic value were identified and their approximate frequency determined in a selected group of 475 patients. These features were: Exposed location site, pairing or clustering of lesions, skin crease orientation, volcanic nodules, satellite papules, subcutaneous nodules and “iceberg” nodules. A combination of three or more of these forms also occurred in Sudan and had the same diagnostic value. Lymph node enlargement was documented in both Sudan and Saudi Arabia. The affected lymph nodes were generally solitary in form, firm, mobile, non-tender, only moderately enlarged appearing to persist beyond the associated skin lesions.

Sporotrichoid CL is the spread of the parasite from the primary cutaneous lesion to the regional lymph nodes. Sporotrichoid spread occurred in 20% and 10% of patients in the Sudan and Al-Ahsa respectively. Sporotrichoid form of the lesion is characterized by linear nodules along the course of the lymphatic. The nodules are better felt than seen. Between nodules, the lymphatic may be thickened and palpable. Pathologically, the nodules are due to an inflammatory infiltrate containing parasites.

As mentioned above, during epidemics all the age groups were affected. After the epidemics abated, the disease became endemic and children were the most affected up to 90% of the population developed a positive leishmanin skin test as a result of healed lesions or sub-clinical infection.

The basic clinical manifestations of CL in Sudan are similar to those in Al-Ahsa with a few differences. Some features reported in Sudan were not described in Saudi Arabia. The less common clinical forms in Sudan included Leishmanial dactylitis which is due to infiltration of the back of the finger or toe by parasites and chronic inflammatory cells. Mycetoma-like lesion is characterized by multiple papules and nodules resembling mycetoma (maduromycosis) Some Sudanese patients developed thick infiltrative lesions and nodules in the face and other parts of the body, which were usually mistaken for leprosy. Recidivans-like lesions are due to *L. major* were reported in two patients who presented with lesions consisting of a central scar and an active ulcerated growing edge. However, they differed from recidivans lesions due to *L. tropica* in that the lesions did not heal first by scarring followed by recurrence in the scar. The healing and ulceration developed simultaneously, a feature that was not previously described in zoonotic CL due to *L. major*. Leishmaniasis in the nose was characterized by an ugly crust lesion. We recently published a case of CL from Darfur in an 18-year-old male who had extensive lesions in the face from the age of 1 year. The parasite was typed as *L. major* and he responded dramatically to intravenous Pentostam.

**THE RESERVOIR HOST, THE PARASITE AND VECTOR IN AL-AHSAS AND THE SUDAN**

The reservoir of zoonotic cutaneous leishmaniasis (ZCL) in Al-Ahsa is *P. obesus*. *L. major* and *L. arabica* were isolated from animals. *L. major* was the predominant species in animals.

Information in the reservoir host of CL in Sudan is limited in 1990. Badi found leishmania parasites in 13 *Arvicanthis* species.
niloticus and in one Genetta genetta in Khartoum district.[19] One isolate from A. niloticus was typed as L. major Zymoderme LON-1. In the outbreak of CL on Tuti island, sandflies were infected with L. major.[20]

After the first survey mentioned above,[18] another major survey of the phlebotomine fauna in a focus of ZCL in the Al-Ahsa Oasis, revealed only one species of Phlebotomus (P. papatasi) and three of Sergentomyia


Figure 7: Recidivans like lesions in the forearm of a female

Figure 8: Heavily crusted lesion in the nose

Eleven specimens of *P. papatasi* from six sites in the oasis were found to have promastigotes in the midgut. An isolate from one of the sandflies was typed by the examination of isoenzymes and was identified as *L. major*, zymodeme LON-4 (=Montpellier zymodeme 26), the principal zymodeme of *L. major* isolated from patients with ZCL in the oasis. Three isolates from leishmania lesions at the sites of the bites of wild specimens of *P. papatasi* were also identified as the same zymodeme of *L. major* as the isolate from the sandfly.

Pathology

The pathology of leishmaniasis in Saudi Arabia and Sudan are essentially the same, despite the different zymodemes of the parasites. We used Ridley’s classification system to classify material from Sudan and Saudi Arabia. There were some differences between our system of classification and Ridley’s. Lesions in Sudan and Saudi Arabia could be grouped into five types: A, B, C, D and E. Type A, which was the least common, consisted of sheets of macrophages containing many parasites. Lymphocytes and plasma cells were few. Type B consisted of macrophages, lymphocytes, plasma cells, but unlike Ridley’s Type B, there was no obvious necrosis of individual macrophages. Despite this difference, we retained Ridley’s Type B nomenclature. Type C showed focalized areas of necrosis. The necrosis affected large numbers of parasitized macrophages. When this occurred near the epidermis, it caused ulceration. We identified a new type that we called Type D. It was composed of a heavy lymphocytic infiltrate, activated macrophages, poorly-formed granulomas and scanty parasites. Patients with this reaction had an exaggerated Leishmanin skin response, reaching 17 mm in diameter. Type D was uncommon in Sudan and Al-Ahsa. Type E consisted mainly of well-formed epithelioid granulomas.
with a virtual absence of plasma cells and relatively scanty lymphocytes. Subcutaneous nodules showed necrosis associated with neutrophils. Histologically, the nodes draining skin lesions showed follicular hyperplasia with prominent germinal centers and expansion of the paracortex. Necrosis was seen in some lymph nodes. Parasites were scanty or not demonstrable in these nodes, although Leishmania antigen was found in the follicular dendritic cells and interdigitating cells of the paracortex.

**IMMUNOLOGY AND IMMUNOPATHOLOGY**

Elimination of parasites in CL involves 2 mechanisms.[22,23] The first is a necrotizing process in which the macrophage and the parasites it contains are destroyed. In the second, the macrophage is activated by T lymphocytes to destroy the parasite in intact macrophages through the production of interferon by the T cells. Tumor necrosis factor Alpha was considered to play a central regulatory cytokine in the introduction of macrophage antimicrobial activities.[22,23] In an ultrastructural study, Veress et al.[24] showed that parasites were destroyed in activated macrophages. El-Hassan et al. reported on the cell phenotypes in lesions of CL caused by *L. major*.[25] T-cells were abundant in CL lesions. In an active lesion, the majority of T-cells were T-helper cells or the T-helper and T suppressor cells were present in almost equal numbers, while in a regressing lesion, the majority was of the CDB cytotoxic/suppressor phenotype.

Apart from T-cells, other cells in the infiltrate include B cells, plasma cells, lysozyme-positive macrophages and S-100 protein antigen-containing cells.[25] The Lysozyme positive cells included parasitized macrophages, epithelioid and multi-nucleated giant cells. The S-100 protein antigen-positive cells were the Langerhans cells of the epidermis and cells with a similar morphology were within the dermal infiltrate. Both lysozyme-positive cells and the S-100 antigen-positive cells were also positive for histocompatibility antigen human lymphocyte antigen (HLA)-DR and had Leishmania antigen on their surfaces. They, therefore, acted as antigen presenting cells (ABC) to T lymphocytes both in the skin lesions and the draining lymph nodes. The keratinocytes of the normal skin were HLA-DR negative, but became HLA-DR positive when they covered the dermal infiltrate of a CL lesion.[22]

Another immunohistochemical study of APC in human CL, identified ABC as Langerhans cells, macrophage follicular dendritic cells and interdigitating reticulum cells of the paracortex of lymph nodes.[26] Peripheral mononuclear cells proliferated in response to leishmania antigen.[27] The response was less in patients with a severe disease than in those with mild disease. When Interferon gamma and IL-4 were measured in the peripheral blood of *L. major* infected individuals, two types of cytokines were found. In some patients, there was INF gamma without IL-4 (TH 1 response). In others, there was IL-4 and little INF gamma (TH-2 like response). The TH-1 response was in patients with mild disease, in which, their lesions eventually healed spontaneously. Those with the TH-2 response had severe disease, ran a prolonged course of the disease and required treatment.[27]

**Diagnosis**

**Smears**

Smears from the lesion are usually used to demonstrate parasites. Several techniques are used, including slit smears, needle aspiration, surface abrasion with sandpaper and the dental broach method.[28] The material is smeared on a clean glass slide, fixed in methanol and stained with Giemsa’s stain. The success rate of these methods was between 50% and 80%, depending on the technique used, the parasite load and the skill of the investigator. The slit smear was the most reliable.[28]

**Demonstration of parasites in sections**

Parasites were identified in about 70% of the cases in paraffin sections stained with hematoxylin and eosin.[29] It was possible to estimate the parasite load by counting the parasites in the sections.[30]

**Culture and animal inoculation**

Material from the lesion can be inoculated into NNN medium, Schneider’s insect medium or other media.[31] The major limiting factor is bacterial and fungal contamination. This can best be overcome by inoculating the material into hamsters of BALB/c mice.

**Serology**

Complement fixation, hemagglutination, indirect fluorescence test, immune-electrophoresis and enzyme-linked immunosorbent assay (ELISA) have been used, but were found to be of little value in the diagnosis of CL.[29] The direct agglutination test and ELISA were used in patients with a CL from Sudan, but were also found to be of no diagnostic value.[12] An ELISA using a 28 amino acid sequence of the repetitive element of gene B protein (GBP) from *L. major* was developed for serodiagnosis of CL.[12] The sensitivity of the test was 82%, higher than ELISA using crude amastigote or promastigote antigens. Significantly higher levels of
antibodies were found in Sudanese patients who had the disease for more than 8 weeks than in patients with a shorter clinical history. The test was negative in healthy normal controls and in patients with malaria. It was concluded that the GBP ELISA could be used as a supplementary test in the diagnosis of CL.\textsuperscript{[33]} It can be used to support a provisional diagnosis in early disease and to confirm it in those with a history of more than 8 weeks.

**Molecular biology**

Using the polymerase chain reaction (PCR), \textit{L. major} was identified in material from Sudanese patients with a CL.\textsuperscript{[34]} Direct microscopy of slit smears or histological sections to demonstrate \textit{L. major} parasites in Sudanese patients with CL was compared with the PCR in frozen sections from the same patients. PCR had a sensitivity of 86% when used alone and 93% when combined with Southern blotting. In contrast, microscopy of histological sections had a sensitivity of 76% and microscopy of slit and impression smears were only 55% and 48% sensitive, respectively. PCR had the added advantage of identifying the species of the parasite.

**Leishmanin skin test**

This is carried-out by the intradermal injection of killed promastigotes.\textsuperscript{[12]} The test is read 48-72 h later. A positive reaction is an induration of 5 mm or more. The test is of little value in the diagnosis of individual cases, but is useful in epidemiological surveys.

**Treatment**

ZCL is a self-limiting disease. In most patients self-healing occurs within 6 months, although lesions may persist for a year or more (chronic CL). There is at present, no uniform protocol for treating CL in Sudan. In our own practice, patients with minor lesions are reassured and lesions are left to heal spontaneously.\textsuperscript{[12]} Patients with severe or multiple lesions (>5), diabetics with lesions and patients with sporotrichoid spread are treated. Large lesions on the face, particularly on the eyelid, which may cause scars that interfere with closing the eye, should also be treated. Leishmaniasis of the nose is unsightly and should be treated even if it is the only site affected. Several treatment schedules are available. One treatment which has been used for a long time in Sudan is the pentavalent antimony compound (Pentostam, Welcome laboratories UK). This drug was given intravenously or intramuscularly at a dose of 10 mg/kg/day for 3-4 weeks). We have now increased the dose to 20 mg/kg/day for 3 to 4 weeks. The response is good. The drug is generally well-tolerated. Possible side-effects include reaction at injection site, allergic skin eruption, anorexia, nausea and vomiting, headache, myalgia and, with high doses, Electrocardiogram (ECG) changes and elevation of transaminase levels.\textsuperscript{[29]} Three antifungal drugs, ketoconazole, itraconazole and terbinafine were used in the treatment of CL. Ketoconazole which is effective and well-tolerated is given orally at a dose of 400 mg daily (for adults) for 4 weeks.\textsuperscript{[35,36]} No idiosyncratic hepatitis has been encountered in Saudi Arabia.\textsuperscript{[29]} Itraconazole has been used to treat \textit{L. major} infection in Iran.\textsuperscript{[37]} The authors concluded that itraconazole cannot be used as a single agent for the treatment of \textit{L. major} infection. Intralesional infiltration with pentostam may be effective and is recommended in early lesions, but is very painful.\textsuperscript{[58]}

Topical paromomycin was successfully used for an acute and chronic CL in Palestine and was reported to cure the disease when used for 20 days.\textsuperscript{[59]}

**Cryotherapy**

Cryotherapy was reported to have a high cure rate without any complication,\textsuperscript{[40]} but using cryotherapy, Al-Gindan \textit{et al.} found a low cure rate and severe complications.\textsuperscript{[41]}

**Control**

A study tested the efficacy of zinc phosphide and fenacoum against the reservoir host \textit{P. obesus} rat. The results showed that both the rodenticides were significantly \((P < 0.01)\) effective in reducing the number of active holes during 1 year of application.\textsuperscript{[42]} It is doubtful if this was followed by a significant reduction in the number of cases in the population. There is no control program in Sudan.

\*In memory of the late Dr. Sabir Elbihari: A great Parasitologist and a wonderful person.

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