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Concomitant natural infection with L. donovani and L. major: A case report from Iraq

Summary

This is a case report of kala-azar with cutaneous leishmaniasis. Upon admission, the patient had fever, hepatosplenomegaly and an ulcer on her cheek. The patient responded to Pentostam. Isoenzyme studies of parasite isolates from the the bone marrow and from the cutaneous lesion revealed that these were L. donovani and L. major, respectively. This is the first report in Iraq of a proven concomitant infection with two species of leishmania parasites.

Cutaneous and visceral leishmaniasis are both endemic in Iraq. The visceral form of the disease, caused by the *L. donovani* complex, is mainly endemic in the central part of the country. In contrast, the cutaneous form, caused by *L. tropica* or *L. major*, is more widespread[1–5]. Overlapping foci of cutaneous and visceral leishmaniasis (kala-azar) have been reported from Iraq[6] as well as from other parts of the world[7–9]. This picture is further complicated by the fact that any given *Leishmania* species can cause different clinical manifestations[10–14].

Simultaneous visceral and cutaneous leishmaniasis has been observed in Rio de Janeiro. The parasites were identified as *L. donovani* and *L. braziliensis*, respectively[15]. In East Africa, both cutaneous and mucocutaneous lesions have been described in the presence of visceral infection. These cutaneous lesions could not be clinically differentiated from bona fide cutaneous leishmaniasis (oriental sore). Though some cutaneous and mucocutaneous lesions have been attributed to *L. donovani* in the Sudan, the majority of cutaneous lesions in this region are caused by *L. major*[16].

This study reports, for the first time in Iraq, a case of kala-azar with concomitant cutaneous leishmaniasis which was due to a mixed infection of the patient by *L. donovani* and *L. major*.

Materials and methods

Case report

A three year old girl from Hafriya, 60 km south east of Baghdad, was admitted to Ibn Al-Baladi Hospital on January 23, 1989 with hepatomegaly (5 cm) and splenomegaly (3 cm) below the costal margin, as well as a history of fever for the last month. The fever peaked at night (38.8 °C), and the pulse frequency was 120/min. The patient had a round ulcer on her left cheek (2 × 2 cm). She had lost weight during the last month, and her present weight was 10 kg. The serum level of leishmania-specific antibodies was 1/32 as determined by immunofluorescence antibody titration. Examination of the bone marrow showed amastigote leishmanias in the macrophages, and parasites could be isolated after culturing the bone marrow cells for 6 days in vitro. The leishmania infection was treated with pentostam (10 mg/kg body weight/day) for 15 days. The patient concomitantly received garamycin (gentamycin, Schering) in order to treat a urinary infection. She became afebril on the 12th day of pentostam treatment. At the time of discharge, her liver was 4 cm and her spleen 2 cm below the costal margin.

The patient was readmitted on March 8 with the complaint of fever of the last two weeks, associated with rigor and sweating. On admission, she was feverish with a temperature of 39.5°, and

she had lost 1.4 kg since her last admission. The skin lesion on her left cheek was still present. The liver was 3 cm and the spleen was 5 cm below the costal margin. The patient received intravenous fluid, and pentostam was given at 15 mg/kg/day for 10 days. During this period, the skin lesion began to heal. The girl was reexamined one month later. At this time, she was afebrile, her liver was 2 cm, and her spleen 1 cm below the costal margin, and she had gained 2.4 kg. The skin lesion on the left cheek had healed, leaving a small scar.

Parasite isolation and cultivation

Bone marrow aspirates were inoculated in modified Tobie's medium[17] supplemented with gentamycin (200 µg/ml). Cutaneous aspirates were obtained according to the method of Urjel et al.[18] and inoculated into modified Tobie's medium. Culture tubes were incubated at 25° and examined after 5 days. At this time, extracellular promastigotes were detected in both aspirate cultures. Once primary cultures were established, subcultures were made. Direct smears from bone marrow and cutaneous aspirates were stained with Giemsa.

Animal inoculation

Inoculation by two different routes (intradermal and intraperitoneal) was performed for each of the two isolates after 3 weekly passages in culture. Golden hamsters and BALB/c mice were injected with 0.5 – 1.0 ml of suspensions containing $4.5 - 7.4 \times 10^7$ promastigotes/ml. Intradermal inoculations were done near the root of the tail (in mice) and into the posterior footpads (mice and hamsters). Samples taken from spleen, liver and skin lesions of infected animals were inoculated into Tobie's medium, and were also used for direct smear preparations.

Isoenzyme electrophoresis

Cultured promastigotes were processed for isoenzme analysis as described[1,6]. Electrophoresis was carried out in an LKB 2117 Multiphor system, using 200 mM Tris-glycine, pH 7.8 as gel buffer and 50 mM Tris-glycine, pH 7.8 as electrode buffer. The acrylamide stock solution contained 22.2% acrylamide and 0.6% bis-acrylamide in H_2O. The final gel (dimensions of $115 \times 245 \times 1$ mm) contained 7.5% acrylamide in 0.1 M Tris-glycine, pH 7.8 and was stored overnight at 4° before use.
The following enzymes were analyzed for strain identification: Glucose phosphate isomerase (GPI), glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase (MHD), malic enzyme (ME) and 6-phosphogluconate dehydrogenase (6PGDH).

Leishmania reference stocks

The following international reference stocks were used as standards for isoenzyme analysis: *Leishmania donovani*: stock MHOM/ET/67/HU3 (LV9), isolated from a human visceral infection in Ethiopia; *L. donovani infantum*: stock MHOM/TN/80/IPT1 (LEM235), isolated from a human visceral infection in Tunisia; *L. tropica*: stock MHOM/SU/58/ Strain OD (LV357) and stock MHOM/SU/74/K27, both isolated from human cutaneous infections in the USSR; *L. major*: stock MHOM/IL/67/JERICHO-II (LV561), isolated from a human cutaneous infection in the Jordan valley.

Results

When promastigostes isolated from the bone marrow aspirates of the patient were injected intraperitoneally into mice or hamsters, parasites were detected 5 days after

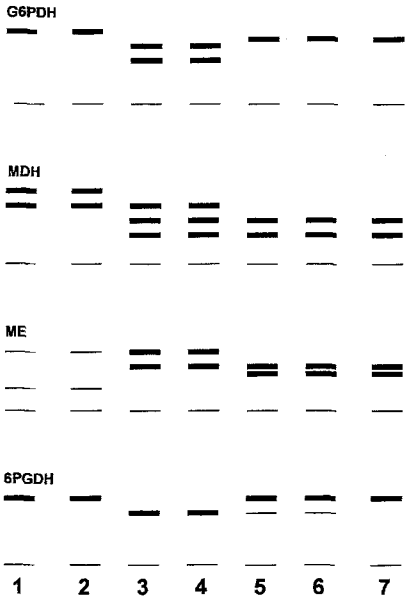

Figure 1. *Diagrammatic representation of the electrophoretic enzyme patterns of Leishmania reference strains and of the isolates from the cutaneous and the bone marrow aspirates.*
Left to right: 1 = LV561 (cutaneous L.major reference strain); 2 = RRLL (cutaneous aspirate); 3 = K27 (cutaneous L. tropica reference strain); 4 = LV357 (cutaneous L. tropica reference strain); 5 = RRLL (bone marrow aspirate); 6 = LV (visceral L. donovani reference strain); 7 = LEM235 (visceral L. infantum reference strain).

infection both in spleen and liver by culturing. No cutaneous lesions were observed on any of the animals. The isoenzyme patterns produced by this isolate were identical to those obtained with the visceral reference stock LV9 for all enzymes tested (Fig. 1). Also, they were similar to those of the reference *L. infantum* stock LEM235 (identical for G6PDH, MDH and ME, but different for GPI and 6PGDH). Both parasite location in the host animals and the isoenzyme patterns demonstrate that the parasites isolated from bone marrow aspirates are *L. donovani*.

Promastigotes isolated from the cutaneous lesion of the patient induced a swelling at the site of inoculation in both mice and hamsters. Some, but not all, of the infected mice developed granulomatous lesions on the ears and small nodular lesions on the back of the body or on the face 54 to 65 days after inoculation. In all hamsters, no symptoms other than the swelling of the inoculated footpads were observed. However, parasites could be reisolated from the footpads as well as from spleen and liever both in hamsters and in mice 54 to 65 days after inoculation. The isoenzyme patterns produced by the promastigotes from the cutaneous lesion of the patient were identical to those of the cutaneous *L. major* reference strain LV561, and they were entirely different from those of the *L. tropica* reference strain. In conjunction, these observations demonstrate that the parasites recovered from the cutaneous lesion of the patient are *L. major*.

Discussion

This is the first documented case of concomitant cutaneous and visceral leishmaniasis caused by two species of parasites, *L. major* and *L. donovani*. Jawad et al.[19] had earlier observed the notable absence of such double infections in Iraq. Dermal lesions had been observed in a patient after subsidence of a systemic infection[20] and in an untreated kala-azar patient from Al-Dawir (Suwaira Quada, about 60 km from Baghdad)[21]. The dermal lesions were attributed to post-kala-azar dermal leishmaniasis. Rassam et al.[6] isolated and characterized the visceral species *L. donovani* from a month old nodule on the face of a 6 year old boy with a history of kala-azar. In another incident, dry cutaneous lesions and visceral infection were observed in a 2 year old girl from Al-Hindiya (about 100 km south of Baghdad). The stock isolated from her bone marrow was identified as *L. donovani*. However, no parasites could be isolated from the dermal lesion[1,6].

Simultaneous infection by more than one species of *Leishmania* in Iraq is not surprising, as foci of cutaneous leishmaniasis caused by *L. major* or *L. tropica* frequently overlap with kala-azar endemic foci. In the Suwara Quada area, two *L. tropica* and one *L. major* stock were isolated from two brothers and a relative living in a house in which another inhabitant had contracted kala-azar only a few months earlier[5,6,22].

The region where our patient lives is endemic for kala-azar, and it contains various species of sandflies which can act as transmission vectors: *Phlebotomus papatasi, P. alexandri* and *Sergentomya* spp. Atempts to infect *P. papatasi* with the cutaneous isolate RRLLL24 in the laboratory were successful (unpublished observations), and a more detailed study of this work is in preparation.

Zusammenfassung

Simultane Infektion mit L. donovani und L. major: Eine Fallstudie im Irak

Wir präsentieren eine Fallstudie von Kala-Azar mit gleichzeitiger kutaner Leishmaniose. Die Patientin hatte Fieber, Hepatosplenomegalie und einen Ulcus an der einen Wange. Sie sprach auf Pentostam an. Die Analyse der Isoenzym-Profile der Parasiten aus einer Knochenmarks-Punktion sowie aus dem Ulcus wiesen nach, dass es sich dabei um L. donovani und um L. major handelte. Diese Studie ist die erste Beschreibung einer simultanen Infektion mit L. donovani und mit L. major im Irak.

Résumé

Infection simultanée par L. donovani et L. major: Une étude rapporte en Iraq

Notre étude rapporte un cas de Kala-Azar accompagné d'une leishmaniose cutanée. La patiente avait la fièvre, une hépatosplénomégalie et un ulcère à une de ses joux. L'infection répondait bien à la Pentostame. L'analyse des isoenzymes montrait que les parasites isolés de la moile osseuse représentaient L. donovani, pendant que ceux de la lésion cutanée représentaient L. major. Notre rapport constitue la première documentation d'un cas d'une infection simultanée par L. donovani et L. major en Iraq.

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