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Detection of Leishmania parasites in the blood of patients with isolated cutaneous leishmaniasis

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SUMMARY

Background: The consequences of the spread of Leishmania parasites to the blood from lesions in patients with cutaneous leishmaniasis are numerous. To assess the magnitude of this invasion we conducted the present study on patients referred to the American University of Beirut Medical Center for cutaneous leishmaniasis.

Methods: Patients referred for the management of cutaneous leishmaniasis were included in the study. Skin and blood cultures for Leishmania were taken from these patients.

Results: One hundred sixty-two patients were proven to have cutaneous leishmaniasis by pathology; 52% were males and 44% females (gender information was missing for 4%). Patient age ranged from 5 months to 70 years. None of the patients had received treatment for Leishmania. We obtained parasite isolates from 85 patients (52.5%), proven by cultures from skin and blood/blood components. Interestingly, the parasite was isolated in the blood and blood components of 50 patients (30.9%). Isoenzyme analysis confirmed the fact that the organisms in blood and skin were the same; from the 28 isolates that were positive in both skin and blood, eight isolates were *Leishmania major* and two were *Leishmania tropica*. The remaining isolates, whether positive in both blood and skin or in either of these tissues, skin or blood and its products, were *Leishmania infantum* sensu lato.

Conclusions: In the current study, the detection rate of parasites in the blood of patients with cutaneous leishmaniasis was high. This illustrates the invasive characteristic of the parasite that has escaped the skin. Testing should be considered in areas other than Lebanon, especially around the Mediterranean basin. Whether these findings support the administration of systemic treatment for cutaneous leishmaniasis or not needs to be confirmed in larger prospective studies.

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1. Introduction

Leishmania major, *Leishmania tropica*, and *Leishmania infantum* sensu lato usually cause cutaneous leishmaniasis, but hematogenous spread can occur.[1,2] Several new foci have been described in different parts of the world,[3–7] but concern about its spread is moderate since the damage is limited and superficial. The pathology of cutaneous leishmaniasis is restricted to the dermis and the overlying epidermal layer,[8–11] and this constitutes the basis of a large number of studies that describe the body reaction to this parasite, especially the reaction of the immune system.[12–15] Furthermore, the classification of this disease as a dermal infection or a localized event limits the evaluation of the efficacy of any treatment modality, especially as many lesions heal spontaneously.[1]

People have occasionally resorted to systemic treatment of cutaneous leishmaniasis in order to obtain better results, particularly after failure of topical treatment. So far only sporadic reports have described cases where the parasite has spread to the blood stream and is circulating carried by blood leukocytes from one organ to another.[16,17] However, the general impression has been that these cases are atypical.[18,19] Included among these unusual cases are situations in which healed scars of the Aleppo button recurred several years later.[20] The recrudescence at the scar site was viewed as a local event, especially when the reactivation happened in areas geographically free of leishmaniasis. In such instances the source of the parasite was thought to be residual organisms limited to the skin lesion.

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In view of the extreme importance of detecting these parasites in blood and the serious implications of such findings, we decided to undertake the current study. Our objective was to evaluate the prevalence of Leishmania parasitemia in patients with different types of cutaneous leishmaniasis and coming from different geographic regions inside and outside Lebanon.

2. Materials and methods

This project received approval from the Institutional Review Board of the American University of Beirut Medical Center (AUBMC) as part of our epidemiologic surveillance study initiated in 1994, when the Leishmania study group was established. The project objectives were re-approved in 2000. Patients with skin lesions were referred to us for tissue diagnosis and histological confirmation of Leishmania. Informed consent was obtained from all patients enrolled in this study. A detailed history was recorded on a special form. A proforma was completed for every patient; information collected included patient demographics, age, gender, history of the skin lesion, and the therapeutic regimens used until referral. We also recorded whether similar lesions were noted in any member of the family or in any subject in the same neighborhood.

A biopsy of the skin lesion was obtained at the dermatology department under local anesthesia; part of this sample was shredded to go on a biphasic culture medium and the rest was used for histological diagnosis. Next, about 2 ml of blood were drawn from the patient in a citrated tube. Whole blood was inoculated in culture flasks, and the rest was separated into plasma, buffy coat, and erythrocyte layers, each of which was placed in a culture tube with a slant of Nicole–Novy–McNeal (NNN) medium covered with overlay and left at 24–26 °C for observation.[21]

We did not use centrifugation. The isolated parasites were subcultured on Schneider's Drosophila medium[22] and then collected and analyzed on starch gel for classification by isoenzyme electrophoresis.[23,24]

3. Results

Only patients who were positive for Leishmania on histology sections were included in this study. One hundred sixty-two patients were proven to have cutaneous leishmaniasis by pathology, of which 52% were males and 44% were females (gender information was missing for 4%). The number of referred cases over the same period was around 380 cases. The patients ranged in age between 5 months and 70 years, and their age distribution was 1–3 years in 2%, 4–13 years in 13%, 15–20 years in 12%, and more than 20 years in 65% (information missing for 8%). The number of skin lesions varied between one and eight. Most of the patients (88%) had a single lesion that was mainly found (in 73% of cases) on the upper half of the body. None of the patients had received treatment for Leishmania; some of them had received antibiotic treatment for the skin lesions.

The size of the lesions, recorded as the widest diameter, ranged from 2 mm to 8 cm. They were described as small button-like lesions (Figure 1), sometimes excoriated or covered by a scab, with very little reaction at the base. A complete physical examination performed on each patient was normal, except for the skin lesions. No data included in the proforma implied the presence of the parasite in blood; these included the absence of fever or any abnormalities on blood counts.

In the current study, we obtained parasite isolates from 85 patients (52.5%) proven by skin and blood cultures. A total of 62 (38.3%) positive cultures were obtained from blood and blood products; these were equivalent to 50 patients. The yield from the skin lesions was equally successful in 62 cases. There were several overlap cases between blood and buffy coat (11 cases), buffy coat and skin (eight cases), blood and plasma (one case), and also blood and its products on the one hand and skin on the other (27 cases). All of these findings are summarized in Figure 2. Leishmania parasite isolation was successful in some instances only from one tissue, to the exclusion of the rest: effectively from whole blood in 19 cases, from buffy coat in three patients, and from skin biopsies in 36 cases.

Furthermore, the classification by isoenzyme electrophoresis revealed that the parasites analyzed were invariably the same in skin and in blood in each case of the 28 patients from whom isolates were obtained from both skin and blood. Eight isolates were *L. major* and two were *L. tropica*. All the rest of the isolates, whether positive in both blood and skin or in each of the two tissues, skin or blood and its products, were *L. infantum* sensu lato.

4. Discussion

Leishmaniasis is endemic in more than 80 countries, with a worldwide prevalence of 12 million.[25] It occurs as a range of syndromes that include cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis. Cutaneous leishmaniasis is the most common form of leishmaniasis in the Middle East.[26] It comprises skin infections caused by a single-celled parasite that is transmitted by sandfly bites.[27] Its annual incidence is estimated at 1–1.5 million cases.[25] There are about 20 different species of Leishmania that may cause cutaneous leishmaniasis.

The gold standard in the diagnosis of cutaneous leishmaniasis is parasitological diagnosis. This includes histopathological examination and sometimes, when available, cultures of biopsied sections.[28] Cultures are a valuable means of allowing species identification, but they require some technical expertise and this may not be readily available, and also the parasite may fail to grow.[29,30] Molecular diagnosis for cutaneous leishmaniasis has developed greatly during the past decade.[31] However, applying

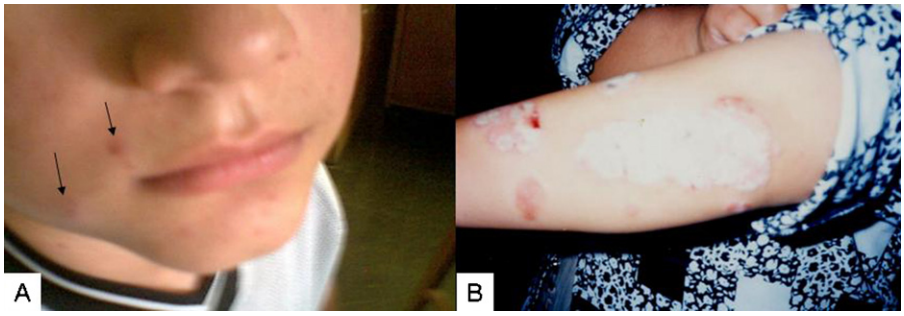

Figure 1. Lesions (arrows) on the face (A) and forearm (B) to illustrate the variety in the size, appearance, and degree of inflammation that characterizes these patients.

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