Cutaneous Leishmaniasis: A Case Confirmed by Both Histopathologic Examination and Polymerase Chain Reaction

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Leishmaniasis is caused by intracellular protozoa of the genus *Leishmania* and transmitted by the bite of female sandflies. Clinically, it is classified into cutaneous, diffuse cutaneous, mucocutaneous, and visceral forms. We report an imported case of cutaneous leishmaniasis. A 50-year-old male international constructor who had worked in Burkina Faso developed two red papules on right upper arm one month after he left Burkina Faso. The cutaneous lesions gradually progressed into erythematous plaques with central ulceration in three months. Another erythematous papule ensued on his right forearm. Histopathologic examination of the biopsy specimen revealed numerous *Leishmania* amastigotes in macrophages. Polymerase chain reaction of the biopsy specimen further confirmed the diagnosis. Itraconazole was given for 2 weeks, and then all the three lesions were excised due to the absence of obvious clinical response and the patient's request. No recurrence was noted after an eight-month period of follow up. (Dermatol Sinica 24: 58-62, 2006)

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皮膚萊什曼原蟲病：同時藉由組織病理檢查及聚合酶連鎖反應確定診斷的病例

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萊什曼原蟲病是由胞內寄生的萊什曼原蟲所引起，經由雌性白蛉叮咬而傳染。臨床上可分為皮膚、廣泛性皮膚、黏膜皮膚，及內臟四型。我們報告一例境外移入的皮膚萊什曼原蟲病。一位50歲男性國際建築商人曾在布吉納法索工作，離開該國一個月後在右上臂出現兩處紅色丘疹，並在三個月內逐漸進展成中央有潰瘍的紅腫結節。接著又在右前臂出現另一紅色丘疹。切片病理檢查在巨噬細胞內發現許多萊什曼原蟲的無鞭毛體，並進一步由切片組織的聚合酶連鎖反應確定診斷。投予itraconazole二星期後，並無明顯的臨床進步，因此在病患要求下，將這三處病灶全部切除。經八個月後的追蹤並無復發。 (中華皮誌 24: 58-62, 2006)
INTRODUCTION

Leishmaniasis is a protozoan disease caused by several species of the genus *Leishmania* and transmitted via the bite of infected female sandflies of the genera *Phlebotomus* or *Lutzomyia*. Clinically, leishmaniasis is classified into cutaneous, diffuse cutaneous, mucocutaneous, and visceral forms. Based on its geographical distribution, cutaneous leishmaniasis (CL) can be divided into Old World (Asia, Africa, Europe) and New world (America) CL. The most common species of Old World CL are *L. major*, *L. tropica*, and *L. aethiopica*, and the most common species of New World CL are the *L. mexicana complex* and *L. braziliensis complex*. While Old World species mostly cause benign and self-limited cutaneous disease, New World species cause a broad spectrum of conditions including mucosal involvement. Traditionally, CL is diagnosed by smear, culture, or histopathology of a skin biopsy specimen. Polymerase chain reaction (PCR) of the specimen by using primers specific for *Leishmania* has been developed as a rapid and sensitive diagnostic tool. We report a case of CL confirmed by both conventional method and PCR technique in Taiwan.

CASE REPORT

A 50-year-old male international constructor had worked in Burkina Faso in May, 2004. One month after leaving Burkina Faso, he developed two asymptomatic red papules on the extensor side of his right upper arm. The cutaneous lesions enlarged gradually and progressed into two erythematous plaques, 3 x 2 cm and 1 x 1 cm respectively, with central ulceration in three months (Fig. 1A). Another erythematous papule about 0.5 cm in diameter appeared on the extensor side of his right forearm in September, 2004 (Fig. 1B). Topical fusidic acid cream and oral minocycline had been given for one month, but the lesions kept deteriorating. Meanwhile, neither fever nor constitutional symptoms were noted.

The laboratory studies including complete blood counts, differential counts, and liver function tests were all within normal limits. VDRL and HIV tests were negative. A skin biopsy specimen was taken from the border of the ulcer. Histopathologic examination of the biopsy specimen revealed epidermal focal ulceration and pseudoeptiheliodmatous hyperplasia. The dermis was densely infiltrated by macrophages, lymphocytes, plasma cells, and neutrophils (Fig. 2A). Numerous amastigotes were noted in the cytoplasm of macrophages (Fig. 2B). They were negative for periodic acid-Schiff and Gomori's methenamine silver stains. A further Giemsa-stained smear of the dermal scrapings from the ulceration didn’t demonstrate amastigotes. Tissue cultures of bacteria, mycobacteria, and fungi were all negative.

Itraconazole 400 mg per day was given for 2 weeks and no obvious clinical improvement was noted. The patient was too busy to be followed up regularly, so he requested excision of...
the cutaneous lesions. The three lesions were all excised in October, 2004. The histopathologic findings of the excised lesions showed similar conditions including epidermal hyperplasia, dermal edema, and diffusely mixed inflammatory infiltrate. Interestingly, only a few intracytoplasmic *Leishmania* parasites could be observed in the histiocytes and multinucleated giant cells in comparison with numerous amastigotes in the initial biopsy. DNA was extracted from paraffin sections of the three specimens and PCR amplification of the extracted DNA with *Leishmania*-specific primer 174 (5’GGTTCCTTTCCTGATTACG 3’) and 798 (5’GGCCGGTTAAAGGCCGA ATAG 3’) was performed according to a previously described method.3 The PCR products were visualized on an 2% agarose gel and a 100 bp DNA ladder was used as a marker. Two of the three excised lesions yielded products with a size of 600 bp and was scored as positive for *Leishmania* (Fig. 3). The wounds all healed well and no recurrence was noted after eight months.

**DISCUSSION**

Leishmaniasis is endemic in 88 countries with a prevalence of 12 million people and threatens 350 million people. The annual incidence of CL worldwide is 1-1.5 million people.4 In the literature, there were four sporadic imported cases of CL in Taiwan.5-7 Three cases contracted CL in Saudi Arabia and one in Thailand.

Leishmaniasis is transmitted via the bite of infected female sandflies. The parasite lives as an extracellular, flagellated promastigote in the gut of the insect. Following inoculation into the
skin of a mammalian host, the parasite exists within lysosomes of mononuclear phagocytes as an intracellular amastigote, which is 2-4 μm long and characterized by a larger eccentric nucleus and a smaller mitochondrial DNA called kinetoplast. CL begins with a nonspecific insect bite-like erythematous papule at the site of the sandfly bite after an average incubation period of 1 week to 3 months. The papule enlarges into a nodule or plaque over a period of 4-12 weeks. A shallow ulcer with seropurulent discharge and crust formation develops in the center of the nodule/plaque. The skin lesions are usually asymptomatic and heal spontaneously over several months, leaving a discolored atrophic scar. Natural resolution leads to partial resistance to re-infection.

Old World CL is mostly self-limited, while New World CL has a more variable clinical course ranging from self-limited to mucosal involvement. In general, most of those lesions caused by L. major or L. mexicana resolve in 6 months, those caused by L. tropica resolve in about 10 months, and those due to L. braziliensis persist much longer. L. braziliensis infection carries a risk of mucosal involvement (2-10%). In most cases mucosal disease becomes evident within several years of resolution of the original cutaneous lesions, but it can ensue while the lesions are present or decades after they heal.

Histopathologic studies of CL reveal epidermal and dermal changes. The epidermal changes are variable, including hyperkeratosis, parakeratosis, atrophy, and pseudopitheliomatous hyperplasia. In the dermis of an early lesion, there is diffuse inflammatory cells infiltration composed predominantly of macrophages with a mixture of lymphocytes and multinucleated giant cells. As the lesion progresses, epithelioid granulomas appear in the upper dermis and may fill the entire dermis. Amastigotes (Leishman-Donovan bodies) are found in the cytoplasm of macrophages.

The clinical differential diagnoses of CL include a list of inflammatory, infectious, and neoplastic diseases. Histopathologic examination of a biopsy specimen may help to shorten the list. The histopathologic differential diagnoses of CL include sporotrichosis, penicilliosis, histoplasmosis, yaws, syphilitic gumma, tuberculosis cutis, anthrax, granuloma inguinale, rhinoscleroma, and furunculosis. Rhinoscleroma, granuloma inguinale, histoplasmosis, and penicilliosis may also show parasitized macrophages. Rhinoscleroma is marked by a large number of plasma cells with formation of numerous Russel bodies, which are usually few in CL. Granuloma inguinale is marked by a formation of a small abscess containing neutrophils. The organisms of penicilliosis and histoplasmosis are stained with fungal stains.

Conventionally, CL is confirmed by the presence of amastigotes in histopathology of a biopsy specimen or Giemsa-stained smear, or by the isolation of promastigotes in the culture. Meredith et al. have developed a method of applying PCR with Leishmania-specific primer 174 and primer 798 for the detection of Leishmania. The primers were constructed with the 3' ends complementary to the specific point mutations present in the small subunit rRNA (SSU rRNA) genes of all Leishmania species. Samples were scored as positive when a product with a size of 600 bp could be detected. According to the study of Faber et al., the sensitivities of diagnosing CL by smear, culture, histopathology, and PCR were 54%, 70%, 69%, and 96%, respectively. PCR appears to be the most sensitive single test for CL and is highly specific. We have confirmed the diagnosis of CL in our case by both histopathologic examination and this PCR technique.

A wide variety of topical and systemic treatments have been proposed for CL. Systemic treatments include pentavalent antimonial, pentamidine, anti-fungal azoles, amphotericin B, miltefosine, allopurinol, rifampicin, dapsone, and oral zinc sulphate. Topical treatments include local infiltration with pentavalent antimony, ointment containing 15% paromomycin, cryotherapy, local heat, cautery, and surgical excision. Blum J. et al. have pro-
posed indications for local and systemic treatments of CL based on the risk of developing mucosal disease and the extent of disease. Systemic treatment is used in patients with multiple lesions, large lesions (> 5 cm), and lesions unresponsive to local treatment. Not all systemic regimens are available currently in Taiwan. One of the useful medications is itraconazole. Clinical studies have shown promising results with itraconazole. Although there was also a report which showed itraconazole was not effective than placebo in the treatment of CL recently, it markedly decreased the number of *Leishmania* parasites in our patient after two-week therapy. A longer therapeutic period with anti-inflammatory regimen may obtain more clinical benefits. Surgical excision of the solitary lesion was another alternative choice. Early surgical treatment of CL was suggested to attain better cosmetic result; however, this can pose a substantial risk of relapse. Therefore, patients of CL treated by surgical excision should be followed up for several months.

The prosperity of international traveling has brought CL to non-endemic areas. Although CL is not endemic in Taiwan, it should be included in the differential diagnoses of patients with long-lasting erythematous plaques or nodules and travel history in endemic areas. It is known from studies in endemic countries that a considerable number of clinically diagnosed CL cases cannot be confirmed when only traditional diagnostic techniques of smear, culture, and histopathology of skin biopsy specimens are used. In nonendemic areas, there might be a higher percentage of patients in whom the diagnosis will be missed. The recently developed PCR technology allows a rapid and sensitive diagnosis for CL and may help clinicians to treat CL correctly.

**REFERENCES**