Pediatric Dermatology Vol. 13 No. 6 455–463, 1996

Tegumentary Leishmaniasis in Childhood

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Abstract: Very little has been published about tegumentary leishmaniasis in children and there are many controversies about this disorder in the literature. Therefore, we discuss the pathogenesis, clinical aspects, means to diagnosis, and treatment of this endemic disease.

Leishmaniasis is an endemic infection that occurs predominantely in tropical and subtropical regions of the world. There are an estimated 400 million individuals exposed to leishmanial infection and 600,000 new cases per year (1). Cutaneous leishmaniasis and its mucosal involvement represent a major health problem in South and Central America, where disfiguring mucosal involvement can occur in children.

Tegumentary leishmaniasis has a wide spectrum of clinical manifestations varying from the responsive—localized cutaneous and mucosal leishmaniasis (CL and MCL)—to the unresponsive—diffuse cutaneous leishmaniasis (DCL). Intermediate forms of this disease are also observed. The main differences between the polar forms of leishmaniasis are shown in Table 1. This spectrum is dependent on the species of leishmania and on the efficiency of the cell-mediated immune (CMI) response to the parasite. Post-kala-azar dermal leishmaniasis (PKDL), which represents the involvement of the skin after treatment of visceral leishmaniasis (VL), is caused by viscerotropic species of leishmania (*L. donovani* and *L. chagasi*) (2).

The parasites that cause tegumentary leishmaniasis are grouped under the *L. braziliensis* and *L. mexicana* complexes. Parasites of the *L. braziliensis* complex generally cause CL and MCL, whereas parasites of the *L. mexicana* complex generally do not involve the mucosae and cause only limited self-healing cutaneous lesions. The majority of the cases of MCL in the New World are caused by *L. braziliensis*. However, MCL may also be caused by *L. panamensis* and *L. amazonensis* (2). On the other hand, CL is a rare sequela of infection with *L. chagasi*, the agent of New World VL (3). *L. mexicana* and *L. amazonensis* are responsible for the rare cases of DCL (4).

L. tropica, *L. aethiopica*, and *L. major* are the etiologic agents of Old World CL. However, *L. tropica* may also cause VL, and *L. aethiopica* is responsible for Oriental DCL. The disease caused by *L. major* constitutes the rural "wet" form of Old World CL; *L. tropica* is responsible for the urban "dry" form of CL. However, the differences between these two types of disease are not always observed. "Dry" lesions may be caused by *L. major*, and *L. tropica* may occur as a rural disease (2).

A prospective study of CL in an endemic rural area (Corte de Pedra) of Bahia, a state in northeast

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This article was supported by the Brazilian National Research Council (CNPq) and United States Public Health Service grant AI30639.

TABLE 1. *Main Differences Among Disseminated Cutaneous Leishmaniasis, Diffuse Cutaneous Leishmaniasis, and Classical American Cutaneous Leishmaniasis*

Clinical and Immunopathologic Findings	Clinical Forms of Leishmaniasis		
	Disseminated	DCL	CL and MCL
Clinical presentation	Papules and acneiform lesions	Nodules	Ulcers
Number of lesions	Many	Many	One or few
Destructive mucosal involvement	Frequent	Absent	Infrequent
Tissue parasitism	Rare or absent	Abundant	Rare or absent
Follicular pattern	Present	Absent	Absent
Granulomatous pattern	Present or absent	Absent	Present or absent
Antileishmanial CMI in vitro and in vivo	Positive or negative	Negative	Positive
Antibody titers	Mainly high	High	Low

Brazil, allowed us to observe early manifestations of the disease and cases with spontaneous healing. In this area most of the cases are due to *L. braziliensis*. In 2076 cases of cutaneous leishmaniasis, 35% were children 15 years old or younger (57% male and 43% female); 1.6% of these children presented with mucosal involvement. Figure 1 shows the frequency of CL in different age groups.

Cutaneous leishmaniasis caused by *L. venezuelensis* affects children less than 6 years of age in 19% of the cases (5). In Saudi Arabia, 66% of the patients with CL were younger than 10 years of age (6), and *L. aethiopica* occurs in the age group of 0 to 10 years in 37% of the cases (7). DCL begins in childhood in approximately 66% of the cases in the Old World and in 61% of the cases in the New World (8–24).

PATHOGENESIS

Following inoculation in the skin, the flagellated promastigotes are transformed into amastigotes inside the macrophages, where they replicate within vacuoles. Leishmanicidal activity is probably due to the increased capacity of the macrophages to produce toxic oxygen and nitrogen radicals in response to IFN-γ (2). Upon entry leishmania induces the macrophage production of TNF-α, which potentiates the action of IFN-γ and promotes macrophage activation (25), and TGF-β (26), which is linked to macrophage deactivation and inhibition of IFN-γ (27,28). Initial survival of leishmania inside the macrophage may depend critically on which of these opposing cytokines predominate in the microenvironment of infection.

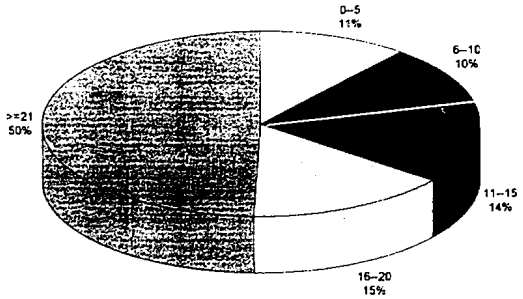

Figure 1. Age distribution (years) of 2,076 cases of cutaneous leishmaniasis of an endemic area of Bahia, Brazil.

Some observers suggest a role for Langerhans cells and keratinocytes in the modulation of CMI in leishmaniasis. Hyperplasia of Langerhans cells has been observed in human CL lesions (2). Also, these cells transport leishmania to the draining lymph nodes and induce an antigen-specific T cell response (29).

In several immunologically mediated skin diseases, MHC class II-positive keratinocytes are observed to be associated with IFN-γ production by surrounding T cells. These cells appear to be able to produce cytokines and maintain clonal expansion of activated lymphocytes. MHC class II-positive keratinocytes have been observed in CL but not in DCL lesions. This finding suggests that there is a lower IFN-γ production in DCL lesions. Epithelial hyperplasia is observed in CL and MCL, but not in DCL, and allows an increased contact between keratinocytes and inflammatory cells. This phenomenon is also considered to be related to IFN-γ production. These aspects, together with the observation of an epidermotropism of T lymphocytes in CL and MCL lesions, suggest a role for Langerhans cells and keratinocytes in the modulation of CMI in CL and MCL (2).

CLINICAL ASPECTS

In New World CL, the most frequent lesion is an ulcer with an elevated border and sharp crater. The disease may also manifest as papules, nodules, tu-

bercles or infiltrated plaques, diffuse infiltration (Fig. 2), and less frequently as verrucous lesions or vegetations. Multiple lesions occur in one-third of patients (2). In Brazil the lesions are generally located on the lower limbs, but in children they are more frequently situated on the head (67.6%) and upper limbs (26%) (30). Irrespective of the age, *L. mexicana* involves the external ear in 40% to 93% of patients and sometimes causes destruction and mutilation (4); and 51% of the lesions caused by *L. venezuelensis* are located on the face (5).

Mucosal lesions develop from the primary lesion by hematogenous spread and occur a few weeks to many years after the onset of infection. Usually the lesion begins in the nasal mucosa, but it can spread to the hard and soft palate, uvula, pharynx, gums, and upper lips. In cases of longer evolution the infiltration can cause enlargement of the upper lip and nose, conferring a tumoral appearance (Fig. 3). Nasal septal perforation is observed in many patients. Involvement of the larynx and vocal cords causes hoarseness and sometimes dysphonia. Mucosal involvement is most frequently caused by *L. brasiliensis* (2). Rarely MCL caused by *L. tropica* has been described in children (31). Retrospective studies have shown that the risk for mucosal disease is associated with inadequate treatment and multiple lesions above the waist.

The cutaneous lesions of Old World leishmaniasis heal spontaneously in 90% to 95% of patients. The lesions caused by *L. tropica* appear as single or multiple soft red papules that develop a central serous crust (dry type). The lesions caused by *L. major* are larger, 2 to 5 cm in diameter, and generally develop a central necrosis that produces a hemorrhagic crust. These are multiple lesions in 80% of cases (31). *L. aethiopica* causes a single, reddish plaque, 2 to 6 cm in diameter, with a shallow ulcer in the center and papular elements locally (7).

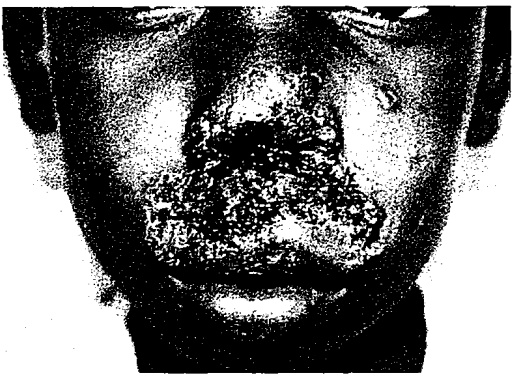

Figure 3. An intense infiltration of the nose and upper lip with destruction of the nasal septum.

Besides the classic aspects of CL, other forms of tegumentary leishmaniasis can be observed in children including recidiva cutis, disseminated, sporotrichoid, and lymphonodal leishmaniases.

Leishmaniasis recidiva cutis or lupoid leishmaniasis is characterized by the appearance of active lesions around leishmanial scars or by peripheral spreading of the lesion with central healing (Fig. 4). It has been described as a manifestation of both Old and New World leishmaniases. The lesions have an indolent course and are resistant to treatment (32).

Disseminated leishmaniasis is due to the hematogenous dissemination of parasites, and many lesions can be observed simultaneously. The lesions appear as acneiform, small papules that sometimes transform into small ulcers. This form of disease is completely different from DCL (Table 1), an entity that also presents with many lesions that are nodular and nonulcerated (33,34).

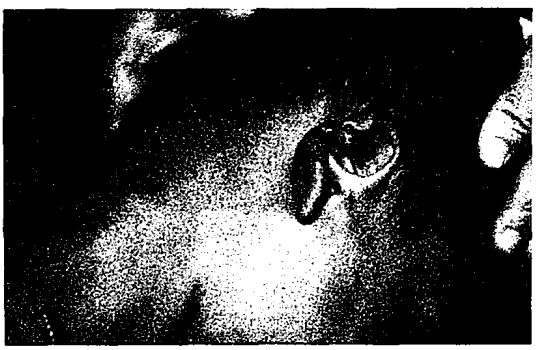

Figure 2. Diffuse infiltration and deformation of the ear in an 11-year-old child, a rare lesion of leishmaniasis caused by *L. braziliensis*. Note the regional lymphadenopathy.

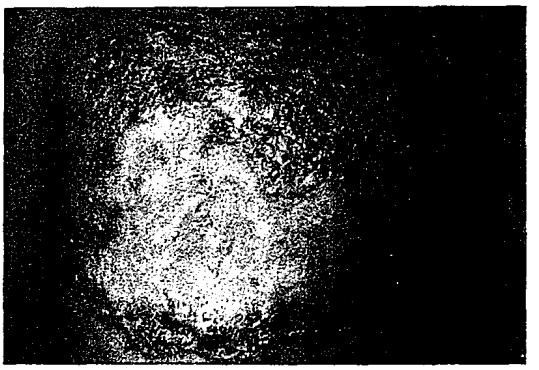

Figure 4. A 2-year-old child with an extensive scar and active lesions at the periphery caused by *L. braziliensis*.

The lymphatic form of CL, with a sequence of nodules along the lymphatic draining route, may mimic sporotrichosis. In the New World, lymphatic CL has been caused by *L. braziliensis*, *L. guyanensis*, and *L. panamensis* (2).

Regional lymphadenopathy with early resolution is a common aspect of New World CL (lymphonodal form of CL), especially in the infection caused by *L. braziliensis*. This presentation of CL is frequent in childhood. In 36 patients with CL with lymph node enlargement, 44% were patients less than 16 years of age. Patients with lymphadenopathy had higher antileishmanial CMI responses as well as humoral antibody responses (35). In some cases of CL caused by *L. braziliensis*, lymphadenopathy represents the first sign of disease and sometimes the only manifestation of infection (35). In CL caused by *L. major*, 10% of the patients have lymph node enlargement (36).

Diffuse CL presents with widespread erythematous nodules and infiltrated plaques (Fig. 5). Between the polar forms of tegumentary leishmaniasis, a borderline or intermediate disease has been rarely reported (8,37,38). Recently, cases of an intermediate form of DCL situated in the spectrum between the classic DCL and the borderline form have been described, some of them beginning in infancy (38).

Post-kala-azar dermal leishmaniasis (PKDL) occurs in about 20% of VL cases 1 to 5 years after the clinical cure of the disease, but it can appear during or shortly after treatment (39). It occurs as multiple hypochromic macules that develop into nodules. Usually an erythematous macular eruption on the cheeks is the first manifestation of the disease.

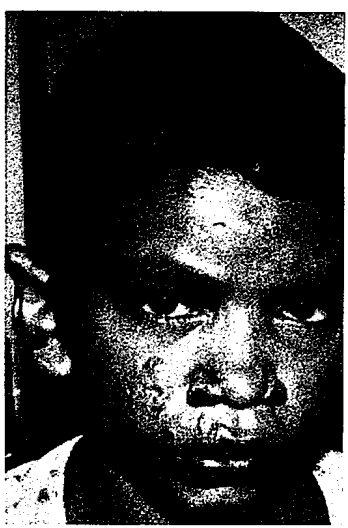

Figure 5. Diffuse leishmaniasis. A 12-year-old boy with infiltrated lesions on the face and ears simulating lepromatous leprosy.

IMMUNE RESPONSE

The immune response in tegumentary leishmaniasis is characterized by the important role of the CMI response. The humoral immune response induces the production of specific antibodies. There is suppression of the CMI in DCL and in borderline leishmaniasis (the anergic pole) as demonstrated by the absence of a delayed type of hypersensitivity (DTH) response in vivo in the Montenegro reaction (leishmania skin test), defective lymphocyte proliferative response in vitro, and decreased IFN-γ and IL-2 production. The defective response in vitro is observed when the peripheral blood mononuclear cells of DCL patients are cultivated with leishmania antigen. However, there is a good response to other antigens, such as PPD and candida, and lectins, such as phytohemoaglutinin or concavalin A. In active MCL the intradermal skin test and lymphocyte proliferative responses are strong and lymphocytes from these patients can produce IFN-γ, which inhibits intracellular replication of leishmania (40). Mucosal CL may represent a hypersensitivity state to leishmania.

The humoral immune response in the DCL patients shows a high production of specific antibodies in titers that range from 1/124 to 1/16,000 as detected by an immunofluorescent assay using intact promastigotes (38).

PATHOLOGY

At the initial stages of human infection, there is an accumulation of polymorphonuclear neutrophils and to a lesser degree eosinophils. These cells are gradually replaced by a monotonous infiltration of vacuolated and parasitized macrophages (macrophagic phase). The lesions of Old World CL remain in this phase longer than those of New World CL (2).

In New World CL and MCL, the inflammatory infiltrate is generally diffuse and consists of plasma cells (the predominant cells), macrophages, and lymphocytes. It is frequently associated with a granulomatous reaction represented by scattered giant and epithelioid cells. These cells are disposed in a disorganized pattern and may be associated with caseous necrosis (Fig. 6). Well-organized granulomas may also be observed (Fig. 7). The frequency

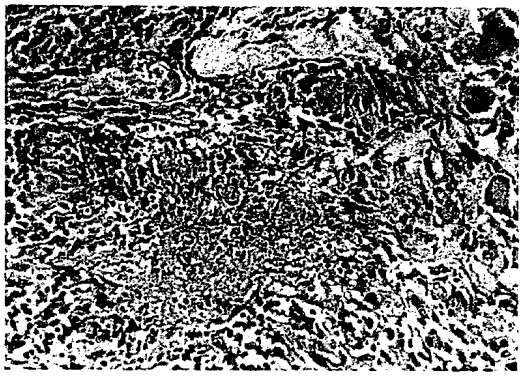

Figure 6. Cutaneous leishmaniasis. Caseous necrosis surrounded by giant cells. (Hematoxylin and eosin; magnification 160×.)

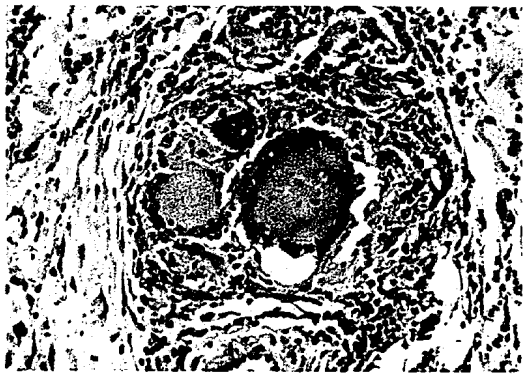

Figure 7. Cutaneous leishmaniasis. A well-organized granuloma with giant and epithelioid cells. (Hematoxylin and eosin; magnification 64×.)

of granulomatous reactions in CL and MCL varies from 50% to 82% depending on the number of biopsy specimens examined from a single patient. In American CL granulomatous reactions may be observed in lesions that have evolved in only a few weeks (2).

In lesions of New World CL and MCL, the parasites are generally scarce or absent. They are more frequent in recent lesions. Amastigotes are more numerous in the lesions of Old World CL, especially in the "dry" type of disease (2).

The necrosis observed in New World CL is generally caseous and simulates cutaneous tuberculosis. However, this type of necrosis is not observed in Old World CL and cytolytic necrosis is frequently reported (41). Cytolytic necrosis results from macrophagic disruption in lesions with a parasite index between 2 and 4 (between 100 and 10,000 parasites per standard section). It is usually associated with an infiltration of neutrophils—many are in karyorrhexis. In Brazilian CL, this type of necrosis is only seen in cases of CL with areas of marked parasitism or in DCL during treatment (2).

New World CL and MCL present with varied histopathologic findings in different lesions of a single patient and sometimes within the same lesion. For this reason classifications of CL and MCL based on histopathologic characteristics have no value (42).

In New World CL and MCL the surface epithelium is generally hyperplastic, assuming a pseudocarcinomatous appearance with deep downgrowths infiltrating the dermis or the corium. Intraepidermal abscesses, epidermotropism, and spongiosis are also frequently seen in the epidermis and represent transepidermal elimination of the dermal infiltrates as described in subcutaneous mycoses. Epidermal hyperplasia is less frequently observed in Old World leishmaniasis (2). In lymph nodes, a granulomatous reaction with caseous necrosis may be observed and sometimes is associated with parasitism (2).

Microscopically, DCL consists of a monotonous infiltrate of vacuolated and heavily parasitized macrophages that replace the dermis and sometimes the hypodermis. This macrophagic aspect is frequently associated with epidermal atrophy. In borderline leishmaniasis there is an association of the macrophagic aspect with a granulomatous reaction (2).

HISTOPATHOLOGIC DIFFERENTIAL DIAGNOSIS

In patients with parasitism a differential diagnosis must be made with histoplasmosis. Although *Histoplasma capsulatum* lacks the kinetoplast, it may often resemble amastigotes. However, with the Gomori methenamine silver stain, the budding forms of the fungus can be easily visualized. Additionally, an identification in situ of leishmania using antiamastigote antibodies can confirm the diagnosis of leishmaniasis.

Histologically cases of CL with a granulomatous reaction and microabscesses may mimic sporotrichosis, a mycotic disease where fungal forms are difficult to find. The diagnosis can be made isolating *Sporothrix schencki* by culture. In addition, positive results of serology, culture, and skin testing for leishmaniasis may provide the correct diagnosis of leishmaniasis.

In cases where a granulomatous reaction with caseous necrosis exists and amastigotes are not demonstrated histopathologically, the differential

diagnosis must include tuberculosis. As acid-alcohol resistant bacilli are not commonly found in cutaneous tuberculosis, it is mandatory to culture the lesion, perform skin testing for both etiologic agents, and conduct serologic tests for leishmaniasis.

Diffuse cutaneous leishmaniasis must be differentiated from PKDL because both conditions may present with nodules and a large number of amastigotes. For histopathologic diagnosis, it is necessary to take into account the presence of hypochromic macules (observed only in PKDL) and the clinical history.

LABORATORY DIAGNOSIS

The diagnosis is made by direct examination of smears, histopathology, serologic tests, and skin testing with leishmanin (Montenegro reaction). Direct observation of parasites can be made in Giemsa-stained smears of aspirate specimens, superficial scrapings of the ulcers, or imprints of biopsy specimens. The amastigotes are oval or round and 2 to 3 μm in length. Giemsa-stained preparations of amastigotes show a pale blue cytoplasm with two eosinophilic-stained structures—a round nucleus and a rod-shaped kinetoplast (Fig. 8). Considering the scarcity of parasites in lesions of New World CL and MCL, it is recommended to search for leishmania in more than one stained smear. The biopsy material or aspirates may also be used for culture and animal inoculation. Indirect immunofluorescence assay and ELISA are the most commonly used serologic techniques, and they demonstrate a good sensitivity and specificity. The Montenegro skin test is positive in only 51.6% of cases of *L. amazonensis* infection, whereas a positivity of 87% is observed in infections with other species of leishmania (2). When no parasitologic evidence of infection is demonstrated, diagnosis can be made using the polymerase chain reaction (PCR), which is much more sensitive than the conventional parasitologic methods and also enables identification of the species of leishmania (43,44).

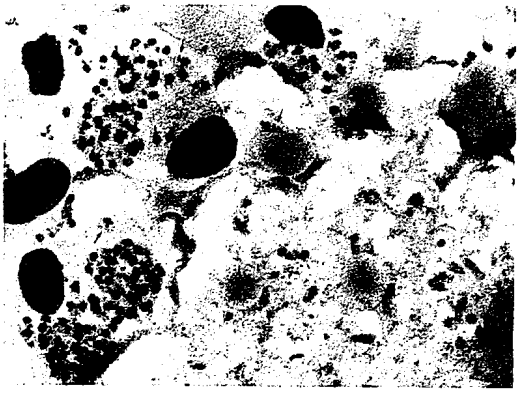

Figure 8. Diffuse cutaneous leishmaniasis (a smear of aspirate specimen). Many amastigotes lie free or within macrophages. Observe the round nucleus and the kinetoplast of amastigotes (arrows). (Giemsa stain; magnification 1200×.)

The diagnosis of DCL and borderline leishmaniasis can be made through smears or histopathology because the lesions are rich in parasites. In both DCL and borderline leishmaniasis, the Montenegro test is negative, but serology is positive, exhibiting higher titers in DCL than those observed in CL (2).

In all forms of leishmaniasis it is important to obtain the species characterization of the parasite. Characterization in situ can be performed by immunocytochemistry using appropriate monoclonal antibodies in sections of paraffin-embedded tissue (45).

TREATMENT

Pentavalent antimony is the drug of choice for the treatment of CL and MCL in childhood. However, it sometimes requires repeated doses, and side effects are frequent. Sodium stibogluconate (Pentostam®) or meglumine antimoniate (Glucantime®) are drugs administered by intramuscular or intravenous routes. The mechanism of action is through interference with the ATP synthesis of the parasites (46). The side effects are cumulative and dose dependent. Antimony can affect the organs in the following order of frequency: heart, kidney, liver, and brain. The single dose toxic limit for man is about 30 mg/Sb^{v}/kg (47). The more frequent side effects are musculoskeletal pain, anorexia, elevation of hepatocellular enzyme levels, alterations of the electrocardiogram (T wave flattening and/or inversion), and renal dysfunction (46,47).

Systemic treatment for CL is also indicated to prevent mucosal involvement, especially in the infections caused by the *L. braziliensis* species complex (47). For the intravenous route, dilution is not necessary and the administration should be done slowly with a fine needle over 5 minutes.

The recommended dose for CL is 10 to 20 mg/Sb^{v}/kg/day for 20 consecutive days, but this dose may vary according to the morphology and frequency of the lesions and the clinical response (46,47). For simple skin ulcers caused by *L. braziliensis*, 10 mg/Sb^{v}/kg/day for 20 days continuous

treatment is the recommended schedule. For MCL, a dose of 20 mg/Sb^{v}/kg/day is recommended for 30 days or more depending on the clinical response (47). The treatment of DCL with pentavalent antimony is not curative. In the initial phase of treatment some clearing of the lesions may be observed, but relapses always occur (48).

The treatment of CL must be maintained until complete clinical and parasitologic cure is achieved. Patients should be reevaluated 4 to 6 weeks after therapy. If the lesions do not regress, continuation of treatment with the same schedule should be considered. Pentavalent antimonials should not be used in children with cardiopathy, nephropathy, or hepatic disease.

Amphotericin B and pentamidine are used as second-line therapy in patients who do not respond to the antimonials. These drugs are potentially toxic and require intramuscular or intravenous administration. Amphotericin B is more often used in MCL, but a few cases relapse after this therapy (47). The recommended dose is 0.5 to 1.0 mg/kg/day up to a total dose of 20 to 30 mg/kg (46), but some patients have been cured with lower doses (46,47). The recommended dose of pentamidine is 2 mg/kg on alternate days for a period of 1 week for cutaneous lesions or 5 weeks for MCL and DCL. These drugs are not recommended for children with diabetes and renal, hepatic, or cardiac diseases (49).

A great number of drugs have also been employed for the treatment of tegumentary leishmaniasis. Systemic therapy with furazolidone, aminosidine, allopurinol, nifurtimox, and others have been tried, with variable results. Many of these drugs need only oral administration which simplifies the treatment (46,50). In limited forms of leishmaniasis intralesional glucantime has been used with partial or complete clearing of lesions (51,52). The aminoglycoside paramomycin has been tried as an ointment at 16% with 12% methylbenzethonium chloride in Oriental and American leishmaniasis with favorable results (53,54). However, Asilian et al (55), using an ointment containing 15% aminosidine and 10% urea in petrolatum in a 2-week regimen did not observe clearing of the lesions. Other local therapy like cryosurgery, localized controlled heat, and carbon dioxide laser alone or associated with systemic drugs have been attempted in some cases (46, 56,57).

The use of cytokines, which stimulate leishmanicidal macrophage activity, has also been tried in antimony-resistant cases. A recent study reports complete clearing of lesions in two patients with antimony-resistant DCL after combined systemic administration of IFN-γ and pentavalent antimony (58).

The criteria of cure are the clinical clearing of lesions along with the absence of or a marked drop in serologic titers. An effective and early treatment of CL is mandatory mainly in areas where *L. braziliensis* is the predominant agent in order to avoid mucosal involvement (47).

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