

ATYPICAL DISSEMINATED CUTANEOUS HISTOPLASMOSIS IN AN IMMUNOCOMPETENT CHILD, CAUSED BY AN "ABERRANT" VARIANT OF *Histoplasma capsulatum* var. *capsulatum*

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SUMMARY

A case of atypical disseminated cutaneous histoplasmosis in a five-year old, otherwise healthy child, native and resident in São Paulo metropolitan area is reported. Cutaneous lesions were clinically atypical. Histologic examination disclosed a granulomatous reaction but no fungal structures could be demonstrated by specific staining nor by immunohistochemical reaction. The fungus was isolated from biopsy material on two different occasions, confirming diagnosis of an unusual fungal infection. The fungus, originally thought to be a *Sepedonium* sp. due to the large sized, hyaline or brownish colored tuberculated macroconidia and to lack of dimorphism (yeast form at 37 °C) produce H and M antigens, visualized by the immunodiffusion with rabbit anti-*Histoplasma capsulatum* hyperimmune serum. Patient's serum sample was non reactive with *H. capsulatum* antigen by immunodiffusion, counterimmunoelectrophoresis and complement fixation tests, and immunoenzymatic assay failed to detect the specific circulating antigen. This serum was tested negative by double immunodiffusion when antigen obtained from one of the isolated samples was used. Both cultures were sent to Dr. Leo Kaufman, Ph.D. (Mycoses Immunodiagnostic Laboratory, CDC-Atlanta/USA), who identified them as *H. capsulatum* by the exoantigen and gen-probe tests. Both clinic and mycologic characteristics of the present case were atypical, suggesting the fungus isolated is an "aberrant variant" of *H. capsulatum* var. *capsulatum*, as described by SUTTON *et al.* in 1997¹⁹. Treatment with itraconazole 100 mg/day led to cure within 90 days

KEYWORDS: *Histoplasma capsulatum*; Cutaneous histoplasmosis; Aberrant strain.

INTRODUCTION

Classic histoplasmosis also called as Darling's disease, is caused by *Histoplasma capsulatum* var. *capsulatum*. The association histoplasmosis-AIDS (WHEAT *et al.*, 1990²⁰; ROCHA & SEVERO, 1994¹⁶; ALVES, 1996¹; BORGES *et al.*, 1997³) became so frequent that this fungal infection was included in the definition of diagnostic criteria of this immunodeficiency syndrome by the CDC. The usual clinical manifestation is an acute self limited lung disease. However, immunosuppressed patients may develop systemic infection with hepatoesplenomegaly, skin lesions, diffuse lung infiltrates and pancytopenia. In African histoplasmosis, caused by *H. capsulatum* var. *duboisii*, cutaneous lesions such as large abscesses predominate and pulmonary compromise is rare. Asymptomatic histoplasmosis infection can be detected by positive intradermal test with histoplasmin, an antigen which possesses both H and M antigenic fractions. In 1976 STANDARD & KAUFMAN¹⁷ standardized immunochemical tests to differentiate both varieties of *H. capsulatum*. The authors performed double immunodiffusion (DID) using serum obtained from hyperimmune rabbits and

standard sample antigens. PADHYE *et al.*¹⁴ developed a specific DNA-probe which is being currently used for diagnostic purposes. The present paper presents an atypical case of disseminated cutaneous histoplasmosis in a 5 year-old immunocompetent child. Specific antibodies and circulating polysaccharide antigens were not detected in this patient's serum. No fungal structures were observed in histologic examination of cutaneous lesions.

Fungal samples were isolated from biopsy material in two different times. Both samples were identified as *H. capsulatum* var. *capsulatum* by means of specific exoantigen test and gen-probe assay.

CASE REPORT

A 5 year-old, Caucasian child, native and resident in São Paulo metropolitan area was admitted at the Dermatology Clinic of "Hospital das Clínicas", University of São Paulo, presenting 5 mm, erythematoviolaceous, slightly infiltrated cutaneous nodules, surrounded by a hypopigmented halo. Lesions had been present for approximately 18 months and were first noted on the buttocks (Fig. 1). During the next few months

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Fig. 1 - Patient G.M.L. Before (a) and after (b) treatment.

lesions progressively extended to upper limbs (Fig. 2), outer ears (Figs. 3 and 4), face and knees. Except for fever at the onset of the disease, no systemic signs nor symptoms were observed.

Physical examination of this child showed an otherwise healthy and weighted 21 kg. Chest X-ray and abdominal ultrasonography were normal, as well as blood biochemistry exams and blood cell count.

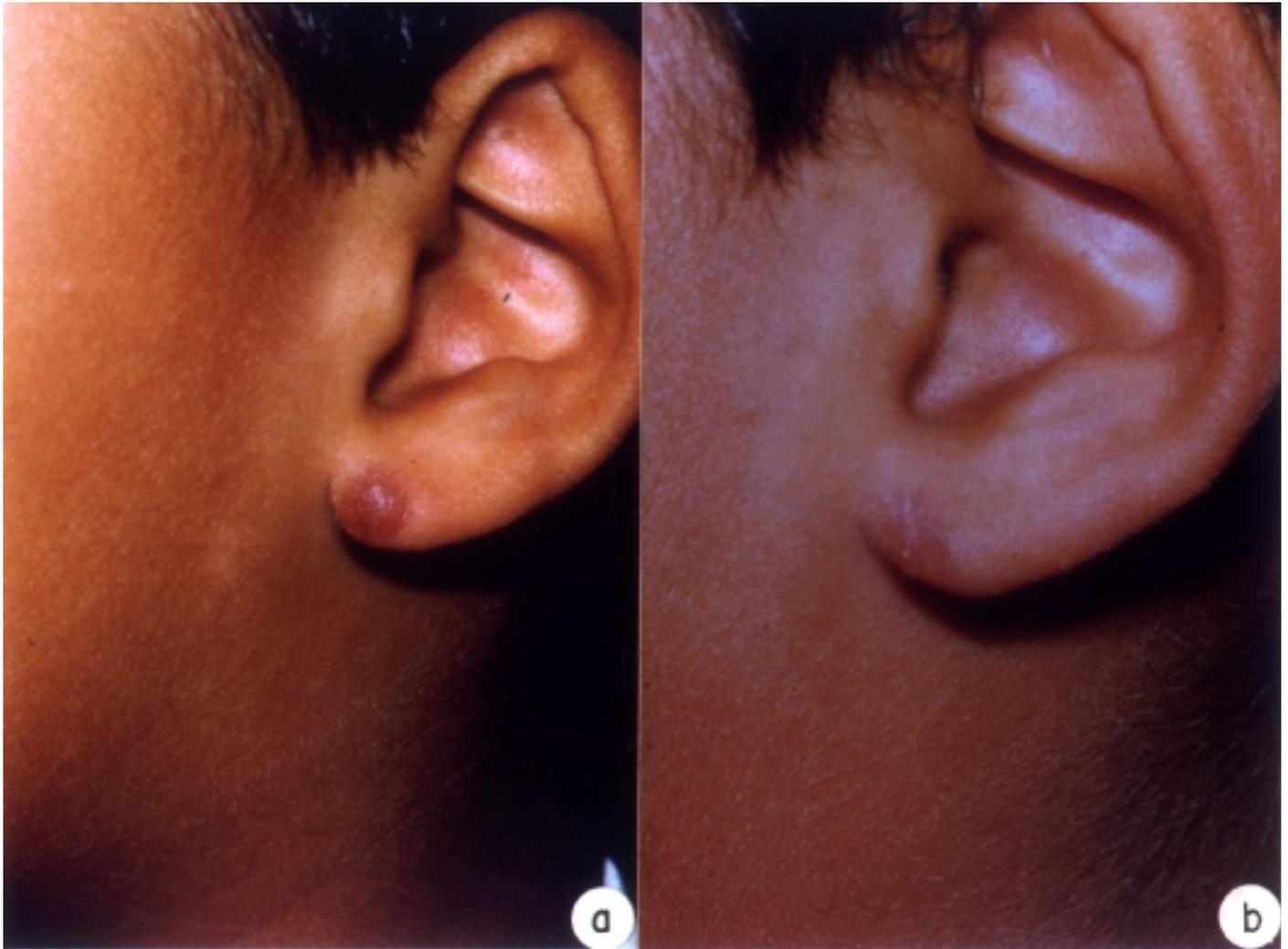


Fig. 2 - Patient G.M.L. Before (a) and after (b) treatment.

Clinical diagnosis included numular eczema, lymphomatoid papulosis, sarcoidosis and persistent reaction due to insect bite.

Histologic examination of five different lesions, excised on different occasions, disclosed similar findings (Fig. 5), *i.e.* acanthosis, spongiosis and lymphocyte exocytosis. Intense dermal inflammatory infiltrate with casts of epithelioid and multinucleated Langhan's giant cells, surrounded by a variable number of lymphocytes, characterizing a granulomatous response was present. Grocott and acid-fast stainings, as well as immunohistochemical ABC-peroxidase reaction using antibodies against *H. capsulatum* antigens resulted negative in all specimens. Diagnosis of a chronic granulomatous dermatitis was established, leading to new diagnostic hypothesis: paucibacillar leprosy, sarcoidosis, cutaneous tuberculosis, cutaneous T-cell lymphoma "slack skin" type and some kind of deep seated mycosis.

Mitsuda (10 x 10 mm), histoplasmin (10 x 7 mm) and candidin (7 x 5 mm) reactions were positive, while PPD, Montenegro's and trichophytin reactions were negative. Antibody detection tests (double immu-

nodiffusion and counterimmunoelectrophoresis) for *Paracoccidioides brasiliensis*, *Histoplasma capsulatum* and *Aspergillus fumigatus* were negative.

Two different biopsy specimens grew on Sabouraud agar, on different times, a white cotton-like colony. The fungus, originally thought to be a *Sepedonium sp.* sample due to the large sized, hyaline or brownish colored tuberculated macroconidia and to lack of dimorphism (37 °C) produced H and M antigens, which are specific for *H. capsulatum*.

Both cultures were sent to CDC, where Dr. Leo Kaufman tested them by exoantigen and gen-probe. Diagnostic confirmation of infection by an unusual fungus suggested the need for a wide spectrum antifungal. Previous positive experience with itraconazole for treatment of children with tinea capitis led the authors to choose this drug. Treatment with oral itraconazole 100 mg/day was started and after three months all lesions had disappeared leaving residual hypochromic macules.

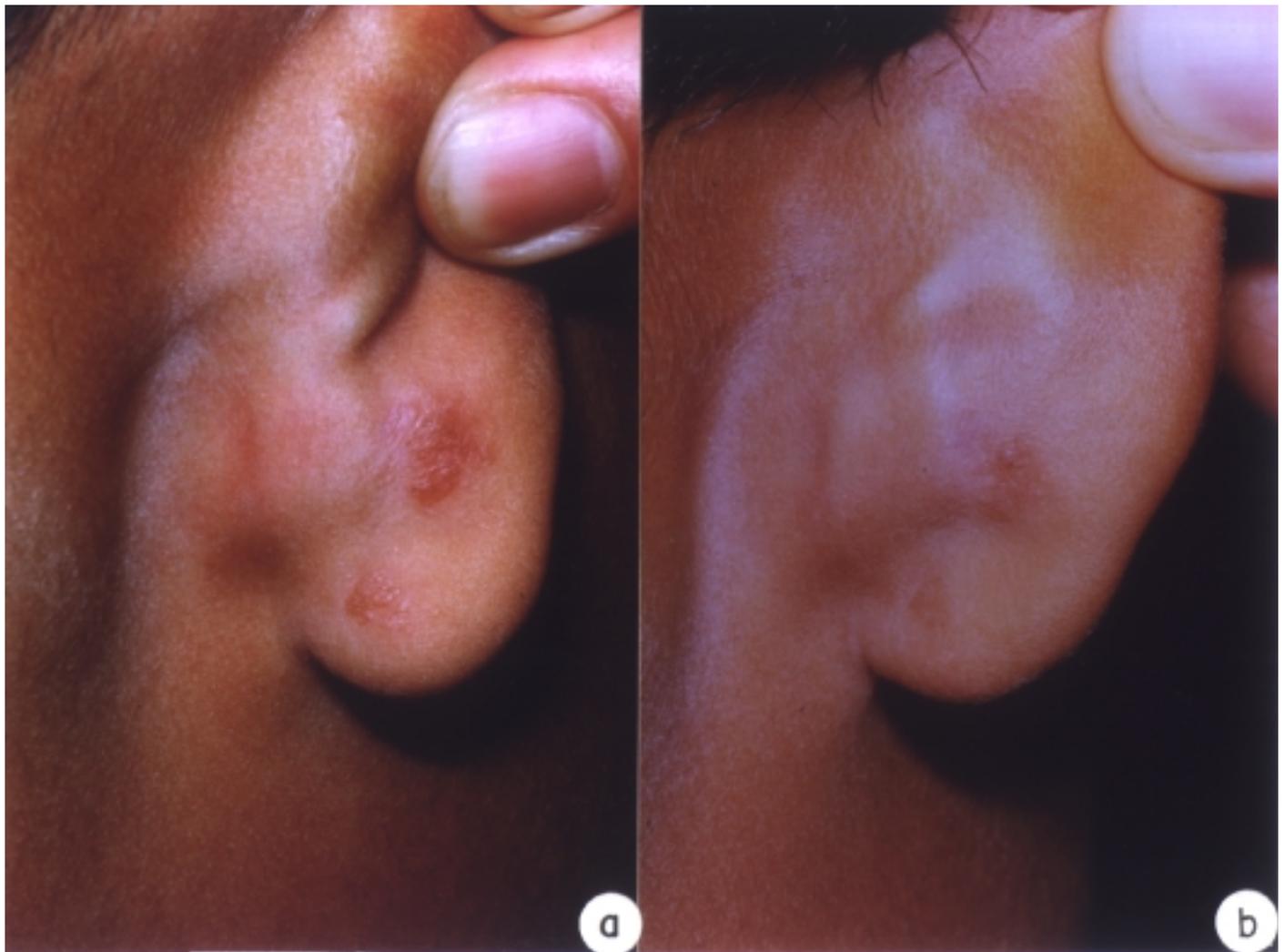


Fig. 3 - Patient G.M.L. Before (a) and after (b) treatment.

DISCUSSION

Clinic, histologic and mycologic characteristics of the present case are unusual. Disseminated cutaneous lesions observed in an immunocompetent child suggest the occurrence of secondary dissemination, most probably from a pulmonary primary site, not identified by clinical nor radiologic examination. In 1955, LACAZ *et al.*¹² reported a case of histoplasmosis in a child from Bahia/Brazil. At that time only nine cases of histoplasmosis had been diagnosed in Brazil. The child presented hepatosplenic lesions and fungi were found in liver and spleen at autopsy.

According to STUDDARD *et al.*¹⁸, cutaneous histoplasmosis normally presents two types of lesions: small papules which gradually become ulcerated and surrounded by erythematous and scaly skin and ulcers with elevated borders. In the present patient erythematoviolaceous, infiltrated, pea-sized cutaneous nodules surrounded by a hypochromic halo were observed. Lesions were present on the upper and lower limbs, outer ear, face and buttocks. General health was otherwise

unchanged. This clinical manifestation, atypical for cutaneous histoplasmosis, led to different diagnostic hypothesis, such as numular eczema, lymphomatoid papulosis, sarcoidosis and persistent reaction to insect bite. Diagnosis was made even more difficult because of the absence of parasitic elements on histologic slides. It is well known that cutaneous histoplasmosis is easily diagnosed through skin biopsy.

Immunohistochemical reaction with anti-*H. capsulatum* antibodies is a valuable tool in the diagnosis of this disease because of its high sensitivity, as well as the quickness with which it may be carried out. PIRES D'AVILA, 1997¹⁵ reported on the high sensitivity of this method, when analyzing 12 tissue samples obtained from patients with cutaneous histoplasmosis.

Isolation of the agent on Sabouraud agar was achieved on two occasions (Fig. 6), excluding the possibility of contamination. The isolated fungus was first thought to be *Sepeconomium sp.*, a contaminant. Impossibility to demonstrate fungal structures in histologic examination contributed to that hypothesis. Tuberculated, hyaline or brownish col-

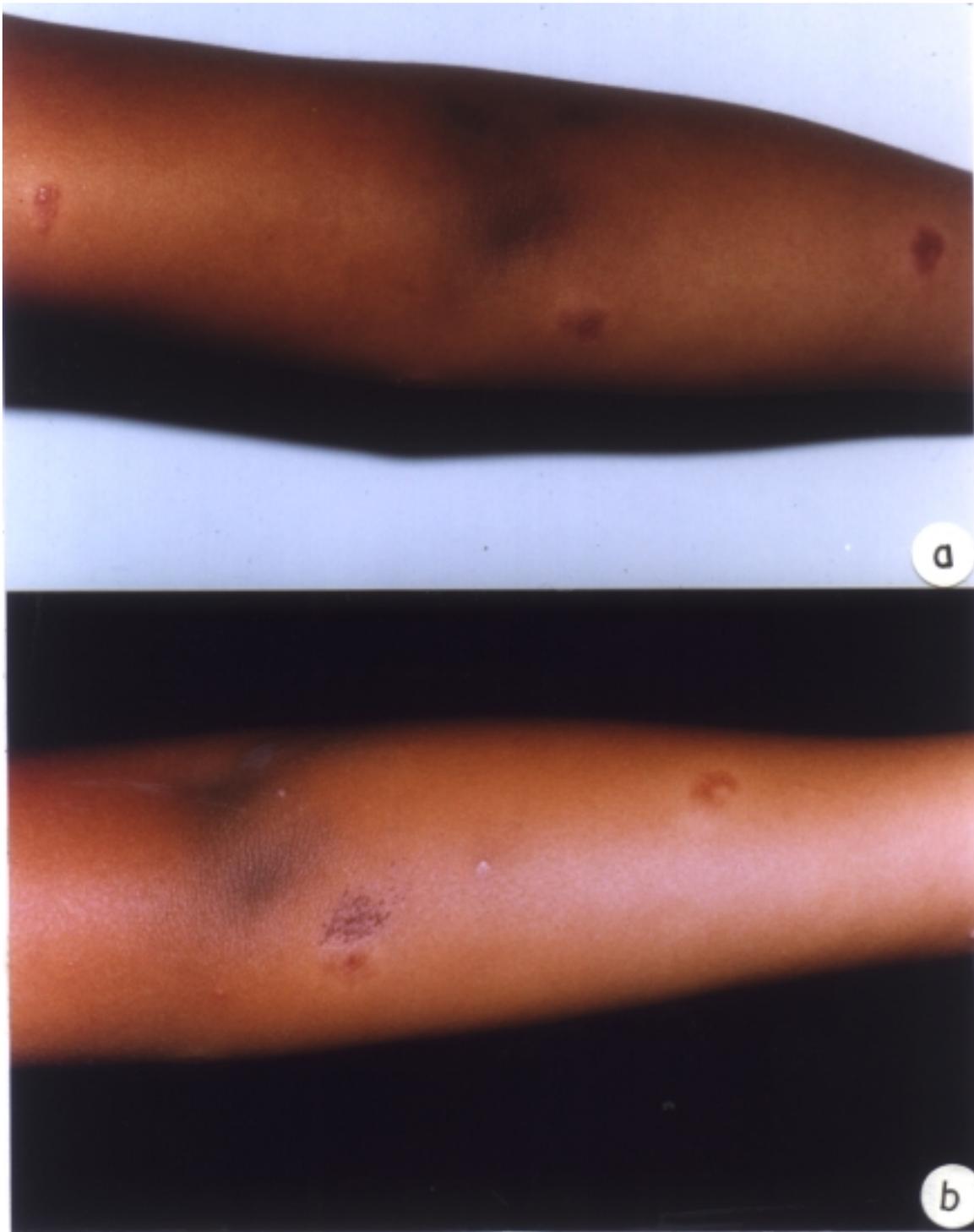


Fig. 4 - Patient G.M.L. Before (a) and after (b) treatment.

ored macroconidia, larger than would be expected for *H. capsulatum* were observed (Fig. 7).

Specific immunochemical tests to differentiate fungi from *Histoplasma* genus were developed by STANDARD & KAUFMAN, 1976¹⁷,

who used filamentous forms of *H. capsulatum* var. *capsulatum*, *H. capsulatum* var. *duboisii*, *H. capsulatum* var. *farcinosum*, *Arthroderma tuberculatum*, *Chrysosporium keratinophilum*, *Corynascus (Thielavia) sepedonium* and their specific antisera. DID showed that *Histoplasma* spp. samples were unique in producing H and M antigens.

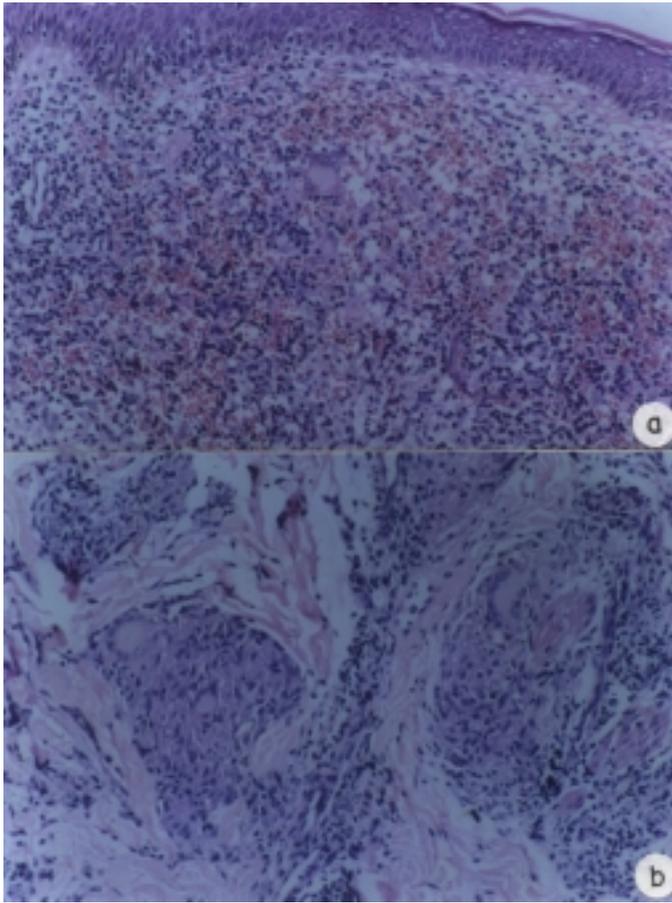


Fig. 5 - a) HE: skin biopsy – Superior dermis with well defined diffuse granulomatous inflammatory infiltrate. b) HE: skin biopsy – Deep dermis disclosing well organized tuberculoid granulomas.

Detection of a precipitation band in one case of acute adiaspiromycosis observed in Bahia/Brazil, by BARBOSA *et al.*, 1997² was suggestive to Dr. Leo Kaufman of concomitant histoplasmosis and adiaspiromycosis infection or an early infection by *H. capsulatum* var. *capsulatum*.

Antigen from one of the isolated samples was obtained by KAUFMAN & STANDARD's technique⁸. It was tested by DID against serum from the patient and rabbit anti-*H. capsulatum* var. *capsulatum* hyperimmune serum. Two precipitation bands identical to the ones observed with *H. capsulatum* antigen were detected using rabbit serum, while no bands were observed with the patient's serum (Fig. 8).

Immunoblotting of patient's serum disclosed a fraction of approximately 94 kDa. According to ZANCOPE *et al.*²¹, this fraction corresponds to a glycoprotein present in histoplasmin. Studies of FAVA, 1996⁶, using the polysaccharide antigen from *H. capsulatum* obtained according to NORDÉN's technique, 1951¹³, demonstrated that this fraction is responsible for the positivity of histoplasmin reaction. According to DEEPE JR & DUROSE, 1995⁴, this band can stimulate a cellular type immune response in Balb c mice, otherwise not providing protection against infection by *H. capsulatum*.



Fig. 6 - *Histoplasma capsulatum* var. *capsulatum*. Macroscopic appearance in Sabouraud agar after 30 days at room temperature.

GOMEZ *et al.*, 1997⁷, utilizing ELISA to detect a specific antigen in the histoplasmosis serum, showed that this technique has great sensitivity and high specificity, giving yet another methodology helpful in diagnosis of this fungal infection.

In 1978, KAUFMAN & STANDARD⁹ extracted antigens from *Coccidioides immitis*, *H. capsulatum*, *Sepedonium sp*, *Chrysosporium karatinophilum*, *Malbranchea sp* and *Arthroderma tuberculatum*. Immunodiffusion test disclosed high sensitivity and specificity for diagnosis of coccidioidomycosis and histoplasmosis. In the present work, patient's serum did not react when tested against antigens obtained from the infecting fungal sample, a noteworthy finding.

Several reports point out the emergence of fairly atypical strains of *H. capsulatum* var. *capsulatum*. These strains that show phenotypic changes could interfere on host-parasite interaction (EISSENBERG &

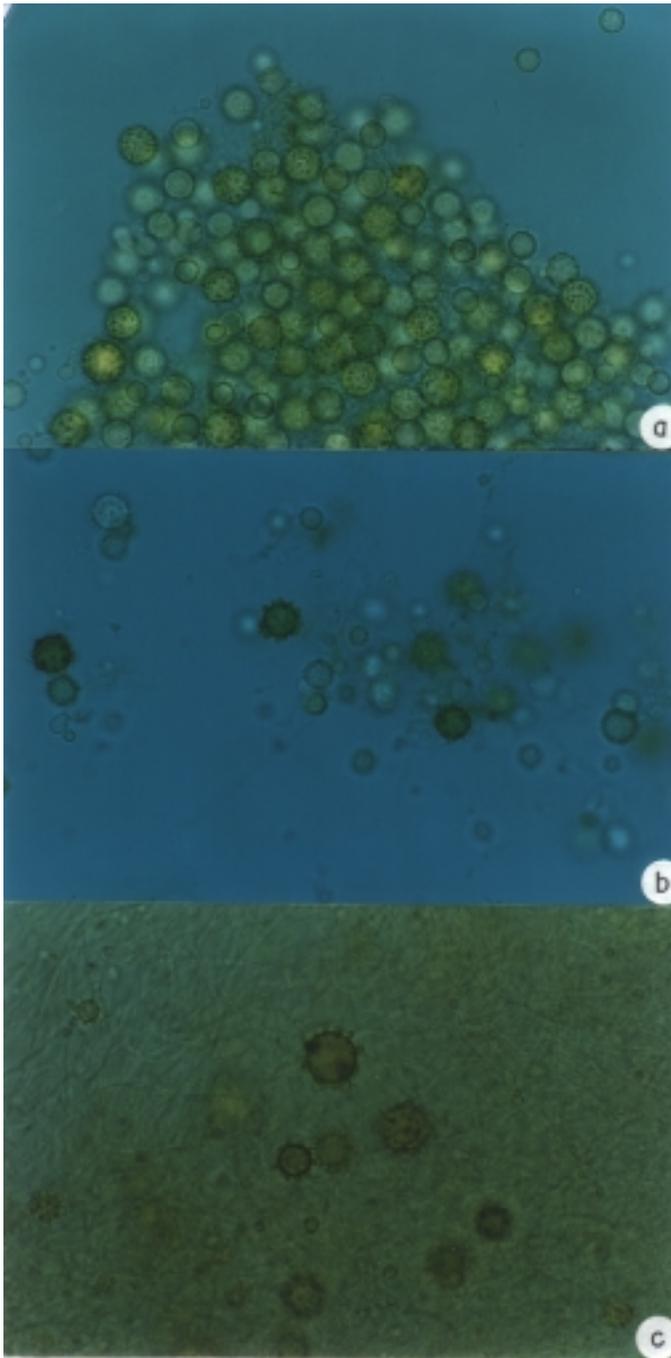


Fig. 7 - Microscopic characteristics in Sabouraud agar after 30 days, growth at room temperature. Hyaline and some pigmented tuberculate macroconidia. a), b) 400X; c) 630X.

GOLDMAN, 1991⁵, KEATH *et al.*, 1992¹⁰ and KERSULYTE *et al.*, 1992¹¹).

SUTTON *et al.*, 1997¹⁹ referred an "aberrant" variety of *Histoplasma capsulatum* var. *capsulatum*. This isolate was obtained from right elbow synovial liquid of a patient with histoplasmosis from Kansas, USA. Colonies grown on Sabouraud agar were smooth, not possessing

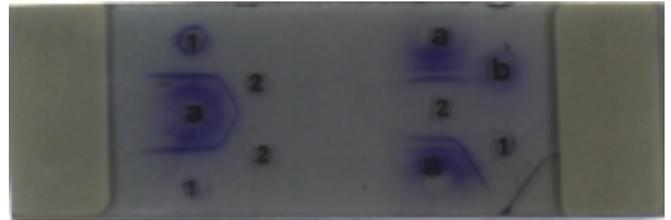


Fig. 8 - Double Immunodiffusion test, exhibiting identical H and M bands in both *H. capsulatum* antigens.

- 1) *H. capsulatum* metabolic antigen with H and M fractions.
- 2) Exoantigen obtained from the sample isolated from patient's biopsy.
 - a) Polyclonal anti-*H. capsulatum* rabbit serum.
 - b) Patient's serum.

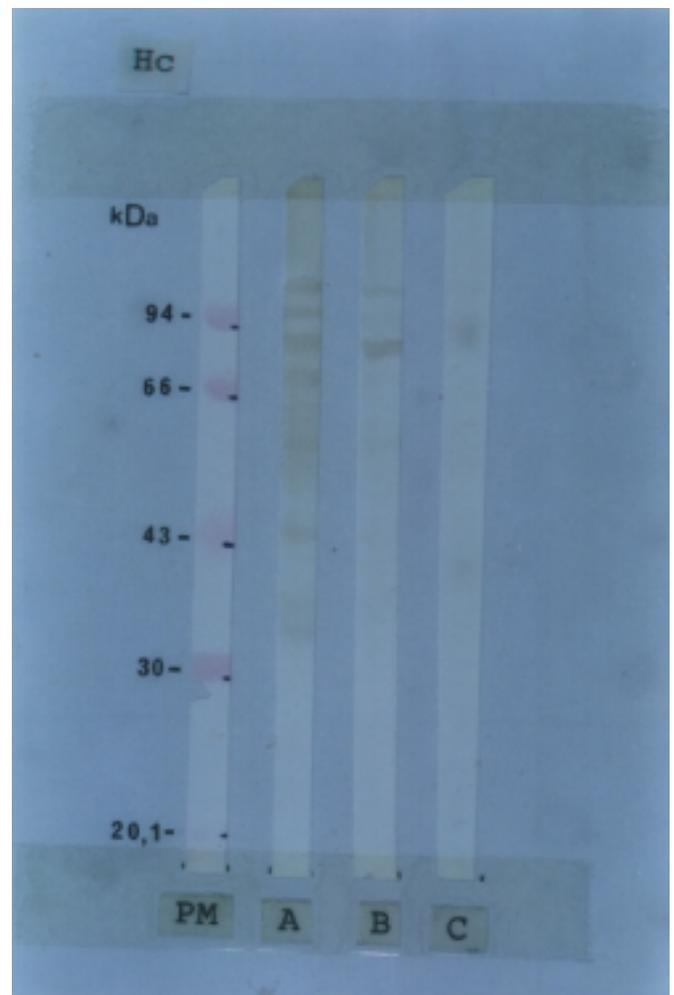


Fig. 9 - Immunoblotting with *H. capsulatum* metabolic antigen showing the presence of 94 kDa band.

- a) Patient's serum.
- b) Control serum presenting positive reaction for histoplasmosis.
- c) Negative control serum.
- PM) Molecular weight pattern.

characteristic macroconidia of *H. capsulatum* var. *capsulatum*. Identification was confirmed by DNA-probe.

RESUMO

Histoplasmose cutânea disseminada atípica em criança imunocompetente, causada por uma variante "aberrante" de *Histoplasma capsulatum* var. *capsulatum*

O presente trabalho registra caso de histoplasmose em paciente de 5 anos, HIV negativo, natural e procedente da cidade de São Paulo, com lesões cutâneas não diagnosticadas clinicamente. Exame histopatológico negativo para infecção fúngica. Cultivos em duas ocasiões, positivos para *Histoplasma capsulatum* var. *capsulatum* (amostras 361 e 387). Sorologia negativa para anticorpos anti-*Histoplasma capsulatum* e *Paracoccidioides brasiliensis* pelas provas de Imunodifusão dupla e Contraímunoelctroforese. Ensaio imunoenzimático pesquisando o antígeno polissacarídico específico, negativo. A obtenção do exoantígeno de uma das amostras permitiu, através de provas de Imunodifusão, com soro de coelho anti-*Histoplasma capsulatum* var. *capsulatum*, detectar duas bandas de precipitação. Registre-se a negatividade da reação de Imunodifusão dupla do soro do paciente face ao exoantígeno produzido com a amostra dele isolada. Através da reação de Immunoblotting foi identificada uma fração com peso molecular de aproximadamente 94 kDa, além de outras. Os dois cultivos foram enviados ao Prof. Leo Kaufman, CDC, Atlanta, USA, o qual identificou as duas amostras como *Histoplasma capsulatum* var. *capsulatum*, através de provas de Imunodifusão dupla com os respectivos exoantígenos e sonda específica do fungo (DNA-probe). As colorações específicas para fungos, nos tecidos, foram negativas, incluindo prova de Imunoperoxidase para *Histoplasma capsulatum* var. *capsulatum*, a negatividade das provas sorológicas para histoplasmose clássica e o próprio aspecto clínico das lesões cutâneas eritemato-violáceas infiltradas, mostram que o caso em apreço é realmente inusitado, correspondendo o cultivo a uma "variante aberrante" do *Histoplasma capsulatum* var. *capsulatum*. Face ao diagnóstico micológico de histoplasmose clássica, o paciente foi tratado com itraconazol 100 mg/dia, durante três meses, com regressão total das lesões.

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