Cutaneous Histoplasmosis —Report of a Case, with Special Reference to the Mycology of the Isolated *Histoplasma capsulatum*

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A Japanese woman with cutaneous histoplasmosis is presented. The patient developed cutaneous lesions consisting of grouped and confluent papules on several parts of the body after settling in Santa Cruz City, Bolivia. The histoplasmin test was positive. Histological sections of the skin showed a few intracellular yeast forms of the organism in the dermis. *Histoplasma capsulatum* was isolated from biopsy specimens of the skin. The morphological characters of the fungus were described in detail. There was no focus of infection in the lung or other internal organs.

Key words: cutaneous histoplasmosis, *Histoplasma capsulatum*, histoplasmin

Histoplasmosis, a systemic fungus disease, is widely distributed throughout the world in temperate and tropical zones of both hemispheres1). In Japan, however, only few cases2,7-10) documenting this disease have been reported. Significant in these reported cases is the lack of adequate cultural data due to either failure to perform a culture initially or to subsequent inexperience of the laboratory identification after cultures have been taken.

A Japanese woman who developed cutaneous lesions of histoplasmosis in Bolivia, South America, where she had emigrated was examined at Toyama Prefectural Central Hospital after she returned to Japan. *Histoplasma capsulatum* was repeatedly isolated in pure culture as a causative agent from the skin lesions.

Case Report

A 38-year-old female patient, born in Toyama City, was seen at the Dermatology Clinic of Toyama Prefectural Central Hospital on February 21, 1986. She emigrated to Santa Cruz City in the Republic of Bolivia, South America, in 1960 with her family when she was 12 years old, and returned to Toyama City in 1985. Her father and elder brother who lived together were found to have a positive histoplasmin test but had neither a history nor symptoms of histoplasmosis. In addition, she has been diagnosed as having Crouzon’s disease, craniofacial dysostosis. In 1976 when she was 28 years old, she first noted a cutaneous lesion consisting of grouped papules on the right elbow which gradually increased in number. Similar lesions appeared on the skin of other parts of the body later. She complained of no subjective symptoms.

On examination, the skin lesions were scattered on the forehead, right cheek, both sides of the back, both arms and right hand (Fig. 1). Each of the lesions was composed of grouped and/or confluent papules, 1 to 2 mm in diameter (Figs. 2-5). There was no ulceration on the surface of the lesions.

She had no fever, no lymphadenopathy, and no hepatosplenomegaly. Laboratory data showed a WBC count of 6,900/cu mm, with 35% lymphocytes. The serum protein level was 7.4 g/dl, with a γ-globulin level of 0.9 g/dl. ESR was 4 mm/hr. SGOT, SGPT, and alkaline phosphatase levels were normal. Electrolytes, BUN, glucose, and hemoglobin values were within normal limits. A chest roentgenogram, bone radiography, and abdominal CT scan were unremarkable. The tuberculin skin test was positive. The sensitization test with DNCB was positive. Fungal cultures of urine, sputum, and blood were all negative. The histoplasmin skin test was positive (induration of 10 × 12 mm). A histoplasmosis complement fixation titer was less than 1:8. Biopsy specimens taken from papules on the right forearm and right cheek were submitted for fungal culture test and histopathology.

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Fig. 1. Distribution of cutaneous lesions.

Fig. 2. Papules on the right cheek.

Fig. 3. Grouped small papules on both sides of the back.

Fig. 4. Grouped and confluent papules on the right elbow to the forearm.

Fig. 5. Grouped papules arranged in a circular fashion on the back of left hand.
Histoplasma capsulatum grew in the fungal culture test. Therapy with intravenous miconazole (200–800 mg per day) was started as soon as diagnosis was made and continued for nine weeks. The skin lesions were simultaneously treated with topical thermotherapy using a pocket-warmer. The lesions have gradually flattened. Histopathology. —The biopsy specimens showed focally a marked granulomatous infiltrate composed largely of histiocytes intermingled with lymphocytes in the upper and middle dermis (Fig. 6). Periodic acid-Schiff staining indicated a few yeast-like organisms within some macrophages (Fig. 7). In addition, several round bodies, 1–3 μm in diameter, strongly stained with hematoxylin and surrounded by a clear space were found within some histiocytes (Fig. 8); these bodies, however, were not stained with the PAS reaction.

Mycological Study

Material and Methods: Approximately 10 inocula of about 1 mm cube of the biopsy specimens for fungal culture test were seeded onto slants of Sabouraud’s dextrose agar (SDA), which contained no antibiotics and incubated at 25°C for 10 days. Totally 6 fungal colonies were grown without any contamination and transferred to SDA for further study. Two representative strains, NHL 2966-1 and 2966-2, from these isolates, were used for this study.

For identification, cultures were grown on three media; SDA (Nissui Seiyaku Co., Ltd.), potato-carrot agar (PCA)11), and oatmeal agar (OA) which had the following constitution: Quaker’s white oats 15.0 g; Nutritional Biochemical’s special wheat germ 15.0 g; agar 20.0 g; distilled water 1 liter. Each plate culture was incubated at 25°C and 37°C, and the growth-rate, colony characters and microscopic morphology were examined after 2 and 3 weeks. Slide cultures grown on PCA and OA were also used for the observation of microscopic morphology.

Tests for converting to the yeast phase were performed as follows. The inoculum was made from colony of the isolate which was embedded into 1% glucose added Brain Heart Infusion agar (BHI; BBL Microbiology Systems), and then mice were inoculated in the peritoneal cavity with a block of the mycelial fragments about 5 mm cube. The block was recovered from the mice 14 days after inoculation, and cut into small pieces. The small pieces were again inoculated on 1% glucose added BHI agar and incubated at 37°C, one in a 5% CO₂ atomosphere and the other under usual aerobic conditions.

Results

The mycological results are summarized in the following description and illustrations (Figs. 9-12):
Cryptococcus capsulatus (Darling) Castellani & Chalmers, 1919.
Torulopsis capsulatus (Darling) Almeida, 1933.
Posadasia capsulata (Darling) Moore, 1934.

Colonies on SDA attaining 20—21 mm diameter in 14 days at 25°C, velvety to cottony, composed of a thick basal felt, slightly furrowed, with abundant aerial hyphae and sparse conidia, white; reverse Saffron to Salmon (Rayner). Colonies on OA or PCA attaining 20—23 mm diameter in 14 days at 25°C, thin, flat, more or less floccose to powdery, with abundant conidia, white or Rosy Buff (Rayner); reverse uncolored or Rosy Buff to Vinaceous Buff (Rayner). At 37°C, growth is rather reduced, attaining 4 mm diameter in 14 days.

Mycelium composed of hyaline to pale yellow, branched, septate, smooth-walled, 1—3.5 μm diameter hyphae; racquet mycelium present, swollen up to 4.5—5 μm at septum. Conidiophores micronematous to semimacronematous, mononematous, straight or slightly flexuous, hyaline, unbranched or irregularly branched, smooth-walled, 25—35 μm long, septate. Conidiogenous cells monoblastic, cylindrical, narrow, sometimes swollen at the upper part, 5—12 × 1—3 (—3.5) μm, not tapered, often reduced to small protuberance on the hypha. Macroconidia dry, simple, borne terminally on the conidiogenous cell, singly or rarely successively in two-three chains, hyaline to pale yellow, globose to subglobose, sometimes pyriform, 8—14 (—17) μm diameter, thick-walled, tuberculate, verrucose or smooth, often with finger-like projections up to 5—7.5 μm long. Microconidia numerously produced and borne singly on short conidiogenous cells or almost sessile on the sides of hypha, hyaline, round to pyriform, 2—3.5 μm diameter, with walls smooth to roughened.

In vitro, cultures on tubes of BHI agar enriched with 6% blood not converted to the yeast phase. Induction of mycelial to yeast phase conversion of the strains was achieved with recovery from mouse after intraperitoneal inoculation. Yeast-like cells are hyaline, subglobose to...
Fig. 11. Yeast-like cells of *Histoplasma capsulatum*, NHL 2966-1, induced by intraperitoneal inoculation into a mouse (original magnification ×1,000).

Fig. 12. *Histoplasma capsulatum*, NHL 2966-1.
A. Conidiogenous cells and macroconidia. B. Microconidia.

Living cultures of the strains have been deposited in the culture collection at the National Institute of Hygienic Sciences, Tokyo, and Research Center for Pathogenic Fungi and Microbial Toxicooses, Chiba University, Chiba.

The identification of isolates was readily confirmed because *H. capsulatum* is characterized by large (7—15 μm), spherical aleurioconidia bearing spines or finger-like projections (1—7 μm long) over the surface13-16. *Histoplasma capsulatum var. duboisii* (Vanbreuseghem) Ciferri17, the cause of large-form African histoplasmosis, is morphologically indistinct in usual culture to *H. capsulatum var. capsulatum* and, in fact, there is little difference between these two varieties. According to Kwon-Chung’s report in 197518, isolates of *H. duboisii* produced a sexual state identical to *Emmonsia capsulata* Kwon-Chung and successfully mated with reciprocal mating types of *H. capsulatum*. These results support Ciferri’s taxonomic treatment that *H. duboisii* is regarded as a variety of *H. capsulatum*. In tissue, the yeast-like forms of *H. capsulatum var. duboisii* are characterized by large (7—15 μm), globose to ovoid cells; hence our isolates are identical to the variety *capsulatum*.

Discussion

So far, five cases2-10 of histoplasmosis have been reported in Japan. None of these five cases had any cutaneous lesions. Cutaneous manifestations of histoplasmosis are uncommon19-21. When present, they are usually associated with progressive disseminated histoplasmosis. Primary cutaneous histoplasmosis22,23, in which the fungal elements are confined to the skin, is extremely rare. The clinical features of cutaneous

ovoid or slightly wedge-shaped, 2—6 × 2—3 μm, often one end pointed, and single or budding (bipolar).

Strains examined: NHL 2966-1 and 2966-2, isolated from biopsy materials at the Toyama Prefectural Central Hospital, Japan, April and May 1986, by T. Kaji.
lesions associated with disseminated histoplasmosis are various. Goodwin et al.\textsuperscript{30} divided the cutaneous lesions into the following three groups: (1) indurated plaques that tend to develop shallow, punched-out appearing ulcers and are seen in acute disseminated disease, (2) nodules progressing to ulcers with heaped up margins, that are seen in chronic disseminated disease and are characterized histologically by a granulomatous reaction, and (3) subcutaneous, erumpent, erythema nodosum-like nodules. Soo-Hoo et al.\textsuperscript{39} reported two cases of widespread mucocutaneous histoplasmosis with papular or nodular lesions on the skin. They assumed that a primary cutaneous site was the source for dissemination to the skin and mucous membrane as there was no focus of infection in the lung or other internal organs. The following criteria to establish the diagnosis of primary cutaneous histoplasmosis are available\textsuperscript{22,23}: (1) history of traumatic inoculation with subsequent development of a chancriform lesion within three to four weeks at the site of trauma, (2) evidence that the wound was contaminated with the causative fungus, (3) development of lymphangitis and regional lymphadenopathy, (4) no history or clinical or laboratory evidence of previous pulmonary or systemic infection, and (5) conversion of the histoplasmin skin test from negative to positive. Cutaneous histoplasmosis in our case is still suspected to have originated on the skin, because the cutaneous lesion primarily developed in the right elbow area and no internal lesions were demonstrated.

In our case, although the fungus was easily cultured from the biopsy specimens, only a few fungal cells with positive PAS staining were found within some macrophages. The reason for these contradictory facts is unknown. The nature of the small round bodies stained with hematoxylin, not stained with the PAS reaction, was thought worthwhile to describe the case and the isolates obtained. Stained preparations from tissues taken from the skin lesions scarcely showed the presence of intracellular yeast cells typical of \textit{H. capsulatum} var. \textit{duboisii} than var. \textit{capsulatum}.

Our case demonstrates the first record of primary cutaneous histoplasmosis in Japan. Even though it originated from an endemic area of South America, it was thought worthwhile to describe the case and the isolates obtained. Stained preparations from tissues taken from the skin lesions scarcely showed the presence of intracellular yeast cells typical of \textit{H. capsulatum} var. \textit{capsulatum}. Moreover, the two isolates considered to be \textit{H. capsulatum} were difficult to convert to the yeast-like form \textit{in vitro}.

\textit{Emmonsella capsulata} Kwon-Chung, the teleomorph of \textit{H. capsulatum}, is heterothallic, and therefore on the basis of its sexuality, the strains of \textit{H. capsulatum} are divided into two mating types which are referred to as “plus (+)” and “minus (−)”. Kwon-Chung et al.\textsuperscript{26}, in their study on the mating behaviour of \textit{H. capsulatum}, found an equal distribution of (+) and (−) types in 1939 soil isolates, whereas in 184 isolates from clinical material tested, the frequency of (−) mating types was 7 times higher than that of (+) ones. Furthermore, in the \textit{in vitro} assays, there was a significant difference between the two mating types of this fungus in their ability to convert into yeast-like cells. It is of special interest that most (+) strains are hardly changeable to the yeast phase at 37°C on blood-glucose-cystine agar, while the strains of the (−) type readily converted. Kwon-Chung \textit{et al.}\textsuperscript{26}, in fact, observed this resistant profile on the mycelial to yeast phase conversion for all 22 (+) isolates from clinical specimens. Although there are many factors affecting their morphological conversion, the very limited occurrence of the mycelial to yeast phase conversion in our clinical isolates strongly suggest that they are sexually of the (+) type. It is a subject deserving of further study on the pairing of our isolates with the authentic strains of \textit{H. capsulatum}.

Finally, we would like to emphasize the need to take adequate cultures for laboratory identification in suspected cases of rarer mycoses in Japan, so that potential histoplasmosis introduced by travellers or immigrants from endemic areas will not be overlooked or misdiagnosed.

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References


