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Case Report

Primary localized histoplasmosis with lesions restricted to the mouth in a Chinese HIV-negative patient

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SUMMARY

Histoplasmosis is a deep mycosis caused by *Histoplasma capsulatum*, which is endemic in many areas of the world but is relatively rare in China. Although the majority of cases present as a mild to moderate flu-like disease requiring only supportive therapy, approximately 1% of patients experience more serious pulmonary and extrapulmonary disease, which can be life-threatening if diagnosis is delayed or the treatment is not initiated rapidly. Definitive diagnosis is usually made by a combination of culture, detection of the organism in tissues, measurement of antibodies, and detection of antigen. We present the case of a 51-year-old patient who presented with histoplasmosis only, with several ulcerated lesions in the oral cavity and without HIV infection, who did not show any detectable signs and symptoms of systemic disease or extra-oral manifestations. Histopathological analysis indicated a chronic inflammatory process with granulomas with yeast-like organisms. Isolation of *H. capsulatum* and molecular identification provided the definitive diagnosis. Treatment with oral itraconazole led to remission of the oral lesions. This is the first Chinese case report of localized histoplasmosis with lesions restricted to the mouth in an HIV-negative patient.

1. Introduction

Histoplasmosis, also known as Darling's disease, is a systemic fungal infection caused by *Histoplasma capsulatum*, which is a dimorphic pathogenic fungus that grows in soil and material contaminated with bat of bird droppings. There are three varieties of *H. capsulatum*: *capsulatum* and *duboisii*, which are pathogenic to humans, and *farciminosum*, which is an equine pathogen. *H. capsulatum* is endemic in certain areas of the USA, South America, Malaysia and Indonesia. Histoplasmosis is clinically classified into three forms: (1) a primary acute pulmonary form that is usually asymptomatic; (2) a chronic pulmonary form that often occurs in the presence of underlying pulmonary disease; and (3) a disseminated form, which is characterized by the progressive spread of infection to extrapulmonary sites.[1]

Disseminated histoplasmosis mainly affects the mononuclear phagocyte system, with bone marrow involvement resulting in changes in the blood picture. It often occurs in immunosuppressed patients.[1] Oral manifestations of histoplasmosis, although usually associated with the chronic disseminated form of the disease, constitute a rare event in HIV-negative patients without underlying clinical disorders. The oral lesions are frequently located on the tongue, palate, or lips.[2] Oral lesions, when present, can manifest in a variety of forms, such as ulcers and erythematous or vegetative nodules. Frequently, in cases of disseminated infection, such lesions appear to be the primary or only manifestation. Nevertheless, few cases of apparently initial mucocutaneous histoplasmosis in patients without detectable systemic involvement have been reported since 1946.[2]

We report herein a case of histoplasmosis in an HIV-negative patient with lesions exclusively in the oral cavity but without other subjacent underlying disease.

2. Case report

A 51-year-old country veterinarian from the suburb of Chongqing, a city located in southwest China, presented to the Department of Dermatology of Southwest Hospital with a two-month history of a painful oral lesion. At that time, the patient was not taking any medication and had no history of systemic disease; however he had suffered a weight loss of approximately 3 kg over the course of about two months. The patient did not report previous blood transfusions or surgeries. He did not report a history of traveling and never went out of the local district. Importantly, he related that he had had direct contact with the excreta of poultry, equines, and pigs during his clinical work and had taken inadequate precautions. A general clinical examination demonstrated a patient with normal

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skin color. He was afebrile, well-hydrated, noncyanotic, and without edema. No lymphadenopathy or hepatosplenomegaly was palpated on physical examination. The abdomen was soft and non-rigid with normal bowel sounds. Cardiac auscultation revealed a regular rate and rhythm, and normal breath sounds. Neurological examination did not reveal any abnormalities.

Examination of the oral cavity revealed two crater-like ulcers, with inflamed base and regular, elevated, well demarcated and hard borders. The right lesion was 3 cm in diameter at its widest and was located in the right soft palate, extending to the hard palate. It was friable and bled upon scraping and was mildly painful to palpation. Another regular, elliptic, ulcerated lesion with the same characteristics was observed in the left soft palate (Figure 1). Examination of the neck did not reveal lymphadenopathy. X-ray of the paranasal sinuses and chest and abdominal ultrasonography did not demonstrate alterations. Because the patient did not respond to the usual treatment for the oral ulcer, we considered the possibilities of primary chancre, squamous cell carcinoma, herpes simplex virus infection, and histoplasmosis. Antibodies to HIV and syphilis were negative. A leukopenia (2.9×10^9/l; normal $4–10 \times 10^9$/l) was detected on blood count. Levels of urea, creatinine, bilirubins, alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase were all normal. Direct sputum examination was negative in three samples for fungus and alcohol–acid-resistant bacillus. Blood cultures were also negative. Both direct smear examination and fungal culture of bone marrow were negative.

A biopsy was performed on the large lesion in the palate. The tissue fragments were sent for histopathological and microbiological examination. Histopathology revealed a granulomatous chronic inflammatory process, and a few yeast-like cells were found when stained by periodic acid-Schiff stain (Figure 2A). After

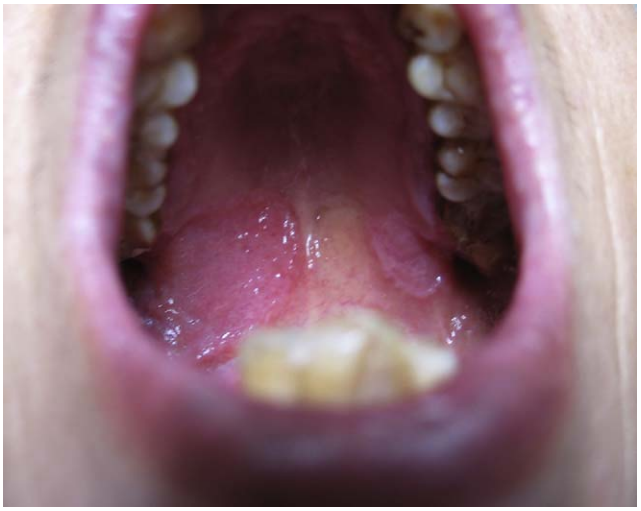

Figure 1. Clinical aspects of the oral lesions. Lesions due to *Histoplasma capsulatum* on the palate of the oral cavity, crater-like ulcers, with inflamed base and regular, elevated, well demarcated, and hard borders.

15 days of culture on potato dextrose agar at 25 °C, white filamentous colonies were observed on the slants. Microscopic examination of slide cultures on potato dextrose agar under appropriate biosafety conditions, revealed septate hyaline hyphae and globose macroconidia, the latter clearly shown under the scanning electron microscope (Figure 2B). Phase conversion of yeast and hyphae confirmed the *H. capsulatum* identity when cultured at different temperatures (Figure 2C).

As histoplasmosis is comparatively rare in China and the patient, who had no HIV infection, showed no detectable signs and

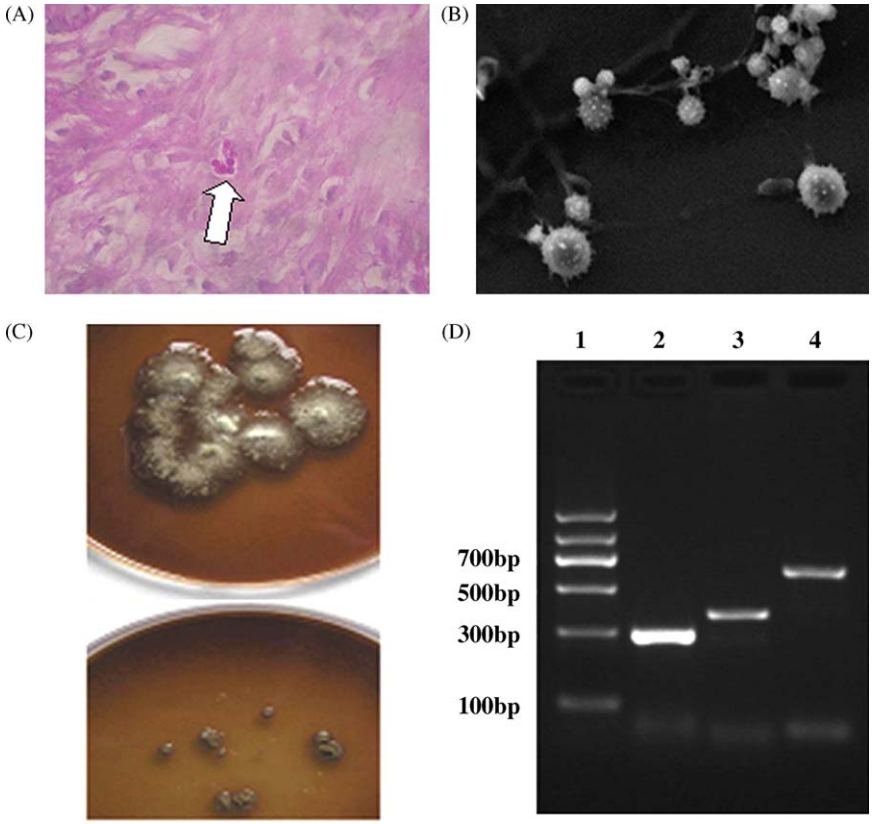

Figure 2. Experimental diagnosis. (A) Histopathology revealed a granulomatous chronic inflammatory process, and a few yeast-like cells were found when stained with periodic acid-Schiff stain (PAS × 100). (B) Scanning electron microscope image of the slide culture (×1000). (C) Upper plate, mould phase on blood plane cultured at 25 °C; lower plate, yeast phase on blood plane cultured at 37 °C. (D) Gel electrophoresis of ITS1, ITS2, D1/D2 PCR product on 1.2% agarose.

symptoms of systemic disease or extra-oral manifestations, the isolate was further evaluated by molecular analyses. DNA was extracted from a loopful of the yeast phase by proteinase K digestion, followed by chloroform–phenol extraction and isopropanol precipitation. Two segments of the nuclear rRNA gene that included the internal transcribed spacer 1 (ITS1) region and the D1/D2 region were amplified with primers (ITS1, upper primer 5′-TCCGTAGGTGAACCTGCGG-3′, down primer 5′-GCTGCGTTCTTCATCGATGC-3′; D1/D2 upper primer 5′-GCATATCAATAAGCGGAGGAAAAG-3′, down primer 5′-GGTCCGTGTTTCAAGACGG-3′), which were previously designed for amplification of pathogenic fungi[3,4] (Figure 2D). The resultant products were sequenced from both the 5′ and the 3′ ends with an automated sequencer (Invitrogen 3730XL DNA sequencer).

A 291-base pair segment was produced by amplification with primers ITS1. The sequence of our strain showed 98.3% (286/291) identity with *Ajellomyces capsulatus* var. *duboisii* and *A. capsulatus* isolate H64. By amplification with primers D1/D2, a 573-bp segment was obtained. The sequence of our strain showed 99.5% (570/573) identity with *A. capsulatus* isolate UAMH 3536.

The patient was treated with oral itraconazole 200 mg twice daily. After 60 days, the oral lesion had completely resolved. Six months after cessation of the antifungal treatment, the patient had no signs of recurrent oral lesions. There was no evidence of persistent *H. capsulatum* infection.

3. Discussion

Histoplasmosis was first described in 1906 by an American pathologist, Samuel Darling.[5] It took 30 years to prove that histoplasmosis was caused by *H. capsulatum*. *H. capsulatum* is a true dimorphic fungus that exists as a mold in the environment and as a true yeast in tissues at 35–37 °C. There are two varieties of *H. capsulatum* that are pathogenic to humans, *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*, and a third variety that is an equine pathogen, *H. capsulatum* var. *farciminosum*, which exists in Africa.[6]

Histoplasmosis is primarily a pulmonary disease, and the environmental reservoir is soil. People acquire the infection through the inhalation of conidial forms present in the environment, such as in caves with resident bats and soils inhabited by chickens. The endemic area includes the Ohio and Mississippi river valleys, Central and South America, and microfoci in the eastern USA, southern Europe, Africa, and southeastern Asia. The first case of histoplasmosis in China was reported in 1958, in a traveler returning from the USA. About 100 cases of histoplasmosis have been reported in China in the 50 years since the first patient. Most of the diseases are sporadic, primarily affecting the lungs or disseminating to other organs. Interestingly, the number of male patients is higher than that of female ones.

A recent investigation into its epidemiology indicates that the rate of *H. capsulatum* infection is high in China. In the central south region (Shaoyang city), the positive skin reaction rates were 22.4% in the normal population and 31.6% in tuberculosis (TB) patients. In the east of China (Nanjing city), the rates were 15.1% in the normal population and 17.7% in TB patients. In the southwest of China (Chengdu city), these rates were 21.8% and 54.4%, respectively. The data suggest that the rate of *H. capsulatum* infection in the southeast of China is higher than that in the northwest, and that the infection rate in patients with pulmonary TB is higher than in normal people and other pneumonopathy patients.[7]

Like most other fungal diseases, predisposing factors for histoplasmosis remain unclear. It is likely that exposure to the infectious particles leads to a positive skin test in most individuals. However, such exposure has not been recorded in all individuals with the disseminated state. There is recent experimental evidence demonstrating that susceptibility to *H. capsulatum* strongly depends on genetic predisposition.[8] The outcome of infection with *H. capsulatum* is determined by dynamic interactions between innate immunity, adaptive immunity and fungal virulence factors.[9] The microconidia formed in the mold phase of *H. capsulatum* are easily aerosolized, inhaled into the lungs, and then phagocytized by alveolar macrophages. Moreover, control of *H. capsulatum* infection is largely based on activation of cellular immunity in concert with innate responses.[10] If cell-mediated immunity is deficient because of underlying illnesses or immunosuppressive drugs, the organisms remain viable within macrophages and cause progressive infection.[11]

In all reported cases, disseminated forms are more associated with immunodeficient subjects, notably those with HIV infection. Oral histoplasmosis has frequently been reported in HIV-seropositive patients.[12] To our knowledge, this is the first reported case of oral histoplasmosis in an HIV-negative patient without detectable systemic involvement described in China.

Patients infected with *H. capsulatum* show a variety of clinical manifestations, which cause difficulties in diagnosis. The differential diagnosis of histoplasmosis with other ulcerative oral diseases is often made by a tissue biopsy or culture of *H. capsulatum*. No gender difference has been observed in the incidence of this disease, although most of the reported cases are male. Oral histoplasmosis is usually diagnosed after the discovery of lesions in the upper aero-digestive tracts in the absence of pulmonary signs. These lesions may remain the only location for a long period of time,[13] and can be misinterpreted as aphthous and/or traumatic ulcers, ulcerative necrotic gingivitis or stomatitis, other mycoses, squamous cell carcinoma, and lymphomas[2,14] The oral lesions are initially budding or wart-like and then develop into ulcerating, indurated and painful lesions.[14] We considered the case presented in this paper to have a primary localized infection of *H. capsulatum* as no systemic signs or symptoms could be detected and treated as such.[15] In fact, the patient was cured with oral antifungal agents and there was no recurrence after six months of follow-up. Although our patient lives in a nonendemic area, he reported frequent contact with the excreta of poultry, equines, and pigs during his clinical work as a veterinarian, which is the possible cause of the infection.

Amphotericin B is the choice of treatment for immunocompromised patients with disseminated or limited disease. However, treatment with itraconazole has also been reported as favorable.[15] These data, combined with other reports,[16] suggest that, with localized lesions without detection of systemic signs or symptoms, drugs with less toxicity, such as the itraconazole, should be chosen for therapy.

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Ethical approval: This study was approved by the Human Studies Committee and Ethics Committee of Southwest Hospital, Third Military Medical University.

Conflict of interest: No conflict of interest to declare.

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