

Disseminated histoplasmosis in immunocompetent patients from an arid zone in Western India: A case series



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ABSTRACT

Histoplasmosis is a systemic fungal disease caused by dimorphic fungus *Histoplasma capsulatum* and is more common in immunocompromised patients. We report two cases of disseminated histoplasmosis in immunocompetent individuals from a non-endemic zone in Western India. Rapid diagnostic tests like urinary antigen detection and molecular assays comprise the need of the hour as early initiation of antifungal therapy can be life-saving. Clinicians need to be aware of this entity to prevent misdiagnosis and initiate prompt effective management.

1. Introduction

Histoplasmosis is a systemic fungal infection caused by dimorphic fungus, *Histoplasma capsulatum* and presents most commonly as pulmonary, primary cutaneous and progressive disseminated (PDH) forms, the latter being more common in immunocompromised patients such as those with HIV infection [1]. In India, the disease is endemic in eastern part of the country [2]. PDH usually presents with fever, malaise, hepatosplenomegaly and lymphadenopathy. Other manifestations include pancytopenia, disseminated intra vascular coagulation, skin lesions, gastrointestinal manifestations like diarrhea and vomiting, encephalopathy, focal parenchymal lesions, renal failure and adrenal insufficiency [3]. We report two cases of disseminated histoplasmosis in immunocompetent individuals from an arid zone in the Western Indian state of Rajasthan. These cases merit discussion so as to create awareness among clinicians regarding this disease as disseminated histoplasmosis is rare in immunocompetent individuals.

2. Cases

Case 1. A 47 year old female from Nagaur district in Rajasthan, India, was admitted in medicine ward of All India Institute of Medical Sciences (AIIMS), Jodhpur, with complaints of high grade intermittent fever, generalized weakness and body aches for two months. She had history of non-productive cough, oral ulcers and several episodes of non-bilious vomiting for seven days. The patient was diagnosed with brucellosis at a private hospital based on IgM-positive anti-Brucella antibody serology one month back and had received treatment with

doxycycline and rifampicin. Clinical examination revealed multiple erythematous papules over the nape of neck and white plaque over right buccal mucosa. Her hematological and biochemical parameters (including renal and liver function tests) were within normal limits except for elevated erythrocyte sedimentation rate (62 mm 1st hour) and high sensitivity C-reactive protein (60.43 mg/L). Ultrasound of whole abdomen revealed mild hepatosplenomegaly. Contrast enhanced computed tomography of chest and abdomen revealed mosaic attenuation with sub-segmental and sub-centimetric mediastinal lymph nodes likely due to small airway disease and hepatomegaly. Sputum smear microscopy was negative for acid fast bacilli. Her serum sample was non-reactive for anti-HIV antibodies.

Case 2. A 59 year old male from Nagaur district in Rajasthan, India, was admitted in medicine ward of AIIMS Jodhpur, with complaints of high-grade fever, painful oral ulcers, mild headache and hypopigmented macular lesions predominantly involving the upper part of face and extensor aspect of bilateral upper limbs for two months. He had significant weight loss over past six months and developed nodular lesions over the nape of neck for two weeks. He was under follow up in Dermatology Department for evaluation of suspected Hansen's disease and referred to Medicine Department for evaluation of hypertension. Physical examination revealed enlarged submandibular lymph node measuring 1 cm × 1 cm and raised, pink, non-tender, nodular lesions on face, nape of neck, shoulders, forearm and thighs (Fig. 1). Tender indurated ulcers were seen over the left buccal mucosa and lower lip. His blood pressure on admission was 180/100 mm Hg. Systemic examination revealed no significant abnormality. Hematological parameters were within normal limits except for mildly elevated erythrocyte

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Fig. 1. Raised, pink, non-tender, nodular skin lesions of disseminated histoplasmosis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sedimentation rate (37 mm 1st hour) and high sensitivity C-reactive protein (47 mg/L). Kidney function tests revealed raised levels of serum urea (69 mg/dL) and serum creatinine (3.58 mg/dL). The albumin to globulin ratio was reversed (Total protein- 8.49 g/dL; Albumin- 3.74 g/dL; Globulin- 4.75 g/dL and alkaline phosphatase- 290 U/L). Urinalysis revealed nephrotic range proteinuria (3.42 g in 24 hours). Slit skin smear was negative for acid fast bacilli. Computed tomography (CT) scan of abdomen revealed bilateral adrenal hypertrophy, atrophic right kidney with left renal artery stenosis and infra-renal aortic thrombus. High resolution CT scan of chest showed bilateral upper lobe pulmonary infiltrates. Serum cortisol (basal and stimulated) levels were within normal range. Antinuclear antibody (ANA) test was positive.

In both cases, punch biopsies were obtained from the lesions over the nape of neck and buccal mucosa (day +2) and sent for histopathological and microbiological examination. Histopathological examination of specimens (day +5) from both the sites showed histiocytes studded with small intracellular encapsulated yeast forms with small narrow based budding. Periodic acid-Schiff (PAS) and Gomori methanamine silver (GMS) staining of the specimens showed many intracellular fungal elements. Twenty percent potassium hydroxide mount and calcofluor white staining of the specimens did not reveal any fungal elements. The specimens were inoculated in two sets of Sabouraud Dextrose agar (SDA) with and without cycloheximide and incubated at 25 °C and 37 °C. After 2 weeks (day +16) of incubation, both SDA tubes at 25 °C showed white dense cottony colonies without any pigment on the reverse (Fig. 2a). There was no growth in any of the SDA tubes at 37 °C. Lactophenol cotton blue (LPCB) mount prepared from the mould colonies revealed large, rounded, thick-walled, single-celled, tuberculate macroconidia (7–15 µm in size) formed on short, hyaline, undifferentiated conidiophores and small, round to pyriform microconidia (2–5 µm in size) borne along the sides of septate hyphae (Fig. 2b). The fungal isolates were presumptively identified as *Histoplasma capsulatum* based on characteristic microscopic morphology. For conversion of mycelial form to the yeast phase in order to demonstrate thermal dimorphism, the mycelial phase was inoculated on Brain Heart Infusion (BHI) agar with 10% sheep blood and incubated at 37 °C. Smooth, moist, brownish, yeast-like colonies appeared after 4 weeks (day +44) of incubation (Fig. 3a). Microscopically, numerous, small, round to oval budding yeast-like cells with hyphal elements representing mycelial- to yeast-phase transition forms, were observed (Fig. 3b). All these features were consistent with *Histoplasma capsulatum var. capsulatum*. The phenotypic identification was further confirmed by ribosomal DNA Internal Transcribed Spacer (rDNA ITS) sequencing performed at National Culture Collection of Pathogenic Fungi (NCCPF), Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.



Fig. 2a. Colony morphology of mycelial phase of *Histoplasma capsulatum var. capsulatum* on SDA showing white dense cottony mould without any pigment on the reverse after 2 weeks of incubation at 25 °C.

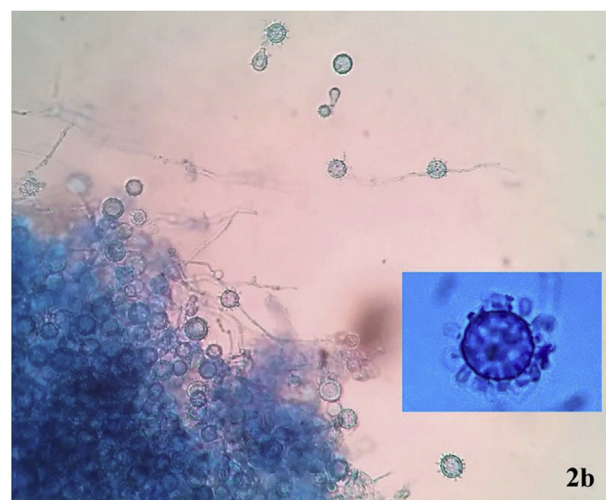


Fig. 2b. Microscopic morphology of the mycelial phase showing large, rounded, thick-walled, single-celled, tuberculate macroconidia (inset) formed on short, hyaline, undifferentiated conidiophores (x 400).

Chandigarh, India.

The histopathology reports in both the cases being suggestive of histoplasmosis, the patients were administered intravenous Amphotericin B deoxycholate (day +5) in a dose of 1mg/kg/day for 14 days followed by oral itraconazole (day +19) 200 mg three times daily for 3 days and then 200 mg twice daily. They became afebrile and the lesions over the nape of neck and buccal mucosa started resolving after 1 week of initiation of treatment (day +12). The patients were discharged with oral itraconazole 200 mg twice daily (day +22) with a plan to continue for 12 months. The second patient being a case of renovascular hypertension was started on anticoagulants, antiplatelet drugs and statins, in addition. Subsequent follow-up visits revealed marked clinical improvement. Kidney and liver functions were monitored throughout the course of treatment with antifungals and were found to be within normal limits.



Fig. 3a. Colony morphology of yeast phase of *Histoplasma capsulatum* var. *capsulatum* on BHI agar with 10% sheep blood showing smooth, moist, brownish, yeast-like colonies after 4 weeks of incubation at 37 °C.

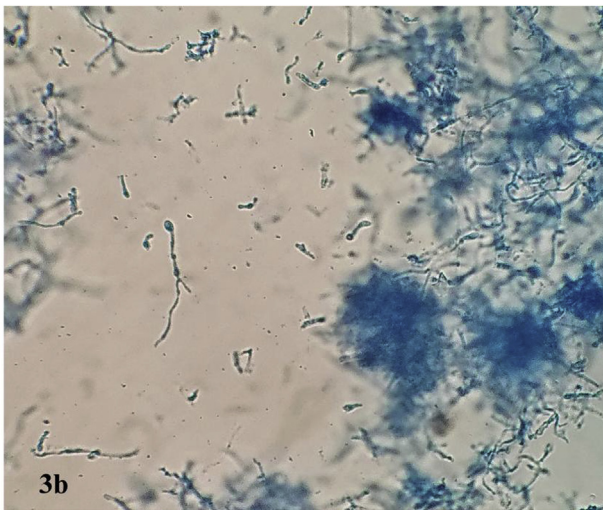


Fig. 3b. Microscopic morphology of the yeast phase showing small, round to oval budding yeast-like cells with hyphal elements representing mycelial- to yeast-phase transition forms (x 400).

3. Discussion

We report two cases of disseminated histoplasmosis in apparently immunocompetent individuals from an arid zone in Western Rajasthan, India. Both the patients showed oral involvement in the form of ulcers over the buccal mucosa which was unique as disseminated histoplasmosis with oral involvement has rarely been reported in immunocompetent individuals.

Histoplasmosis or Darling's disease is a systemic fungal infection caused by thermally dimorphic fungus, *Histoplasma capsulatum* which exists as mycelial form in warm and humid environment contaminated by avian excreta or bat guano and as yeast form in tissues. It was first

described by Samuel Taylor Darling in 1906 in the viscera and bone marrow of a patient suspected to have died from tuberculosis [4]. The first case of histoplasmosis in India was reported by Panja and Sen from Kolkata in 1954 [5]. Histoplasmosis in humans has two clinical entities: Histoplasmosis capsulati caused by *H. capsulatum* var. *capsulatum*, endemic in Eastern United States and Latin America and Histoplasmosis duboisii caused by *H. capsulatum* var. *duboisii*, prevalent in Africa and South East Asia [2].

In India, the disease is endemic in eastern part of the country with most cases reported from gangetic West Bengal [2]. In this case series, both patients hailed from Nagaur district of Western Rajasthan, India and have not traveled outside the district before their illness started. Nagaur has its three-fourth area covered with Thar Desert and lacks vegetation. It has hot summer and in general is dry except during the monsoon season from July to September when 80% of the yearly rainfall is received. Both the patients became symptomatic during February–April when the average temperature of the district ranged between 18 °C and 30 °C with average relative humidity of 34% and average precipitation of 4 mm. Such arid conditions are unfavorable for acquiring histoplasmosis as the fungus requires temperate moist climate with relative humidity of 67–97% for optimum growth [6]. This highlights the need for further studies to know the epidemiology and pathogenesis of the disease in non-endemic areas.

Infection is acquired by inhalation of infectious microconidia during activities like cleaning of chicken coops, visiting bat-infested caves, excavation, demolition of old buildings, and cutting of dead trees which lead to disruption of soil containing the organism and aerosolization of microconidia [2]. There could be three major clinical presentations of histoplasmosis: pulmonary, progressive disseminated (PDH), and primary cutaneous [7]. The extent of disease is determined by the immune status of the host. In immunocompetent hosts, majority (50–90%) of the infections result in self-limiting flu-like illness [2]. PDH develops in approximately 10% of the patients and may manifest as chronic disease in immunocompetent hosts or acute progressive disease in immunocompromised individuals who are unable to mount an effective cell-mediated immune response against the organism [3]. Moreover, immunocompetent individuals may have endogenous reactivation of the disease which may present at a later stage of life, as observed in tuberculosis [8]. Oral involvement in the form of chronic non-healing ulcer or mass lesion of the buccal mucosa, tongue, and palate are seen in 25%–40% of patients with PDH [9]. Oral manifestation of histoplasmosis, though usually associated with chronic disseminated form of the disease, constitutes a rare entity in immunocompetent individuals. The present case series is unique as none of the patients had underlying immunodeficiency.

Though mucocutaneous histoplasmosis is more common in immunocompromised patients, higher mucocutaneous involvement has also been observed in immunocompetent individuals, particularly from Asia [10]. Kathuria et al., in a large case series involving 61 immunocompetent patients with histoplasmosis from India, reported mucocutaneous involvement to be 36% [11]. Skin lesions are not specific and are characterized by their polymorphism, ranging from papules, plaques, pustules, and nodules to lesions resembling molluscum contagiosum, acneiform eruptions, erythematous papules and keratotic plaques. The most commonly affected areas are the face, trunk and extremities [12]. In some cases, the skin lesions may mimic erythema nodosum as was observed in our second case.

PDH is a disease of the reticuloendothelial system and can involve the liver, spleen, lymph nodes, bone marrow and adrenal glands. Adrenal involvement is particularly common in immunocompetent patients. Bilateral symmetrical adrenomegaly, central hypodensity with peripheral enhancement and presence of calcification have been described on CT scan [13]. Kathuria et al. [11] reported that adrenal glands were the most common organs involved in PDH followed by liver, skin and mucosa, spleen, and lymph nodes and that the adrenals were enlarged in all the patients with adrenal involvement. Our second

case had adrenal involvement in the form of bilateral adrenal enlargement. Such cases need to be followed carefully as they may progress to adrenal insufficiency, a life-threatening condition. Histoplasmosis and other systemic fungal infections should be considered in the differential diagnosis of unilateral or bilateral adrenal masses detected on imaging [13].

Direct microscopy of wet mounts or stained tissue sections of clinical specimens has very low sensitivity [2]. In our case, biopsy tissue specimens obtained from both the patients were negative for fungal elements by direct microscopy. Histopathology remains an important diagnostic modality despite low sensitivity as a positive result permits initiation of specific antifungal therapy. Other pathogens that should be considered while making the histopathologic diagnosis of histoplasmosis include *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Candida glabrata*, *Pneumocystis jirovecii*, *Coccidioides* spp., *Talaromyces marneffei*, *Leishmania* spp., *Trypanosoma cruzi*, and *Toxoplasma gondii* [14]. The use of specific histochemical stains like GMS and PAS facilitates the differentiation of these pathogens.

Culture remains the gold standard for the diagnosis of histoplasmosis because it allows the isolation and characterization of the fungus; however it requires prolonged incubation, may take up to 8 weeks and there are significant biosafety concerns. Mould colonies showing tuberculate macroconidia in LPCB mount though highly suggestive of *H. capsulatum*, other fungi, including *Sepedonium* species, can also produce such structures. Demonstration of thermal dimorphism plays a key role in phenotypic confirmation of such cultures but the rate of mould to yeast conversion is low and therefore impractical as a diagnostic tool. However, in both the cases, we have been successful in demonstrating thermal dimorphism in 4 weeks. Furthermore, the sensitivity of cultures depends on the clinical type, host's immune status, and the burden of disease. Patients with disseminated histoplasmosis have higher culture positivity (74%) than patients with acute pulmonary histoplasmosis (42%) [14]. Therefore, more specific and reliable tests are needed for definitive diagnosis of histoplasmosis. Urinary antigen detection is rapid, non-invasive, highly sensitive and a useful marker of treatment response. Even low positive (quantitative) and false positive test results have clinical significance because of cross-reactivity with antigens of other fungi causing endemic mycoses [15]. Moreover, this test is cost effective and can be performed in routine laboratories in resource poor countries. Molecular methods offer the advantage of high analytical specificity and shorter turnaround times compared to other diagnostics. Currently, there are no FDA-approved molecular tests to detect *H. capsulatum* directly from clinical specimens [14]. In our case, the identities of both the isolates were confirmed as *Histoplasma capsulatum* var. *capsulatum* by rDNA ITS sequencing performed at NCCPF, PGIMER, Chandigarh, India.

Treatment of disseminated histoplasmosis includes an induction phase to achieve clinical remission and a maintenance phase to prevent relapse. Amphotericin B is the antifungal agent of choice for induction therapy of moderate to severe cases. Itraconazole is the preferred oral azole in patients who can receive oral medication. Maintenance therapy with itraconazole (200 mg, once or twice daily) is given for at least 12 months, may be lifelong [12]. In our case, both the patients responded well to treatment as evidenced by defervescence, disappearance of skin lesions and marked clinical improvement on subsequent follow-up visits.

To conclude, disseminated histoplasmosis poses a diagnostic

challenge due to its highly protean clinical manifestations, more so in immunocompetent individuals. These cases highlight the importance of high index of clinical suspicion regarding a differential diagnosis of histoplasmosis to prevent misdiagnosis and initiate early antifungal therapy. Over the past few years, cases of disseminated histoplasmosis have been increasingly reported worldwide. This is attributable to increased awareness among clinicians and advancement in diagnostic facilities. With patients having no history of travel to endemic areas of histoplasmosis, it is likely that the disease is endemic in the Indian subcontinent. However, supportive evidence like demonstration of natural reservoirs of the pathogen and the occurrence of the disease in local animal populations remain largely obscured. Systematic and comprehensive studies are required in this field to know the exact burden of histoplasmosis in India.

Conflict of interest

There are none.

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