Case Report: Histoplasmosis Accompanying Disseminated Tuberculosis in an Immunocompetent Adolescent Boy

Ashis Kumar Choudhury,¹ Ankit Kumar Mishra,¹ Deepak Kumar Gautam,¹ Ragini Tilak,² Vijai Tilak,³ Indrajeet Singh Gambhir,⁴ and Sankha Shubhra Chakrabarti^{4*}

¹Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; ²Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; ³Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; ⁴Department of Geriatric Medicine (Previously Department of General Medicine), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Abstract. The incidence of tuberculosis in India is quite high. In such a situation, empirical antitubercular therapy (ATT) is often resorted to, when some of the investigation findings are clearly diagnostic of tuberculosis. This may mean missing out on coinfections. Whereas this is particularly true for immunosuppressed patients, rarely even immunocompetent patients may present with such diagnostic dilemmas. We present the case of an adolescent boy who had been previously asymptomatic and who presented with fever with lymphadenopathy, splenomegaly, and pancytopenia. Initially, ATT was administered based on the detection of acid-fast bacteria in lymph node, caseating granulomas with Langhans giant cells, and a positive cartridge-based nucleic acid amplification test specific for *Mycobacterium tuberculosis*. However, when the patient failed to respond fully to the treatment, additional investigation in the form of bone marrow fungal culture led to the diagnosis of histoplasmosis.

INTRODUCTION

The incidence of tuberculosis in India is around 204 per 100,000 population per year.¹ Diagnostically proven cases of tuberculosis with typical symptoms are seldom explored for coexistence of other infections, especially in an immunocompetent host. We present the case of an adolescent boy who presented with a fever with lymphadenopathy, splenomegaly, and pancytopenia. Although initially diagnosed as tuberculosis, persistence of fever despite antitubercular therapy (ATT) led us to further investigate the patient, which resulted in the detection of coexistent histoplasmosis.

CASE REPORT

A 15-year-old boy from Azamgarh city in the eastern part of Uttar Pradesh state of northern India presented with a high-grade fever (up to 103.5°F) for one and a half months. He also complained of easy fatigability and exertional breathlessness on less than normal exertion, but no cough. On examination, a single firm and freely mobile, non-tender right lower jugular lymph node of size 1 × 1 cm was palpable. The spleen was also palpable 2 cm below the left costal margin.

At presentation, he had a hemoglobin level of 9.2 g/dL, a mean cell volume of 101 fL, a total leukocyte count of 1,590/μL (55% neutrophils and 39% lymphocytes), and a manual platelet count of 67,000/μL. The peripheral blood smear microscopy revealed marked leucopenia and moderate thrombocytopenia with no blast cells, toxic granules, or malarial parasites, and his corrected reticulocyte count was 1.02. No abnormalities were observed in the morphology of white blood cells on microscopy. Further diagnostic workup for febrile illnesses with pancytopenia was performed. HIV serology was negative. Ultrasonography of the abdomen revealed mild hepatomegaly and moderate splenomegaly. An ostium secundum type of

atrial septal defect was evident on transthoracic echocardiography but without any evidence of vegetations. Four blood cultures performed were sterile. Histological examination of bone marrow biopsy specimen revealed numerous caseating granulomas in the intra-trabecular spaces (Figure 1). Histopathology of the biopsied enlarged cervical lymph node showed extensive caseation necrosis with epithelioid histiocytes with Langhans giant cells. Ziehl–Neelsen staining of the lymph nodal tissue was performed and was positive for acid-fast bacilli. Three samples from the patient were sent for cartridge-based nucleic acid amplification test (CBNAAT)—bone marrow aspirate, lymph node biopsy specimen, and pus from lymph node. The CBNAAT is highly sensitive and specific for *Mycobacterium tuberculosis* (MTB).^{2,3} Whereas the first two samples were negative for MTB, the pus sample was positive for MTB which was sensitive to rifampicin. *Mycobacterium tuberculosis* culture was performed using liquid culture system only for bone marrow sample, which was negative. A diagnosis of disseminated

FIGURE 1. Histopathology of bone marrow biopsy specimen (Hematoxylin & Eosin stain, ×40 magnification) showing caseating granuloma with arrow showing Langhans-type giant cell. This figure appears in color at www.ajtmh.org.

* Address correspondence to Sankha Shubhra Chakrabarti, Department of Geriatric Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India. E-mail: sankha.geriatrics@gmail.com

tuberculosis was made as per operational guidelines of the Indian Tuberculosis Control Programme, and ATT was started with standard four-drug regimen of rifampicin, isoniazid, pyrazinamide, and ethambutol.⁴ Vitamin B12 deficiency was ascribed to the patient's almost entirely vegetarian food habit. Injectable vitamin B12 was started for macrocytic anemia. The pancytopenia responded within 1 week, and the patient was discharged on ATT and oral vitamin B12.

After 2 months of follow-up, the boy again presented with a fever (measured up to 102°F) with evening rise of temperature and significant loss of weight. Multidrug-resistant tuberculosis was suspected. A repeat bone marrow aspiration was performed, and the aspirate was subjected to CBNAAT again. *Mycobacterium tuberculosis* culture, fungal staining, and fungal culture were also performed. Tests for MTB were all negative, presumably because the patient had received 2 months of ATT. Fungal staining of bone marrow aspirate revealed few budding yeast forms. Microscopy of the bone marrow fungal culture revealed spiked spherical conidia suggestive of *Histoplasma capsulatum* (Figure 2). The mold phase was successfully cultured in the brain heart infusion agar with blood, to isolate the yeast phase of the fungus. The patient was administered injectable lipid amphotericin B 5 mg/kg body weight per day for 2 weeks. The fever responded in 1 week along with dramatic improvement in the general condition of the patient. The patient was discharged on oral itraconazole 200 mg twice daily and ATT. The patient has been on follow-up for more than 14 months. He has been free from any illness during this period and has gained weight.

DISCUSSION

The incidence of histoplasmosis in India is underappreciated. Reports of *H. capsulatum* being isolated from the soil of the Gangetic plain exist since 1975.⁵ Many cases have been reported from the eastern part of India such as the state of

West Bengal, traversed by the river Ganges.⁶ Incidentally, the patient in the current case belonged to Azamgarh in the eastern part of Uttar Pradesh in northern India, which also lies on the Ganges plain. Histoplasmosis is of late being reported as an endemic infection in developing Asian countries such as China, and hence endemicity of this fungus in India is a strong possibility.⁷ It has been suggested that histoplasmosis in India may not be rare but instead just underdiagnosed.⁸ The predominant presentation of histoplasmosis in the Indian subcontinent is as progressive disseminated histoplasmosis.^{8,9} Most patients present with fever, weight loss, oral ulcerations, hepatosplenomegaly, and lymphadenopathy. Adrenal insufficiency may occur in some.^{8,9} The clinical similarity of tuberculosis and histoplasmosis is probably the strongest reason for underdiagnosis of the latter. In previous retrospective case series, it was noticed that often patients had been empirically started on ATT and the suspicion of non-tubercular infection arose only after failure to respond.⁹ This is true for most low- and middle-income countries. Misdiagnosis as tuberculosis and late suspicion of histoplasmosis have been reported from Cameroon, where awareness and facilities for fungal detection are quite low.¹⁰ A study from northern Tanzania reported seven of 870 febrile inpatients to be positive for *Histoplasma* by urine antigen testing. These included both persons living with HIV and immunocompetent individuals. Poor physician awareness and failure to make a definitive diagnosis because of unavailability of resources were also highlighted.¹¹ Even in geographical areas hyper-endemic for histoplasmosis such as Guatemala, diagnosis is often compromised, despite histoplasmosis and tuberculosis being the most important AIDS-defining illnesses.¹² It is quite natural that in zones endemic for tuberculosis, histoplasmosis either mimicking the features of the former or coexisting with it may be a missed diagnosis, especially in apparently immunocompetent individuals. Apart from diagnostic dilemma, coinfection also affects therapeutic strategy. Rifampicin is an

FIGURE 2. Lactophenol cotton blue mount from bone marrow culture with thick black arrows showing tuberculate macroconidia indicative of *Histoplasma capsulatum* ($\times 40$ magnification). This figure appears in color at www.ajtmh.org.

TABLE 1
Lymphocyte subtypes in patient

Cell	Count	Reference range
Absolute lymphocyte count	1,375/ μ L	660–4,600/ μ L
B lymphocytes (CD19 ⁺)	23%	6–23%
T lymphocytes (CD3 ⁺)	68%	56–84%
CD4 ⁺ T cells	40%	31–52%
CD8 ⁺ T cells	25%	18–35%
CD4 to CD8 ratio	1.62	≥ 1
Natural killer cells (CD3 ⁻ CD16 ⁺ CD56 ⁺)	8%	3–22%

All percentage values are expressed as percentage of total lymphocytes. Reference range is provided by laboratory. All measured values are also within normal limits of adolescent lymphocyte estimations provided by Valiathan et al., 2014.

inducer of itraconazole metabolism through CYP3A4 activation and results in decreased therapeutic concentrations. Treating physicians need to watch out for signs of therapeutic failure. Furthermore, serum itraconazole concentration needs to be monitored in setups where such facilities exist.¹³

Coexistence of histoplasmosis and tuberculosis is common in people living with HIV and patients on biological therapy.^{14,15} In the current case, the patient tested negative for HIV, had not received any immunosuppressant medications, and had never had a major illness or frequent minor infections till the current hospitalization. Even on follow-up over 14 months, he stayed asymptomatic. Microscopic evaluation of his blood smear for abnormalities in neutrophil morphology was unremarkable at initial hospitalization. Additional testing for possibly undetected immune dysfunction was performed at 14 months follow-up. Table 1 shows his lymphocyte counts.¹⁶ His total serum immunoglobulin level (IgG + IgA + IgM) was 858 mg/dL (cutoff for immunodeficiency states < 600 mg/dL), with a normal distribution of individual subtypes.^{17,18} Thus, the patient could be considered immunocompetent, with reasonable certainty.¹⁹ It is possible that disseminated tuberculosis itself is a risk factor for histoplasmosis. Disseminated tuberculosis is a common entity in India, even among immunocompetent individuals, and it may have created a state of temporary immunocompromise, which predisposed the patient to develop histoplasma infection.

The current case highlights that rarely even an apparently immunocompetent patient may have tuberculosis coexisting with histoplasmosis, making it easy to miss the rarer diagnosis. The utility of bone marrow examination in patients with HIV who have pyrexia of unknown origin has been emphasized.²⁰ This may hold true even in immunocompetent individuals and should be backed up by fungal culture facilities. Although not possible at the periphery, a fungal culture of easily accessible biological samples such as sputum or tissue aspirate may be routinely performed, at least in referral medical centers, so that the few cases are not missed.

Received June 2, 2019. Accepted for publication October 29, 2019.

Published online November 25, 2019.

Acknowledgment: We thank Dr. Lal PathLabs, New Delhi, for providing complimentary laboratory service for immunodeficiency panel. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

Authors' addresses: Ashis Kumar Choudhury, Ankit Kumar Mishra, and Deepak Kumar Gautam, Department of General Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, E-mails: ashischoudhury91@gmail.com, ankitm964@gmail.com, and dgautam@bhu.ac.in. Ragini Tilak, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar

Pradesh, India, E-mail: raginijain29@rediffmail.com. Vijai Tilak, Department of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, E-mail: vijaitilak@rediffmail.com. Indrajeet Singh Gambhir and Sankha Shubhra Chakrabarti, Department of Geriatric Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, E-mails: iac2k10@gmail.com and sankha.geriatics@gmail.com.

REFERENCES

1. World Health Organization, 2018. *WHO Global Tuberculosis Report*. Available at: https://www.who.int/tb/publications/global_report/en/. Accessed September 11, 2019.
2. World Health Organization, 2014. *Xpert MTB/RIF Implementation Manual, Technical and Operational 'How To' Practical Consideration*. Available at: http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf?ua=1. Accessed September 11, 2019.
3. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, Sharma R, Singh BK, 2015. Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. *PLoS One* 10: e0141011.
4. Central Tuberculosis Division, Government of India, 2016. *Technical and Operational Guidelines for TB Control in India*. Chapter 3: Case Finding & Diagnosis Strategy. Available at: <https://tbcindia.gov.in/showfile.php?lid=3216>. Accessed September 11, 2019.
5. Sanyal M, Thammayya A, 1975. *Histoplasma capsulatum* in the soil of Gangetic Plain in India. *Indian J Med Res* 63: 1020–1028.
6. Goswami RP, Pramanik N, Banerjee D, Raza MM, Guha SK, Maiti PK, 1999. Histoplasmosis in eastern India: the tip of the iceberg? *Trans R Soc Trop Med Hyg* 93: 540–542.
7. Pan B, Chen M, Pan W, Liao W, 2013. Histoplasmosis: a new endemic fungal infection in China? Review and analysis of cases. *Mycoses* 56: 212–221.
8. Gopalakrishnan R, Senthur Nambi P, Ramasubramanian V, Abdul Ghafur K, Parameswaran A, 2012. Histoplasmosis in India: truly uncommon or uncommonly recognized? *J Assoc Phys India* 60: 25–28.
9. Subramanian S, Abraham OC, Rupali P, Zachariah A, Matthews MS, Mathai D, 2005. Disseminated histoplasmosis. *J Assoc Phys India* 53: 185–189.
10. Mandengue CE, Denning DW, 2018. The burden of serious fungal infections in Cameroon. *J Fungi (Basel)* 4: E44.
11. Lofgren SM et al., 2012. Histoplasmosis among hospitalized febrile patients in northern Tanzania. *Trans R Soc Trop Med Hyg* 106: 504–507.
12. Medina N, Samayoa B, Lau-Bonilla D, Denning DW, Herrera R, Mercado D, Guzmán B, Pérez JC, Arathoon E, 2017. Burden of serious fungal infections in Guatemala. *Eur J Clin Microbiol Infect Dis* 36: 965–969.
13. Drayton J, Dickinson G, Rinaldi MG, 1994. Coadministration of rifampin and itraconazole leads to undetectable levels of serum itraconazole. *Clin Infect Dis* 18: 266.
14. Agudelo CA, Restrepo CA, Molina DA, Tobón AM, Kauffman CA, Murillo C, Restrepo A, 2012. Tuberculosis and histoplasmosis coinfection in AIDS patients. *Am J Trop Med Hyg* 87: 1094–1098.
15. Munoz-Oca JE, Villarreal Morales ML, Rodriguez AN, Martinez-Bonilla L, 2017. Concomitant disseminated histoplasmosis and disseminated tuberculosis after tumor necrosis factor inhibitor treatment: a case report. *BMC Infect Dis* 17: 70.
16. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D, 2014. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of south Florida. *Immunobiology* 219: 487–496.
17. Sorensen RU, Moore C, 2000. Antibody deficiency syndromes. *Pediatr Clin North Am* 47: 1225–1252.
18. Agarwal S, Cunningham-Rundles C, 2007. Assessment and clinical interpretation of reduced IgG values. *Ann Allergy Asthma Immunol* 99: 281–283.
19. Bousfiha A et al., 2018. The 2017 IUIS phenotypic classification for primary immunodeficiencies. *J Clin Immunol* 38: 129–143.
20. Quesada AE, Tholpady A, Wanger A, Nguyen AN, Chen L, 2015. Utility of bone marrow examination for workup of fever of unknown origin in patients with HIV/AIDS. *J Clin Pathol* 68: 241–245.