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Case Report

Disseminated Emergomycosis in an HIV and HBV Co-Infected Immunocompromised Patient: A Case Report and Review of Literature

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Abstract

The differential diagnoses in patients with advanced Human Immunodeficiency Virus (HIV)/ acquired immunodeficiency syndrome (AIDS) infection presenting with fever and systemic illness are wide and warrant both infectious and non-infectious considerations. Early and accurate diagnosis is essential to provide appropriate therapy and to improve outcome. We describe a case of disseminated *Emergomyces pasteurianus* (*E. pasteurianus*) in an HIV and HBV co- infected patient. The patient presented with fever, cough, weight loss, and multiple scattered non-tender skin lesions over the face and body for six weeks. He was a newly diagnosed case of HIV on anti-retroviral therapy. He was referred with a clinical suspicion of tuberculosis. On admission, he was diagnosed with chronic HBV infection. The high-resolution chest computed tomography (CT) revealed consolidations and mediastinal lymphadenopathy. Disseminated emergomycosis was diagnosed on fungal culture of skin biopsy and needle aspirate of lung consolidation. Isolate was identified as *E. pasteurianus* by sequencing the internal transcribed spacer region of ribosomal DNA. Amphotericin B and itraconazole therapy helped in resolution of lesions and patient recovery.

Introduction

Recently, the global emergence of emergomycosis, a systemic fungal infection caused by a novel dimorphic fungus *Emergomyces* species has been observed among imunocompromised individuals [1]. Though initially classified under the genus Emmonsia, a taxonomic revision in 2017 based on DNA sequence analyses placed five Emmonsia-like fungi under a separate genus *Emergomyces*. A taxonomic revision in 2020 added two more fungi under the genus *Emergomyces* [1-2]. A novel, non-adiaspore producing dimorphic fungus, *Emergomyces pasteurianus* (*E. pasteurianus*) was first reported from an Italian patient in 1998 [3]. Subsequently, more cases were reported due to similar fungi from other parts of world [4]. We report a case of disseminated emergomycosis by *E. pasteurianus* in an human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infected patient. To best of our knowledge, the present case is the third case of emergomycosis from India in HIV infected patient. The fungal isolate was identified by culture and genomic sequencing of internal transcribed spacer (ITS) region of ribosomal DNA.

Case Report

A 38-years-old man presented with six weeks history of low-grade intermittent fever and cough with generalised weakness and weight loss (Day 01). He also complained of multiple scattered non-tender skin nodules. He was diagnosed case of HIV, with recent CD4 counts of 110 cells/mm^3. He was on anti-retroviral therapy (tenofovir, emtricitabine and lamivudine) with poor compliance. He was referred with clinical diagnosis of tuberculosis (TB). He was afebrile, dyspnoeic at rest, and had bilateral consolidation with crepitations. Rest systemic examination was normal. Skin nodules were 1-2 cm in diameter, firm, non -tender, non-discharging with central ulceration on face, right shoulder, right chest wall and back.

Laboratory investigations (Day 02) revealed CD4 count of 98 cells/mm^3. C- Reactive protein (CRP) was raised to 48.9 mg/dl. Renal function test and urine routine examination were within normal limits. Liver enzymes abnormalities led to suspicion of viral hepatitis. Chronic HBV infection was diagnosed with serology (Abbott Architect, USA) and HBV DNA PCR (Roche Diagnostics GmbH, Mannheim, Germany). He was HBeAg-positive with HBV DNA viral load of 2000 IU/ml. Sputum sample was negative for acid fast bacilli on Ziehl Neelsen staining. Xpert® MTB/RIF Ultra (Cepheid Germany) and MTB/NTM real time PCR (3B BlackBio, India) were negative. The high-resolution chest computed tomography (CT) revealed bilateral peripheral upper lobes consolidations, lobular enhancing masses with a necrotic centre and peripheral consolidation in the left lower lobe and few nodules in the right lung with mediastinal lymphadenopathy. The CT-guided fine needle aspiration from lung consolidation and skin nodules biopsy were subjected to direct microscopy, histopathology and aerobic, anaerobic bacterial and fungal cultures. Aerobic, anaerobic bacterial cultures were sterile (Day 04, Day 07). Giemsa stain of both the lung and skin aspirate revealed intracellular budding yeast cells. Histopathology of skin biopsies demonstrated similar intracellular budding yeast cells (Day 08). Patient was started on intravenous amphotericin B deoxycholate (50 mg/day) and methyl prednisolone (1 mg/kg) for 2 weeks (Day 09).

Growth was observed on Sabouraud's dextrose agar (SDA) plates incubated at 25°C, from both skin nodule biopsy (Day 18) and lung needle aspirate (Day 20). Colonies were white velvety, slightly raised and furrowed with ochraceous-buff to warm buff reverse (Fig. 1a, 1b). The slide culture of the growth showed thin (1–2 um diameter) hyaline septate hyphae, perpendicular slender conidiophores with terminal conidia or cluster of 1–4 conidia in a floret arrangement. Occasional intercalary chlamydoconidia were noticed (Fig. 1c). The grown fungus could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) (bioMérieux, France) (Day 22). The isolate was therefore sent for genomic sequencing. Molecular identification of the isolate was done by panfungal DNA polymerase chain reaction (PCR) and sequencing of amplified products. The PCR sequencing targets multicopy genes, the ribosomal DNA (rDNA), genes (18S, 28S, and 5.8S), and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2). DNA was extracted. The amplified and purified product was sequenced on 3500 DX Analyzer (Thermo Fisher Scientific, Massachusetts, USA). The sequences were then run through GenBank Basic Local Alignment Search Tool (BLAST) searches (http://www. ncbi.nlm. nih.gov/BLAST/Blast.cgi) for species identification [5]. BLAST searches confirmed the isolate as *E. pasteurianus* with 99.2% identification (GenBank accession no. KP260922) of ITS1, ITS2, 5.8S gene, and rRNA gene (Day 25).

The isolate from SDA plates was sub-cultured on brain heart infusion (BHI) agar plate containing 5% sheep blood and incubated at 350C to obtain a yeast form of a fungus. Yellowish-white to tan, pasty, cerebriform colonies appeared after 03 weeks of incubation (Fig. 1d) (Day 46). Gram-stained smear prepared from culture reveals small, oval yeast cells with narrow based budding. Antifungal susceptibility test (AFST) was done by the CLSI (M27-A3) recommended broth micro-dilution (BMD) method for azoles (fluconazole, voriconazole, posaconazole and itraconazole), amphotericin B, and echinocandins (caspofungin, micafungin and anidulafungin) on yeast phase of an isolate. Breakpoints observed were fluconazole (02 µg/mL), voriconazole (0.25µg/mL), posaconazole (0.125µg/mL), itraconazole (0.015µg/mL), amphotericin B (0.06 µg/mL), caspofungin (01 µg/mL), micafungin (02 µg/mL) and anidulafungin (02 µg/mL) (Day 53).

Figure 1A: SDA, Obverse Showing White to Tan, Slightly Raised, and Furrowed Colony

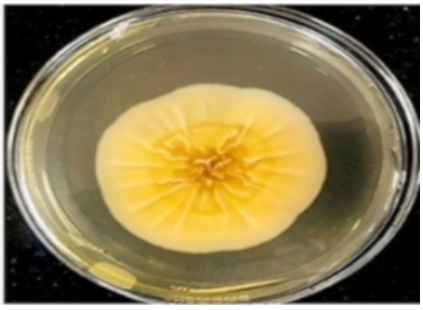

Figure 1B: SDA, Ochraceous-Buff to Warm Buff Reverse

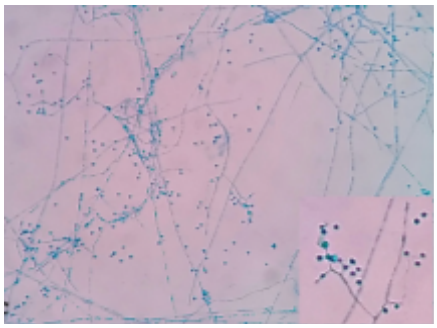

Figure 1C: Microscopic Morphology in Lactophenol Cotton Blue Preparation Showing Florets of Conidia Borne on Slender Conidiophores

Figure 1D: BHI Agar, Showing Yeast-Like, Pasty, Cerebriform Yellowish-White to Tan Colonies

Treatment and Clinical Course

Upon completion of 02 weeks of amphotericin B therapy, patient's lung opacities improved and skin lesions started healing (Day 22). ART with tenofovir, emtricitabine and efavirenz to manage his HIV-HBV co-infection was initiated (Day 23). He was discharged (Day 30) with antiviral therapy and oral itraconazole (400 mg). At 01-month follow-up, improvement was noticed in skin lesions, which further gradually improved. Basis AFST result and patient's clinical response, itraconazole was continued. At 03 months follow-up visit, almost 50% clearance of pulmonary consolidation was noted and by the end of 12 months, skin and lung lesions were completely cleared. Antifungal therapy was discontinued after 12 months, and he continued with antiviral drugs only.

Discussion

Based on molecular phylogenetic analyses, currently seven species are placed in the genus *Emergomyces* namely *E. pasteurianus*, *E. africanus, E. canadensis, E. orientalis, E. europaeus, E. crescens* and *E. sola*. Except for *E. sola*, the other pathogenic *Emergomyces* species are known to cause invasive diseases in human [1,2]. Till date, globally, total 79 proven cases of emergomycosis either with culture or with direct detection using molecular methods are reported in literature (Table 1) [4,7-32]. A highest case burden has been reported from Africa (57), followed by Asia (07), North America (07), Europe (06), and South America (02) as mentioned in Table 1. However, considering the increasing prevalence of HIV/AIDS, it is safe to presume a global distribution of emergomycosis with many cases going undetected [1]. *E. africanus* (53) was predominantly reported followed by *E. pasteurianus* (13), *E. canadensis* (05), *E. crescens* (02), *E. orientalis* (02), and *E. europaeus* (01). The *Emergomyces* species identification was not available in three cases. (Table 1) Majority of *Emergomyces* infections were reported in immunocompromised patients. The associated risk factors were HIV infection, transplant recipients, malignancies, renal diseases and diabetes mellitus. However, occasionally, infections were reported in apparently healthy immunocompetent hosts as well. Most common manifestations were cutaneous lesions/nodules and pulmonary consolidations. However, sepsis, ataxia, severe disseminated disease was also reported. (Table 1) Little is known regarding the virulence factors of this group of dimorphic fungi and the pathogenesis of emergomycosis. Infection with *Emergomyces spp*. is presumed to occur through inhalation of conidia present in soil, followed by *in vivo* transformation to a yeast-like phase that is capable of extrapulmonary dissemination in susceptible hosts [1]. In the present case, patient was immunocompromised and there is a possibility of incidental inhalation of conidia from contaminated soil. However, it is contingent.

Table 1: Characteristics of Previously Published Cases of Infections with *Emergomyces Spp*

Ref No. in Text	References	No. of Cases	Year	Age / Sex	Country	Clinical Presentation	Risk Factor	Diagnosis Method	Species	Antifungal Drugs	Outcome
3	Gori S, et al.	1	1998	40/F	Italy	Skin Ulcerations	HIV/AIDS	NA	*E. pasteurianus*	Amphotericin B	Died, Unrelated cause
8	Wellinghausen N, et al	1	2003	64/M	Germany	Diffuse Pulmonary	RA	Culture + Molecular	*E. europeus*	Itraconazole	Chronic Relapsing
9	Dot JM, et al	1	2009	30/M	France	Pulmonary	None	Molecular	*E. crescens*	Itraconazole	Survived
10,11	Pelegrín I, et al	1	2011, 2014	46/M	Spain	Skin Ulcerations + Pulmonary	HIV, Liver Transplant	Molecular	*Emmonsia spp*	Amphotericin B	Died
12	Kenyon C, et al	13	2013	08-M, 05-F	South Africa	Skin Ulcerations	HIV	Molecular	*E. pasteurianus*	Amphotericin B	06 Died, 01 Lost to follow up, 06 Survived
13	Fielli M,et al	1	2014	42/M	Argentina	Pulmonary	Smoking	Culture	*E. crescens*	Amphotericin B, Itraconazole	Relapsed
14	van Hougenhouck-Tulleken W, et al.	3	2014	Male	South Africa	Skin Ulcerations + Pulmonary	HIV	Molecular	*E. africanus*	Fluconazole, Amphotericin B	02- Died 01- Survived
15	Heys I, et al	1	2014	52/M	South Africa	Headache, Ataxia, Raised ICT	None	Molecular	*E. africanus*	Amphotericin B, Itraconazole.	Survived
		1	2014	48/M	South Africa	Skin Ulceration, + Pulmonary	Transplant	Molecular	*E. africanus*	Itraconazole	Died
16	Feng P, et el	1	2015	43/M	China	Skin Ulcerations + Pulmonary	Transplant	Molecular	*E. pasteurianus*	Amphotericin B, Voriconazole Caspofungin	Survived

17	Lochan H, et al	1	2015	03/M	South Africa	Skin Ulcerations	HIV	Culture + Molecular	*E. pasteurianus*	Amphotericin B	Survived
18	Mutyaba A, et al	1	2015	36/M	South Africa	Skin Ulcerations	HIV, HBV	Molecular	*E. pasteurianus*	Amphotericin B	Survived
19	Tang XH, et al.	1	2015	30/F	China	Skin Ulcerations	CMV	Molecular	*E. pasteurianus*	Voriconazole	Survived
7	Swartz IS, et al.	54	2015	32-M 22-F	South Africa	Skin Ulcerations + Pulmonary	51 HIV, 01Transplant 02- None	Molecular	*E. africanus*	Amphotericin B Itraconazole. Fluconazole	26- Died 28- Survived.
20	Malik R, et al.	1	2016	38/F	India	Skin Ulcerations + Pulmonary	HIV	Molecular	*E. pasteurianus*	Amphotericin B, Itraconazole	Survived
21	Wang P, et al.	1	2016	64/M	China	Skin Ulcerations + Pulmonary	Diabetes Mellitus	Molecular	*E. orientalis*	Fluconazole Amphotericin B, Itraconazole.	Survived
22	Koneru H,et al	1	2017	52/M	North America	Pulmonary	NA	Culture	*Emmonsia spp*	Itraconazole	NA
23	Crombie K, et al.	1	2018	42/M	South Africa	Pulmonary	HIV	Culture + Molecular	*E. africanus*	Amphotericin B, Itraconazole	Died of HIV
24	Swartz IS, et al.	1	2018	39/M	North America	Pulmonary Skin Ulcerations	DM, Transplant	Culture + Molecular	*E. canadenesis*	Fluconazole, Amphotericin B	Survived
		1		68/M	Canada	Pulmonary	HIV	NA	*E. canadenesis*	NA	Died
		1		75/M	USA	Sepsis	NA	NA	*E. canadenesis*	NA	Died
		1		40/M	Mexico,	Skin Ulcerations, Sepsis	HIV	NA	*E. canadenesis*	NA	Survived

25	Gast KB et al.	1	2019	80/M	Netherlands	Pulmonary	B CLL	Molecular	*E. pasteurianus*	Posaconazole	Died
		1	2019	62/F	Netherlands	Pulmonary	B NHL	Culture + Molecular	*E. pasteurianus*	Posaconazole	Survived
26	Moodley A, et al.	1	2019	31/F	South Africa	Skin Ulcerations	HIV	Culture + Molecular	*E. africanus*	Fluconazole	Survived
27	Rooms I, et al.	1	2019	38/F	Uganda	Skin Ulcerations	HIV	Culture + Molecular	*E. pasteurianus*	Fluconazole, Itraconazole	NA
4	Capoor MR, et al.	1	2019	27/F	India	Skin Ulcerations	HIV	Culture + Molecular	*E. pasteurianus*	Amphotericin B, Itraconazole	Survived
28	Chik KK et al.	1	2020	61/M	China	Pulmonary	Transplant	Culture + Molecular	*E. pasteurianus*	Amphotericin B, Voriconazole	Survived
29	He Da, et al.	1	2020	41/M	China	Pulmonary	Transplant	Molecular	*E. orientalis*	Fluconazole, Posaconazole	Survived
30	Kuzyk AC, et al.	1	2021	52/M	Canada	Skin Ulcerations	Transplant	Culture + Molecular	*Emmonsia spp*	Amphotericin B, Posaconazole	Died
31	Mah J, et al.	1	2022	17/M	Canada	Pulmonary	NA	Molecular	*E. canadensis*	NA	NA
32	Pierce J, et al	1	2023	65/M	South America	Pulmonary	HIV	Culture + Molecular	*E. pasteurianus*	Voriconazole	Survived
Present Case		1	2023	38/M	India	Skin Ulcerations, Pulmonary	HIV	Culture + Molecular	*E. pasteurianus*	Amphotericin B, Itraconazole	Survived

For the clinicians and microbiologists, diagnosis of emergomycosis is challenging. Studies indicate that three-quarters of patients with emergomycosis get misdiagnosed as TB and receive treatment for the same as pulmonary lesions on chest radiograph may mimic pulmonary TB [1]. The cutaneous lesions of emergomycosis may simulate cutaneous lesions of secondary syphilis, drug reactions, guttate psoriasis, Kaposi sarcoma, papular eruption of HIV, pyoderma gangrenosum, scrofuloderma or varicella [1]. Additionally, morphological intracellular yeast form of *Emergomyces* resembles to *Histoplasma capsulatum* [4]. In skin biopsy specimens, it can be mislabelled as *Sporothrix schenckii* [4]. Histopathology can detect yeasts, but cannot differentiate between the different fungal genera or give a species level identification. Therefore, fungal culture and isolation are imperative for differentiating between these dimorphic fungi followed by molecular identification to confirm the diagnosis [1,4]. In most reported cases, identification of the species was confirmed by sequencing (Table 1). In the present report, fungal culture and genomic sequencing helped us to reach a species level identification. MALDI TOF MS could not identify the causal fungus. Possibly, a library update can help with this.

At present no treatment guidelines are available for emergomycosis. The IDSA recommendations for management of histoplasmosis and blastomycosis are being followed for emergomycosis [1]. Multiple treatment courses have been used with varying outcomes (Table 1). Maphanga TG et al, studied AFST by BMD for 50 isolates of *E. africanus* in order to guide clinical management. Amphotericin B was reported to be a best therapeutic option, followed by the azoles (itraconazole > voriconazole/posaconazole). Fluconazole was found to be relatively less potent. Echinocandins showed almost no activity with higher minimum effective concentrations (MECs) [33]. Similar results were obtained by Dukik et al, for anidulafungin and micafungin. However, they observed posaconazole to be most effective, followed by amphotericin B, itraconazole, voriconazole, and isavuconazole [34]. A disseminated emegomycosis by *E. pasteurianus* in Netherlands was also reported to be successfully treated with 14 months of posaconazole therapy indicating that posaconazole could be a possible treatment option [35]. However, for isavuconazole, is a novel triazole, studies have reported comparatively higher MIC values [34,35] and therefore, not a preferred treatment option. Our AFST findings are in accordance with the previously published findings of Maphanga TG et al, [33]. Though fluconazole was considered as relatively less potent agent, our isolate was susceptible. The current case was successfully treated with amphotericin B deoxycholate followed by itraconazole. The optimal timing of ART initiation following diagnosis of emergomycosis has not been established [4]. In the present case, ART was started 2 weeks after antifungal therapy and deterioration of cutaneous or lung lesions after ART therapy was not noted.

Despite the good susceptibility data, high death rates had been reported in emergomycosis (Table 1, 2). The mortality rate in emergomycosis patients ranges from 48 to 51% [7,36]. Late clinical suspicion after the appearance or deterioration of widespread cutaneous lesions after initiation ART may be the reason. Additionally, comorbid factors, poor control of the underlying condition, late or missed diagnosis, drug to drug interactions, emergence of resistance and intolerance to the available antifungals might be the proximate causes. Considering the host toxicity profile of currently available antifungal drugs and the endogenous resistance of dimorphic fungi to the less toxic echinocandins, new and alternative antifungal drugs, preferably with novel modes of action (to avoid cross-resistance and/or cross-toxicities) need to be explored. Also, the availability of oral formulations would enable ambulatory treatment resulting in improved patient compliance and adherence to treatment.

Table 2: Antifungal Drugs Breakpoints of *Emergomyces Spp* Isolates in Previously Published Cases Report

Ref No	References	AMB	MICA	ANID	FLC	ITC	VOR	POS	ISA	5 FC
10	Pelegrín I, et al	0.031	0.031	0.5	>64	0.125	0.25	0.125	2	NA
16	Feng P, et el.	0.125	0.063	0.0031	>64	0.25	0.25	0.063	1	NA
20	Malik R, et al.	1	0.05	NA	2	0.125	0.25	0.125	NA	NA
25	Gast KB et al.	0.031	<0.008	NA	64	0.063	0.25	0.063	1	NA
4	Capoor MR, et al.	1	0.5	NA	4	0.125	0.25	0.125	NA	NA
32	Pierce J, et al.	0.25	0.03	<0.015	>64	0.06	0.25	0.25	1	>64
Present Case		0.06	2	2	2	<0.015	0.25	0.125	NA	NA

AMB: amphotericin B; FLC: fluconazole; ITC: itraconazole; VOR: voriconazole; POS: posaconazole; ISA: isavuconazole; ANID: anidulafungin; MICA: micafungin, NA- Not Available.

Conclusion

Emergomycosis is a fatal systemic fungal disease among immunocompromised patients in endemic regions and diagnosis is challenging, particularly in resource limited settings. A high index of suspicion is needed, especially in countries where tuberculosis infections are endemic. Fungal culture and isolation are imperative followed by molecular identification to confirm the diagnosis. Close scrutiny of histoplasmosis cases diagnosed only by histopathology in immunosuppressed patients is required. For the management of emergomycosis, amphotericin B appeared to be the best therapeutic option, followed by the azoles.

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