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Original Article

Successful treatment of human eumycetomas caused by Aspergillus species with voriconazole

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Abstract

Background Mycetoma is a chronic, granulomatous infection of skin, soft tissues and bones which developed after traumatic inoculation of pathogenic spores deep in to the tissues. Classical triad consists of swollen tissues, draining sinuses and coloured grains.

Objective This study aimed to document clinical impact, side effects and safety profile of voriconazole in Aspergillus eumycetomas.

Methods Retrospective study of clinical, histopathological and culture proven cases of Aspergillus eumycetomas treated with voriconazole between October 2017 to March 2020. Voriconazole 400 mg/day was given for six to 15 months.

Results Six patients were selected for the study. Male to female ratio was 1:1. Mean age of cases was 45.33 years; five belonged to rural areas; most common occupation was farmer; feet were involved in five while knee was involved in one case only. Mean delay in diagnosis was 9.33 years. KOH mount showed filamentous, septate, acute-angled, dichotomously branched hyphae in all cases. Histopathology was diagnostic in four cases. Culture was positive in all cases and reported Aspergillus flavus in three, Aspergillus niger in two and Aspergillus fumigatus in one case. Bone involvement was none to minimal. Cultures became negative after three months of therapy while clinical improvement required at least six months of drug treatment. Surgical intervention was required in four cases. None of the case ended up for amputation. Five cases achieved complete clinical and microbiological cure.

Limitations Single center study, small sample, lack of molecular identification facilities.

Conclusion Voriconazole is safe and efficacious for treatment of eumycetomas caused by Aspergillus species.

Key words
Eumycetoma, voriconazole, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus.

Introduction

Mycetoma is chronic, suppurative & granulomatous infection of soft tissues and bones. It can involve any body part but feet are the site of predilection. Although, it has been reported from almost every part of the world but there are certain endemic countries from where a vast majority of cases had been reported. These include Sudan, Mexico, India, Somalia, Senegal,

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Yemen and Venezuela.[1] All these countries fall in a typical geographical zone, known as 'mycetoma belt', which exists in between 15° South and 30° North on the World map. This zone has abundance of different plant species, especially Acacia, with long, sharp and firm thorns. These thorns, in turn, promotes deposition of spores deep in to the tissues upon penetrating injuries.[2]

Rural areas are most affected all across the world and those who work barefoot are predominant victims like farmers, shepherds, dairy farm workers, gardeners, livestock workers and daily labourers.[3] Mycetoma has been reported in all age groups from infancy to old-age but young males between 20 to 40 years of age are particularly affected perhaps because of their occupational exposure. The male to female ratio in mycetoma is 3:1 which also supports that males are more vulnerable to this disease due to various reasons.[3,4]

Mycetoma has no documented zoonotic transmission, neither vector nor any animal reservoir.[3] Besides higher numbers of cases; Sudan and Mexico have most aggressive forms of mycetoma as well, and the disease which is generally regarded as benign in most of the world, is fatal in many cases in above mentioned two countries. Various genetic, hormonal, immunological and environmental factors play their role in a complex fashion for this heterogenic behaviour.[3,5]

The well-known triad of mycetoma is characterized by formation of multiple draining sinuses, presence of coloured grains in the discharge and development of painless subcutaneous swellings.[6] Bacteria and fungi both are responsible for this ailment and causing 'actinomycetoma' and 'eumycetoma' respectively. Aerobic actinomycetes; various Streptomyces and Nocardia species are responsible for the former variety. Fungi from diverse classes, orders and groups are accountable for the later type. The two types of mycetomas can coexist together in a patient but it's extremely rarely reported in the literature.[7]

In 1729, Aspergillus fungus was discovered and named by an Italian biologist Pier Antonio Micheli.[8] The word Aspergillus came from a Latin word 'spargere' which means 'sprinkling of holy water'. Aspergillus is a genus which consists of hundreds of species that belong to Order: Eurotiales, Class: Eurotiomycetes, Division: Ascomycota, Kingdom: Fungi.[8] These are true filament-forming, ubiquitous, climate-adapting, opportunistic fungi. Aspergillus species are present in air, soil, dust, deserts, water, forests, farms and fields, as well as over plant surfaces and animals' furs.[9,10]

Mycetoma is generally a neglected disease in many tropical and subtropical countries including Pakistan. In developing countries; lack of expert facilities, improper treatment, poor compliance of patients, late diagnosis and amputation in many cases create severe physical and psychological consequences in sufferers and their families. The exact incidence, prevalence, type and species pattern for mycetoma in Pakistan remains to be elucidated. Here, authors shared their experience of diagnosing, confirmation through histopathological examination, culturing of grains to identify causative species and management of mycetoma cases.

Methods

This was a retrospective, longitudinal study conducted at a tertiary care center in Karachi, Pakistan. Authors decided to include all Aspergillus species induced eumycetoma cases of either gender who attended dermatology outpatient department (OPD) between October

2017 to March 2020, for this study. Cases which have culture proven Aspergillus species as a cause of eumycetoma and treated with oral voriconazole 400 mg per day in two divided doses were included. Patients who had completed treatment from other hospitals, amputated cases, and those having concomitant tuberculosis, Human Immunodeficiency Virus (HIV) & Acquired Immunodeficiency Syndrome (AIDS) were excluded.

All mycetoma cases were clinically diagnosed by a dermatologist. None of them was on therapy for at least four weeks before undergoing any procedure. Two or more biopsies were done in each patient and sent for histopathology and tissue culture. Extraction of grains, followed by 10% potassium hydroxide (KOH) mount, Gram's staining, acid fast staining and extensive microbiological cultures were performed in all cases. Culture media used for growth of different Aspergillus species were Potato dextrose agar, Sabouraud dextrose agar, Czapek-Dox agar, and Czapek yeast extract agar media. Antifungal drugs susceptibility testing can't be performed because of unavailability at the institute.

Complete blood count, urea, creatinine, electrolytes, liver function tests and urine detailed report were performed at baseline, follow up visits (four to eight weeks) and upon cessation of drug therapy. Chest x-rays, Hepatitis B & C and HIV screening were performed before starting voriconazole. X-rays and magnetic resonance imaging (MRI) with contrast were done as a baseline investigation to detect extent of disease and bony involvement, and also performed subsequently to monitor response and cure in all cases.

Only six patients were included who met the selection criteria. Permission from the institutional ethical review committee was taken prior to conduct the study.

Side-effects profiles were evaluated clinically and biochemically at each visit. "Surgical debulking" was considered as adjuvant to chemotherapy and performed, if required, at least six months after starting voriconazole therapy.

"Clinical cure" was defined as no relapse and no signs of active disease at follow up after 12 months of cessation of chemotherapy. "Microbiological cure" was defined as two or more consecutive negative cultures of discharged or extracted grains during chemotherapy. "Radiological cure" was defined as no relapse or signs of active disease in bones (if previously present) on MRI scans at follow up after 12 months of cessation of chemotherapy.

Results

In this study, out of six patients; three were male and three were female with an equal ratio of 1:1. Mean age of patients was 45.33 years. All patients belonged to rural areas except one. Most common profession of affected patients was farmer (four patients) followed by housewife (one patient) and livestock worker (one patient). Right foot, left foot and right knee were involved in three, two and one patients respectively **(Figures 1 & 2)**. Mean duration of delay in diagnosis was 9.33 years **(Table 1)**.

KOH mount of grains in all cases showed filamentous, septate, acute-angled, dichotomously branched hyphae suggestive of Aspergillus species. Histopathology was diagnostic of fungal mycetoma **(Figure 3)** in four cases and also confirmed with Periodic acid-Schiff-diastase (PASD) and Grocott-Gomori's methenamine silver (GMS) stains

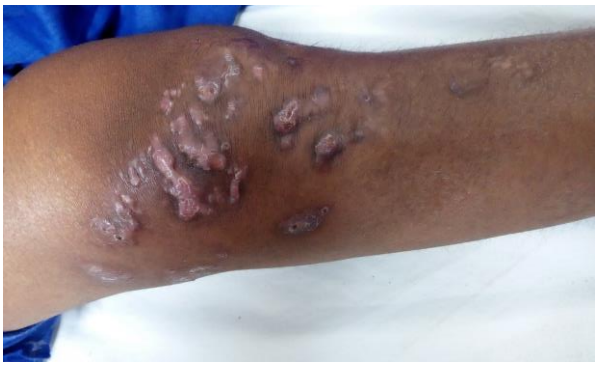

Figure 1 Mycetoma of right knee showing diffuse swelling, nodules and sinuses.

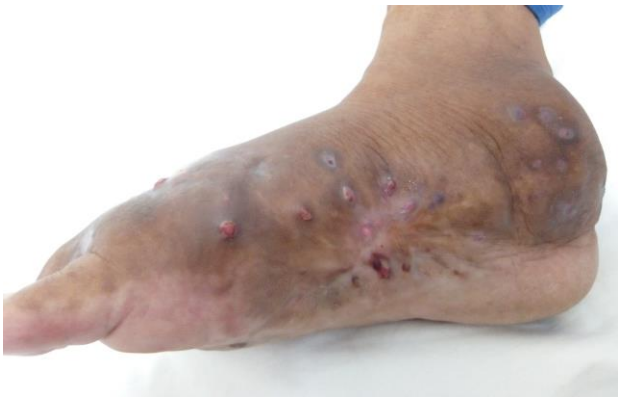

Figure 2 Mycetoma in a livestock worker showing gross involvement of the right foot.

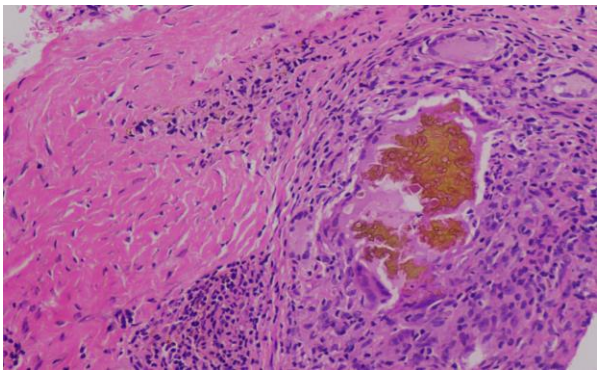

Figure 3 Septate fungal hyphae with hyaline budding surrounded by multinucleate giant cell reaction, neutrophils, eosinophils and lymphocytes (H&E,x40).

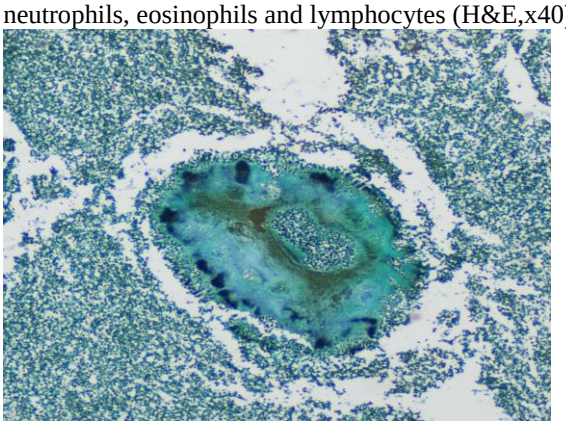

Figure 4 Fungal colony stained positively with GMS stain.

Table 1 Demographics of aspergillus eumycetoma patients.

Case No.	*Sex (M/F)*	*Age (years)*	*Locality*	*Anatomical region*	*Profession*	*Duration (years)*
1	F	44	Urban	Right foot	Housewife	13
2	M	48	Rural	Right foot	Farmer	8
3	F	60	Rural	Right foot	Livestock worker	10
4	M	41	Rural	Left foot	Farmer	12
5	M	49	Rural	Left foot	Farmer	7
6	F	30	Rural	Right knee	Farmer	6

(Fig*ure* 4). In rest of the cases, organisms were not identified amongst dense chronic granulomatous inflammation. Culture was positive in all cases and reported Aspergillus flavus in three, Aspergillus niger in two and Aspergillus fumigatus in one case **(Figures 5a-5c)**. All cultures were positive in at least two different growth media with morphological confirmation by an expert microbiologist.

White and black coloured grains were reported in four and two patients respectively. Both black-coloured grains were detected as "Aspergillus niger" while white-coloured grains were detected as "Aspergillus fumigatus" in one and "Aspergillus flavus" in three cases of eumycetoma **(Table 2)**.

Despite being late diagnosis, years after acquiring the infection, there was no bone involvement in two cases and minimal bone

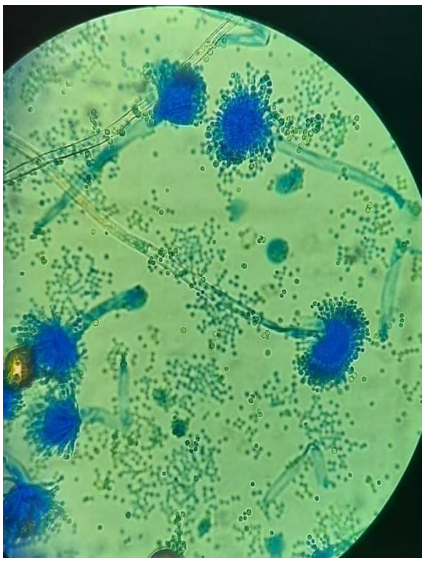

Figure 5a Aspergillus flavus in LPCB mount at 100X.

Figure 5b Aspergillus fumigatus in LPCB mount at 100X.

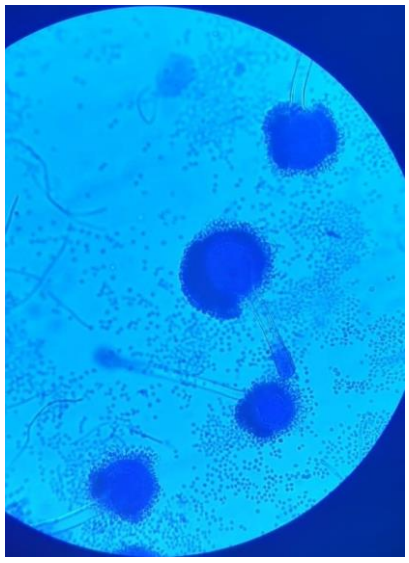

Figure 5c Aspergillus niger in LPCB mount at 100X.

LPCB: Lactophenol cotton blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue.

Table 2 Diagnosis and management of aspergillus eumycetoma cases

Case No.	*Colour of grains*	*Biopsy findings*	*Species on culture*	*Bone involvement*	*Need for surgical management*	*Duration of drug therapy*
1	White	Fungal mycetoma	Aspergillus fumigatus	Minimal	Sequestrectomy once	15 months
2	Black	Fungal mycetoma	Aspergillus niger	Minimal	No surgical procedure	12 months
3	White	Non-conclusive	Aspergillus flavus	Minimal	Sequestrectomy once	6 months
4	Black	Fungal mycetoma	Aspergillus niger	None	Surgical debulking procedures twice	12 months
5	White	Fungal mycetoma	Aspergillus flavus	Minimal	Surgical debulking procedure once	6 months
6	White	Non-conclusive	Aspergillus flavus	None	No surgical procedure	6 months, under treatment

involvement (up to three bones) in rest of four cases **(Figures 6a & 6b)**. Need for surgical management was required in four cases and limited to sequestrectomy and surgical debulking procedures with preservation of foot structure as much as possible. None of the case was ended up for amputation in this series **(Table 2)**.

Voriconazole 400 mg/day in two divided doses was given orally in all cases. As there were no specific guidelines for treatment of Aspergillus eumycetomas, authors decided to give chemotherapy till complete clinical and microbiological clearance. Cultures of discharged and extracted grains became negative after three months of voriconazole therapy. All patients had reduced swelling, decrease in girth of affected region, healing of all sinuses, complete absence of discharging grains, minimal to no pain and returned to all routine and occupational activities after six months of therapy.

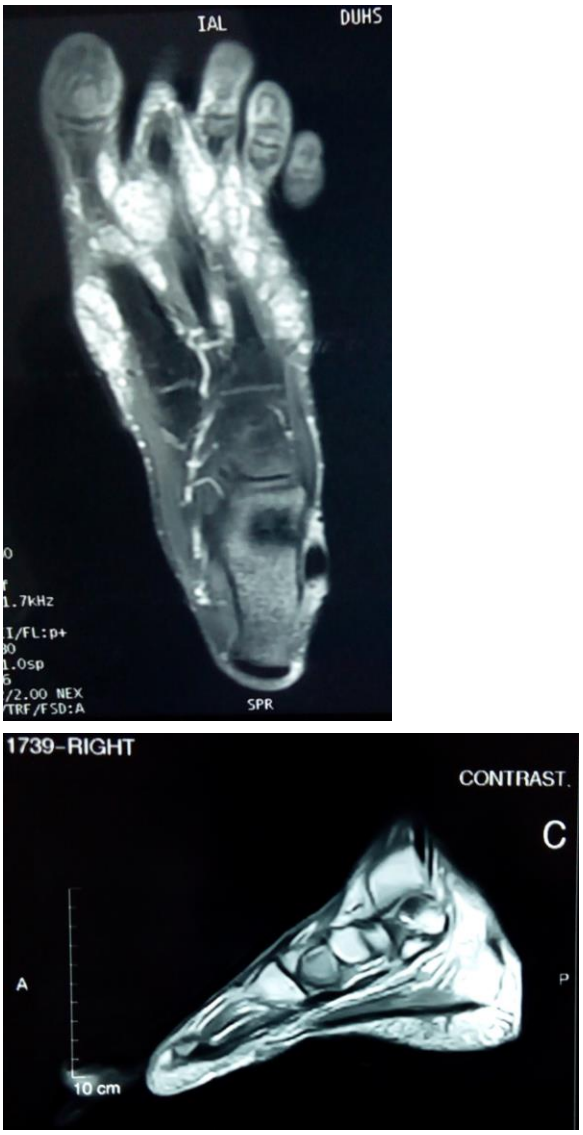

Figure 6 MRI scans: a) Classical dot-in-circle sign of mycetoma b) Post-contrast enhancement of fungal invasion in medial cuneiform bone of right foot.

All patients experienced repeated flu like symptoms, tiredness, photophobia, myalgia and muscle spasms during the course of therapy. Photosensitivity and actinic cheilitis was reported by one patient each. No serious clinical or biochemical side effects were reported and laboratory tests (mentioned above) were remained within normal limits during therapy. Duration of therapy was variable and adjusted according to response **(Table 2)**.

Five cases achieved complete clinical and microbiological cure with voriconazole 400 mg/day. Follow ups at 6th and 12 months after cessation of chemotherapy did not show any relapse. Repeat MRI scans did not detect any signs of recurrence or disease activity. One patient was still on therapy after six months although she became culture negative.

Discussion

Aspergillus are responsible for production of various diseases in humans ranging from primary and secondary cutaneous aspergillosis to invasive pulmonary aspergillosis. Others notable illnesses caused by various species of aspergillus are allergic bronchopulmonary aspergillosis, chronic pulmonary aspergillosis, pulmonary aspergilloma, maxillary sinus mycetoma, sinusitis, severe asthma with fungal sensitization, otitis externa, osteomyelitis and eumycetoma.[11-13] The literature is loaded with eumycetoma cases caused by different Aspergillus species like A. fumigatus, A. flavus, A. nidulans, A. terreus, A. ustus, and A. niger but therapeutic use of voriconazole for its treatment is limited to merely case reports.[10]

Voriconazole is a broad-spectrum antifungal drug belongs to triazole group. It is commonly used to treat invasive fungal infections caused by Aspergillus, Candida, Scedosporium and Fusarium species especially in immunodeficient and organ transplant recipients.[11] However, it has also been used to treat eumycetoma especially caused by Aspergillus species.[10]

Voriconazole has good bioavailability of 96% after oral administration. The drug binds to an enzyme of CYP 450-dependent system known as 14-alpha sterol demethylase (14-ASD) and inhibits its activity. Inhibition of 14-ASD leads to reduced production and depletion of ergosterol in fungal cell membrane which causes

cellular instability and subsequent swelling, lysis and death of fungal cells.[14]

Ahmed *et al*. from Sudan, Hopps *et al*. from USA and Mallick *et al*. from Pakistan reported very good results of oral voriconazole 400 mg/day in Aspergillus eumycetoma cases.[9,10,15] Ahmed *et al*. delineated a case of eumycetoma of left foot due to Aspergillus flavus in a type 2 diabetic patient. Patient used several courses of systemic ketoconazole and itraconazole but no improvement was appreciated. Oral voriconazole 400 mg/day displayed very good outcomes within few weeks of treatment but drug was prematurely stopped due to unavailability in their region.[9]

Hopps *et al*. described eumycetoma of right thigh due to Aspergillus and Penicillium species in an immunocompromised patient. They used intravenous infusion of liposomal amphotericin B for 2 weeks followed by oral voriconazole 400 mg/day for one year. In between, patient developed drug induced hepatitis and dose was reduced to 200 mg/day for few weeks.[15] Mallick *et al*. reported eumycetoma of right foot due to Aspergillus niger in an immunocompetent patient. They used oral voriconazole 400 mg/day for one year without any interruption. No serious side effects were noted.[10]

Porte *et al*. and Padhi *et al*. used voriconazole in eumycetomas caused by different fungal species and documented good efficacy.[16,17] Tendolkar *et al*. from India treated a case of Madurella mycetomatis mycetoma of forearm in a child with intravenous voriconazole (6 mg/kg/day) and reported very good outcome after 6 weeks with complete healing of wounds but patient was lost to follow up after that.[18]

In 2016, Infectious Diseases Society of America recommended use of voriconazole in cutaneous aspergillosis in their "Practice guidelines for the diagnosis and management of Aspergillosis".[11] Furthermore, Rudramurthy and colleagues in their recent review of "Invasive Aspergillosis by Aspergillus flavus" published in 2019, emphasized use of voriconazole as a first line agent in cutaneous and subcutaneous aspergillosis including eumycetomas in both immunocompromised and immunocompetent cases.[19]

Consensus statement of international experts suggested use of voriconazole in all regions, institutes and departments across the world where reliable data showed <5% cases with azoles resistance in invasive aspergillosis.[20] Similar guidelines don't exist in dermatology literature regarding management of eumycetoma cases.

There is a high need for training of clinics and primary care centers of commonly reported mycetoma zones of Pakistan along with formation of a proper referral system to well-equipped tertiary care centers for proper diagnosis and management of all mycetoma cases. This will decrease economic burden, save patient from amputation and prevent subsequent psychological consequences. Moreover, national-level guidelines should be prepared with especial focus on management of different types of mycetomas. Furthermore, there is immense need for availability of existing treatment options all across Pakistan and development of novel treatment options for eumycetomas.

Conclusion

Voriconazole is safe, highly effective and tolerable with minimal side effects in the treatment of aspergillus eumycetoma with or without bone involvement.

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