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Research Article

Nasal Fungal Pathology and Trichothecenes Associated with Water-Damaged School and Home

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Abstract

A 52 year old immunocompetent woman exposed to fungi in a water-damaged classroom and possibly in her home was evaluated for rhinosinusitis. CT scan of the sinuses revealed a nodular mass in the left ethmoid. Swab of the nasal mucosa cultured on SDA agar plate identified bacteria (TNTC), Candida (TNTC) and at least 10 other genera of fungi. A brown halo developed around the nasal mucosa on the SDA agar. The halo was sampled and revealed the presence of trichothecenes at 0.28 ppb. The surgical removal, fungal IgG antibodies and treatment of the fungal nodule, culture and identification of the trichothecenes are described in full in this communication.

Keywords: Fungus; Rhinosinusitis; Hypersensitivity; *Aspergillus*; Ethmoid sinus mucosa

Introduction

Chronic Fungal Rhinosinusitis (CRS) is relatively common, but often it is a misdiagnosed disease process of the nasal mucosa and paranasal sinuses [1,2]. It has been suggested that eosinophilic major basic protein may be involved in the inflammatory response of CRS [2,3]. However, the main diagnostic approach to identify fungal rhinosinusitis and CRS is an allergic condition related to Type I (IgE) hypersensitivity [4,5]. IgE fungal hypersensitivity occurs in 30 % of CRS patients, but elevated IgG fungal antibodies are present in about 90 % of CRS cases [1]. The incidence of the disease is 37 million cases that encompass a wide range of pathological and immune responses involving the innate immune system (Th-2 chemokines and cytokines), dysfunction of the nasal epithelial immune response and actual mucosal invasion by bacteria and fungi [6-10]. Pathological responses include invasive, chronic granulomatous and allergic conditions. A recent attempt was made to classify the various types of fungal sinusitis [11]. The current schema still includes 1) invasive diseases (acute invasive, granulomatous invasive and chronic) FRS and 2) noninvasive disease (saprophytic fungal infections, fungal ball and fungus related eosinophilic FRS that includes AFRS (allergic fungal rhinosinusitis). Thus, FRS results from multiple fungal genera, including *Aspergillus* species [12-16]. *Aspergillus* species are involved in invasive CRS in immunocompetent individuals [17-20]. In all cases the condition is refractory to antibiotic regimens and is improved with intranasal antifungals [2,19,20]. The use of corticosteroids should be limited because of the potential for suppression of the neutrophil migration and killing action of fungal spores and hyphae by both neutrophils and macrophages [21-23]. In addition, *Aspergillus* sinusitis can mimic malignant disease and even appear as pituitary tumor and a neuroblastoma [24-27]. Mimicry of a variety of disease conditions is common to individuals who have developed Sarcoidosis [28, 29]. This is raised because recent reports have identified Sarcoidosis in fungal exposed patients [30-32]. Presented herein is a case of a 52 year old immunocompetent woman who, following exposure to the bio-contaminants present in her water-damaged classroom and house, developed bacterial, Candida and several genera of fungi in a nasal mucosa and ethmoid sinus infection. Trichothecene mycotoxins in the nasal mucosa were symptomatic, not responding to medical therapy and required endoscopic sinus surgery to improve symptoms.

Materials and Methods

Patient history

The patient is a 52 year old woman seen on 07/29/14. She had chronic nasal and sinus congestion consistent with chronic rhinosinusitis and sought diagnostics and treatment. She had no history of chronic sinusitis, use of antibiotics but had positive sinus pressure in her ear and head and post-nasal drip. Her home and school had water intrusion. Environmental tests revealed Stachybotrys and *Penicillium/Aspergillus* spores in the indoor air of home and school. She had shortness of breath that was improved by Itraconozole provided by another physician.

Mold assessment

The inspection and assessment for fungi and water damage in the school classrooms was done by Quality Environmental Solutions and Technologies, Inc., Wappinger Falls, NY according to the guidelines of the New York City Department of Health.

Nasal surgery and procedures

Endoscopic Sinus Surgery was done under general anesthesia, the

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ethmoid sinuses were entered, and the mucosa was a yellowish brown with some areas of normal color. All affected mucosa was removed and some specimens were sent to Peachtree Laboratory & Associates, Atlanta GA that resulted in the diagnosis of Chronic Sinusitis (left and right ethmoid, and frontal). No hyphae, eosinophils, or mucosal invasion was seen on microscopic exam. Some of the mucosa was placed in a SDA agar plate to grow out mold, but no mold grew, a brown halo developed around the sinus mucosal tissue on day 4 and it was sent to Real Time Laboratories for mycotoxins testing of the brown substance in the agar. At conclusion of the procedure, all paranasal sinuses were irrigated with Amphotericin-B solution of 50mg in 500ml sterile water. And following patient nebulizer Amphotericin-B at 3mg per 30ml of sterile water in through nose and out through mouth for 6 weeks; Itraconozole 200mg 2x day for 2 months. Nystatin 1, 2x day for Candida control; Hydrocortisone 5mg 2x day for adrenal insufficiency, Thyroid 50mg AM, Levothyroxine 50mg pm.

Additional medications from other physicians included: Chorella 8 tabs 2x day, Grape Seed extract 500mg po bid (unknown amount) Cholestyramine 4gm po bid, monolaurin 600mg po bid means 1 2x day; Intramax liquid vitamin, 1 oz daily (Drucker labs); Transfer factor sublingual spray for antifungal & bacterial immune support 5 sprays sublingual 2x day and 1-3 Beta Glucan (Microbalance Health Products); Methyl B12, Folic acid, Probiotic (VSL#3); and Xymogen I5 to detoxify phase I and II of liver detoxification pathways (Xymogen.com). None of these supplements were evaluated for this communication.

CT scan

The CT scan was performed at MRI and Imaging at Midtown, Atlanta, Georgia on 8-8-14 using coronal view at 1mm cuts resolution.

SDA agar plates

Sabouraud Dextrose agar plates were purchased from BD product center, San Diego, CA. A sterile cotton colgi swab was taken from the nasal cavity. The SDA plate was then inoculated, sent to Immunolytics, Albuquerque, NM, and cultured for 5 days in the dark at room temperature. The colonies of fungi were identified by Immunolytics.

IgG fungal antibodies

The serum from the patient was sent to Commonwealth Medical laboratories, Inc., Warrenton VA. IgG antibodies against 11 fungi and Brewer's yeast was ordered. The results were expressed as µg/ml.

Mycotoxin identification

The SDA agar plate and urine specimen were sent by overnight carrier to Real Time Laboratories, Carrollton, TX. The urine and the brown halo on the SDA agar around the ethmoid mucosa (Figure 1) were tested for Aflatoxin, Ochratoxin A and Trichothecenes as previously published [33].

Sinus mucosa pathology

Sinus mucosa was sent to pathology at Peachtree Laboratory Associates, Atlanta, GA. Left and right ethmoid sinus and right frontal sinus mucosa was submitted for GMS stain for microscopic exam for hyphae and eosinophil identification and histological examination.

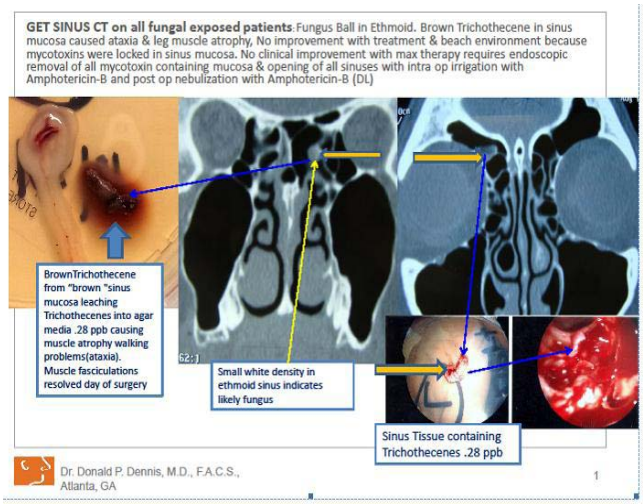

Figure 1: This figure summarizes the pathology and CT scan of the fungal ball in the ethmoid sinus as follows:
(1) On the left side the blue arrows point the dark brown ethmoid sinus mucosa and the brown halo around the ethmoid tissue in the SDA agar is mycotoxins leaching out of the ethmoid tissue, was identified as Trichothecenes at 0.28 ppb. (2) In the center the yellow arrows point to the ethmoid fungal ball. (3) The blue arrows are pointing at the sinus tissue that contained the trichothecenes note in (1) above.

Results

Inspection and assessment

The inspection and assessment was limited to visual observations for water intrusion and mold growth in the HVAC ducts, total airborne spores (Three Aero-Spore-Trap cassettes) and one tape lift sample. Water intrusion was noted in several places with the presence of suspected microbial growth. Sampling was not done on the suspected microbial growth. The HVAC system was visually inspected, but sampling of dust was not done. Airborne spores included Ascospores, Basidiospores, *Chaetomium, Aspergillus/ Penicillium spores, Curvularia, Rhizopus and Stachybotrys.* One air sample had Stachybotrys at 66.2 % of the total of 7,100 spores per cubic meter. No attempt was made to identify species of fungi. However, *Chaetomium and Stachybotrys* are hydrophilic fungi and require ≥90 % water content. Thus, based upon the visual observation revealing water intrusion and the detection of Chaetomium and Stachybotrys, the school rooms had experienced serious intrusion events.

Patient evaluation

Physical examination revealed muscle wasting in her hands and legs, ataxia from weakness of the legs. She stated that she did not feel better at the beach. She had a low energy level of 4 with 10 being normal. She experienced an allergic complex to foods, stomach pressure, leaky gut syndrome, gluten sensitivity, muscle and joint pain, weakness, memory loss, concentration problems, blurred vision, numbness and tingling, anxiety, depression, irritability, and urticaria. She did not experience improvement with maximal medical therapy, including moving out of the house and not taking any household belongings with her.

Results of CT scan and Figure 1

CT scan revealed a right ethmoid fungus appearing mass with

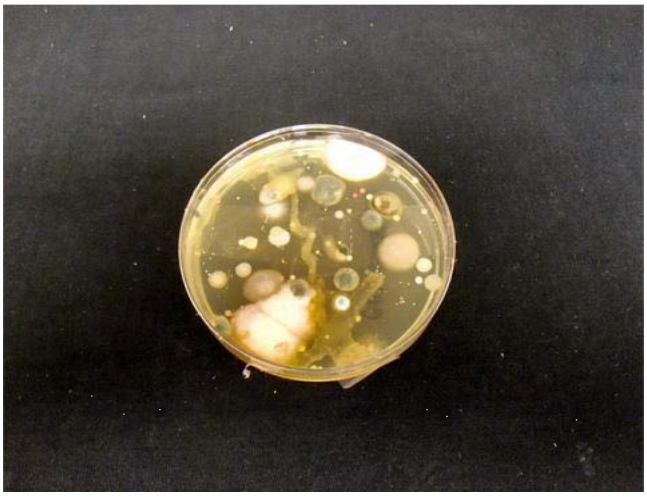

Figure 2: This figure shows the SDA agar plate demonstrating the fungal colonies that appeared following culturing of the nasal swab sample for 5 days in the dark at room temperature. The number of colonies of each fungus ranged from as low as one (*Microsporium*) to nine (*Rhodotorula*). The bacteria and Candidia were TNTC.

Table 1: Bacteria and Fungi cultured from the nasal mucosa of the ethmoid sinus. The number of colonies of each fungal colony observed on the SDA agar plate ranged from 1 to TNTC, while the bacteria were too numerous to count.

Cultured Organism	Number of Colonies
Bacteria	TNTC
Candida	TNTC
Cladosporium	5
Penicillium	2
Epicoccum	2
Microsporium	1
Rhodotorula	9
Aspergillus	2

mucosal thickening of the left posterior ethmoid sinus, thickening in the right frontal and ethmoid sinuses (Figure 1). The infundibula were narrowed and the left frontal sinuses appeared clear. A deviated nasal septum to the right with turbinate hypertrophy was present.

SDA Culture results

The results of the culture of the ethmoid specimen are shown in (Figure 2) and summarized in (Table 1). The bacteria were not identified because it was a fungal identification culture. However, colonies of fungal genera were identified. The number of colonies ranged from 1 to TNTC as follows: Candida (TNTC); Fusarium (3); *Penicillium* (3); *Helminthosporium* (2); *Aspergillus* (2); *Alternaria* (2); and other fungi at (1).

IgG antibodies in serum

The IgG serum antibodies detected by Commonwealth Laboratories showed an immune response to the eleven species of fungi is summarized in (Table 2). IgG antibodies were directed against all species of fungi with the exception of *Cladosporium herbarum* and Brewer's yeast.

Table 2: This table summarizes the IgG antibodies to fungi detected in the serum of the patient with a comment regarding interpretation of the degree of sensitivity as recommended by the Diagnostic Laboratory.

Test	Class	Concentration (µg/ml)
Yeast Brewer's	Neg	0.46
Penicillium. notatum/chrysogenum	III	12.37
Cladosporium herbarum	Neg	0.81
Aspergillus Fumigatus	II	7.14
Mucor racemous	I	1.08
Candida albicans	IV	52.01
Alternaria. ternius (alternata)	II	4.43
Helminthosporium halodes	II	4.97
Curvularia lunata	I	2.10
Fusarium oxsporum	III	24.92
Epicoccum nigrum	I	2.18
Acremonium killense (cephalsosp)	I	1.22

Mycotoxins in urine and SDA plate

The results of the mycotoxins present in the urine sample and the halo area around the ethmoid sinus mucosa in (Figure 1) are summarized in (Table 3). Trichothecenes were detected at 0.52 ppb with a limit of detection of 0.2 ppb. Ochratoxin A was detected at 1.77 ppb, which is below the detection limit of 2 ppb. However, the patient had received prior treatment with Sporanox, which can result in decreased concentration of urine mycotoxins. The halo around the ethmoid sinus mucosa on the SDA agar plate in (Figure 1) contained trichothecenes at 0.28 ppb.

Pathology

Histological examination: Left and right ethmoid sinus mucosa, and right frontal sinus mucosa was submitted. GMS stain microscopic exam did not show fungi. Histology showed chronic sinusitis in all specimens. No eosinophils were seen. The most likely Fungal Chronic Rhinosinusitis (FCRS) classification was AFRS because it was non invasive on histology exam. Fungus was cultured from the nose prior to treatment and the Sinus CT scan shows a small polyp density usually associated with eosinophils. Although after treatment with antifungals orally and via nebulization with steroids, as expected, no fungi or eosinophils were seen in the surgical specimen.

Results of surgery and prescribed medications: Prior to surgery she had leg muscle atrophy, weakness, ataxia, and leg fasciculations nightly while sleeping for approximately 10 years. After surgery she experienced resolution of the leg fasciculations during sleep and the weakness and ataxia improved.

The medical protocol improved her symptoms very slightly by getting her energy level from a 4 to a 5 with normal of 10. Improvement was minimal. Medical protocol was: Chorella 8 tabs 2x day, Grape Seed extract 500mg po bid, Cholestyramine 4gm po bid, monolaurin 600mg po bid means 1 2x day; Intramax liquid vitamin, 1 oz daily (Drucker labs); transfer factor sublingual spray for antifungal & bacterial immune support 5 sprays sublingual 2x day and 1-3 Beta Glucan (Microbalance Health Products); Methyl B12, Folic acid, Probiotic (VSL#3); and Xymogen I5 to detoxify phase I and II of liver detoxification pathways (Xymogen.com); Itraconozole

Table 3: This Table presents the concentrations in ppb of mycotoxins in the urine sample from the patient and trichothecene detected in fungal colony in Figure 1.

Sample	Aflatoxin	Ochratoxin A	Trichothecene
Urine	N.D.	1.77	0.52
Brown Halo – SDA Plate	N.D.	N.D.	0.28

N.D. = Not Detected
Aflatoxin Limit of Detection = 1.0 ppb
Ochratoxin A Limit of Detection = 2.0 ppb
Trichothecene Limit of Detection = 0.2 ppb

100-200 mg bid, Amphotericin-B nasal nebulization bid, saline nose irrigation bid. None of these supplements were evaluated for this communication.

Discussion

The patient in this case study was exposed to the microbial conditions that existed in her classroom and home. As a result she did develop health conditions that made her seek medical attention. An initial trial of Sporanox prescribed by another physician lessened her shortness of breath. However, her conditions of fatigue, muscle fasciculations and congestion in her head and nasal cavity resulted in her consult. The SDA culture from a nasal swab resulted in identifying bacteria at TNTC and Candida (TNTC) as well as several genera of fungi (Figure 2 and Table 1). In addition, IgG antibodies were positive for 10 species of fungi present in water-damaged indoor environments. The CT scan revealed a dense nodule in her left ethmoid sinus. Surgery was undertaken to remove the foreign nodule. At surgery the sinus mucosa representing the ethmoid nodule was placed on the SDA agar plate. Following 5 days of incubation a brown halo developed around the ethmoid tissue. The halo on the SDA plate tested positive for trichothecenes at 0.28 ppb that apparently leached from the mucosa. In addition her urine contained trichothecenes at 0.52 ppb and ochratoxin at 1.77 pbb (Figure 1, Table 3). She was then placed on intranasal amphotericin-B nebulization, which killed the mucosal fungus. The surprise of this procedure was the detection of leached trichothecenes in the absence of fungal growth on the SDA plate. The absence of fungal growth may have resulted from a short culture time and lack of optimum temperatures of 35-37 °C for *Aspergillus* species (55, 56). Although the detection of trichothecene in the brown halo was an accidental discovery, the discovery should alert other ENT physicians to look for the presence of mycotoxin in affected nasal mucosa.

The role of fungi in chronic rhinosinusitis was initially introduced by Ponikau *et al.* [1]. Since then, numerous studies have been published demonstrating the role of fungi in Type I IgE Hypersensitivity, chronic inflammation, innate immunity and invasion of the orbit and central nervous system [1-27]. The invasion of surrounding tissues occurs in immunocompetent individuals [17,19,20,26,34,35]. Recent publications have presented evidence that fungi are present in brain regions of Alzheimer's patients and in the cerebrospinal fluid and brain tissue from patients with amyotrophic lateral sclerosis [36,37]. We suggest that the classification of invasive fungal infections outlined by Chakrabarti *et al.* be seriously considered with respect to patients with fungal sinusitis [11]. Individuals exposed to fungi and bacteria in water-damaged buildings develop multiple symptoms and upper and lower respiratory infections, sarcoidosis, asthma and asthma-like symptoms [38-45]. Thus, it appears that fungal and bacteria along with their secondary metabolites are important factors in illness of occupants in water- damaged homes, schools and office buildings. Recent case reports on families and office employee are supportive of this conclusion [26,30-32, 43-45].

The patient in this case presentation was exposed to fungi in a water-damaged classroom. The environmental investigation revealed elevated levels of ascospores, Basidiospores and spores of *Aspergillus, Penicillium* and *Stachybotrys*. Fungal fragments less than one micron containing trichothecene mycotoxins have been identified in the indoor air of water-damaged buildings [46,47]. The fungal fragments are aerosolized from mold colonies by air current simulating the HVAC system [48,49]. The aerodynamic characteristics and respiratory deposition of these fungal fragments occur in the nasal cavity and lungs [50]. In addition, mycotoxins are readily deposited in HVAC systems [51-53].Thus, it is not surprising occupants of water-damaged buildings and homes have trichothecene mycotoxins in their sera, nasal cavity, tissues, and urine [19,20,33,43-45,54].

Conclusion

This case report illustrates that since most airborne mycotoxins enter through the nose, some remain in concentrations high enough to cause systemic symptoms and therefore must be removed via Endoscopic Sinus Surgery (ESS) to relieve symptoms in those who fail environmental and medical therapy. In this case accompanied with symptoms of leg muscle atrophy, ataxia, muscle weakness, and muscle fasciculation, the symptoms improved after endoscopic sinus mucosal removal and irrigation with Amphotericin-B. In addition, the presence of Candida at TNTC in the nasal cavity may have resulted from the immunosuppressive effects of mycotoxins, including trichothecenes. This may also account for the overgrowth of bacteria detected on the SDA plate. Although the detection of trichothecene in the brown halo was an accidental discovery, the discovery should alert ENT physicians to the presence of mycotoxin in affected nasal mucosa. Sinus surgery should be considered for patients who fail medical and environmental treatment.

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