Research Article

Morphological spectrum of fungal infections: a retrospective study

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Abstract

Background: Over the past few years, major advances in health care have led to an unwelcome increase in the number of life-threatening infections due to true pathogenic and opportunistic fungi. The population at risk includes transplant recipients, cancer patients, and other individuals receiving immunosuppressive treatments. Various diagnostic modalities are used to diagnose fungal infections. These include KOH preparation, culture, histopathology, antigen and antibody assay, metabolite detection, and polymerase chain reaction methods. Histopathologic examination permits rapid, presumptive identification of fungal infections. Also, histopathologic examination reveals many significant prognostic evidences such as host tissue reaction, extent of invasion by fungi, and also tissue response to treatment, which cannot be assessed by any other methods. One or combination of these methods can be employed to know the etiologic agent and so determining therapy.

Objective: To detect the type of fungal infections and their distribution according to age, sex, and organ in histopathologic specimens.

Materials and Methods: This was a retrospective study carried out in the department of pathology for a period of 4 years from January 2011 to January 2015. All the histopathologic specimens diagnosed to have fungal infections during this study period were included in this study.

Result: During the study period, of 15,501 total histopathologic specimens, 33 with fungal infections were received, accounting for 0.08% of total histopathologic specimens. Of these, 76% were males, 24% were females with male preponderance. The most common type of fungal infection was mucormycosis (19) followed by candidiasis (5), others were zygomycosis (2), pigmented fungosis (2), and one case each of rhinosporidiasis, cryptococcosis, and aspergillosis were found. The most common site of infection was maxillary sinus.

Conclusion: Histopathologic examination can offer prompt provisional identification of infectious fungal organisms and remains the only available reliable means to identify certain pathogens. However, significant morphological overlap in fungal organisms, a desire to provide unequivocal fungal categorization owing to pressure from clinical colleagues, and idiosyncratic language in surgical pathology reporting contribute to errors.

KEY WORDS: Histopathology, fungal organisms, special stains, culture

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Introduction

Fungi have been recognized as the causative agents of human disease earlier than bacteria. Michele (1729) described aspergillosis and named it because of its resemblance to "rougher head."[1] Since then many mycologists have identified approximately 400 species of the family.

Modern trends of therapy with widespread, prolonged, and indiscriminate use of broad spectrum antibiotics; use of

cancer chemotherapeutic agents; use of immunosuppressive drugs; irradiation; and increased use of radiological screening procedures, hyperalimentation, organ transplantations, and acquired immunodeficiency have led to increase in incidence of fungal infections.[2] Immunosuppression and breakdown of anatomical barriers such as the skin are the major risk factors for fungal infections.[3,4]

Some fungal infections are difficult to diagnose by microbiological examination, more over microbiological examinations can be misguided by contamination of other fungi. Some fungi cannot be cultured. Serological reactions lack complete specificity. Some fungi are so adherent and tightly bound that they are rarely visible in body fluids and exudates. In this scenario, histopathologic examination remains one of the major diagnostic tools in mycology, because it permits rapid, presumptive identification of fungal infections.[5,6] Although culture is considered as gold standard for etiologic diagnosis of fungal infection, it is a slow method and also some fungi cannot be cultured. Although histopathologic method may not detect fungal infections in all the instances due to sparse organisms, morphological overlapping, and observer inexperience, it helps in etiologic diagnosis in very good number of cases.[7] It can detect fungal infections in clinically unsuspected cases when tissue is sent to rule out malignancy or to know the cause of inflammation. It is the only method for etiologic diagnosis of some organisms such as *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*), *Loboa loboi*, and *Rhinosporidium seeberi*.[7–10] The demonstration of tissue invasion or an inflammatory reaction can help to determine whether an organism represents contamination, colonization, or true infection.[11] The aim of this study is to detect the type of fungal infections and their distribution according to age, sex, and organ in histopathologic specimens received in the Department of Pathology, MS Ramaiah Medical College, Bengaluru, Karnataka, India. In this study, the histomorphology of various fungi has been stressed upon, this leads to a better diagnosis and awareness, so as to diagnose the fungal infections in histopathologic specimens.

Materials and Methods

This was a retrospective study carried out in the department of pathology for a period of 4 years from January 2011 to January 2015. All the histopathologic specimens diagnosed to have fungal infections during this study period were included in this study. Specimens obtained from autopsy cases were excluded. The sections stained with routine hematoxylin and eosin [H&E] and special stains such as periodic acid–Schiff (PAS) and Gomori methenamine silver (GMS) were retrieved and reviewed. Both clinically suspected cases and incidentally detected fungal infections were included in this study. All medical records were reviewed and clinical details including age, sex, and predisposing conditions were obtained.

Results

During this study, of 15,501 total histopathologic specimens, 33 specimens with fungal infections were received, accounting for 0.08% of total histopathologic specimens. Of these, 76% were males, 24% were females with male preponderance. The cases showed wide range of age incidence between 11 and 70 years. So no age predilection for incidence of fungal infections was noted in this study. But no cases were seen below 10 years and above 70 years [Table 1].

The most common type of fungal infection was mucormycosis (19) followed by candidiasis (5), others were zygomycosis (2), pigmented fungosis (2), and one case each of rhinosporidiasis, cryptococcosis, and aspergillosis were found. In two cases, particular etiologic diagnosis was not possible and labeled as fungal granuloma based on granuloma formation, histological tissue reaction, and poorly made out fungal elements [Table 2].

The most common site of infection was maxillary sinus (10) followed by nasal cavity (7)], esophagus (4), orbit (3), frontal abscess (2), and palate (2). Single case was found in each of the following sites: skin, foot, kidney, and soft tissue around bone and bronchus [Table 2].

Mucormycosis was the most common type of fungal infection in maxillary sinus and also one case of fungal granulomas was seen. In nasal cavity, six cases of mucormycosis with one case of rhinosporidiosis were found. Four cases of fungal infections were seen in the esophagus all of which were candidiasis. In two cases, candidiasis was associated with adenocarcinoma and in one case it was associated with squamous-cell carcinoma. Three cases of fungal infections were noted in the orbit, two of which were caused by mucormycosis and one case was due to zygomycosis. In central nervous system, two cases were seen, both of which were caused by pigmented fungi. In palate, two cases were seen, one case of mucormycosis and the other by candidiasis. In the kidney, one case of cryptococcosis and in the bronchus, one case of aspergillosis was found.

Table 1: Age- and sex-wise distribution of fungal infections

Age (years)	Male	Female	Total
11–20	1	1	2
21–30	2	0	2
31–40	2	1	3
41–50	8	1	9
51–60	6	5	11
61–70	6	0	6
Total	25	8	33

Table 2: Organ-wise distribution of different fungal infections

Type of lesion	Nasal cavity	Nasal sinus	Foot	Frontal abscess	Palatal ulcer	Orbit	Kidney	Esophagus	Soft tissue around bone	Bronchus	Total
Rhinosporidiosis	1	0	0	0	0	0	0	0	0	0	1
Mucormycosis	6	9	0	0	1	2	0	0	1	0	19
Cryptococcosis	0	0	0	0	0	0	1	0	0	0	1
Candidiasis	0	0	0	0	1	0	0	4	0	0	5
Zygomycosis	0	0	0	0	0	1	0	0	1	0	2
Fungal granuloma	0	1	1	0	0	0	0	0	0	0	2
Pigmented fungosis	0	0	0	2	0	0	0	0	0	0	2
Aspergillosis	0	0	0	0	0	0	0	0	0	1	1
Total	7	10	1	2	2	3	1	4	2	1	33

Discussion

In this 4-year retrospective study, all histopathologic specimens received in our department, which were diagnosed with fungal infections, were reviewed and categorized according to age, sex, and organ-wise involvement. An attempt is made in our study on restressing the morphological identification and diagnostic difficulties that are faced while reporting fungal infections. The most common infection in our study was mucormycosis in maxillary sinus, which was identified based on broad, non-septate hyphae branching irregularly and the angle of branching is greater than other organisms, and usually approaches 90°. Candidiasis was the next common infection that was seen in esophagus and organisms appeared as mats of yeasts measuring 3–5 μm in diameter intermingled with pseudohyphae or filaments. Histopathologic examination of specimens is very important to define invasion of tissues and vessels, because growth from skin, lung, and the gastrointestinal or genitourinary tract is only indicative of colonization. Two cases of pigmented fungi were identified both of which presented as frontal abscesses, which had dark-pigmented hyphae and two cases of zygomycosis that were characterized by aseptate hyphae. Also, we found single cases of *R. seeberi*, *Cryptococcus*, and *Aspergillus*.

Although culture studies are considered as the gold standard for the identification of etiologic agents, they may not always be available or positive.[12–14] Moreover, differentiating colonization and contamination from pathogens may be difficult. Also, the studies by Sundaram et al.[12] and Guarner and Brandt[15] have shown the difficulties in differentiating colonization and contamination of fungi. Accurate diagnosis of the etiologic agent is important as the in vitro susceptibility to antifungal agents of different species and the emerging pathogens are variable.[15,16] Histopathology provides rapid and cost-effective means of providing diagnosis. Mucormycosis species was the most common organism identified in biopsy in our study. In most of the studies, *Aspergillus* spp. were found to be more common.[2,6,16,17] The most common differential diagnosis of mucormycosis is with *Aspergillus* spp. that are thinner, septate, with regular branching, and they branch at acute angles (45° as opposed to 90°). *Candida* spp. can be confused with *Aspergillus* spp. and *Trichosporon* spp. Elongated *Candida* pseudohyphae can appear to be branching but are differentiated because pseudohyphae are slender and do not have septations. Germinating *Candida* blastospores can also appear to be branching but can be distinguished by the absence of a constriction between the base of the blastospore and the germ tube. Although typically present extracellularly, intracellular *Candida* spp. can mimic *Histoplasma* spp. Clues for the differentiation of *Candida* include the variably sized yeast cells, lack of a pseudocapsule, and better staining with H&E and Gram's stain. Furthermore, *Candida* spp. typically generate a suppurative tissue reaction, whereas *Histoplasma* spp. tend to elicit a more granulomatous reaction. Luna has shown pitfalls of morphological identification of *Candida* spp. [6,17] *R. seeberi*, a mesomycetozoan parasite that causes palate and nasopharyngeal polyps, produces large sporangia with multiple internal endospores. The most common differential diagnosis *R. seeberi* is coccidioidomycosis spherules, but *R. seeberi* sporangia and endospores are larger than *Coccidioides* spherules, and its inner sporangial wall stains with mucicarmine stain.[18] Pigmented fungi have dark-pigmented hyphae, spores, or both. They cause primarily two groups of infections: chromomycosis and Phaeohyphomycosis. The brown pigment in the fungi is a melanin, which can be clearly demonstrated in tissue section by the stains for melanin. The organisms are round, golden brown in color, and thick walled. These are known as sclerotic bodies, muriform cells, or medlar bodies, and are 5–12 mm in diameter. Melanin stain can be used to confirm dematiaceous fungi. Gomez and Nosanchuk[19] has referred melanin to as "fungal armor" due to the ability of the polymer to protect microorganisms against a broad range of toxic insults.

H&E is a versatile stain that enables the pathologist to evaluate the host response, including the Splendore–Hoeppli phenomenon, and to detect other microorganisms. It is the stain of choice to confirm the presence of naturally pigmented fungi, and to demonstrate the nuclei of yeast-like cells. However, there are drawbacks to using just the H&E stain for fungal diagnosis. It is often difficult to distinguish poorly stained fungi from tissue components, even at higher magnifications. Also, sparse fungi are easily overlooked in H&E-stained sections. The morphological features may not be evident and sometimes may be misleading. Histopathology usually cannot provide the fungal genus and species, which are very important for treatment. Infections with more than one fungus, the morphological diversity may be subtle and not appreciated. Thus, other tests should be used to determine if more than one organism is present. Several studies have demonstrated the usefulness of special stains in the identification and classification of fungal organisms in tissue sections.[20–23] Most fungi can be readily demonstrated with the common special stains, GMS, Gridley's fungus (GF), and PAS, also referred to as "broad spectrum" fungal stains. The GMS is preferred for screening, because it gives better contrast, and stains even degenerated and nonviable fungi that are sometimes refractory to the other two stains. The disadvantage of GMS and GF stains is that they mask the natural color of pigmented fungi, making it impossible to determine whether a fungus is colorless, hyaline, or dematiaceous (pigmented). The PAS stain performs almost as well as GMS, in screening for fungi. It actually demonstrates fungal morphology better than the silver stains. The PAS can stain degenerated fungi that may not be visible on H&E stain.

Conclusion

The histopathologic examination can offer prompt provisional identification of infectious fungal organisms and remains the only available reliable means to identify certain pathogens. However, significant morphological overlap in fungal organisms, a desire to provide unequivocal fungal categorization owing to pressure from clinical colleagues, and idiosyncratic language in surgical pathology reporting contribute to errors.

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