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Original Research Article

Clinicopathological Spectrum of Fungal Infections in a Tertiary Care Centre of Bihar

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Abstract:

Background: Fungal lesions are frequent in immunocompromised patients, including those with diabetes, cancer patients receiving chemotherapy or radiation, HIV positive people, and transplant recipients. Although tissue culture is the preferred method for identifying fungi, histopathology analysis provides a more accurate picture of tissue invasion.

Methods: This study covered 47 cases over a 6-months period. This study examined the histological spectrum of fungal infections, their distribution by age, sex, and site of involvement, and their morphology in our hospital's setting.

Results: Aspergillosis is most prevalent fungus in this investigation, and the majority of patients of this condition manifested as ganglion and dermoid cysts. The foot was the most typical site.

Conclusion: We aimed to identify the etiological diagnosis of fungal infections in histopathological specimens acquired in our department in this investigation.

Keywords: Fungal lesions, Dermoid cyst, Ganglion, Gomori methenamine silver stain, Immunocompromised patients, Phaeohypomycosis.

Introduction

Nowadays, fungi infections are more frequent, which leads to a wide spectrum of human disorders. They range from systemic infections affecting the brain, heart, lungs, liver, spleen, and kidneys to superficial infections affecting the stratum corneum of the skin. Patients with invasive fungal lesions are more likely to be transplant recipients, cancer patients receiving chemotherapy and immunosuppressive treatments, HIV patients, premature newborns, elderly patients, and people having major surgery. Invasive fungal infections are a serious problem for these people.[1,2] Fungal infections can range from superficial, which only affects the stratum corneum, to cutaneous, which affects the appendages of the skin, subcutaneous, or deep, which affects the abdominal viscera, lungs, bones, and central nervous system. They can also be divided into groups according to how they were acquired and their level of pathogenicity. Either primary pathogenic or opportunistic fungal pathogens are responsible for deep mycoses. Opportunistic pathogens infect immunocompromised patients and invade through the respiratory tract, alimentary tract, or intravascular devices, as opposed to primary pathogenic fungi, which can infect a normal host and enter through the respiratory system.[1,3]

The gold standard for identifying fungal species is tissue culture. However, there are a select few cases where the physical traits of fungi are distinctive, such as in Pneumocystis and Rhinospoidiosis, which can only be identified through histological analysis and not through culture. Histopathology is still a quick and inexpensive diagnostic method for making a firm diagnosis of an invasive fungal infection. Similar to this, histopathology is essential for assessing tissue invasion and host response to the organism.[4,5] As a result, histologic examination of tissue sections provides a quick and simple method for identifying fungal species and an effective auxiliary to microbiologic culture in the diagnosis of fungal infections.[1,6]

Typically, there won't be any inflammation with superficial mycosis. In contrast, inflammation is induced in the epidermis and around the hair and nail in cutaneous

infections. Additionally, when there is a subcutaneous infection,the inflammation first appears in the subcutaneous tissue before it may subsequently spread to the epidermis. Other methods, including immunohistochemistry, in situ hybridization, and PCR, may be employed to identify the precise agent present in the histopathologic specimen.[2,7]

Materials and Methods

The pathology department at Sri Krishna Medical College in Muzaffarpur, Bihar, carried out this investigation for six months, from November 2022 to April 2023. In this investigation, a total of 47 cases were examined. Each case's sex, lesion site, and clinical diagnosis were recorded. All of the small biopsy specimens were embedded, and maximum number of bits were removed from large specimens. Hematoxylin and eosin (H&E) was used to stain all of the sections, along with Gomori methenamine silver (GMS) and Periodic acid Schiff stains as necessary to identify fungi. All patients underwent a histological examination to determine the morphology of the fungus, as well as whether necrosis, granulomas, calcification, and inflammation were present.

Ziel Neilson's stain was employed in every case of the histologic assessment of granulomatous lesions to rule out thepresence of acid-fast bacilli and special stains to identify fungus. Fungi typically appear in the tissue section as hyphae, budding yeast, spherules, or a combination of these. We can assess the host reaction and find other microorganisms using the hematoxylin and eosin stain. In H&E-stained sections, there is a potential of missing fungus when they are thinly dispersed or weakly stained. The morphologic characteristics of some fungal lesions, such as Histoplasmosis and Blastomycosis, may be deceptive and difficult to interpret because they may exhibit cytoplasmic retraction artifacts or size variations that make morphologic evaluation challenging. Some fungus may occasionally have pseudohyphae, or the therapy may change the morphology of the fungi in tissue sections. Hence For the histopathologic analysis of unexplained inflammatory lesions, special fungal stains are crucial. The two most popular special stains used to identify fungi are Gomori Methenamine Silver (GMS) and Periodic acid-Schiff (PAS). "Broad spectrum" fungal stains include periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS). Evenin case where fungi are degenerated and non-viable, GMS gives better contrast hence it is highly preferred for screening.[4,8,9]

Results

Out of these 47 cases, 30 cases were male with a male predominance and 17 cases were female. With a mean age of 45 years, the age range included 10 to 80 years. In 17 cases of our study, aspergillosis accounted for a sizable portion of all fungal infections, followed by 9 cases of candidasis, and 3 cases each of Actinomycosis and Dermatophytosis, Maduramycosis and Rhinosporidiosis as well as 2 cases of Mucormycosis. Specific etiology was not possible in five instances, which were classified as having fungal elements in four cases and subcutaneous mycosis in one case, one case of phaeophypomycosis and Sporotrichosis.

The foot was the location of infection in 13 of the cases, followed by the hand and the nasal cavity. There was necrosis in 14 cases, granuloma formation in 24, significant inflammation in 18, and calcification in one case. The clinical manifestations, histological diagnosis, and host response to mycosis are summarized in Tables 1, 2, and 3. The morphologies of several fungi are compiled in Fig. 1 to 5.

Table 1: Spectrum of clinical presentation

	No. of cases	Percentage
CNS	2	4.3%
Nasal Cavity	7	13%
Maxilla	1	2.3%
Paranasal Sinus	3	6.8%
Wrist	3	6.5%
Elbow	2	4.3%
Forearm	2	4.3%
Hand	7	13%
Foot	13	27.6%
Leg	4	8.6%
Thigh	1	2.1%
Ankle	1	2.1%
Skin Plaque	1	2.1%
Total	47	100.0%

Table 2: Histopathological diagnosis

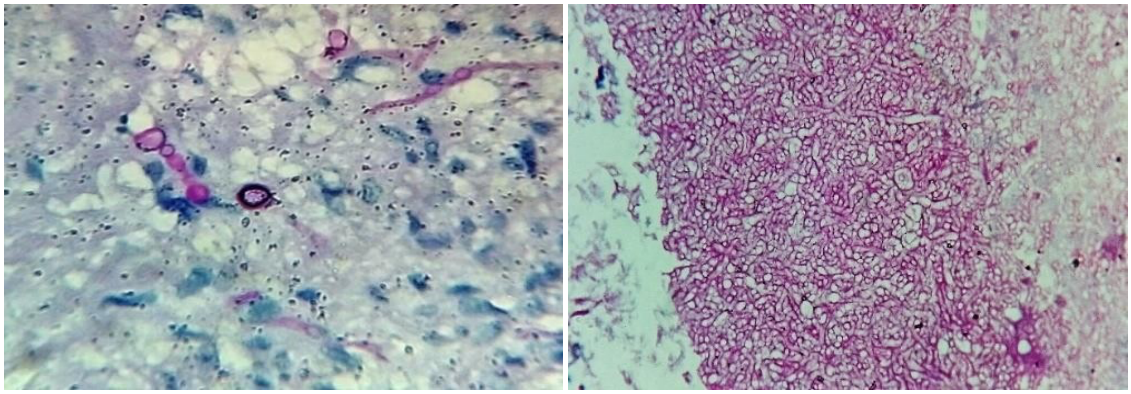
	No. of cases	Percentage
Phaeophypomycosis	1	2.1%
Aspergillosis	17	36.0%
Candida	9	18.8%
Dermatophytosis	3	6.5%
Maduramycosis	3	6.5%
Mucormycosis	2	4.3%
Actinomycosis	3	6.5%
Positive For Fungal Elements	4	8.6%
Rhinosporidiosis	3	6.5%
Sporotrichosis	1	2.1%
Subcutaneous Mycosis	1	2.1%
Total	47	100.0%

Table 3: Host response to Mycosis

	No. of cases	Percentage
Necrosis	14	29.7%
Granuloma	24	51%
Marked inflammation	18	38.29%
Calcification	1	2.1%

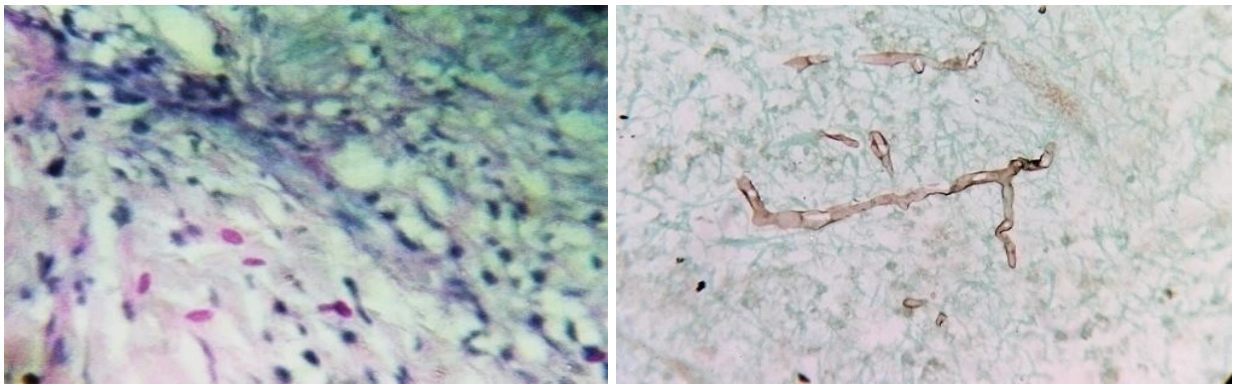
Table 4: Clinical diagnosis of Aspergillosis

Clinical Diagnosis	No. of Cases
Ganglion	6
Dermoid Cyst	5
Abscess	1
Dermatofibrosarcoma	1
Sebaceous Cyst	2
Cyst	1
Swelling	1
Total	17



A B

Figure 1: (A) Pheohypomycosis, pigmented hyphae and yeast forms 400x (H&E); (B) Aspergillosis, thin acute angled septate hyphae forming fruiting body 400X (PAS)



C D

Figure 2: © Candidiasis, Yeast forms 400x (PAS); (D) Mucormycosis, non-septate right angled broad branching hyphae 400x (GMS)

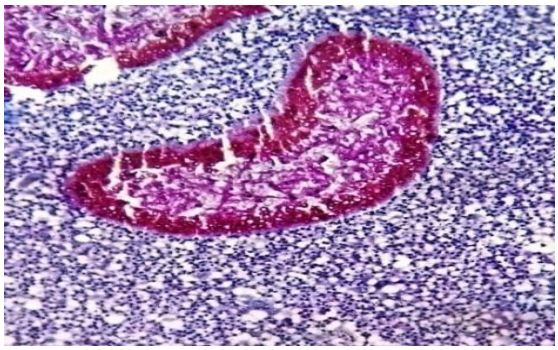


Figure 3: Neutrophilic abscess with center granule of Maduramycosis bordered with Splendore-Hoeppli material400X (PAS).

Phaeohyphomycosis is caused by dematiaceous fungi with cytoplasmic melanin like brown to black pigments fungi.

Discussion

The evaluation of the histopathology remains the accurate way of identifying specific pathogens and aids in the identification of infectious fungal species. A microbiological analysis cannot distinguish between fungi that are contaminated and harmful fungi that are colonizing. Not all fungi are cultivable. The challenges in separating fungi that are colonizing and those that are contaminating have been demonstrated by Guarner, Brandt, and Sundaram et al.[2,10]

Exophiala jeanselmei, E. spinifera, and Wangiella dermatitidis are the most often isolated dematiaceous fungi that cause a heterogeneous collection of fungal illnesses known as phaeohyphomycosis. The presence of pigmented yeast-like and hyphae forms, but not sclerotic cells, muriform cells, or copper pennies as observed in chromoblastomycosis (Picture A), helps to identify them as the slowly progressing infections of the superficial, cutaneous, subcutaneous, and deep visceral tissues. These infections, which can be fatal, can affect a variety of immunocompromised patients.[1,3,5,11-14]

The histopathological cyst wall in phaeohyphomycosis is composed of fibro-collagenous tissue and granulomatous inflammation with several large cells. The lesions contained yeast-like entities and septated hyphae, both of which were fungi. They typically manifested as sebaceous and ganglion cysts, and histological examination revealed tissue reaction in the form of necrosis, inflammation, granuloma, and calcification. The use of histopathology in etiologic diagnosis enables the detection of fungi in clinically undetected instances.

Aspergillosis was the most frequent fungus in our analysis, appearing in seventeen cases. Two cases of aspergillosis manifested as a brain lesion that took up area. Thin, acute-angled, dichotomous branching hyphae with septations are indicative of aspergillosis (Picture B). Degenerated or necrotic hyphae will seem hyaline or eosinophilic, but healthy hyphae would typically be deeply basophilic. The most prevalent species of aspergillosis is aspergillosisfumigatus, and immunocompromised hosts can become unwell when exposed to A. niger. Allergy-related bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis/aspergilloma, and invasive or systemic aspergillosis are the three types of aspergillosis.[2,5,15]

In ABPA, the lesions may exhibit invasive aspergil-

losis in the form of microabscesses with sparse hyphae, vasculitis, interstitial fibrosis, and granulomatous inflammation. A mass of fungus hyphae with a necrotic background is called an aspergilloma. They produce a fungal ball or fruiting body that develops in the lung cavity that already exists as a result of tuberculosis. Typically, fibrosis surrounds these holes. Granulomas occasionally accompany the Splendore-Hoeppli syndrome in Actinomycosis. Other fungi, such Coccidioides immitis and Pseudallescheria boydii, can occasionally create fungus balls. Aspergillus species' typical hyphae are homogeneous, narrow, and uniformly septated. They also have regular, dichotomous, and acutely angled branching. The lungs and paranasal sinuses are affected by invasive aspergillosis, which also affects the brain, liver, kidneys, heart, and bones. As shown in Picture C, we had five cases of candidiasis, four of which were in middle-aged females and one of which was in a nasal cavity. The most frequent species that causes candidiasis is Candida albicans. Typically, it colonizes the oropharynx and vagina. In cases of microbial imbalance brought on by the use of antibiotics, hormones, and in people with immunocompromised conditions like diabetes, cancer, or HIV, it can result in either a superficial or deep invasive infection. Candida can be seen under a microscope as yeasts mixed with pseudohyphae that occasionally constrict. Pseudohyphae are not formed by C. glabrata. Invasive candidiasis is distinguished from colonization by its invasion of tissues and blood vessels.[1,2,4,5,15]

Three cases of mucormycosis were observed in our investigation (Picture D). Out of which one instance had a clinical maxillary cancer diagnosis. They can be identified by the broad, crooked hyphae that branch at a straight angle. Hyphae can occasionally be septate or nonseptate, as it does most frequently. Due to their lack of structural stability, the hyphae are frequently folded, twisted, wrinkled, or collapsed. In the tissues, chlamidoconidia can occasionally be observed in ovoid forms. Clinical manifestations of mucormycosis include cutaneous, gastrointestinal, pulmonary, rhino-orbito-cerebral, pulmonary, and cutaneous manifestations. Following trauma, mucormycosis is uncommon in immunocompetent hosts but prevalent in immunocompromised patients.[2,4,5,15] Three Maduramycosis cases were reported by us (Picture E). Out of which, one instance had an odd wrist appearance. Mycetoma is often divided into two categories: eumycotic and actinomycotic mycetoma. Maduramycosis is characterized by draining sinus tracts that include visible pigmented grains that range in color from brown to black and are made up of the fungal microcolonies that are the source of the illness.[1,2,15]

Three cases of rhinosporidiosis were observed. The most prevalent species causing this rhinosporidiosis is R. seeberi, which is characterized by polyploid tumors in the nose, nasopharynx, and eye area. Their spherical sporangium, which has endospores in various phases, serves as an identifying feature. They may very rarely spread to other mucous membranes, skin, or viscera.[2,4,15]

Two Dermatophytosis instances were reported by us. The spores (arthroconidia) and branched, septate hyphae that infiltrate the stratum corneum, hair follicle, hair shafts, and nails are used to identify dermatophytes in tissue sections. They consist of the following three organisms: Epidermophyton, Trichophyton, and Microsporum.

The dermatophytes can best be seen with GMS and PAS stains because they have hyaline hyphae. They can be found in the stratum corneum between the upper normal and lower aberrant stratum corneum. The pattern of the hair invasion may be ectothrix, endothrix, or endoectothrix. They can result in either severe mononuclear inflammation in the form of Majocchi's granuloma or severe neutrophilic inflammation of the hair follicles and shafts known as kerion.[1,2,4]

Sporotrichosis, which is brought on by the sporothrix schenckii fungus, happened to us once. When viewed under a microscope, they resemble round, oval, or cigar-shaped yeasts and may exhibit tube-like budding. Because S. schenckii yeasts are difficult to identify using H&E stains, GMS and PAS stains should be used instead. Asteroid bodies, which are star-shaped masses of eosinophilic material surrounding the yeasts, are sometimes detected in sporotrichosis instances. Using immunohistochemistry, Sporothrix has been shown to exist in these asteroid bodies.[1,2,5]

Conclusion

As part of this investigation, we sought to identify the etiological diagnosis of fungal infections in histopathological specimens received by our department. Thus, a reliable method of identifying pathogenic fungi and the host response is the histopathologic investigation.

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