

Fungal infection in surgical pathology – Our institutional experience

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ABSTRACT

Background: Fungal infection has a worldwide prevalence and it affects almost all age groups and both the sexes. Due to neoteric treatment modalities nowadays, people are enjoying longevity of life. However, on the other hand, immune status is compromised that has resulted in increased prevalence of fungal infection in society. Fungi reported by surgical pathologist, either incidentally or in clinically suspicious cases, greatly improve morbidity and outcome of the patients.

Objective: The objective of this study is to study the prevalence of fungal infection in surgical pathology tissue and frequency and spectrum-based analysis in relation to age, gender, and systemic involvement. **Materials and Methods:** Ethically approved retrospective observational secondary data analysis is done based on histomorphological and cytomorphological evaluation of 17 fungal cases diagnosed at the Department of Pathology, New Civil Hospital, Surat, from January 2015 to January 2017. **Results:** Out of 17 cases, most common fungus seen was *Candida albicans* mainly affecting gastrointestinal tract. Common age group affected was 41–60 years and few rare fungi, i.e., mucormycosis and tinea corporis were also reported. **Conclusion:** Distribution of fungi in tissue section in relation to frequency, age, sex, various organs, and its correlation with clinical symptoms is analyzed in our study and results are found to be yielding that provided outlook in the prevalence of infectious agent.

KEY WORDS: *Aspergillus*; *Candida albicans*; Eumycetoma; Fungi; Periodic Acid-Schiff; Grocott's Methenamine Silver

INTRODUCTION

Fungal infection has a worldwide prevalence and it affects almost all age groups and both the sexes. Fungal infections can be encountered by many measures, i.e., aerosols, soil contact, water contact, or even by decrease in immunity level. In patients, with or without suspected clinical diagnosis, cytology smears are imperative in diagnosing fungal infections. Being an easy and rapid method, it helps clinician to start treatment immediately. Moreover, the dilemma of whether it is a contamination, colonization, or true infection can be solved by histopathological examination

where tissue invasion and inflammatory reaction can be easily demonstrated. Henceforth, for the diagnosis of certain pathogen, i.e., *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) and *Rhinosporidium seeberi*, histopathology examination is essential.^[1-3]


MATERIALS AND METHODS

A hospital-based retrospective observational study of secondary data analysis of 3 years duration (January 2015–January 2017) is done based on histomorphological and cytomorphological evaluation of 17 cases of fungi diagnosed at the Department of Pathology, New Civil Hospital, Surat.

Inclusion Criteria

The following criteria were included in the study:

- Fungal infections diagnosed histopathologically in tissue sections

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- Fungal infections diagnosed cytomorphologically in FNA smears.

Exclusion Criteria

The following criteria were excluded from the study:

- Fungal infections diagnosed in brush and exfoliative cytology smears (*Papanicolaou* [PAP])
- Smears from fluid cytology
- Autopsy cases.

RESULTS

Analysis of 3-year data from histopathology section and cytopathology section was done.

As shown in Table 1, the prevalence of fungi in tissue section was <0.5% and it varied in different years. In our study of 3-year duration, maximum number of fungal infection were seen in 2016 (42.8%) which decreased in 2017 (19%). In 2016, fungal infections were more than parasitic one.

Table 1: Prevalence of fungi

Diagnosis by	Number of specimens	Parasite	Fungi	Total number	%
Histopathology	11,432	23	11	34	0.3
Cytopathology	10,005	02	06	08	0.07

Table 2: Age groups affected by fungi

Age group (years)	Number	%
0–20	02	11.8
21–40	04	23.5
41–60	09	52.9
61–80	02	11.8
Total	17	100

Table 3: Clinical presentation

Symptoms	Number of cases
Peptic perforation	06
Soft tissue swelling	05
Skin lesion	03
Nasal polyp/mass	02

Table 4: System wise distribution of various fungi

Fungi	Number (%)	Gastrointestinal tract	Soft tissue	Skin	Respiratory
<i>Candida albicans</i>	07 (41.17)	04	01	00	02
<i>Aspergillus</i>	03 (17.6)	00	00	00	03
Eumycetoma	02 (11.7)	00	01	01	00
Rhinosporidiosis	02 (11.7)	00	00	00	02
Mucormycosis	02 (11.7)	02	00	00	00
Tinea corporis	01 (5.8)	00	00	01	00
Total (%)	17 (100)	06 (35.3)	02 (11.7)	02 (11.7)	07 (41.1)

As per Table 2, commonly affected age group by fungi was adults. Females (59%) were more commonly affected than males (41%). As per Tables 3 and 4, commonly affected systems were gastrointestinal tract (GIT), skin, and soft tissue. In GIT, cases of mucormycosis affecting esophagus in HIV-positive patient, *Candida albicans* affecting pylorus (presenting as perforation – a surgical emergency) were noteworthy. The most common fungus found in our study was *C. albicans*. Multisystem affection by various fungi was seen. Fungi *Aspergillus* and rhinosporidiosis were affecting respiratory system. Cases of eumycetoma in patient having discharging granules from foot ulcer and tinea corporis in arm swelling were also reported.

DISCUSSION

Fungi are broadly classified into two types: Superficial and deep mycosis. In pathology, mostly, we encountered deep fungi in tissue section. *Candida* spp. affect both skin, appendages, subcutis, and various organs, whereas *Aspergillus*, actinomycosis, histoplasmosis, rhinosporidiosis, etc., are deep fungi causing systemic infections. Patients with invasive and non-invasive fungal infection can present with various complains, i.e., destructive mass lesions and other mimickers of malignancy. In pre-operative cases, cytology diagnosis can prevent the need for biopsy which saves time and money of both, the patient and the clinician. Specific tissue reaction, i.e., eosinophilic infiltration, xanthogranulomatous reaction, and foreign body granuloma with or without necrosis provide clue to look actively for them in tissue section other than incidental diagnosis. Identification of specific fungal profile can solve the cases many times. Fungi can be present both intra- and extracellularly. There are few mimickers of fungal profile in surgical pathology. For instance, organic or non-organic fibers, stained bare nuclei, erythrocytes, and lymphocytes. They can sometimes misguide the pathologist. In such cases, few ancillary techniques can help in reaching the diagnosis. For example (1) special staining with periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) stain which stains hyphae of fungi in magenta and black color respectively, and (2) autofluorescence under fluorescent microscope.^[4-7]

The main strength of this study is that it gives the most comprehensive picture of the state of the prevalence of

fungal infection in surgical pathology tissue and specimens (histopathology and fine-needle aspiration cytology [FNAC]) in South Gujarat Zone. In general, many studies have been conducted for the detection of fungi in swabs, sputum, culture media, and other specimens in microbiology. However, studies done about fungi in tissue section are limited till date. Hence, ours is a novel study stating that fungi can be the main pathology affecting various systems. In many cases, fungi were incidentally found, without any clinical suspicion.

The most common fungi found in our study was *C. albicans*. In our study, we have excluded positive cases in PAP smear because it biased our study due to great increase in number of *Candida* spp.

The most common age group affected was 41–60 years, females were more affected in our study. The most commonly affected system was gastrointestinal system. The most common fungus in the study of Gochhait *et al.* was aspergillosis.^[8] The appearance of non-pathological fungi, i.e., zygomycetes in tissue section in our study is justified due to increase in number of immunocompromised patients. This finding correlates with a study of Jeannette and Brandt.^[9] These alterations in distribution of fungal infections can be due to myriads of factors. To exemplify, changes in habitat, ease of traveling, migrating population, and changing human life habits are few such factors. Besides, recently, due to increase in number of transplant receiving patients, immunosuppressive and chemotherapeutic therapies, HIV infections, premature infants, elderly population, and patient undergoing major surgeries at-risk population for opportunistic fungal infections are increasing which are responsible for shift in the mycoses, encountered recently in the health-care sector.^[9]

Nevertheless, limitation of the study is that there are many interpersonal discrepancies in reporting of fungi. Few cases were broadly given as “Fungal infection.” Recent advancement like molecular-based techniques is not used for further confirmation and classification of fungi due to lack of resources.

We encountered a total of seven cases of *Candida* spp., out of which four were in GIT, presenting as peptic perforation, two cases reported in lung biopsy, and one case of soft tissue swelling. Two patients were post-operated who presented with peptic perforation later and one patient who presented with lung abscess was intubated for the past 5 days. These reasons explained finding candida at unusual sites. In tissue section, *Candida* fungi appear as matted yeast forms of 3–5 mm diameter which are budding at places and intermingled with pseudohyphae. These hyphae may show periodic constrictions [Figure 1]. It is also difficult to differentiate *Candida glabrata* from *C. albicans* which does not produce filaments. Histopathological specimen examination is very important to define invasion of tissue and vessels since growth from skin, lung, GIT, and genitourinary tract is only indicative of colonization.^[10]

We diagnosed two cases of *Aspergillus* in lung biopsy and one case in lung FNAC. Out of three, two were of old age and both were chronic smoker. Fungus *Aspergillus* is also a mold that reproduces by asexual method, producing non-branching chains of conidia from a bulbous structure, which is called vesicle [Figure 2]. In tissue, *Aspergillus* infection manifests in three different manners: (1) Allergic bronchopulmonary aspergillosis, (2) Chronic pulmonary aspergillosis/aspergilloma, and (3) Invasive or systemic aspergillosis.^[11] Besides, *Actinomyces*, which are recognized by their radiating filaments, may also appear in bronchoalveolar lavage specimens which can be due to oral contamination.^[8,9]

In our study, two young patients presented with polypoidal nasal mass and diagnosed as rhinosporidiosis. Causative agent for this chronic infective disorder is *Rhinosporidium seeberi*. Most commonly patients come with soft, fleshy, sessile/pedunculated polypoidal mass in nose/nasopharynx and to a lesser extent into conjunctiva, maxillary sinus, penis, and urethra. Definitive diagnosis depends on identification of sporangia in various stages of maturity, enclosed in a thin chitinous wall. The diameter of sporangia is 50–1000 um, whereas the diameter of numerous endospore is 5–10 um each [Figure 3]. These thick-walled sporangia and endospores stain positively with various special stains, i.e., PAS, mucicarmine, GMS stain, etc. As a reactive process, hyperplasia of overlying epithelium and infiltration by lymphocytes, macrophages, plasma cells, and even polymorphonuclear cells within fibrovascular stroma can be seen. Occasionally, rupture of sporangia can elicit giant cell reaction. Eosinophils, which are common in other fungal infection, not so commonly encountered in rhinosporidiosis.^[6]

In our study, we found two cases of zygomycetes. Finding of mucormycosis molds should be considered the cause of disease. Out of two, one was an incidental finding and another patient was patient living with HIV presented with difficulty in swallowing. His esophageal biopsy showed broad-based, non-septate hyphae. Focally, fungi having pauciseptate hyphae with non-dichotomous branching were also seen which was reported as zygomycetes. However, one always needs to keep in mind close differential diagnosis – *Aspergillus* spp. which has septate hyphae with dichotomous branching.^[12,13]

In our study, we did not find any case of cryptococcosis, however, it is common to find in today's scenario as immunocompromised states have increased. *Cryptococcus* spp. includes two categories: (1) *Cryptococcus neoformans* and (2) *Cryptococcus gattii*. On FNAC, it appears as numerous spherical encapsulated yeast cells. Few macrophages can be seen in background. More precisely, special stain such as pas and mucicarmine can highlight the capsule of fungi. However, the diagnosis needs high index of suspicion because the main histological presentation as granulomatous disease can be seen in plethora of other conditions.^[1,13,14]

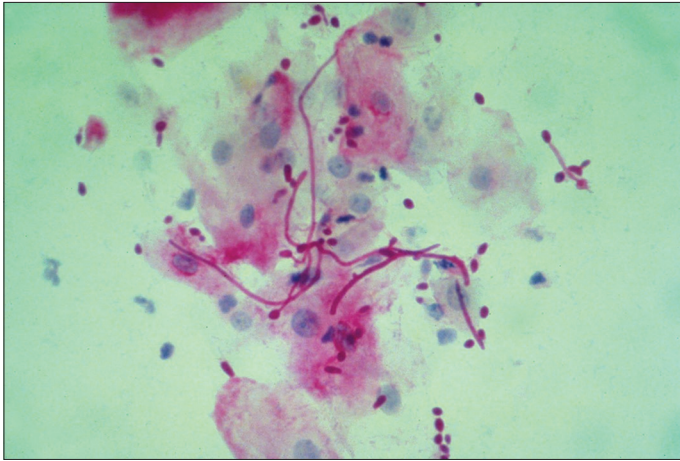


Figure 1: *Candida albicans*

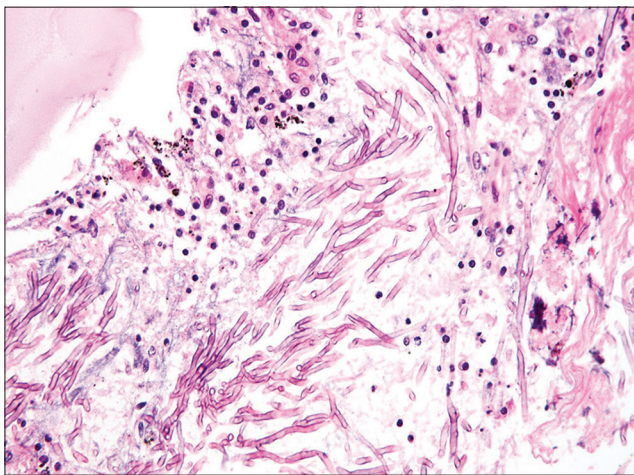


Figure 2: *Aspergillus*

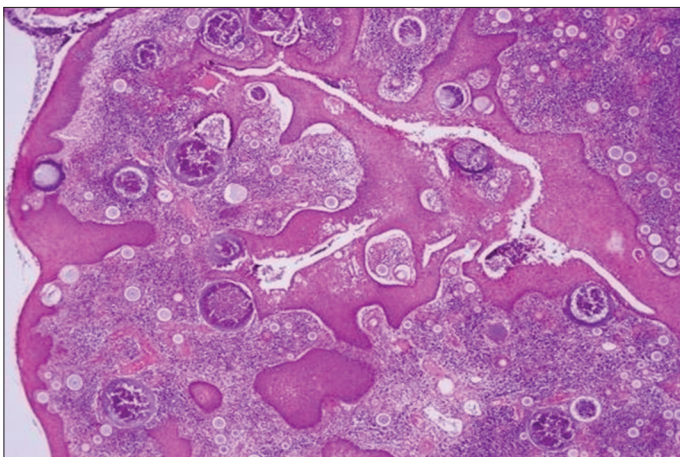


Figure 3: Rhinosporidiosis

As reflected in our study, incidence of deep fungal infection is increasing in today's era due to multiple causes. Histopathological diagnosis of fungal infection is a herculean task as morphology of many fungi is similar. There are many close differential diagnoses for histopathological reporting of fungi like differentiation between *Candida* and *Histoplasma*, *Candida* and *Cryptococcus* and so on (due to

tangential cutting) which are really challenging. For that, few specific points should be kept in mind, i.e., (i) *C. glabrata* lack pseudohyphae production, (ii) *Candida* spp. typically generate a suppurative tissue reaction, (iii) *Histoplasma* spp. is likely to elicit more granulomatous reaction, and (iv) *Cryptococcus* is recognized by mucicarmine positive yeast which is surrounded by a distinctive empty space that indicates the presence of a thick capsule.^[13,15-20] When compared with conventional histology, some recent methods such as immunochemistry, fluorescent antibody staining, molecular methods, and/or *in situ* hybridization have proved to be better in identification and classification of yeast and hyphal organisms.^[19,20] However, most of these technical advancements are not easily commercially available and may also require technical and interpretative proficiency. Henceforth, there are still not in widespread use today.^[1,15]

For universal homogeneity and standardization for reporting of fungal infection in tissue, few basic implementations are required in reporting format. For instance, deemphasizing attempts for definitive species identification and providing notes and advice. One such template is exemplified in ensuing paragraph.^[13]

For yeast-like organisms:

1. Diagnosis – Yeast with pseudohyphae (refer comment)
2. Comment – Morphologic findings are suggestive of *Candida* spp. The differential diagnosis includes other molds (septate/non-septate hyphae)
3. Note – As accuracy of correct fungal identification on tissue sections is averagely estimated at 70–80% based on recent studies, correlation with cultures is recommended if clinically needed.

In some cases, comments about invasion/extent/tissue necrosis can be added if possible.

CONCLUSION

Increasing change in life habits, overuse of antibiotics, and immunocompromised states are major concerns in today's time, calling for cautious eye to look for fungi in tissue sections. In cases of higher clinical suspicion, even with no fungal evidence in hematoxylin and eosin slides, special stain may highlight the organisms. As there is no vaccine treatment for such lesions, active search followed by systemic medications and/or topical applications can lead to great decrease in morbidity and mortality.

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