Clinicopathological Study of Fungal and Parasitic lesions in Histopathology - An institutional experience; Are special stains Obligatory?

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Abstract

The incidence of fungal and parasitic lesions has been on the rise over the decades. In our study, we assessed and studied the spectrum of fungal and parasitic lesions retrospectively for one year and their clinicopathological correlation which was diagnosed on histopathological examination in a tertiary health care center. The present study has been done to analyze the incidence of fungal and parasitic infections obtained in the histopathology laboratory and study its clinical correlation and significance. Clinical details of the cases were accessed from biopsy requisition forms and included tissues from various sites in the body. Special stains were done which included periodic acid Schiff (PAS) and Grocott’s methenamine silver (GMS) for the identification of parasites and fungus. A total of 49 cases including 39 fungal infections and 10 parasitic cases. The fungal infections were dominated by 19 cases of phaeohyphomycosis followed by 8 cases of candidiasis, 4 cases each of mucormycosis, eumycetoma, and 2 cases of aspergillosis, and in the case of each rhinosporidiosis and sporotrichosis. The parasitic category included 3 cases of Hydatid cyst, 2 cases of Enterobiusvermicularis, and one case of filariasis, tapeworm, and neurocysticercosis. The histopathological diagnosis of fungal and parasitic lesions is of utmost importance as it is more reliable than culture in circumstances of reduced tissue availability. Early reporting is extremely necessary to reduce complications related to fungal and parasitic lesions.

Keywords: Parasites, Fungi, special stains, culture.

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Introduction:

Fungi (from the Latin fungus, meaning mush-room) are eukaryotic saprotrophic organisms. Fungal infections or mycoses may be caused by pathogenic fungi that infect healthy individuals or by opportunistic fungi in immunocompromised individuals. Mycoses are encountered more frequently due to the increasing proportion of the at-risk population in the society are post-transplant patients, premature infants under chemotherapy or immunosuppressive treatment, the elderly, debilitating illnesses and infections like HIV(1)

The pathologist plays a crucial role in the diagnosis of both routine and new upcoming infectious diseases. The fungal infections can be diagnosed by many methods like clinical features, histopathological examination of tissues, fungal culture, and other techniques like direct fluorescence antibody, immunohistology, enzyme immunoassay, in situ hybridization, PCR, etc. Even though culture is a gold standard for identification of fungus, the histopathological examination of tissues is more diagnostic than the culture because of the inadequate tissue samples in formalin, and also delay in the isolation of the organisms by the presence of unwanted material like fibers, dead tissue. For the diagnosis of fungal infections, the pathologist should be knowledgeable about fungal morphology in tissue and their reactions in response to the infection in various tissues(2).

Most of the time special stains are required to identify the morphology of fungus in tissues because unable to identify in the hematoxylin and eosin stains. The morphological features are more important to differentiate different types of fungi based on the presence or absence, thick or thin capsule, budding yeast forms either single or multiple, hyphal forms which are septate or non-septate, branching or non-branching, the wide-angle or acute angle of the septa, etc. The yeast form of dimorphic fungi can be formed and present in human body temperature and mold forms at room temperature(3)

In addition to fungal infections, Parasitic infections are a major public health problem. Most of the developing countries are more prone to intestinal and extra-intestinal parasitic diseases(4)The frequency and incidence of parasites also vary depending upon the age, sex, and geography(5)In developing countries the morbidity and mortality increased due to the increased prevalence of nutritional deficiencies, chronic diarrhea, immunocompromised states, and impaired physical development in children(6,7) the parasitic infections also produce Specific tissue reaction i.e. eosinophilic infiltration, foreign body granuloma and xanthogranulomatous reaction which gives clue to search the organisms in tissue section other than incidental diagnosis. If the parasite is dead/calcified, there is no evidence of tissue reaction.

So the incidence of fungal and parasitic infections is increasing steadily, hence active search of these organisms in tissue sections in correlation with clinical history is important for early diagnosis, treatment, and complications (8). The study aimed to detect the various types of fungal and parasitic infections and their distribution according to age, sex, clinical presentation, and site of infection in histopathological specimens received in the pathology department, Saveetha Medical College.

Materials and Methods:
The study was carried out in the department of pathology, Saveetha Medical College, for a period of one and a half years from 2018 January to 2019 June. in this study, we included all histopathological specimens received during the study period in both clinically suspected cases and incidentally detected fungal and parasitic infections Along with specimens, the relevant clinical data was obtained from the request form and the tissues were fixed in 10% formalin, processed and the sections were stained with hematoxylin and eosin stain and special stains like Periodic acid Schiff and Gomori's methenamine silver stain were used whenever required. The study was approved by the institutional ethics committee (IEC)

Results:

This study was done from 2018 January to 2019 June. The total number of fungal and parasitic organisms is 48. Out of 48 cases, 38(79.2%) cases were fungal and 10(20.8%) cases were parasites in various tissues of different sites, as shown in Tables 1 and 2. The commonest fungal lesion was phaeohyphomycosis (19 cases 46.3%). The next most common fungal lesion was candidiasis (8 cases 19.5%), followed by mucormycosis(4cases,10.5%), eumycetoma (Madura mycosis 4 cases10.5.%), aspergillosis (2 cases,5.3%) followed by each case of rhinosporidiosis and sporotrichosis in different sites.(Fig/Table-5 , 6)The most common site of infection was the upper limbs and lower limbs (26) followed by the Nasal cavity (4). The most common sites of infection in the upper limb were elbow, forearm, wrist, legs, foot, and knee in the lower limb. The other sites were skin and lungs. The most common type of infection in the nasal cavity was Mucormycosis followed by Rhinosporidiosis, and Aspergillosis and the other types of infections were not seen in the nasal cavity

The most important parasites diagnosed in our study are hydatid cyst (3 cases,30.%) followed by Enterobiusvermicularis (2 cases,20%), cestode (1case,10%), and neurocysticercosis(1 case,10%), cutaneous myiasis(2 cases,20%). The sites of hydatid cyst were the liver, spleen, and lung. The rare case of cestode (tapeworm) was in the eye and neurocysticercosis in the 4th ventricle. Cutaneous myiasis was found in diabetic foot and pinworm in the appendix(Fig/Table – 7))

Infectious diseases are more common in males than females and no cases were seen below 10 years of age. Fungal and parasitic infections occur in the age group of 11 to 80 years and from 11 years to 60 years.

In fungal infections the males to female ratio was (23 cases)60.5% , to(15 cases) 39.5% followed by (8 cases)80% to (2 cases) 20 % in parasitic infections (Table3,4).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Fungal infections</th>
<th>Total number of cases</th>
<th>Site</th>
<th>Clinical presentations</th>
</tr>
</thead>
</table>

http://doi.org/10.36295/ASRO.2020.2323129
| 1. | Phaeohyphomycosis | 19 | i. Leg(4)  
   ii. Toe(2)  
   iii. Knee  
   iv. Forearm(3)  
   v. Elbow(6)  
   vi. Wrist(3)  
   vii. Thigh |
|---|---|---|---|
|   |   |   | a) Abscess(2)  
   b) Implantation  
   c) Swelling(4)  
   d) Infected  
   e) Ganglion(7)  
   f) Bursitis (2) |
| 2. | Mucormycosis | 4 | i. Nasal cavity (2)  
   ii. Middle meatal mucosa  
   iii. Thigh |
| | | | a), Chronic fungal sinusitis(2)  
   b) Mass  
   c) Ulcer |
| 3. | maduramycosis | 4 | i. Leg  
   ii. Forearm  
   iii. Left foot,1st web space |
| | | | a) Soft tissue tumor  
   b) Swelling  
   c) Corn foot |
| 4. | Candida infection | 8 | i. Scalp  
   ii. Antrum  
   iii. Below knee amputation  
   iv. 4th toe  
   v. Skin  
   vi. Aryepiglottic fold  
   vii. Foot  
   viii. Thigh |
| | | | a) Non healing ulcer  
   b) D1 growth  
   c) DM ulcer  
   d) 4th toe Ulcer  
   e) Drug induced lichenoid dermatitis  
   f) Hypopharyngeal growth  
   g) Ulcer foot  
   h) sebaceous cyst |
| 5. | Aspergillosis | 2 | Nasal cavity  
   Bronchial |
| | | | a) Fungal sinusitis  
   b) Left middle lobe lesion with hemoptysis |
| 6. | Rhinosporidiosis | 1 | Nasal cavity |
| | | | epistaxis |
| 7. | Sporotrichosis | 1 | Rt forearm |
| | | | Ulcerated plaques and papules |

Table 1. Clinical Presentation and site of infection of Fungal organisms

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http://doi.org/10.36295/ASRO.2020.2323129
<table>
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<th>Total number of cases</th>
<th>Site</th>
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<td>1.</td>
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<td>3</td>
<td>Liver, Abdomen, Spleen</td>
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<td>2.</td>
<td>Enterobiusvermicularis</td>
<td>2</td>
<td>Appendix</td>
</tr>
<tr>
<td>3.</td>
<td>Filariaisis</td>
<td>1</td>
<td>Spermatic cord</td>
</tr>
<tr>
<td>4.</td>
<td>Cestode</td>
<td>1</td>
<td>Eye</td>
</tr>
<tr>
<td>5.</td>
<td>neurocysticercosis</td>
<td>1</td>
<td>4th ventricle, Cystic lesion</td>
</tr>
<tr>
<td>6.</td>
<td>Cutaneous myiasis</td>
<td>2</td>
<td>Diabetic foot</td>
</tr>
</tbody>
</table>

Table 2. Clinical Presentation and site of infection of Parasitic organisms

<table>
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<th>Age</th>
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<th>Female</th>
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<td>3</td>
</tr>
<tr>
<td>21-30</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>31-40</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>41-50</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>51-60</td>
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<td>Total</td>
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<td>10</td>
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</table>

Table(3). Age and sex distribution of parasitic infections

<table>
<thead>
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<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21-30</td>
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<td>6</td>
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<tr>
<td>31-40</td>
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<td>7</td>
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<td>41-50</td>
<td>5</td>
<td>4</td>
<td>9</td>
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<tr>
<td>51-60</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>61-70</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>71-80</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>15</td>
<td>38</td>
</tr>
</tbody>
</table>

Table(4). Age and sex distribution of fungal infections

Discussion:

In developing countries like India, various demographic factors like increasing population, socioeconomic status culture, and poor hygiene are risk factors for the development of infectious diseases. The incidences of fungal infections are increased because of HIV, the intake of immunosuppressive drugs, etc (9).

There are many diagnostic methods to arrive at an etiological diagnosis for treatment. The methods are histopathology, culture, KOH preparation, antigen and antibody assay, and PCR methods. Among these cultures was considered the gold standard. But culture cannot help in identifying certain fungal organisms like rhinosporidiosis and also other organisms due to inappropriate sample. Where histopathological method is used for etiological diagnosis. The PCR is considered to be highly sensitive and specific but it has disadvantages like limited availability, high cost, and difficulty in maintaining, and required proper technique for the procedure (10).

In our study, the most important fungal organism was phaeohyphomycosis. The term phaeohyphomycosis was introduced by Ajello in 1974s. In our study, it was an incidental finding, clinically presented as ganglion, abscess, infected sebaceous cyst, bursitis, and swelling in the elbow, leg, forearm, and wrist location. Initially, it was diagnosed as fungal cyst or abscess in H & E staining sections, using special stains, periodic acid Schiff reagent (PAS), and Gomori's methenamine silver stain, these organisms were found within the cyst cavity or abscess wall (Fig-5) Abraham LK et al., (11) and Sidhalingreddy et.al (12) studies showed a lower incidence of phaeohyphomycosis in seven cases and one case respectively, but our study showed a higher incidence (19 cases).

The second most fungal organism in our study was candida. These are benign commensal in the skin, mouth, gastrointestinal tract, and vagina. If the skin or mucosal barrier is breached, then it may cause infection (3). The most common species are Candida albicans. In our study, the diagnosed candida infection (Fig-5) is also an incidental finding in patients clinically presented as nonhealing ulcers and malignant growth from esophagus. These organisms can be visualized in H&E, GMS, and PAS stains (13) as oval budding yeast forms or as pseudohyphae in this study also we identified candida by H&E, GMS, and PAS.

Mucormycosis is most commonly caused by Rhizopus or Mucor and frequently involves the sinuses, brain, or lungs. In our study mucormycosis (Fig-6) was seen in the nasal cavity, middle meatus, and ulcer thigh, with vascular invasion in one case. A study done by Vijay Kumar Gupta et al (14), showed mucormycosis 9/22 cases clinically presented as a mass in the sinus tract and ulcer, and Sidhalingreddy et.al (12) had 5/30 cases that occurred in the nasal cavity and one case in the foot which is compatible to our study.

Mycetoma is a chronic progressive infectious disease involving skin and subcutaneous tissue. The sites most affected are a foot, hand, and less frequently knee, arm, head, neck, thigh, chest, and the perineum. The characteristic symptom is swelling with an ulcer, draining sinuses, and extrusion of characteristic colored grains in the exudates. Rajan Shah et al (15) showed on a case of maduramycosis in the knee presented as swelling and Sidhalingreddy et.al (12) study showed 3 cases in the foot.

In this study, maduramycosis was commonly found in the leg, forearm, and foot which is correlating with the above studies. Microscopic examination by H&E, and special stains Periodic Acid Schiff (PAS) and Gomori stains were used.
Methenamine Silver (GMS) showed hyphae with transverse septae and the surrounding tissue shows Splendore Hoeppli material with acute inflammation (Fig-6)

The next fungal organism in this study was aspergillosis and a case of rhinosporidiosis and sporotrichosis.

Aspergillosis is the second most common opportunistic mycoses. The most common species is A. fumigatus. The most commonly involved organs are the lungs, nasal cavity, and sinuses (3,13). On microscopic examination (fig 6), the hyphae are uniform, narrow non-pigmented regularly septated, and branching at an acute angle on H&E stain.

Rhinosporidiumseberi is endemic in India, Sri Lanka, and parts of Africa (4) and causes mainly mucosal and cutaneous infections. It cannot be isolated on synthetic culture media, so histopathology is important for its diagnosis. A study done by Vinay Kumar Gupta et al showed 3/22 cases of cutaneous rhinosporidiosis. Sidhalingreddy et.al 12 cases of rhinosporidiosis, which was the commonest fungal organism and nasal cavity are the commonest site in their study (14,12). Our study showed one case of rhinosporidiosis in the nasal cavity.

Sporotrichosis is a rare, acute, or chronic fungal infection caused by the dimorphic fungus sporothrixschenkii. Also known as rose-gardener disease. The first case was reported in 1898 by Schenck [4]. Infection commonly results from percutaneous inoculation of infected wood splinters or thorns. Farmers, florists, and gardeners are most usually affected. In our case also the patient was a farmer by occupation. Brisa et al. presented a rare case of sporotrichosis presenting as an ear pinna lesion (16) Similarly Henry T. Lederer reported a case of osteoarticularsporotrichosis. In this study, it is clinically presented as ulcerated plaques and papules in the forearm.

The diagnosis of Sporotrichosis is established by culture and histopathology findings. In our case, the H&E sections were showing granulomatous inflammation and confirmed Sporotrichosis fungal infection with PAS and GMS special stains. (Fig 6)

Parasitic infections are a major public health problem in developing countries. Many studies have reported the prevalence of parasites in stool samples in various regions of India. But very few studies have been conducted on the presence of different parasites in the tissue section.

The most common parasites in our study were hydatid cyst followed by Enterobiusvermicularis, cutaneous myiasis, Filariasis, tapeworm, and neurocysticercosis. The most common parasite in a study by Manoharan et al. was cysticercosis and hydatid cyst, and studies by Gochhat, PranabDey, et al., was cysticercosis followed by hydatid cyst (8,17). This difference is due to geographical variation and climate changes in the distribution of parasite and life habits.

Most of the time symptoms are non-diagnostic and these parasites are diagnosed incidentally. Hydatid cyst (Fig-7) is a zoonotic helminth caused by Echinococcus. It is most prevalent in sheep grazing areas. Humans are intermediate hosts. The common sites of involvement are liver followed by lung and involvement of Spleen is rare. According to Gupta et al., cysts are found in the liver (55%--60%), lungs (30%), kidney (2.5%), heart...
(2.5%), bones (2%), muscles (1%), brain (0.5%) and spleen (1.5%) [18]. In our study, 3 cases of hydatid cyst seen in the liver, abdomen, and spleen.

In our study, a case of a larval form of filarial was seen in the spermatic cord. But a study done by A Manoharan(8) Filariasis affecting the genitourinary region is common. Diagnosis of the filarial parasite in the blood smear and Buffy coat is relatively easy, but detection of the parasite in the tissue section requires more focus where microfilaria or larval form or both can be seen (19). The diagnosis of E. vermicular, being the most common parasite found in appendix specimen (Fig- 7) operated for acute appendicitis, matches that of the study of Aydin et al. and Da Silva et al(20,21)

In our study, a case of neurocysticercosis(fig-7)in the 4th ventricle was reported. Human cysticercosis is caused by the pork tapeworm, Taeniasolium. It occurs due to fecal-oral contamination. The most common sites of occurrence of cysticercus are skeletal muscle, subcutaneous tissues, brain, and eye but can occur in lung, brain, The incidence is higher in developed countries, due to travel/immigration of NCC patients and/or tapeworm carriers from endemic zones by Hawk et al. (22)

In our study, two cases of myiasis were reported as diabetic foot ulcer. Myiasis an infestation of fly larvae (from the Greek word for fly, myia). In diabetic patients, myiasis is seen in wounded skin. In wound myiasis, fly larvae (maggots) are deposited on necrotic flesh and the larvae generally remain superficial, are usually alive, and can be identified grossly at the time of biopsy. A study done by SerhatUysa showed the largest reported series of 18 cases of diabetic foot with myiasis(23)

Ocular parasitosis in humans is more prevalent in geographical areas where environmental factors and poor sanitary conditions favor the parasitism between man and animals. In our study, a case of cestode in an eye was reported. The clinical diagnosis was a tapeworm. Microscopic examinations showed only parasite, no tissue reaction seen (fig-7).
**Fig/Table 5:** A – Fungal Abscess (H&E,40X), B – Fungal cyst wall (H&E,40X), C – Fungal septate Hyphae - Phaeohyphomycosis (PAS,40X), D – Fungal septate hyphae – Phaeohyphomycosis (GMS, 40X), E – Candida in eosophagus (H&E, 40X), F – Candida in eosophagus (PAS, 40X), G – Candida in diabetic foot ulcer (PAS,40X), H – Candida in diabetic foot ulcer (GMS,40X)

**Fig/Table 6:** A – Mucormycosis – Wide angled aseptate hyphae (H&E, 40X), B – Mucormycosis (PAS,40X), C – Mucormycosis (GMS, 40X), D – Madura mycosis (H&E,40X), E – Madura mycosis (PAS,40X), F – Madura mycosis (GMS, 40X), G – Aspergillosis, acute angled hyphae (40X, H&E), H – Rhinosporidiosis, sporangium with sporangiospores, (40X, H&E), I – Sporotrichosis, round to oval spores with in the dermal granuloma, (40X, GMS stain)
Conclusion: The fungal and parasitic diseases vary from study to study due to geographical area, environmental factors, and food habits. In our study, the commonest fungal and parasitic organisms are phaeohyphomycosis and hydatid disease. Early reporting is extremely necessary to reduce complications related to fungal and parasitic lesions.

The role of the pathologist is vital for the diagnosis of common as well as upcoming infectious diseases. The role of histopathology for the diagnosis of infectious diseases is well established. It requires a thorough knowledge of pathogens, the use of special stains, etc. It is useful particularly when culture has not been obtained.

Ethical Clearance – Institutional Human ethical committee were obtained from Saveetha Medical College and Hospital (SMC/IEC/2018/11/436)

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Conflict of Interest – None

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References


