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Post-COVID-19 fungal rhinosinusitis – Correlation between histopathological and microbiological findings

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ABSTRACT

Background: COVID-19 was a global pandemic. In the late December 2019, several patients presented with symptoms of pneumonia. Coronavirus is the pathogen frequently affecting respiratory system and gastrointestinal system. There was surge in the number of cases of COVID-19 associated with the fungal rhinosinusitis, mainly by Mucormycosis and *Aspergillus*. This study was done to analyze and correlate various fungal etiologies in post-COVID-19 patients presenting with fungal infections and correlating histopathological findings with frozen section, KOH mount findings, and fungus culture. **Aims and Objectives: (**1) The aims and objectives of the study are to identify the causative fungal species in biopsy specimens of clinically suspected post-COVID-19 fungal rhinosinusitis and (2) to correlate histopathology findings with frozen section, KOH mount, and fungus culture. **Materials and Methods:** A prospective study conducted over a period of 4 months between May 2021 and August 2021. Total of 30 nasal biopsies were included. Patients' data were obtained from department of pathology, Hassan Institute of Medical Sciences, Hassan. **Results:** Out of 30 cases, 66.67% were males and 33.33% were females with male-to-female ratio of 2:1. Majority of the cases were belonged to 41–50 years of age with mean age of 50 years. On histopathological examination, 56.67% cases were *Mucor*, 10% cases were mixed *Mucor* and *Aspergillus* infection. Out of 30 cases, 24 cases showed good correlation between histopathology and fungus culture. **Conclusion:** In our study, we found good correlation of histopathology with fungus culture findings.

Key words: COVID-19; Fungal rhinosinusitis; *Mucor* mycosis

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INTRODUCTION

COVID-19 was a global pandemic.[1] In the late December 2019, several patients presented with symptoms of pneumonia, with an epidemiological background of sea foods market and wholesale market in Wuhan, China.[2] Later, it was named as 2019 Novel (New) Coronavirus outbreak and the WHO coined the term COVID-19, on February 11, 2020.[2] By the end of January 2020, COVID-19 cases were reported in many parts of the world.[2] The case fatality rate was 2.2%.[3] Earlier to COVID-19, there were outbreaks of coronavirus which includes the severe acute respiratory syndrome-CoV and the middle east respiratory syndrome-CoV.[2]

Coronavirus is the pathogen frequently affecting respiratory system and gastrointestinal system.[2] Cases of neurological diseases have also been seen.[4] These viruses are large, enveloped, single-stranded RNA viruses affecting humans and domestic animals.[4] Symptoms starts appearing after an incubation period of 4–5 days. The symptoms include fever, rhinorrhea, cough, sneezing, sore throat, fatigue, sputum production, headache, hemoptysis, diarrhea, dyspnea, and lymphopenia.[2] Infiltrates in the upper lobe of the lungs are associated with dyspnea with hypoxemia.

Person-to-person transmission is the route of spread and occurs primarily through direct contact or through droplets spread by talking,[4] coughing, or sneezing from an infected

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individual. In severe COVID-19 infection, fulminant activation of coagulation and reduction of clotting factors occurs. Inflamed lung tissues and pulmonary endothelial cells may result in microthrombi formation and resulting in deep venous thrombosis, pulmonary embolism in critically ill patients. The development of viral sepsis, caused by a dysregulated host response to infection, further contributes to multiorgan failure.[4]

Paranasal sinus fungal infections have been considered uncommon and were previously thought to occur only in immune-compromised and diabetic individuals. India is the most affected country by mucormycosis, includes 44.3% of the worldwide cases, followed by the USA and Australia.[5] Inflammation of sinus mucosa is caused by fungi such as *Aspergillus*, *Candida*, *Mucor*, and *Penicillium*.[1] There was surge in the number of cases of COVID-19 associated with the fungal rhinosinusitis, mainly by Mucormycosis and *Aspergillus*.[6] The *Mucor* species is a commensal in the nasal mucosa of healthy people. In the presence of risk factors such as immunosuppression and diabetes, they tend to grow within the nose and paranasal sinuses and can progress up to rhino-orbito-cerebral-mucormycosis (ROCM). In COVID-19, CD4+T and CD8+T cells are decreased, resulting in impaired cell-mediated immunity and an increased predisposition to fungal infections.[6]

Symptoms in these patients include nasal stiffness, foul smell, epistaxis, blackish nasal discharge, nasal mucosal discoloration, locoregional pain or swelling, facial paresthesia, proptosis, ptosis, sudden loss of vision/diplopia, facial palsy, altered sensorium, and focal seizures.[6] Computed tomography is another modality to look for extent of tissue damage. Non-contrast CT is performed in many institutions and can show the extent of the mucosal involvement.[6]

This study was done to analyze and correlate various fungal etiologies in post-COVID-19 patients presenting with fungal infections and correlating histopathological findings with frozen section, KOH mount findings, and fungus culture.

Aims and objectives

1. To identify the causative fungal species in biopsy specimens of clinically suspected post-COVID-19 fungal rhinosinusitis
2. To correlate histopathology findings with frozen section, KOH mount, and fungus culture.

MATERIALS AND METHODS

Study place

Department of Pathology, Hassan Institute of Medical Sciences, Hassan.

Study design

Prospective study.

Study subjects

30.

Inclusion criteria

Nasal biopsies of clinically suspected post-COVID-19 fungal rhinosinusitis patients.

Exclusion criteria

Patients with missing or partial clinical history and autolyzed specimens were excluded.

Methodology

Our study was a prospective study conducted over a period of 4 months between May 2021 and August 2021. A total of 30 nasal biopsies were included in this study. Patient demographic data and clinical data were obtained from the request forms.

Specimens were received in the Department of pathology, HIMS Hassan. Specimens sent in saline were subjected to frozen sectioning and slides were stained with rapid hematoxylin and eosin. Specimens sent in formalin were subjected to routine histopathology processing and stained with hematoxylin and eosin. Periodic acid–Schiff stain was done for all formalin fixed tissues. All the slides were viewed to identify presence of fungal elements. KOH mount and culture results of the same lesions were obtained from the microbiology department. Results were correlated with KOH mount findings and fungus culture reports and also correlated with the frozen section findings wherever available.

RESULTS

Total of 30 cases were analyzed. Out of 30, 20 cases (66.67%) were males and 10 cases (33.33%) were females. Male-to-female ratio was 2:1 (Table 1 and Graph 1).

Out of 30 cases, the minimum age of the patient was 30 years and maximum age was 72 years with mean age of 50 years. Majority of the cases were belonged to 41–50 years of age (30%), followed by 51–60 years (26.67%), 30–40 years (23.33%), 61–70 years (16.67%), and 71–80 years (3.33%) (Table 2 and Graph 2).

Specimens received were mainly from the maxillectomy specimens and FESS contents. On histopathology examination, 17 cases were positive for *Mucor* (56.67%), and 3 cases were positive for mixed fungal infections, i.e., *Mucor* and *Aspergillus* (10%). 10 cases were negative for *Mucor* (33.33%); among this, 1 case was diagnosed as

malignancy, i.e., adenoid cystic carcinoma (Table 3 and Graph 3). *Aspergillus* shows septate hyphae with branching at acute angles with surrounding chronic inflammation (Figure 1a and b) and also seen conidiophores-forming fruiting bodies[1,7] (Figure 1c). Mucormycosis shows broad aseptate hyphae with right-angled branching[1] (Figure 2). Mucormycosis was identified on routine H-and-E stain and periodic acid-Schiff stain (Figure 3), and in majority of cases, areas of necrosis were noted.

Out of 30 cases, 4 cases were positive for fungal colonies in frozen sectioning (Figure 4) and correlated with histopathology, and 2 cases were negative for fungus on frozen sectioning and came positive in histopathology. 5 cases were negative for fungus both in frozen sectioning and histopathology.

Out of 30 cases, 13 cases were positive for fungal colonies on KOH mount and correlated with histopathology, 6 cases were negative for fungal colonies on KOH mount and came positive in histopathology. 1 case was positive for fungal colonies in KOH mount and came negative on histopathology. 9 cases were negative for fungus both on KOH mount and histopathology (Table 4 and Graph 4).

Table 1: Distribution of sex

Sex	Number of cases	Percentage
Female	10	33.33
Male	20	66.67

Table 2: Distribution of age in years

Age range (years)	Number of cases	Percentage
30–40	7	23.33
41–50	9	30
51–60	8	26.67
61–70	5	16.67
71–80	1	3.33

Table 3: Distribution of histopathology findings and number of cases

Histopathology findings	Number of cases	Percentage
Mucormycosis	17	56.67
Mucor and *Aspergillus* mixed infection	03	10
Negative for fungal colonies	09	30
Adenoid cystic carcinoma	01	3.33

Table 4: Correlation between histopathology and KOH findings

Tests	KOH mount findings	
	Positive	Negative
Routine histopathology findings		
Positive	13	06
Negative	01	09

Out of 30 cases, fungal culture was positive for 20 cases and negative for 10 cases. 11 out 20 cases (55%) were positive for *Mucor*, 4 out of 20 cases (20%) were positive for *Aspergillus*, and 3 out of 20 cases (15%) were positive for mixed *Mucor* and *Aspergillus* fungal elements. 1 out 20 cases (5%) was positive for mixed *Mucor* and *Candida* elements and 1 out of 20 cases (5%) was positive for mixed *Candida* and *Penicillium* elements (Table 5 and Graph 5). *Candida* species were identified as budding yeasts. Mycelial elements show Pseudohyphae.[1]

Out of 30 cases, 17 cases were positive on both histopathology and culture, 3 cases were positive on histopathology and negative on culture, and 3 cases were negative on histopathology but came positive on culture. 7 cases were negative both on histopathology and culture findings (Tables 6 and 7 and Graphs 6 and 7).

DISCUSSION

Fungal rhinosinusitis cases have been increased since the COVID-19 pandemic causing higher mortality rate. *Mucor* and *Aspergillus* are the most common species causing an angioinvasive fungal infection. Total of 30 cases, for all the cases, we did histopathological examination, KOH mount studies, and fungus culture. 11 out of 30 specimens were sent for frozen section.

Sebastian et al.,[8] study showed that the COVID-19 infection is a predisposing factor for acute invasive fungal rhinosinusitis. In the present study also, we found that the COVID-19 infection is the main predisposing factor for fungal rhinosinusitis.

Suresh et al.,[9] studied 30 cases and showed that the prevalence of fungal rhinosinusitis was 30%. *Mucor* was

Table 5: Distribution of number of cases with fungal culture findings

Positive for fungal culture	
Species identified	Number of cases positive by fungus culture (n=20) (%)
Mucor	11 (55)
Aspergillus	4 (20)
Mucor and *Aspergillus*	3 (15)
Mucor and *Candida*	1 (5)
Candida and *Penicillium*	1 (5)

Table 6: Correlation between histopathology and fungus culture findings

Tests	Fungus culture findings	
	Positive	Negative
Routine histopathology findings		
Positive	17	03
Negative	03	07

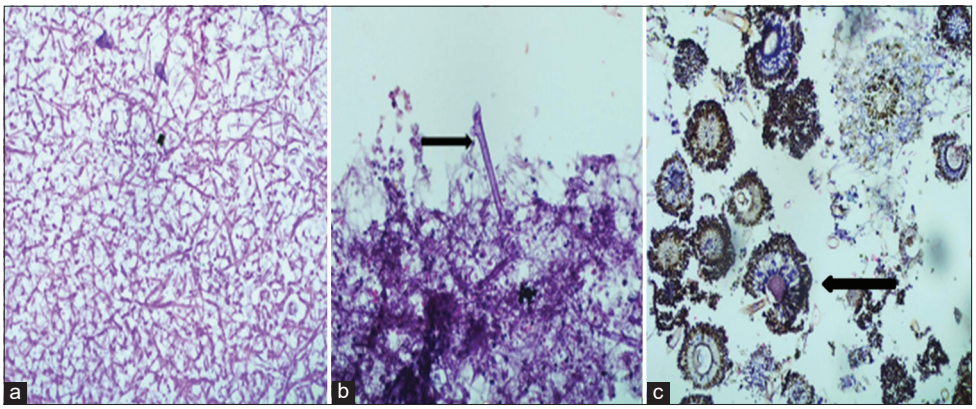


Figure 1: Narrow, thin, acute branching septate hyphal forms of *Aspergillus* species (a) and higher magnification of septate fungal hyphae (black arrow) (b). Fruiting bodies of *Aspergillus* species (black arrow) (c). H and E

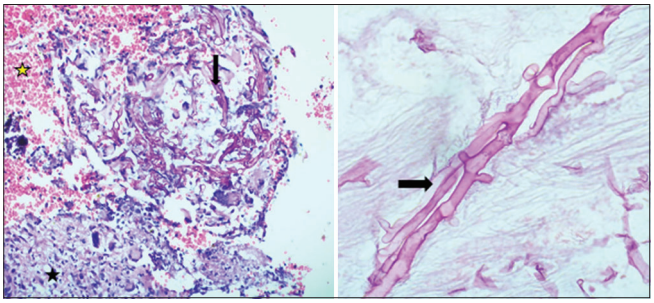


Figure 2: A broad aseptate with irregular branching (90°) hyphal forms (*Mucor* species), surrounding areas showing necrosis (black star) and hemorrhage (yellow star) (left) and higher magnification of fungal hyphae (black arrow) (right). H and E

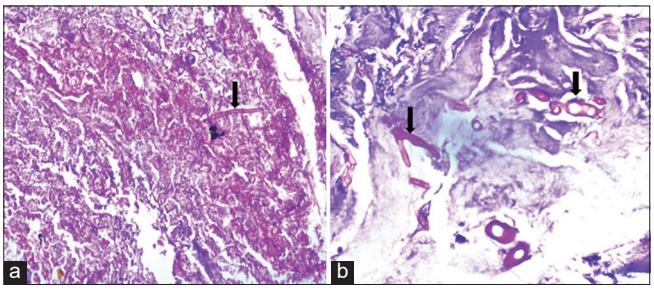


Figure 3: Fungal hyphae (*Mucor* mycosis- black arrow) (periodic acid–Schiff staining, ×40)

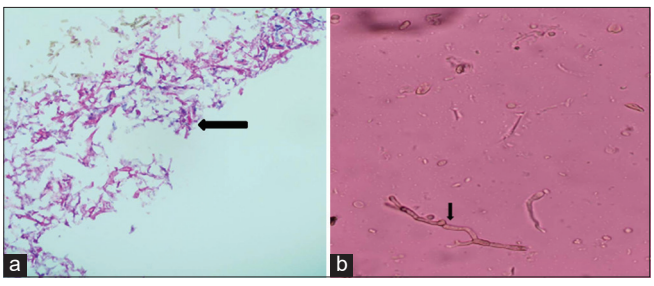


Figure 4: (a) Frozen section image showing *Mucor* species (black arrow) (H and E, ×10). (b) KOH mount image showing *Aspergillus* species (black arrow) (×10)

the most commonly isolated species (n=15 [50%]) of fungus in their study. In the present study also, we found that most common species isolated was *Mucor* (n=17, 85%) on histopathology.

Singh et al.,[10] study concluded that the diagnosis of fungal rhinosinusitis should not be based on the single method as every method has its own advantage. Histopathology is important in classifying the disease but it may lack the sensitivity. Therefore, all the tests such as KOH microscopy, fungal culture, and PCR must be used in conjunction with histopathology, especially for those cases which are difficult to diagnose. In the present study, Histopathology and fungus culture showed good correlation.

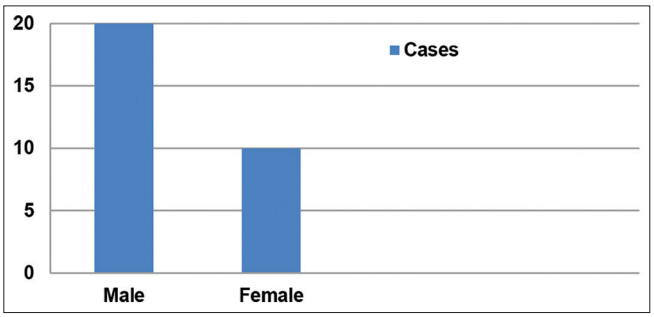
Das et al.,[11] study concluded that the acute fungal rhinosinusitis is the most common type of fungal rhinosinusitis. Cases with mixed reaction pattern suggest that different types of fungal rhinosinusitis represent a progressive spectrum of disease. An exact histopathological categorization of FRS is important as regard treatment. In the present study also, we found that fungal infections were the leading cause of fungal rhinosinusitis with *Mucor*, *Aspergillus*, *Candida,* and *Penicillium* species.

Alkhateb et al.,[12] study concluded that the decision to undertake surgical debridement in patients with acute invasive fungal rhinosinusitis requires definitive histopathologic diagnosis, for which intraoperative consultation with frozen section may be requested. Analysis has demonstrated overall high accuracy of frozen sections in this scenario, and this information is valuable in the surgical management of these patients. In the present study, 11 specimens were received for frozen, among 9 cases were correlated with the histopathology findings. Moreover, it helped the surgeons with margins involvement. False-negative case was due to smaller size of the lesion and site of the lesion (margins).

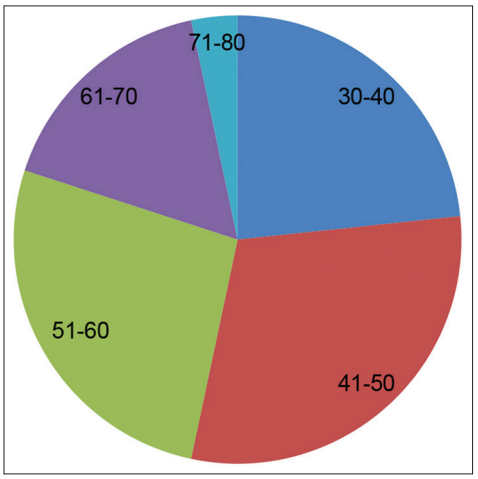
Vaghasiya and Bhalodia[13] study concluded that COVID-19 is associated with secondary infections, such as bacterial

Table 7: Number of cases with histopathology, fungus culture, KOH mount, frozen section findings

Total cases	Histopathology findings	Fungus culture findings	KOH mount findings	Frozen section findings
Case 1	*Mucor*	*Mucor*	*Mucor*	NA
Case 2	Negative	Negative	Negative	NA
Case 3	*Mucor*	*Mucor*	*Mucor*	Positive for *Mucor*
Case 4	Negative	Negative	Negative	Negative
Case 5	*Mucor*	*Mucor*	*Mucor*	NA
Case 6	*Mucor*	*Aspergillus*	*Aspergillus*	Negative
Case 7	*Mucor*	*Mucor*	*Mucor*	NA
Case 8	*Mucor*	*Aspergillus*	*Aspergillus*	NA
Case 9	*Mucor*	*Mucor* and *Aspergillus*	*Mucor*	NA
Case 10	*Mucor*	Negative	Negative	NA
Case 11	*Mucor*	Negative	Negative	NA
Case 12	Negative	Negative	Negative	Negative
Case 13	*Mucor*	*Mucor*	Negative	Positive for *Mucor*
Case 14	*Mucor*	*Mucor* and *Aspergillus*	*Mucor*	NA
Case 15	Negative	*Aspergillus*	*Aspergillus*	Negative
Case 16	Negative	*Mucor*/*Candida*	Negative	NA
Case 17	*Mucor*	Negative	Negative	Positive for *Mucor*
Case 18	*Mucor*	*Mucor*	*Mucor*	NA
Case 19	*Mucor* and *Aspergillus*	*Aspergillus*	*Aspergillus*	NA
Case 20	Negative	Negative	Negative	NA
Case 21	*Mucor* and *Aspergillus*	*Mucor* and *Aspergillus*	*Mucor*	NA
Case 22	*Mucor* and *Aspergillus*	*Mucor*	*Mucor*	Positive or *Mucor*
Case 23	*Mucor*	*Mucor*	Negative	Negative
Case 24	Negative	*Candida* and *Penicillium*	Negative	Negative
Case 25	Negative	Negative	Negative	NA
Case 26	Negative	Negative	Negative	Negative
Case 27	*Mucor*	*Mucor*	*Mucor*	NA
Case 28	*Mucor*	*Mucor*	*Mucor*	NA
Case 29	*Mucor*	*Mucor*	Negative	NA
Case 30	Negative	Negative	Negative	NA



Graph 1: Distribution of sex and total number of cases



Graph 2: Distribution of age in years

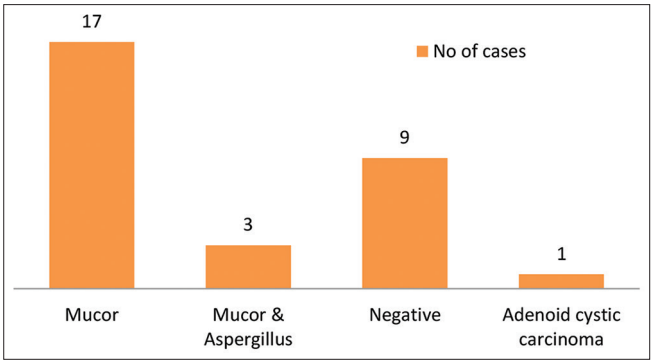
and fungal mainly due to dysregulation of immune system. The use of steroids, broad-spectrum antibiotics, or monoclonal antibodies in the treatment of COVID-19 leads to the exacerbation of pre-existing fungal diseases. In our study also, we found patients who had COVID-19 infection and treated for the same with steroids and antibiotics. Based on literature rate, steroid use is higher about 88%.[14]

The risk of COVID-19 associated mucormycosis is very high in India. Based on literature, the yearly incidence of mucormycosis is very less around 1.7 per 1 million people.[12,15] Incidence is 80 times higher in India, affecting 0.14/1000 population.[13] ROCM is the most common type presenting in 30–50% of cases.[13,16] In our study, we found 1 case out of 20 positive cases with rhino-orbito-cerebral involvement (Table 8).

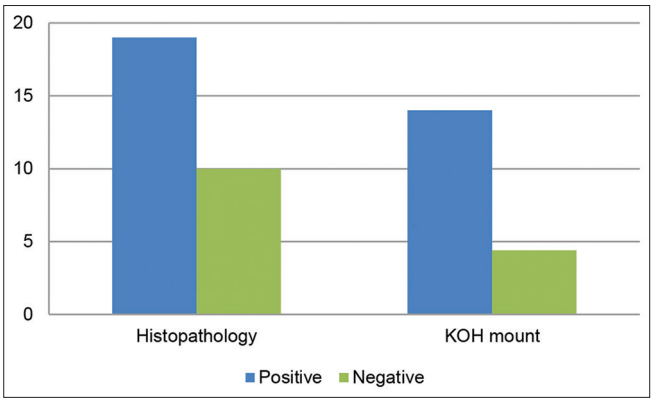
Our study includes 30 cases, with mean age of 50 years. El-Kholy et al.,[17] study includes 36 cases with mean age of 52 years, Vaghasiya and Bhalodia[13] study includes 50 cases with mean age of 67 years, and Chaganti et al.,[1] study includes 20 cases with mean age of 50 years. Age

Table 8: Comparison of parameters of the present study and other similar studies

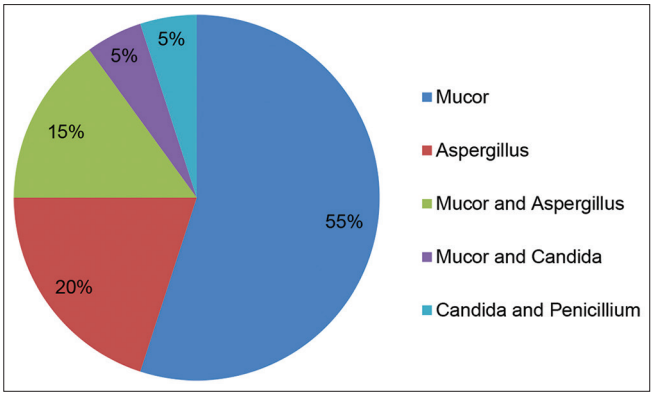
Parameters	Our study	El-Kholy et al.[17]	Vaghasiya and Bhalodia[13]	Chaganti et al.[1]
Total cases	30	36	50	20
Mean age (in years)	50	52	67	50
M: F	2:1	1.1:1	2.5:1	Females only
Positive for fungus	20 (66.6%)	36 (100%)	43 (86%)	20 (100%)
Mucor identified	17 (85%)	77.8%	23 (53.4%)	4 (20%)
Only *Aspergillus*	0%	30.6%	3 (6.97%)	11 (55%)
Mixed infection	3 (15%)	8.3%	3 (6.97%)	5 (25%)



Graph 3: Distribution of histopathology findings and number of cases

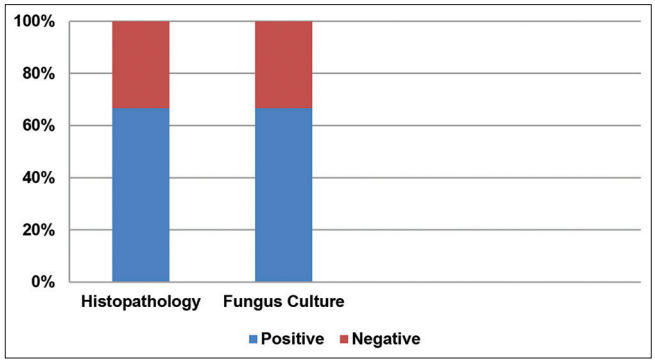


Graph 4: Correlation between histopathology and KOH findings

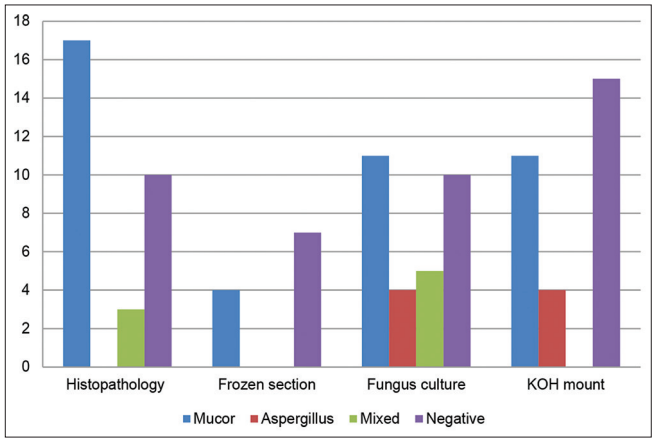


Graph 5: Distribution of cases and fungal culture findings

distribution of our study was comparable with the EL-Kholy et al.,[17] and Chaganti et al.,[1] studies.



Graph 6: Correlation between histopathology and fungus culture findings



Graph 7: Number of cases with histopathology, fungus culture, KOH mount, frozen section findings

Our study showed male predominance and it was comparable with the EL-Kholy et al.,[17] and Vaghasiya and Bhalodia[13] study.

Our study showed 85% (n=20) of *Mucor* only cases and 15% (n=20) of mixed *Mucor* and *Aspergillus* cases. Other studies also showed an increased number of *Mucor* cases, EL-Kholy et al.,[17] study shows 77.8% *Mucor* only cases, and 8.3% of mixed fungal infection. Vaghasiya and Bhalodia[13] study showed 53.4% of *Mucor* only cases and 6.97% of mixed fungal infections. Chaganti et al.,[1] study showed 20% of *Mucor* only cases and 25% of mixed fungal infections. In our study, no exclusive *Aspergillus* only cases were found.

Limitations of the study

Small sample size was the limitation of the study.

CONCLUSION

In our study, we found good correlation of histopathology with fungus culture. Histopathology examination is needed for diagnosing invasive mucormycosis. In our study, we observed 17 cases of mucormycosis and 3 cases of mixed fungal infection (*Mucor* species and *Aspergillus* species). Frozen section was helpful in 4 cases. Inspite of faster results by frozen section, accurate results were obtained by routine histopathology.

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Authors Contribution:
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