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A stitch in time saves nine - rapid diagnosis of mucormycosis on crush cytology smears

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Abstract

Second wave of covid 19 pandemic in India and worldwide was followed by an alarming increase in the cases of opportunistic bacterial and fungal infections. Mucormycosis is one such fatal Angio invasive fungal disease that presents as a medical emergency requiring early diagnosis and treatment.

Present study evaluated the cytopathological spectrum of the cases of fungal infections in covid positive/suspected patients, received over a period of one-monthalong with their histopathological correlation.

Biopsy tissue from the patients with suspected mucormycosis was received in normal saline and crush/imprint smears were prepared for rapid cytological evaluation, remaining tissue was fixed in formalin and processed as histological specimen. The crush smears were stained with H&E, PAP and PAS stains and examined for any fungal pathogen.

Total 32 specimens from patients with suspected mucormycosis were received in the department of pathology, JNMC, over a period of one month. In cytopathology 12 cases (37.5%) were positive for fungus, 17 cases (53%) were reported negative while 3 cases (20%) were suspicious for fungal infection. Amongst the positive cases maximum cases (9/12, 75%) were of isolated mucormycosis.

one case was positive for candida (8.3%) and one case each of mucor with candida (8.3%) and mucor with aspergillus (8.3%) coinfection.

Histopathological examination conformed with the cytological spectrum of fungal pathogens diagnosed as positive on crush smears.

We thus came to a conclusion that preparation of crush smears from biopsy sample is an effective method for rapid diagnosis which correlates well with the histopathological diagnosis.

Keywords: Angio invasive, Crush smears, Covid 19, Mucormycosis

Introduction

Mucormycosis has emerged as a dreaded opportunistic infection in people with SARS coronavirus (SARS-CoV-19) infection with increasing number of cases being reported worldwide and in India.

The immunosuppressive state occurring as a result of coronavirus infection together with the incesstant use of glucocorticoids in its treatment predispose an individual to a number of opportunistic bacterial and fungal infections.

Aspergillus and candida were the main fungal infections reported to be associated with COVID 19 infection.[1] But the second wave of COVID 19 pandemic in India caused a massive surge in the cases of mucormycosis infections.

Recent studies have demonstrated the prevalence of mucormycosis to be 80 times higher in India (0.14 per 1000) during 2019-2020 as compared to developed countries. [2,3]

Mucor belongs to the order Mucorales of the class zygomycetes and is responsible for majority of human infections, hence the terms mucormycosis and Zygomycosis are often used interchangeably.The other order in class zygomycetes isentophmothorales, which are uncommon pathogens affecting immunocompetent hosts causing mainly cutaneous or subcutaneous infections.

Mucorales on the other hand cause an angioinvasive disease in immunocompromised patients causing widespread tissue infarction and necrosis which can be frequently fatal if untreated. The common mucorales species to be encountered in clinical specimens belong to the generarhizopus, lichthemia and mucor.[4] Mucorales are ubiquitous in nature with modes of infection being inhalation of fungal spores or inoculation through disrupted skin/mucosal surfaces.[5]

Depending upon the organ of involvement disease can be classified as rhino-orbital-cerebral (ROCM; most common type), pulmonary, gastrointestinal, cutaneous or disseminated.[6]

The main risk factors implicated in mucormycosis include diabetes mellitus, Haema to logical and other solid organ malignancies, transplant recipients, immunosuppressive states due to underlying autoimmune diseases, HIV, use of corticosteroids and malnourished.[7]

Recent studies on covid associated mucormycosis have highlighted the pathogenic mechanisms of the disease itself (hypoxia, hyperglycemia, increased ferritin, reduced phagocytic activity of WBCs) to provide a conducive environment for the mucorale spores to germinate along with certain other risk factors like use of humidifiers and mechanical ventilators.[8]

Material and methods

This study was conducted on clinical specimens from suspected mucormycosis patients which were received over a period of one month in cytopathology laboratory, department of Pathology, JNMCH, AMU with the aim to

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study the spectrum of fungal infections on crush cytology smears. Correlation with histopathological diagnosis was also done after routine histological processing of the specimen from which crush smears were prepared. The cases were also analysed with respect to RT-PCR positivity, age and sex distribution, socioeconomic status and presence of other risk factors like diabetes mellitus, history of hospitalization, oxygen requirement and intake of steroids.

Biopsy tissue from the patients with suspected mucormycosiswas received in normal saline and crush/imprint smears were prepared for rapid evaluation, remaining tissue was fixed in formalin and processed as histological specimen. The crush smears were stained with H&E, PAP and Periodic acid Schiff (PAS) stains. Cytological diagnosis was mainly based upon morphological characteristics of the fungus which were highlighted by PAS stain.

Results

Total 32 specimens from patients with suspected mucormycosis were received in cytopathology laboratory. Out of these 12 (37.5%) cases were positive for fungal infection, 3 (25%) were reported as suspicious and 17 (53.1%) cases were negative for fungal infection. Amongst the positive cases mucor was the most common fungus to be identified (9/12 cases, 75%), 1 case (8.3%) each of mucor coinfection with aspergillus and candida and 1 case (8.3%) of isolated candida infection was also diagnosed.

3 cases showed mostly necrotic tissue with some degenerated hyphae like structures whose morphology was not clear and hence were reported as suspicious for fungal infection. Histopathological examination of the same biopsy specimen confirmed the cytological spectrum of fungal pathogens in all cases diagnosed as positive on crush smears, except 1 case of isolated candida infection on crush cytology which also revealed mucor hyphae on histopathology. Additionally, 2/3 cases reported as suspicious and 8/17 cases reported as negative on crush cytology also turned out to be positive for mucor hyphae on careful histopathological examination. (Table 1).

Mucormycosis affected patients over wide age ranges (21-80 years). The youngest patient was of 22 years and the oldest was 80 years. Male to female ratio was 1.4. The most common presenting complaint was facial pain and swelling (22/32), followed by eye pain and swelling (13/32). Other complaints included Di munition of vision, headache, dental problems (tooth loosening and pain), nasal obstruction and discharge.

14/32 (43.7%) patients had history of positive RT-PCR in past two months, 10/32 (31.2%) had negative RT-PCR results, while 8/32 (25%) patients did not get themselves tested but gave history of fever and sore throat in recent past thus putting them under category of suspected covid. (Table 2).

Careful enquiry into the presence of other risk factors for mucormycosis showed that 20/32 (62.5%) patients (included 14 RT-PCR positive patients and 6 patients who were RT-PCR negative but treated as covid) had history of hospitalization and steroid intake. History of supplemental oxygen requirement was present in 13/32 (40.6%) patients. (Table 3). Diabetes was the most common comorbid condition (19/32, 59.3%) followed by hypertension (8/32, 25%).

Table 1: Spectrum of fungal pathogens on crush smears with histopathological correlation

Type of fungus	Cytopathology (%)	Histopathology (%)
Mucor	9 (60%)	20 (86.96%)
Mucor +	1 (6.67%)	1 (4.34%)

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candida		
Mucor + aspergillous	1 (6.67%)	1 (4.34%)
Suspicious for Mucor	3 (20%)	1 (4.34%)
Candida	1 (6.67%)	0
Negative	17 (53%)	9 (28%)
Total	32	32

Table 2: relation to RT- PCR status

RTPCR	No. of cases	Percentage
Positive	14	43.75%
Negative	10	31.25%
Suspected	08	25.0%
Total	32	

Table 3: Presence of risk factors and comorbid conditions

Risk factors/comorbidities	No. of cases
Hospitalization	20/32(62.5%)
Steroid intake	20/32(62.5%)
Supplemental Oxygen	13/32(40.62%)
Diabetes	19/32 (59.38%)
Hypertention	8/32 (25.0%)
Hypothyroidism	1/32 (3.1%)
Heart disease	0/32 (0%)

Fig 1: Broad aseptate mucor hyphae with obtuse angle braching on crush smear (H&E 40X)

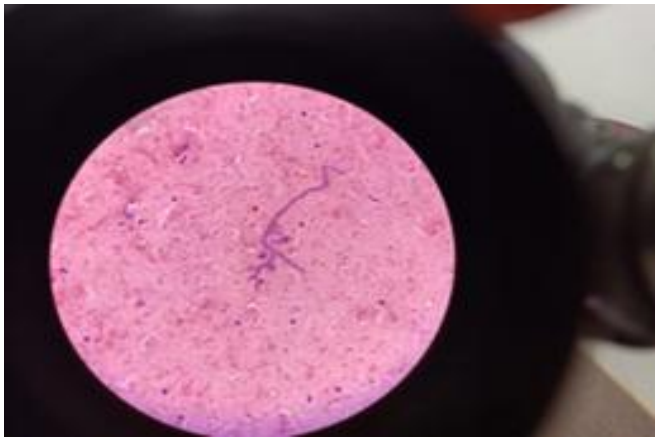


Fig 2: Mucor sporangia on crush smears (H&E 40X)

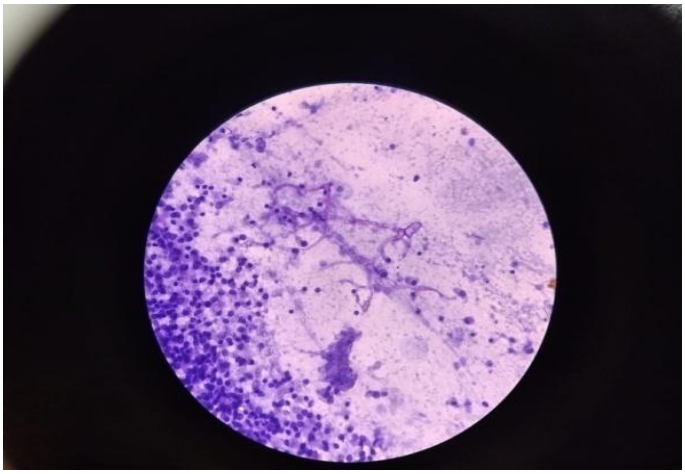


Fig 3: Aspergillus septate hyphae with acute angle branching on crush smear (PAS 40X)

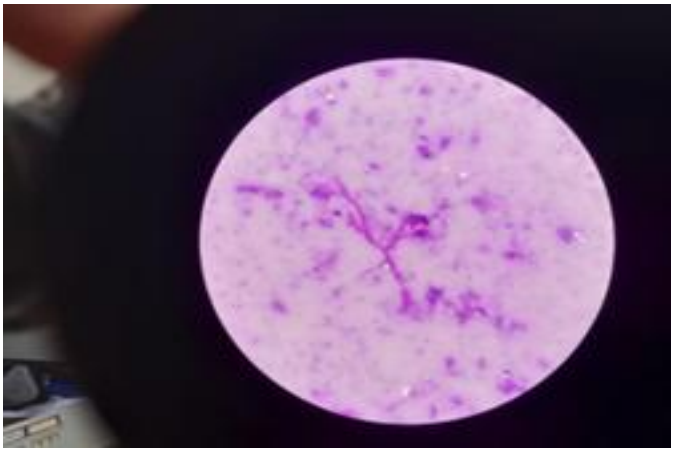
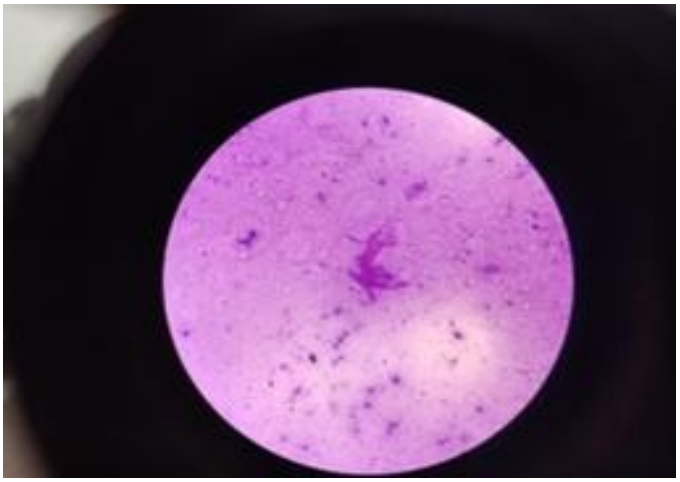


Fig. 4: Mucor along with candidalpseudohyphae seen on crush smear (H&E 40X)



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Discussion

Second wave of covid 19 pandemic in India was followed by a sharp rise in the cases of covid associated mucormycosis which is an angioinvasive opportunistic infection and could prove fatal if not diagnosed and managed at an early stage.[9]Present study evaluated the role of crush cytology smears as a rapid diagnostic aid. Preparation and evaluation of crush cytology smears reduced the turnaround time to a maximum of 2-3 hours as opposed to a minimum of 4 days required for the histopathological diagnosis. The spectrum of fungal pathogens diagnosed on crush smears correlated well with histopathological diagnosis in the cases which were reported as positive for fungal infection.Mucormycosis was seen to affect people over wide age range with greater incidence (62.5%) among people with history of hospitalization and steroid intake.

Conclusion

Mucormycosis is an angioinvasive fungal infection which presents as a medical emergency. The initiation of antimicrobial therapy depends upon the demonstration of fungal pathogen in the clinical specimen which can take anywhere between 3-5 days by microbiological culture and histopathological examination. Preparation of crush smears is a rapid and effective method to evaluate biopsy tissue from cases with suspected mucormycosis which can be used for demonstration of fungal pathogen within 2-3 hours thus helping in early initiation of effective antifungal therapy.

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