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Histopathology! Gold Standard To Diagnose Mucormycosis In Covid-19 Patients And Its Microbiological Correlation

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Abstract:

Background: The pandemic of coronavirus disease 2019 (COVID-19) continues to be a major problem worldwide. Although COVID-19 cases are declining worldwide, an emerging new problem, mucormycosis, commonly known as 'black fungus' is a challenge to healthcare professionals.

It is a serious, but rare opportunistic fungal infection that spreads rapidly and hence prompt diagnosis and treatment is necessary. Mucormycosis is caused by the mucormycetes, a group of molds, with Rhizopus and Mucor as the most common species.

Methodology: We took six months data of proven COVID-19 patients on treatment along with complete clinical profile, radiological investigations including NCCT head, nose and paranasal sinuses with histopathological findings including gross examination of the resected biopsy specimen, microscopic examination and special stains like periodic acid schiff and methenamine silver used for the identification of the fungus and microbiological findings including KOH mount for the fungus and the fungal culture.

Results: Total 50 cases of mucormycosis were included in the study. Out of which all (100%) cases were positive on histopathology while (52%)cases showed correlation of histopathology and microbiology and the rest (48%)cases did not show positivity on microbiology.

Conclusion: Histopathology is the gold standard investigation for the diagnosis of mucormycosis.

Keywords: Mucormycosis,COVID-19, Molds, Rhizopus, Mucor.

Introduction:

Mucormycosis is a rare, invasive fungal infection, caused by mucormycetes, a group of molds. Mucormycetes belongs to the order Mucorales.[1]The most common species being Aspergillus and Candida.[2] The exact incidence/prevalence is not known due to very few population based studies.[3] However, the prevalence of mucormycosis is approximately 0.14 cases per 1000 population, which is about 80 times the prevalence in developed countries.[4]It affects the sinus, brain, gastrointestinal tract and lungs and can be life-threatening in severely immunocompromised individuals.[5] The early diagnosis of this deadly black fungus is very important to improve the outcome as it may increase the survival, may also reduce the need for or extent of surgical resection.[6]The prerequisites for the diagnosis of mucormycosis are a high index of suspicion, recognition of risk factors, assessment of clinical manifestations and prompt initiation of diagnostic methods.[7] The diagnostic methods include imaging procedures, histopathological examination and microbiological examination.

Since very few studies are available on diagnosis of mucormycosis, in this study we evaluated the histopathology for the detection of the fungi and

compared it with the microbiology.

Materials And Methods:

Study design

The present study was a prospective study undertaken at Mahatma Gandhi Medical College, Jaipur, India, over a period of six months, from May to October 2021 on proven Coronavirus disease 2019 (COVID-19) patients. A written and informed consent was taken from each study participant before enrolling him/her in the present study.

Sample size

In the present study 50 COVID 19 patients on treatment were enrolled along with their complete clinical profile, radiological investigations including MRI,NCCT head nose and paranasal sinuses.

Study participants

All the COVID 19 positive patients who were suspected positive for mucormycosis on radiological examination were subjected for biopsy and sent for histopathological examination.

Sample collection

Tissue samples from all suspected sites were received in formalin for histopathological examination and were used for KOH smear and fungal culture. The various types of fungi were confirmed by histopathological examination.

Histopathology: All histological tissue obtained in formalin were fixed with 10% neutral formaldehyde for 24 h, routinely dehydrated and embedded with paraffin, 4 µm sections were serially cut on albumin coated slides and stained by Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) stain.

KOH Microscopy and Culture: Tissue was examined in 20% KOH. Culture was done on Sabouraud Dextrose Agar (SDA) with chloramphenicol and incubated at 25ºC and 37ºC respectively and were examined until 28 days.

Statistical Analysis

Statistical analysis was done using excel and analysis was done in the form of percentages, contingency tables and test of significance. A significance level of 0.05 was regarded for interpretation of analysis.

Results:

A total of 50 patients of mucormycosis were studied. Out of which 32 (64%) patients were male and 18(36%) patients were female with ages ranging from 31 to 75 year (Table 1). The majority (86%) of patients were aged over 40 years. The most common (70%) co-morbidity in our study was diabetes mellitus. Other co-morbidity found was prolonged steroid use (48%). Among 50 cases, all (100%) the cases were positive on histopathology while 26 (52%) cases were positive for mucormycosis on both histopathology and microbiology. And the rest 24(48%) cases were negative by KOH.

On histopathology, the fungal hyphae of mucormycosis seen were broad, ribbon like, irregular and aseptate with branching at right angle. Hyphae were evident with Hematoxylin and Eosin stain and PAS stain. (Figure 1 and Figure 2)

Nose and paranasal sinuses were involved in 46 cases. In 4 cases, there was orbital involvement along with nose and paranasal sinuses while no cases under study had intracranial extension (Table 2).

Discussion:

Total 50 patients of mucormycosis were included in our study aiming to find the correlation between histopathology and microbiology for the diagnosis of mucormycosis. COVID-19 has been associated with a wide range of opportunistic bacterial and fungal infections.[8] In our study maximum no of cases were above the age group of 40 years which is similar to the study done by Poorna et al[9] and Ashina et al.[10] The most important predisposing factors of mucormycosis include diabetes mellitus, malignancies, transplantation, and corticosteroids.[11]

Dr. Priyanka et al[12] reported 50 cases of mucormycosis; 34(68%) of them had diabetes which is similar to our study where also 35 (70 %) cases were diabetic. Ashina et al in his study of 33 mucormycosis patients found that maximum no of patients were immunocompromised with diabetes mellitus which is again similar to our study. Poorna et al in the study of 38 cases found that maximum no of cases 29 cases (76.3%) presented with diabetes mellitus which is similar to the results of our study.

The fungal spores enter the human organism by inhalation, ingestion or direct inoculation.[13] Through inhalation the fungal spores reach the nasal cavity and germinate into hyphae. The germination is

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favoured by low oxygen concentration, high glucose, acidic medium and high iron levels. Extension of the disease into the maxillary and ethmoid sinus can lead to orbital involvement. It can also spread intracranially through superior orbital fissure, ophthalmic vein and cribriform plate.[12] Dr Priyanka et al in her study of 50 cases found that most of the (30 %) patients had a primary disease infection involving the maxillary group of sinuses which is similar to our study where maxillary sinus was involved in 56% of cases.

The diagnosis of mucormycosis is more difficult than other fungal infections. Under normal laboratory conditions, sporulation fails and culture results from the biopsies are often negative due to unviable organism in necrotic tissues making its treatment challenging[13]. However the histopathological study using H & E , PAS and MS stain makes the diagnosis of the organism definitive. The time taken for small biopsy report is usually within 3 days whereas the culture report takes 3-4 weeks. Dr Priyanka et al in her study of 50 cases found that 43(86%) cases were positive on histopathology while only 31(62%) cases showed positivity on KOH as compared to our study where all the 50(100%) cases were positive for histopathology and only 26(52%) cases were positive on KOH.[12]

Conclusion:

Histopathology is the gold standard investigation for the diagnosis of mucormycosis as in our study all the cases showed positivity on biopsy irrespective of its microbiological positivity. Histopathological examination distinguishes the presence of fungus as a pathogen in the specimen from a culture contaminant and is indispensable to determine angioinvasion. Thus it helps in early intervention by clinicians resulting in good prognosis and less fulminant disease course.

Uncontrolled diabetes and over-zealous use of steroids in COVID-19 can also suppress immunity allowing opportunistic fungal infections to grow which might aggravate the illness and so both of these information must be properly collected from history.

The data pertaining to cases of mucormycosis in developing countries is dwindling. Studies from different COVID pandemic affected states can help us produce a cumulative incidence / prevalence rate.

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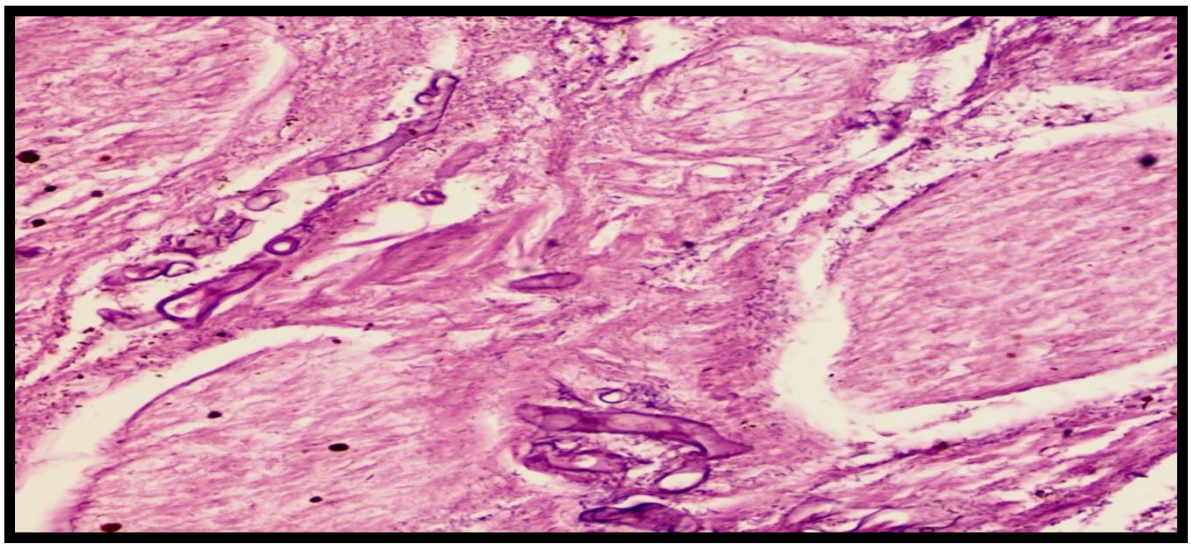

Fig1 : Hematoxylin and eosin staining showing fungal hyphae

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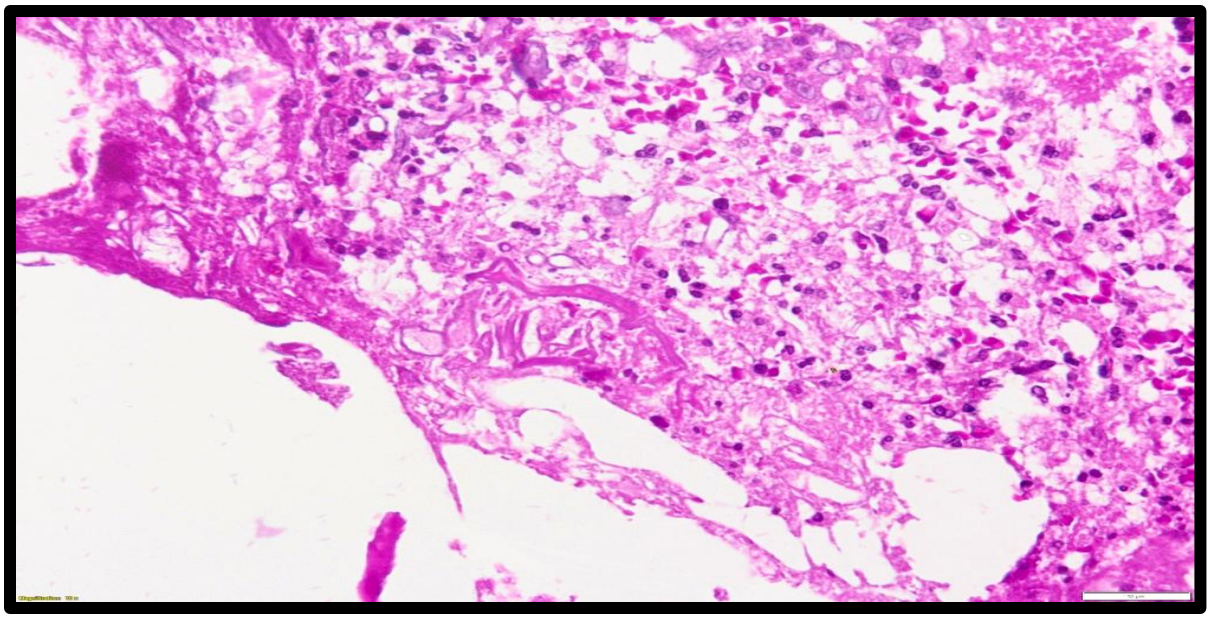

Fig 2 : PAS stain positive hyphae

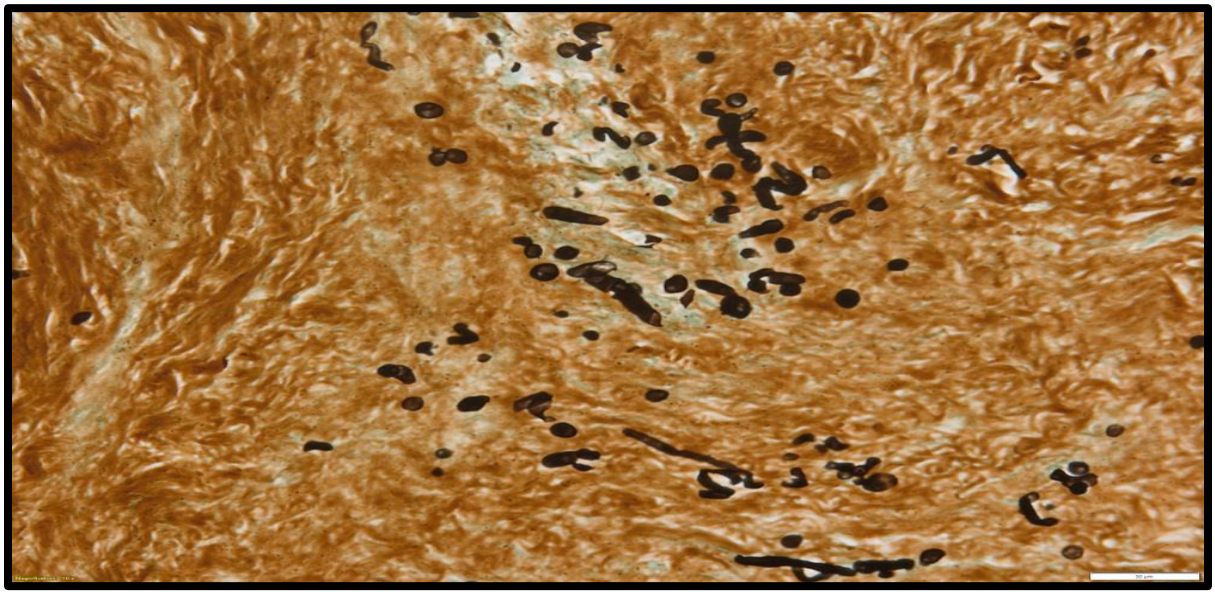

Fig 3 : Methanamine silver stain positive hyphae

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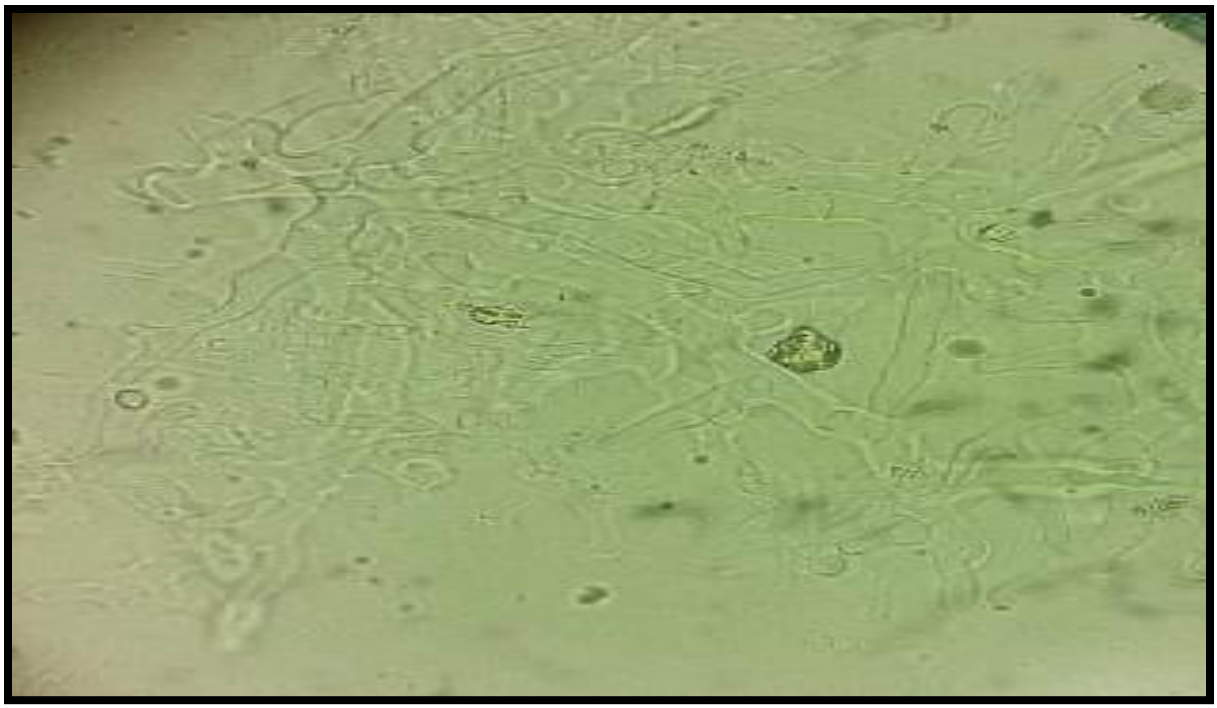

Fig 4 : KOH mount showing septate fungal hyphae with branching

Table 1: Age wise incidence of cases

AGE (YEARS)	NO OF CASES (%)
1-10	0 (0%)
11-20	0(0%)
21-30	0(0%)
31-40	07(14%)
41-50	15(30%)
51-60	16(32%)
61-70	09(18%)
71-80	03(6%)

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Table 2: Incidence of sites affected

Site affected	**No of cases(%) n=50**
Maxillary sinus	28 (56%)
Ethmoid	20 (40%)
Turbinate	15 (30%)
Sphenoid	12 (24%)
Orbit	12 (24%)
Frontal sinus	10 (20%)

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