



Research Article

RAPID DIAGNOSIS OF INVASIVE FUNGAL INFECTION DURING COVID PANDEMIC ERA: ROLE OF POTASSIUM HYDROXIDE PREPARATION

PATANKAR M.C., SONI S.T.*, SHAH H.S., PATEL V.R., UPADHYAY S.C., PATEL P.M. AND ROHIT P.P.

Department of Microbiology, B. J. Medical College, Gujarat University, Ahmedabad, Gujarat, 380016, India

*Corresponding Author: Email - drsumeetasoni@gmail.com

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Abstract- Introduction: Mucormycosis is a life-threatening angioinvasive infection caused by fungi of the order Mucorales and class of Mucormycetes. It is common in covid and diabeticketoacidosis patients. Main sources of mucormycosis in hospital settings are mechanical supply of oxygen and environmental contamination. It is rapid grower, angioinvasive and destructive for human body, so rapid diagnosis and treatment is necessary. Methods: This study was conducted for suspected samples received at microbiology laboratory of civil hospital during May and June 2021. Samples are treated with KOH preparations and findings were noted down. All findings are compared with biopsy findings and radiological findings and results are noted down. Result: A total of 1070 samples were screened from which 204 samples were found positive for fungal elements resembling mucormycosis showing that it was more prevalent among all fungal infections in that time period. Highest number of positive samples are tissue samples. More number of samples are of male patients than female patients. Age group of 31-60 years are more affected from this disease. More number of positive patients are diabetic considering it as a risk factor for this disease. Conclusion: After pandemic wave of covid 19 there was increasing prevalence of mucormycosis patients showing some relation of it to covid 19 infection. Mucormycosis was most prevalent fungal infection among all fungal infections in that post covid 19 pandemic wave. As it was fatal to human beings it was necessary to detect this fungal infection by any rapid proven method and KOH preparation method was proven as best method for rapid detection.

Keywords- Mucormycosis, Covid 19 infection, KOH preparation

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Introduction

Mucormycosis is a life-threatening angioinvasive infection proven to be fatal for many patients caused by the ubiquitous fungi of the Order Mucorales and class of Mucormycetes. The order Mucorales includes several species which are involved in rhinocerebral, pulmonary, cutaneous, and gastrointestinal and other less frequent infections in immunocompetent and immunocompromised individuals, and all are characterized by tendency to disseminate [1].

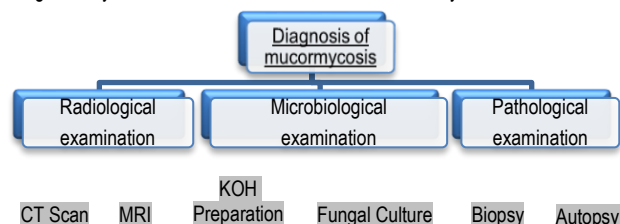
Mucormycosis (sometimes called zygomycosis) was considered as rare fungal infection in last few years, but now a days it is considered as serious and common in covid and diabeticketoacidosis patients. People get mucormycosis from contamination through the pipes and prongs used for the mechanical supply of oxygen to patients in hospital settings and from environmental contamination with fungal spores [2]. As it is rapid grower, angioinvasive and destructive for human body, early and necessary steps should be taken for rapid diagnosis, treatment, and prevention of this fungal infection.

For rapid diagnosis of this infection KOH mount preparation (Potassium hydroxide preparation) is one of the best methods to be used. Mucormycetes are characterized by the presence of broad aseptate hyphae (coenocytic mycelia) in KOH preparation and formation of zygosporangia in culture. KOH is a cheaper than DMSO (Dimethyl sulfoxide) and readily available alkali. It is a strong base which softens, digests, and clears cellular and keratin debris but leaves fungal hyphae cell wall intact which is resistant to digestion by KOH, thus clearing the background and allows visualization of fungal elements under a microscope [3]. The present study was carried out to know the prevalence of mucormycosis and other yeast like fungus in Covid 19 pandemic era by KOH preparation method.

Material and methods

Study groups

Present study was carried out at Mycology laboratory of tertiary care teaching hospital in Gujarat [5]. Patients having signs and symptoms suggesting of invasive fungal infection among the Out Patient Department, indoor and ICU of the hospital during 17 May to 8 June 2021 were enrolled in this study.



Enrolled patients comprised both immunocompromised and immunocompetent individuals.

Sample processing

Samples like nasal swabs, nasal tissue, crusts were collected by ENT surgeons and were sent to the Mycology laboratory of Microbiology Department for microscopy by KOH slide preparation method. Two types of KOH 10% (10 g KOH crystals in 100 ml of distilled water) and 40% (40 g KOH crystals in 100 ml of distilled water) were prepared from KOH crystals. In swab samples 10% KOH was added and were kept for 5-10 minutes. In tissue samples 40% KOH was added and then incubated at 37°C temperature for 2-3 hours.

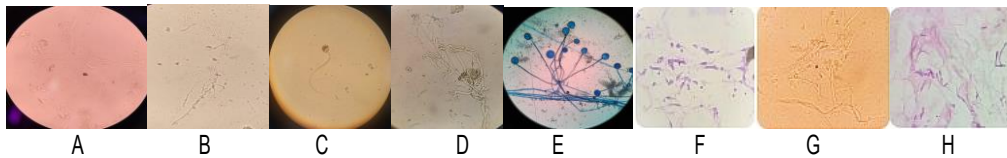


Fig-1: A, D-KOH preparation(40X and 10X) showing broad, aseptate, branched, irregular ribbon like hyphae. B- Pseudohyphae with yeast cells, C-Sporangium with sporangiospore in KOH preparation E-LCB(Lactophenol cotton blue) preparation of fungal culture showing well developed sporangium with sporangiospore inside it and sporangiospore with stolon. F, H- are showing fungal elements of mucorales in tissue section of biopsy material stained with H & E stains. G-is showing fungal elements of mucorales and pseudohyphae.

In crust samples 40% KOH with few drops of DMSO (dimethylsulfoxide) was added and incubated at 37°C temperature for 2-3 hours[4].KOH mount was prepared from that samples and observed under 10X and 40X of microscope. Diagnosis of mucormycosis was made if, in KOH preparation broad, aseptate, branched, irregular ribbon like hyphae were observed. If budding yeast cell with or without pseudohypha seen then reported as candida species seen. Thin, branched, septate hyphae, which rule out mucormycosis was also reported. Result was noted down and reports were generated in LIS system. Radiological and pathological reports of the same patient were also studied (for comparison).

Results

A total of 1070 samples were screened for KOH slide preparation method. From which 232 samples were found positive for invasive fungal infections, So, overall prevalence of positivity by KOH preparation is 22% (232/1070). [Table-5] shows prevalence of invasive fungal infection by KOH slide Microscopy. prevalence of mucormycosis among positive samples by KOH preparation was 88% (204/232) and among 204 positive samples for mucormycosis, 50 were positive from swab samples, 115 were positive from tissue samples, 90 were positive from crusts samples and 9 was positive from sputum sample. So, KOH positivity for mucormycosis is 25 % for swabs, 56% for tissue 15% for crusts and 4% for sputum, which is shown in [Table-1]. [Table-2] shows Covid and non covid status of the patients. [Table-3] shows age, gender and risk factor distributions among KOH positive patients. [Table-4] shows comparison of KOH slide Microscopy with histopathological biopsy and radiological evidence.

Table-1 shows mucormycosis KOH positivity from different samples

Type of Sample	Total No of Samples	No of Positive Samples
Tissue	322	115 (56%)
Crusts	90	30(15%)
Swabs	599	50(25%)
Sputum	47	9(4%)
Total	1058	204

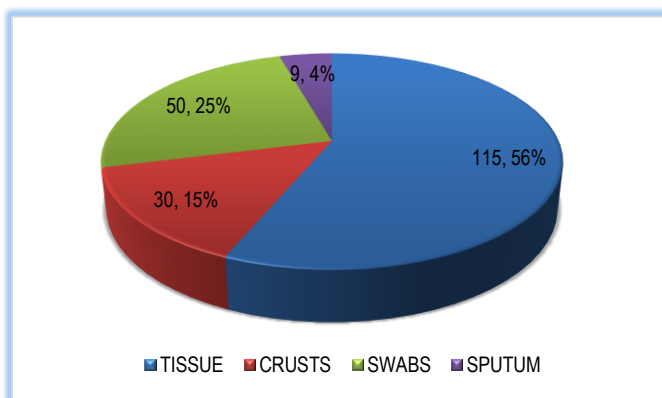


Fig-2 Tissue samples are more ideal for diagnosis of mucormycosis as 56% positive samples for mucormycosis were detected from tissue samples while 15% were isolated from crusts, 25% were isolated from swabs and only 4% was isolated from sputum.

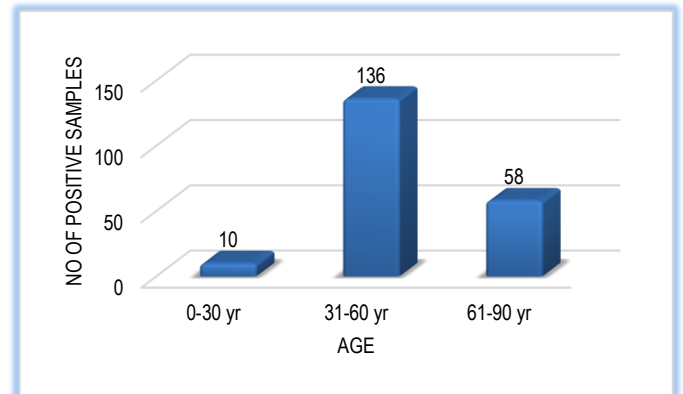
Table-2 shows Covid and non-Covid status of the mucormycosis patients

Covid Status	Total No of Samples	No of Mucor Positive Samples
COVID Positive	609	132
COVID Negative	461	72
Total	1070	204

There was more number of mucor cases in covid positive (132) patients than covid negative (72) patients which indicates significant role of corticosteroids as a risk factor of mucormycosis in COVID-19 era.

Table-3 age, gender and risk factor distribution among mucormycosis positive patients

	Age Wise Distribution			Gender Wise Distribution		No of Diabetic Patients
	0-30 Yr	31-60Yr	61-90Yr	Male	Female	
Mucormycosis	10	136	58	138	66	141
Candida infection	0	16	3	14	5	0
Thin filamentous, septate hyphae infections	1	7	1	6	3	2
Total	11	159	62	158	74	143



From all mucormycosis positive patients 69% patients were diabetic which shows there is association of diabetes as a risk factor of mucormycosis as it is angioinvasive. There were more cases(67%) in age group 31-60 years which shows mucormycosis is more affecting younger people than older. There were more cases(68%) in male which shows males are more prone than females for mucormycosis.

Table-4 shows comparison of KOH slide preparation method in diagnosis of mucormycosis with other methods like biopsy and radiological investigations.

	Positive	Negative
Biopsy Findings of KOH Positive Samples	161	33
Radiological Findings of KOH Positive Samples	55	9

In our study 27% KOH positive samples were also positive for radiological findings and 79% samples were also positive for biopsy findings which shows KOH preparation is more reliable for rapid diagnosis of mucormycosis and gives accurate result as biopsy, CT Scan and MRI when sample collection is proper and slide is examined by expert microbiologist.

Table-5 shows KOH positivity for common fungal infections.

Fungal Infections	No of Positive Samples
Mucorales	204(88%)
Thin filamentous fungal hyphae	7(3%)
Pseudohyphae with budding yeast cells	19(8%)
Mucorales and Pseudohyphae with budding yeast cells	0
Mucorales and Thin filamentous fungal hyphae	2(0.9%)

Highest number of KOH preparations were positive for mucormycosis(204) and second highest numbers were for pseudohyphae with budding yeast cells(19) so now a days mucormycosis is more prevalent than other fungal infections in COVID 19 era.

Discussion

After pandemic of COVID-19, there has been a dramatic increase in occurrence of invasive fungal infections observed worldwide largely [1]. Our study was carried out for measuring that increased prevalence of mucormycosis and other yeast like fungus in Covid-19 pandemic era by KOH preparation method.

In study carried out by Skiada A at Department of Medicine, Laiko Hospital, National and Kapodistrian University of Athens, 11527 Athens, Greece diabetes has been reported as a risk factor for mucormycosis in 73.5% of cases in India while in current study diabetes has been reported as a risk factor for mucormycosis in 69% cases[6]. In study carried out by Hariprasath Prakash a total of 388 proven/probable mucormycosis cases were reported during the study period with overall mortality at 46.7% while in current study 204 cases were reported positive for mucormycosis among all 232 KOH positive cases[7]. As per current study from all KOH positive samples 88% samples were positive for mucormycosis so now a days mucormycosis is more prevalent than other fungal disease. Highest detection of mucormycosis fungal elements were from tissue samples than crusts and swabs so tissue samples are more ideal to avoid false negative result. Only 4% mucormycosis fungal elements were isolated from sputum sample which is suggestive of less prevalence of pulmonary mucormycosis. While 96% fungal elements were isolated from nasal samples which is suggestive of more prevalence of rhinocerebral mucormycosis. 65% patients with mucormycosis were also positive for covid-19 by RTPCR, rapid antigen test or outside positives. while 35% patients were negative for covid 19 which shows more chances of mucormycosis infection in covid 19 positive patients due to exposure to mechanical ventilation devices and corticosteroids during treatment of covid-19 infection and also, we can conclude that corticosteroid therapy for covid-19 infection has a significant role as risk factor for mucormycosis. 69% patients were diabetic in which 72 patients were positive for mucormycosis but negative for covid-19 so we can consider diabetes as major risk factor for mucormycosis. Results of all (204) KOH mucor positive samples were compared with radiological and pathological findings in which results of 171 samples were perfectly matched so we can conclude that KOH test gives as accurate result as other methods. From 232 total positive samples 204 samples were positive for mucormycosis suggestive of more prevalence (88%) of mucormycosis than yeast cells infections (19%). In some cases, mucormycosis was in coexistence with thin filamentous fungal infections.

Conclusion

From current study we can conclude that mucormycosis is a serious problem in public health concern with more prevalence in diabetic 31-60 years age group male patients. Hence keeping in view of rapid and devastating course of mucormycosis, every institution should resort on to its local mandatory protocol in Emergency department, where in KOH wet mount is to be done in half an hour of the arrival of suspected patient so that antifungal regimen can be instituted as soon as possible [8].

Now a days mucormycosis infection is more prevalent than yeast cells and thin filamentous fungal infections while among mucormycosis patients rhinocerebral mucormycosis is more prevalent than pulmonary and other types of mucormycosis. As per diagram (diagnosis of mucormycosis) shown in introduction radiological examinations like CT Scan and MRI cannot detect which fungal infection is there, while biopsy takes 3-5 days for procedure and result, even fungal culture takes time of 1-10 days to grow so only KOH preparation is there which can diagnose mucormycosis early in 2-3 hrs and patient can get early treatment with good prognosis.

Application of research: Mucormycosis was most prevalent fungal infection among all fungal infections in that post covid 19 pandemic wave. As it was fatal to human beings it was necessary to detect this fungal infection by any rapid proven method and KOH preparation method was proven as best method for rapid detection

Research Category: Invasive Fungal Infection

Abbreviations: KOH- potassium hydroxide

H & E stain- hematoxylin and eosin stain, COVID-19- coronavirus disease

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Study area / Sample Collection: Mycology Laboratory, Tertiary Care Teaching Hospital, Gujarat

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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