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COMPARATIVE ANALYSIS OF HISTOPATHOLOGICAL EXAMINATION IN BIOPSIES OF ALL SUSPECTED MUCOR PATIENTS, IN RELATION TO KOH MOUNT SMEARS AND TISSUE CULTURES.

Histopathology

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with a wide range of opportunistic bacterial and fungal infections [1]. More cases of Mucormycosis were increasingly reported in both people with covid and post covid state in india during the second wave. So as a diagnostic tool though, tissue culture is considered to be Gold Standard. some tend to be negative as there is predominantly necrotic tissue and unviable organism. In such cases biopsies for histopathological examination prove effective in diagnosis, as early diagnosis helps in early medical management. **Aim:** To highlight that the histopathological examination of biopsied tissues as a cheap, quick and early diagnostic tool where KOH & Tissue culture fails especially in highly necrotic tissues. **Method:** A total number of 47 new suspected cases of invasive fungal infection were selected. Tissue samples were collected along with patient age ,sex, associated Co-morbidities, COVID Status, Ferritin level, Duration of stay in Hospital and Outcome of the patient. Tissues were subjected to routine histopathological Processing, Paraffin embedded and cut into 5µ thickness section in Department of pathology, Stanley Medical College & Hospital. Sections are stained with Haematoxylin & eosin, specials stains like Gomori's methenamine silver for examination. Data analysis was done by SPSS Version 20 and correlation was considered with p value <0.05. **Results:** Invasive fungal infection was found commonly in the age group of 40-50 years. Men were commonly affected with most of the patients were in the post covid state. The common site of involvement of invasive fungal infection was rhino orbital region and Diabetes is found to be most common underlying predisposing factor . We found that histopathological examination of tissue specimen is a valuable tool in diagnosing mucormycosis than KOH mount and tissue culture, there is no stastistical significance found between HPE diagnosis and KOH mount, HPE diagnosis and tissue culture since the sample size was not adequate in our study. **Conclusion:** More further studies in this regard has to be done in future, as it is a cheapest and sensitive method which may help the treating physician for early diagnosis and treatment.

KEYWORDS

Invasive fungal infection, Histopathological Examination , Culture

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) is associated with a wide range of opportunistic bacterial and fungal infections [1]. Among those, Mucormycosis cases were identified in increase number in people with COVID and post COVID state during the second wave in India.

A primary reason being as it provides ideal environment for the fungal spores to germinate. The risk factors that provide media for the organisms to grow include uncontrolled glycemic levels, hypoxia, metabolic acidosis, increase levels of iron, impaired engulfment of leukocytes and prolonged hospital stay with or without mechanical ventilators.

The causative agent includes *Rhizopus, Mucor, Rhizomucor, Cunninghamella* and *Absidia* of Class- Zygomycetes [2]. Most common etiological agent being *Rhizopus Oryzae.* It is responsible for 60% cases in humans and accounts for 90% of the Rhino-orbital-cerebral mucormycosis [3]. Mode of infection occurs through the inhalation of fungal spores.

The prevalence of mucormycosis varies from 0.005 to 1.7 per million population worldwide. The prevalence in india is 80 times more when compared to developed countries [4-6]. Globally though Diabetes remains the leading predisposing factor for mucormycosis , with an overall mortality of 46% [7] , use of prolong and short term corticosteroids has often been associated with several opportunistic fungal infection including aspergillosis and mucormycosis.

These findings made the necessity of relooking the context of covid 19 where most patients were treated with corticosteroids as it is life saving. Because of high mortality and rapid progression, early diagnosis and effective treatment of invasive fungal infection are important.

Many different modalities are available for the diagnosis of invasive fungal infection, which includes signs and symptoms, serologic tests, culturing and microscopic examination, imaging procedures, histopathological methods, and polymerase chain reaction. The diagnosis requires either the presence of broad pauci septate hyphae in the involved tissues or growth of the fungus in tissue cultures (8). The diagnosis of invasive fungal infection remains difficult because under normal laboratory conditions, sporulation fails and culture results from the biopsies are often negative due to unviable organism in necrotic tissues (13).

Moreover, morphological identification of the hyphae can help distinguish between different fungal species(14,15). Although culture is considered the gold standard for determining the causative agent, (16) some tend to be negative as there is predominantly necrotic tissue and unviable organism. In such cases histopathological examination of necrotic tissue prove effective in diagnosis, as early diagnosis helps in early medical management.

OBJECTIVE

To highlight that the histopathological examination of biopsied tissues as a cheap, quick and early diagnostic tool where KOH & Tissue culture fails especially in highly necrotic tissues.

METHODS

The study was conducted in Stanley Medical College and Hospital as a prospective study among patients who were clinically suspected cases of Mucormycosis. The study period was three months.

Inclusion Criteria

1. Clinically suspected mucor patients RT-PCR POSITIVE/ NEGATIVE
2. Patients with High Ferritin Level
3. Patients with co-morbidities
4. Patients with/without O2 Support/Steroids

Exclusion Criteria

1. Organ transplanted individuals
2. Cancer patients on Chemotherapy
3. PLHA
4. Chronic autoimmune disorder on long term steroids
5. Recurrent mucormycosis

METHODOLOGY :

Institutional ethics committee approval was obtained. A total number of 47 new suspected cases of invasive fungal infection were selected. Tissue samples were collected along with patient age ,sex, associated Co-morbidities, COVID Status,Ferritin level, Duration of stay in Hospital and Outcome of the patient . Tissues were subjected to routine Histopathological Processing, Paraffin embedded and cut into 5µ thickness section in Department of pathology, Stanley Medical College & Hospital. Sections are stained with Haematoxylin & eosin, specials stains like Gomori's methenamine silver for examination.

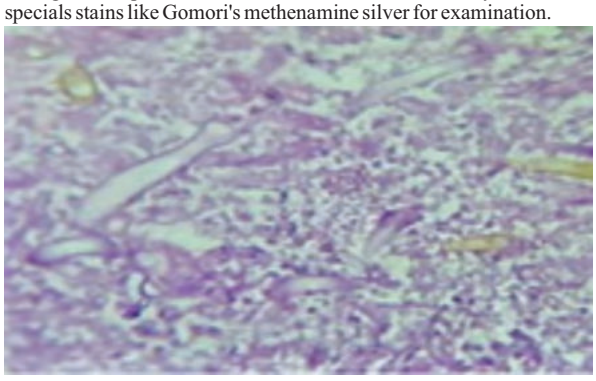

Figure1: H & E

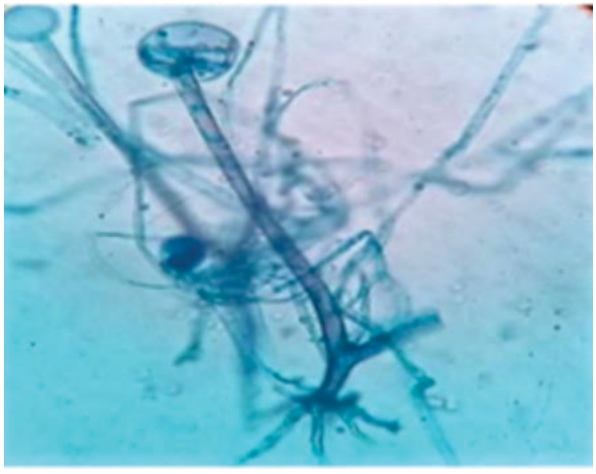

Figure 2: KOH MOUNT

Statistical Analysis:

Data will be summarized using percentages, mean and standard deviation. Categorical variables will be analyzed using Chi-square test and Fisher's exact test. Significance will be assessed at p value □0.05. All data will be entered into Excel format and Statistical analysis will be done through SPSS software version 26.0. Charts and Graphs will be expressed using Origin Pro v9.1.

RESULTS:

This study was done to find a cheap, quick valuable tool for diagnosing invasive fungal infection in suspected cases.

According to the study conducted by Parisa Badiee et al (8) and Awadhesh Kumar Singh et al (1) the most common affected age group were in the fourth decade and men were two to three times commonly affected. In our study, around 29.8% of cases were found to be between the age group of 41-50 years and 27.7% of the study patients were below 40 years of age. Invasive fungal infection were more common in males (66%).

Table 1: Age Distribution

AGE DISTRIBUTION		
	Frequency	**Percent**
Upto 40 yrs	13	27.7
41 - 50 yrs	14	29.8
51 - 60 yrs	10	21.3
61 - 70 yrs	8	17.0
Above 70 yrs	2	4.3
Total	47	100

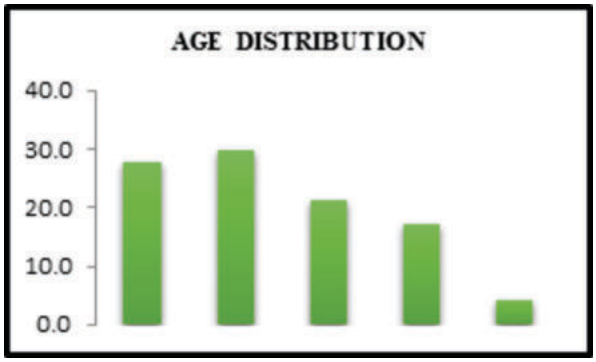

Figure 3: Age Distribution

Table 2: Gender Distribution

Gender Distribution		
	Frequency	**Percent**
Female	16	34
Male	31	66
Total	47	100

Table 3: Biopsy Site Distribution

Biopsy Site		
	Frequency	**Percent**
B/L Nasal Cavity	3	6.4
B/L Sphenoid Sinus	1	2.1
Left Maxillary Sinus	5	10.6
Left Nasal Cavity	11	23.4
Left Orbital Contents	2	4.3
Left Sphenoid Sinus	1	2.1
Right And Left Nasal Cavity	1	2.1
Right Cheek Nodule	1	2.1
Right Lateral Wall And Septum	1	2.1
Right Maxillary Antrum	3	6.4
Right Maxillary Sinus	3	6.4
Right Nasal Cavity	15	31.9
Total	47	100

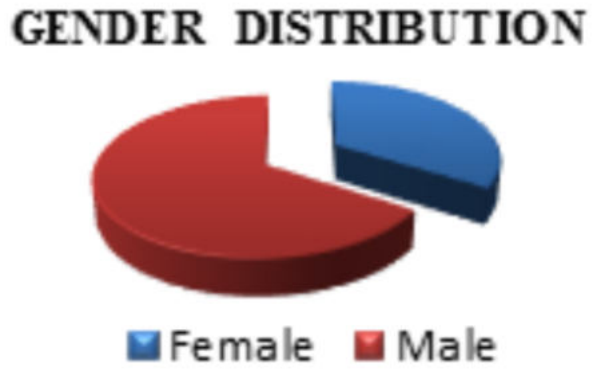

Figure4: Gender Distribution

According to Roden M.M et al and Jeong W et al (11,7) mucormycosis is grouped into rhino-orbito-cerebral (ROCM), pulmonary, gastrointestinal, cutaneous, renal, disseminated and other miscellaneous forms based on site of involvement and ROCM is the most common form .In our study , all the 47 cases were found to involve Rhino orbital region and 31.9% cases were seen in nasal cavity.

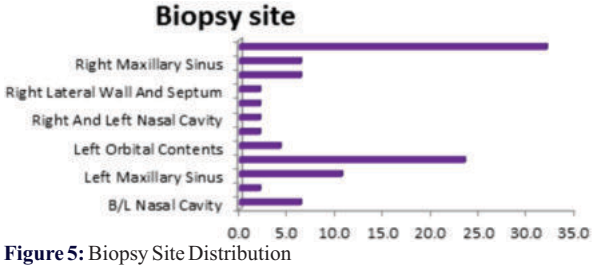

Figure 5: Biopsy Site Distribution

According to Hariprasad et al (16) there is specific association between underlying disease/predisposing factors with clinical types of mucormycosis. In a study conducted by Roden M.M et al and Jeong W et al (11,7) more number of ROCM form seen in patients with diabetic ketoacidosis or with uncontrolled diabetes mellitus. In our study, diabetes mellitus were found in 95.7% cases.

Table 4: Diabetes Distribution

Diabetes		
	Frequency	**Percent**
Absent	2	4.3
Present	45	95.7
Total	47	100

Figure 6: No Of Diabetes Case

According to Manjusha Nambiar et al (9) post covid 19 patients are at increased of developing invasive fungal infection. In our study, increase frequency of mucormycosis cases were seen in post covid patients (80.9%).

Table 5: COVID Status Distribution

COVID STATUS		
	Frequency	**Percent**
Covid Positive	2	4.3
Negative	5	10.6
Non Covid	2	4.3
Post Covid	38	80.9
Total	47	100

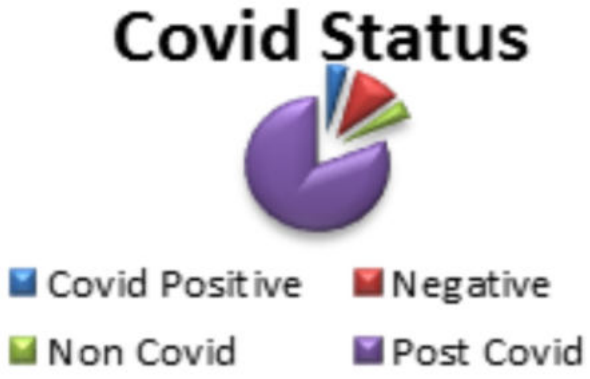

Figure 7: Covid Status

According to the study conducted by Parisa Badiee et al, non septate hyphae was detected histopathologically in 25% cases and by tissue culture in 17% cases. In our study, it was found histopathologically in 72% cases , by tissue culture in 47% cases and by KOH mount in 43% cases.

Table 6: Culture Distribution

Culture		
	Frequency	**Percent**
Present	22	46.8
Absent	25	53.2
Total	47	100

Figure 8: Culture Positive

Table 7: KOH Mount Distribution

KOH Mount		
	Frequency	**Percent**
Positive	17	36.2
Negative	30	63.8
Total	47	100

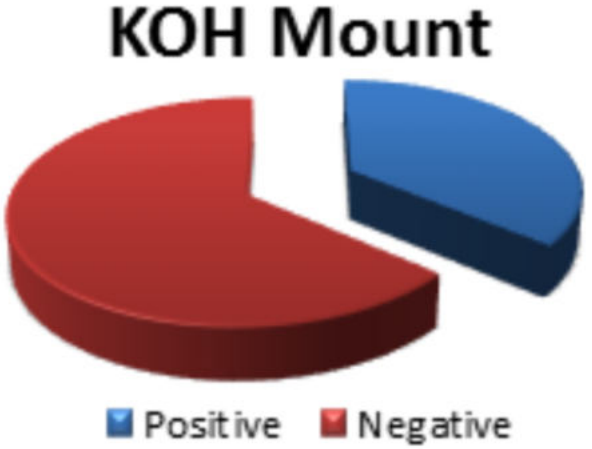

Figure 9: KOH Mount Positive

According to the study conducted by Awadhesh Kumar Singh et al (1) Corticosteroid intake for the treatment of COVID-19 was recorded in 76.3% of casesin our study it was 85%.

Table 8: Steroid Distribution

Steroids		
	Frequency	**Percent**
Not in steroids	7	14.9
In steroids	40	85.1
Total	47	100

Figure 10: %On Steroids

In patients with COVID-19, increase in cytokines especially interleukin-6, increases free iron by increasing ferritin levels. Free available iron is an ideal growth media for mucormycosis. In our study, increase in serum ferritin levels were found in 68% of cases.

Table 9: Serum Ferritin Distribution

KOH Mount with HPE Diagnosis						
		HPE Diagnosis		Total	Sensitivity	38.1
		Positive	Negative		Specificity	80.0
KOH Mount	Positive	16	1	17	PPV	94.1
	Negative	26	4	30	NPV	13.3
Total		42	5	47	Accuracy	42.6

Figure 11:% Of Increase Serum Ferritin

In our study we did a comparative analysis between histopathological examination & KOH mount using Receiver Operating Characteristic curve (RoC), which shows the area of the curve is 0.590, p- value= 0.512>0.05 with 95% C.I 0.341 to 0.840, which is no statistical significance with the Sensitivity is 31.8%,Specificity 80.0%, PPV 94.1%,NPV 13.3% and accuracy is 42.6%.

Table 10: Comparison Of KOH Mount With HPE Diagnosis By Receiver Operating Characteristic Curve

Area Under the Curve			
Area	**p-value**	**95% C.I**	
		LB	UB
.590	0.512 #	.341	.840
# No Significant at p > 0.05 level			

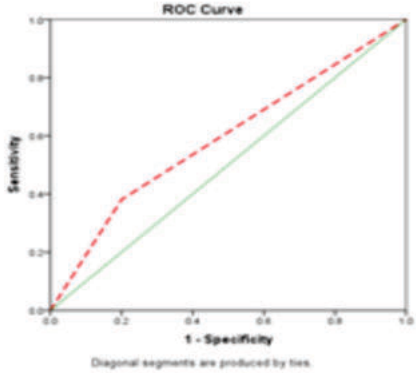

Figure 12

DISCUSSION

Invasive fungal infections are more common in immunocompromised individuals , though it is uncommon in healthy individuals. The predisposing factors are hyperglycaemia, leukaemias and other malignant tumors, organ transplantation, prolonged neutropenia, immunosuppressive therapy, iron overload , iron therapy, severe burns, acquired immunodeficiency syndrome , intravenous drug abusers, malnutrition (7) .

The site of predilection for Mucormycosis includes nasal and paranasal sinuses, orbital, rhinocerebral , lung , gastrointestinal tract , skin, jaw bones, joints, heart, kidney, and mediastinum (1). Based on underlying predisposing factor, the site of involvement may varies (1). ROCM commonly occur in association with poorly controlled diabetes and metabolic acidosis, whereas lung involvement frequently occur in neutropenia, bone marrow and organ transplant, and hematological malignancies, while GIT in malnourished individuals.

There are many methods available for diagnosing fungal infections. In 1950 Smith and Krichner [12] criteria for the clinical diagnosis of mucormycosis are still considered to be gold standard and include: (i) Black, necrotic crust, facial pain, nasal discharge , peri-orbital or peri-nasal swelling with discoloration , Ptosis , proptosis , complete ophthalmoplegia and cranial nerve palsies .

For confirmatory diagnosis , our study emphasis the requirement of cheapest and sensitive method of diagnosing mucormycosis when KOH mount and tissue culture fails to identify the organism and in a set up where serological methods and molecular PCR methods are not available.In our study we have found that histopathological examination is a valuable tool in diagnosing invasive fungal infection

The pathological hallmark of invasive fungal infection includes Giant cell reaction, arterial thrombosis , necroinflammatory tissue, aseptate ribbon like hyphae with right angled branching differentiates it from other fungal infections (2).

CONCLUSION

In this present study , invasive fungal infection was found commonly in the age group of 40-50 years. Men were commonly affected. Most of the patients were presented in the post covid state. The common site of involvement of invasive fungal infection ,in our study was rhino orbital region. Diabetes is found to be most common underlying predisposing factor than others.

Though steroids are considered to be live saving drug in treatment of COVID 19 patients, it has been found that it has a strong association in developing mucormycosis as it leads to a immunosuppressive state. We also found that increase in ferritin levels also predispose to mucormycosis, as it provides a good growth media for the organism by increasing free iron levels in our study.

In the present study, though we found that histopathological examination of tissue specimen is a valuable tool in diagnosing mucormycosis than KOH mount and tissue culture, there is no stastistical significance found between HPE diagnosis and KOH mount, HPE diagnosis and tissue culture since the sample size was not adequate. More further studies in this regard has to be done in future, as it is a cheapest and sensitive method which may help the treating physician for early diagnosis and treatment.

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