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Original Research Article

A study on the utility of KOH mount and HPR in the diagnosis of post COVID mucormycosis

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ABSTRACT

Background: Mucormycosis is a deadly disease that mostly affects the immunocompromised. Steroid therapy following COVID-19 infection has led to an alarming rise in the rates of this rare infection. Key to management of this disease is early diagnosis. The most common modalities used for diagnosis are KOH mount and histopathological examination. In our study we compare the efficacy of these two diagnostic modalities.
Methods: Nasal endoscopy was done in all suspected cases of the disease. Nasal discharge, crusts were collected in a sterile manner and sent for KOH mount examination. Specimens collected following surgical debridement was sent for HPR examination. The results of both are compared.
Results: In our study, HPR was positive in 82.2% cases while KOH mount showed positive results in 43.5% cases only.
Conclusions: Histopathology helps in confirming the diagnosis of mucormycosis. It also helps to demonstrate tissue invasion and identification of species of fungus. But it is time consuming. KOH mount examination is rapid and inexpensive but the test shows high false negative values. Thus, KOH mount examination can be used as a screening tool while histopathological examination can be used as a confirmation test for post covid mucormycosis.

Keywords: Mucormycosis, KOH mount, Histopathological examination, Nasal endoscopy

INTRODUCTION

Mucormycosis has been long recognized as a fulminant life-threatening disease of fungal etiology belonging to Mucorales.[1] It may present in many forms which include rhino orbito cerebral, pulmonary, cutaneous, gastrointestinal disease. Acute or invasive fungal rhinosinusitis is usually present in immunocompromised patients with impaired neutrophilic response like uncontrolled diabetes mellitus, AIDS, iatrogenic immunosuppression, hematological malignancies.[1] This condition is characterized by the presence of hyphal invasion of sinus tissue within a time span of 4 weeks.[1] Rhino orbito cerebral mucormycosis is a rare disease with a high mortality rate of 30% to 70%. The most pathogenic species of the family Mucoraceae is Rhizopus oryzae which accounts for 90% of all rhinocerebral cases.[2] In developed countries, mucormycosis occurs primarily in severely immunocompromised hosts like those with organ transplantation, severe hematological malignancies. But, in developing countries, most cases occur in persons with poorly controlled diabetes mellitus. It poses an important burden in immunocompromised patients due to high mortality. The development of newer, more effective immunosuppressive drugs has been associated with an increase in incidence of this disease in developing countries.[2] The human resistance to fungal infections rests on the body's ability to restrict the availability of iron to the invading fungus. The reduced ability of serum to bind iron at low pH causes high iron concentration in blood

which when associated with glucose rich acid mileu facilitates fungal growth.[3] Steroid therapy which has evolved as an integral part of COVID-19 management leads to impaired glycemic state causing waning immune response. Common reported clinical symptoms include fever, crusting of nasal mucosa, epistaxis, visual disturbances and headaches. In early stages, nasal endoscopic findings may be as subtle as the presenting symptoms. Without early treatment, rapid progression of the disease with high mortality rates from intra orbital and intracranial complications have been reported. Moreover, the background of poorly controlled diabetes further jeopardizes the host response. Rapid investigation results translate into more rapid administration of antifungal therapy and better outcomes for the patient. So early diagnosis is the key to management. Nasal endoscopy with biopsy, swabs for KOH mount and fungal culture are the most economical and easily available tools for diagnosis. Potassium hydroxide is a keratin digestion reagent that will dissolve proteins, lipids, and lyse epithelium. The fungus element will withstand the KOH solution (10%-30%), because it contains chitin and glycoproteins in the cell wall.[4] This clearing agent provides a significant difference in brightness between fungal cells and the sample background and helps to improve quality of results.[5] KOH mount examination is a rapid means of diagnosis but it is not confirmative.[6] Gold standard for diagnosis of invasive fungal sinusitis is histopathology. It helps in identification of fungal species and to demonstrate fungal invasion of tissues. But it is time consuming and requires trained professionals. In our study, we compare the utility of KOH mount and HPR in diagnosis of mucormycosis.

Aim and objectives

Aim and objectives were to compare the results of HPR and KOH mount in diagnosis of post COVID mucormycosis and to assess the efficacy and diagnostic validity of KOH mount in post COVID mucormycosis.

METHODS

This is a cross sectional study done in 154 patients admitted in the Department of Otorhinolaryngology and Head and Neck surgery of NSCB Medical College, Jabalpur following fungal sinusitis during June to July 2021. Purposive sampling was done for this study with sample size of all admitted patients in the study period. All data was coded, entered, and analyzed by using statistical package for the social sciences (SPSS) version 23.0. Data was cleaned before subjected to analysis. All data concerning the variables was analyzed using frequency, percentage and graph.

Inclusion and exclusion criteria

All clinically diagnosed cases, patients who had a history of covid infection presenting with symptoms of sinusitis and patients giving consent for study were included. Patients with no history of COVID infection and patients not giving consent for study were excluded.

Procedure

After taking written informed consent, patients underwent a thorough history taking and comprehensive ear, nose, throat, and neck examination. Nasal endoscopy was done in all cases. The specimen for KOH mount was taken at the time of admission as soon as the patient came into the ward. Operated specimen was labeled and sent to the lab in normal saline for fungal microscopy & KOH. The specimen is placed on a slide and a drop of 20% KOH was added, cover slip applied and incubation done for 2 hrs. The slides are then microscopically examined for fungal hyphae. Specimens for histopathology were collected at the time of surgery. The specimens were sent to labs under sterile precautions. On microscopic examination, broad aseptate to sparsely septate wide angled branching fungal hyphae are considered belonging to Mucorales.

RESULTS

In current study, the maximum number of patients were in the age group of 41-50 years (38.9%).

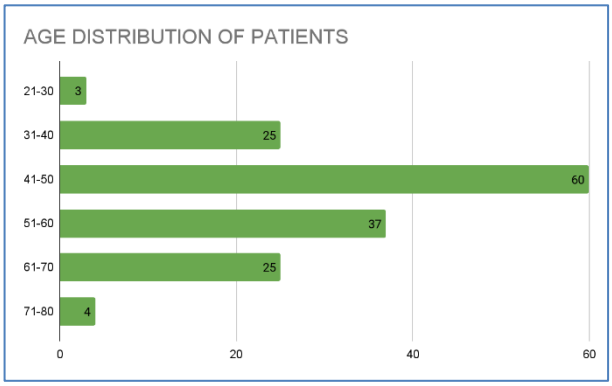

Figure 1: Age distribution of patients.

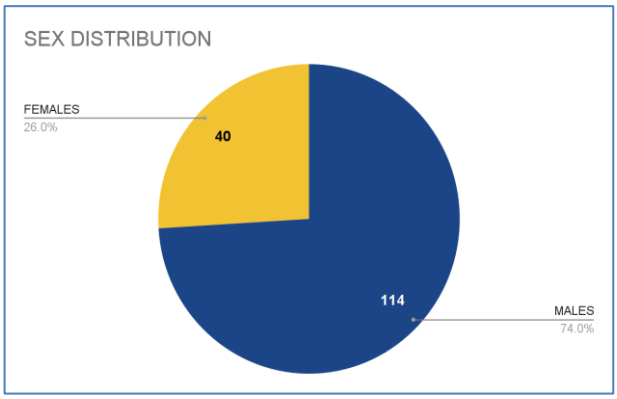

Figure 2: Sex distribution of patients.

Maximum patients were males (74%). Male to female ratio is 2.8:1. 151 patients (98%) were diabetic and maximum in the age group of 41-50 years (33.7%).

Figure 3: KOH mount showing broad aseptate wide angled fungal hyphae.

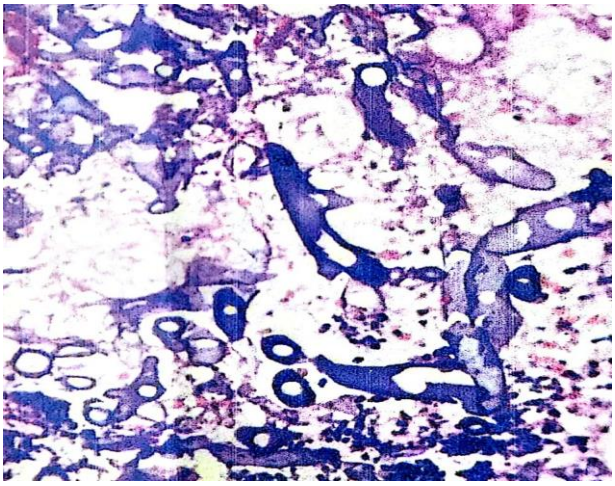

Figure 4: Histopathological specimen showing fungal hypahe.

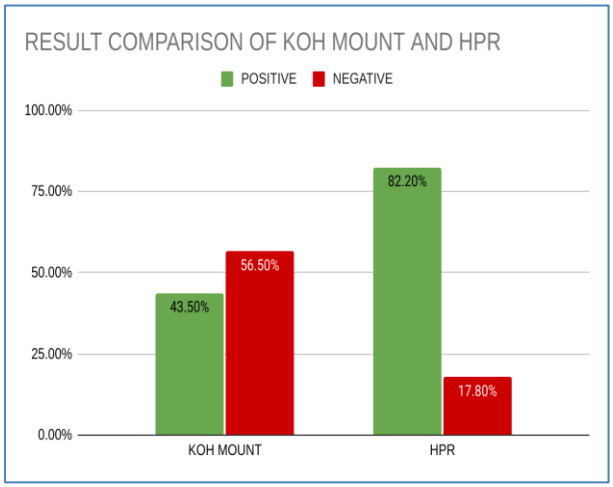

Figure 5: Result comparison of KOH mount and HPR.

All patients had a history of covid infection and associated steroid treatment. In our study, the most common clinical symptom with which the patient presented was swelling of eyes (45%) and orbital pain (20%).

Table 1: Comparison of results of KOH mount and HPR.

Test	Positive (%)	Negative (%)
KOH mount	43.5	56.5
HPR	82.2	17.8

Figure 6: Palatal erosion in a patient with maxillary sinus mucormycosis.

Figure 7: Patient of rhinoorbitocerebral mucormycosis showing periorbital edema.

The other symptoms were facial pain (8.1%), headache (11.5%), visual disturbances (6.3%), erosion of palate (7.1%) etc. KOH mount results were positive in 43.5% while HPR was positive in 82.2%.

DISCUSSION

In our study, the maximum patients were of the age group 41-50 years. This is similar to the study of Nilam et al in which the average age group was 49.9%.[7] Maximum patients were males with male to female ratio 2.8:1. Except 3 patients who were of the age group of 21-30, rest all patients were diabetic. This finding is consistent with the findings of Chakrabarti et al which shows that type 2 diabetes is the major risk factor for mucormycosis.[8] There are many factors that predispose a patient with diabetes to mucormycosis. Firstly, dysfunction of phagocytes in the presence of diabetes and ketoacidosis. Both neutrophils and phagocytes exhibit defective killing and phagocytosis under such conditions. Secondly, patients with diabetic ketoacidosis show acidic pH and elevated levels of free iron that is favorable for fungal growth. Also, elevated levels of glucose and iron, increases the expression of GRP 78 which is a glucose-regulated protein belonging to the HSP-70 family present in endoplasmic reticulum where mediates several cellular processes as a chaperon, including protein folding, degradation of misfolded proteins, regulation of calcium homeostasis and sensing the endoplasmic reticulum stress. A fraction GRP-78 is also translocated to the cell surface in many cell types, wherein it acts as the receptor mediating penetration and damage of endothelial cells by Mucorales, leading to the observed angioinvasion.[9] Mice with diabetic ketoacidosis have an increased expression of GRP-78 in sinus, lungs and brain, and anti-GRP-78 serum can protect such mice from mucormycosis, indicating a plausible role of GRP-78 overexpression in susceptibility of diabetics to this disease.[10] As the majority of Indian patients have diabetes and metabolic acidosis as the major risk factors, the principal management modalities in such cases include a control of hyperglycaemia and prompt reversal of ketoacidosis, along with surgical debridement and amphotericin B therapy.[11] In our study, the most common clinical symptom with which the patient presented was swelling of eyes (45%) and orbital pain (20%). The other symptoms were facial pain (8.1%), headache (11.5%), visual disturbances (6.3%), erosion of palate (7.1%) etc.

In current study, all patients underwent nasal endoscopy and samples were collected from nasal cavity, maxillary and ethmoidal sinuses. Maxillary sinus was the most commonly involved sinus. All samples were analyzed by KOH mount and histopathological examination and we found that KOH mount examination showed positive results in 43.5% while HPR was positive in 82.2%. These results are coinciding with those reported by Desai et al where biopsy suggestive of invasive fungal sinusitis was reported in 100% of the cases. In another study conducted by Singh et al found that out of 37 (48.68%) positive cases 17 (22.3%) cases were found to be positive by direct microscopy, 14 (18.42%) by histopathology with PPV and NPV of 47% and 88% respectively when histopathology is taken as a gold standard investigation, other studies conducted by Singh et al and Bala et al more fungal elements presence was observed on by KOH mount microscopy (22.3%) than histopathology (14.8%).[12] This difference may be due to differences in professional expertise, differences in preparation of samples. Histopathological examination is a very important diagnostic tool since it distinguishes the presence of the fungus as a pathogen in the specimen from a culture contaminant. There are many preanalytic parameters that are important for the optimal recovery of fungi from the clinical specimens. Proper sealing and packing of the sample must be done to prevent contamination. Next to be considered is optimal specimen transport or processing. A lapse in any of these may result in a failure of the test. Although KOH mount examination is rapid, inexpensive and may be highly specific when used by an experienced microscopist, it cannot identify tissue invasion by fungus and also, it is difficult to identify fungal species by KOH mount. On the other hand, histopathology is more specific and can demonstrate tissue invasion by fungus and also is cost effective. The disadvantage is that it is time consuming. In a deadly disease like mucormycosis, in which early treatment is the only way to save the patient, a delay in diagnosis cannot be afforded. In our institution all patients were treated by surgical debridement and liposomal Amphotericin B at a dose of 3-5 mg/kg/day.

Limitations

The limitation of the study is short duration of the study and that the results of fungal culture were not considered for this study.

CONCLUSION

The KOH mount is a rapid investigation to demonstrate the presence of fungus. It cannot identify species of fungus. So, it can be used as a screening tool for mucormycosis. Histopathology can demonstarte tissue invasion by fungus and also identify the fungal species. This is the gold standard investigation of diagnosis of rhino orbito cerebral mucormycosis. Since the test is time consuming, Liposomal Amphotericin must be started immediately on clinical suspicion rather than waiting for investigation results. The treatment regime can be later modified based on the test results. Both the investigations are helpful in the diagnosis of post covid mucormycosis, however, histopathology is more diagnostic of the disease than KOH mount examination.

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