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STUDY OF COMPARISION BETWEEN KOH AND HISTOPATHOLOGY REPORT IN POST COVID19 FUNGAL INFECTION; OUR EXPERIENCE

^{*1}Dr. Nilam U. Sathe, ²Dr. Lal Pek Thangi, ³Dr. Kamini Chavan and ²Dr. Saad Ahmed

¹Head of Unit and Associate Professor, Department of ENT, KEM Hospital, Mumbai ²Senior Resident, Department of ENT, KEM Hospital, Mumbai ³Assistant Professor, Department of, KEM Hospital, Mumbai

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*Corresponding author: Dr. Nilam U. Sathe

ABSTRACT

Objectives: This study is aimed to compare the diagnostic modalities of detecting fungus; KOH mount and Histopathological evaluation in cases of invasive fungal rhinosinusitis. Invasive fungal rhinosinusitis, i.e. fungal invasion of sinus mucosa, submucosa, blood vessels, bone, etc.has emerged as one of the most debilitating sequelae of corona virus infection. This sudden rise in fungal infection may be multifactorial with reasons ranging from low immunity status to uncontrolled diabetes mellitus to incessant steroid use to hyperferritinemia due to Covid Infection. While most of the diagnosis rests on clinical and radiological grounds, the nature of the species of fungus to provide tailor made treatment, as well as differentiation from non fungal sinusitis, is also of utmost importance. Here in this study of 85 patients who presented with Post Covid infection, a comparison is being made between KOH and HPR to determine which a better suited test is in the diagnostic dilemma. Material & Methods: Nasal discharge or crusting are collected in a sterile manner from Nasal, Rhino orbital, Rhiniorbital cerebral Post covid 19 invasive fungal sinusitis patients. KOH Microscopy & Histopathological examinations were done. Conclusion: In post covid 19 invasive fungal infections KOH & HPR were very helpful diagnostic treatment. However HPR was more definitive in diagnosing the invasive fungal sinusitis which also helped in prompt treatment & reduced mortality & morbidity.

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INTRODUCTION

Rhinosinusitis refers to inflammation of the nose and paranasal sinus mucosa due to infectious or non-infectious causes (Chatterjee, 2009). In fungal rhinosinusitis, as the name implies, different species of fungi are implicated in the pathogenesis. As fungi are ubiquitous in nature, these fungi are inhaled into the respiratory system. However, they do not cause disease in everyone. The development of fungal rhinosinusitis is thus multifactorial, depending on the immune status of the patient, environment, the nature of fungus and the genetic factors. The presentation of rhinosinusitis can range from both acute and chronic rhinosinusitis and from non-invasive to invasive, acute fulminant rhinosinusitis (Watkinson, 2018). Invasive fungal sinusitis is commonly seen in haematological neoplasia and is a major cause of death in patients with haematological cancer and in patients receiving long-term immunosuppressive therapy (Lamoth and Thierry Calandra, 2017). However, since the emergence of the novel corona virus, there has been an overwhelming rise in theincidence of invasivefungal rhinosinusitis, in post covid patients, an intriguing presentation which compels further study of the disease and its

association with corona virus. The reason for this can be multifactorial. Incessant use of corticosteroids in Covid patients has caused significant immunosuppression. This superadded with diabetes and use of Immunomodulators such as Tocilizumab along with hyperferritinemia becomes the perfect broth for the fungus to grow.¹² With this superfluous rise, the need for accurate diagnosis becomes imperative, as the nature of fungus, the line of management, medicolegal documentation, depends on the diagnosis. Early diagnosis and treatment is crucial to reduce the mortality and morbidity associated with this condition. KOH mount microscopy and histopathological examination are one of the most the important diagnostic investigations.

KOH Microscopy: KOH mount is one of the main methods of investigating fungal infections. It is a strong alkali and makes the fungus visible by dissolving the surrounding tissues. It is used as a primary screening tool and demonstrates fungal elements present but may not necessarily identify the species of the fungi (Archarya Taneshkar). It serves as a primary screening method as it is easy to perform and gives rapid results.



Fig. 1. KOH microscopy showing fungal hyphae

However, it does not help to demonstrate tissue invasion by the fungus. In our study, tissues were examined using 3% KOH.

ADVANTAGES (Archarya Taneshkar; Manjyoti Gautam, 2020)

- It is cheap.
- It requires minimum infrastructure.
- It is rapid.
- It is easy to perform.
- It is minimally invasive procedure.
- It can be used for screening test.

DISADVANTAGE (ArcharyaTaneshkar; Manjyoti Gautam, 2020)

- It is difficult to identify the species of fungus.
- It does not demonstrate tissue invasion by the fungus.
- Presence of cotton fibres may give rise to false positive result.

HISTOPATHOLOGY

Histopathology is the gold standard investigation for invasive fungal sinusitis⁴. It helps to demonstrate fungal invasion of the sinus mucosa and surrounding tissues and also helps in identifying the species of fungus. All the histological sections were stained with haematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) and Gomoric Methamine Silver Stain (GMS).

Advantages of HPR (Guarner, 2011)

- It can identify the species of fungus.
- It is more specific.
- It is more sensitive.
- It is cost effective

Disadvantage of HPR (Guarner, 2011)

- It is time consuming.
- Requires trained personnels.
- It requires a proper laboratory set up.

MATERIALS AND METHODS

A total of 85 post covid infection cases clinically suspected for invasive fungal sinusitis were included in this study.

Inclusion Criteria- Patients with diabetes mellitus, systemic compromised diseases with covid 19 infection

Age: Patients above 18 yrs of are included in the study

Sex: Both sexes are included in the study

Clinical features: Nasal, Rhino orbital, Rhiniorbital cerebral Post covid 19 invasive fungal sinusitis patients

Exclusion criteria- Patients with diabetes mellitus, systemic immunocompromised diseases without Covid 19 infection. Investigations- Diagnostic nasal endoscopy, MRPNS & Brain, CT PNS with Brain

Nasal discharge or crusting are collected in a sterile manner, kept in a saline containing sterile container and sent to Microbiology Department for KOH mount. A piece of the crust is placed on a clean glass slide and a drop of 3% KOH solution is placed over it and is covered with a coverslip. The tissue is then allowed to dissolve while care is taken to make sure that the slide does not dry up and is then examined under the microscope and findings duly noted. And subsequent diagnostic nasal endoscopy was done for all patients and biopsy taken from suspicious tissue and the specimen is kept in formalin containing sterile container and sent to Pathology Department where Formalin is changed and fixed for a day. Then it grossed and put into cassette the next day. The tissue is processed and then slides (3-5 slides) are prepared. Slides are then stained by haematoxylin and eosin and examined under microscope. If fungal elements are seen, they are further stained by GMS or PAS stain for species identification.

RESULTS

Age of the cases ranged from 18 - 73 yr with average being 49.4 yr in which 76.5 % were male and females constituted 23.5 %.



Fig. 2. H&E stain showing plenty of budding yeast cells with pseudo hyphae

PROFILE OF HPR REPORT

ASSOCIATION BETWEEN KOH MOUNT AND HPR: Our study states that out of 85 cases, 41.2% of the cases were KOH mount positive while 63.5% of the cases were HPR positive. This table shows that more patients were diagnosed as invasive fungal sinusitis on HPR. Out of 63.5% positive cases only 46.3% were positive for KOH mount microscopy while 53.7% were negative for KOH mount microscopy.



Fig. 3. Gender distribution among the study cases



Fig. 4. Representation of KOH report



Fig 5. Representation of HPR report

Fig 6. Comparison of KOH mount and HPR reports.

		POSITIVE	NEGATIVE
KOH MOUNT	41.2%		58.8%
HPR	63.5%		36.5%

Taking histopathology as gold standard, KOH mount microscopy showed an accuracy of 54.1% with PPV and NPV of 71.4% and 42.0% respectively.

Fig.7 a Showing Follow up results Morbidity & mortality %

Table 7: FOLLOW-UP (DNE)				
Outcome	No. of cases	%		
Expired	5	4.2		
Recovered	84	70.0		
Recurrence	31	25.8		
Total	120	100		



Fig. 7b. Showing Follow up results

DISCUSSION

In our study median age group was found to be 49.4(+/- 12.6). This may be due to comorbidities associated with age. The youngest case was reported to be 22 years of age while Desai et al reported that no cases of mucormycosis post covid 19 infection was found below 30 years of age (Desai, 2021). Male to female ratio was found to be 3.2:1. Similarly White et al reported Similar results were reported by White et al.in his study of 135 adults with median age 57 years and male to female ratio 2.2/1 (White et al., 2020). All our study cases were suffering from diabetes mellitus. Apart from diabetes mellitus hypertension was the most common comorbidities (35.5%) after diabetes mellitus which was similar to findings reported by Desai et al and Yohai et al, Ferry et al (Desai, 2021; Yohai, 1994; Ferry, 1983). Debridement of necrotic tissues was done for all patients. Even though surgery alone is not curative, it has been reported that surgery has shown to improve the disease outcome. It helps to decrease fungal loads which slows down the disease progress and improves the effectiveness of antifungals (Invasive Fungal sinusitis). Out of 83 cases, 70 % of our cases are found to be free of disease on follow up diagnostic nasal endoscopy, 25.8% are found to have residual disease while 4.2% died due to extensive disease and comorbidities. All patients were given injection Amphotericin B which is the drug of choice. Liposomal Amphotericin B was used for patients not tolerating conventional Amphotericin B. Posaconazole was used for maintenance therapy after Amphoterin B treatment. HPR suggestive of invasive fungal sinusitis was seen in 63.5 % of our cases where as KOH mount revealed presence of fungal elements in 41.2% of our cases. These results are co-inciding to those reported by Desai et. where biopsy suggestive of invasive fungal sinusitis was reported 100% of the cases. However, other studies conducted by Ajay Kumar Singh et al and Bala et al, higher fungal elements presence was observed on by KOH mount microscopy(22.3%) than histopathological examination (14.8%). Taking histopathology as gold standard, our study showed that KOH mount microscopy has an accuracy of 54.1% with PPV and NPV of 71.4% and 42.0% respectively. In other study conducted by Ajay Kumar 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 NVP of 47% and 88% respectively when histopathology is taken as a gold standard investigation¹. Also a study by Kiran Bala et the mucormycosis cases were positive by KOH (84%), histopathology (58%) (Kiran Bala, 2015). This difference could be due to difference in laboratory preparation, expertise of the microbiologist and the sample collected for microscopy.

CONCLUSION

- KOH mount is one of the main methods of investigating fungal infections. It is used as a primary screening tool and demonstrate fungal elements present but may not necessarily identify the species of the fungi. It serves as a primary screening method as it is easy to perform and gives rapid results. However, it does not help to demonstrate tissue invasion by the fungus.
- Histopathological examination helps to demonstrate fungal tissue invasion and also helps in identifying the species of fungus. It can identify the species of fungus It is more specific & Sensitive also cost effective.
- In post covid 19 invasive fungal infections KOH & HPR were very helpful diagnostic treatment. However HPR was more definitive in diagnosing the invasive fungal sinusitis which also helped in prompt treatment & reduced mortality & morbidity.

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