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Research Article

Multiple and Appropriate Sampling Proved as Key for Early Diagnosis of COVID Associated Mucormycosis

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Abstract

Mucormycosis is a life-threatening, opportunistic infection, caused by a group of saprophytic fungi belonging to taxonomical Order Mucorales. Severe COVID-19 disease is associated with an increase in pro-inflammatory markers, this, increases the susceptibility to various infections. One such opportunistic fungal infection involved is mucormycosis, an angio-invasive disease which is often linked to the use of high dose steroid therapy for longer duration.

In this study samples were taken from post COVID-19 patients suspected of mucormycosis, requested for direct KOH microscopy and fungal culture. A total of 699 samples were processed (including repeat samples) received from 361 post COVID-19 patients. Out of the 361 patients screened by KOH/Calcoflour white preparation, 101(27.9%) came to be positive after first time sampling. On repeat sampling, 36 patients (9.97%%) were additionally picked as positive. A total of 137 patients were positive on screening by KOH. Hence, the positivity rate was increased from 27.9% to 37.87% on multiple and appropriate sampling.

Diagnosis of Mucormycosis remains a challenging task. Multiple sampling of the accessible site of infected area can be a key to early diagnosis of CAM in emergency. The mortality as well as morbidity can be reduced by prompt diagnosis and treatment initiation.

Keywords: COVID Associated Mucormycosis (CAM); Multiple Sampling; Early Diagnosis

Abbreviation

CAM: COVID Associated Mucormycosis

Introduction

Mucormycosis is a life-threatening, opportunistic infection, caused by a group of saprophytic fungi belonging to taxonomical Order Mucorales. It mostly causes systemic and subcutaneous infection in patients who are immunocompromised because of diabetic ketoacidosis, neutropenia, immunosuppressive therapy, organ transplantation, and/or increased serum levels of available iron [1]. In susceptible individuals, the clinical presentations of mucormycosis are classified on the basis of anatomic localisation, such as rhino-orbital-cerebral (ROCM), pulmonary, gastrointestinal, cutaneous, renal, and disseminated mucormycosis [2]. Among these ROCM is the commonest form [2].

The global pandemic of COVID-19 has infected >18 million people internationally and there have been increasing reports of bacterial and fungal co-infections occurring in COVID-19 patients [3]. Severe COVID-19 disease is associated with an increase in pro-

inflammatory markers, this, increases the susceptibility to various infections. One such opportunistic fungal infection involved is mucormycosis [4], an angio-invasive disease which is often linked to the use of high dose steroid therapy for longer duration. Mucormycosis is a disease difficult to diagnose in clinical and laboratory settings. A high index of suspicion on the clinician side with the need to send an appropriate sample to the laboratory is a prerequisite. It is not easy to isolate and maintain them in the laboratory since their poorly septate hyphae can lose the vital cytoplasm at the least manipulation [5].

Since ROCM is the most common form of the mucormycosis [2,6] nasal secretions, were taken to screen for the disease. In this era of pandemic where the burden of COVID-19 patients are rising exponentially, adequately taken nasal secretion or scrapping of the lesion can be helpful in early detection of ROCM. As the disease is an invasive fungal infection with an exceedingly high mortality and few therapeutic options, a less invasive method of sampling with repetition can be done in a short span of time for prompt diagnosis and treatment initiation. Hence patients highly suspected of mucormycosis clinically, should be subjected to direct microscopy with KOH or Chalcoflor white for presumptive diagnosis.

Materials and Methods

Primary objective is laboratory detection of Mucormycosis in post COVID-19 patients. Secondary objective is to evaluate the importance of repeat sample for confirmatory laboratory evidence.

In this prospective observational study conducted over a period of two months (15th April - 15th June, 2021), we included all the samples received in the department of Microbiology at AIIMS, Raipur. These samples were taken from post COVID-19 patients suspected of mucormycosis, requested for direct KOH microscopy and fungal culture. We excluded samples sent as dry swabs or saline flooded swabs. Depending upon the site of infection, the specimen was obtained and samples (tissues, biopsies, fluids, nasal secretions etc.) were processed in biosafety cabinet [7]. All samples were initially treated with 10-20% potassium hydroxide (KOH) and direct microscopy was done including fluorescent brightener, Calcofluor. Repeat specimen were obtained when initial screening by direct microscopy was found to be negative.

We looked for an important diagnostic feature of mucormycosis for presumptive diagnosis i.e. wide angle of non-dichotomous branching (≥45-90 degree) and greater hyphal diameter as compared to other filamentous fungi, ranging from 6 to 25 µm (and even larger).

Culture is necessary for identification to genus or species level hence we had inoculated the sample on two sets of Sabouraud dextrose agar (SDA) with 10% sugar and chloramphenicol incubated at 25°C and 37°C.

Cultures of Mucorales were identified by their rapid growth rate within 3-5 days, characteristic morphologic features like cotton candy like or floccose greyish or brown coloured colonies. The macro and micro characteristics of the fungal growth were examined by lactophenol cotton blue (LCB). Identification to genus level was done by observing sporulation, specific morphological features such as presence of rhizoids, columellae, shape and size of sporangia and sporangiospores. Heat tolerance test was also observed for presumptive genus identification.

Results and Discussion

Results

This study included a total of 699 samples (including repeat samples) received from 361 post COVID-19 patients. The most affected age group (Figure 1) of patients were mostly in the range of 18-60 years (292). 71% of these patients were male and the male-female ratio came out to be 2.4 males per female.

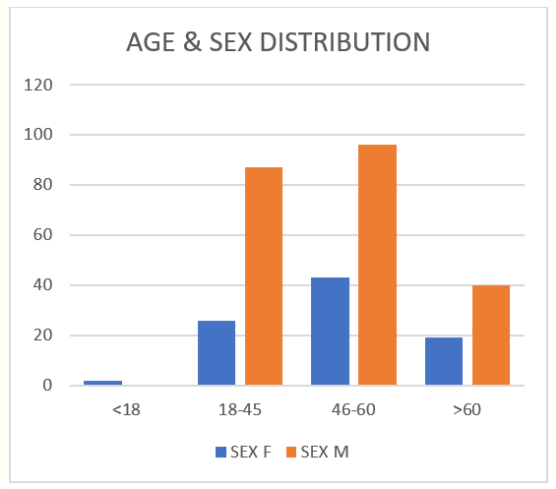

Figure 1

Figure 2

Among these patients 83.65% (302) were diabetic. 57% of the patient had history of steroid therapy of more than 5 days. Medical records were referred for other relevant history and associated risk factors. Distribution of specimen in this study is shown in figure 2. Nasal discharge/swab was the most common specimen received in our lab which comprised of more than 50% of the total samples received. Out of the 361 patients screened by KOH/Calcoflour white preparation, 101(27.9%) came to be positive after first time sampling. On repeat sampling, 36 patients (9.97%%) were additionally picked as positive. A total of 137 patients were positive on screening by KOH. Hence, the positivity rate was increased from 27.9% to 37.87% on multiple and appropriate sampling (Figure 3).

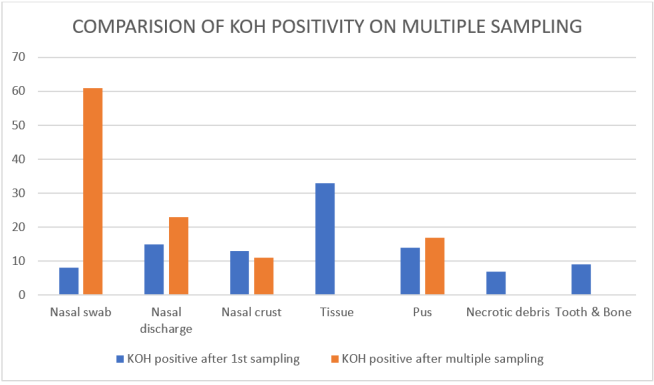

Figure 3

89 (24.65%) specimen were culture positive of which 36 were initially screened negative by KOH. Hence culture showed only 14.68% positivity rate (Figure 4). Of the total 89 culture isolates, genus identified were *Rhizopus* (76), *Lichtheimia* (5), *Rhizomucor* (2), *Apophysomyces* (2), *Syncephalastrum* (1). In three of the isolates mixed growth of Rhizopus was seen with *Aspergillus fumigatus, Fusarium spp. and Lichtheimia* each. (Figure 5).

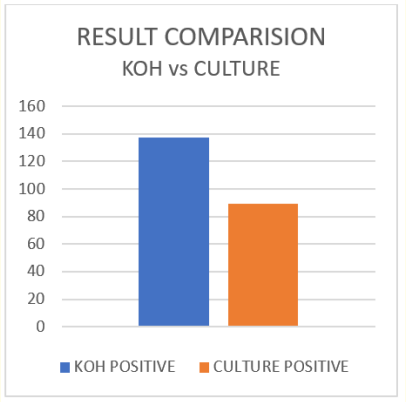

Figure 4

Figure 5

Discussion

In this study we found an increase in mucormycosis cases in the month of May-June 2021 which can be attributed to the surge in

second wave of COVID-19. Our study showed male preponderance as 71% of the affected patients were male as seen in other studies [8,9].

In our study diabetes mellitus was by far the most commonly associated risk factor for the disease. Although, we could not collect glycosylated haemoglobin values of the patients in this short duration of study. SARS-CoV-2 has been shown to affect the beta cells of the pancreas, resulting in metabolic derangement, possibly causing diabetes mellitus [10,11]. Also, concurrent glucocorticoid therapy probably heightens the risk of CAM. Study conducted by Patel., *et al*. showed significant correlation of CAM with glucocorticoid therapy for COVID-19 [8]. In another study on a large review of 929 patients from 1940 to 2003 identified poorly controlled diabetes mellitus as the commonest risk factor (36%), followed by hematologic cancers (17%), and hematopoietic stem cell or solid organ transplant (12%) [12]. Subsequently, the RetroZygo study done over three years in France has shown hematologic malignancy as the risk factor in almost half of the patient population followed by diabetes mellitus (23%) and trauma (18)% [13]. We could not find or correlate any other risk factor which could be associated with CAM probably because short duration of study and lack of proper history.

Rate of mortality in CAM is very high which is of major concern to the health sector [14]. In this study mean age of patients with CAM was 49.5 years Evidence suggests that older age imparts increased risk for hospitalization, respiratory failure, ICU admission, and attendant glucocorticoid therapy in COVID-19 [4,8]. In a study by Patel., *et al*. the mean age of CAM patients was 56.4 years showing association of older ager with CAM [8]. Our study showed a lower mean age in CAM which could be because COVID-19 mostly affected this age group in this part of the country or could be a small sample size in a short duration of time.

Mucormycosis in the debilitated patient typically involves the rhinofaciocranial area, lungs, gastrointestinal tract, skin, or, less commonly, other organ systems. Infections are rare in the immune-competent patient and usually present as cutaneous lesions after traumatic inoculation. In our study, the most common presentation was ROCM. Accordingly the most frequent sample received was nasal swab, secretion or scrapping followed by tissue and pus. This is also shown in various other studies [2,6,12,15].

For microbiological investigations appropriate sampling, always remains a key for early and accurate diagnosis. But as there was acute surge in the cases of Mucormycosis, endoscopic sampling of every patient in the emergency was not possible whereas easily accessible nasal swab, secretion had to be collected as a first sample. The swabs usually collect the unnecessary materials and hold extremely less amount of the specimen but with proper technique appropriate specimen collection, yields better diagnosis. Therefore, repeat appropriate specimen was collected for the negative samples in patients with high index of suspicion. This improved the clinical diagnosis by 10%. Although samples like biopsy tissue, necrotic debris, pus from the affected area, yield more accurate results due to invasive nature of the disease. In these samples lab result of the primary specimen was in correlation with the clinical features. This is shown in our study as these samples gave positive results on multiple preparations from single sample. The clinician should obtain several samples for examination from a single large lesion or from each of several smaller lesions to get optimum result and proper diagnosis.

Since the culture is falsely negative in up to half of cases of mucormycosis due to loss of viability of the non-septate, fragile hyphal forms of these fungi, we avoided grinding of specimens and slicing of tissue was performed as per the recommendations.

Eighty-nine samples were isolated on culture, while in others, the diagnosis was made on the basis of direct KOH wet mount or Calcoflor preparation. The increased KOH sensitivity is also shown by other study Chander., *et al*. [5]. Hence it is rapid and found to be more useful method.

Fungi belonging to the order Mucorales are distributed into six families, all of which can cause infections. Species belonging to the family Mucoraceae are isolated more frequently from patients with mucormycosis than any other family. Among the Mucoraceae, *Rhizopus oryzae* is by far the most common cause of infection [15]. It is associated with all the clinical forms except cutaneous form of the disease. ROCM is frequently associated with *R. oryzae* (most common), *Absidia corymbifera, Cunnighamella bertholletiae, Saksenaea vasiformis, Apophysomyces elegans, Mucor ramosissimus* [2].

Rhizopus was the most common finding in our study as seen in other studies in India and globally. It was followed by *Lictheimia, Rhizomucor, Apophysomyces* and *Syncephalastrum.* The pattern

shows similar prevalence to other studies with *Rhizopus* being the most common occurrence [5,12]. Second most common genus in our study was *Lictheimia* as against *Apophysomyces* in these studies.

Lichtheimia is renamed from genus *Absidia* and only two species have been reported to cause human infections. In the microbiology laboratory, a fungus of this group with abundant circinate side branches on the sporangiophores and pleomorphic giant cells with finger-like projections can give sufficient clues to suspect *Lichtheimia* as a possible isolate which is an emerging cause of Mucormycosis [16].

Apophysomyces cause cutaneous mucormycosis in the form of necrotising fascitis post traumatic inoculation [15]. Rarely, the agent can cause ROCM. An aeromycological survey showed the presence *of Apophysomyces* species in air samples, which may explain the source in ROCM Mucormycosis [2,17].

We isolated single strain of *Syncephalastrum*, from pus of nasal lesion. In the literature, fewer than 10 cases of infections by *Syncephalastrum* have been reported. Recently, a case of subcutaneous mucormycosis due to *Syncephalastrum* was reported [5]. Extensive study is needed to find out the ecological niche of these mucorales.

Other growths were of *Rhizomucor* and three specimens showed mixed growth of *Rhizopus* with *Aspergillus fumigatus, Fusarium spp.* and *Lichtheimia* each.

Conclusion

Diagnosis of Mucormycosis remains a challenging task. Appropriate sampling, rapid diagnosis, early surgical debridement, to lower mortality are linked with each other. With the rapid surge in SARS-CoV-2 infection, prevalence of CAM has reached a new height. Multiple sampling of the accessible site of infected area can be a key to early diagnosis of CAM in emergency. The mortality as well as morbidity can be reduced by prompt diagnosis and treatment initiation.

Acknowledgements

None.

Conflict of Interest

Nil.

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