Prevalance of fungal disease among SARS CoV-2 patients

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Abstract

The prognosis of fungal infections, specifically mucormycosis depends on early diagnosis and timely medical and surgical intervention. Hence we did this study to estimate the incidence of various fungal infections during the COVID 19 pandemic and to evaluate the merits of conventional diagnostic approach

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INTRODUCTION

COVID-19 is a primary respiratory viral illness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). It proved to be a serious warning to humankind, given its easy and fast route of spread and its high rate of morbidity and mortality, particularly during the second wave. It causes respiratory symptoms ranging from mild to severe pneumonia. To worsen the situation, there was a sudden rise in secondary fungal infections emerging as severe threat to the health of people already fighting COVID 19. There were many reports of mucormycosis from different parts of India, where patients presented with involvement of various organs like nose, eyes, brain, GIT, skin and kidney. The factors that were attributed to this sudden upsurge of fungal infections included uncontrolled diabetes, prolonged hospitalization, overuse of corticosteroids leading to hyperglycemia and unjudicious use of antifungal agents.[1,2]

The clinical and radiological diagnosis have low sensitivity and specificity, however, they prompt to raise suspicion and trigger laboratory investigation. Histopathology, direct examination and culture still remain the mainstay of diagnosis especially in low socio-economic countries like India where molecular testing availability is not always feasible due to cost constraints.

The prognosis of fungal infections, specifically mucormycosis depends on early diagnosis and timely medical and surgical intervention. [5] Hence we did this study to estimate the incidence of various fungal infections during the COVID 19 pandemic and to evaluate the merits of conventional diagnostic approach**.**

Method:

It was a retrosprospective observational study done in the Department of Microbiology COVID-19 pandemic. Nasal swabs and endoscopic biopsy samples received from COVID positive or COVID suspected patients were collected and

the details of all the patients were recorded. Consent were obtained from all patients included in the study. Samples were processed in the laboratory by conventional methods including direct microscopy and culture. The swab samples were kept in normal saline and biopsy samples after cutting into small pieces in were placed in 20% KOH , after which KOH wet mounts were prepared. All the samples were cultured on Sabouraud's Dextrose agar (SDA) without cycloheximide and and incubated at 25°C in BOD. Once the growth was obtained, identification was done by standard mycological procedures including colony characteristics, morphological features on lactophenol cotton blue mount (LPCB).

Results:

A total of 400 samples from suspected mucormycosis patients were received during the study period including 200 nasal swabs and nasal biopsies each, 2 sample each of nasal fluid, BAL and pus along with 4 sputum samples.

The positivity rate in direct microscopy of swabs was 27.6%, where maximum (38) were positive for *Mucorales* (hyaline, broad, pauciseptate, ribbon like hyphae), followed by *Aspergillus spp.*(thin, hyaline, septate, acute branching hyphae) (27), dematiaceious fungi (coloured, septate hyphae)(10) and C*andida spp.* (budding yeast cells and pseudohyphae) (08). The positivity rate in direct microscopy of tissue samples was 73%, where maximum (120) were positive for *Mucorales*, followed by *Aspergillus spp.* (26), dematiaceious fungi (04) and *Candida spp.* (08). Culture positivity rate was 48% amongst swabs with 20 *Mucorales*, 42 *Aspergillus spp.*, 23 dematiaceious and 22 *Candida spp*. Culture positivity rate amongst biopsy samples was 41 with 36 for *Mucorales*, 37 for *Aspergillus spp.*, 9 for dematiaceious The confirmation of culture positive and direct microscopy negative findings was done on the basis of repeat culture positivity.

The majority of culture isolates from a total of 200 culture positive samples belonged to the *Aspergillus* spp. (78) followed by *Mucorales* (55), dematiaceious fungi (29), *Candida spp* (27). Amongst the *Mucorales* were 19 *Rhizopus spp*. followed by 17 *Mucor spp*., 11 *Lichthemia spp.* Amongst the *Aspergillus* spp, maximum isolates were *A. fumigatus* (40), followed by *A. flavus* (35), followed by *A. niger* . Amongst the dematiaceious fungi, 2o were *Curvularia spp*, The nasal fluid and BAL samples were both positive by culture and direct microscopy and grew *Rhizopus spp*, whereas amongst the sputum samples, two were positive by culture for *Aspergillus spp*.

Discussion:

Mucormycosis is a fulminant disease which if not diagnosed at the right time can prove to be devastating and can affect various organs such as the eyes, upper part of the nasal cavity, skin and brain. It is very often misdiagnosed due to the non specific findings, resulting in poor prognosis of the disease, hence a very high index of suspicion and timely diagnosis is required for a better outcome.[6] In low- and middle socio-economic countries including India, diagnoses mainly relies on identification of phenotypic characteristics such as growth rate, colony morphology, and reproductive structures on the basis of direct microscopy and culture .[7]

The maximum number of samples that we received were nasal swabs and tissue biopsies. Although it has been stated that nasal swabs is not an appropriate specimen, as the cotton fibres would interfere with the cultivation of pathogenic fungi.[9] But in our study, culture positivity was found to be more in case of nasal swabs rather than tissue biopsies. This culture positive finding was confirmed by repeat sample culture positivity. During times of emergency, when timely diagnosis is the decisive point and every sample is precious, the significance of cotton swabs should not be knocked back.

As evident from our study, direct microscopy is more sensitive in detecting the *Mucorales* as compared to culture. This microscopy positivity and culture negative finding may be attributed to the *Mucorales* hyphae which are quiet vulnerable and may get destroyed while processing the sample. [8] Nevertheless,

Aspergillus was better detected in culture as compared to microscopy. Also the nasal swabs had better positivity in culture as compared to direct microscopy in this study.

Sensitivity via microscopy depends on the source, method of collection and quality of specimens obtained.[8] Direct microscopy alone in our study detected 73 % cases in tissue specimens in contrast to 39% in nasal swabs delineating the importance of biopsy samples. Direct Microscopy enables a wide, easy, economical application as well as a rapid processing time to give a presumptive diagnosis. [3,8] The turnaround time for direct microscopy was maximum one hour enabling the clinician to start the empirical treatment timely, compared to culture findings which took almost a week. It has been observed that delayed treatment of invasive mucormycosis was an independent predictor of poor outcome in multivariate analysis.[10]

Maximum isolates in our study belonged to *Aspergillus spp* followed by *Mucorales*, dematiaceious and *Candida spp*. It clearly depicts that *Aspergillus* was also an understated but equally prominent secondary complication during the COVID-19 times, which has been highlighted by few other studies.[11,12] This is in contrast to a some studies, where maximum isolates belonged to *Mucorales.*[13,14]

Conclusion: The maximum number of samples that we received were nasal swabs and tissue biopsies. Although it has been stated that nasal swabs is not an appropriate specimen, as the cotton fibres would interfere with the cultivation of pathogenic fungi. But in our study, culture positivity was found to be more in case of nasal swabs rather than tissue biopsies.

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