COMMITTEE REPORT

Microbiological Surveillance

for

COVID-19 Associated Mucormycosis

In

Healthcare Settings

Submitted by:

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Committee on COVID-19 Associated Mucormycosis,

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Background

COVID-Corona Virus Disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2 has affected most of the countries with a few nations reporting surge of cases in the succeeding waves. India is currently experiencing second wave with higher caseload as compared to the first wave. COVID-19 associated mucormycosis (CAM) is a life-threatening complication noted among patients with COVID-19. The incidence of mucormycosis in the preceding weeks has been a cause of concern.

In this context, Government of Karnataka has formed a task force of clinical microbiology experts with Dr. Ambica R, State Nodal Officer, VRDLs, Karnataka, Professor & Head, Department of Microbiology, Bangalore Medical College & Research Institute, Bengaluru as the chairperson with Dr. Nagarathna S. (NIMHANS), Dr. Jayanthi Savio (St. Johns' Medical College) and Dr. Anjana Gopi (Kempegowda Institute of Medical Sciences) as members to address the issue of susceptibility/ source of infections among COVID-19 patients and suggest recommendations for to prevent the same.

Methodology

The committee held a series of discussions on mucormycosis among COVID-19 patients & formulated Standard Operating Procedures for data collection. A standardized approach was initiated after consensus was reached among the task force members. Epidemiological and clinical details of COVID-19 patients with suspected/ diagnosed mucormycosis were noted. History of diabetes mellitus, use of steroids for COVID-19 management, history of other immunosuppressive disorders and risk factors for mucormycosis were noted and discussed with clinical colleagues.

In addition to these, infection prevention & control practices (IPC) in the hospital were monitored for compliance to the prescribed guidelines. Microbiological surveillance for determination of zygomycetes was done as per the SOP devised for the purpose as mentioned in the subsequent pages. These guidelines were also approved by the Government of Karnataka for application in health care settings in the preceding days.

The findings of microbiological surveillance were deliberated upon to devise recommendations for prevention of CAM in health care settings.

Microbiological Surveillance for COVID associated Mucormycosis in Healthcare settings

Sample locations:

Intensive Care Unit (ICU)/ High Dependency Unit (HDU)/ Wards. Preference was given to areas from where mucormycosis cases were reported.

Method of sampling:

1 The SDA plates were examined for contamination, prior to use.

2. The infection control nursing officer doing the procedure wore PPE and entered COVID ICU/ HDU/ ward. The plates were assembled and ensured that the correct information was written on the base of the plate (the part containing the media) with ink or other marker. The following details were marked on each plate or recorded separately:

• Date and time of day sample taken

• Area/ location of sample

• Position/ sample number

3. The plates were kept in the ICU/ HDU/ Ward where they were exposed. Four side tables next to beds were identified so as to ensure it falls within 10 cu.ft

4. The plates were placed in the appropriate positions with the lids still on.

5. Lids were raised to expose the surface of the medium; the lid was rested on the very edge of the plate so that the entire agar surface was completely exposed.

6. The plates were exposed for one hour. The exposure time recorded before sending the plates for incubation.

7. After exposure: All plates exposed were collected, and transported in sample transportation container to department of Microbiology

8. The sterile swabs were labeled appropriately and following items were swabbed

a. Patient linen (dress, bedspread and blanket)
b. Patient cot railings and IV Stand
c. Inner surfaces of Nasal cannulas or oxygen mask
d. Inner surface of Oxygen humidifiers

9. 50- 100 mL of water used for oxygen humidifiers were collected in a sterile container after proper labelling

10. SDA plate were exposed to oxygen outflow 2 L/min for 5 minutes

In Department of Microbiology:

Once received at Mycology section, Department of Microbiology,

Incubation conditions:

Settle plates:

1. Plates checked for appropriate label
2. Plates placed with lids upright at 25^0C in an incubator
3. Checked next day and subsequent days for initial growth & also to rule out plate contamination
4. Final reading taken on day 5 for fungal growth, speciated and counts recorded as CFU/cu.ft

Swabs:

Each swab was inoculated on separate SDA plate (appropriately labelled), incubated at 25^0 C and further processed similar to settle plate and any fungal growth was speciated and recorded

Water:

50-100 mL was passed through membrane filter (0.4 / 0.2 micrometer) using 10 ml syringe followed by membrane being removed aseptically using sterile forceps and area of membrane facing upwards inverted and placed on SDA agar plate and further reading taken as in settle plate method and any fungal growth was speciated and recorded.

Oxygen:

Exposed SDA plates were incubated and further reading taken as in settle plate method and any fungal growth was speciated and recorded.

Laboratory Diagnosis of Mucormycosis:

Clinical specimens (in order of preference):

1. Biopsy from Functional endoscopic sinus surgery (FESS)
2. Biopsy from suspected lesions
3. Exudates from nares, hard palatal lesions, sinus material or from any suspected lesion

Following investigations were carried out for the clinical specimens:

1. In Microbiology laboratory: KOH (10 %) and Fungal Culture (Sample in sterile saline)
2. In Pathology laboratory: HPE (Sample in formalin).

KOH mount: KOH helps in screening and has short Turn Around Time.

Sample is put in 10% KOH and observed after 30 min to 1 hour to look for broad, aseptate hyphae with obtuse angle branching.

Sabouraud's Dextrose Agar (SDA) with antibiotics were used for culture. The plates were checked for appropriate label & placed with lid upright at 25^0 in an incubator.

Reading was taken every day up to 5 days to look for growth suggestive of any fungal growth and in particular Mucorales.

Findings

1. Most of the cases of CAM were noted among patients with uncontrolled diabetes mellitus or who were administered steroids in the 1st week of COVID management.

2. It was noted that there was NO growth of Zygomycetes in any of the environmental samples taken from patient care areas or water used in humidifiers or liquid medical oxygen during the current exercise.

3. A few settle plates mainly from the wards showed evidence of *Aspergillus spp.* & *Penicillium spp.* in the settle plates.

4. Fungal growth evidenced among surveillance samples collected from ICU/ HDU were significantly less when compared to those from wards.

5. KOH & culture positivity of zygomycetes were noted only among clinically suspected cases and the same was correlated with histopathological examination (HPE).

6. It was noted that air conditioners in Victoria Hospital, a designated COVID hospital were not being used as a preventive measure since the beginning of the pandemic to minimise chances of transmission.

Recommendations for healthcare settings

1. Fungi are ubiquitous. No construction/ renovation activities to be carried out within the premises of COVID hospitals as such activities significantly raise the microbiological burden specially of fungal spores
2. Seepage/ dampness & visual findings of fungi in health care settings are to be addressed on priority.
3. **Engineering controls:** Air handling units (AHU) and Air conditioners (AC) are to be maintained appropriately and audited as per protocol. On receipt of oxygen cylinders, the same should be wiped & placed in well lit, dust-free areas. SOPs for ICPs are to be framed in line with the standard guidelines and effectively monitored in all health care settings**.** Strict adherence to infection prevention & control practices is to be enforced in all health care settings. **Audit of ICP at least once a week and targeted microbiology surveillance during outbreak is recommended**
4. Multi-disciplinary teams involving Physician, ENT specialist, Ophthalmologist, Anaesthestist/ Critical care physician, Microbiologist & Pathologist should be formed to address the epidemic of CAM in healthcare settings. Hospital guidelines should be framed by this committee keeping in view management of CAM cases & surveillance activities
5. Maintain overall cleanliness in the patients surroundings
6. Wet mopping of floor, patient surroundings /articles with appropriate disinfectant to be ensured at least once every shift
7. Restricted entry inside COVID wards (Similar to ICU practices)
8. Sterile distilled water to be used for oxygen humidifiers and changed every day
9. Respiratory hygiene to be maintained in all patients on oxygen and ventilator as per infection control protocol
10. Host factors and patient parameters need to be evaluated
    a. Patients need to be evaluated for Diabetes Mellitus, even if patient is not a known diabetic (irrespective of COVID status)
    b. Other co-morbid conditions (malignancy, long term steroids for any medical condition)
    c. In patients unaware about their COVID status (asymptomatic / mild symptomatic patients), symptoms suggestive of COVID-19 in recent past and history of contact with known COVID-19 positive patients to be noted
    d. History of vaccination for COVID-19 among CAM cases to be recorded
    e. Close monitoring of blood profile (CBC, LFT, S.ferritin, CRP, LDH, RBS) during active infection is recommended

11. Any oral ulcer should be treated immediately
12. Judicious usage of steroids for COVID management
13. Early clinical indicators of CAM are to be noted and effectively followed up aggressively with laboratory & radiological evidence
14. Biopsy and tissue scrapings are recommended for KOH examination and fungal culture in suspected cases of CAM. KOH mount reports can be made available within 60 minutes of sample receipt in the lab and would form strong evidence for clinical management of CAM. Molecular diagnosis of Mucormycosis is not known to have an edge over conventional methods (microscopy and culture in cases of Rhino cerebral mucormycosis), as per opinion of experts in the field of Mycology
15. Microbiologist should identify & speciate the fungi grown from CAM cases and provide suitable feedback to the treating clinicians
16. Post COVID Work up for CAM: Patients should be alerted about warning signs of CAM during online consultation or during discharge from hospital. Continuous monitoring of blood sugar required even after quarantine period. If patients seek medical consultation with warnings symptoms of CAM, patient should be immediately evaluated by ENT Physicians/ Ophthalmologists and managed accordingly. **KOH of nasal swabs in all discharged cases of COVID -19 is unwarranted**
17. **Nomenclature of fungal diseases as Black fungus, White fungus, Yellow fungus disease is to be strongly discouraged as the names are misleading**.
18. Some unique features noted in CAM are mostly rhinocerebral ocular mucormycosis is being reported in contrast to other forms
19. The factors that appears to be facilitating Mucorales spores to germinate in people with COVID-19 is an ideal environment of low oxygen (hypoxia), high glucose (diabetes, new-onset hyperglycemia, steroid-induced hyperglycemia), acidic medium (metabolic acidosis, diabetic ketoacidosis (DKA), high iron levels (increased ferritins) and decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background co-morbidities) coupled with several other shared risk factors including prolonged hospitalization with or without mechanical ventilators. The pathogenesis of mucormycosis among COVID -19 patients to be studied in much more detail
20. High index of suspicion, early diagnosis and management of CAM is the key to reduce mortality and morbidity.

Recommendations for general public

1. Cleanliness & hygiene to be maintained at all times within the homes & surroundings
2. Cloth masks should be washed daily or whenever visibly soiled before reuse. Disposable masks should not be reused.
3. Oral hygiene should be maintained as per recommended guidelines or as per doctor's advice; However, excessive use of mouthwash & steam inhalation to be avoided
4. In case of co-morbidities like diabetes mellitus, malignancies & chronic diseases, the family physician should be consulted and their advice followed accordingly
5. In case of mild to moderate or asymptomatic persons with history of contact with known COVID positive cases, doctor's consultation should be sought & managed according to the prevailing guidelines
6. COVID-19 patients under home isolation or in the aftermath of discharge post-treatment in hospitals should report early warning signs of CAM as it is a medical emergency and should consult doctor without loss of time
7. Cleanliness of accessories used by the patients for oral care or oxygen delivery should be maintained at all times.
8. Accessories used by the patients for oral care or oxygen delivery should be single-use and should not be shared with others at any cost
9. Self medication by patients & general public should be discouraged
10. Patients are advised to stay away from construction/ renovation sites.

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