



DETECTION OF RISK FACTORS AND PATIENT CHARACTERISTICS ASSOCIATED WITH MUCORMYCOSIS TO DESIGN SCREENING TOOLS HELPFUL IN PREVENTING ADVERSITIES AT PRIMARY CARE LEVEL: A CASE CONTROL STUDY.

Dr. Sumit Rawat	MD H.N. 27 , Phase II Sunrise Town, Tili Road Sagar M.P.
Dr. Shoib Akhtar Khan	MD BMC campus, Sagar M.P.
Dr . Talha Saad	MD BMC campus, Sagar M.P.
Dr. Nitu Mishra*	MDS H.N. 23 , Phase II Sunrise Town, Tili Road Sagar M.P. *Corresponding Author
Devkinandan Kurmi	Msc Near Novel College Makroniya Sagar M.P.
Dr. Pratima Verma	MD Rose Residency, Sanjay Drive Road, Sagar M.P.
Dr. Jyoti Tiwari	MD BMC campus, Sagar M.P.
Dr Amardeep Rai	MD BMC campus, Sagar M.P.

ABSTRACT **Background:** Timely screening, early suspicion and accurate diagnostic measures are needed at primary care level to prevent catastrophe by events such as the recent and sudden emergence of COVID-19 associated mucormycosis (CAM). This entity which was observed during the second wave of this pandemic in India had caused severe chaos by its sudden appearance and frequent devastating outcomes. **Objectives:** To identify the underlying risk factors, clinical characteristics and presentation in CAM cases enabling an early diagnostic approach by use of screening tools at primary care. **Methods:** A retrospective case-control study (April to June 2021) among those fulfilling the diagnostic criteria of mucormycosis with a prior COVID-19 infection. **Results:** 50 patients with mucormycosis as cases and 100 without as controls were enrolled in the study. The median duration from COVID-19 till the onset of CAM was 15 days. The mean age was 50 years and male preponderance, with most commonly reported infection sites nose and sinus (90%) and pansinusitis a predominant finding in CECT (contrast enhanced computed tomography). About 84% (42/50) CAM patients had diabetes mellitus and 60% had received corticosteroid treatment for COVID-19. 13/50 (26%) cases had history of hypertension. History of previous hospitalization was present in 60% (30/50) patients during COVID-19 infection and 26% patients had received oxygen therapy. Serum ferritin levels were available for 19, with elevated levels in 8/19 cases, 11/19 had normal range, 5/50 cases had received Remdesivir injection, only 10/50 CAM patients had received a single dose of COVID-19 vaccine, others were non-vaccinated. **Summary :** Current study unveiled that uncontrolled diabetes mellitus and those who inadvertently receive corticosteroid therapy are at increased risk of CAM. With the ongoing pandemic and increasing number of CAM cases, patients positive for these risk factors during COVID management need regular screening at primary care level in order to prevent this deadly and often fatal secondary infection.

KEYWORDS : COVID-19; Diabetes; Hypertension; Hospitalized; Mucormycosis;

INTRODUCTION

Coronavirus disease 2019 (COVID-19), was associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been declared as global pandemic by the World Health Organisation (WHO) in March 2020 [1–3]. This pandemic continues to be the biggest public health concern causing millions of deaths globally [4]. With the upsurge of its cases worldwide, a myriad of resulting complications including enhanced vulnerability to secondary bacterial and fungal infections are being increasingly appreciated [5–8].

The concomitant medical conditions such as diabetes mellitus and hypertension, and the widespread use of immunosuppressive agents and broad-spectrum antibiotics further aggravated the immune dysregulation caused by COVID-19. In addition, COVID-19 patients are more susceptible to develop secondary infections if they have invasive mechanical ventilation or decompensated pulmonary functions. The rate of in-hospital secondary bacterial and fungal infection has been reported to be approximately 8% [5].

Mucormycosis is known to affect immunocompromised patients especially those with diabetes mellitus, hypertension, prolonged corticosteroid use, neutropenia and haematological malignancies [9–11]. It is an opportunistic infection leading to invasion of blood vessels by fungal hyphae, causing infarction and necrosis of a variety of host tissues. [10]

During COVID-19 pandemic, the upsurge in the cases of mucormycosis occurred either coexisting with or following recovery from viral infection, justifying it as a separate entity: COVID-19 associated mucormycosis (CAM) [8].

As of November 29, 2021, the country has recorded 51,775 cases of

mucormycosis from 28 states. [12] Hospitals around the country continue to report a growing trend of mucormycosis cases in COVID-19 positive patients and this disease was declared an epidemic. Hence, the Government of India has made mucormycosis as a notifiable disease as per the epidemic act 1897 in May 2021 [13].

The current pandemic continues to be a significant public health issue globally and in Indian population, there needs to be increased awareness at general care level about mucormycosis, its diagnosis, and challenges in managing and treating the advanced stage of infection. It is necessary to prevent such secondary infection in future since both conditions in combination may lead to significant morbidity and mortality in Indian communities.

We postulated that diabetes mellitus, hypertension, use of systemic corticosteroids in the treatment of COVID-19, prolonged hospital stay, use of oxygen cylinders and ventilators, might predispose to increased incidence of mucormycosis and these could be useful screening tools at initial level of healthcare. We present a series of 50 cases, by far the one of the largest prospective case series to date, with the objective of highlighting the risk factors associated with the occurrence of mucormycosis in COVID-19 cases.

METHODOLOGY

Overview

This prospective case control surveillance was conducted at Bundelkhand medical college and hospital, Sagar, Madhya Pradesh, April to June 2021 after obtaining due permission to conduct this study by ethical committee of the institute (IEC BMC/108/21/03/09/21).

Case Definitions

It included 50 patients (as cases) who were hospitalized in a special

dedicated 50 bedded Mucormycosis ward at the institute. The control group included 100 patients without mucormycosis. The case definition of mucormycosis was determined on the basis of clinical judgement by the team consisting of one ENT surgeon, one Ophthalmologist, a Dentist, one Pathologist and a Microbiologist, affecting nose, paranasal sinuses, orbit, periorbital area and palate. Computed tomographic (CT) scan reports of PNS, report of microbiological culture done on specialized media for fungus i.e. Sabouraud Dextrose Agar from the scrapping of lesions and the histopathological report of biopsied tissues was considered.

Inclusions Criteria

1. Patients having a laboratory proven COVID-19 either prior to or at the time of development of mucormycosis
2. Patients having coinfections of mucormycosis and aspergillosis were also included.

Exclusion criteria

1. Patients having fungal infections other than mucormycosis were excluded.
2. Patients without a confirmed lab evidence of COVID-19 were excluded.
3. Patients without evidence of invasive mucormycosis.

Data Abstraction And Processing

Demographic and clinical data was collected through the set of questionnaires and previous hospital records. This data was recorded onto a standardized form and also into an electronic data base REDCap (Research Electronic Data Capture, a secure web application) online registry [14]. In our study we have considered and correlated various factors which may commence or augment the infection of mucormycosis. Cases and matched controls were compared in terms of the following variables: age and gender, hospitalized for same or home isolated, history of oxygen support, remdesivir injection during hospitalization. The detailed description of medications from patients previous medical record like steroids, zinc and iron supplements, associated comorbidities like diabetes mellitus, hypertension or any immunosuppressive therapy and current vaccination status as well were included.

Statistical Analysis.

Cases and matched controls were analysed in terms of the demographic features using chi square test and clinical variables using odds ratio ($p < 0.05$ was considered statistically significant).

RESULTS

It is a case control study to identify high risk factors for mucormycosis. Our study included 50 patients (as cases) with confirmed diagnosis of mucormycosis along-with history of COVID-19 and age/gender matched 100 controls (persons with same duration history positive SARS-CoV-2 infection without mucormycosis). The mean duration from onset of COVID 19 to diagnosis of CAM was 15.28 days. All individual enrolled in the study were aged between 17 years to 87 years. The mean age in cases and control group was 50 years and 41.75 years respectively. The case contains 16 Females and 34 Males and control group had females 40 and 60 Males (fig 1,2).

In the case group 60% had diabetes and 26% hypertension while in control group 18% had diabetes and 19 % had hypertension. Diabetes was found to be statistically significant in association with cases (OR= 3.18- 14.63, $P < 0.001$). The study showed that 60% of cases consumed steroids while in control group 36% individual consumed steroids (OR= 10.69 to 2979.3, $p < 0.05$ is significant). Hospitalization was associated with higher odds of mucormycosis compared to control (OR= 3.006 to 13.60, $p < 0.001$) and 26% CAM patients was on oxygen support and none of the control required it. 10 % cases and 4% control were on remdesivir injection during COVID period (OR = 2.66). There was no increased risk of mucormycosis associated with Ferritin level ($p=0.30$) and zinc supplementation ($p=0.73$) in our study. In case and control group 20% and 72% were vaccinated and we found no association with case (OR= 0.2100 to 1.07, $p= 0.0752$). Among our study cases, two were discharged with no reported mortality, with 42 /50 requiring surgical intervention and on continuing anti-fungal therapy for 3 months and a follow up check-up every month. The analysed data have been summarised in Table 1 and Table 2.

DISCUSSION

The current study set-up being in the middle of the strongest waves of COVID-19 ever witnessed, our study tries to uncover the predisposing factors behind the surge of CAM among such cases which posed a

strong challenge to safe outcomes. As far as the patient characteristics are concerned we found the mean duration from the onset of COVID-19 symptoms to the diagnosis of CAM to be 15 days. The mean age of CAM patients in our study was 50 (range 30 to 84) years, with male preponderance. These findings relating to patient characteristics were in agreement to that of some earlier studies [15,16,17].

The most common sites affected with mucormycosis in our cases were Sino-nasal (90%) followed by rhino-orbital (53.7%) and ROCM (rhino orbit cerebral mucormycosis in 22%). Interestingly, these findings were in contrast to that reported by some recent studies in which ROCM were the more commoner to get affected [18, 19].

Imaging with CECT demonstrated predominantly pansinusitis (38%) which is a unique finding revealed by the current study.

Diabetes mellitus with poor glycaemic control (none having diabetic keto-acidosis) in about 84% CAM (42/50) patients appeared to be most frequent and important risk factor in our study. The previously postulated reasons like compromised chemotaxis and phagocytic effect of macrophages and neutrophils by oxidative and non-oxidative pathways, in states of hyperglycaemia might be responsible [20,21].

Steroid administration stood out as another important risk factor in the majority 30/50 (60%) of our patients with CAM. Steroids are an admirable treatment, if used judiciously in management of COVID-19. The widespread, inappropriate or may be an overuse of steroids here very well served the purpose of predisposing our patients, as it has been suggested that steroids function mainly through interaction with glucocorticoid receptors, or by causing defects in the function of macrophages and neutrophils, which downregulates the expression of pro-inflammatory cytokines (such as (TNF)- α , IL-6, IL-8, and IL-12) secreted by macrophages thereby leading to immunosuppression aiding the development of CAM. Majority of our patients but had taken steroids for more than a week, which was quite less for the controls. However, the accurate duration and dose administered to all the patients could not be determined as it varied due to physician preference and intake of OTC (over the counter) steroid by the patients, which has been seen earlier [15,16,22].

Hypertension (26%) among our cases of CAM emerged as important co morbidity associated with development of CAM. Most studies failed to consider its role to be associated with development of mucormycosis.

Hyperglycemia along with administration of high dose of steroid, and other related risk factors, in situations of weakened immune system (during COVID-19) is the plausible explanation for high incidence of association of these factors with mucormycosis, which was highlighted by our study [23].

Among the various other associated risk factors studied, recent hospitalization for COVID-19 infection was present in 30/50 (60%) cases and 26% patients received oxygen therapy. Various underlying suspected factors include unhygienic delivery of oxygen, the use of oxygen cylinders with unclean masks, prolonged usage of same mask for more than two patients, low-quality tubing system to these patients at the hospital, or use of contaminated tap water in humidifiers [24,25,26].

Risk factors like prolonged ventilation and extended stay in hospital were not found in our cases, not suggesting their usefulness as a screening tool. However, these factors have been suggested as significant causes for such secondary infections in previous studies (Mishra et al).

Injection Remdesivir administration, showed higher odds for developing the disease and was found in 10% cases, highlighting its role as risk factor in causation of mucormycosis [22].

History of Zinc intake was present with all cases and controls in our study. In an attempt to prevent severe COVID-19 infection an increasing number of people have been taking Zinc disproportionately through oral medications or dietary supplements. However, we did not find any link between Zinc intake and susceptibility of mucor infection.

Serum ferritin levels of only 19 CAM cases could be retrieved from the hospital record. Out of which 8 cases had elevated serum ferritin levels

and 11 had normal range. Elevated iron may increase expression of host receptor glucose regulator protein 78 (GRP78), spores adhere to and invade endothelial cells by specific recognition of GRP 78 and thus facilitates angio-invasion of mucorales[27].

In our patients with CAM, only 10 patients had received a single dose of COVID 19 vaccine, rest were non-vaccinated. The type of vaccines could not be determined as patient was not aware of the type they were administered.

The overall picture emerging from our study findings suggest that Mucorales spores might germinate in people with COVID-19 due to favourable environment of low oxygen (hypoxia), high glucose, decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background comorbidities) along with other risk factors including induced hospitalization, ventilation and incomplete dose of vaccination.

Treatment outcomes could be determined in all the cases. The standard treatment included liposomal amphotericin B on clinical suspicion of mucormycosis followed by endoscopic debridement. In 42/50 (84%) cases endoscopic debridement of sinus was done. During the entire duration of study all the patients were continued treatment, only 2 could be discharged and the rest were in hospital on parenteral antifungals.

No mortality was witnessed in our study perhaps due to early diagnosis along-with timely management and surgical interventions. However, it should be noted that we did not have any patients with cerebral/disseminated mucormycosis. Nor did we have any case with haematological malignancy or organ transplant with immunosuppression as observed in some other studies with higher adverse outcome.

The large sample size in our study for this rare and deadly disease, the inclusion of control group (i.e. COVID-19 without CAM), helped to delineate the risk factors that predict the chances of development of CAM in COVID-19 and assessment of mortality due to CAM.

Limitations Of The Study

The study also has few limitations like, the data of dose and duration of glucocorticoid therapy were infrequently reported, hence they could not be included in the analysis. There could be some recall bias as some cases did not know their diabetic status before COVID-19 infection or whether they were newly diagnosed at the time of COVID-19. Nevertheless, these findings should be able to lay a strong foundation to the path in preventing these dreaded complications in areas showing rising infections of SARS-CoV-2.

CONCLUSION

Among patients with COVID-19, mucormycosis is an important hurdle to achieve long-term safety and outcomes. COVID-19 has further complicated the pre-existing and infamous mucormycosis situation. Current study highlights the need for early identification and optimally reduce this deadly disease complication through screening for risk factors via screening tools like obtaining history of steroids/Remdesivir administration during COVID-19 treatment and regular blood glucose monitoring in patients treated for COVID-19, thus facilitating timely management, and preventing adversities in such situations.

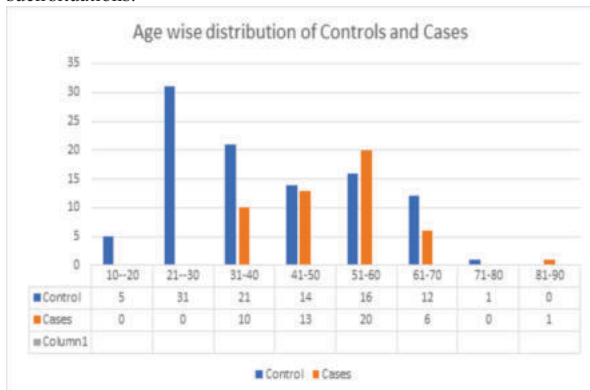


Figure 1

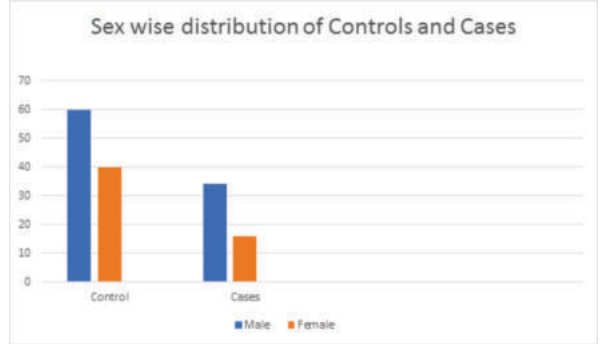
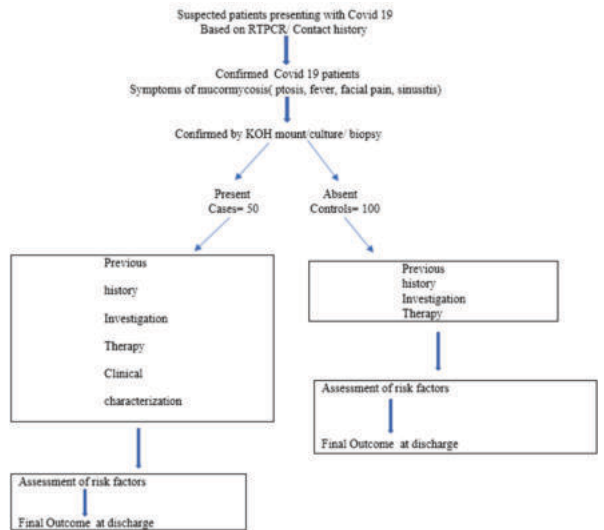


Figure 2



Flow Chart 1

Table 1

Factors	Case (n= 50)	Control (n=100)	Odds Ratio	P Value	Confidence Interval
Hospitalization	30	19	6.39	p<0.0001	95%
Oxygen Requirement	13	0	98.67	p=0.0016*	95%
Remdesivir	5	4	2.66	P=0.158	95%
Steroid intake	50	36	178.4	p=0.0003*	95%
Diabetes	30	18	6.83	P=<0.0001*	95%
HTN	13	19	1.49	p=0.325	95%
Ferritin	8	55	0.595	p=0.305	95%
Zinc	50	100	0.502	p=0.731	95%

* Significant

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