Comparative Evaluation of COVID-19 Associated Mucormycosis (CAM) and Non-COVID-19-associated Mucormycosis (non-CAM)

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ABSTRACT

Objective: To compare COVID-19 associated mucormycosis cases (CAM) with non-COVID-19 associated mucormycosis (non-CAM) cases followed as in-patients.

Study Design: Observational Study.

Place and Duration of Study: Department of Infectious Diseases and Clinical Microbiology, Adana City Training and Research Hospital, Health Sciences University (HSU), Adana, Turkey, between January 2018 and March 2022.

Methodology: Patients with a diagnosis of mucormycosis (proven and probable) were dichotomised as COVID-19 associated mucormycosis and non-COVID-19 associated mucormycosis cases. Both groups were compared for underlying malignancy, chemotherapy, antifungal therapy related side effects and overall survival.

Results: Of the 35 cases enrolled in the study, 17 (48.6%) had CAM and 18 (51.4%) had non-CAM. A statistically significant difference was detected between non-CAM and CAM cases in terms of haematological malignancy, receiving chemotherapy, and antifungal therapy related side effects (p=0.019, p=0.019, and p=0.027 respectively). Steroid use was found as a risk factor for the diabetic CAM cases (p<0.0001). The difference between the CAM and non-CAM cases in terms of overall survival was not statistically significant (p=0.088).

Conclusion: Because of the ongoing COVID-19 pandemic and the increasing number of critical patients, treatment of COVID-19 should be performed cautiously in patients who have the risk of developing CAM, particularly those with diabetes and immunosuppression (haematologic malignancy, receiving steroid or chemotherapy, etc.) and these patients should be monitored closely.

Key Words: Mucormycosis, COVID-19, Mucormycosis associated with COVID-19, Diabetes mellitus, Turkey.

INTRODUCTION

Mucormycosis is a rare, rapidly-progressive fungal infection associated with angio-invasion and has a mortality rate of 40-80% unless diagnosed.¹ Mucormycosis is classified into six clinical categories as rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated, and other localisations depending on the clinical presentation and the anatomic localisations.²

Uncontrolled diabetes mellitus (with or without ketoacidosis), neutropenia, haematological malignancies, organ transplantation, the use of immunosuppressant drugs such as corticosteroids, excessive iron load (desferrioxamine use, etc.), and impaired skin integrity (due to trauma, burnt, etc.) are among the risk factors for mucormycosis.³⁻⁴ Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is a new coronavirus that has been first identified in 2019 in China, has led to a worldwide pandemic resulting in 510,270,667 confirmed cases and 6,223,526 confirmed deaths to date.⁶ In India, cases of invasive mucormycosis, also known as black fungus infection, increasing in number up to 9000 have been reported synchronously with the rising second wave of COVID-19.⁷ During SARS-CoV-2 pandemic, steroids have been prescribed by clinicians at an amount and for a duration quite exceeding those recommended by the World Health Organisation (WHO). As a consequent, patients’ immune system has been suppressed, blood glucose regulation has been impaired, and patients have

biopsy materials obtained from clinically suspected mucormycosis and treatment methods were retrieved from electronic patient microbiological characteristics, comorbidities, and diagnostic or 12 weeks before the diagnosis of mucormycosis in combination with related symptoms. Non-COVID-19 associated mucormycosis (non-CAM) meant absence of the positivity of SARS-CoV-2 polymerase nucleic acid chain reaction (PCR) test at the time of or 12 weeks before the diagnosis of mucormycosis in combination with absence of related symptoms.

Patients’ demographic, radiological, histopathological and microbiological characteristics, comorbidities, and diagnostic and treatment methods were retrieved from electronic patient records and were recorded in the case report forms. Tissue biopsy materials obtained from clinically suspected mucor patients were first inoculated into SGA medium. Thereafter, the samples were kept in 10% potassium hydroxide for 15 minutes and then subjected to direct microscopic examination. Non-septate or pauciseptate hyphae structures with irregular ribbon-like appearance and 90-degree branching that vary in width was searched. The plates were incubated for 2-5 days both at 30°C and at 37°C. Mucorales tended to reproduce rapidly and filled the entire petri dish within a few days. After macromorphological and micromorphological evaluation of the fungi grown in the medium with lactophenol cotton blue dye, matrix assisted laser desorption ionization time of flight (MALDI-TOF; Vitek MS BioMérieux-IVD; Liyon, France) were used to identify the fungus.

Sections obtained from the clinical materials were stained with haematoxylin eosin and PAS. In the microscopic examination of stained preparations, and detection of non-septate hyphae branching at right angles was defined as mucor.

Descriptive statistics were presented as frequency, percentage, mean and standard deviation, median, minimum, and maximum. Categorical variables were analysed by Fisher’s Exact test if the percentage of cells, where the expected value was <5, was greater than 20%, whereas, Pearson Chi-Square test was used if it was smaller than 20%. The assumption of normality was checked with the Shapiro-Wilk test. The difference between the numerical data of the two groups was analysed by independent samples t-test if the data were in accordance with the normal distribution. Analyses were done using SPSS 23.0 program. Level of statistical significance was considered to be p<0.05.

RESULTS

A total of 35 mucormycosis cases were evaluated. Of the cases, 23 (65.7%) were males, 12 (34.3%) were females and the mean age was 59.54±15.32 (22-84) years. According to the EORTC/MSG criteria, 31 cases (88.6%) had proven mucormycosis and 4 cases (11.4%) had probable mucormycosis. Thirty-four cases were clinically classified as rhinocerebral (5 cases rhinoorbital, 5 cases nasal, and 24 cases rhinoorbitocerebral) and one was classified as disseminated mucormycosis. Diabetes mellitus 80% (n= 28) was the most common comorbidity. Among the cases with diabetes, 18 (64.2%) had ketoacidosis.

There was statistically significant difference between non-CAM and CAM cases in terms of haematological malignancy, receiving chemotherapy, and antifungal drug-related side effects p=0.019, p=0.019, and p=0.027 respectively). Steroid use was found to be a risk factor for the diabetic CAM cases (p<0.001). Comparison of demographic characteristics, site of clinical involvement and treatment outcomes between CAM and non-CAM patients is summarised in Table I.

All cases underwent paranasal tomography, with additional magnetic resonance imaging (MRI) of the cranium and orbita performed in the patients considered to have cranial and orbital involvement. An appearance suggestive of mucormycosis was detected in 12 (34.3%) of the cases that underwent cranial MRI
and in 15 (78.9%) of the cases that underwent orbital MRI. Radiological findings of the cases are demonstrated in Table II.

Endoscopic sinus surgery was performed in 74.3% (n=26) of the cases, and all cases received systemic antifungal therapy (Liposomal amphotericin B, 5 mg/kg IV). Sixteen cases had undergone orbital exenteration. It was detected that only three cases were healed without sequel, while two cases were healed with loss of vision and one case was healed with hemiplegia and loss of vision.

Table I: Characteristics of CAM and non-COVID-19 associated mucormycosis patients.

<table>
<thead>
<tr>
<th></th>
<th>CAM= 17 N (%)</th>
<th>Non-CAM=18 N (%)</th>
<th>p 1,2,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (year)</td>
<td>62.12±14.26</td>
<td>57.11±16.29</td>
<td>0.342†</td>
</tr>
<tr>
<td>Gender (Female/Male)</td>
<td>(23.5%)/4/13</td>
<td>(44.4%)/8/10</td>
<td>0.289§</td>
</tr>
<tr>
<td>Diabetes mellitus (DM)*</td>
<td>16 (94.1%)</td>
<td>12 (66.6%)</td>
<td>0.088†</td>
</tr>
<tr>
<td>Diabetic ketoacidosis (DKA)</td>
<td>9 (52.9%)</td>
<td>9 (%)/50</td>
<td>0.862†</td>
</tr>
<tr>
<td>Haematologic disease**</td>
<td>0</td>
<td>6 (33%)</td>
<td>0.019†</td>
</tr>
<tr>
<td>Kidney transplantation</td>
<td>1 (7%)</td>
<td>0</td>
<td>0.486†</td>
</tr>
<tr>
<td>Steroid use</td>
<td>17 (100%)</td>
<td>0</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Chemotherapy use</td>
<td>0</td>
<td>6 (33%)</td>
<td>0.019†</td>
</tr>
<tr>
<td>Site of involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal</td>
<td>4 (23.5%)</td>
<td>15 (5.6%)</td>
<td>0.252†</td>
</tr>
<tr>
<td>Rhinocerebral***</td>
<td>13 (76.5%)</td>
<td>16 (88.8%)</td>
<td></td>
</tr>
<tr>
<td>Disseminated</td>
<td></td>
<td>1 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Pathologic diagnosis</td>
<td>16 (94.1%)</td>
<td>15 (83.3%)</td>
<td>0.603†</td>
</tr>
<tr>
<td>Growth in the culture</td>
<td>1 (7.7%)</td>
<td>2 (20%)</td>
<td>0.589†</td>
</tr>
<tr>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor spp</td>
<td>9 (69.2%)</td>
<td>5 (50%)</td>
<td></td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>3 (21.3%)</td>
<td>3 (30%)</td>
<td></td>
</tr>
<tr>
<td>Surgical intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exenteration</td>
<td>8 (47.1%)</td>
<td>8 (44.4%)</td>
<td>0.877†</td>
</tr>
<tr>
<td>Antifungal drug side effect</td>
<td>9 (52.9%)</td>
<td>16 (88.9%)</td>
<td>0.027†</td>
</tr>
<tr>
<td>Result</td>
<td>12 (70.6%)</td>
<td>17 (94.4%)</td>
<td>0.088†</td>
</tr>
<tr>
<td>Exitus</td>
<td>5 (29.4%)</td>
<td>1 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Healing/healing with sequel</td>
<td></td>
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</tbody>
</table>

*3 cases of acute myeloid leukemia, 1 case of aplastic anemia, and 1 case of plasma-cell cancer; **3 cases of chronic kidney disease and 1 case of chronic renal failure; ***Rhino-orbital cerebrovascular involvement in 24 and rhino-orbital involvement in five case; †Independent t test; §Pearson Chi square test; ††Fisher’s Exact test were used.

DISCUSSION

COVID-19 caused numerous cases and deaths occurring during the first wave in 2020. In addition, during the next wave of COVID-19, mucormycosis, called as black fungus disease, caused worldwide concern. The majority of the cases occurred during the second wave of the active SARS-CoV-2 outbreak in India and Indian Central Government announced a mucormycosis outbreak in May 2021. In this study of mucormycosis affected patients, the gender and mean age comparison showed no statistically significant difference between the two groups. Similar to the study by Eker et al., this study determined that CAM cases were seen more frequently in older males compared to non-CAM cases.

In literature, the majority of cases presented with rhino-orbital-cerebral (ROC) mucormycosis; pulmonary mucormycosis was the most common clinical involvement in the cases reported from European countries such as France, England, and Italy. which was different from the large case series reported from India. The number of CAM cases in the present study were higher than that reported from Europe, with similar patient characteristics to the case series reported from India. This can be explained by suboptimal blood glucose control in diabetic population in Turkey as compared to Europe, as well as by missing cases of pulmonary and gastrointestinal mucormycosis since it is more difficult to diagnose them.

In line with the WHO recommendations, guideline from the Ministry of Health in Turkey also recommends the use of corticosteroid therapy in COVID-19 to suppress hyper-inflammation. The presence of diabetes mellitus in combination with steroid therapy increases the risk of mucormycosis by means of contributing to immunosuppression and hyperglycemia. In the present study, COVID-19 cases with uncontrolled diabetes, who received steroids for the treatment of COVID-19 were complicated with mucormycosis. Steroid therapy in diabetic patients with COVID-19 should be used only in cases recommended by the guidelines, and these patients should be followed closely also after discharge from the hospital for blood glucose regulation and development of mucormycosis.

Haematological malignancy and chemotherapy are among the predisposing factors for the development of ROC mucormycosis particularly in non-diabetic patients. The rates of chemotherapy and haematological malignancy were statistically significantly higher in non-CAM cases (p=0.019 and p=0.019, respectively). In this patient group, mucormycosis must be considered in the differential diagnosis since prompt diagnosis and treatment is associated with reduced mortality and morbidity.

The prevalence of ROC in non-CAM diabetes cases was reported to be 88% by Nithyanandan et al., 51% by Jeong et al., and 64% by Vaughan et al. In two case series from Turkey, the most common clinical form was rhino-cerebral form (94-96%). ROCM was detected in 88.9% of the non-CAM cases. Although ROC involvement in CAM cases shows variation between studies, it is seen at a rate of 74-86%. Consistent with the literature, ROC was the most common type of involvement in our CAM cases with a prevalence rate of 76.5%. The groups showed no statistically significant difference (p=0.656) regarding the site of involvement, with ROC being the most common site in both groups. Since mucormycosis is an aggressive infection that usually spreads over the orbital or cranial area from the paranasal sinuses, radiological imaging is critical in the diagnosis. Appropriate scanning should be performed in the shortest time to detect the margin and the extension of the
Mucormycosis in hematopoietic cell transplant recipients and in patients with haematological malignancies was significantly higher in the non-CAM cases (p=0.027). The present authors believe this to be due to the fact that non-CAM cases of haematological malignancy patients receiving chemotherapy constitute the vast majority. The most common side effects were acute renal failure and hypokalemia. Every patient who is receiving LAmB therapy requires close monitoring.

Tissue necrosis reduces the penetration of antifungal therapy into the infected area, and therefore the most critical step in treatment is the removal of necrotic tissue by surgical debridement. Besides, surgical debridement prevents the extension of infection to the other organs. In a meta-analysis, it was demonstrated that mortality remarkably decreased with the combination of surgery and antifungal therapy compared to antifungal therapy alone. This can be explained by the fact that the transient immunosuppression of the patients can be corrected with adequate supportive treatment and high doses of liposomal amphotericin-B. Only three cases were healed without a sequel, while 2 cases were healed with loss of vision, and one case was healed with hemiplegia and loss of vision.

The small sample size and retrospective study design are the main limitations of the present study. However, it is the first study on this subject covering different patient groups from Turkey with the largest sample size to date.

CONCLUSION

Mucormycosis is an angioinvasive fungal infection with increasing incidence also in COVID-19 cases. Unnecessary corticosteroid use is the main risk factor for mucormycosis, especially in diabetic patients during the COVID-19 pandemic. Mortality can be reduced by early diagnosis and treatment achieved by clinical suspicion. In order to suppress hyper-inflammation in COVID-19 patients, it would be reasonable to use the corticosteroids in the patient groups defined in the guidelines and to monitor the patients who are likely to develop CAM cautiously and closely.

ETHICAL APPROVAL:
The protocol of the study was approved by the ethics committee of Adana City Training and Research Hospital, Health Sciences University (HSU), Adana, Turkey (Approval No. 1927).

PATIENTS’ CONSENT:
In keeping with the policies for a retrospective review, the informed consent requirement was waived.

COMPETING INTEREST:
The authors declare no competing interest.

AUTHORS’ CONTRIBUTION:
EO: Conception and design of work. The acquisition, analysis, and interpretation of data. Drafting the work and reviewing it critically for important intellectual content. Revision of the manuscript.
TAG: Data curation, literature reviewing, and statistical analysis.
TT: Data curation, literature reviewing, and visualisation.
NYE: Data curation and literature review.
NU: Literature reviewing and drafted the manuscript.
All authors contributed to the study conception and design. All authors have read and approved the final version of the manuscript.

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