COVID-19-associated mucormycosis in Iran: A retrospective study on 52 patients

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Abstract

Background: COVID-19-associated mucormycosis (CAM) has become one of the most severe post-COVID-19 comorbidities.

Objectives: To describe CAM cases, identify possible risk factors, and report outcomes of patients.

Methods: This retrospective study was performed in Amir-Alam Hospital, Tehran University of Medical Sciences, Tehran, Iran between February 2020 and September 2021. Patients with an active or previous diagnosis of COVID-19 have been included.

Results: Of 94 patients with mucormycosis, 52 (33 men and 19 women; mean age:57.0±11.82 years) were identified with the confirmed diagnosis of COVID-19. Rhino-orbital, rhinomaxillary, rhino-orbital cerebral subtypes were detected in 6(11.5%), 18(34.6%), and 28(53.8%) patients. As a control, 130 (69 men and 61 women; mean age:57.0±11.82 years) random RT-PCR-confirmed COVID-19 patients without mucormycosis have been included. The mean interval between COVID-19 diagnosis and initial mucormycosis symptoms was 16.63 ±8.4 days (range 0-51). Those in the CAM group had a significantly more severe course of COVID-19 (P-value<0.01). Diabetes mellitus (DM), high-dose corticosteroid pulse therapy, smoking were found as risk factors (P-value<0.01 for all). New-onset post-COVID-19 hyperglycemia was lower in the CAM group (46.2% vs. 63.8%; P-value=0.028). After treatment of the CAM group, 41 (78.8%) of patients recovered from mucormycosis. The mean ages of the expired patients were significantly higher (66.18±9.56 vs. 54.56±11.22 years; P<0.01); and COVID-19 disease was more severe (P=0.046).

Conclusion: smokers, COVID-19 patients with a history of DM, and those with severe disease are at a high risk of CAM, and the mortality rate is higher in older patients. More studies are required to clarify risk factors.

Introduction

From the beginning of the novel Coronavirus pandemic, announced by WHO on 11 March 2020, the number of individuals with a history of infection with SARS-CoV-2 is more than 350 million people worldwide, with more than 5 million related deaths as of Jan 2022. Coronavirus disease-19 (COVID-19) is a highly contagious and fatal infectious disease, caused by the SARS-CoV-2 virus (1). After the identification of the SARS-CoV-2 sequence, the Wuhan version, six SARS-CoV-2 variations of concern have been identified so far. As the most important variations, according to WHO, Alpha (B.1.1.7) and Beta (B.1.351), were reported in the United Kingdom and South Africa, respectively in late 2020. Subsequently Gamma (P.1), first reported in Brazil, and Delta (B.1.617.2), first described in India have been reported. Recently, another variation, called B.1.1.529 was reported to WHO from South Africa on 24 November 2021, which has affected many counties in a short time (2). New SARS-CoV-2 strains could make the virus more contagious, pathogenic, and even help the virus to evade vaccines or be diagnosed by regular testing strategies (3, 4).

Apart from mortality, COVID-19 is associated with increased morbidity and accounts for a significant proportion of the use of healthcare staff and resources in many countries, especially developing ones. Recently, an increasing number of studies are reporting the association of COVID-19 with a very rare fungal infection, known as Mucormycosis, also known as ‘Black Fungus’ (5–10). It is a rare opportunistic and serious fungal infection caused by Mucomycetes (11, 12). As Mucormycosis has critical outcomes and can be life-threatening in some situations, appropriate identification and characterizing of individuals at high risk is extremely important.

A comprehensive range of risk factors for Mucormycosis has been identified previously, such as poorly controlled diabetes mellitus (DM), cancer chemotherapy, organ transplantation, immunosuppressive therapies, and any other condition with immune system disruption (13). Knowing these risk factors helps to treat patients at early stages, which minimizes the risk of mortality and irrevocable tissue damages. However, concomitant COVID-19 and mucormycosis might complicate the situation. During the pandemic, especially within the second wave of spreading of new variants of SARS-CoV-2 in India, several reports of emerging cases of. mucormycosis as in those with resolved COVID-19 or patients with active disease have been published. Although some hypotheses are suggested for the probable association of these two conditions, it has remained a mystery. In Iran, especially during the fifth wave of the pandemic, the number of patients with mucormycosis has increased. Some studies have suggested that the association might be because of glucocorticoid usage, hyperglycemia, delta variation, etc. variation (14–17).

Here, we designed a retrospective study to assess the patients with an active or documented history of COVID-19 before and after the dominance of Delta variation; who have been admitted for mucormycosis. We aimed to find risk factors for the development of mucormycosis in the Iranian population with active or history of infection with SARS-CoV-2 infection. Additionally, clinical signs, symptoms, and outcomes of included patients with COVID-19 associated mucormycosis (CAM) were collected and analyzed.

Material And Methods

Study design

This is a retrospective study of all the admitted patients to Amir-Alam Hospital, Tehran University of Medical Sciences, Tehran, Iran between February 2020 and September 2021, who had been diagnosed with mucormycosis, regardless of the subtype have been included. Those with active or with a history of COVID-19 were considered for in-depth analyzes. Demographic data, diseases' characteristics, comorbidities, treatments, and outcomes were recorded to evaluate to find any possible correlation between COVID-19 and mucormycosis.

All procedures were approved by the relevant independent ethics committees and were done in accordance with the Helsinki Declaration. Informed consent was obtained from all subjects.

Patients' inclusion and diagnosis

Patients with any of the symptoms and signs of mucormycosis were evaluated for confirming or excluding the diagnosis. Initial diagnosis of mucormycosis has been made based on histopathological examination of biopsy specimens and radiological findings (CT, MRI), which had been confirmed by two pathologists and one radiologist. Patients under 18 had been excluded. Regarding COVID-19, For those patients, in addition to looking for typical signs and symptoms of COVID-19, reverse transcriptase-polymerase chain reaction (RT-PCR) and chest computed tomography (CT) have been done to confirm the disease. Those with a history of COVID − 19 in the last 6 months and also those with active COVID-19 have been included. As the control group, 130 patients were selected with the COVID-19 diagnosis, but without mucormycosis had been included retrospectively.

Clinical and radiological evaluation for mucormycosis

For each patient, main clinical manifestations related to COVID-19, either revolved ones or in the active form have been collected in an electronic database. Regarding the mucormycosis, initial symptoms, the subtype, and affected anatomical regions have been recorded. Patients with suspected mucormycosis were assessed by ENT specialists. A thorough head and neck examination including anterior rhinoscopy, oral cavity, oropharyngeal examination, and otologic examination were all done. All patients underwent endoscopic nasal examination under local anesthesia, and tissue biopsy was taken for pathologic assessment. All patients underwent ophthalmologic evaluation as well.

Radiological assessments have been done using magnetic resonance imaging (MRI) and CT. Imaging points that suggest acute invasive fungal sinusitis (AIFS) are sinus wall erosion and peri sinus fat stranding which are usually observed in preantral fat, buccal and infratemporal spaces, and orbital intra and extraconal spaces. Also, we scrutinized nasal cavity mucosal thickening, and if present, a clinical exam was suggested. Non-enhancing regions, perineural spread, vessel wall invasion, orbital apex, and superior orbital fissure syndrome, cavernous sinus thrombosis, epi/subdural empyema, cerebritis, cerebral abscess formation, and cerebral watershed territory infarct were other findings that imaging especially MRI can help us to find them.

COVID-19 severity assessments

Regarding the COVID-19, patients were categorized based on these criteria of their COVID-19 disease to mild, moderate, and severe. In the mild category, the patients were symptomatic but did not show dyspnea or imaging abnormality in the lung, Moderate group had shortness of breath or imaging abnormalities, but o2 saturation was equal or more than 94% on room air and patients with any of the following complication were categorized as a severe disease: O_2 saturation < 94% on room air, a respiratory rate of > 30 breath per minute or lung involvement > 50% (18, 19).

Treatment

Regarding COVID-19 treatment in those with active disease, depending on the symptoms and severity, different strategies were employed. For the mild cases or without hypoxia, conservative management was commenced. In the cases with hypoxia, they were hospitalized and received oxygen therapy along with

parenteral low dose dexamethasone and remdesivir. COVID-19 patients might receive high-dose of pulse methylprednisolone or low-dose corticosteroids (dexamethasone), depending on clinical judgment and disease severity. In smaller numbers of the patients, Interferon alfa and/or Tucilizumab were prescribed.

Regarding mucormycosis, intravenous amphotericin administration, along with surgery in some cases were considered. The surgical debridement was planned as soon as possible in our center, according to the discretion of the anesthesiologists to tolerate the general anesthesia by the patients. The plan of surgery included the endoscopic debridement of necrotic tissues and bones. In the case of detection of necrotic tissue at endoscopy, without waiting for pathologic confirmation, surgical debridement was planned as soon as possible for all the cases.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software (version 27) and RStudio version 8.17. Quantitative data were presented as mean and standard deviation (SD) and qualitative data were presented as percentages and frequencies. The comparisons of the quantitative data were statistically evaluated using the two independent sample t-test, according to the normal distribution assessed by the Shapiro–Wilk test. The comparisons of qualitative data were evaluated using the Chi-square test. The univariable and multivariable logistic regressions were used to investigate the risk factors for the Cam group, and the odds ratio (OR) with a 95% confidence interval (95% CI) was calculated. Variables were excluded in the multivariable logistic regression if: 1-The univariate analysis demonstrated the probability of $P > 0.15$; 2-Variable had obvious collinearity with other variables resulting in variance inflation factor (VIF) of more than 5 (20). (Because of the similarity between history of DM, Hyperglycemia, Familial history of DM, and Blood Sugar; We only use the history of DM in the models). The quality of multivariable logistic regression was evaluated by Cox & Snell, and Hosmer Lemeshow Chi-square goodness-of-fit tests.

Penalized logistic regression analysis was used for multivariable analysis to get reliable odds ratios (ORs) and 95% CIs (21). because some variables, such as Cardiovascular, history of smoking, and ASK had few cell counts.

Based on types of risk factors we calculate two logistic regression models. In model 1 it has been used age, History of smoking, COVID-19 (severe), history of DM, and cardiovascular disease; Also, Laboratory Parameters (AST, Na, Mg, CR, LDH, and lymphocytes number) added together with Age, Covid-19 (Severe), and history of DM as risk factors in model 2. Because we had a Smaller − 2log-likelihood and narrower confidence intervals in model 2 with multivariable Penalized logistic regression method, we have considered adjusted ORs as the most trustable ones.

Results

Patients' characteristics

In total, between February 2020 and September 2021, 94 patients with mucormycosis have been identified in our center. Forty-two (44.7%) of them did not have any complaint about COVID-19-related signs and symptoms, or showed negative results with our diagnostic tools, including both qPCR and CT scan; none of them have COVID-19 during the last six months. They had been excluded from our study. For the other 52 patients (55.3%), all were adults who had a history of diagnosis with symptomatic COVID-19, ranging from mild to severe within the last few months of admission. First, we tried to evaluate if there is any association between Delta variation (B.1.617.2) of SARS-CoV-2 and CAM. Considering the fact that identification of each subtype based on the genomic sequencing was inapplicable, we have divided the included patients into two groups, which could be most likely the variants before Delta (i.e., alpha, beta, gamma) or Delta variation according to the period of time and available local reports regarding the predominance of each variation. Accordingly, 23 (44.2%) and 29 (55.8%) were categorized as Delta and Alpha variations in CAM, respectively, which did not show any significant association (P-value = 0.157). Although it might not be accurate, this allowed us to do all the analysis regardless of variation type. Figure 1 and Fig. 2 show imaging and pathological findings of patients with CAM, respectively.

In the control COVID-19 group, 69 were men and 61 were women; the CAM group comprises 33 men and 19 women. No significant difference between the groups in respect to gender has been recorded (P-value = 0.202). The mean ages of the patients in the control COVID-19 and CAM were 53.1 ± 14.49 years (range 19–92) and 57.0 ± 11.82 years (range 27–82), respectively; this suggests no significant difference between the groups (P-value = 0.085). Most of the patients (n = 45; 86.5%) showed first signs/symptoms of mucormycosis in less than 4 weeks from COVID-19 diagnosis, with the mean of 16.63 ± 8.4 days (ranged 0–51) after the confirmation of COVID-19 diagnosis. In the CAM group, 24,(46.2%), and 28 (53.8%) had a history of severe and moderate COVID-19, respectively. In contrast, in the control group, 25 (19.2%), 105 (80.8%), were categorized into severe, and moderate groups, respectively. We found that COVID-19 disease was more severe in the CAM group in comparison to the control group (P-value < 0.01).

The demographic profile and the clinical characteristics of patients have been shown in Table 1.

Table 1
Demographic data and univariable analysis of included probable risk factors associated with CAM (before CAM development)

	COVID-19 (n = 130)	CAM (n = 52)	OR [CI 95%]	P-Value
Gender				
Female	61 (46.9)	19 (36.5)	Ref	N/A
Male	69 (53.1)	33 (63.5)	1.535 [0.793–2.974]	0.202
Age (Mean ± SD)	53.10 ± 14.495	57.02 ± 11.816	N/A	0.085
Variant				
Alpha	87 (66.9)	29 (55.8)	Ref	N/A
delta	43 (33.1)	23 (44.2)	1.60 [0.831–3.098]	0.157
history of DM (FBS > 126)	19 (14.6)	29 (55.8)	7.37 [3.542–15.321]	**< 0.01**
Hyperglycemia	83 (63.8)	24 (46.2)	0.485 [0.253–0.932]	**0.028**
hypertension	21 (16.2)	11 (21.2)	1.393 [0.618–3.140]	0.423
Cardiovascular diseases	5 (3.8)	6 (11.5)	3.261 [11.201]	0.060
history of smoking	5 (3.8)	8 (15.4)	4.545 [1.412–14.630]	**< 0.01**
Severe COVID-19	25 (19.2)	24 (46.2)	3.60 [1.791–7.237]	**< 0.01**
Familial history of DM	18 (13.8)	17 (32.7)	3.022 [1.408–6.487]	**< 0.01**

Clinical manifestations and pathological/radiological findings of mucormycosis

We categorized the patients with CAM into three distinct groups based on the main localization of mucormycosis; 1) rhino orbital (RO), 2) rhinomaxillary (RM), and 3) rhino-orbito cerebral (ROC), 6 (11.5%), 18 (34.6%), and 28 (53.8%) patients were in each group, respectively. Regarding the clinical characteristics, the most common presenting symptoms were facial paresthesia, visual impairment, and nasal obstruction. Remarkably, we had frozen eye syndrome, facial paralysis, dental loosening, palatal necrosis, cavernous sinus thrombosis, and internal carotid artery occlusion among our patients.

Evaluation of possible risk factors for CAM development

Of the 52 patients with CAM, 29 (55.8%) had a known history of diabetes mellitus (DM) at the time of COVID-19 admission with a mean of BS (300.24 ± 68.24 mg/dl), and previous DM was detected in 19 (14.6%) of the patients in the control group with mean BS of 280 ± 105.41 mg/dl. Familial history of DM

was also correlated with the incidence of mucormycosis and regarding this, there was a significant difference between CAM and control groups. (P-value < 0.01).

Regarding the medications, in the CAM group, five of the patients had received corticosteroid pulse therapy (methylprednisolone 500mg/day for 4 days, intravenous injection), and no one in the control group had received pulse therapy (P-value = 0.022). Regarding low dose corticosteroid, 113(86.9%) and 46(88.5%) had received Dexamethasone as the anti-inflammatory agent, at the constant dose of 6–8 mg/d for five days; this does not show any significant difference (P-value = 0.778).

Another identified factor that was significantly associated with CAM development in COVID-19 patients was the history of smoking. Indeed, the number of active smokers was significantly higher in the CAM group (P-value < 0.01). We found that smoker COVID-19 patients were 6.73 times more susceptible to developing mucormycosis. Table 1 shows the summary of possible risk factors for CAM development in our study. Tables 2 and 3 are showing the risk factors associated with mucormycosis based on multivariable logistic and multivariable Penalized logistic regression models, respectively.

Table 2
multivariable logistic models for risk factors of mucormycosis

	Model 1		**Model 2**	
	OR [95% CI]	**P-value**	**OR [95% CI]**	**P-value**
Age	1.014 [0.98–1.044]	0.338	0.991 [0.951–1.033]	0.682
History of smoking	7.384 [1.925–28.319]	**< 0.01**	-	-
COVID-19 (Severe)	2.799 [1.708–7.251]	**0.014**	4.332 [1.209–15.53]	**0.024**
History of DM (FBS > 126)	7.784 [3.446–17.584]	< 0.01	5.906 [1.998–17.46]	**< 0.01**
Cardiovascular	6.369 [1.537–26.384]	0.011	-	-
AST High level (> 35U/L)	-	-	0.074 [0.017–0.320]	**< 0.01**
Na Low level (< 135 meq/L)	-	-	7.099 [2.048–24.608]	**< 0.01**
Mg Low level (< 1.46mg/dl)	-	-	6.672 [1.780–25.01]	**< 0.01**
High Cr (GFR < 60 cc/m2/min)	-	-	5.786 [2.078–16.11]	**< 0.01**
High level of LDH > 280U/L	-	-	2.289 [0.693–7.561]	0.174
Low Lym (< 1000cells/microlitre)	-	-	0.085 [0.015–0.471]	**< 0.01**
-2 log-likelihood	165.526		103.23	
Cox & Snell	0.25[a]		0.47[b]	
Hosmer and Lemeshow	$\chi^2 = 5.73, \mathrm{P} = 0.677$		$\chi^2 = 9.52, \mathrm{P} = 0.3$	
a Variables in model1 explained 25% of the variance (Cox & Snell = 0.25).				
b Variables in model2 explained 47% of the variance (Cox & Snell = 0.47).				

Table 3
multivariable Penalized logistic regression models for risk factors of mucormycosis

	Model 1		Model 2	
	aOR [95% CI]	**P-value**	**aOR [95% CI]**	**P-value**
Age	1.013 [0.99–1.042]	0.348	0.992 [0.953–1.032]	0.719
History of smoking	6.73 [1.88–25.83]	**< 0.01**	-	-
COVID-19 (Severe)	2.69 [1.21–6.05]	**0.016**	3.703 [1.166–13.15]	**0.026**
history of DM	7.21 [3.302–16.34]	**< 0.01**	4.95 [1.84–14.23]	**< 0.01**
Cardiovascular	5.96 [1.51–24.04]	**0.012**	-	-
High level of (AST > 35U/L)	-	-	0.106 [0.024–0.352]	**< 0.01**
Low level of (Na < 135 meq/L)	-	-	5.64 [1.85–19.14]	**< 0.01**
Low level of (Mg < 1.46mg/dl)	-	-	5.49 [1.66–20.28]	**< 0.01**
High level of (GFR < 60 cc/m2/min)	-	-	4.85 [1.92–13.13]	**< 0.01**
High level of (LDH > 280U/L)	-	-	2.149 [0.70–6.84]	0.181
Low level of (Lyn < 1000cells/microlitre)	-	-	0.117 [0.020–0.510]	**< 0.01**
-2 log-likelihood	165.526		106.04	

Serological markers associated with CAM

Although DM was found as a possible risk factor, new-onset post-COVID-19 hyperglycemia was higher among the control group than CAM. In fact, it was detected in 24 patients (46.2%) in the CAM group with the mean BS of 259.46 ± 68.11 mg/dl, while in the control group, this type of hyperglycemia was shown in 83 (63.8%) of the patients and mean BS was 197.45 ± 83.68 mg/dl. Regarding other serological markers associated with CAM, we have found that there are some associations between the CAM and high creatinine levels (P-value < 0.01; aOR = 4.85). In contrast, the number of patients with the high levels of some important inflammatory markers was not statistically different between the groups, (i.e., CRP: P-value = 0.418, OR = 1.373; ESR: P-value = 0.245; OR = 1.5). However, liver enzymes level ALT (P-value < 0.01; aOR = 0.136), and AST (P-value < 0.01; OR = 0.106) were significantly higher in control group than patients with CAM in our patients. Hyponatremia (P-value < 0.01), hypokalemia (P-value < 0.01), hypomagnesemia (P-value < 0.01), hypoalbuminemia (P-value < 0.01) and microcytic anemia (P-value <

0.01) were more prevalent in the control group. A summary of the serological markers for patients with CAM and controls are brought in the Table 4.

Table 4
univariable analysis of factors evaluated after CAM development and starting of treatment

	COVID-19 (n = 130)	CAM (n = 52)	OR [CI 95%]	P-Value
ALT High level[1]	57 (43.8)	5 (9.6)	0.136 [0.051–0.365]	**< 0.01**
AST High level (> 35U/L)	60 (46.2)	3 (5.8)	0.071 [0.021–0.241]	**< 0.01**
Alk High level[2]	7 (5.4)	5 (9.6)	1.87 [0.565–6.181]	0.299
ESR High level[3]	78 (60.0)	36 (69.2)	1.50 [0.756–2.977]	0.245
Na Low level (< 135 meq/L)	14 (10.8)	25 (48.1)	7.672 [3.528–16.683]	**< 0.01**
K Low level (< 3.6 mmol/L)	5 (3.8)	38 (73.1)	N/A[⊥]	**< 0.01**
Mg Low level (Mg < 1.46mg/dl)	9 (6.9)	20 (38.5)	8.40 [3.49–20.22]	**< 0.01**
Hb Low level [4]	11 (8.5)	38 (73.1)	N/A[⊥]	**< 0.01**
Alb Low level (< 4 mg/l)	1 (0.8)	28 (53.8)	N/A[⊥]	**< 0.01**
Cr High level (GFR < 60 cc/m2/min)	32 (24.6)	37 (71.2)	7.554 [3.675–15.528]	**< 0.01**
LDH High level (> 280U/L)	30 (23.1)	19 (36.5)	1.919 [0.956–3.851]	0.064
CRP High level (> 10 mg/L)	95 (73.1)	41 (78.8)	1.373 [0.636–2.966]	0.418
WBC Low level (< 4000)	17 (13.1)	5 (9.6)	0.70 [0.243–2.022]	0.510
WBC High level (> 10,000)	13 (10.0)	5 (9.6)	0.916 [0.307–2.731]	0.875

[1]ALT High level (> 33 for males, > 25 for females IU/L), [2] Alk High level (> 240U/L for females, > 270 U/L for males), [3] ESR High level (> 22 for males > 29 for females), [4] Hb Low level (< 14.0 for males, < 12.3 for females)

OR, odds ratio; CI, confidence interval; ALT, alanine transaminase; AST, aspartate transaminase; ALK Alkaline Phosphatase; ESR, Erythrocyte Sedimentation Rate; Na, sodium; K, potassium; Hb, hemoglobin; ALBO, Albumin; Cr, creatinine; CRP, c-reactive protein; WBC, white blood cell; LDH, lactate dehydrogenase; PLT, platelet; Lym, lymphocytes;

[⊥] Not reported) because these confidence intervals are fairly wide, the point estimates are somewhat unreliable).

	COVID-19 (n = 130)	CAM (n = 52)	OR [CI 95%]	P-Value
PLT Low level (< 100,000)	19 (14.6)	8 (15.4)	1.619 [0.766–3.422]	0.207
Lym Low level (< 1000cells/microlitre)	29 (22.3)	4 (7.7)	0.249 [0.083–0.753]	**0.014**
pulse therapy	0 (0.0)	5 (9.62)	N/A	**0.022**
dexamethasone	113 (86.9)	46 (88.5)	N/A	**0.778**
Blood Sugar	203.99 ± 93.699	274.42 ± 73.72	N/A	**< 0.01**
[1]ALT High level (> 33 for males, > 25 for females IU/L), [2] Alk High level (> 240U/L for females, > 270 U/L for males), [3] ESR High level (> 22 for males > 29 for females), [4] Hb Low level (< 14.0 for males, < 12.3 for females)				
OR, odds ratio; CI, confidence interval; ALT, alanine transaminase; AST, aspartate transaminase; ALK Alkaline Phosphatase; ESR, Erythrocyte Sedimentation Rate; Na, sodium; K, potassium; Hb, hemoglobin; ALBO, Albumin; Cr, creatinine; CRP, c-reactive protein; WBC, white blood cell; LDH, lactate dehydrogenase; PLT, platelet; Lym, lymphocytes;				
[⊥] Not reported) because these confidence intervals are fairly wide, the point estimates are somewhat unreliable).				

Treatments, outcomes, and prognostic factors

Regarding patients in the CAM group, treatment has been started for all the patients immediately after diagnosis of mucormycosis. All of the patients received antifungal therapy (mainly amphotericin monotherapy or combination therapy) with surgical debridement as soon as possible.

In the course of treatment of the CAM group, 41 (78.8%) of patients recovered from mucormycosis. We have analyzed the data between expired patients and survived ones in the CAM group to find the possible prognostic factors for outcomes. We realized that the mean ages of the patients in the CAM who expired and survived were 66.18 ± 9.558 years and 54.56 ± 11.221 years ($P < 0.01$). Moreover, we found that the severity of COVID-19 was associated with mortality in the patients, significantly ($P = 0.046$). Other variables had no significant association with mortality.

Discussion

Here, we performed a retrospective study to assess the patients with an active or documented history of COVID-19. In total, we reported 52 patients with CAM with symptomatic active/history of COVID-19 and compared them to 130 randomly selected patients with COVID-19, but not CAM. Based on our analysis, although the mean age of the CAM group was higher than the control group, we did not find any significant association between the age and the risk of CAM. Meanwhile, the mean age in our study (57-

year-old) was similar to the reported number in the recent meta-analysis (22) (54.6-year-old), which was composed of 17 studies (101 patients). This might be because of the higher age of regular COVID-19 in the study period of time in our center. The mean age of COVID-19 patients varies in a wide range, while in a study with more than 7 thousand of COVID-19 patients in Iran, the mean age of infection was reported 41.48 ± 16.35 (23). According to the same meta-analysis (22), the male gender is commonly affected by CAM; we observed a higher number of males than controls (63.5% vs. 53.1), but not in a statistically significant manner in comparison to the regular included COVID-19 patients. This might be because of a different pattern in Iran since the other study from Iran has reported 66% as the percentage of affected men in the reported group with CAM (24). Although in the literature, the mean interval between CAM clinical manifestation and the time of COVID-19 diagnosis has been reported in the range of 15–24 days, (mean of 20 days), we found this interval in a wider range, 0–51 days, but with similar mean, 16.63 ± 8.4 days. This period of time could be longer for some cases since confirmation usually happened 2–3 days after the initial signs/symptoms. In our study, we detected more severe COVID-19 among those in the CAM group in comparison to regular COVID-19. Although choosing control patients from COVID-19 patients who survived the diseases might affect the results, approximately half of the patients in the CAM group were categorized in the severe group.

Considering the fact that the number of reported CAM have has increased since the emergence of Delta-variation, some studies have suggested that the association might be because of the dominance of the last variation of concern, the Delta variation (15). We considered this speculation in our analysis, based on an estimated time period of variations dominancy, but not genotyping of viruses. Although it might not be fully trustable, it could be speculated that variations might not be involved, but with increasing in the cases of patients, and allocating attention to the CAM more cases are being reported. However, studies on the confirmed variations of viruses might be helpful to clarify.

It has been shown that DM is a possible risk factor for the development of CAM (16), which is in line with our results. 29 of 52 (55.8%) patients in the CAM group had a known history of DM, which was observed in 19 (14.6%) in the control group. However, new-onset post-COVID-19-related hyperglycemia was detected 46.2% in the CAM group, and 63.8% in the control patients. It has been speculated that COVID-19 could lead to hyperglycemia, although the exact underlying mechanism remained unknown (25). Patients will remain hyperglycemic in the absence of proper treatment and close monitoring, which could easily happen during the collapse of the healthcare system due to pandemics. A higher percentage of post-COVID-19 hyperglycemia in patients with CAM could be explained by close monitoring of patients in the CAM group in comparison to the regular COVI-19 patients. In our cases, hyperglycemia was probably controlled better in the CAM group as they were hospitalized and were monitored in a daily manner. In our study, not only DM in individuals but also a familial history of DM was associated with CAM development. This only shows the higher risk of DM in individuals who have familial history of DM as well, as was shown in previous studies (26). Thus, we need to have close monitoring of such patients. The history of corticosteroid therapy was also another mentioned risk factor for CAM development in COVID-19 patients. In our study, we found that 5 patients in the CAM group, but no one in the control, have received high dose pulse therapy of corticosteroids. This suggests a high-dose corticosteroid as a

possible risk fact. However, since most of our patients had a history of treatment with low-dose corticosteroid (i.e., dexamethasone), we did not find any significant association. Moreover, we found smoking might be a risk factor for development, which is in line with the fact that smoking can increase the risk of invasive fungal infections (27). In addition to the risk factors, we found some serological markers, such as creatinine and liver enzymes that are associated with CAM. Since these factors were evaluated after CAM development and might be affected with disease and treatments, we only can consider them as factors associated with CAM, but not risk factors.

In this study, we have noticed that the percentage of CAM among the patients with mucormycosis unrelated to COVID-19 was 55.3%. This suggests that the development of mucormycosis is more frequent among the patients with active and resolved COVID-19 patients. In the meantime, the number of real patients with CAM might have been underestimated, since some COVID-19 patients might have missed identifying due to the asymptomatic nature of the disease in some patients. More precisely, they might have experienced asymptomatic COVID-19, while due to lack of symptoms, they were not evaluated for the COVID-19 test.

We have selected the control group, COVID-19 patients, from the patients who had at least follow-up to make sure that the possible risk factors are more trustable. Random selection of COVID-19 patients has been done in only surviving patients, because one of our goals was to identify risk factors for the development of CAM, and we needed to have at least three months of follow-up of patients to make sure that CAM has not happened. Thus, we were unable to compare the mortality rate between COVID-19 alone and CAM; according to the literature, the mortality for COVID-19 in Iran is ~ 5% (23). Additionally, only inclusion of surviving patients might affect the accuracy of some reported markers which are associated with CAM development, since some of these factors might be associated with mortality. In order to evaluate this, we excluded the expired patients in the CAM group and repeated the analysis. We found that none of the variables, except hyperglycemia (became statistically insignificant) and lymphocyte number (became significant) had been changed. It is worthy to note that because the number of patients who were evaluated for Interleukin-6 (IL-6) was less than the acceptable number for statistical analysis, then in this regard we could not differentiate between the two groups.

In conclusion, it is obvious that patients with either active or resolved COVID-19, especially smokers with a history of DM are at a higher risk of mucormycosis development. Those with risk factors should be closely monitored within the few months after recovering from COVID-19. CAM looks to be more fatal in older patients with more severe COVID-19. However, proper antifungal treatment along with doing surgery during its golden time could significantly decrease the mortality of CAM.

Declarations

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Author Contributions:

ST, Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing

ShI, Investigation, Methodology, Writing – original draft;

MSY, Formal Analysis, Software

MA, Formal Analysis, Investigation, Writing – original draft, Software

MH, Resources, investigation

HA, Resources, investigation

MAK, Resources, investigation,

ML, investigation

HSh, Writing – original draft, Visualization

NANY, Resources, investigation,

SHS, Resources, investigation,

MRF, Resources, investigation

FaF, investigation, Writing – original draft

MRMG, investigation, Writing – original draft

FiF, Resources, investigation, Writing – original draft

FS, Methodology, Resources, investigation, Writing – original draft, Writing – review & editing, Supervision

References

1. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, et al. Coronavirus disease 2019–COVID-19. Clinical microbiology reviews. 2020;33(4):e00028-20.
2. Fernandes Q, Inchakalody VP, Merhi M, Mestiri S, Taib N, Moustafa Abo El-Ella D, et al. Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. Annals of Medicine. 2022;54(1):524 – 40.

3. Bian L, Gao F, Zhang J, He Q, Mao Q, Xu M, et al. Effects of SARS-CoV-2 variants on vaccine efficacy and response strategies. Expert review of vaccines. 2021;20(4):365 – 73.
4. Rubin R. COVID-19 vaccines vs variants—determining how much immunity is enough. Jama. 2021;325(13):1241-3.
5. Mishra N, Mutya VSS, Thomas A, Rai G, Reddy B, Mohanan AA, et al. A case series of invasive mucormycosis in patients with COVID-19 infection. Int J Otorhinolaryngol Head Neck Surg. 2021;7(5):867 – 70.
6. Moorthy A, Gaikwad R, Krishna S, Hegde R, Tripathi K, Kale PG, et al. SARS-CoV-2, uncontrolled diabetes and corticosteroids—an unholy trinity in invasive fungal infections of the maxillofacial region? A retrospective, multi-centric analysis. Journal of maxillofacial and oral surgery. 2021;20(3):418 – 25.
7. Sarkar S, Gokhale T, Choudhury SS, Deb AK. COVID-19 and orbital mucormycosis. Indian Journal of Ophthalmology. 2021;69(4):1002.
8. Satish D, Joy D, Ross AB. Mucormycosis co-infection associated with global COVID-19: A case series from India. Int. J. Otorhinolaryngol. Head Neck Surg. 2021;7:815 – 20.
9. Sen M, Lahane S, Lahane TP, Parekh R, Honavar SG. Mucor in a viral land: a tale of two pathogens. Indian journal of ophthalmology. 2021;69(2):244.
10. Sharma S, Grover M, Bhargava S, Samdani S, Kataria T. Post coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum. The Journal of Laryngology & Otology. 2021;135(5):442-7.
11. Reid G, Lynch III JP, Fishbein MC, Clark NM, editors. Mucormycosis. Seminars in respiratory and critical care medicine; 2020: Thieme Medical Publishers.
12. Skiada A, Pavleas I, Drogari-Apiranthitou M. Epidemiology and diagnosis of mucormycosis: an update. Journal of Fungi. 2020;6(4):265.
13. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2021;15(4):102146.
14. Bari MS, Hossain MJ, Akhter S, Emran TB. Delta variant and black fungal invasion: A bidirectional assault might worsen the massive second/third stream of COVID-19 outbreak in South-Asia. Ethics, Medicine and Public Health. 2021;19:100722.
15. Arakeri G, Rao V, Mendes RA, Oeppen RS, Brennan PA. COVID-associated mucormycosis (CAM): is the Delta variant a cause? The British Journal of Oral & Maxillofacial Surgery. 2021.
16. Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, et al. Coronavirus disease (Covid-19) associated mucormycosis (CAM): case report and systematic review of literature. Mycopathologia. 2021;186(2):289 – 98.
17. Pal R, Singh B, Bhadada SK, Banerjee M, Bhogal RS, Hage N, et al. COVID-19-associated mucormycosis: an updated systematic review of literature. Mycoses. 2021;64(12):1452-9.

18. Health NIo. Coronavirus disease 2019 (COVID-19) treatment guidelines. 2020.
19. Health NIo, Health NIo. Clinical spectrum of SARS-CoV-2 infection. Retrieved April. 2021;12:2021.
20. Akinwande MO, Dikko HG, Samson A. Variance inflation factor: as a condition for the inclusion of suppressor variable (s) in regression analysis. Open Journal of Statistics. 2015;5(07):754.
21. Devika S, Jeyaseelan L, Sebastian G. Analysis of sparse data in logistic regression in medical research: A newer approach. Journal of postgraduate medicine. 2016;62(1):26.
22. Bhattacharyya A, Sarma P, Kaur H, Kumar S, Bhattacharyya J, Prajapat M, et al. COVID-19–associated rhino-orbital-cerebral mucormycosis: A systematic review, meta-analysis, and meta-regression analysis. Indian Journal of Pharmacology. 2021;53(6):499.
23. Azarbakhsh H, Jokari K, Moftakhar L, Ghojogh MG, Karimyan A, Salmanzadeh S, et al. Epidemiological characteristics of patients with COVID-19 in Southwest of Iran from February 19 to June 20, 2020. Medical Journal of the Islamic Republic of Iran. 2021;35:116.
24. Pakdel F, Ahmadikia K, Salehi M, Tabari A, Jafari R, Mehrparvar G, et al. Mucormycosis in patients with COVID-19: a cross-sectional descriptive multicentre study from Iran. Mycoses. 2021;64(10):1238-52.
25. Montefusco L, Ben Nasr M, D'Addio F, Loretelli C, Rossi A, Pastore I, et al. Acute and long-term disruption of glycometabolic control after SARS-CoV-2 infection. Nature Metabolism. 2021;3(6):774 – 85.
26. Annis AM, Caulder MS, Cook ML, Duquette D. Family history, diabetes, and other demographic and risk factors among participants of the National Health and Nutrition Examination Survey 1999–2002. Prev Chronic Dis. 2005;2(2):A19-A.
27. Pourbaix A, Lafont Rapnouil B, Guéry R, Lanternier F, Lortholary O, Cohen JF. Smoking as a risk factor of invasive fungal disease: systematic review and meta-analysis. Clinical Infectious Diseases. 2020;71(4):1106-19.

Figures

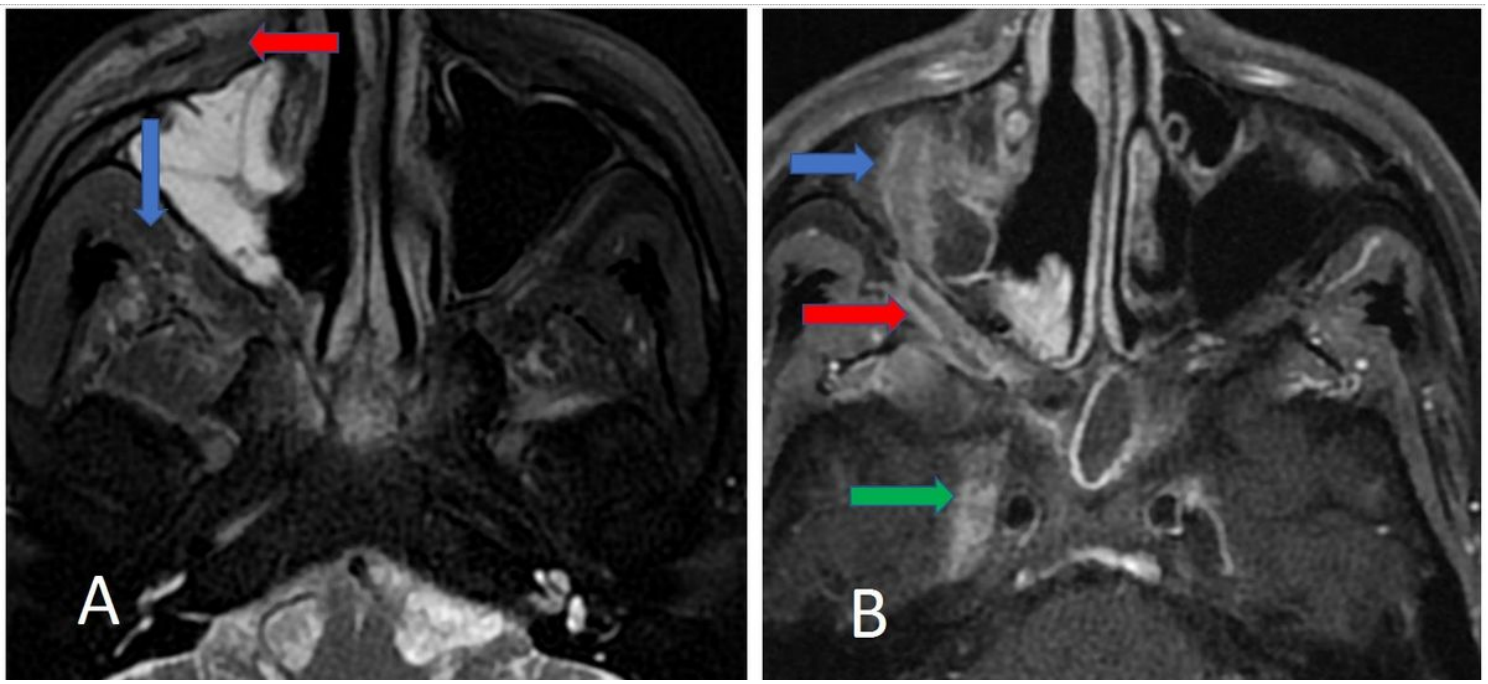

Figure 1

(A) Axial Short tau inversion recovery (STIR) of a 47 year old shows right maxillary sinus mucosal thickening with preantral (red arrow) and infratemporal (blue arrow) fat stranding suggestive of acute invasive sinusitis. **(B)** Axial fat sat T1 C+ of the same patient shows trigeminal maxillary branch (V2) perineural spread of infection to the right cavernous sinus (green arrow). V2 pathway involvement in the infraorbital canal and groove (blue arrow) and inferior orbital fissure (red arrow) are visible.

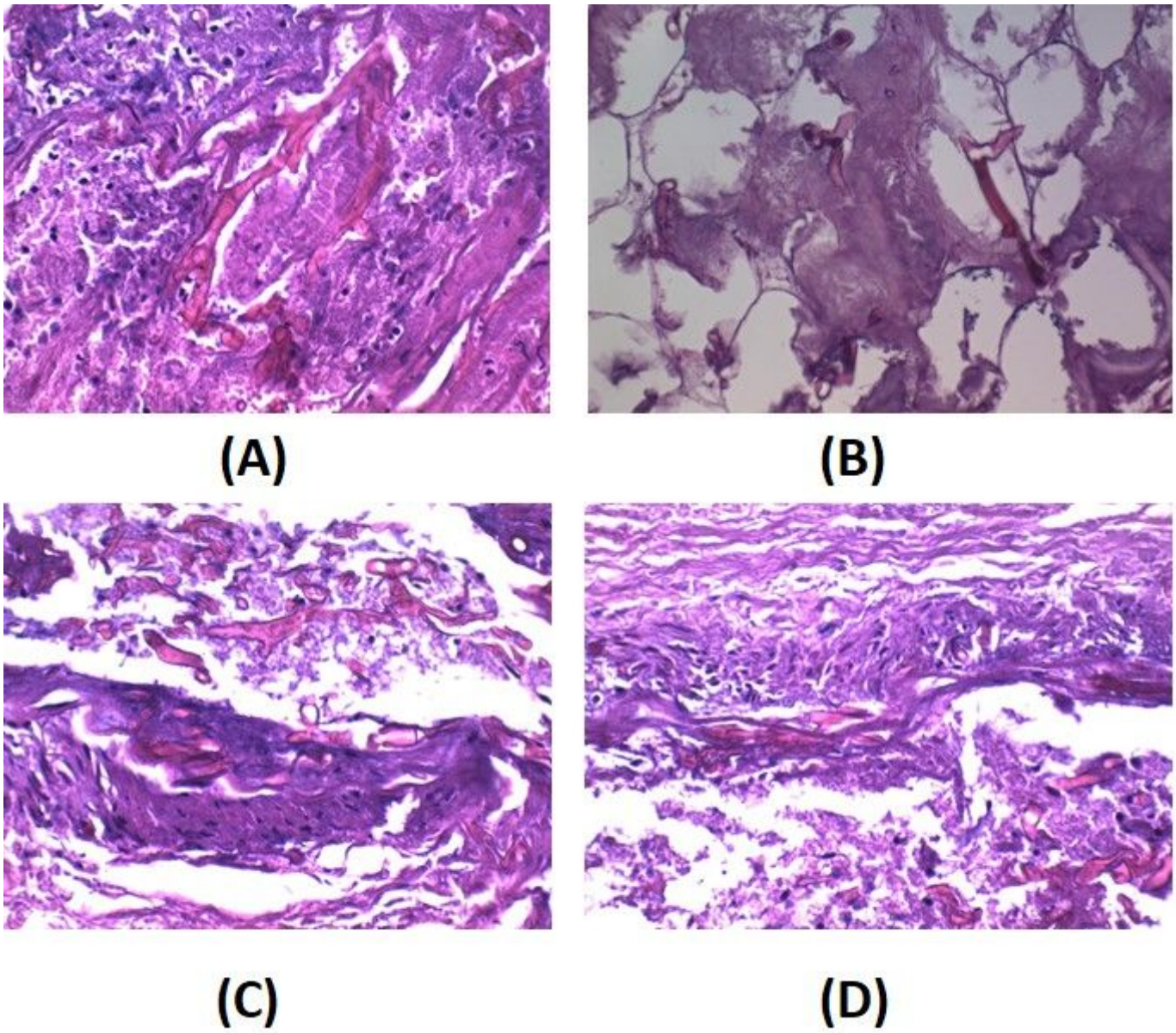

Figure 2

Pathologic figures of the patients with CAM. **(A)** 50-year-old woman with a history of poorly controlled diabetes mellitus type 2 and recent covid-19 infection,underwent nasal endoscopy and a biopsy was taken from the left inferior turbinate. Microscopic examination of H&E stained slides depicts branching nonseptate hyphae with variable width in a necroinflammatory background. **(B)** 56 -year-old man with a history of diabetes mellitus type 2 who presented with acute visual loss of right eye following covid-19 disease. Histologic evaluation of the periorbital adipose tissue showed broad branching nonseptate hyphae of mucormycosis in the background of necrotic adipose tissue. **(C and D)** 71- year-old man with a history of covid-19 was referred to our center due to the necrosis of the palate. Microscopic examination of the necrotic tissue showed that the arterioles wall was invaded by broad aseptate fungal hyphae.