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ORIGINAL ARTICLE

Risk Factors of COVID-19 Associated Mucormycosis (CAM) in Iranian Patients: A Single-Center Retrospective Study

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Abstract

Background COVID-19 associated mucormycosis (CAM) has been known as one of the most severe post-COVID morbidities.

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, Iran between February 2020 and September 2021. Patients with mucormycosis who had 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 an active or history of COVID-19. Rhino-orbital, rhino maxillary, rhino-orbito cerebral subtypes of mucormycosis were detected in 6 (11.5%), 18(34.6%), and 28(53.8%) patients. As a control group, 130 (69 men and 61 women; mean age: 53.10 ± 14.49 years) random RT-PCR-confirmed COVID-19 patients without mucormycosis have been included. The mean interval between COVID-19

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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 (OR = 3.60, *P*-value < 0.01). Known history of previous diabetes mellitus (OR = 7.37, *P*-value < 0.01), smoking (OR = 4.55, *P*-value < 0.01), and history of receiving high-dose corticosteroid pulse therapy because of more severe COVID-19 (*P*-value = 0.022) were found as risk factors. New-onset post-COVID hyperglycemia was lower in the CAM group (46.2% vs. 63.8%; OR = 0.485, *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 in the CAM group were significantly higher than those who recovered from mucormycosis (66.18 ± 9.56 vs. 54.56 ± 11.22 years; $P < 0.01$); and COVID-19 disease was more severe ($P = 0.046$).

Conclusion Either active or history of COVID-19 can cause an increase in the risk of mucormycosis development. Some of the most important risk factors are the medical history of diabetes mellitus, smoking, and high-dose corticosteroid therapy. CAM is important possible comorbidity related to COVID-19, which could make the post-COVID conditions more complicated. More research and studies with greater sample sizes among different ethnicities are needed to explore the association between COVID-19 and mucormycosis.

Keywords Mucormycosis · COVID-19 · SARS-CoV-2 · CAM

Introduction

From the beginning of the novel coronavirus pandemic, which was announced by World Health Organization (WHO) on March 11, 2020, the number of individuals with a history of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported to be more than 350 million people worldwide, with more than 5 million related deaths (as of January 2022). Coronavirus disease 2019 (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, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and recently, another variation, called Omicron B.1.1.529 [2]. New SARS-CoV-2 strains could make the virus more contagious, and pathogenic, and even help the virus to evade vaccines or be diagnosed by regular testing strategies [3, 4].

Recently, an increasing number of studies, most of them from India have reported the association of COVID-19 with a very rare fungal infection, named mucormycosis, also known as 'Black Fungus infection' [5–10]; known as COVID-19 associated mucormycosis (CAM). It is an opportunistic and serious infection caused by *Mucorales* [11, 12]. A wide range of risk factors has been proposed for mucormycosis, including poorly controlled diabetes mellitus (DM), cancer chemotherapy, organ transplantation, immunosuppressive therapies, and any other condition with immune system disruption [13–17].

Here, we designed a retrospective study to assess the patients with mucormycosis who had an active or a recent history of COVID-19 and compared them with a control group who had experienced COVID-19, but not mucormycosis within the next six months. We aimed to find more relevant risk factors and the possible association between mucormycosis and COVID-19 in the target people of our study.

Material and Methods

Study Design

This retrospective study was conducted among admitted COVID-19 patients to Amir-Alam Hospital, Tehran, Iran, between February 2020 and September 2021. Amir-Alam is the main center for education and treatment of Ear-Nose and Throat (ENT) in Iran and one of the referral centers for COVID-19 patients.

In this study, demographic data, diseases' characteristics, comorbidities, treatments, and outcomes were recorded to evaluate and 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.

Participants

Patients older than 18 years, with an active or a documented history of COVID-19 in the previous 6 months, who presented with symptoms and signs suggestive of mucormycosis, were considered for in-depth analysis. For diagnosis of COVID-19, both reverse transcriptase-polymerase chain reaction (RT-PCR) and chest computed tomography (CT) have been done to confirm the diagnosis. For RT-PCR detection, after taking the nasopharyngeal swab sample, RNA extraction, and cDNA synthesis using random primers (Fermentas, Lithuania), RT-PCR was performed following "Real-time RT-PCR assay for the detection of SARS-CoV-2 " protocol, recommended by World Health Organization (WHO) [18].

Regarding the diagnosis of mucormycosis, suggestive findings which had been detected by an experienced radiologist via imaging techniques (CT and/or MRI), followed by histopathological examination of biopsy specimens and confirmed by two expert pathologists in this field, separately. Regarding the definitive diagnosis of mucormycosis, it is preferred to do a culture and or immunohistopathology test for confirming mucormycosis, but because of cost issues for our patients and time limitation, in the setting of high turnover of patients with COVID-19, it was not possible to follow that for all the patients. In addition, a few significant characteristics between mucormycosis and aspergillosis such as hyphal width and branching pattern were mentioned to differentiate them by histopathologic examination, efficiently [19].

Recovery from mucormycosis is mostly related to the absence of necrotic tissues found in serial endoscopic surgeries along with completion of the antifungal therapeutic dose, which almost is accompanied by improvement in patients' general condition, confirmed by at least two ENT specialists and one ophthalmologist, mainly via clinical and/or radiological improvement in serial follow-up visits.

In the control group, 130 patients with a medical history of COVID-19, but not mucormycosis who had been admitted to Amir Alam hospital between February 2020 and September 2021 and discharged without complication were included retrospectively. For this control group, two follow-up visits were scheduled by an ENT specialist 6 months after recovery from COVID-19, to exclusion of possible superimposed mucormycosis. As the aim of the study was the evaluation of only the adult population, and because most of our COVID-19 patients were in the adult range, those who were under 18 have been excluded.

Clinical and Radiological Assessments

For each patient, the 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, and otological examination were all done. All patients underwent endoscopic nasal examination under local anesthesia, and tissue biopsy was taken for pathologic assessment. All patients underwent were consulted and examined by an ophthalmologist as well. Patients without contraindication underwent MRI with gadolinium. The protocol included axial and coronal short tau inversion recovery (STIR), T1, and T2, sagittal T2, and axial DWI sequences. Post-contrast T1 fat-saturated axial, coronal, and sagittal images were added. Axial images of paranasal sinuses CT scan with coronal reconstruction were performed without contrast with a 16-detector CT scanner. Images were constructed with bone and soft tissue protocol. 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, 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 Classification

Regarding 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 O_2 saturation was equal to or more than 94% on room air and patients with any of the following complication were categorized as a severe disease: O2 saturation $< 94\%$ on room air, a respiratory rate of > 30 breath per minute or lung involvement $> 50\%$ [20, 21].

Treatment

Regarding COVID-19 management in those with active disease, depending on the severity, different strategies were employed. For the mild mild patients who did not have hypoxia, conservative management was commenced. Patients who showed hypoxia were hospitalized and received, high–flow oxygen by a nasal cannula or non-rebreather mask along with intravenous (IV) dexamethasone 3 mg twice per day and parenteral remdesivir, for 5 days were used. The dosage of remdesivir was an IV, loading of 200 mg on the first day, followed by 100 mg IV daily for the next 4 days. Also, there was a retrospective history of taking IV methylprednisolone as pulse therapy for 4 days in five of our patients with CAM, during their COVID-19 admission (Table 4). The indication of methylprednisolone was no response of patients to dexamethasone regarding hypoxia and progressive COVID-19 signs and symptoms despite receiving remdesivir. After discharging the patients, no one was on oral corticosteroid for more than 5 days.

In smaller numbers of patients, interferon alpha and/or tocilizumab were prescribed.

Regarding mucormycosis, intravenous liposomal amphotericin as an antifungal medication was commenced and when needed, surgical debridement was planned as soon as possible.

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 [22]. (Because of the similarity between the 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 [23]. Because some variables, such as the history of cardiovascular disease, smoking, and aspartate aminotransferase (AST) level had few cell counts.

Based on types of risk factors we calculated 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 count) were 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 (CI) in model 2 with the 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 report having any history of COVID-19 during the last 6 months, and had negative results with our diagnostic tools, including both qPCR and CT scan at the time of presentation. As a result, they had been excluded from our study. For the other 52 patients with mucormycosis (55.3%), all were adults who had a history of

symptomatic COVID-19, within the last few months of mucormycosis, consequently, they were categorized as CAM group.

Demographic data and characteristics of patients are summarized in Table 1. Figures 1 and 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 (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 (P-value = 0.085).

Most of the patients (n = 45; 86.5%) showed the first signs/symptoms of mucormycosis less than 4 weeks after their COVID-19 diagnosis, with the mean of 16.63 ± 8.4 days (range: 0–51). 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.

Clinical Manifestations and Pathological/Radiological Findings of Mucormycosis

Patients with CAM were categorized into three distinct groups based on the main localization of mucormycosis; (1) rhino orbital (RO), (2) rhino-maxillary (RM), and (3) rhino-orbito-cerebral (ROC), 6 (11.5%), 18 (34.6%), and 28 (53.8%), respectively. There was no any pulmonary subtype of mucormycosis among our patients. Regarding the clinical characteristics, the most common presenting symptoms were facial paresthesia, visual impairment, and nasal obstruction. Remarkably, we had cases with 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 Mucormycosis Development in the CAM Group

Of the 52 patients with CAM, the first and most prominent risk factor was a previous history of diabetes mellitus (DM) at the time of COVID-19 admission in 29 of them (55.8%), the mean blood sugar (BS) was 300.24 ± 68.24 mg/dl. Previous DM was detected in 19 (14.6%) of the patients in the control group with a mean BS of 280 ± 105.41 mg/dl. Familial history of DM, as another risk factor was

Table 1 Demographic data and univariable analysis included probable risk factors associated with CAM (COVID-19 Associated Mucormycosis)

	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
History of DM (FBS > 126)	19 (14.6)	29 (55.8)	7.37 [3.542–15.321]	**< 0.01**
Post-COVID-related 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**

Bold values indicate statistical significance at the $p < 0.05$

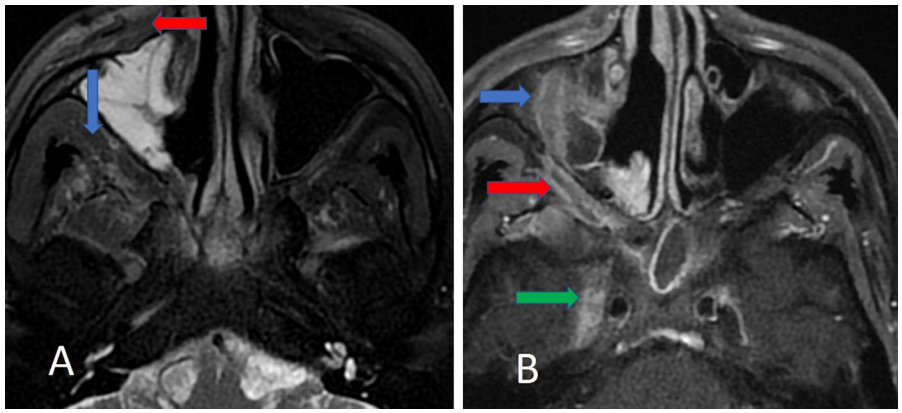

Fig. 1 **A** Axial Short tau inversion recovery (STIR) of a 47-year-old shows right maxillary sinus mucosal thickening with preantral (red arrow) and infratemporal (blue arrow) fat stranding suggestive of acute invasive sinusitis. **B** Axial fat sat T1 C + of the same patient shows trigeminal maxillary 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

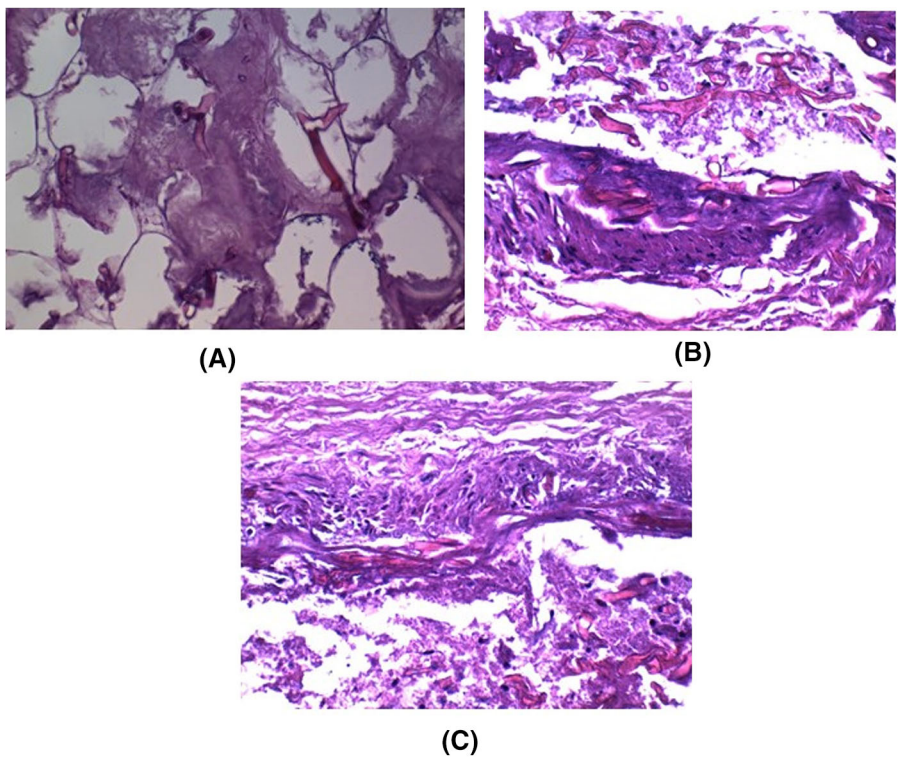

Fig. 2 Pathologic figures of the patients with CAM. **A** 56 -year-old man with a history of diabetes mellitus type 2 who presented with acute visual loss of right eye following covid-19 disease. Histologic evaluation of the periorbital adipose tissue showed broad branching nonseptate hyphae of mucormycosis in the background of necrotic adipose tissue. (**B** and **C**) 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

correlated with the higher incidence of mucormycosis in this regard (*P*-value < 0.01).

Regarding the medications, in the CAM group, five of the patients had received corticosteroid pulse therapy (intravenous methylprednisolone 500 mg/day for 4 days), but no one in the control group (*P*-value = 0.022). Also, 113 (86.9%) and 46 (88.5%) patients had received dexamethasone, at the constant dose of 6–8 mg/d for five days in CAM and control groups, respectively; which did not show any significant difference (*P*-value = 0.778).

Another identified factor that was significantly associated with mucormycosis development in COVID-19 patients, was the history of smoking (*P*-value < 0.01); COVID-19 patients who had a history of smoking were 6.73 times more susceptible to developing mucormycosis. Table 1 shows the summary of possible risk factors for mucormycosis development in the CAM group in our study. Tables 2 and 3 are demonstrating the risk factors associated with mucormycosis based on multivariable logistic and multivariable Penalized logistic regression models, respectively.

Biochemical Parameters

New-onset post-COVID-19 hyperglycemia was detected in 24 patients (46.2%) in the CAM group with a mean BS of 259.46 ± 68.11 mg/dl and in 83 (63.8%) of the control group with a mean BS of 197.45 ± 83.68 mg/dl.

Regarding other biochemical markers, we have found that creatinine level (Cr) was higher in CAM than in the control group (*P*-value < 0.01; aOR = 4.85). In contrast, the number of patients with high levels of some important inflammatory markers was not statistically different between the two groups, (i.e., CRP: *P*-value = 0.418, OR = 1.373; ESR: *P*-value = 0.245; OR = 1.5). However, liver enzymes alanine aminotransferase (ALT) (*P*-value < 0.01; aOR = 0.136), and AST (*P*-value < 0.01; OR = 0.106) were significantly higher in the control group than in patients with CAM. 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

Table 2 Multivariable logistic models for risk factors of mucormycosis and significant biochemical and electrolyte disturbances in CAM group

	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**
Known history of previous DM	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	–	–
High AST (> 35U/L)	–	–	0.074 [0.017–0.320]	**< 0.01**
Hyponatremia (Na < 135 meq/L)	–	–	7.099 [2.048–24.608]	**< 0.01**
Hypomagnesemia (Mg < 1.46 mg/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 and Snell	0.25[a]		0.47[b]	
Hosmer and Lemeshow	$\chi^2 = 5.73, P = 0.677$		$\chi^2 = 9.52, P = 0.3$	

[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)

Bold values indicate statistical significance at the $p < 0.05$

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 disease	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 < 1.46 mg/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	

Bold values indicate statistical significance at the $p < 0.05$

serological markers for patients with CAM and controls is brought in Table 4.

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 immediately received antifungal therapy (mainly liposomal amphotericin) with or without surgical debridement.

In the CAM group, 41 (78.8%) of patients recovered from mucormycosis. 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 ($P < 0.01$), respectively. Moreover, we found that the severity of COVID-19 was associated with higher mortality in these patients, significantly ($P = 0.046$).

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 a symptomatic active/history of COVID-19 and compared them to 130 randomly selected patients with COVID-19, but without mucormycosis. 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 age and the risk of COVID-19-related mucormycosis development. Meanwhile, the mean age in our study (57-year-old) was similar to the reported number in the recent meta-analysis (54.6-year-old), which was composed of 17 studies (101 patients) [24]. This might be because of the higher age of regular COVID-19 during 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 COVID-19 patients in Iran, the mean age of infection was reported as 41.48 ± 16.35 [25]. According to the same meta-analysis [24], 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 [26]. 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

Table 4 Univariable analysis of factors evaluated after mucormycosis development in the CAM group 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$^{\perp}$	**< 0.01**
Mg low level (Mg < 1.46 mg/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$^{\perp}$	**< 0.01**
Alb low level (< 4 mg/l)	1 (0.8)	28 (53.8)	N/A$^{\perp}$	**< 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
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 lU/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;

$^{\perp}$Not reported) because these confidence intervals are fairly wide, the point estimates are somewhat unreliable)

Bold values indicate statistical significance at the $p < 0.05$

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 (OR = 3.60). Although choosing control patients from COVID-19 patients who survived the disease might affect the results, approximately half of the patients in the CAM group were categorized as severe one. It has been shown that DM is a possible risk factor for the development of mucormycosis in COVID-19 patients [16], which is in line with our results. Twenty-nine 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 (OR = 7.37). However, new-onset post-COVID-19 hyperglycemia was detected in 46.2% of the CAM group, and 63.8% of the control patients. In fact, because of the higher prevalence of past medical history of diabetes mellitus in the CAM group, new onset hyperglycemia was not prominent among them. It has been speculated that COVID-19 could lead to hyperglycemia, although the exact underlying mechanism remained unknown [27]. 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.

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 [28]. Thus, we need to have close monitoring of such patients. The history of high-dose corticosteroid therapy was also

another mentioned risk factor for mucormycosis development in the CAM group in COVID-19 patients. In our study, we found that five patients in the CAM group, but no one in the control, have received pulse therapy of corticosteroids. This suggests a high-dose corticosteroid as a possible risk factor for developing mucormycosis in patients included in this study. 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 (OR:4.55), which is in line with the fact that smoking can increase the risk of invasive fungal infections [29]. 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 mucormycosis development in the CAM group and might be affected by disease and treatments, we only can consider them as factors associated with CAM, but not risk factors.

In this study, we 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 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 identification 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 a 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 $\sim$ 5% [25]. Additionally, only the 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. 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.

One of the limitations of this study is lack of confirmatory test for the diagnosis of mucormycosis by fungal culture tests. Because of the importance of making quick decision about starting treatment during the COVID-19 pandemic, we made our diagnostic decisions based on the microscopic examination and clinico-radiological evidence. Low number of included patients and lack of follow-up data for patients are other limitations of this study.

In conclusion, those with either active or a history of COVID-19, who have a medical history of diabetes mellitus, high dose corticosteroid therapy during COVID-19 management, and smokers should be closely monitored within the few months after recovering from COVID-19. Since CAM looks to be more fatal in older patients with more severe COVID-19, such groups need more special attention in the case of mucormycosis development. Proper antifungal treatment along with surgery during its golden time could significantly decrease the mortality of CAM.

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Declarations

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