Original Article

Distribution of Acute Phase Proteins in Post-SARS CoV2 Mucormycosis Infection

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Abstract

Background

This study was aimed to estimate the serum levels of acute phase reactants/proteins (APR) which includes serum ferritin, C Reactive Protein, D-dimer and Procalcitonin in mucormycosis infection following SARS CoV2 infection and to determine the clinical, biochemical and histopathological findings in the same.

Methods

It was an observational cross-sectional study. All cases of mucormycosis following SARS CoV2 infection were reviewed. Demographic and clinical details with comorbidities and relevant laboratory investigations which included serum glucose, serum ferritin, C Reactive Protein, D-dimer, Procalcitonin were noted at the time of diagnosis of mucormycosis. Diagnosis of mucormycosis was based on frozen section examination and histopathological examination.

Results

Thirty-two cases of mucormycosis following SARS CoV2 infection confirmed on histopathology were studied. Majority of the cases (77%) were with comorbidity especially having diabetic mellitus type 2. C-Reactive protein, Procalcitonin and serum Ferritin were raised in almost all cases with mean of 80.92±62.5mg/l, 17.99±26.87ng/ml. and 464.9±422.03ng/ml respectively. D Dimer was raised in less than 50% cases with a mean of 828.5±245.9 ng/ml.

Conclusion

Acute phase proteins like C reactive Protein, Procalcitonin, Serum ferritin and D Dimer were raised in Post-SARS CoV2 infected individual who presented now with mucormycosis infection. These markers were raised markedly in critically ill patients thus indicated its pathogenetic role in severe morbidity, thus estimation of serum acute phase reactants can help in predicting the course and severity of illness.

Keywords:

SARS CoV2, Mucormycosis, Acute Phase Proteins

Introduction

Infections play a major role in morbidity and mortality in developing countries. Acute phase reactants (APRs) are proteins which have noticed in high levels during inflammation and infection and hence there has been increasing focus on the use of acute-phase reactants (APRs) in the management of infections.[1] Acute phase reactants are a heterogeneous group of plasma proteins, the level of which increase or decrease in response to inflammatory stimuli such as infections, trauma, acute arthritis, systemic autoimmune disorders and neoplasms.[1]

The liver plays a significant role in production of APRs. Parallelly the production of other plasma proteins is reduced and these are referred to as negative acute-phase proteins, and proteins which increase in circulation are called positive acute phase proteins. [2]

C-Reactive Protein (CRP), serum amyloid A (SAA) protein, erythrocyte sedimentation rate (ESR), procalcitonin (PCT), fibrinogen, ferritin, alpha-1 antitrypsin, haptoglobin, alpha-1 acid glycoprotein, ceruloplasmin, and complement proteins: C3 and C4 are important APRs. Erythrocyte Sediment Rate (ESR) and C-Reactive Protein (CRP) are most commonly used acute-phase proteins as serum markers in clinical practice. Procalcitonin has created abundant interest in the previous years as an acute phase protein in bacterial infections, and there are lot of evidence to support its usefulness in specific infections. [3]

World Health Organization (WHO) declared Coronavirus disease 2019 (COVID-19) as a global pandemic in March 2020. The pandemic continues to be a public health concern even today. Coronavirus disease 2019 (COVID-19) is caused by virus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causing primarily pulmonary infection and has been associated with a wide range of opportunistic bacterial and fungal infections. [4] Both Aspergillosis and Candida have been reported as the main fungal pathogens for co-infection in people with SARS CoV 2 infection. Recently, several cases of mucormycosis in people with COVID- 19 have been increasingly reported world-wide particularly in South Asian countries. [4]

The prevalence of mucormycosis varies from 0.005 to 1.7 per million population worldwide and its prevalence is nearly 80 times higher in developing countries compared to developed countries, as observed in recent literature (estimate year being 2019-2020). Few Asian countries have highest cases of the mucormycosis in the world compared to other south Asian countries. These countries have also noticed highest prevalence of diabetes and thus the burden of opportunistic infections in diabetes and immunocompromised state is known to occur in the population of developing countries compared to developed countries.[4] Acute phase reactants like CRP, D-dimer and procalcitonin increase in inflammatory conditions including mucormycosis. [5]

This study was aimed to estimate the serum levels of acute phase proteins in mucormycosis cases following SARS CoV2 infection and to determine the clinical, biochemical and histopathological findings in post covid mucormycosis cases.

Materials and Methods

It was an observational cross-sectional study where all the cases of Post-SARS CoV2 mucormycosis infection, diagnosed through histopathological examination of the biopsy specimens (frozen sections and formalin fixed tissue) from January 2021 to December 2021 were studied. Ethical clearance was obtained from institutional ethical committee (SDMCDS IEC.No.2021/ Medical/ Pathology/ S/ 15). Demographic and clinical details with comorbidities and relevant laboratory investigations which included Serum Glucose, Serum Ferritin, C Reactive Protein, D-dimer and Procalcitonin were retrieved from medical record department at the time of diagnosis of mucormycosis. Data regarding fungal growth in KOH media was retrieved from microbiology laboratory.

Cases in which estimation of acute phase reactants were not done were excluded from the study.

Diagnosis of mucormycosis was based on frozen section examination and routine histopathological examination. The routine formalin fixed paraffin embedded slides were stained with hematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) and viewed under light microscope. Patient outcome was also noted at the time of discharge. Descriptive statistical analysis was performed with mean, standard deviation, percentages and ratios.

Results

In present study, specimens of 85 suspected cases of mucormycosis following SARS CoV2 infection were received in the laboratory out of which 35 cases were confirmed as mucormycosis by frozen section examination/ paraffin embedded H&E sections of the specimen. In 3 cases, estimation of acute phase reactants was not performed and were excluded from the study. Thus, 32 cases were considered for the study. The duration of mucormycosis following post SARS CoV2 infection was 2 months to 4 months interval.

Age group of the patients ranged from 28 to 73 years with a mean age being 51.9 years. Males were more affected with male: female ratio of 2.6:1. Biochemical profile of diabetes was reported in 23 cases (77%) with mean HbA1c levels of 9.5±2.23% while one patient had both diabetes mellitus and hypertension.

Most common site of involvement was sinonasal involvement in 17 (53%) followed by only sinus involvement in 13 (40%) cases. Other sites involved were frontal bone, oral cavity, maxilla, mandible and orbit. Headache was the most common clinical presentation followed by facial, pain and swelling, blurring of vision, toothache, nasal obstruction and paresthesia being the other presenting symptoms (Table 1).

Table 1: Clinical presentation of mucormycosis infection in Post SARS CoV2 patients

Clinical presentation	**Number of cases (%)**
Headache	14 (46.7)
Facial pain/ swelling	6 (20)
Blurring of vision	2 (6.7)
Tooth pain	2 (6.7)
Paresthesia	3 (10)

Frozen section examination was performed in all the cases and revealed non or pauci-septated broad, ribbon like hyphae suggesting fungal elements and diagnosed as mucormycosis (Figure 1a & 1b).

27 cases were diagnosed as mucormycosis while 3 cases were suspicious for mucormycosis (histological features of fungal infection without mucor elements) while rest 2 was reported as negative for mucormycosis in frozen section examination. In paraffin embedded H&E sections, necrosis was the most consistent finding reported in 100% of the cases with 31% (10 cases) showing granulomatous reaction with multinucleated giant cells. Majority of the cases (63%) showed mixed inflammatory cell infiltrates comprising of lymphocytes, neutrophils, histiocytes and plasma cells with few showing exclusive neutrophilic (31%) and lymphocytic (6%) infiltrations (Table 2). 16% of the cases showed angioinvasion. KOH culture was performed on 30 cases and was positive for fungal growth in 17 (57%) cases including the cases which were suspicious on histopathological examination.

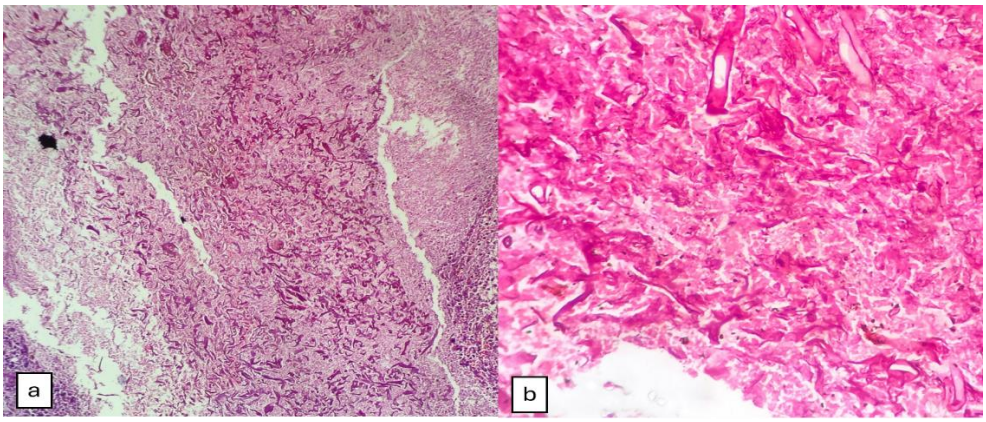

Figure 1: a: 10X H&E stain Microphotograph shows area of necrosis and pauci-septate broad, ribbon like hyphae of Mucormycosis. b: 400X H&E microphotograph showing high power view of the fungus embedded in necrotic material.

Table 2: Morphological findings on histopathology of Mucormycosis infection.

Histopathological features	**Number of cases (%)**
Necrosis	30 (100)
Inflammatory cells: Mixed	16 (53.3)
Inflammatory cells predominant neutrophils	9 (30)
Inflammatory cells predominant lymphocytes	5 (16.7)
Giant cells with granulomatous reaction	9 (30)
Angio-invasion	5 (16.7)

CRP was raised in 25 cases with mean serum levels of 80.92±62.5mg/l. Serum ferritin was raised in 18 cases with a mean of 464.9±422.03ng/ml. D Dimer was raised in 18 cases with a mean of 828.5±245.9 ng/ml. Procalcitonin was raised in 19 cases with a mean of 17.99±26.87ng/ml (Table 3). Culture on KOH media was positive in 57% (performed in 30 cases) of the cases.

Table 3: Distribution of acute phase proteins/reactants in the Mucormycosis cases

Parameter (normal range)	**Mean**
CRP (<6mg/l)	61.4±63.8
Ferritin (22-322ng/ml)	476.8 ±446.7
D-dimer (<500ng/ml)	784.7 ± 215.6
Procalcitonin (<0.1ng/ml)	3.6 ± 11.6
HbA1c (<6.5%)	9.6 ± 2.5%
CRP: C Reactive Protein, HbA1c: glycated hemoglobin	

All the patients except one recovered while one patient, 70 years male diabetic expired.

Discussion

Covid-19 caused by SARS-CoV-2 has been reported to be associated with various opportunistic bacterial and fungal infections. Worldwide it is noticed there is rapid rise in mucormycosis infection in patients recovered from Covid 19. [6]

Mucormycosis is a fatal fungal infection and an uncommon infection that usually affects patients with altered immunity. Mucormycosis is an angio-invasive fungal infection caused by mold fungi of the genus Rhizopus, Mucor, Rhizomucor,

Cunninghamella and Absidia of order- Mucorales, class- Zygomycetes. The Rhizopus oryzae is most common type noticed and responsible for nearly 60% of mucormycosis cases in humans and accounts for 90% of the rhino-orbital-cerebral mucormycosis (ROCM) infection. [7] Mode of contamination occurs through the inhalation of fungal spores. [8]

Fungal spores of the mucor organism enter the humans through inhalation, local inoculation through skin lesion or through the gastrointestinal tract by ingestion. It is easily aerosolized and regardless of the point of entry, the fungi require to undergo certain important steps to establish and inoculate in human body which includes inoculation of spores into host tissue which may be skin or alveoli or intestinal mucosa. The spores succeed evading phagocytosis by macrophages and neutrophils, and the germinate into hyphae which is the angio-invasive form of the fungus. The fungi invade, grow and the virulence of the organism depending upon the individual host health status. Conditions like hyperglycemia, ketoacidosis, iron overload, functional or quantitative neutropenia favor viral growth. The fungi attach the endothelial cells of blood vessels through specific unique receptors subsequently inducing endocytosis and causing endothelial damage thus causing clinically apparent disease through hemorrhage, thrombosis, and tissue necrosis. Finally, the fungi enter the circulation and disseminating through circulation to various tissue organs leading to systematic disease and multi-organ involvement. [7,9]

The innate immunity plays significant role and this barrier is basically 2-fold as explained, [10] Tissue macrophages facilitate phagocytosis of spores. Spores that escape phagocytosis and germinate to hyphae lead to chemotaxis of neutrophils, which exert their oxidative cytotoxic effect.

The neutrophils and macrophages directly cause damage to and phagocytose both spores and hyphae through the production and release of reactive oxygen metabolites, cationic peptides, perforin, and other antimicrobial enzymes. In addition, they produce proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, IFN-γ, and IL-1b, which facilitate activation and recruitment of other immune cells. Any lack or delay in this early inflammatory response can rapidly lead to tissue destruction and disseminated disease.10,11 Other mechanisms explained are, pathogen-associated molecular patterns where in the fungi bind to pattern-recognition receptors of phagocytes and cause activation and transduction of intracellular signals. Toll-like receptor 2 is one such significant receptor involved in early proinflammatory response. [11]

High prevalence of type 2 diabetes mellitus (8.9% of adults) is a known independent risk factor for developing mucormycosis. [12] Increased incidence of mucormycosis in patients recovered from SARS CoV2 infection may be attributed to an ideal environment for the mucormycosis to germinate. The ideal environment for viral growth includes pulmonary low oxygen levels, plasma high glucose due to diabetic status, new-onset hyperglycemia, steroid-induced hyperglycemia, acidic medium like metabolic acidosis, diabetic ketoacidosis, high iron levels and decreased phagocytic activity of leucocytes due to immunosuppression. [6]

Various studies have reported varying age of involvement with a mean of 18 years to 55 years. [13] Golushankar et al noted male preponderance which was similar observation in the present study. However, Badiee et al have reported female preponderance. Majority of the patients were having history of diabetes mellitus which was in concordance with the present study with 78% diabetic cases. Sino-nasal site was the most common site of involvement by mucormycosis similar to the present study. [6] (Table 4)

In critically ill patients it is markedly noticed that there are characteristic signs of hyperinflammation, which consist of elevated serum C-reactive protein, procalcitonin, D-dimer and hyperferritinemia. These findings suggest a possibly crucial role of a cytokine storm in COVID-19 pathophysiology. [14]

Table 4: Comparison of laboratory parameters of present study with other recent studies

Laboratory Parameters	Present study (n=32)	Parisa Badiee study (n=1058)	Lav Selarka et al (n=47)	Awadhesh Kumar Singh (n=101)
Age (mean age in years)	51.9	18	55	
Male:female(% males)	72	54.3	74.5	78.9
Diabetes	78	--	76.6	80
Most common Site	Nasal cavity+ sinuses	--	--	Nasal cavity+ sinuses
S. CRP (mg/l) (mean)	80.92±62.5	57	76.4 ± 55.6	
S. ferritin (ng/ml) (mean)	464.9±422.03		357.0± 280.3	
D-Dimer (ng/ml) (mean)	828.5±245.9		305 ± 335.9	
Procalcitonin (ng/ml) (mean)	17.99±26.87			

S. CRP: Serum C Reactive Protein.

Release of APRs is a systemic response to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or surgery, or immunological disorders. [15] Liver plays major role in production of acute phase proteins, there is reduction and increase production of proteins called negative and positive Acute Phase Proteins (APP). Negative acute phase reactants are albumin, transferring, transthyretin, transcortin, and retinol-binding protein. Positive acute phase reactants are CRP, D-dimer protein, mannose-binding protein, α-1 antitrpysin, α-1 anti-chymotrypsin, α-2 macroglobulin, fibrinogen, prothrombin, factor VIII, Von Willebrand factor, plasminogen, complement factors, ferritin, Serum Amyloid P component, Serum Amyloid A (SAA), ceruloplasmin, and haptoglobin. [2]

Raised serum levels of APRs including ferritin, CRP, D Dimer levels have been reported in various studies in mucormycosis cases similar to present study. [16] Increased serum levels of procalcitonin have been reported in various case reports similar to present study. It is observed that APRs like CRP, D-dimer and procalcitonin are also raised in non-specific inflammatory conditions. [16,17]

Increased serum ferritin level is also an ideal environment for propagation of mucormycosis fungi. Hyperglycemia causes glycosylation of transferrin and ferritin, and reduces iron binding allowing increased free iron. Increase in cytokines (especially interleukin-6) in SARS CoV2 infection, increases free iron by increasing ferritin levels due to increased synthesis and decreased iron transport. Concomitant acidosis increases free iron by the same mechanism and additionally by reducing the ability of transferrin to chelate iron. [6 As free iron plays an important role in pathogenesis of mucormycosis, it has been hypothesized that iron chelation therapy might act as a preventive therapy for mucormycosis. [18]

Acute phase reactants like CRP, D dimer, Procalcitonin and ferritin have been shown to indicate poor patient prognosis in SARS CoV2 infected patients and susceptibility to bacterial co-infections. [19,20] The following table (Table 4) compares present study findings with various studies found in literature.

Very few studies were found in literature search, where distributions of APRs were studied in Post SARS CoV2 mucormycosis cases. Hence present study emphasizes the role of various APRS in mucormycosis infections.

Limitations

Present study had less sample size as it was acute rise of cases post covid infection, so in present study duration the sample studied were few. Secondly all cases diagnosed with mucormycosis fungal infection following SARS CoV-2 infection were not tested for

serum acute phase proteins levels hence they were excluded adding to small size of samples. Thirdly follow up of these patients couldn't be done due to difficult times of pandemic.

Conclusion

Frequency of mucormycosis has increased during covid 19 pandemic following SAR CoV2 infections accusing significant morbidity. Presence of diabetes mellitus should have high index of suspicion. Estimation of serum acute phase proteins can help in predicting the course and severity of illness. Serum ferritin levels may indicate increased susceptibility to mucormycosis in post SARS CoV 2 infection.

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Informed Consent: Patient participating in the study, consent for the study was taken in written consent form.

Ethical clearance: the study had undergone institutional ethical clearance; the study proposal was presented and IEC was obtained with reference number as (SDMCDS IEC.No.2021/ Medical/ Pathology/ S/ 15).

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