Original Article

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Estimation of Ferritin and D-Dimer Levels in COVID-19 Patients with Mucormycosis: A Cross-sectional Study

Biochemistry Section

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ABSTRACT

Introduction: There are increasing reports of the occurrence of fungal co-infections in Coronavirus disease-2019 (COVID-19) patients resulting in severe morbidity among predisposed individuals. Mucormycosis is an Invasive Fungal Infection (IFI). Early anticipation and identification of fungal co-infections can significantly reduce morbidity rate among COVID-19 infected patients.

Aim: To determine quantitatively the levels of ferritin and D-dimer in COVID-19 infected patients with mucormycosis.

Materials and Methods: This cross-sectional study was conducted on 84 Real Time Polymerase Chain Reaction (RT-PCR) positive for COVID-19 in oropharyngeal swab patients from June 2021 to August 2021 at Sri Devaraj Urs Medical College, Kolar, Karnataka, India. D-dimer and ferritin levels were measured in the patient's blood sample using Latex Enhanced Immunoturbidimetric method in Vitros 5.1 FS and Vitros Eci Immunodiagnostics, respectively. Continuous data represented as mean and standard error of mean, Kruskal-Wallis test and Mann-Whitney U test was used to test significance, p-value <0.05 was considered as statistically significant.

Results: Of the 84 COVID-19 Infected patients, 40 were included in group 1, 25 patients in group 2 and 19 patients in group 3. A total of 21 patients were aged between 20-40 years, 48 patients between 41-60 years age group and 15 patients were in 61-80 years of age group. The number of male patients was 63 and female patients were 21. The D-dimer levels were 1259.37±258.9, 2632.60±472.6 and 229.53±18.4 (p-value <0.001) in group 1, 2 and 3, respectively and ferritin levels were 528.58±45.03, 511.48±74.4, and 256.89±51.8 (p-value <0.007) in group 1, 2 and 3, respectively.

Conclusion: Serum ferritin and plasma D-dimer were significantly elevated in COVID-19 patients with mucormycosis. Mucormycosis in COVID-19 patients without pre-existing co-morbidities may be attributed to the use of steroid therapy in these patients for COVID-19 infection. Thus, serum ferritin and plasma dimer levels may have a significant predictive role in the risk assessment for the development of mucormycosis among COVID-19 infected patients.

Keywords: Acute phase protein, Coronavirus disease-2019, Fibrin degradation products, Fungal disease, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

Mucormycosis is an Invasive Fungal Infection (IFI) caused by a group of saprophytic environmental fungi-*Rhizpous, Mucor, Cunninghamella, Aposphysomyces, Licitheimia (Absidia), Saksenaea, Rhizomucor* [1]. Mucormycosis was previously called zygomycosis. The clinical manifestations of mucormycosis can be rhinocerebral, pulmonary, cutaneous, gastrointestinal, and disseminated. Rhinocerebral mucormycosis is the most common manifestation accounting for between one-third and one-half of all cases of mucormycosis [2]. The recent pandemic Coronavirus Disease-2019 (COVID-19) due to the novel Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) has caused more than 110 million cases and more than 2.4 million deaths globally [3]. There are increasing reports of the occurrence of bacterial and fungal co-infections in COVID-19 patients resulting in severe morbidity among predisposed individuals [4].

The common identifiable risk factors of mucormycosis are diabetes mellitus, patients on immunosuppressive therapy, leukaemias, neutropenias, neutrophil dysfunction, haematopoetic stem cell transplantation, diabetic ketoacidosis, iron-overload and Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS) [5].

The mold gains entry into the host through the respiratory tract and has a remarkable affinity for the internal elastic lamina of arteries and subsequently causes thrombosis and infarction [6,7]. The disease progression from nose and sinuses is both by direct or through vascular occlusion. Intracranial spread occurs by invasion through superior orbital fissure, ophthalmic vessels, cribriform plate, carotid artery and perineural route [8,9]. COVID-19 infection involves the pulmonary parenchyma resulting in diffuse alveolar damage, hyaline membrane formation, interstitial lymphocyte infiltration and vascular micro thrombi [10]. These pulmonary changes take weeks to resolve and may serve as a nidus for fungal infection [11].

Precious time would be wasted in the initiation of treatment to these predisposed individuals waiting for culture reports. Previous studies have shown that there is significant elevation of D-dimer levels in patients with COVID-19 infection. An elevated level of D-dimer indicates enhanced coagulation leading to thrombus formation, a nidus for fungal infection, and also an elevation in serum ferritin indicates the immune dysregulation in severe COVID infection [12-14]. There are hardly any studies available that have compared the levels of these key mediators ferritin and D-dimer in COVID-19 patients with mucormycosis, early anticipation and identification of fungal co-infections can significantly reduce morbidity rate among COVID-19 infected patients [15].

Hence, this study aimed to determine quantitatively the levels of ferritin and D-dimer levels in COVID-19 infected patients developing mucormycosis.

MATERIALS AND METHODS

This cross-sectional study was conducted in a tertiary care hospital and research centre attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India, from June 2021 to August 2021. Ethical

Clearance was obtained from the Institutional Ethics Committee (IEC Ref No: DMC/KLR/IEC/102/2021-2022 dated 29-06-2021). Informed consent was obtained from the participants.

Inclusion criteria: All COVID-19 positive patients, diagnosed on admission by RT-PCR of oropharyngeal swabs with or without respiratory symptoms, and COVID-19 positive patients diagnosed with mucormycosis by fungal culture were included in the study.

Exclusion criteria: Patients younger than 18 years, critically ill patients like acute myocardial infarction during hospitalisation, diabetes mellitus with acute complications, acute pancreatitis, chronic kidney disease were excluded.

A study with a total of 84 RT-PCR positive for COVID-19 virus in oropharyngeal swab patients were included in the study and were divided into 3 groups:

- **Group 1-** 40 COVID-19 positive patients with confirmed mucormycosis infection by fungal culture and microbiological identification by Lacto-phenol Cotton Blue (LCB) were included in group 1 and were mild to moderately symptomatic. Among 40 COVID-19 positive patients with Mucormycosis infection, two patients developed mucormycosis within seven days of COVID-19 infection, two patients within 14 days,10 patients within 21 days and rest of the 26 patients developed mucormycosis in the next 45 days of being positive for COVID-19 infection.
- **Group 2-** 25 patients with severe symptoms of COVID-19 infection with Respiratory Rate (RR) >30/min (or) SpO_2 <90% at Room Air (or) less than 94% with oxygen, Acute Respiratory Distress Syndrome (ARDS) and without symptoms or clinical history related to Mucormycosis infection were included.
- **Group 3-** 19 patients with mild to moderate symptoms of COVID-19, RR 24-30/m (or) SpO_2: 90-94% at room air were included [16].

Procedure

A 4 mL of blood was collected from the patients, 2 mL blood was collected in plain red vacutainer and assayed for Serum Ferritin automated clinical biochemistry analyser (Vitros Eci immunodiagnostic systems, Ortho clinical diagnostics, United Kingdom), and 2 mL blood was collected in sodium citrate vacutainer, assayed for plasma d-dimer estimated by Latex Enhanced Immunoturbidimetric method (Vitros 5.1 FS, Ortho clinical diagnostics, United Kingdom) [17-19]. Serum ferritin and plasma D-dimer values are expressed in ng/mL.

A detailed clinical history of co-morbidities like diabetes mellitus, hypertension, cardiac disease, tuberculosis, and bronchial asthma if any were recorded.

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and analysed using Statistical Package for the Social Sciences (SPSS) 22.0 version software. Continuous data represented as mean and standard error of mean. Kruskal-Wallis test was used to test significance among the categorical variables between the groups, Mann-Whitney U test was used for pair wise comparison to compare variables which do not conform to normal distribution, p-value <0.05 was considered as statistically significant.

RESULTS

Total 84 patients were included, of which 40 patients in group 1, 25 patients in group 2 and 19 patients in group 3. A total of 21 patients were aged between 20-40 years, 48 patients between 41-60 years age group and 15 patients were in 61-80 years of age group. The number of male patients was 63 and female patients were 21. The number of patients with history of co-morbidities like type 2 diabetes mellitus, hypertension, cardiac disease like Congestive Cardiac Failure (CCF) or Ischaemic Heart Disease (IHD) in each group were as shown in [Table/Fig-1]. In the study, all the COVID-19 infected patients had received steroid therapy as a part of treatment protocol for COVID-19 infection.

Co-morbidities	Group 1 (n=40)	Group 2 (n=25)	Group 3 (n=19)
Diabetes mellitus	20	6	3
Hypertension	4	-	-
Diabetes mellitus and hypertension	11	9	2
Cardiac disease- Congestive cardiac failure/ Ischaemic heart disease/Myocardial infarction	1	-	-
Respiratory diseases- Tuberculosis/Bronchial asthma	-	-	-
No co-morbidities	4	10	14

[Table/Fig-1]: Shows the number of patients with history of co-morbidities under group 1, group 2 and group 3.

The D-dimer levels were significantly higher in group 1 and group 2 compared to group 3 subjects. Serum ferritin levels were significantly increased in group 1 compared to group 2 and group 3. The variables d-dimer and ferritin checked for normality and does not satisfy normality conditions among the groups. The skewness, Shapiro-Wilk and normality plots test, non parametric kruskal-wallis test was used to test the significance of difference. The difference in mean levels of D-dimer and ferritin across 3 groups was statistically significant [Table/Fig-2].

Parameter	N	D-dimer (ng/mL) Mean±SE	Ferritin (ng/mL) Mean±SE
Group 1	40	1259.37±258	528.58±45.03
Group 2	25	2632.60±472.6	511.48±74.4
Group 3	19	229.53±18.4	256.89±51.8
*p-value		<0.001	<0.007

[Table/Fig-2]: Shows the mean±SE and Kruskal-Wallis test of significance for D-dimer and Ferritin levels in group 1, group 2 and group 3 subjects.
*Kruskal Wallis test

Pairwise comparison among group 1 and group 2, showed the D-dimer levels to be significantly different and increased in group 1 subjects compared to group 2 subjects, there was however no significant changes in the levels of ferritin levels among group 1 and group 2. Pairwise comparison among group 1 and group 3, shows the D-dimer levels were highly increased in group 1 compared to group 3 which was also statistically significant. Even the ferritin levels were decreased in group 3 compared to group 1 which was also statistically significant. Pairwise comparison among group 2 and group 3 shows the D-dimer levels were significantly increased in group 2 compared to group 3. And the serum ferritin levels were significantly decreased in group 3 compared to group 2 [Table/Fig-3].

Pairwise comparison of groups		D-dimer	Ferritin
Group 1 and Group 2	Mann-Whitney U	257.000	480.500
	Z	-3.277	-0.263
	p-value	0.001	0.792
Group 1 and Group 3	Mann-Whitney U	11.000	174.500
	Z	-5.986	-3.334
	p-value	<0.001	0.001
Group 2 and Group 3	Mann-Whitney U	10.000	149.500
	Z	-5.628	-2.086
	p-value	<0.001	0.037

[Table/Fig-3]: Shows the pairwise comparison of serum ferritin and plasma D-dimer levels among the groups using Mann-Whitney U test.
p-value <0.05 considered significant

DISCUSSION

This study included 84 RT-PCR confirmed COVID-19 infection cases presenting with mild symptoms to severe pneumonia at our

hospital. Forty COVID-19 infected patients with mucormycosis had Rhino orbital mucormycosis. In this study, authors observed that the group 1 patients had significantly higher levels of ferritin as compared to group 2 and group 3 patients. Among group 2 and group 3, the group 2 patients with severe COVID-19 infection had higher levels of ferritin compared to group 3 subjects. The study findings are in par with observations made by Zhou F et al., where ferritin levels of more than 400 correlated significantly with severe infection and mortality due to COVID-19 [20]. This can be attributed to the fact that the hyperglycaemia due to co-existing co-morbid condition like diabetes mellitus or secondary to steroid therapy in COVID-19 patients, is known to cause glycosylation of protein transferrin and ferritin, reducing the iron binding with these proteins and thus causing elevated free iron which serves as an ideal source for mucor infection by facilitating the fungal heme oxygenase to uptake iron for its metabolism [21,22]. Further, in COVID-19 infection there is a release of excessive of ferritin from cells due to cytokine stimulus by the interleukins especially IL6, as an account of viremia there is significant activation of macrophages. This hyperferritinemia is seen in severe infections of COVID-19 and its levels correlates with high mortality [23,24]. Further studies have shown that iron overload to predispose individuals to mucormycosis infection [25,26]. In this study also the group 1 COVID-19 patients with mucormycosis have higher ferritin levels.

D-dimer is a fibrin degradation product, a protein fragment present in the blood after a blood clot is degraded by fibrinolysis [27]. In the present study, authors found that the D-dimer levels were high in group 1 compared to group 3 patients, the levels of D-dimer in group 2 patients was significantly higher compared to the other groups. The elevation in D-dimer in COVID-19 patients has been attributed to the endothelialitis, endothelial damage and dysfunction of the haemostatic system leading to hypercoagulable state induced by the virus [28].

An alarming finding in this study was that four patients with no co-morbidities with COVID-19 infection had developed mucormycosis, this may be attributed to the steroid therapy given to COVID-19 positive patients, that is known to cause stimulation of intravascular coagulation [29]. As a consequence, the microthrombi formed may serve as a nidus for Mucormycosis infection in these patients. Recent studies have reported that the high glucose levels, free iron, and lowered pH, with reduced phagocytic activity of White Blood Cell (WBC) due to steroid use makes the COVID-19 patients susceptible to Mucormycosis infection [20].

Limitation(s)

The influence of the duration of co-morbid conditions specifically diabetes mellitus and a baseline levels of HbA1c in these patients prior to COVID-19 infection needs to be considered and associated treatment that might have effect on the levels of D-dimer and ferritin should also be considered in future studies with larger sample size to ascertain the cut off limits and independent use of these biomarkers for assessment of the risk of developing mucormycosis in COVID-19 infected individuals.

CONCLUSION(S)

Serum ferritin and plasma D-dimer were significantly elevated in COVID-19 patients with mucormycosis. Mucormycosis in COVID-19 patients without pre-existing co-morbidities may be attributed to the use of steroid therapy in these patients for COVID-19 infection. Thus, serum ferritin and plasma D-dimer levels may have a significant predictive role in the risk assessment for the development of mucormycosis among COVID-19 infected patients. Further considering the findings of this study, the use of the above biomarkers along with judicious use of steroid therapy and maintenance of optimum blood glucose levels would reduce the burden of secondary infection mucormycosis among COVID-19 patients.

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