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Research Article

STUDY OF D-DIMER AND SERUM FERRITIN LEVELS AS PROGNOSTIC BIOMARKERS IN COVID-19 PATIENTS

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ABSTRACT

Objective: Coronavirus disease 2019 (COVID-19) is primarily a respiratory illness causing thrombotic disorders. Pro-inflammatory cytokines are one of the responsible causes of cytokine storm syndrome in patients with COVID-19. Coagulopathy and inflammation are associated with COVID-19 severity. The coronavirus spike protein facilitates the entry of the virus into the target cells causing coagulopathy and inflammation.Other infections include direct viral toxicity, endothelial cell damage, inflammation, and deregulation of the immune response and renin-angiotensin-aldosterone system. The study aims to estimate levels of D-Dimer and Serum Ferritin in symptomatic and asymptomatic COVID-19 patients and its comparison with healthy controls.

Methods: The study includes 30 healthy control and 30 symptomatic and 30 asymptomatic COVID-19 patients of both sexes. Analysis of serum ferritin was done on a fully automated immunology analyzer-SIEMENS based on the principle of chemiluminescence. D-dimer was estimated on mLab which is cartridge-based.

Results: We observed that the levels of D-Dimer and Serum Ferritin significantly increased in symptomatic COVID-19 patients as compared to asymptomatic COVID-19 positive patients and healthy non-COVID-19 controls.

Conclusion: The elevated serum ferritin and D-dimer were associated with a poor outcome and poor prognosis and could predict the worsening of COVID-19 patients. The significant increase showed that D-Dimer and serum ferritin accurately predicts patients developing severe COVID- infection.

Keywords: COVID-19 patients, Cytokine storm, D-dimer, Ferritin, Hyperinflammation, Hyperferritinemia, Pro-inflammatory cytokines.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) disease is one of the most unforgettable and memorable pandemics of the 21st century that was caused by a novel coronavirus that causes severe acute respiratory syndrome [1]. It was first recorded in Wuhan, the capital of Hubei Province of China in December 2019 [2]. On March 11, 2020, the World Health Organization (WHO) has declared COVID-19 disease a global pandemic [3]. The disease mainly affects the respiratory system and spreads through aerosols released during coughing and sneezing [4]. The major symptoms include fever, running nose, nasal congestion, shortness of breath, and headache [5]. Majority of patients with COVID-19 have a mild influenza-like illness or they may show no symptoms but a small proportion of patients develop severe pneumonia, acute respiratory distress syndrome (ARDS), and a multi-organ failure that includes renal, hepatic, and cardiac systems [6].

The pandemic of COVID-19 in India

On January 30, 2020, the first case of COVID-19 infection was reported in Kerala, India. With more than 9,000,000 recorded instances of COVID-19 infection and more than 100,000 deaths per day, India, showed the highest number of confirmed cases in Asia and was the second highest number of confirmed cases in the world after the United State. The cases peaked in mid of September when the data showed about 90,000 cases per day but soon it dropped to under 40,000 in December. Soon many cases were reported in many cities of India such as Delhi, Mumbai, Chennai, Ahmedabad, etc.

D-Dimer

D-dimer is a cross-linked fibrin degradation breakdown fragment that shows enhanced thrombin production. Hence, it is widely used as a biomarker for thrombotic disorders. Seeing the outbreak of the COVID-19 pandemic, D-Dimer has been identified as a potential indicator for COVID-19 patients.

Serum ferritin

Serum ferritin is an iron storage protein that plays a key role as an immune modulator with both pro-inflammatory and immunosuppressive properties. It is also an acute phase reactant and its level increases in an acute inflammation state whether it is infectious or non-infectious.

Thus, our study aimed to evaluate the levels of D-Dimer and serum ferritin in symptomatic and asymptomatic COVID-19 patients and their comparison with healthy controls.

METHODS

Study design

The study was carried out on COVID-19 asymptomatic and symptomatic patients admitted to PIMS, hospital, Udaipur. Sixty patients (Thirty symptomatic and thirty asymptomatic) with confirmed COVID-19 positive patients according to ICMR guidelines. Thirty healthy ages (20-60yrs) and sex-matched non-COVID-19 controls were included in the study.

Ferritin and D-dimer laboratory testing was performed in the Clinical Biochemistry Laboratory of PIMS on the day of hospital admission. Ferritin value was determined using a Sysmex 5100 analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) and D-dimer was determined using an m-Lab-micro point machine which is cartage based. Institutional Ethics Committee approved this study. Verbal informed consent was obtained from all patients for blood testing, including D-dimer and ferritin.

The exclusion criteria included subjects with any systemic or metabolic diseases, immunosuppressive disorders, liver, renal and vascular disease, alcoholics, pregnant females, patients on ferritin treatment, or those who are on anticoagulant therapy, cancer, and diabetic patients, patients with any history of thromboembolism, patients who are receiving Vitamin D.

The inclusion criteria included subjects showing symptoms such as fever, running nose, headache, body ache, and weakness. Patients who had vomiting, diarrhea, or whose oxygen saturation level was below 94% were included in the study. Furthermore, those people were included who returned to the country from an international destination or where the infection was endemic in the 14 days before the symptom's onset.

Sample collection

5 ml of venous blood was collected with an aseptic measure. The blood sample was transferred into two vials-one for ferritin (red top plane vial for serum) and the other for D-dimer (blue top-sodium citrate vial). For ferritin, the blood was clotted and centrifuged for 30 min and the supernatant (serum) was taken. The D-Dimer sample was used as whole blood that was mixed with sodium citrate anticoagulant.

Study duration

The duration of the study was from April 2020 to November 2020.

Statistical analysis

The mean and standard deviation has been used to define data in each group. These data were compared and significance was calculated between rural and urban neonates and also between male and female neonates using the unpaired "t" test. $p<0.05$ was considered as significant and values <0.001 were considered highly significant. GraphPad Prism version 6 software was used for analysis**.**

RESULTS

The results are categorized as Group 1 (symptomatic) and Group 2 (asymptomatic) COVID positive cases, with Group 3 as healthy controls are depicted in Table 1. The study included 30 subjects in each group. The results were expressed in mean ± SD and showed that D-Dimer and ferritin were lowest in healthy controls (Table 2).

The levels were highest in symptomatic (7329.3±1660 ng/ml D-Dimer and ferritin = 943.9±194.1 ng/ml) patients as compared to asymptomatic (396.5±164.80 ng/ml D-Dimer and ferritin = 290.7±96.68 ng/ml) and healthy controls (55.9±19.1 ng/ml D-Dimer and ferritin = 231.7±7.34 ng/ml). The statistical significance was calculated using online graph pad software. The comparison of D-Dimer and ferritin values in symptomatic with asymptomatic was highly significant ($p<0.0001$). The comparison with healthy subjects also was highly significant ($p<0.0001$).

Table 1: Group categorization of the present study

Group 1	Symptomatic COVID-19 positive patients (n=30)
Group 2	Asymptomatic COVID-19 positive patients (n=30)
Group 3	Healthy Non-COVID-19 Controls (n=30)

DISCUSSION

COVID-19 seems to be a very serious public health threat since it causes asymptomatic or mild illness to severe life-threatening infection and sustained person-to-person transmission [7]. The WHO has declared COVID-19 as an international public health emergency [8]. Hence, it is important for health professionals to be fully aware of this new 2019-nCoV so that time and with proper coordination and with effective actions and management, proper actions can be taken to prevent additional cases and further to control poor health outcomes [9].

The changes in the levels of different blood indicators are connected to the severity, illness, and death of COVID 19 patients. In the early stages of infection, D-dimer and serum ferritin levels are abnormally high which reflects excessive inflammation and hypercoagulability [10]. Further, uncontrolled inflammation combined with hypoxia and the direct cytotoxic effects of the virus on endothelial cells contribute to thromboembolic complications [11].

A hypercoagulable state was reported in COVID-19 patients. Thrombotic complications and coagulopathies including disseminated intravascular coagulopathy (DIC), venous thromboembolism (VTE), and pulmonary embolism are common in COVID-19 patients with high mortality rates and likely reflect activation of the coagulation cascade due to cytokine storm, or possibly due to superinfection and organ dysfunction [12,13]. The result of our study concluded that the levels of D-dimer in symptomatic COVID-19 positive patients were statistically significantly high with frequent clotting disorders and microthrombotic formation in peripheral blood vessels as compared to asymptomatic and healthy Non-COVID-19 control. This was in agreement with the studies of other authors [14,15]. Thromboembolic complications are found to be associated with low platelet levels with increased levels of prothrombin time and high D-dimer. Hence, highly increased D-dimer is likely to be associated with persistent clotting disorders, acute myocardial infarction, pulmonary embolism, and microthrombotic formation which may be the cause of respiratory failure, DIC, and death [16]

Hyperferritinemia is a cardinal characteristic and contributes to cytokine storm syndrome by exerting direct immunosuppressive and pro-inflammatory effects. Fatal results from cytokine storm syndrome are reported in COVID-19 patients that indicated the severity of the disease [17]. COVID-19 severity and worse prognosis mean that mortality is due to virally induced hyperinflammation that is associated with high ferritin levels [18]. In COVID-19-infected patients, elevated serum ferritin levels were observed to have a strong correlation with illness severity. Our study reveals that symptomatic COVID-19 positive patients had greater ferritin levels than asymptomatic COVID-19 positive patients and healthy non-COVID-19 control according to a previous study [19]. Studies reveal that higher ferritin levels were seen in COVID- 19 death cases compared with survivors and in COVID-19 patients with thrombotic complications [20]. The findings of our study showed high serum ferritin level in symptomatic COVID-19 positive patients than asymptomatic COVID-19 positive patients and healthy non-COVID-19 control that was independently associated with ARDS, mortality, and severe COVID-19 infection and was in association with another authors [21,22].

Table 2: Comparison of mean and SD values among Groups 1, 2, and 3

Parameters	Symptomatic covid-19 Group 1 (n=30)	Asymptomatic Covid-19 Group 2 (n=30)	Healthy Control Group 3 (n=30)	p-value Groups 1 and 2	p-value Groups 1 and 3	p-value Groups 2 and 3
D-Dimer (ng/ml)	7329.3±1660	396.5±164.8	55.9±19.1	<0.0001	<0.0001	<0.0001
IL-6 (pg/ml)	397.9±291.1	140.3±22.06	25.9±2.84	<0.0001	<0.0001	<0.0001
Ferritin (ng/ml)	943.9±194.1	290.7±96.68	231.7±7.34	<0.0001	<0.0001	<0.0001
CRP (mg/dl)	194.1±76.9	111.8±14.9	7.51±3.38	<0.0001	<0.0001	<0.0001

CONCLUSION

High levels of serum ferritin suggest that liver injury and severe illness are quite common in COVID-19 patients as ferritin is released from hepatocytes. D-dimer helped physicians in the diagnosis of DIC in COVID-19 patients as the incidence of pulmonary embolism is highly observed in COVID patients as D-dimer is the product of degradation of a blood clot formed out of fibrin protein. D-Dimer and serum ferritin biomarkers should be included in future studies to predict the severity in the patients diagnosed with COVID-19 disease.

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CONFLICT OF INTEREST

None.

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