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Original Research Article

Impact of Inflammatory Markers in Predicting Outcomes of SARS-CoV-2 Patients: The why's and how's of Diabetic Hypertensive Patients

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Abstract

Background: Hypertension and Diabetes Mellitus, the most frequent co-morbidities in SARS-Cov-2 patients are considered as risk factors for disease severity and mortality. And the role of inflammatory biomarkers on these patients is still under evaluation. ***Methods***: Retrospective data was collected study in 3 independent groups each with 24 patients: diabetes, hypertension and with concomitant diabetes and hypertension respectively of critically ill SARS-CoV-2 patients admitted in the CCM. The data of age, gender, diabetic and hypertensive history, inflammatory marker, duration of stay was obtained from electronic patient data repository of the hospital and compared with Mortality. ***Results:*** The mean CRP levels in diabetes was 78.81 ± 16.8, in hypertension 82.23 ± 13.98 and in group with both the co morbidities was 79.05 ± 16.38. Serum ferritin levels were high in hypertensive group 731 ± 621.12, were as in diabetes population it was 560.31 ± 319.81, and 629.37 ± 350.8 in both diabetic and hypertensive population. The mean and SD of D-dimer it was 3726.4 ± 2411.86 in diabetic group, 2861.28 ± 2041.36 in hypertensive group and 2755.6 ± 1980.67. CRP levels and D-dimer were positively correlated with mortality and duration of stay. ***Conclusion***: Our study concluded inflammatory markers CRP and D-dimer levels were elevated in both comorbid patients and this was statistically significant. Correlation of ferritin to the outcome was not significant and understanding the molecular mechanism of infection in co-morbid patients and assessing the inflammatory markers can provide necessary assistance at earliest, for a better clinical outcome.

Trial Registration No: CTRI/2020/12/030070

Keywords: Hypertension, Diabetes Mellitus, Inflammatory Markers, D-dimer, Ferritin , SARS-CoV-2.

INTRODUCTION

In December 2019, the rapid outbreak of corona virus disease(COVID-19), was declared as a global pandemic and a Public Health Emergency of International Concern (PHEIC)[1].221 Countries and Territories around the word have reported a total of 235,077,026 confirmed cases of the corona virus that originated from Wuhan, China and a death toll of 4,805,890 deaths. India being the second top country affected with more than 33,700,000 confirmed cases and more than 445,000 deaths as of 1st October 2021, and this pandemic has an enormous adverse impact in all aspects of life globally.

According to latest reports, the clinical manifestations of COVID-19 are heterogeneous. Analysis has indicated that hypertension and diabetes mellitus, the most frequent co-morbidities in Covid-19 patients are considered as risk factors for disease severity and mortality [1-3]. Patients critically ill, will develop hyper inflammatory response, leading to pathological dysfunction of innate host defense mechanisms, causing complications like multiple organ failure (MODS) and or cytokine release syndrome [4]. An elevation in serum C-reactive protein (CRP), D-dimer and Ferritin, the inflammatory biomarkers was associated with a poor outcome in Covid-19patients [5].

Covid-19 patients with uncontrolled hyperglycemia are at high risk of thrombotic events and they may suffer a hypercoagulable state with a worse prognosis thus increased D-dimer levels are found

[6].The detection of CRP levels is of great value in assessing the severity of their condition [7]and many individuals with diabetes exhibit elevated serum Ferritin levels[8]. Hypertension is reported to occur in over two-third of patients with diabetes [9] where it impairs the inflammatory blood biomarkers level and has been reported to increase the severity and mortality of the patients with infection [10].Yet, no study has, thus far addressed the individual effect of diabetes, hypertension and their combination on inflammatory markers and outcomes in patients affected with Covid-19 infection along with the pathogenesis.

The aim of this study was to determine the impact of inflammatory biomarkers (CRP, D-dimer and Ferritin) in diabetes, hypertension individually and with concomitant diabetes and hypertension together, and their outcomes in patients with SARS-CoV-2.

MATERIALS AND METHODS

Study design

This retrospective study was done on 3 independent groups, Diabetes, Hypertension and patients with both diabetes and hypertension admitted at the critical care unit, from March 2020 - December 2020.

STUDY SUBJECTS

72 critically ill patients tested RT-PCR positive of nasopharyngeal and oropharyngeal swabs of age 18 and above and of any gender were enrolled in this study, 24 from each 3 independent group. The first three days of the registration period were analyzed in the study and patients name was kept anonymous.

STUDY VARIABLES

After obtaining Institutional Ethical Committee Clearance, data of Age, Gender, history of Diabetes Mellitus, Hypertension, CRP, D-dimer, Ferritin, and Duration of stay and Mortality was obtained from electronic patient data repository of the hospital.

The index test for diagnosis of SARS-CoV-2 was Real Time-PCR for detection of RNA virus in the nasopharyngeal and oropharyngeal swab was done according to the WHO guidelines for diagnosis of COVID-19.Serum CRP levels were estimated by Vitros micro slide in Vitros 5600. Serum D-dimer levels were estimated by Immunofluorescence technology with Standard F Analyzer in F2400. Ferritin is estimated by Vitros microwell method, an immunometric technique in Vitros 5600.

STATISTICAL ANALYSIS

Data entered in excel sheet were statistically analyzed using, IBM SPSS software version 23.Normal distribution of continuous date was checked by Shapiro-wilk test. The Pearson's correlation co-efficient was used to correlate Inflammatory markers to mortality and length of stay. Mean difference with 95% Confidence Interval was calculated for inflammatory markers. Independent T test was applied as tests of significance and p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 72 RT-PCR positive patients were enrolled and categorized into 3 groups with 24 patients each. The mean age was 61.8 ± 12.7 years. Numbers of males were 37 (51.4%) and numbers of females were 35 (48.6%). Table 1: Inflammatory markers in study population

The mean CRP levels in diabetes was 78.81 ± 16.8, in hypertension 82.23 ± 13.98 and in group with both the co morbidities was 79.05 ± 16.38. Serum ferritin levels were high in hypertensive group 731 ± 621.12, were as in diabetes population it was 560.31 ± 319.81, and 629.37 ± 350.8 in both diabetic and hypertensive population. The mean and SD of D-dimer it was 3726.4 ± 2411.86 in diabetic group, 2861.28 ± 2041.36 in hypertensive group and 2755.6 ± 1980.67.

Its graphically depicted in Figure 1, serum CRP and Ferritin levels were high in hypertensive group whereas D-dimer levels were high in diabetic group.

Table 2 shows the Pearson Correlation between the inflammatory markers and sara-cov-2 outcomes. P-value less than 0.05 were considered statistically significant. CRP levels were positively correlated with mortality and duration of stay, even serum d-dimer levels were positively correlated with mortality and duration of stay. But we could not find any correlation with ferritin levels in mortality and duration of stay.

Table-1: Inflammatory markers in study population

Variables	diabetes n=24	Hypertension n=24	Diabetes and hypertension n=24
CRP	78.81 ± 16.80	82.23 ± 13.98	79.05 ± 16.38
FERRITIN	560.31 ± 319.81	731 ± 621.12	629.37 ± 350.38
D-DIMER	3726.40 ± 2411.86	2861.281 ± 2041.36	2755.6 ± 1980.67
MORTALITY	79.16%	79%	87.5%
LENGTH OF STAY	8 days	10.5 days	9.2 days

Table-2: Pearson correlation between the inflammatory markers and sara-cov-2 outcomes.

Inflammatory markers	Pearson correlation	P value
CRP	0.179	0.040
D-DIMER	0.531	0.020
FERRITIN	-0.486	0.019

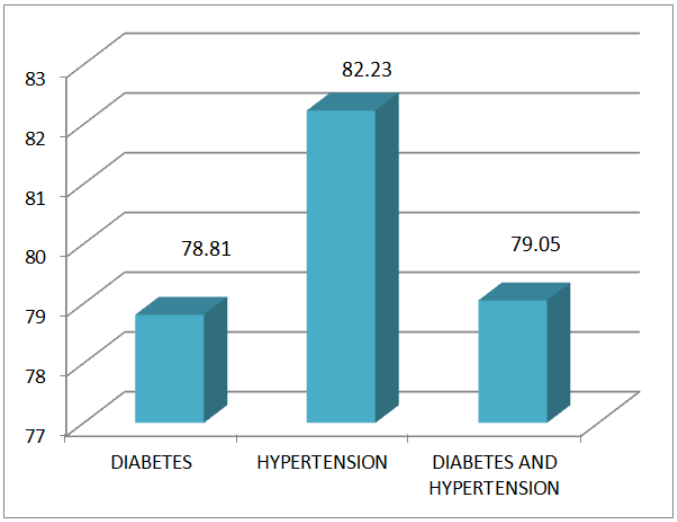
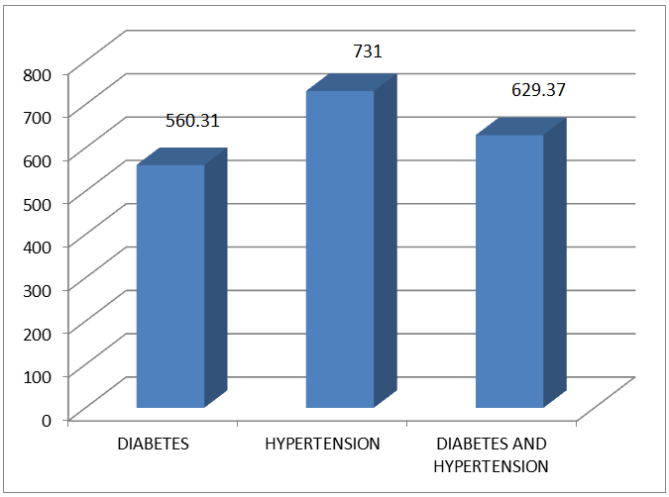
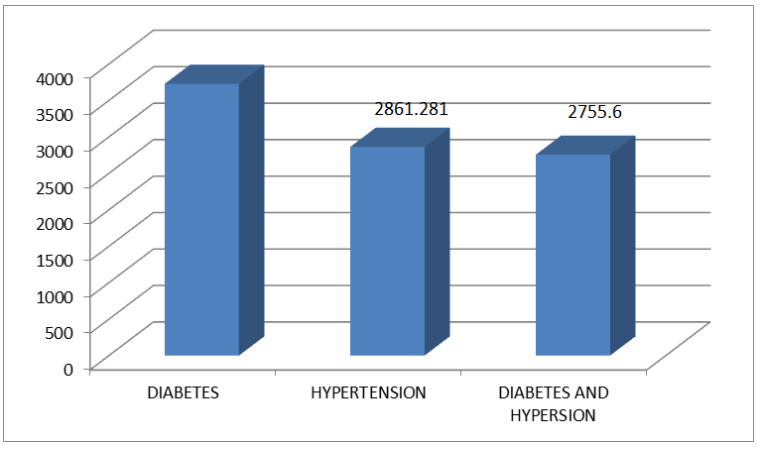


Fig-1: Mean levels of CRP, ferritin and d-dimer CRP

Ferritin



D-DIMER



DISCUSSION

SARS-CoV-2,the single stranded enveloped RNA virus have a three dimensional spike glycoprotein on its surface, which can closely bind to the human ACE 2 receptor ,which is expressed in lung, heart, kidneys and intestines and they are shown to be the co-receptor for the corona virus entry[11]. This S protein binds to the ACE2 with affinity and potentially, twenty time's greater affinity than the SARS virus (Severe Acute Respiratory Syndrome), and is the reason of spreading so easily. Thus cells with ACE2 expression may act as a target cells and may correlate with severity of disease in each tissue [12]. This S protein on its virion surfaces resembles crown-like shape and thus the virus is named in Latin "CORONA" meaning Crown [13]. By binding to the ACE 2 receptor, sialic acid receptor, transmembrane serine protease 2, extracellular matrix metalloproteinase inducer, catepsin B and L, the virus access the human host cell. The virus SARS-CoV-2, infect goblet secretory cells of the nasal mucosa and alveolar type II pneumocytes by binding membrane bound angiotensin-converting enzyme. The human protease enzyme cleaves ACE2 receptor and thus virus gets internalized into the cell and viral replication begins progressive infection of alveolar pneumocytes with significant viral shedding results in apoptosis and necrosis [14].it has been shown that SARS-CoV-2 has induced endothelial dysfunction or damage and is a major determinant [12]. Endothelial cells release substance that control vascular relaxation and contraction as well as enzymes that control blood clotting, immune function and platelet.

HYPERTENSION, DIABETES AND SARS-cov-2

Life threatening complication of SARS-CoV-2 in hypertensive and diabetic patients is due to the pre-existing endothelial dysfunction [13]. In our study mortality was high for the patients having both the co-morbidities and was found to be 87.5% whereas mortality in diabetes group was 79.16% and in hypertension group it was 79%. Guan *et al.* in a cohort study, reported that patients having hypertension and diabetes reached the composite endpoint of ICU admission, mechanical ventilation and death [15].High-affinity cellular binding and efficient viral entry is easier in diabetic individuals with decreased viral clearance. These patients have decreased T-cell function and thus cause dysfunction of innate host defense mechanisms and cytokine release syndrome. With all these factors in patients with cardiovascular disease, hypertension, severity of the disease worsens. Lvliang Lu *et al.* in a Meta-analysis showed, patients with SARS-CoV-2co morbidities, diabetes, and hypertension were at complications and ended in death [16].Hypoglycemic agents like glucagon like peptide-1 agonists classically called as glp-1 agonist up regulate ACE2. The expression of ACE2 is substantially increased in patients with Type 1 or Type 2 Diabetes Mellitus and Hypertension who are treated with ACE inhibitors and angiotensin II type-1 receptor blockers (ARBs) when compared to healthy individuals. As a result, increased expression of ACE would facilitate infection with SARS-CoV-2.This warning is supported by analysis of 1099 patients in China, treated with SARS-CoV-2 from December 2019–January 2020 [17].

Thus Hypertension and diabetes are associated with high risk of severe SARS-CoV-2 [18]. The angiotensin-converting enzyme is the link between hypertension, diabetes and SARS-CoV-2.This has been supported by many studies [19, 17].

In our study serum CRP and Ferritin was high in patients with hypertension than those with both the co-morbidities, increased D-dimer levels were found in the diabetic population as it's associated with an imbalance between clotting factors and fibrinolysis [20].The elevated levels of CRP and Ferritin are associated with overproduction of inflammatory cytokines.CRP, an acute phase protein, synthesized by liver under inflammatory conditions of the body, like viral infection, bacterial infection and tissue destruction appears in blood within 6-10 hours of tissue damage [4]. High CRP level is associated with worse prognosis [2] and thus it may be a valuable early biomarker in predicting the possibility of disease prognosis in non-severe patients infected with SARS-CoV-2, which help in early treatment. Ferritin is the storage form of iron in humans; also an acute phase protein. In infections iron gets released into the ER due to decrease transport capacity of spleen and liver due to damage [4]. Serum Ferritin levels were high for patients with poor composite outcome [5].The plasmin mediated, cross linked fibrin degradation results in multiple peptide fragments called D-dimer. Patients with SARA-CoV-2 often show clotting disorders with multi organ dysfunction and coagulopathy, resulting in high mortality [20].Endothelial dysfunction is often said to play a key role in such a hypercoagulopathy state.

CONCLUSION

Our study concluded inflammatory markers CRP and D-dimer levels were elevated in both comorbid patients and this was statistically significant. Since, India is the second top country affected by the corona virus, understanding the molecular mechanism of infection in co-morbid patients and assessing the inflammatory markers can provide necessary assistance at earliest, for a better clinical outcome.

Limitation

The sample size was small and the study was done only at single centre

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