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Evaluation of CRP, Hb, LDH and other Biomarkers of Severity in Iraqi Patients with COVID-19 Infection.

A Scientific dissertation submitted to the College of Medicine /Diyala University in partial fulfilment of the requirement of M.B.Ch.B.

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Abstract:

Although many Iraqi patients infected with coronavirus disease 2019 (COVID-19) only experience mild symptoms, in some cases a patient's condition deteriorates, resulting in a poor outcome. Our study investigates the behaviour of biomarkers in patients with moderate to severe COVID-19.

Methods: The disease severity of 100 COVID-19 patients was classified into moderate, and severe, and the behaviour of laboratory biomarkers was examined across these moderate and sever conditions.

Results: The median age and male/female ratio increased with severity. Underlying diseases, which were not observed in 45% of mild stage patients, increased with severity. A ROCH analysis showed that C-reactive protein (CRP), haemoglobin (Hb) A1c, and lactate dehydrogenase (LDH) levels were significantly useful for the differential diagnosis of mild/moderate I stage and moderate II/severe stage. In the severe stage, Hb levels, coagulation time were significantly different on the day of worsening from those observed on the day of admission. The frequency of haemostatic biomarker abnormalities was high in the severe disease stage.

Conclusion: The evaluation of severity is valuable, as the mortality rate was high in the moderate II and severe stages. The levels of CRP, and LDH were useful markers of severity, and haemostatic abnormalities were frequently observed in patients in the severe disease stage.

Keywords: COVID-19; biomarkers; moderate; severe

Introduction

Since the first outbreak of coronavirus disease 2019 (COVID-19) in China [1,2], COVID-19 infections have spread worldwide, generating a pandemic [3]. The mortality rate of COVID-19 is approximately 2%, with 5–10% of patients developing severe and life-threating disease [3]. COVID-19 infection predominantly displays hyperinflammation and immune dysregulation, which induce multiorgan damage. The primary cause of mortality due to COVID-19 is severe acute respiratory distress caused by epithelial infection and alveolar macrophage activation in the lungs [4]. Therefore, immune modulation and suppression therapy may prevent the deterioration of the condition of COVID-19 patients [5]. Some COVID-19 patients develop severe disease or die [6],

while many more develop mild or moderate disease [7]. The risk factors for severe disease are reported to include an age 65 years [8], malignant tumor [9], chronic obstructive pulmonary disease [10], chronic renal disease [11], diabetes mellitus [12], hypertension [13], hyperlipidemia [8], obesity [14], smoking [13], and immunodeficiency after transplantation [15]. Biomarkers including white blood cell (WBC) count, lymphocyte count, platelet count; albumin, ALT, lactate dehydrogenase (LDH), D-dimer, ferritin, interleukin-6, and procalcitonin (PCT) levels; and prothrombin time (PT) have been reported as specific biomarkers of severity [16].

The real challenge for the clinicians is to quickly identify CoVID-19 patients at high risk for ARDS. Old age, comorbidities (hypertension, diabetes), lymphocytopenia, elevated inflammatory indices (C-reactive protein, serum ferritin, erythrocyte sedimentation), and organ dysfunction (aspartate aminotransferase, creatinine, lactate dehydrogenase) are risk factors for ARDS in CoVID-19 patients [17], [18]. Unfortunately, the pathogenesis of CoVID-19 has not been completely understood. Certainly, inflammatory cytokine storm and viral evasion of cellular immune responses play a central role in disease progression and severity [19]. Many laboratory abnormalities have been described to be associated to an adverse outcome in COVID-19 patients [20]. In a meta-analysis by Henry et al., biomarkers of inflammation, cardiac and muscle injury, liver and kidney function and coagulation measures were significantly elevated in patients with both severe and fatal COVID-19, in particular Interleukin (IL) -6, IL-10 and serum ferritin were strong discriminators for severe disease [21].

In our current study, specific biomarkers of severity and haemostatic abnormalities were examined in 100 patients of COVID-19 in Iraq.

Materials and Methods

One hundred patients with COVID-19 infection (median age, 45 years; 33–75th percentile, 38.0–70.0 years) were admitted to DAR AL-SALAM for COVID-19 isolation in Baghdad/ Iraq from 1 August 2020 to 30 December 2021.

The severity of COVID-19 was evaluated based on the Iraqi and WHO Medical Care Guidelines [15]. There were some of patients with moderate disease

(pneumonia with oxygen therapy without mechanical ventilation), while the other patients were suffering with severe disease (pneumonia with mechanical ventilation).

5 ml of blood was drawn from each patient. It was divided into two types of gel tubes for separating the serum purpose, and the other part was placed in an EDTA tube for complete blood count purpose.

All analysis were done by Cobas 8000 e602 (Roche Diagnostics K.K., Tokyo, Japan). The WBC count, haemoglobin (Hb), total neutrophil count, and total lymphocyte count were measured using a fully automatic blood cell counter XN-3000 (Sysmex Co., Kobe, Japan).

Statistically, The data are expressed as the median (25–75th percentiles). The difference in the frequency was analysed by the chi-squared test. The significance of differences between groups was examined using the Mann-Whitney U test. p-Values < 0.05 were considered to indicate statistical significance. Cut-off values were determined by a receiver operating characteristic (ROC) analysis. A multivariate analysis with a stepwise regression test was performed. All statistical analyses were performed using Stat-Flex software (version 6; Artec Co. Ltd., Osaka, Japan).

Results

The median age and male/female ratio increased with severity (Table 1). The mortality rate was 12.5% in both the moderate II and severe stages. Comorbidity, which was not observed in 45% of mild stage patients, increased with severity (Table 1).

Table 1. Relationship between age

Stage	Moderate/ n=100	Sever/ n=100
Number	52	48
Age, years	66.5 ***	50.5 *
(25–75th percentile)	(29.3–57.8)	(38.0–64.0)

Hypertension, hyperlipidaemia, or diabetes mellitus were observed 40% of patients with moderate or severe disease. Heart failure, other pneumonia

complications, and cerebrovascular accident were frequently observed among the patients who died within 45 days.

Regarding the laboratory data (Table 2), the neutrophil, lymphocyte, and platelet counts levels in the moderate and severe stages were significantly different from those in the mild stages. The CRP levels in the moderate and severe stages were significantly higher than the mild disease stages. In the moderate and severe stages the levels of LDH, and hemoglobin A1C levels were significantly higher in comparison with the mild disease stages. * $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ COVID-19 in these patients, CT indicated other pneumonia, or their culture showed a bacterial or fungal infection

Table 2. Laboratory data on the day of admission

	Moderate n=100	Sever n=100
WBC ($X10^9$ /L)	5.7 (4.0–7.6)	5.5 (4.0–6.7)
Neutrophil ($X10^9$ /L)	4.4 ** (2.2–5.9	3.2 (1.9–4.1)
Lymphocyte ($X10^9$ /L)	9.8 ** (0.6–1.3)	1.3 (1.0–1.7)
Haemoglobin (g/dL)	13.4 (12.4–14.9)	14.3 (13.5–15.0)
CRP (mg/mL)	5.59 *** (2.69–8.11)	9.21 ***, (7.53–13.0)
LDH (U/L)	254 ***, (216–333)	428 *** (318–731)
Haemoglobin A1c (%)	6.45 *** (5.90–7.45)	6.70 *** (6.55–8.43)

WBC, white blood cells CRP, C reactive protein; LDH, lactate dehydrogenase; Moderate, pneumonia with oxygen therapy; Severe, pneumonia with mechanical ventilation. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Discussion

in our present study, the mortality rate was 0% in the moderate disease stages of COVID-19 infection, 12.5% in severe disease stages, suggesting a mortality rate similar to other reports [3,16]. These findings suggest that four patients without mechanical ventilation died, and the direct cause of death was not COVID-19 in these patients. In addition to the management of severe COVID-19,

the prevention of progression from mild/ moderate to severe disease stages is important. Comorbidity, diabetes mellitus, hypertension, and cerebral vascular accident were related to the disease severity of COVID-19 infection.

The elevation of Hb A1c and onset of hypertension may have occurred before the onset of COVID-19 infection, suggesting the importance of antihypertensive

therapy and glycemic control before the onset of COVID-19 infection. An increased WBC, especially increased neutrophil counts and decreased lymphocyte counts, was useful for predicting the disease severity of COVID-19. A decrease in lymphocytes due to apoptosis of CD4+ lymphocytes is correlated with the elevation of inflammatory cytokines [22]. CRP and ferritin were also useful biomarkers for predicting the disease severity of COVID-19. Ferritin elevation is believed to be caused by the cytokine storm and secondary hemophagocytic lympho histiocytosis [23].

Elevation of CRP and a reduction in lymphocytes may occur in association with a cytokine storm [24].

LDH levels have been reported to be associated with Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA), and computed tomography (CT) semiquantitative rating scores [25], and LDH is suggested to be a useful marker for multiple organ failure. TP and albumin levels, A/G ratio, and eGFR are also related to multiple organ failure. Thus, these biomarkers are associated with organ failure or renal function, indicating a correlation with the disease severity of COVID-19 infection.

There are several limitations associated with the present study. Specifically, this study was observational and thus affected by comorbidities and varying therapies. Several patients died of old age or due to comorbidities rather than due to the severity of their COVID-19 infection. For most patients without severe disease, blood sampling was performed on the day of admission. In addition, the study population was relatively small.

Conclusion

The mortality rate was high in COVID-19 patients with moderate /severe disease. CRP, and LDH levels were useful markers of severity, and hemostatic abnormalities were frequently observed in patients with severe disease.

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