The Role of Serum Cathepsin S as Predictors of COVID-19 severity

Ymam sabah derea[1], Ahmed Sabah Noori[2], Raid J.M. AL-Timimi[3]
[1] Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq
[2] Consultant Hematologist/ Internist / AL- Imamein AL- Kadhimein Medical city/Department of Medecine Hematology / Oncology division
Baghdad, Iraq
[3] Assistant Professor, Ph.D. in Medical Chemistry/Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq
Email: draayamam@gmail.com
Email: ahmed.noori@meciq.edu.iq
Email: dr.altimimi@colmed-alnahrain.edu.iq

Abstract
COVID-19 is a rapidly growing pandemic with its first case identified during December 2019 in Wuhan, Hubei Province, China. Due to the rampant rise in the number of cases in China and globally, WHO declared COVID-19 as a pandemic on 11th March 2020. The disease is transmitted via respiratory droplets of infected patients during coughing or sneezing and affects primarily the lung parenchyma. The spectrum of clinical manifestations can be seen in COVID-19 patients ranging from asymptomatic infections to severe disease resulting in mortality. Although respiratory involvement is most common in COVID-19 patients, the virus can affect other organ systems as well. The systemic inflammation induced by the disease along with multisystem expression of Angiotensin Convertin Enzyme 2 (ACE2), a receptor which allows viral entry into cells, explains the manifestation of extra-pulmonary symptoms affecting the gastrointestinal, cardiovascular, hematological, renal, musculoskeletal, and endocrine system. To date, many biomarkers reflecting the main pathophysiological characteristics of the disease have been identified and associated with the risk of developing severe disease. Proteolytic enzymes, or proteases, are known to play important roles in the maintenance of pulmonary homeostasis. However, during disease, proteolytic activity can become dysregulated and cause damage to the lung, contributing to the pathology of conditions like cystic fibrosis, chronic obstructive pulmonary disease, asthma, pulmonary fibrosis and ARDS. we first evaluated the status of CTSS in the context of ARDS and models of ARDS. These investigations revealed that CTSS levels and activity were elevated in the lungs of patients with ARDS, and that elevated CTSS activity was also detectable in the plasma of these patients. Altogether, these findings support a role for CTSS in the pathogenesis of ARDS and the fact that Corona virus infects the respiratory system and the severity of the infection increases with the increase in the severity of the inflammation.
Keywords: Cathepsin S, COVID-19, severity

1. Introduction

The infection caused by the new severe acute respiratory syndrome coronavirus (SARS-CoV-2), associated to Coronavirus disease-2019 (COVID-19), characterized by severe pneumonia in a variable proportion of cases, was first reported in the city of Wuhan China, in December 2019 and then spread across continents. [1-3] Although most patients experience mild to moderate disease, 5 to 10% progress to critical pneumonia and acute respiratory failure [4]. The high morbidity and mortality of COVID-19 is associated with dysregulated immune responses as evidenced by the presence of high levels of inflammatory markers including C-reactive protein, inflammatory cytokines and chemokines, and Prostaglandin E2 (PGE2) in the circulation [5-7]. The hyperactive immunopathology is postulated as a major cause of morbidity and mortality in COVID19.

Cathepsin S is a member of the cysteine cathepsin protease family. CTSS is one of 15 cathepsin proteases encoded in the human genome that partake in various cellular processes. CTSS is one of 11 cysteine cathepsin proteases, which is the largest cathepsin subclass. Cathepsins B, C, F, H, L, O, and X are expressed ubiquitously in human tissues and cell. CTSS is mainly found inside the lysosomal/endosomal compartments of antigenpresenting cells, such as B cells, macrophages, dendritic cells, but is also produced by epithelial cells, smooth muscle cells, endothelial cells, and neutrophils [8] It is a lysosomal protease which can promote degradation of damaged or unwanted proteins in the endo-lysosomal pathway. Additionally, it has more specific roles such as MHC class II antigen presentation, where it is important in the degradation of the invariant chain. Unsurprisingly, mis-regulation has implicated cathepsin S in a variety of pathological processes including arthritis, cancer, and cardiovascular disease, where it becomes

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secreted and can act on extracellular substrates. In comparison to many other cysteine cathepsin family members, cathepsin S has uniquely restricted tissue expression and is more stable at a neutral pH, which supports its involvement and importance in localised disease microenvironments. [9]

2. Patients and Methods

This is a cross sectional study that was conducted in Department of Chemistry and Biochemistry at the College of Medicine/Al-Nahrain University and the outpatient clinic of internal medicine at Al Immamain Alkadhmain medical city during the period between February to November N 2022. The study protocol was approved by the Ethical Committee of College of Medicine\ Al-Nahrain University.

The participants included (60 patients) Severe_ critical patients who required hospital admission and (60 patients) mild to moderate infection with no need for hospital aged 18–75 years. Each participant was subjected to a physical examination. All individuals had their height and weight measured. The body mass index (BMI) was calculated in kilograms per square meter. Approximately 7mL of venous blood was withdrawn from all participants and divided into two tubes, a serum separated tube (Gel Tube) for Serum Cathepsin S, Ferritin, CRP and Serum LDH measurement, and sodium citrate tube for D-dimer measurement.

3. Results

Demographic characteristics of the study population

One hundred and twenty patients with COVID-19 infection were separated into two groups: those who severe disease, which included 60 patients aged 53.3±17.71 years. The second subgroup consists of 60 individuals who have mild to moderate COVID-19 infection are 44.95±17.23 years old, with statistically significant difference between the two subgroups.

Table 1. Demographic features of the study population.

Variable	Severe COVID-19 (n=60)	Mild – Moderate COVID-19 (n=60)	p-value
Age, years *Mean±SD* *Range*	 53.30±17.71 18-81	 44.95±17.23 14-74	0.010
Gender *Male* *Female*	 41(68.33%) 19(31.67%)	 42(70%) 18(30%)	0.843
BMI, Kg/m^2 *Mean±SD* *Range*	 25.27±4.18 18-35	 25.48±3.79 19-40	0.777
Comorbidities* *Diabetes mellitus* *Hypertension*	 20(33.33%) 16(26.67%)	 11(18.33%) 6(10%)	 0.061 0.018
* patients may have more than one disease; BMI = body mass index			

Inflammatory and Hypercoagulability Markers

Data regarding inflammatory and hypercoagulability markers were found to be non-normally distributed. Accordingly, they were expressed as median, and analyzed with non-parametric Mann Whiney U test. The median level of ferritin, D-dimer CRP and LDH in severe cases was 595.5ng/mL, 3774.5 ng/ml, 36 mg/L and 506.4 U/L, respectively which was higher than that of mild-moderate cases (309.0 ng/ml, 514.67 ng/m, 12.05 mg/L and 319.6 U/L, respectively) with highly significant differences (Table 2).

Table 2. Inflammatory and hypercoagulability markers

Variable	Severe COVID-19 (n=60)	Mild – Moderate COVID-19 (n=60)	p-value
Ferritin, ng/mL *Mean±SD* *Median* *Range*	749.47±514.55 595.5 360-3600	310.04±149.36 309.0 60-672.24	<0.001
D-dimer, ng/mL *Mean±SD* *Median* *Range*	3909.12±3006.17 3774.5 365-9872	714.91±758.44 514.67 310-4500	<0.001
CRP, mg/L *Mean±SD* *Median* *Range*	40.99±17.14 36 12.1-89.3	11.99±8.42 12.05 0.6-36	<0.001
LDH, UL *Mean±SD* *Median* *Range*	525.45±111.85 506.4 310-780.3	328.08±72.31 319.6 159-466.9	<0.001

Serum Level of Cathepsin S

The mean cathepsin-S level was significantly elevated in severe cases relative to the mild to moderate cases (5.0±1.45ng/mL vs 2.37±0.54ng/mL) as indicated in Table 3, figures 1.

Table 3: Serum level of cathepsin S			
Markers	Severe COVID-19 (n=60)	Mild – Moderate COVID-19 (n=60)	p-value
Cathepsin-S, ng/mL *Mean±SD* *Range*	 5.0±1.45 3.21-9.17	 2.37±0.54 0.78-3.39	 <0.001

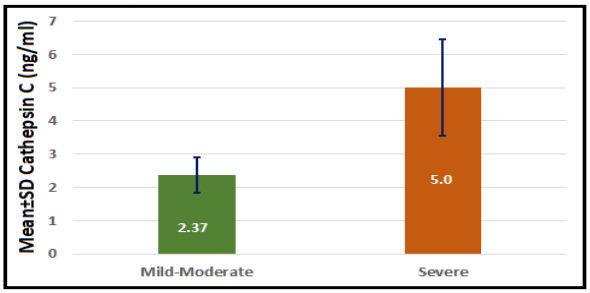

Figure 1: Mean serum level of cathepsin S in mild-moderate and severe cases of COVID-19

Diagnostic Value of Cathepsin-S in Detection of Severe COVID-19.

To assess the diagnostic value of Cathepsin-S, the receiver operating characteristic (ROC) curve was used. The AUC for cathepsin-S level was 0.999, 95% CI= 0.996-1.0, p <0.001. The test's sensitivity and specificity were 98% and 98%, respectively, at a cut-off value of cathepsin-S level = 3.28 ng/ml (Figure 2).

Table 3: diagnostic value of Cathepsin S in the context of discrimination between severe and mild to moderate cases of COVID-19.				
Markers	AUC	Sensitivity	Specificity	Cut off
Cathepsin S	0.999	98%	98%	3.28 ng/ml

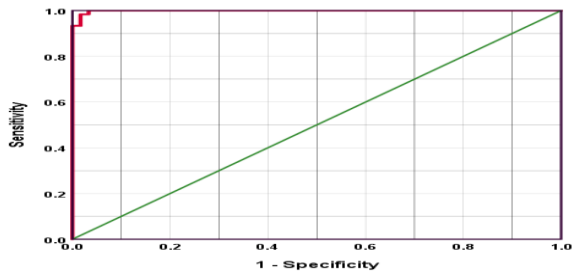

Figure 2: ROC curve for Cathepsin S in the context of discrimination between severe and mild to moderate cases of COVID-19.

Correlation of Cathepsin-S with age and laboratooory markers.

The age show no correlation with Cathepsin-S level. On the contrary, Likewise, Cathepsin-S level show positive correlaton with ferritin (r = 0.599, p<0.001), D-dimer (r = 0.556, p<0.001), CRP (r = 0.675, p<0.001), and LDH (r = 0.710, p<0.001) as shown in Table 4 and Figures 3 to 6.

Table 4: Correlation of Cathepsin-S with age and inflammatory markersis severe COVID-19.		
Variable	Cathepsin-S	
	r	*p*-value
Age	0.072	0.435
Ferritin	0.599	<0.001
D-dimer	0.556	<0.001
CRP	0.675	<0.001
LDH	0.710	<0.001
CRP= C-reactive protein, LDH = Lactate dehydrogenase		

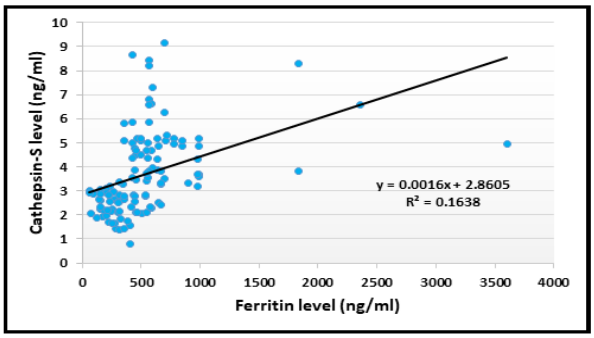

Figure 3:Scatter plot and regression line between ferritin and cathepsin S in patients with COVID-19.

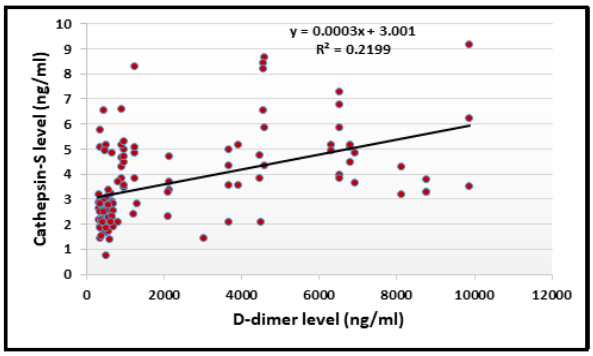

Figure 4: Scatter plot and regression line between D-dimer and cathepsin S in patients with COVID-19.

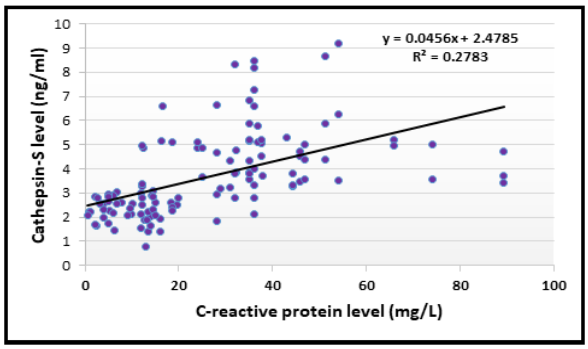

Figure 5: Scatter plot and regression line between CRP and cathepsin S in patients with COVID-19.

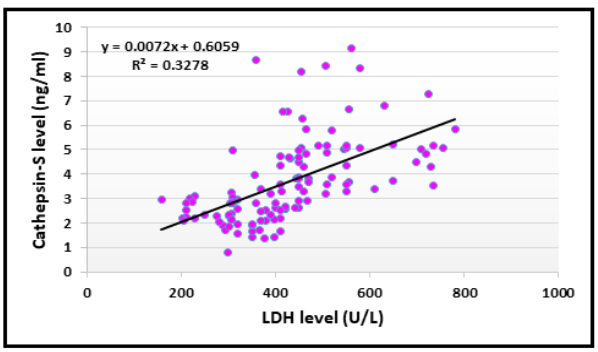

Figure 6: Scatter plot and regression line between LDH and cathepsin S in patients with COVID-19.

Association of Cathepsin-S with gender and comorbid diseases

No association was demonstrated between

cathepsin-S and gender or comorbid diseases (diabetes mellitus and hypertension) as indicated in Table 5.

Table 5: Association of Cathepsin-S with gender and comorbid diseases.

Variables	Cathepsin S, ng/mL
Gender Males Females	 3.75±0.2 3.54±0.24
p-value	0.523
Diabetes mellitus No Yes	 3.68±0.21 3.69±0.2
p-value	0.993
Hypertension No Yes	 3.8±0.22 3.66±0.19
p-value	0.726

4. Discussions

The effect of middle-aged and elderly patients infected with COVID-19 has been observed with more severe symptoms than younger-aged patients in many studies including this study. In this study there was significant difference in age between the two groups (severe groups 53.3±17.71 more than mild groups 44.95±17.23). This study agreement with study done by (Abbas et al.)[10], they found the severity of diseases increased by increasing age.

In this study there was **no significant difference** in gender between the two groups, were identical in the gender distribution (those who severe disease, which 68.33% and 70%, respectively).There was significant difference between the two groups in terms of comorbid hypertension (p = 0.018) this agreement with study done by (Al-Omari et al., 2020)[11] The reason for this is unclear as history of angiotensin-converting enzyme inhibitor or angiotensin II receptor blockers use has exceptionally low evidence in predicting outcomes in COVID-19.

In this study the median level of ferritin, D-dimer CRP and LDH in severe cases was 595.5ng/mL, 3774.5 ng/ml, 36 mg/L and 506.4 U/L, respectively which was higher than that of mild-moderate cases (309.0 ng/ml, 514.67 ng/m, 12.05 mg/L and 319.6 U/L, respectively) with highly significant differences as shown in (Table 2).This study agreement with study done by (Kumar et al., 2022) [12] Hyperferritinemia can serve as predictor of severe and fatal Covid-19 and extent of cytokine strom and its risk factor of poor prognosis in patients with Covid19. Elevated levels of LDH activity served as a predictor for Covid-19 and its activity was increased during acute lung damage. (Tan et al., 2020) [13] and other studies concluded that CRP was associated with disease progression and predicted early severe Covid-19. Higher CRP levels are also linked to development of acute respiratory distress syndrome, higher troponin-T levels, and myocardial injury, which is observed in patients with severe Covid-19. The blood of the patients with COVID-19 appeared to be in a hyper- coagulable state, which resulted in the consumption of coagulation factors, and likely promoted the development of disseminated intravascular coagulation (DIC), resulting in the COVID-19 endpoint events (Han and Yang, 2020, Wu et al., 2020) [14].

In this study was found Cathepsin-S were observably higher in severe COVID-19 patients than in mild COVID-19 patients. These results correspond to a study conducted by the researcher (McKelvey et al., 2019)[15] in patients suffering from acute pneumonia, and it was found that cathepsin-S acts as a mediator of acute pneumonia syndrome and increases with an increase in the severity of the inflammation, and the fact that Corona virus infects the respiratory system and the severity of the infection increases with the increase in the severity of the inflammation. This result makes this study compatible with the researcher (McKelvey et al., 2019) [15].

The positive correlation of D-dimer, LDH, CRP and Ferritin levels with Cathepsin-S with the severity of COVID-19 disease in this study are in agreement with many studies that show the positive association of these parameters with the severity of the disease. Many studies have found that laboratory markers at admission associated with mortality risk in COVID-19 patients with comorbidities. These findings strengthen the understanding of thromboembolism and tissue injury and may help in better management of thromboembolic complications in COVID-19 patients (Gupta et al., 2022) [16].

In this study we found no association was demonstrated between cathepsin-S and gender or comorbid diseases (diabetes mellitus and hypertension) as indicated in Table 5. That disagreement with study done by (Tejera-Segura et al., 2016)[17] when he found cathepsin S was significantly associated with hypertension and diabetes. Cathepsin S was inversely related with male gender.

5. Conclusions

We revealed that baseline serum cathepsin S might serve as a potential inflammatory biomarker, independently predicting poor outcomes for SARS-CoV-2 infection. cathepsin S may thus be of clinical importance in the prognosis for adult patients with COVID-19.

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