Role of Calprotectin and Cystatin C Levels as in Covid-19 Complication Pathogenesis of Iraqi Pandemic

Hawraa Fadhil Abbas¹, Fadhil Jawad Al-Tu'ma¹* and Riyadh Mohei Al-Saiegh²

¹Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala / Kerbala – Iraq
²Section of Nephrology, Department of Internal Medicine, College of Medicine, University of Kerbala / Kerbala – Iraq.

E. mail: f_altoma_56@yahoo.com

Abstract:

Background: Coronavirus disease (Covid-19) causes severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2) was spread to hundreds of countries and it is served as global pandemic. Death occurs as a result of severe Covid-19 due to increasing hypoxemia, acute respiratory distress syndrome (ARDS), and multi-organ failure. Human pathogenic of Covid-19 occurred as a result that coronaviruses (SARS-CoV and SARS-CoV-2) connect to their target cells via angiotensin-converting enzyme 2 (ACE2), which is synthesized by epithelial cells of the lung, kidney, gut and blood vessels. Covid-19 entry to cells of kidney is much higher than lungs by ACE II. Measurement of serum cystatin C levels is play greater role in the estimation of kidney function. Calprotectin is a member of the calcium-binding S100 protein family and it is a heterodimeric protein, found in the cytoplasm of neutrophils and monocytes, both of which play important roles in the inflammatory response in the human body. Calprotectin used an early biomarker in coronavirus disease with bacterial co-infections and patients at risk to develop severe events.

Objectives: The presented work aimed to study the role of calprotectin and cystatin C in pathogenesis of the severe complications associated with Covid-19 and their association with other diagnostic biomarkers.

Materials and Methods: This cross-sectional study included 91 samples patients infected with Covid-19 and they divided into two group, 46 patients of them with severe Covid-19 and remaining 45 patients of them infected with moderate infected Covid-19 include (56 male, 35 female) with a match age ranged between (22-88) years. The study was conducted from Al-hayat unit, Al-Hussein Teaching Hospital, Al-Hussein Medical
City, Kerbala Health Directorate / Kerbala - Iraq during Oct., 2020 to July, 2021). The bio-markers determined include: Ferritin, C-reactive protein (CRP), total lactate dehydrogenase (LDH) activity, creatinine, urea, cystatin C (Cys C), calprotectin (CLP), and complete blood count test (CBC) and they were determined by various biochemical techniques.

Results: According to the presented results there is a significant changes obtained for ferritin, LDH and RDW-CV levels in severe Covid-19 when they compared with moderate Covid-19 group (p<0.05) while CLP, Cys C and the other parameter show a non-significant elevations when compared with moderate Covid-19 group.

Conclusion: The obtained results illustrated that CLP, Cys C were non-significantly increase in severe Covid-19 patients groups, but ferritin, LDH and RDW-CV levels were changes and significantly increased when with moderate group and they are served as predictor parameter and increased mortality risk for Covid-19 patients.

Keywords: Calprotectin, Cystatin C, Severity, Covid-19, Pandemic, Pathogenesis

Introduction:

Coronavirus is one of the major viruses which primarily affecting the respiratory system in human (1). However, coronaviruses have been also diagnosed in animals and can cause a range of severe diseases such as gastroenteritis and pneumonia (2). Previous coronavirus outbreaks have been reported, including severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), which is described as a significant public health threat (3). Clinical features of Covid-19 include dry cough, fever, diarrhea, vomiting, and myalgia. Individuals with multiple comorbidities are prone to severe infection and may also present with acute kidney injury (AKI) and features of ARDS (4). Moreover, circulating mediators could interact with kidney-resident cells resulting in endothelial dysfunction, microcirculatory derangement, and tubular injury (5).

Calprotectin (CLP) is a heterodimeric complex formed by two binding proteins of the calcium ion, which belong to the S-100 protein family, S100A8 and S100A9, having both anti-inflammatory and anti-bacterial properties. The first applied name to CLP was
major leukocyte protein L1 or 27E10 (6). CLP represents almost two-thirds of the soluble cytosolic protein content of neutrophils and may also be detected at various levels in monocytes, macrophages, epithelial cells, and platelets (7). Calprotectin has microbicidal, cytotoxic functions via heavy-metal detoxification (8). The pro-inflammatory mediator calprotectin (S100A8/A9, MRP 8/14) is reported to be an early signal, mediating the cytokine storm associated with an increased severity of Covid-19 (9). Previous studies have reported significantly elevated levels of calprotectin in patients with severe Covid-19 and the possible ability of calprotectin to discriminate between mild and severe form of the disease (10).

There are three distinct types of cystatins that share sequence and structure homology but differ in size, site of action, and disulfide bond topology. Human cystatin C (hCC) belongs to the type 2 cystatins that are generally secreted as extracellular polypeptides. In terms of biological function, hCC is a target of proteolysis, and primarily functions as a protease inhibitor. It is degraded by cathepsin D and elastase (11,12). Renal tubular epithelial cells do not secrete sCys C into the lumen, therefore its serum concentration is mainly determined by glomerular filtration rate which is an important indicator of glomerular filtration (13). Several studies reported kidneys alterations, as reflected by increased serum creatinine, in Covid-19 patients. SARS-CoV-2 could directly infect kidney tubular cells, which express the ACE-2 receptor on their cellular surface (5).

**Materials and Methods:**

This cross-sectional study included 91 samples (56 male, 35 female) infected with Covid-19 divided into two group according to their clinical and biochemical investigations, 46 patients of them were infected with severe Covid-19 and the other 45 patients with moderate Covid-19 with match age ranged between (22-88) years. The patients were admitted Al-hayat unit, Al-Hussein Teaching Hospital, Al-Hussein
Medical City, Kerbala Health Directorate / Kerbala - Iraq during Oct., 2020 to July, 2021). The study proposal was approved by local medical ethics and all participants, information consent before the onset of study. The patients were registered and handed over a file for recording their data such as name, age, gender, weight, height, smoking states, current chronic diseases (diabetes mellitus, hypertension). Serum calprotectin, cystatin C, C- reactive protein, ferritin, creatnine, urea concentration was investigated by ELISA, chemical and spectrophotometric procedure. The complete blood counts were investigated by XP-300™ automated hematology analyzer Sysmex.

The study excluded patients with any chronic or immune diseases like diabetes mellitus, infection and inflammation, and receiving long term oral corticosteroid, anti-IL-6 or anti-TNF therapy and patients had history of vacuities connective tissue disease.

**Results:**

The clinical demographic characteristics and laboratory parameters of both patients groups of Covid-19 were summarized in Table (1).

**Table (1): Demographic characteristics of Covid-19 patients in severe and moderate groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Covid-19 Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>56.82 ± 12.574</td>
</tr>
<tr>
<td>Gender (Male / Female)</td>
<td>(28/17)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
</tr>
<tr>
<td>Smoking (Yes / No)</td>
<td>(6/39)</td>
</tr>
<tr>
<td>DM (Yes / No)</td>
<td>(17/28)</td>
</tr>
<tr>
<td>HT (Yes / No)</td>
<td>(19/26)</td>
</tr>
<tr>
<td>Sat. O₂</td>
<td>91.87 ± 3.181</td>
</tr>
</tbody>
</table>
This table illustrated the mean ± SD of participants age which was within the age group of (22–88) years old that is higher in severe than in moderate. Gender distribution among the studied groups was: 62% male, 38% female for patients group. The diabetes mellitus, hypertension and oxygen saturation were higher in severe than moderate. The patient’s group was divided into (moderate and severe) based on severity of disease based on WHO guideline. The data was collected through self-reported technique (student questionnaire).

Table (2) showed the biochemical changes in the concentrations of different biomarkers in both severe and moderate groups. There were significant increments in levels of Ferritin, LDH and RDW-CV p (0.02) respectively. There was a non-significant level in CLP, Cys, C and others biomarkers.

**Table (2): Change in biochemical parameters between severe and moderate Covid-19 groups**

<table>
<thead>
<tr>
<th>Variable with normal range</th>
<th>Moderate Covid-19 Cases Mean ± SD</th>
<th>Severe Covid-19 Cases Mean ± SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, (0-6 mg/l)</td>
<td>85.58 ±122.14</td>
<td>63.65 ± 59.89</td>
<td>0.27</td>
</tr>
<tr>
<td>Ferritin, (20-350 ng/ml)</td>
<td>598.63 ±502.53</td>
<td>894.99 ±712.82</td>
<td>0.02</td>
</tr>
<tr>
<td>LDH , (240-480 U/l)</td>
<td>409.31 ±217.30</td>
<td>524.31 ±260.63</td>
<td>0.02</td>
</tr>
<tr>
<td>S. Creatinine, (0.7-1.2 mg/dl)</td>
<td>2.67 ± 10.28</td>
<td>1.11 ± 0.69</td>
<td>0.3</td>
</tr>
<tr>
<td>Blood Urea, (12-45 mg/dl)</td>
<td>57.70 ± 35.45</td>
<td>54.45 ± 35.32</td>
<td>0.6</td>
</tr>
<tr>
<td>Calprotectin, (31.64 – 126.97 ng/ml)</td>
<td>78.24 ±130.38</td>
<td>89.27± 49.90</td>
<td>0.5</td>
</tr>
<tr>
<td>Cystatin C, (0.24 – 0.76 mg/dl)</td>
<td>0.49 ±0.104</td>
<td>0.48 ± 0.103</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table (3) showed the hematological changes of complete blood count in the in both severe and moderate groups of Covid-19. There were significant increments in levels d RDW-CV p (0.02) respectively. There was a non-significant level in others biomarkers.
Table (3): Change in CBC investigations between severe and moderate Covid-19 groups

<table>
<thead>
<tr>
<th>Variable with normal range</th>
<th>Moderate Covid-19 Cases Mean ± SD</th>
<th>Severe Covid-19 Cases Mean ± SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDW-CV (11.5-14.5%)</td>
<td>14.01 ±1.26</td>
<td>14.91 ±1.91</td>
<td>0.02</td>
</tr>
<tr>
<td>WBC 4-11(10^9/L)</td>
<td>14.47 ± 7.75</td>
<td>14.99 ± 5.67</td>
<td>0.7</td>
</tr>
<tr>
<td>NEU% (39.3-73.7 %)</td>
<td>82.51 ± 10.44</td>
<td>84.77 ± 8.68</td>
<td>0.3</td>
</tr>
<tr>
<td>LYM% (18-45.3 %)</td>
<td>10.84 ± 7.75</td>
<td>8.77 ± 7.85</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Discussion:

Most data regarding Covid-19 are from China, and although most confirmed cases have been classified into mild or moderate, 14% is severe and 5% critical. Older age, cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancer were all associated with an increased risk of death (14). The most common comorbidities in one report were hypertension (30%), diabetes (19%), and coronary heart disease (8%) (15). In data of the presented work observed that male are more than female infected with Covid-19 and these data was agreed with Zhao et al. who found cell type-specific expression of the ACE2 receptor in type II alveolar epithelial cells is higher in males than in females (16). In Italy, the reported death rate in men was (16.6%) which is significantly higher than that in women 9.1% (17). Among the death population of Covid-19 cases, the elderly accounts for a large proportion (18). With regard to coronaviruses, in particular, smoking is associated with increased susceptibility and mortality in MERS-CoV infection, potentially due to up-regulation of dipeptidyl peptidase-IV, the host receptor for MERS-CoV, in smokers (19). In addition,
a meta-analysis performed failed to find a relationship between active smoking and severe Covid-19 on Chinese patients, and another meta-analysis indicates that active smoking is not a predisposing factor for hospitalization (20). The ratio of oxygen saturation as measured by pulse oximetry divided by the fraction of inspired oxygen is a simple measure, which has been previously used in the setting of acute respiratory distress syndrome instead of more complex variables (21), and thus can be evaluated in each patient with Covid-19 pneumonia to help identify patients at higher risk of severe disease. A strong association between hypoxemia and worse clinical outcomes has been reported (22).

In our study ferritin is increased in severe than moderate group table (2) and it is significant when compare between them and that is agreement with Qin. et al. reported that Covid-19 patients with high levels of ferritin have greater proportions of severe and deceased cases (P=0.0016) (23). Hyperferritinemia caused by the excessive inflammation due to the infection is associated with the admission to the intensive care unit and high mortality, and represents an indication to recognize high-risk patients to guide the therapeutic intervention to control inflammation (24). Ferritin levels greater than 800 g/l were found in 100% of patients with severe disease and 30% of those with non-severe disease (25). Another meta-analysis also recommended serum ferritin as a candidate variable for risk stratification models that may serve as clinical predictors of severe and fatal Covid-19 (26).

Theoretically, elevated serum LDH activity is an important laboratory indicator elevated in Covid-19 infection (27). This study showed a significant elevation in LDH activity levels in severe and moderate groups and that was agreed with Zhou et al. which showed that the levels of serum ferritin, D-dimer, lactate dehydrogenase, and interleukin-6 (IL-6) are increased during the worsening of the disease, providing an indication of the risk of mortality (14). It has been reported that elevated serum LDH activity levels are associated with poor prognosis in various diseases, especially in
tumors and inflammation (28). To date, studies have shown that patients with severe Covid-19 have elevated serum LDH levels (29), but no study has specifically evaluated its effect on the severity and mortality of Covid-19.

Tan et al. and other studies concluded that CRP was associated with disease progression and predicted early severe Covid-19 (30). 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 (31).

In present study there is a non-significant elevation when measure the kidney function biomarkers such as serum (creatinine, urea, Cystatin C and calprotectin). In support of these characteristics, cystatin C has been shown to have superior sensitivity to changes in borderline renal function, and to rise earlier than creatinine in different patient groups, e.g., transplant, surgical, cardiovascular, and diabetic (32), while higher serum concentrations of cystatin C are likely to reflect the presence of kidney dysfunction such as in acute kidney injury associated with Covid-19 infection, they might also be a marker of the excessive systemic inflammatory and pro-oxidant state that characterizes this group (33).

Calprotectin is a protein that is especially secreted by neutrophils secondary to inflammation. It has recently been reported that calprotectin can be used as a biomarker of inflammation for assessing the activity of some inflammatory disorders (34). However, there are few published studies regarding the relationship between serum calprotectin and Covid-19 severity. In addition, according to that study, a significant elevation of serum calprotectin was associated with high mortality in Covid-19 patients (35). In their study with 94 Covid-19 patients, Shi et al. reported that the serum calprotectin levels of patients requiring mechanical ventilation were higher than those who did not need intubation (36). Studies are ongoing, but there is not enough evidence at this time to support this finding and no scientific consensus whether or not calprotectin can serve as a prediction of how serious the virus will be in some patients. Researchers will continue studying calprotectin in Covid-19 patients, but now,
calprotectin is still used primarily as a way for physician to observe if there is an inflammation in their intestines (37). Causes of false positive calprotectin have been listed as infections, malignancy, drugs such as non-steroidal anti-inflammatory diseases, food allergy, coeliac and cirrhosis. However, Crohn’s disease can affect any part of the GI tract and up to 30% of patients may have disease confined to the small bowel. Previous estimates of false positivity from a meta-analysis were 9% (38,39).

It has been previously reported that the WBCs count increased with the severity of the Covid-19 disease (40). Patients with severe and fatal disease had significantly higher WBC count and lower lymphocyte and platelet counts compared with non-severe disease or survivors (26). The presented study found that neutrophil in severe group is less and caused neutrophilia which agreed with the study performed by Zhang et al. which indicate that among 82 dead Covid-19 patients also revealed that 74.3% of them had neutrophilia on admission and this increased to 100% in 24 hours before death (41). Neutrophil count, increased NLR, and thrombocytopenia, were the most common findings observed and positively correlated with disease severity (42). The presented study revealed that mean of RDW-CV in severe cases (14.91%) and is higher than that found in moderate Covid-19 (14.01%) and have significant increase when compare between it (0.02). A higher RDW level indicates abnormal variation in individual red blood cell size, termed anisocytosis, when observed in a blood smear microscopically. An elevated RDW implicates an increased rate of red blood cell (RBC) destruction, dysfunctional erythropoiesis and or shortened RBC lifespan (43). Although RDW appears to be non-specific parameter of the illness that provides general quantitative risk estimation, when used in conjunction with other parameters (MNR, NLR, PLT) to distinguish mortality of Covid-19 patients, it may be particularly useful for Covid-19 prognosis (43). Finally, since an increased RDW value has been shown to significantly predict patient mortality after ICU discharge (44), and the medium- and long-term clinical consequences of Covid-19 patients recovering after severe illness are still
largely unknown (45), urgent studies should be planned to identify whether RDW may also be useful for predicting the post-recovery course of this disease.

**Conclusion:**

There is a non-significant increase in CLP, Cys C and cannot serve as indicator biomarkers that plays a role in disease pathogenesis. Significant increase of RDW-CV enables it to be useful for Covid-19 prognosis and increased mortality risk in Covid-19 patients. Ferritin, LDH and RDW-CV elevated in severe compared to moderate cases in Covid-19 patients. 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 Covid-19. Elevated levels of LDH activity served as a predictor for Covid-19 and its activity was increased during acute lung damage.

**Acknowledgments:**

All authors would like to thank the participated patients and the team of Covid-19 centers for their support during this study.

**References**


19. Seys, Leen JM, et al. DPP4, the Middle East respiratory syndrome coronavirus receptor, is upregulated in lungs of smokers and chronic obstructive pulmonary disease patients. Clinical Infectious Diseases, 2018, 66.1: 45-53.


38. Tharian, Benjamin; Caddy, Grant; Tham, Tony CK. Enteroscopy in small bowel Crohn’s disease: a review. World journal of gastrointestinal endoscopy, 2013, 5.10: 476.


