ORIGINAL ARTICLE

CORRELATION BETWEEN HEPCIDIN AND PROCALCITONIN AND THEIR DIAGNOSTIC ROLE IN PATIENTS WITH COVID-19

Ghusoon AL-Janabi[1], Ali Al-Fahham[2], Alyaa Neamah Najm Alsaedi[3], Ali Yas Khudhair Al-Amery[4]
[1]CLINICAL LABORATORIES DEPARTMENT, APPLIED MEDICAL SCIENCES COLLEGE, KERBALA UNIVERSITY, KERBALA, IRAQ
[2]FACULTY OF NURSING, UNIVERSITY OF KUFA, KUFA, IRAQ
[3]CENTRAL LIBRARY DEPARTMENT, KERBALA UNIVERSITY, KERBALA, IRAQ
[4]DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY, FACULTY OF MEDICAL TECHNOLOGY, ISLAMIC UNIVERSITY, NAJAF, IRAQ

ABSTRACT
The aim: The purpose of this study is to find out the association between procalcitonin and hepcidin in patients with COVID-19, in addition to their role as diagnostic markers.
Material and methods: A total of 75 patients infected with coronavirus were included in the current study, their age is ranging between 20 to 78 years. Those patients was hospitalized in Al-Sadr Teaching Hospital in Najaf, in Iraq. This study also included 50 healthy subjects which are volunteers and considered as a (control group). Biomarker (procalcitonin and hepcidin) measurements were achieved by electrochemiluminescent immunoassay (ECLIA) in the Elecsys immunoassay system.
Results: The present study showed a significant increase the serum cencentration of hepcidin and procalcitonin in patients with COVID-19 as compared tohealthy subjects. There was a highly significant increasing(p < 0.01) in hepcidin and PCT level in patients with severe infection comparing to other catgaries.The current study also revealed that the sensitivity values of the markers were: 0.88%, 0.85 for procalcitonin and hepcidin respectively, which indicate high diagnostic power.
Conclusion: Serum levels of hepcidin and procalcitonin are increased as inflammatory markers in COVID-19 patients with relatively high sensitivity. It seems that these imflammatory markers obviously elevate in the severe cases COVID-19dusease.

KEY WORDS: Covid-19, hepcidin, procalcitonin, imflammatory markers

INTRODUCTION

The novel coronavirus called "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) was appeared in the end of 2019, when it was identified in China [1]. The pandemic status of COVID-19 then distributed extensively overall the world [2]. Both versions of this virus get in to the host cell through their common receptor [3]. The clinical signs and symptoms of coronavirus disease very greatly, including severe, moderate and mild forms. Therefore, the treatment of patients is corresponding to the severity of the clinical maninfestation. Of this clinical biomarker, serum procalcitonin (PCT) has been considered as a significant marker that increase significantly in patients with severe COVID–19 [4, 5]. This disease is not only restricted to the lungs, but the transport of the virus into the bloodstream has resulted in the invasion of almost all the body systems, including the liver, heart, kidney, brain, skin, eyes and intestine [6]. PCT is a protein that composed of 116 aminoacids and is the calcitonin peptide precursor, it

is present in small concentrations in the blood stream (≤0.1 ng/mL) and acts as an proinflammatory marker of bacterial and viral infections. It was observed that serum procalcitonin concentrations to be very elevated (6–53 ng/mL) in clients with strong infections that are caused by bacteria comparing to clients with local mild infections caused by bacteria and viruses (0.1–1.5 ng/mL) [7, 8]. There are increasing evidences that in severly sick patients, there are many markers of strong inflammatory responses, which are composed of high serum levels of procalcitonin (PCT), C-reactive protein (CRP), hyperhepcidinemiaand D-dimer. These results suggested a possible critical role of a cytokine surge in the pathophysiology of COVID-19 [9-12]. PCT is a none of the important inflammatory biomarker, it also plays a crucial role in the development of inflammatory responses. Elevated serum cencentrations of PCT may be due to a cascade of reactions during acute inflammation; PCT concentration ranging between 0.05 and 1.00 ng/mL and used in the identification of chronic

inflammatory with decreased severity [13]"type":"article-journal","volume":"109"},"uris":["http://www.mendeley.com/documents/?uuid=2c7acbbc-ddaa-442e-9bd4-3c1c22015427"]}],"mendeley":{"formattedCitation":"[13]","plainTextFormattedCitation":"[13]","previouslyFormattedCitation":"(Khreiss <i>et al.</i>, 2004. During the clinical manifestation of Covid-19 infection interleukin-6 (IL-6) and PCT increase both drastically [14]. Hyperhepcidinemia is resulted from the strong inflammatory response that is caused by infection, and is correlated with attendence to the COVID-19 isolation unit and high death rate and is considered as an evidence to detect patients with high–risk to direct the therapeutic implementation to manage inflammatory responses [15].

THE AIM

The objective of this study is to find out the association between procalcitonin and hepcidin in COVID-19 patients, in addition to their role as diagnostic markers related to acute inflammatory response.

MATERIAL AND METHODS

STUDY SAMPLE

A total of 75 patients with confirmed diagnosis of COVID-19 have been included in this study, their age is ranging between 20 to 78 years. Those patients was hospitalized in Al-Sadr Teaching Hospital in Najaf, in Iraq. This study also included 50 healthy volunteers used as a control. In the control group, male and female were 28 and 22 respectively, but, in COVID-19 patients, gender distribution was 42 male and 33 female.

MEASUREMENT OF BIOMARKERS (PCT AND HEPCIDIN)

All the patients were classified as "mild", "moderate", and "severe" based on the clinical maninfestation with the assistance of specialized physicians, diagnostic tests, and scanning computed tomography (CT). Moderate and severe cases were identified according to the pneumonia manifestations on CT imaging and had a (%SpO$_2$) of less than 40%. Severe levels were diagnosed as they require mechanical ventilation because of respiratory failure. The healthy voluntreers (control group) consists of 50 healthy people who have not shown previous and signs and symptoms of COVID-19. The study was conducted in the isolation unit at Al-Sadr Teaching Hospital in Najaf in Iraq. Biomarker (procalcitonin and hepcidin) measurements were achieved by electrochemiluminescent immunoassay (ECLIA) in the Elecsys immunoassay system. All biomarker parameters were measured in both patients and the control group (PCT, kit reference range: less than 0.1 ng/mL), (hepcidin, kit reference values: 1.23 – 36.46 ng/mL). An increase in deviation from normal values may be at risk for infection COVID-19. Statistical analysis was done by SPSS program (version 24) in which both descriptive (frequency and percentage) and inferential statistics (independent t-test and
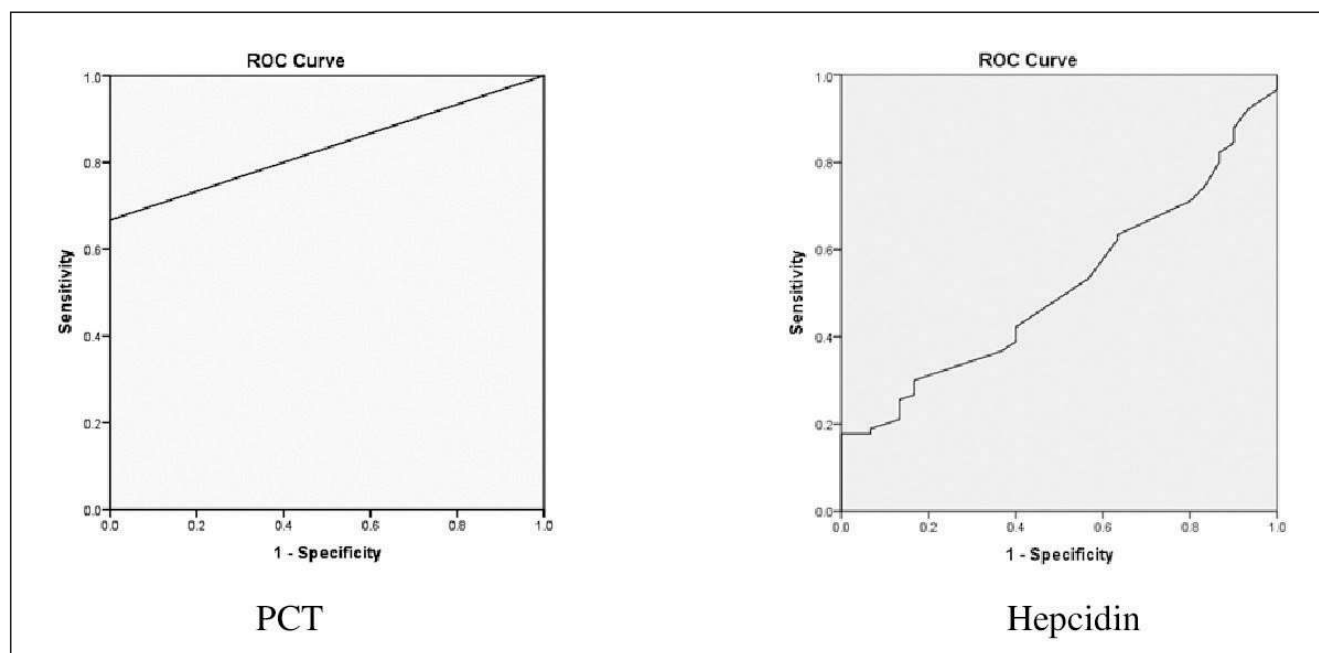
Fig. 1. The areas under the curve (AUC) of the biomarkers for the diagnosis of Covid-19 for PCT (A) and hepcidin (B).

Table I. Differences in procalcitonin and hepcidin between patients with COVID-19 and healthy control subjects.

Statistics	Control group, n = 50	Patients group, n = 75	p
PCT [ng/mL]			
Mean	0.043	1.33	<0.01 HS
SD	0.011	0.34	
Hepcidin [ng/mL]			
Mean	3.23	77.45	<0.01 HS
SD	1.5	6.2	

HS: High Sigmnificant at p<0.01

Table II. ANOVA table for the differences in procalcitonin and hepcidin in patients subgroups classified according to disease severity.

Subgroups	Mild n = 32	Moderate n = 38	Severe n = 24	F Test P value
PCT(ng/L)	1.24±0.12	1.36±0.13	1.56±0.16	14.56 (0.01) HS
Hepcidin	22.87±4.13	67.42±8.13	101.65±12.53	23.55 (0.006) HS

HS: High Sigmnificant at p<0.01

Table III. Differences in procalcitonin and hepcidin between male and female patients.

Biomarkers	Female n = 42	Male n = 33	Independent T Test p-value
PCT(ng/mL)	1.35±0.15	1.39±0.17	1.56 (0.22) NS
Hepcidin(ng/mL)	47.66±6.77	88.69±9.51	9.78 (0.01) HS

NS: Non-Sigmnificant at p>0.05; HS: High Sigmnificant at p<0.01

Table IV. The areas under the curve (AUC) of PCT and hepcidin for the diagnosis of Covid-19.

Characteristic	PCT	Hepcidin
AUC	0.81	0.74
Sig.	0.01	0.05
Cut-off Point	0.043	3.23
Sensitivity [%]	0.88	0.85
Specificity [%]	0.63	0.61
PPV	0.83	0.63
NPV	0.73	0.53

NPV: Negative Predictive Value; PPV: Positive Predictive Value ;

F-test) have been used. Pearson correlation coefficient (r) was utilized to assess correlation between markers. To identify the power of diagnosis of procalcitonin and hepcidin, the estimating of areas under the curve (AUC) of these biomarkers for the diagnosis of Covid-19 has been applied in the current study.

RESULTS

The current study included 75 hospitalized clients with COVID-19, in which 23 cases were mild, 38 cases mod-

erate and 24 cases were severe. They were diagnosed by the doctor based on the patient's condition, computerized tomography scanning the blood test, and the degree saturation of oxygen.

The findings of the current study showed a significant elevation (p <0.01) that was observed in the PCT is levels in patients with COVID-19 (1.33 ng/mL) as compared with control group (0.043 ng/mL) and as it is shown in table I. In this study a significant increase (p <0.01) in serum levels of hepcidin was seen in patients with COVID-19 (77.45 ng/mL) comparing to control group (3.23 ng/mL), as also shown in table I.

The PCT level was significantly increased (p<0.05) in the severe subgroup (0.56±0.16) mg/L as compared with mild and moderate cases (table II.).

The serum hepcidin level was significantly (p<0.05) increased in the male group (88.69±9.51) mg/L as compared to female (47.66±6.77). The was no significant difference in the concentration of PCT between the two genders (table III).

To identify the diagnostic power of procalcitonin and hepcidin, the areas under the curve (AUC) of these biomarkers for the diagnosis of Covid-19 have been shown in table V. This table reveals that the sensitivity values of the markers were: 0.88% and 0.85% for procal-

citonin and hepcidin respectively, which indicate high diagnostic power. However, that the specificity values of the markers were: 0.63% and 0.61% for procalcitonin and hepcidin respectively (Fig. 1A-B).

DISCUSSION

The present study showed a significant elevation of the serum level of hepcidin and procalcitonin in patients with COVID-19 comparing to control group. These results come in agreement with studies that indicated COVID-19 status as an inflammatory conditiond results in high levels of hepcidin and PCT [16-20]. The increaed levels of PCT might be correlated with the increased production of cytokines during inflammation in patients with severe COVID–19 status. PCT synthesis is activated by interleukins which are released by destruction of patientslung tissue infected with COVID–19. Accordingly, a high level of PCT may be a critical proinflammatory predictive marker for diagnosis of disease and its prognosis in patients with mild COVID–19 [21]. There was a highly significant increase in hepcidin level among patients according to severity; the level was high in severe cases as explained in Table 2. These results correspond with the studies [19, 20]. Many recent researches have shown the effect of hyperhepcidinemia in the assessment of the prognosis of disease in patients with COVID-19 and the difference was highly significant (p<0.01) [22, 23]. The inflammatory responses modulate iron metabolism. Hepcidin is an inflammatory marker that is recently identified and modulates iron intake via control of the digestive tract and iron liberation, which are both significant passage ways participated in the control of iron concentration for homeastasis. Hepcidin concentrations are increased in inflammatory responses that are caused by infections [24]. The suggested mode of action for hepcidin production and elevation by pro-inflammatory interleukins such as interleukin-1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α). This cytokine activation resulted in increased inflammation that results in cellular damage and the secretion of hepcidin [25, 26]. It was found that patients with hyperhepcidinemia were to have high death rates, but here is an argument about this increase is due to consequence of the inflammation or because of the pathogenesis the virus [27]. Hepcidin levels have been observed to rise as a result of a cytokine storm, and it has also been seen in severe COVID-19 patients. Many inflammatory cytokines are rapidly produced during the cytokine storm in COVID-19, including IL-6, TNF-, IL-1, IL-12, and IFN-, which activate hepatocytes, Kupffer cells, and macrophages to manufacture hepcidin. Hyperhepcidinemia syndrome is an unregulated and defective immune response linked to macrophage activity. Notably, hepcidin is not only the outcome of excessive inflammation, but it also plays a harmful function in the inflammation process by stimulating the development of numerous pro-inflammatory mediators through its interaction with T-cell immunoglobulin and mucin domain 2 (TIM-2) [23]. As a result, the innate immune system will reduce iron bioavailability in order to inhibit virus multiplication during acute infection. The concentrations of the liver-derived iron hormone hepcidin – the key regulator of iron homeostasis – could increase and block the activity of the transporter ferroportin, which carries iron out of the cells, and thus reducing the quantity of iron absorbed from the diet, causing cellular sequestration of iron, through interleukin-6 and Toll-like-receptor-4 dependent pathways (i.e., principally in hepatocytes, enterocytes, and macrophages). Increased intracellular iron sequestration leads to an increase in cytosolic hepcidin, which polymerizes and stores iron to minimize free radical damage caused by iron [22]. Increased levels of hepcidin due to cytokine storm and secondary hem phagocytic lymphohistiocytosis were recorded in patients with severe COVID-19 [23]. The current study has an agreement with some prevcious studies that have shown a significant elevation in hepcidin in male comparing to female, this difference may be attributied to the hormonal changes between the two sexes that affect iron storage and metabolism [28, 29]. This table reveals that the sensitivity values of the markers were : 0.88%, 0.85 for procalcitonin and hepcidin respectively, which indicate high diagnostic power. This table reveals that the sensitivity values of the markers were: 0.88% and 0.85% for procalcitonin and hepcidin respectively, which indicate high diagnostic power. This result may come in accordance with Zhou et al. which found that hepcidin more than 32.7 ng/mL indicated the severity of COVID-19 and predicted (56.5% for sensitivity, 97.3% for specificity) [19]. To the best kneledge of the researchers, the is no previous study that identified specificity and sensitivity of PCT in COVID-19 patients. The limitation of the current study may be in the small sample size.

CONCLUSION

This research has recorded an association between serum hepcidin levels and Procalcitonin levels in COVID-19 patients, as well as an important link between increased hepcidin and PCT levels and COVID-19 severity. The results can be used as atinflammatory biomarker with a relatively high sensitivity to improve the diagnosis of COVID-19 disease

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ORCID and contributionship:
Ali Al-Fahham: 0000-0002-6316-6281[A, F]
Ghusoon AL-Janabi: 0000-0002-3611-9367[B-C]

Alyaa Neamah Najm Alsaedi: 0000-0002-0269-6473^{C-D}
Ali Yas Khudhair Al-Amery: 0000-0002-3621-1294^E

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Ghusoon AL-Janabi
Clinical Laboratories Department, Applied Medical Sciences College, Karbala University, Kerbala, Iraq,
email: gosoon.ghanim@uokerbala.edu.iq

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article