

ORIGINAL ARTICLE

Medicine Science 2022;11(4):1398-401

Is there a relationship between HMGB1 levels and clinical conditions of patients tested positive for COVID-19?

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Received 07 July 2022; Accepted 30 August 2022

Available online 14.09.2022 with doi: 10.5455/medscience.2022.06.141

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Abstract

Severe COVID-19 may be complicated by acute respiratory distress syndrome, sepsis and septic shock, which can lead to death. These life-threatening conditions are supposed to be the outcome of immune system overactivity, which causes cytokine storm syndrome and multiple organ failure. High mobility group box-1 (HMGB1) is one of the damages associated with molecular model molecules (DAMPs), which plays a role in the pathogenesis of various inflammatory diseases and infections where many studies have been conducted. In this study, the role of HMGB1 in COVID-19 positive cases, which could be considered a potential diagnosis and treatment method, is investigated. COVID-19 patients, who were hospitalized to our clinic in Sanko University Hospital, were included in this study. Blood samples were taken from patients to measure their HMGB1 level which was then compared with their clinical severity. It is observed that HMGB1 levels rise in COVID-19 patients when compared to the control group, but the severity of the illness does not get particularly affected by their HMGB1 levels.

Keywords: COVID-19, HMGB1, symptoms of COVID-19

Introduction

Acute respiratory distress syndrome, sepsis, and septic shock may exacerbate severe COVID-19, which can be fatal. These life-threatening complications are assumed to be the outcome of immune system overactivity, which causes a cytokine storm syndrome and multiple organ failure.

Virally infected or otherwise stressed cells release endogenous damage-associated molecular pattern molecules (DAMPs) to alarm the environment about a loss of intracellular homeostatic balance.

High mobility group box-1 (HMGB1) is one of the DAMPs that contributes to the development of numerous inflammatory

disorders and infections. HMGB1 is a small protein with cytokine activity which has nuclear, cytosolic and extracellular activities. The intranuclear functions involve regulation of gene transcription, chromatin repair, and additional tasks. In addition, HMGB1 may be passively released extracellularly as a prototypical DAMP from dying cells or actively secreted by stressed or activated cells present in any tissue [1-3].

Regulating gene transcription and repairing chromatin are the two other intranuclear functions of HMGB1. HMGB1 is a typical DAMP that can be actively released by means of stressed or activated cells in any tissue or alternatively released by dying extracellular cells in a passive manner [1-3].

It is shown in some previously published studies where the levels of HMGB1 had risen with acute respiratory distress syndrome, sepsis and diseases, inflammation is found to be triggered [4,5,6]. Nevertheless, there is still only limited number of studies where serum HMGB1 level in COVID-19 are available [1,7]. In this regard, some conflicting conclusions were identified where some

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studies show no change in HMGB1 level in COVID-19 cases, whereas some other studies suggest that HMGB1 levels increase and even is associated with severe illness. As a result, it is unclear what function HMGB1 serves in COVID-19 patients. In our study, the role of HMGB1 in COVID-19 patients is investigated to understand whether it could be used as a potential diagnosis and treatment method.

Material and Methods

All patients over 18-years old and diagnosed with COVID-19 admitted to the isolated COVID service of SANKO University Faculty of Medicine, were included in this study. During the admittance process, the demographic characteristics of these patients, their existing complications, duration of these complications, number of days passed by since their first positive PCR test results, all their previous medication given both related and unrelated to COVID-19 condition were recorded. Patients with any chronic inflammatory, psychiatric, malign, neurogenic or collagen tissue diseases were excluded from this study. The patients' daily complaints, concerning illnesses and any related medications were recorded. Patients were clinically classified in three groups; namely, (1) mild (minor symptoms with no imaging findings), (2) moderate (fever and respiratory tract infections, images may show signs of pneumonia) and (3) severe (respiratory rate over 30+ per minute, saturation below 93% in room air, PaO₂/FiO₂ ratio below 300 or lesions in lung imaging increased by more than 50% within the last 1-2 days). The treatments of the patients were done accordingly to the Turkish Ministry of Health's COVID-19 Guide for Patient Management and Treatment [8]. In addition, blood samples were taken from healthy volunteers who have not caught COVID-19 to help with the determination of HMGB1 levels for comparison. In order to analyse the serum HMGB1 levels, approximately 5–6 mL of venous blood samples were obtained from those patients (just before the start of treatment) and also from healthy control participants. Within an hour of collection, blood samples were centrifuged for 8 to 10 minutes at 4000 rpm. The samples were then removed from -80

°C and chilled to 4°C for one night prior to the measurements. The samples were vortexed and placed into the microplate wells after defrosting without any delay. The measurements were performed using the ELISA method, with each sample tested in duplicate. Human HMGB1 levels were determined quantitatively using commercially available Bio-Techne kits (Bio-Techne Ltd. Abingdon-Oxfordshire/United Kingdom; catalog number NBP2-62766) according to manufacturer's instructions. The double-antibody sandwich enzyme immunoassay technique was used for the analysis. All concentration/absorbance curves for human HMGB1 by ELISA tests and related calculations were obtained using the integrated software of the Biotek ELx808 (Winooski, Vermont, USA) absorbance reader.

Statistical Analyses

For statistical analysis, the SPSS for Windows 25.0 software suite (SPSS Inc., Chicago, IL, USA) was utilized. Both visual (histograms and probability plots) and analytical techniques were used to determine whether the variables had a normal distribution or not (Kolmogorov-Smirnov and Shapiro-Wilk tests). The Kolmogorov-Smirnov test revealed a normal data distribution if the p value was greater than 0.05. The Student's t-test was employed to evaluate the variations between the patient and control groups for data with a normal distribution. The chi-square test was performed to compare the two groups for categorical variables as well. Pairwise comparisons were carried out using the Mann-Whitney U test and evaluated using the Bonferroni correction when normal distribution was not found. An approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (2021/16).

Results

The study was carried out on 88 COVID-19 positive patients and 35 healthy volunteers. Demographic and clinical characteristics of the patients are shown in Table-1. In total, 48 of our patients were clinically classified as mild cases, whilst 35 were classified as moderate and 5 as severe.

Table 1. Demographic and clinic information of the COVID-19 patients

	Women (n:43)		Men(n:45)		P
Avg. age ± SS	46.14±15.334		51.31±17.809		0.012*
Educational status	n	%	n	%	
Illiterate	2	4.7	1	2.2	0.53
Elementary School	17	39.5	14	31.1	
Secondary School	12	27.9	11	24.4	
Tertiary School	12	27.9	19	42.2	
(+) Smoking	6	14	17	37.8	0.01
(-)	37	86	28	62.2	
(+) DM	8	18.6	11	24.4	0.50
(-)	35	81.4	34	75.6	
(+) HT	11	25.6	12	26.7	0.9
(-)	32	74.4	33	73.3	
(+) COPD	-	-	3	6.7	0.08
(-)	43	100	42	93.3	
ASTHMA	3	7	1	2.2	0.28
	40	93	44	97.8	
CAH	-	-	4	8.9	0.04
	43	100	41	91.1	

n=number of individuals. The chi square* test was used in statistical comparison Student t test was used. Mean. ± St.Dev.: Mean ± standard deviation. p<0,05 is accepted as statistically significant

Meanwhile, the relationship between HMGB-1 levels of COVID-19 positive patients and other healthy individuals is shown in Table-2.

As it can be seen in Table-1, when COVID-19 positive patients were grouped according to the severity of the case and lung involvement, it is observed that there is no significant change in HMGB1 levels ($p < 0.05$). When comparing the relation between clinic severity and HMGB1 levels, as we have few severe patients, the data from mild patients was compared to that of moderate and severe groups jointly as well. Additionally, a statistically

significant difference in terms of the clinical classification of patients regarding their gender was observed ($p = 0.03$). In the post hoc comparison, it is observed that this difference is caused by patients who are classified as mild and moderate ($p = 0.06$).

In Table-2, the relation between the HMGB1 levels of COVID-19 positive patients and healthy individuals was evaluated. It is observed that, the HMGB1 levels of COVID-19 positive patients has increased significantly compared to the healthy control group ($p < 0.001$).

Table 2. Serum HMGB1 levels of COVID-19 patients and the control group

	Patient n (88)	Control n(35)	p
HMGB1 (ng/mL)Median (min-max)	12.88(4.71-98.77)	9.60(4.70-18.71)	$p < 0.001^{**}$

n=number of individuals The Mann-Whitney U test was used for statistical analysis

Discussion

In our study, it is observed that HMGB1 levels rise in COVID-19 patients when compared to the control group, but severity of the illness does not necessarily get affected by their HMGB1 levels. In a study, as a result of comparison of HMGB1 levels over a total of 121 healthy volunteers as well as patients hospitalised in the ward, no significant change was found, but when the patients hospitalized in the intensive care unit were compared to those in the ward, HMGB1 levels of the patients hospitalized in the intensive care unit were found to be significantly higher [9]. In a different study carried out with patients in ICU, HMGB1 levels of 60 COVID-19 positive patients were found to be significantly higher than those of healthy volunteers, however this higher level of HMGB1 could not be associated with mortality [10]. In another study, the HMGB1 levels of patients in the intensive care unit were compared with the HMGB1 levels of healthy volunteers, individuals who had contracted and recovered from COVID-19, mild or asymptomatic and not hospitalized COVID-19 patients, and HMGB1 levels were found to be higher in patients in the intensive care unit [11]. Meanwhile, in our study, HMGB1 level was found to be significantly higher in COVID-19 positive patients than in healthy volunteers. It is believed that this high level may be due to the presence of 40 patients representing approximately 45% of the cases with moderate and severe clinical status in our service.

Cazzato et al. have observed in their study that there is no difference between CD4 and CD8 levels in skin biopsy samples in healthy volunteers and COVID-19 positive patients. However, in their study, they have also concluded that HMGB1 levels rise significantly in COVID-19 positive patients [12].

Chen et al. in a different study which they conducted with 40 patients (of which 11 severe cases with bilateral lung infection), found that the HMGB1 level in severe patients was higher than both the non-severe group and the healthy control group. The mean serum HMGB1 level was 189.40 ± 40.90 ng/mL in the severe group, 35.51 ± 41.92 ng/mL in the non-severe group, and finally 7.16 ± 2.88 ng/mL in the normal control group [7]. In our study, the HMGB1 value was determined to be 12.88 (4.71-98.77) ng/mL. When this value is compared to the study made by Chen et al., it is still deemed to be significantly lower than both their severe and non-severe groups. This difference between the studies might be

caused by the fact that 34 of our patients had CT normal, while 23 of them had a unilateral mild lung infection. However, with 31 of our patients having bilateral severe illness, no relationship between HMGB1 levels and lung infection were identified ($p > 0.05$).

Bolay et al. have found in a study carried out with 88 patients that the patient group who had headaches and the other group with lung infection have higher serum HMGB1 levels [13]. In contrast, we have observed in our study that lung infections do not have any direct effect on HMGB1 levels. Nonetheless, we have not actually investigated whether there was any relationship between the patients' symptoms and their HMGB1 levels. Studies which correlate lung infections with the date of first symptoms, can relate those HMGB1 levels to any lung infection more accurately. This is because lung infection starts several days after the date of first symptoms and progresses further gradually.

The limitation of our study is that only two of our patients needed intensive care during our study; and therefore, we could not determine whether HMGB1 levels could predict any intensive care admission. Therefore, it is strongly recommended that future studies are necessary to investigate this issue. In addition, since none of our patients were fatal, we could not establish a connection between HMGB1 levels and mortality.

Conclusion

In some studies, anti-HMGB1 agents have been tried in patients with high HMGB1 levels [14,15]. The high level of HMGB1 in COVID-19 patients in our study suggests that HMGB1 may have a role in the pathogenesis of COVID-19. This can be also considered as a further justification to carry out more studies targeting HMGB1 in this highly mortal situation.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

An approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (2021/16).

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