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Research article

The association between high mobility group box 1 (HMGB1) and Interleukin-18 (IL-18) serum concentrations in COVID-19 inpatients

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ABSTRACT

Background: High mobility group box 1 (HMGB1) and interleukin-18 (IL-18) are involved in various non-coronavirus disease pathogenesis and are reported as potential biomarkers for coronavirus disease (COVID-19). However, their association with COVID-19 pathogenesis has not yet been explored.
Aim: This study aimed to investigate the association between HMGB1 and IL-18 concentrations in the sera of COVID-19 patients versus non-COVID-19 patients.
Material and methods: We used stored serum samples obtained from 30 COVID-19 patients and 30 non-COVID-19 patients. We collected data on age, gender, treatment status, principal diagnosis, and comorbidity from patient medical records. HMGB1 and IL-18 concentrations were analyzed in the serum by enzyme-linked immunosorbent assay (ELISA). The swab samples' RT-PCR cycle threshold (CT) values were obtained from the laboratory database.
Results: HMGB1 concentrations were increased in the COVID-19 inpatients and non-COVID-19 inpatients compared to non-COVID-19 outpatients (COVID-19 inpatients vs. non-COVID-19 outpatients: 151.33 (90.27–192.38) vs. 80.75 (54.16–128.72) ng/ml; $p = 0.0316$; non-COVID-19 inpatients vs. non-COVID-19 outpatients: 152.66 (104.04–288.51) vs. 80.75 (54.16–128.72) ng/ml; $p = 0.0199$). IL-18 concentrations were also higher in the COVID-19 inpatients and non-COVID-19 inpatients compared to non-COVID-19 outpatients (COVID-19 inpatients vs. non-COVID-19 outpatients: 620.00 (461.50–849.6) vs. 403.10 (372.70–556.90) pg/ml; $p = 0.0376$; non-COVID-19 inpatients vs. non-COVID-19 outpatients: 835.70 (558.30–1602.00) vs. 403.10 (372.70–556.90) pg/ml; $p = 0.0026$). Moreover, HMGB1 was associated with IL-18 concentrations in the sera of COVID-19 inpatients ($p = 0.0337$; $r = 0.5500$).
Conclusion: The association of HMGB1 and IL-18 in COVID-19 might indicate the potential for a dangerous cycle leading to a cytokine storm to occur.

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1. Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in 14.83 million excess deaths globally over 2020–2021. Excess mortality is defined as the difference between all-cause mortality during the pandemic period and that which was expected under normal conditions [1]. Indonesia was one of the top three Southeast Asian countries with the highest infection and case fatality rate (CFR) in 2020 [2]. Almost 7 million COVID-19 cases had been confirmed in Indonesia by the end of 2022; however, it was reported that the number of cases continued to decline throughout the year [3]. The COVID-19 situation is quite challenging in 2023. Globally, the fatality rate is going down and vaccine coverage is increasing [4]. However, high-risk people are still prone to developing severe COVID-19 [5]. COVID-19 threat also remains for reasons such as the possible emergence of new variants, high transmissibility, unvaccinated people, and not wearing personal protective equipment [6].

SARS-CoV-2 is associated with more severe outcomes compared to other respiratory viruses in a retrospective cohort study of hospitalized adults who tested positive for SARS-CoV-2, influenza virus A/B, RSV, rhinovirus, enterovirus, parainfluenza viruses, metapneumovirus, seasonal coronaviruses, adenovirus, or bocavirus. ICU admissions and complications tend to be higher in COVID-19 patients [7]. The in-hospital mortality was three times higher for COVID-19 patients than for influenza patients [8]. SARS-CoV-2 infection triggers distinct immunological responses in comparison with other respiratory viruses. Pathological immune signatures exclusive to COVID-19 revealed low expression of human leucocyte antigen (HLA) and exhaustive T cells, leading to impaired virus recognition and clearance [9]. In line with these findings, many COVID-19 patients suffer from hyperinflammation with features of cytokine storm syndrome, which causes high morbidity and mortality [10].

Cytokine storms in COVID-19 patients are thought to be triggered by the release of damage-associated molecular patterns (DAMPs) from the infected host cells due to high viral loads, which act as alarmin [11]. One well-known DAMP is a nuclear protein, high mobility group box 1 (HMGB1). Extracellular HMGB1 protein is actively secreted by immune cells and passively released by dead cells due to inflammatory stimuli and tissue damage [12]. The activation of a danger signal by HMGB1 can lead to an overactive inflammatory response in the lungs and weaken the body's ability to defend against lung infections, creating a harmful feedback cycle [13]. Furthermore, HMGB1 was confirmed as a potential biomarker for respiratory virus infection, and the inhibition of virus-induced HMGB1 release ameliorated acute lung injury (ALI) [14].

HMGB1 was released from SARS-CoV-2-infected cells in the in vitro study [15]. Extracellular HMGB1 binds to specific pattern recognition receptors (PRRs) located on the cell surface, such as toll-like receptor (TLR)2, TLR4, and receptor for advanced glycation end-product (RAGE), to activate downstream signal via nuclear factor kappa B (NF-κB), leading to the production of proinflammatory cytokines that have effects both locally and systemically [16]. Clinical evidence described the increased concentration of HMGB1 in the serum or plasma of COVID-19 patients, which was linked to the concentration of proinflammatory cytokines [17–20].

The potent proinflammatory cytokine interleukin-18 (IL-18) plays a role in the host's defense against infections and modulates both innate and acquired immune responses. IL-18 directly augments NK cell and CD8 T cell cytotoxicity by inducing the expression of perforin and Fas ligands [21]. Sepsis and inflammatory lung disease are two inflammatory disorders linked to increased IL-18 production. IL-18 plays a key role in inducing macrophage activation syndrome (MAS), which is a specific type of cytokine storm syndrome [22]. An association between IL-18 and inflammatory markers and organ injury indicators was identified in COVID-19 patients [23]. IL-18 has been proven to be elevated in the lung and spleen of severe COVID-19 patients [24].

HMGB1 could stimulate IL-18 production by interacting with the pathogen-associated molecular patterns (PAMPs) that SARS-CoV-2-infected dead cells release. HMGB1-PAMP complexes stimulate RAGE, followed by its transfer to the endolysosomal system. HMGB1 triggers the lysis of lysosomes, enabling PAMPs to interact with cytosolic inflammatory receptors. This process stimulates the activation of the inflammasome, the production and release of cytokines, and the initiation of pyroptosis cell death [25]. In in vitro research, HMGB1 and its partner molecule induced IL-1β and IL-18 secretion through RAGE. However, HMGB1 alone simply promotes the synthesis of pro-IL-1β and pro-IL-18 [26]. Pro-IL-18, a biologically inactive precursor of IL-18, requires cleavage by caspase-1 to become active. Processing of IL-18 into its active form occurs in the cytoplasmic inflammasome. Caspase-1 also facilitates the release of IL-18 and other cytoplasmic proteins, including HMGB1 [27].

As far as we know, the HMGB1 and IL-18 relationship has not been explored in COVID-19. We hypothesize that HMGB1 might induce the synthesis and release of IL-18 in COVID-19. This pathway could potentially stimulate a dangerous cycle that leads to a cytokine storm. Therefore, this study aimed to investigate the association between HMGB1 and IL-18 concentrations in the sera of COVID-19 patients compared to non-COVID-19 patients.

2. Material and methods

2.1. Study design, samples, and data collection

We conducted a study on serum samples stored in the Laboratory of COVID-19, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. This facility received 33,697 samples from many public health offices and hospitals in Yogyakarta and other provinces from April to December 2020 for COVID-19 confirmation. Of these, 5,734 samples were confirmed positive for SARS-CoV-2, and 27,963 samples were confirmed negative for SARS-CoV-2 in the nasopharyngeal swab sample by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Based on the RT-PCR results from their swap, we grouped the serum into the COVID-19 group (confirmed as a positive result by RT-PCR) and the non-COVID-19 group (confirmed as a negative result by RT-PCR). The samples collected from the hospital in the acute phase were selected. Samples taken from individuals in close contact

with COVID-19 patients (contact tracing), follow-up, and convalescent serum were excluded. A total of 30 COVID-19 and 30 non-COVID-19 samples were randomly selected from serum that met the above criteria. The selected samples were collected from Bhayangkara Hospital Yogyakarta, Dr. S. Hardjolukito Hospital Yogyakarta, Hermina Hospital Yogyakarta, Panti Rini Hospital Yogyakarta, Kota Yogyakarta Hospital, Respira Hospital Yogyakarta, and Dr. Soeradji Tirtonegoro Hospital Klaten.

Based on the laboratory of the COVID-19 registry, we traced each patient's medical record to the hospital where the samples were collected. We collected data on age, gender, the status of treatment, principal diagnosis, and comorbidity from patient medical records in each hospital. RT-PCR's cycle threshold (CT) values from swab samples were obtained from the Laboratory COVID-19 database.

Ethical approval

This study is a retrospective study using stored left-over specimens and medical records that have been approved by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada–Dr. Sardjito General Hospital, Yogyakarta, Indonesia (Ref: KE/FK/0531/EC/2021 and KE/FK/0909/EC/2022). The Institutional Review Board (IRB) had waived the mandatory obtaining of informed consent from subjects.

2.2. RT-PCR

The recorded CT value from each nasopharyngeal sample was obtained from one of the following detection kits for 2019 novel coronavirus (2019 nCoV) RNA: EUL 0493-141-00 Da An Gene Co. Ltd. of Sun Yat-sen University, China (detection for ORF1ab and N genes) or RR-0479-02 Liferiver Shanghai ZJ Bio-Tech Co. Ltd., China (detection for ORF1ab, N and E genes). For both kits, the CT value of the ORF1ab gene considered positive for SARS-CoV-2 infection was $\leq$40. The E gene threshold value for a positive result was $\leq$40. The N gene threshold CT value for the Liferiver kit was $\leq$42, while the Da An kit was $\leq$40. The sample was considered positive for COVID-19 if two or more genes displayed positive results or one gene consistently showed positive results in the re-test.

2.3. HMGB1 and cytokine assay

We used the human HMGB1 (MBS9141311) and IL-18 (MBS175863) ELISA kits (MyBioSource, USA) to estimate the HMGB1 and IL-18 concentrations in serum samples. All serum samples were processed according to the manufacturer's instructions, followed by data analysis using MyAssays Analysis Software Solutions (MyAssays: http://www.myassays.com) under a four-parameter logistic curve fit.

2.4. Statistical analysis

Continuous variables were tested for normal distribution by Shapiro-Wilk tests. When they showed a normal distribution, independent t-tests or one-way ANOVA were performed; when they did not, Mann-Whitney U tests or Kruskal-Wallis tests were performed. Variables were expressed as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR). X^2 tests or Fisher exact tests were used for categorical analysis, and variables were shown as frequency (%). Spearman tests were performed for correlation analyses. The correlation rating is given by Spearman's coefficient of correlation rho (r) and interpreted as negligible (0.0–0.3), low (0.3–0.5), moderate (0.5–0.7), high (0.7–0.9), or very high (0.9–1.0) [28]. P values < 0.05 were considered significant. All statistical analyses were performed in STATA version 17.0.

Table 1
Non-COVID-19 and COVID-19 patient's characteristic.

	Non-COVID-19 (n = 30)	COVID-19 (n = 30)	p-value
Age (year)	32.77 ± 21.13	41.60 ± 16.37	0.0754[a]
Gender:	21 (70.0%)	21 (70.0%)	1.000[b]
Male	9 (30.0%)	9 (30.0%)	
Female			
Patient status:	23 (76.7%)	15 (50.0%)	–
Outpatient	7 (23.3%)	15 (50.0%)	
Inpatient			
Main diagnosis:			
Nonhemorrhagic stroke	1 (3.3%)	–	–
Acute gastroenteritis/diarrhea	4 (13.3%)	–	
Acute myocardial infarction	1 (3.3%)	–	
Bronchitis/bronchopneumonia/pneumonia	12 (40%)	–	
Chronic kidney disease	1 (3.3%)	–	
Dengue hemorrhagic fever	1 (3.3%)	–	
Encephalitis/meningoencephalitis	2 (6.7%)	–	
Sepsis	1 (3.3%)	–	
Unknown	7 (23.3%)	–	
COVID-19	–	30 (100.0%)	

Data are shown in mean ± SD or frequency (%), [a] Independent *t*-test, [b] X^2 test.

3. Results

3.1. The outpatient and inpatient characteristics of non-COVID-19 and COVID-19 patients

A total of 30 serum samples from the non-COVID-19 group and 30 serum samples from the COVID-19 group obtained from the hospitals in the region of Yogyakarta Province and Klaten District, Central Java Province, Indonesia, were included in this study. Patients' ages and genders weren't significantly different among groups. However, the mean age was higher in the COVID-19 patients than in the non-COVID-19 patients (41.60 ± 16.37 vs. 32.967 ± 21.755 years; p = 0.0754). Most of the COVID-19 and non-COVID-19 patients were males (70.0%). Seven patients of the non-COVID-19 patients (23,3%) and 50% of patients in the COVID-19 group visited the hospital only for COVID-19 confirmation by RT-PCR of the nasopharyngeal swab, which is termed outpatients in this study. The patients who stayed at the hospital for treatment are termed inpatients, and their serum has been collected within the first week of admission. The diagnosis of seven outpatients confirmed with RT-PCR negative for COVID-19 in the non-COVID-19 group was unknown. The main diagnosis varied among inpatients in the non-COVID-19 group. Respiratory infections (bronchitis, bronchopneumonia, or pneumonia) were the main diagnosis for 40% of non-COVID-19 inpatients. (Table 1).

Elevated HMGB1 and IL-18 serum concentration in the COVID-19 and non-COVID-19 inpatients.

Comparison of HMGB1 and proinflammatory cytokines IL-18 concentration in the COVID-19 and non-COVID-19 groups were shown as median (IQR) since both variables were non-normally distributed. There was no statistically significant difference between the HMGB1 and IL-18 concentrations of the two groups. HMGB1 concentration in the COVID-19 group was almost equal to that of the non-COVID-19 group (136.33 (90.18–203.79) ng/ml vs. 141.055 (90.27–183.58) ng/ml; p = 0.7338). A higher IL-18 concentration was observed in the serum of the COVID-19 group compared to the non-COVID-19 group (713.00 (471.30–1377.00) pg/ml vs. 649.80 (399.80–808.40) pg/ml; p = 0.1984).

Since there were two types of patients in the non-COVID-19 and COVID-19 groups: outpatients and inpatients, we analyzed HMGB1 and IL-18 serum concentrations in those subgroups. HMGB1 serum concentration was consistently higher in the inpatient compared to the outpatient subgroups in the non-COVID-19 (80.75 (54.16–128.72) vs. 152.66 (104.04–288.51) ng/ml; p = 0.0199) and COVID-19 (104.43 (81.36–171.61) vs. 151.33 (90.27–192.38) ng/ml; p = 0.4306), although the latter one was not significant. In comparison with non-COVID-19 outpatients, HMGB1 concentration was significantly higher in COVID-19 inpatients (p = 0.0316) and non-significantly higher in COVID-19 outpatients (p = 0.1127). We observed that there were no differences regarding HMGB1 concentration in non-COVID-19 inpatients compared to that of outpatients (p = 0.1101) and inpatients (p = 0.5016) COVID-19 subgroups (Fig. 1a).

IL-18 concentrations in non-COVID-19 subgroups were 403.10 (372.70–556.90) vs. 835.70 (558.30–1602.00) pg/ml; p = 0.0026 which was significantly higher in the inpatient subgroup. IL-18 concentrations in COVID-19 subgroups were 660.00 (356.80–808.40) pg/ml vs. 620.00 (461.50–849.6) pg/ml; p = 0.4306 which was slightly lower in COVID-19 inpatients. However, COVID-19 inpatients presented significantly higher levels of IL-18 when compared to COVID-19 outpatients (p = 0.0376). No significant difference was observed between COVID-19 outpatients and non-COVID-19 outpatients regarding to their IL-18 concentration (p = 0.2746). Non-COVID-19 inpatients had higher IL-18 concentrations compared to COVID-19 outpatients (p = 0.0470) but there was no significant difference in the comparison with COVID-19 inpatients (p = 0.0505) (Fig. 1b).

3.2. HMGB1 was associated with IL-18 serum concentration in COVID-19 inpatient

Next, we examined whether HMGB1 correlated with IL-18 concentration in the non-COVID-19 and the COVID-19 subgroups. Since the diagnosis of the non-COVID-19 group was varied, we have specified the next analysis for the non-COVID-19 inpatients with and without the main diagnosis of respiratory infection (bronchitis, bronchopneumonia, or pneumonia), termed non-COVID-19 respiratory

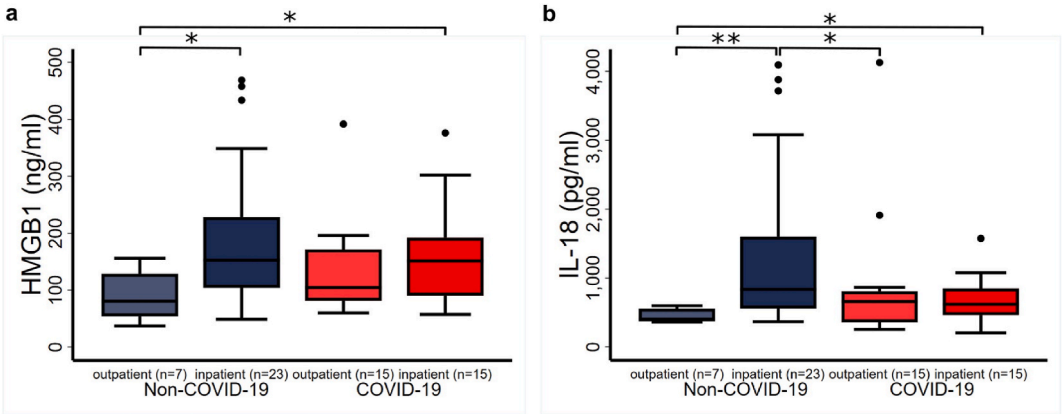

Fig. 1. HMGB1 (**a**) and IL-18 (**b**) serum concentrations in outpatients and inpatients non-COVID-19 and COVID-19 groups. All data are expressed in median (IQR). *p < 0.05; **p < 0.01; HMGB1: high mobility group box 1; IL: interleukin.

inpatients (n = 12) and non-COVID-19 non-respiratory inpatients (n = 11), in comparison with the COVID-19 subgroups. Interestingly, we observed a moderate correlation between HMGB1 and IL-18 concentration in the COVID-19 inpatients (p = 0.0337; r = 0.5500) (Fig. 2d). On the contrary, there was no association between HMGB1 and IL-18 concentration in the non-COVID-19 respiratory inpatients (p = 0.7954; r = −0.0839), non-COVID-19 non-respiratory inpatients (p = 0.6115; r = −0.1727) and the COVID-19 outpatients (p = 0.0736; r = −0.4750) (Fig. 2a–**c**). No significant difference was observed for the intergroup comparison of comorbidities. The average hospital stay was significantly longer for the COVID-19 inpatients compared to the non-COVID-19 respiratory and non-respiratory inpatients (7 (6–8.5) vs 7 (4–18) vs. 14 (8–20) days; p = 0.0398) (Table 2).

3.3. HMGB1 and IL-18 serum concentrations in the COVID-19 subgroups were not associated with the CT value of the nasopharyngeal sample

The correlation of HMGB1 and IL-18 concentrations in the serum with the CT value of real-time RT-PCR from nasopharyngeal samples was analyzed in this study. The CT value of each gene concerning HMGB1 and IL-18 concentration was analyzed separately. Unfortunately, not every sample displayed complete CT values for the three genes, resulting in varying sample numbers for each analysis. Our data showed that there were no significant correlations between HMGB1 and IL-18 concentrations and the CT values of the ORF1ab, N, and E genes (Supplementary Fig. a–l).

4. Discussion

HMGB1 and IL-18 both play a substantial role in COVID-19 and non-COVID-19 diseases. Their relationship could be involved in the disease's pathogenesis. We found no statistically significant difference between HMGB1 and IL-18 concentrations in the sera of COVID-19 and non-COVID-19 patients. The non-COVID-19 main diagnosis was varied, ranging from infectious to non-infectious. Elevated levels of HMGB1 were reported in a variety of infectious diseases, i.e., dengue hemorrhagic fever [29], and many viral respiratory infections, i.e., respiratory syncytial virus (RSV) and SARS-CoV infection [30], and non-infectious diseases, i.e., stroke [31], and acute myocardial infarction [32]. HMGB1 has been involved in the pathological condition of multiple organs and systems, including the central nervous system, cardiovascular system, gastrointestinal system, lung, and kidney [33]. It was confirmed in this study that for the non-COVID-19 group, the inpatients had significantly higher HMGB1 concentrations than the outpatients.

One of the non-COVID-19 groups in this study was diagnosed with dengue fever; thus, it would be worth exploring the potential of HMGB1 and IL-18 as biomarkers for both dengue and COVID-19. The levels of HMGB1 in the serum of dengue-infected individuals

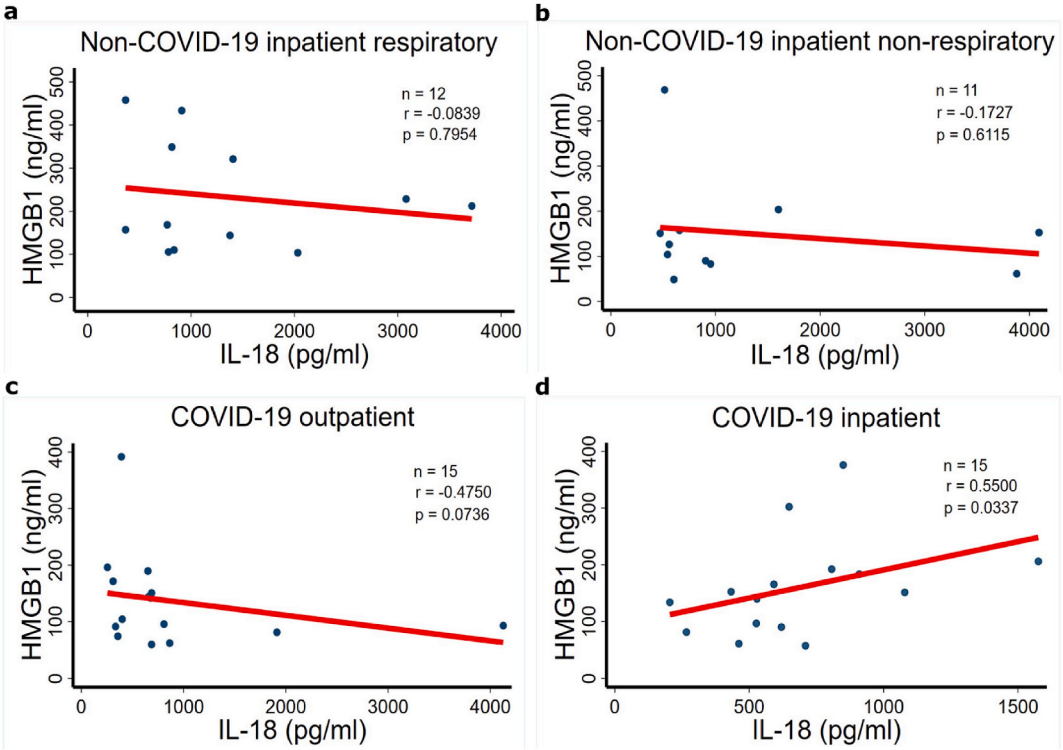

Fig. 2. Spearman's correlation analyses between HMGB1 and IL-18 serum concentrations in non-COVID-19 respiratory inpatients (**a**), non-COVID-19 non-respiratory inpatients (**b**), COVID-19 outpatients (**c**), and COVID-19 inpatients (**d**) groups. The degree of correlation is expressed as Spearman's correlation coefficient rho (r) and p-value. HMGB1: high mobility group box 1; IL: interleukin.

Table 2
Characteristics of non-COVID-19 patients with and without main diagnosis as respiratory infection compared to COVID-19 outpatients and inpatients.

	Non-COVID-19 respiratory inpatients (n = 12)	Non-COVID-19 non-respiratory inpatients (n = 11)	COVID-19 outpatients (n = 15)	COVID-19 inpatients (n = 15)	p-value
Age (year)	30.42 ± 22.94	35.00 ± 25.65	38.80 ± 11.63	44.40 ± 20.07	0.3361[a]
Gender:	6 (50.0%)	10 (90.9%)	13 (86.7%)	8 (53.3%)	0.096[b]
Male	6 (50.0%)	1 (9.1%)	2 (13.3%)	7 (46.7%)	
Female					
Comorbidity:					–
Cardiovascular disease (heart failure/ ischemic heart disease)	2 (16.7%)	1 (9.1%)	–	–	
Dengue fever	1 (8.3%)	–	–	1 (6.7%)	
Diabetes mellitus	1 (8.3%)	–	–	–	
Gastrointestinal disorder (melena)	–	1 (9.1%)	–	–	
Hematologic disorder (anemia, thrombocytopenia)	3 (25.0%)	–	–	1 (6.7%)	
HIV	–	2 (18.2%)	–	–	
Hypertension	1 (8.3%)	–	–	–	
Neurological disorder (hemiparesis, aphasia, febrile seizure)	1 (8.3%)	–	–	1 (6.7%)	
Post-operative (sectio caesarea)	–	–	–	1 (6.7%)	
Respiratory tract infection (bronchitis/ bronchopneumonia/pneumonia)	–	7 (63.6%)	–	–	
Shock (hypovolemic)	–	1 (9.1%)	–	–	
Skin disorder		1 (9.1%)	–	–	
Ulcer (foot ulcer)	1 (8.3%)	–	–	–	
Urinary tract infection	–	–	–	1 (6.7%)	
Unknown	–	–	15 (100.0%)	–	
The number of comorbidities:					
None	7 (58,3%)	3 (27.3%)	–	10 (66,7%)	0.069[b]
1	2 (16,7%)	4 (36.4%)	–	5 (33,3%)	
≥2	3 (25,0%)	4 (36.4%)	–	0 (0%)	
Length of hospital stay (days)	7 (6–8.5)	7 (4–18)	–	14 (8–20)	0.0398[c]
HMGB1 (ng/ml)	190.44 (127.02–344.96)	126 .60 (83.09–157.61)	104.43 (81.36–171.61)	151.33 (90.27–192.38)	0.0791[c]
IL-18 (pg/ml)	873.15 (776.25–1719.50)	655.70 (542–1602)	660 (356.80–808.4)	620 (461.50–849.60)	0.1059[c]

Data are shown in mean ± SD, median (IQR) or frequency (%), [a]One-way ANOVA test, [b]Fisher exact test, [c]Kruskal-Wallis test.

were elevated, and this elevation was also observed during the secondary infection [29]. The presence of HMGB1 in the blood of COVID-19 patients was associated with a higher peak CT score and more severe clinical parameters at the time of ICU admission, further supporting its role in the disease's severity [18,20]. However, evidence regarding HMGB1's contribution to the severity of dengue is limited. The study of dengue fatal cases verified the heightened presence of cytoplasmic HMGB1 in liver, lung, and heart samples [34]. Consistent with this finding, HMGB1 released from dengue-infected cells stimulates vascular leakage in endothelial cells, indicating the contribution of HMGB1 in the crucial process that drives the progression of dengue infection [35]. Similar to its role in COVID-19, IL-18 may serve as a valuable biomarker for determining dengue severity. IL-18 was correlated with the levels of the liver enzymes serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). Elevated levels of IL-18 during the initial phase of dengue infection may serve as an indicator of illness progression [36]. The presence of comorbidity may account for the elevated levels of IL-18 in severe dengue patients as compared to severe dengue patients without comorbidity [37].

COVID-19 inpatients tended to have higher HMGB1 concentrations than COVID-19 outpatients, although this did not reach statistical significance. HMGB1 concentration in both COVID-19 subgroups was higher in comparison with non-COVID-19 outpatients. These findings are in line with the results of another study. Chen et al. [17] and Chen et al. [18] reported that severe or ICU-admission COVID-19 patients had significantly higher HMGB1 concentrations than normal or healthy controls. No significant differences were found with non-severe or non-ICU-admission COVID-19 patients, although the HMGB1 concentrations were higher than the control groups. The present study's findings confirm the previous study's finding that HMGB1 concentrations were increased in COVID-19 and potentially linked to severe outcomes.

IL-18 is a proinflammatory cytokine that has many functions and can interact with specific receptors in many types of cells. Together with IL-2, IL-3, and IL-12, IL-18 enhances IFN-γ production from Th1 cells and stimulates many cells, including NK cells, $CD4^+$ NKT, mast cells, and basophils [27]. IL-18 is involved in the pathogenesis of various infectious, metabolic, or inflammatory diseases, including the life-threatening complications of a systemic inflammation disorder termed MAS [21]. IL-18 concentrations were elevated in the non-COVID-19 and COVID-19 sera in our study. IL-18 was associated with disease severity in mild versus severe and recovered versus fatal cases of COVID-19 [23,38]. However, there were no significant differences regarding IL-18 concentration between inpatients and outpatients of COVID-19 patients in this current study. It was reported that IL-18 concentration was comparable between the asymptomatic and mild COVID-19, and mild COVID-19 had a non-significant IL-18 concentration compared to moderate COVID-19 [39,40], suggesting that many COVID-19 patients in our study were in the asymptomatic, mild, and moderate

categories. Because of the lack of data, we could not classify COVID-19 patients based on the asymptomatic-mild-moderate-severe categories.

According to the Republic of Indonesia Minister of Health Decree (number of decree: HK.01.07/MenKes/413/2020) [41], which was implemented in Indonesia during the period of sample collection, the symptomatic and mild COVID-19 cases were isolated at home (outpatients), while the moderate and severe COVID-19 cases were hospitalized (inpatients). It indicated that the majority of patients in the COVID-19 inpatients group were likely classified as moderate cases, while the COVID-19 outpatients group consisted mainly of asymptomatic and mild cases. Our approach is contextualized to the post-COVID-19 pandemic situation. COVID-19 has become endemic, with predominantly asymptomatic or mild to moderate clinical manifestations. Most cases with symptoms are mild, and severe-critical cases are rare [42–44]. As previously stated, numerous studies have already established the significant role of HMGB1 and IL-18 in severe cases of COVID-19. Identifying potential proinflammatory markers in individuals with mild to moderate COVID-19 could be advantageous in tracking the disease's transitions toward severe COVID-19. This would allow for the exploration of alternative treatment approaches while the body is still able to adjust its immunity to combat the infection [45].

In our study, HMGB1 concentration was positively associated with IL-18 concentration in COVID-19 inpatients. In vitro, extracellular HMGB1 mediated pro-IL-1β and pro-IL-18 synthesis via RAGE and the downstream signal mitogen-activated protein kinase (MAPK) p38 and NF-κB in THP1 macrophages [26]. Caspase-1 cleaves pro-IL-1β and pro-IL-18 into their mature form and induces pyroptotic cell death, which triggers the release of mature IL-1β and IL-18 extracellularly. Caspase activation is triggered by inflammasome assembly, which is a cytoplasmic multiprotein complex consisting of sensor protein, inflammatory caspase, and adapter protein [46].

The presence of IL-18 in the sera of COVID-19 patients suggests inflammasome engagement [47]. The higher level of IL-18 in COVID-19 inpatients compared to non-COVID-19 outpatients in this study supported this idea. Previous in vitro and in vivo studies reported SARS-CoV-2 spike 1 (S1) glycoprotein or receptor binding domain (RBD) protein-triggered NLR family pyrin domain containing 3 (NLRP3) inflammasome activation and IL-18 release [48]. However, based on our findings, the correlation between HMGB1 and IL-18 concentrations supported the important role of HMGB1 in triggering inflammasome-induced IL-18 release in the COVID-19 pathogenesis. Inflammasome-derived products such as caspase-1 and IL-18 were associated with moderate and severe COVID-19 disease severity. Moreover, IL-18 concentrations were significantly increased in the non-survival group compared with survivors [47]. Inflammasome activation can be useful to protect host cells against virus invasion if it is properly regulated. On the contrary, in severe cases, the immune response fails to clear the infection, which triggers inflammasome dysregulation, leading to exacerbated inflammation [49]. Unfortunately, due to insufficient data, we could not analyze the HMGB1-IL-18 relationship based on the COVID-19 severity (mild, moderate, and severe).

HMGB1 alone could not induce activation of the inflammasome, caspase-1, and subsequent IL-1β and IL-18 secretion without its interaction with other molecules [26]. HMGB1 was reported to be able to interact with lipopolysaccharides (LPS), IL-1β, virus nucleoproteins, and ribonucleic acid (RNA) [50–53]. RNA and protein of SARS-CoV-2 were found in the patient's multiple organs [54], allowing possible interaction with HMGB1 released from dead cells and immune cells after viral infection. The association between HMGB1 and IL-18 concentrations in the current study reflected the ability of HMGB1 to induce IL-18 synthesis and secretion, possibly in synergy with other proinflammatory molecules. The interaction between HMGB1 and these molecules in COVID-19 needs to be elucidated.

If the immune system was unable to induce viral clearance, the association between HMGB1 and IL-18 could potentially create a dangerous cycle leading to a cytokine storm. Pyroptotic death-induced IL-18 release also triggers HMGB1 release to the extracellular environment. The HMGB1 isoform strongly influences receptor usage and, thus, its functional consequences. Pyroptosis triggers the release of disulfide HMGB1 with the capability to bind TLR4, a pivotal mediator for proinflammatory cytokine production [55]. Another HMGB1's role in the pathogenesis of COVID-19 was reported. HMGB1 enhanced angiotensin 1-converting enzyme-2 (ACE-2) expression, the primary receptor for SARS-CoV-2, in a RAGE-dependent pathway that might accelerate viral infection [17].

This study had some limitations. We could not assess the level of severity in the COVID-19 group or the comorbidity of outpatients in both the non-COVID-19 and COVID-19 groups. This information could be valuable in determining the HMGB1-IL-18 relationship in more detail. In addition, the sampling was performed only once, so we could not evaluate the dynamics of HMGB1 and the proinflammatory cytokine IL-18. Regarding the CT value, there were two different kits used to obtain this data, which could have affected the results.

5. Conclusions

Our study sheds light on HMGB1 and IL-18 concentrations in a real-world comparable setting between COVID-19 and non-COVID-19 patients. HMGB1 and IL-18 concentrations were increased in COVID-19 and non-COVID-19 inpatients. HMGB1 was associated with IL-18 concentrations in the sera of COVID-19 inpatients. The association of HMGB1 and IL-18 not only confirmed its role in the pathogenesis of COVID-19 but also indicated the potential for a dangerous cycle leading to a cytokine storm to occur.

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Data availability

The datasets collected during the current study are available from the corresponding authors at reasonable request.

CRediT authorship contribution statement

Sri Wulandari: Writing – original draft, Visualization, Investigation, Formal analysis. **Titik Nuryastuti:** Writing – review & editing, Resources, Data curation. **Farida Nur Oktoviani:** Writing – review & editing, Data curation. **Marselinus Edwin Widyanto Daniwijaya:** Writing – review & editing, Resources. **Endah Supriyati:** Writing – review & editing, Resources. **Eggi Arguni:** Writing – review & editing, Resources. **Hartono:** Writing – review & editing, Methodology. **Tri Wibawa:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26619.

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Abbreviations

HMGB1: high mobility group box 1
IL-18: interleukin-18
COVID-19: coronavirus disease-19
ELISA: enzyme-linked immunosorbent assay

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
CFR: case fatality rate
HLA: human leucocyte antigen
DAMPs: damage-associated molecular patterns
ALI: acute lung injury
TLR4: toll-like receptor 4
NF-κB: nuclear factor kappa B
RAGE: receptor for advanced glycation end-product
RT-PCR: real-time reverse transcriptase-polymerase chain reaction
CT: cycle threshold
SD: standard deviation
IQR: interquartile range
RSV: respiratory syncytial virus
MAS: macrophage activation syndrome
MAPK: mitogen-activated protein kinase
RBD: receptor binding domain (RBD)
NLRP3: NLR family pyrin domain containing 3
LPS: lipopolysaccharides
RNA: ribonucleic acid
ACE-2: angiotensin 1-converting enzyme-2