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Coronavirus Pandemic

Demystifying the role of MMP9 and TIMP-1 as markers of lung imaging and functional abnormality of COVID-19

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Abstract

Introduction: This study aimed to analyze the levels of MMP-9 and TIMP-1 as biomarkers for identifying lung anatomical and functional abnormalities in coronavirus disease 2019 (COVID-19).

Methodology: Adult COVID-19 patients hospitalized between October and December 2021 were included in the study. MMP-9 and TIMP-1 levels were measured from the blood. Chest X-ray was categorized using the Brixia index. A blood gas analysis was performed in the emergency room.

Results: MMP-9 was higher in severe COVID-19 patients (1,430.09 ± 1,492.22) than in non-severe cases (819.90 ± 750.13) with $p < 0.05$, but not different between mortality groups. TIMP-1 was lower in non-severe cases (4.88 ± 3.49) than in severe cases (8.61 ± 9.09) with $p > 0.05$. The increase in MMP-9 was correlated to TIMP-1 with a linear regression value $R^2 = 0.945$. Lung abnormalities were categorized as normal (6.9%), mild (23.6%), moderate (29.2%), and severe (40.3%). Brixia score was significantly correlated with FiO_2 (r = 0.547), PaO_2/FiO_2 (r = – 0.317), and SpO_2/FiO_2 (r = – 0.476). MMP-9 and TIMP-1 were significantly correlated with the Brixia score (r = 0.315 and r = – 0.234, respectively), and PaO_2/FiO_2 (r = – 0.291 and r = 0.283, respectively). MMP-9 was significantly correlated with severity (r = 0.313).

Conclusions: MMP-9 and TIMP-1 were related to lung imaging, functional abnormalities, and severity; but were not associated with mortality.

Key words: COVID-19; X-ray; extracellular; matrix; respiratory; infection.

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Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic caused by a beta corona virus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) [1], with varying degrees of severity [2]. The greater the severity of COVID-19, the greater the inflammation [3]. Various pro-inflammatory cytokines released from multiple immune cells damage the lungs and endothelium. Cytokines and chemokines trigger a cytokine storm. The pro-inflammatory cytokine IL-6 can trigger increased angiogenesis, vascular permeability, keratin proliferation, and collagen formation, which impact lung lesions [4,5]. The lung healing process occurs in COVID-19 patients with angiogenesis, fibroblast activation, and collagen deposition [6–8]. When microbes are inhaled into the lungs, the lung tissues are exposed to these microbes which can cause lung damage, and the body repairs this damage by maintaining extracellular matrix (ECM) homeostasis [6]. The balance between matrix metalloproteinases (MMPs) and tissue inhibitor matrix metalloproteinases (TIMPs) contributes to ECM degradation or accumulation [9–11]. ECM accumulation damages alveolar processes in alveolar-capillary diffusion [7].

Radiological images can show lung abnormalities, such as bilateral pneumonia in the basal ground-glass opacity, which causes hypoxia [8]. Bilateral abnormality is dominant in the lower area of parenchyma [9]. COVID-19 pneumonia type H is characterized by acute respiratory distress syndrome (ARDS), hypoxemia, bilateral infiltrates, and decreased lung compliance [10,11]. Plain chest X-rays show lung abnormalities in COVID-19 patients; and even though the sensitivity is low, this examination is cheap and easy [10]. New infiltration on chest X-rays is used to

diagnose pneumonia [12]. Lung infiltration decreases oxygen saturation and increases oxygen demand. Research has shown that peripheral oxygen saturation (SpO_2) to fraction inspiration of oxygen (FiO_2) ratio below 200 can be a potential predictor of COVID-19 mortality [13]. This marker can be used for monitoring lung functional abnormalities during COVID-19 [14]. When SpO_2 falls below 91%, it indicates the need for oxygen supplementation and is related to case fatality rate (CFR). Limited access to supplemental oxygen increases COVID-19 mortality [15]. Spirometry or peak flow cannot be done during COVID-19 due to viral aerosolization.

Several studies mention the occurrence of pulmonary fibrosis in the radiological images of patients during and after COVID-19. Pulmonary fibrosis events often interfere with the patient's breathing. However, there are knowledge gaps about the role of the ECM in COVID-19 cases. Researchers are trying to find biomarkers that have the potential to be a sign of abnormalities in the ECM of the lungs. There has been little research on biomarkers of pulmonary interstitial abnormalities [16], including studies on the association of MMP and TIMP with lung abnormalities in COVID-19 patients [17]. In order to find the biomarkers, it is important to know the potential role of MMP inhibitor and TIMP exogen, in correlation with lung imaging and functional abnormalities, which may prevent the severity or mortality of COVID-19 [18,19].

MMP-9, a complex form of the MMP gelatinase family, can degrade ECM components and induce collagen, cytokine secretion, and fibroblast migration [16,20]. MMP-9 contributes to inflammation and lung tissue damage in lung injuries, and its levels significantly increase; and this is associated with the severity of COVID-19. In addition, MMP-9 levels increase before respiratory failure [21,22]. MMP-9 triggers signaling molecules like cytokines and chemokines, triggering a cytokine storm in COVID-19. It is a better predictor of mortality than sequential organ failure assessment (SOFA) scores and may be a suitable marker for lung damage and systemic progression [23–25]. MMP-9 is positively correlated with IL-6, neutrophils, and monocytes. Targeted therapy of MMP-9 may benefit COVID-19 patients [22]. Circulating TIMP-1 levels are linked to COVID-19 severity, inflammatory status, and SARS-CoV2 replication [23]; and it is a prognostic non-invasive predictor [26]. TIMP-1 is a potent inhibitor of various MMPs, including MMP-9. TIMP-1 binds to latent or pro-MMP-9. The balance of MMP and TIMP is responsible for ECM proteolysis [22,27]. Serum TIMP-1 levels may help identify patients who can benefit from the matrix metalloprotease pathway treatments [26]. However, more research is needed to fully understand these relationships.

This study aimed to evaluate the correlation between the levels of MMP-9 and TIMP-1, and chest X-ray abnormalities, oxygen impairment, severity, and mortality of COVID-19.

Methodology

Study design and setting

We performed a cohort observational study on adult COVID-19 patients hospitalized from October to December 2021. The study complied with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, version 2013) and has been reported following the STROCSS criteria [21].

Participants

The subjects included in the research had tested positive for the SARS-CoV-2 real time reverse transcriptase polymerase chain reaction (RT-PCR) swab test. Inclusion criteria were patients aged 21 to 70 years and who wished to participate in the study after signing the informed consent. Patients with various comorbidities and degrees of severity were included in the study. Patients with chronic lung diseases such as pulmonary tuberculosis or a history of anti-tuberculosis treatment, bronchial asthma, chronic obstructive pulmonary diseases (COPD), and interstitial lung diseases (ILD) were excluded from the research to reduce the bias of X-ray abnormalities. Patients with asthma, COPD, and ILD were also excluded from the study to avoid bias because these cases may have had MMP-9 and TIMP-1 disorders before COVID-19 infection. The investigation did not include pregnant women and human immunodeficiency virus (HIV) / acquired immunodeficiency syndrome (AIDS) patients, because both conditions may have variations in their inflammatory processes. Maternal and placental hormones can influence inflammatory pathways. Interactions between tissue remodeling factors such as MMPs mediate response to inflammation. In addition, pregnant women tend to experience severe degrees of COVID-19 [20].

Variables

The variables analyzed in this study were epidemiological data, clinical data, oxygen use, blood tests (MMP-9, TIMP-1, blood gas analysis), and chest X-ray examination. The severity of patients was

assessed at admission, and follow-up mortality at discharge.

Clinical data

The patients' identity (age, gender), comorbidities, supporting examinations (laboratory and chest X-ray), duration of hospitalization, and condition when discharged from the hospital were recorded. Upon admission to the hospital, a physical exam was performed, which included respiratory rate, and FiO_2 and SpO_2 measurements. The World Health Organization (WHO) divided levels of severity of COVID-19 into non-severe, severe, and critical. The non-severe groups included patients with mild and moderate severity, and the severe group consisted of patients with severe symptoms who were critically ill [1]. The research sample was classified into two groups, namely, non-severe and severe. The samples were also divided into survivor and non-survivor groups.

Laboratory examination

Blood samples were collected from participants 1 to 72 hours after admission and analyzed using a Sysmex XM-1000 Automatic Hematology Analyzer (Sysmex America Inc., Lincolnshire, Illinois, USA), and the plasma or serum were separated to measure MMP-9 and TIMP-1. The serum samples were then examined using an enzyme-linked immunosorbent assay (ELISA) Reader and iMarkTM Microplate Absorbance Reader (Bio-Rad, Hercules, California, USA), using specific kits from Elabscience Biotechnology [28]. Blood samples were centrifuged at 3000 rpm for 15 minutes using an Eppendorf 5720 centrifuge (Eppendorf AG, Hamburg, Germany) to separate out the serum. The resulting serum was taken and stored in a refrigerator at – 80 degrees Celsius. The study followed standard examination protocols and involved careful handling of blood samples [28]. A microtiter plate with standard or sample was taken, and incubated for 90 minutes at 37 °C. Biotinylated Detection Ab Working Solution (Abcam**,** Cambridge, UK) was added to each well, incubated for 60 minutes, and followed by repeated aspiration and washing. The plate was then incubated with antibodies horseradish peroxidase conjugate working solution specific to MMP-9 or TIMP-1 and then read at 450 nm. The absorbance of the color change was measured, and the intensity was proportional to the concentration of MMP-9 or TIMP-1 in the sample. The data was then calculated and analyzed using standard software, comparing absorbance values to a standard curve [28].

Chest X-Ray interpretation

A chest X-ray (using Siemens General X-Ray MultixSwing, Siemens Healthineers, Munich, Germany) was taken in the emergency room to evaluate pulmonary abnormalities and distribution. A junior radiologist and a junior pulmonologist reviewed digital images using the Brixia index. A senior radiology doctor confirmed the differences. Chest X-ray images were divided into 2 × 3 areas and analyzed. The Brixia index, a chest X-ray technique, identified six zones based on lung abnormality detection. The upper zones (A, D) were above the aortic arch's inferior wall; the middle zones (B, E) were between the right inferior pulmonary vein's wall; and the aortic arch's inferior border, and the lower zones (C, F) were under the lung's base. Brixia lung scores were 1, interstitial infiltrates; 2, dominant interstitial infiltrates with alveolar infiltrates; and 3, interstitial infiltrates with predominant alveolar infiltrates [24]. The Brixia scores were then categorized into normal (scores 0), mild (scores 1–6), moderate (scores 7–12), and severe (scores 13–18) categories. The researchers used data from each reviewer to simplify the categories and shorten the gaps between each assessment.

Statistical analyses

Statistical analysis was done using IBM SPSS 26 (IBM Corp, Armonk, NY, USA). Research data were summarized in tables and figures. Interval data were tested for normality using Kolmogorov Smirnov test. The data were tested using an independent t-test for comparison and a Pearson test for correlation to check if the distribution was normal. The differentiation of both groups was analyzed using an independent Mann-Whitey U test and correlation test with Spearman Rho to determine if the distribution was abnormal. Path analysis was done using Lisrel 8.8. Statistical significance was determined at $p < 0.05$ [29].

Results

Characteristics of samples

The 72 patients who participated in the study were divided into groups based on severity (50% severe and 50% non-severe) and mortality (68% survivors and 32% non-survivors). The average age of patients was 50.79 ± 12.29 years, 19.4% were elderly (> 60 years), and 54.16% were males (Table 1). Patients with

Table 1. Characteristics of samples included in the study.

Characteristics	Non-severe (n = 36)	Severe (n = 36)	*p*	Survivor (n = 52)	Non-survivor (n = 20)	*p*
Age (years)	49.86 (14.13)	51.72 (10.25)	0.89	50.08 (12.43)	52.65 (12.05)	0.09
Gender						
Male	22 (61.1%)	17 (47.2%)	0.34	30 (57.7%)	9 (45%)	0.50
Female	14 (38.9%)	19 (52.8%)		22 (42.3%)	11 (55%)	
Geriatric (> 60 years)	9 (25%)	5 (13.5%)	0.37	10 (20.4%)	4 (17.4%)	0.51
Comorbidity						
No	26 (72.2%)	16 (44.4%)	0.02*	34 (65.4%)	8 (40%)	0.03*
1 comorbidity	9 (25%)	13 (36.1%)		14 (26.9%)	8 (40%)	
≥ 2 comorbidities	1 (2.7%)	7 (19.4%)		4 (7.7%)	4 (20%)	
Length of stay (days)	13.08 (8.06)	11.25 (7.78)	0.63	12.29 (7.31)	11.85 (9.52)	0.48
Duration of stay						
< 3 day(s)	0	3 (8.3%)	0.80	1 (2%)	2 (10%)	0.52
3-7 days	6 (16.7%)	9 (25%)		10 (19.2%)	5 (25%)	
>7 days	20 (55.6%)	14 (38.9%)		26 (50%)	8 (40%)	
> 14 days	10 (27.8%)	10 (27.8%)		15 (28.8%)	5 (25%)	
Outcome				-	-	-
Alive	32 (88.9%)	17 (47.2%)	0.00*			
Died	4 (11.1%)	19 (52.8%)				
Severity	-	-				0.00*
Non-severe				34 (65.3%)	2 (10%)	
Severe				18 (34.7%)	18 (90%)	

$p < 0.05$ was considered significant when using the Mann-Whitney U test.

comorbidities had a significantly higher degree of severity (55.5%, $p = 0.002$) and mortality (Table 1).

Statistical analysis

Kolmogorov-Smirnov analysis indicated abnormal data distribution; therefore, we used the Mann-Whitey U test (differentiation) and the Spearman Rho test (correlation). Age, gender, and duration of hospitalization were not related to oxygen demand (FiO_2, PaO_2/FiO_2, SpO_2/FiO_2), Brixia, MMP-9, and TIMP-1.

Oxygen parameters and Brixia score

The group with a severe need for oxygen (FiO_2 65.86%) was significantly larger than the non-severe group (FiO_2 41.03%, $p = 0.000$; Table 2). FiO_2 for non-severe patients was 41.03 ± 18.49% and for survivors it was 47.48 ± 21.51%. The measurement of FiO_2 was equal to 21% + (4 × number of O_2 liters supplementation). FiO_2 of 41.03% and 47.48% were equivalent to 5–7 liters of oxygen per minute. Meanwhile, severe patients (FiO_2 65.86 ± 19.88%) and dead patients (FiO_2 68.95% ± 18.70%) required higher FiO_2, equivalent to oxygen above 10 liters per minute. PaO_2/FiO_2 and SpO_2/FiO_2 in severe and non-survivor groups were significantly lower than in non-severe and survivor groups.

The severe patients had Brixia scores significantly ($p < 0.05$) higher than non-severe (13.94 ± 4.21 vs 6.92 ± 4.42) and dead vs survivor patient (13.95 ± 4.47 vs

Table 2. Comparison of oxygen need, laboratory results, and chest X-ray in severity (severe and non-severe) and mortality (survivor and non-survivor) subgroups.

	Non-severe (n = 36)	Severe (n = 36)	*p*	Survivor (n = 52)	Non-survivor (n = 20)	*p*
FiO_2	41.03 (18.49)	65.86 (19.88)	0.000*	47.48 (21.51)	68.95 (18.70)	0.000*
PaO_2/FiO_2	232.13 (158.77)	142.85 (54.15)	0.004*	188.13 (128.74)	152.89 (72.82)	0.127
SpO_2/FiO_2	270.83 (118.89)	162.97 (68.99)	0.000*	240.80 (116.23)	154.77 (62.76)	0.002*
MMP-9 (ng/mL)	819.90 (750.13)	1,430.09 (1,492.22)	0.041*	886.93 (868.59)	1,216.56 (1,316.77)	0.637
TIMP-1 (ng/mL)	4.88 (3.49)	8.61 (9.09)	0.106	5.24 (3.47)	7.32 (8.01)	0.950
MMP-9/TIMP-1	170.44 (55.06)	169.95 (33.67)	0.338	165.34 (51.23)	171.07 (43.22)	0.204
Brixia	6.92 (4.42)	13.94 (4.21)	0.000*	9.08 (5.38)	13.95 (4.43)	0.001*
Zone A	0.78 (0.83)	2.19 (1.03)	0.000*	1.19 (1.06)	2.25 (1.12)	0.001*
Zone B	1.46 (1.01)	2.47 (0.73)	0.000*	1.75 (1.01)	2.25 (0.76)	0.003*
Zone C	1.50 (0.91)	2.56 (0.80)	0.000*	1.81 (1.01)	2.60 (0.75)	0.003*
Zone D	0.67 (0.71)	1.78 (1.04)	0.000*	1.04 (0.99)	1.70 (1.08)	0.010*
Zone E	1.22 (0.898)	2.42 (0.87)	0.000*	1.62 (1.07)	2.35 (0.87)	0.009*
Zone F	1.33 (0.98)	2.53 (0.77)	0.000*	1.71 (1.07)	2.50 (0.82)	0.005*

$p < 0.05$ was considered significant when using the Mann-Whitney U test. FiO_2, fraction Inspiration of oxygen; MMP, matrix metalloproteinases; PaO_2, partial pressure of oxygen; SpO_2, peripheral oxygen saturation; TIMP, tissue inhibitor matrix metalloproteinases.

Table 3. Correlation among variables.

	Severity	Mortality	Brixia	MMP-9	TIMP-1	MMP-9/TIMP-1
Comorbidity	r = 0.313 *p* = 0.007*	(-)	(-)	(-)	(-)	(-)
Mortality	r = 0.458 *p* = 0.000*	(-)	r = 0.405 p = 0.000*	(-)	(-)	(-)
Brixia score	r = 0.650 *p* = 0.000*	r = 0.405 p = 0.000*	(-)	r = 0.315 p = 0.007*	r = -0.234 p = 0.048*	r = 0.335 p = 0.004*
FiO_2	r = 0.539 *p* = 0.000*	r = 0.471 p = 0.000*	r = 0.547 p = 0.000*	r = 0.263 p = 0.025*	(-)	r = 0.279 p = 0.004*
PaO_2/FiO_2	r = -0.348 *p* = 0.009*	(-)	r = -0.317 p = 0.018*	r = -0.291 p = 0.031*	r = 0.283 p = 0.036*	(-)
SpO_2/FiO_2	r = -0.483 *p* = 0.000*	r = -0.374 p = 0.001*	r = -0.476 p = 0.000*	(-)	(-)	r = 0.252 p = 0.032*
MMP-9	r = 0.242 *p* = 0.041*	(-)	r = 0.315 p = 0.007*	(-)	r = 0.928 p = 0.000*	(-)
TIMP-1	(-)	(-)	r = -0.234 p = 0.048*	r = 0.928 p = 0.000*	(-)	(-)

p value < 0.05 was considered significant. (-) *p* value not significant. The correlation test used the Kendall-Tau test for categorical data (comorbidity, severity, and mortality) and the Spearman correlation test for continuous data (MMP-9, TIMP-1, FiO_2, PaO_2/FiO_2, SpO_2/FiO_2, and MMP-9/TIMP-1). There was no correlation between the age, gender, length of stay, and the various variables (severity, mortality, Brixia score, MMP-9 level, TIMP-1 level, and ratio of MMP-9/TIMP-1). FiO_2, fraction Inspiration of oxygen; MMP, matrix metalloproteinases; PaO_2, partial pressure of oxygen. SpO_2, peripheral oxygen saturation; TIMP, tissue inhibitor matrix metalloproteinases.

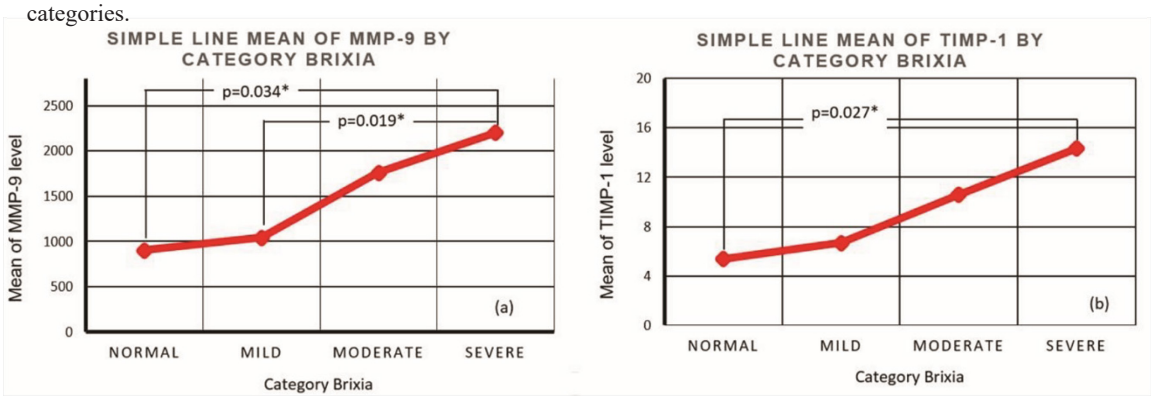
9.08 ± 5.38) (Table 2). Each zone of Brixia in severe patients was also higher than non-severe patients. Brixia scores in each area in non-survivors were significantly higher than in survivors. Basal zones (C and F) scores were higher than other middle and upper zones. Brixia scores had a significant (p = 0.000) positive moderate correlation with severity (r = 0.650) and mortality (r = 0.405; Table 3).

The patients with higher Brixia scores had higher FiO_2 than lower Brixia score groups (Table 4). Patients with wide lung abnormality (high score of Brixia) had significantly higher FiO_2 (60.36 vs. 37.73, p = 0.000) and had significantly lower SpO_2/FiO_2 (180.57 vs. 299.46, p = 0.001). The ratio of SpO_2 to FiO_2 in the moderate-severe Brixia scores groups was lower than in the normal-mild Brixia scores groups

Levels of MMP-9 and TIMP-1, and Brixia scores

The expression of MMP-9 was higher in patients with lung abnormalities. The level of MMP-9 in patients with severe chest X-ray Brixia score was significantly higher than in normal chest X-ray scores (p = 0.034). MMP-9 increased in all COVID-19 hospitalized samples in this study (Table 2). Severe groups had MMP-9 levels that were significantly higher than in non-severe groups. Increasing MMP-9 was higher in non-survivor patients than in survivors, although this difference was not statistically significant. MMP-9 correlated significantly with FiO_2 and Brixia scores (Table 3); and correlated negatively and significantly with PaO_2/FiO_2. The expression of MMP-9 strongly correlated to TIMP-1 levels (r = 0.928, p = 0.000; Table 3).

Figure 1. Simple line diagrams show the means of MMP-9, TIMP-1, and Brixia. A, Mean MMP-9 compared with Brixia category. The mean Brixia of normal and mild were significantly different compared to severe category. B, Mean TIMP-1 compared with Brixia category. There was a significant difference between normal vs. severe Brixia categories.



MMP, matrix metalloproteinases; TIMP, tissue inhibitor matrix.

Table 4. Comparison of oxygen need and laboratory results in the chest X-Ray Brixia categories.

	Normal-mild Brixia n = 22	Moderate-severe Brixia n = 50	*p*
MMP-9	987.67 (1,117.25)	1,437.09 (1,381.53)	0.013*
TIMP-1	5.87 (5.80)	8.72 (9.24)	0.083
MMP9/TIMP-1	163.75 (42.75)	184.86 (48.53)	0.020*
FiO_2	37.73 (20.19)	60.36 (20.46)	0.000*
PaO_2/FiO_2	214.41 (201.70)	168.66 (91.01)	0.496
SpO_2/FiO_2	299.46 (136.58)	180.57 (73.27)	0.001*

p value of < 0.05 was considered significant in the Mann-Whitney U test. FiO_2, fraction Inspiration of oxygen; MMP, matrix metalloproteinases; PaO_2, partial pressure of oxygen; SpO_2, peripheral oxygen saturation; TIMP, tissue inhibitor matrix metalloproteinases.

Levels of MMP-9 and TIMP-1, and oxygen parameters

TIMP-1 decreased in all COVID-19 samples (Table 2). Expression of TIMP-1 in non-severe and survivor patients was lower than in severe and non-survivor patients, although this difference was not statistically significant. TIMP-1 weakly correlated with the Brixia scores (r = – 0.234) and PaO_2/FiO_2 (r = 0.283; Table 3). The increase of MMP-9 was higher in the severe category Brixia (Figure 1A), and the decrease of TIMP-1 was lower in the normal category Brixia (Figure 1B).

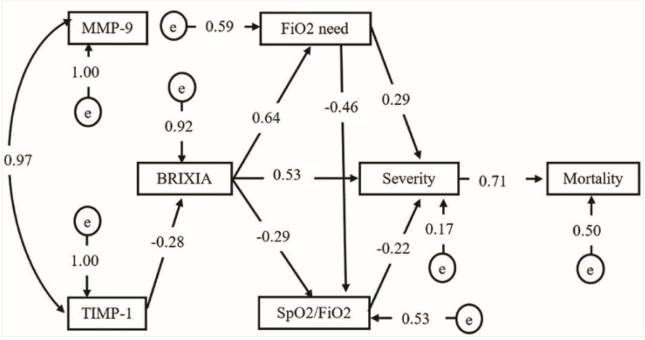
Correlation of variables

The severity significantly correlated with comorbidity (r = 0.313), mortality (r = 0.458), Brixia score (r = 0.650), FiO_2 (r = 0.539), PaO_2/FiO_2 (r = – 0.348), and SpO_2/FiO_2 (r = – 0.483). Comorbidity correlated with severity (r = 0.313, $p = 0.007$). Severity was significantly correlated to mortality (r = 0.458, $p = 0.000$) (Table 3). The addition of pulmonary infiltrates was reflected in the Brixia score which increased with the severity in COVID-19 patients. Mortality significantly correlated with Brixia score (r = 0.405), FiO_2 (r = 0.471), and SpO_2/FiO_2 (r = – 0.374). Brixia score significantly correlated with FiO_2 (r = 0.471), PaO_2/FiO_2(r = – 0.317), and SpO_2/FiO_2 (r = – 0.476). FiO_2 had a significant positive correlation with Brixia (r = 0.547), severity (r = 0.539), and mortality (r = 0.471; Table 3). In contrast, PaO_2/FiO_2 and SpO_2/FiO_2 correlated negatively to Brixia and severity ($p < 0.05$).

Table 5 shows the correlation of MMP-9, TIMP-1, and complete blood count laboratory results. There was no correlation between MMP-9, TIMP-1, leucocyte, lymphocyte, neutrophil, and neutrophil to lymphocyte ratio (NLR). A significantly low correlation existed between lymphocyte and the ratio of MMP-9/TIMP-1 (r = 0.179). There was a significant reverse low correlation between leucocyte (r = – 0.196) and NLR (r = – 0.180) with the ratio of MMP-9/TIMP-1.

Figure 2 delineates the hypothetical path analysis correlation of MMP-9 and TIMP-1 with lung abnormalities and oxygen demands that influenced the severity and mortality of hospitalized COVID-19 patients. MMP-9 directly correlated with TIMP-1 and indirectly correlated with Brixia scores (lung abnormalities). Chest X-ray abnormalities were directly related to oxygen demand (FiO_2 and SpO_2/FiO_2) and severity. Chest X-ray abnormalities and oxygen demand indirectly correlated with mortality through the severity pathway.

Figure 2. Hypothetical path analysis of correlation of MMP-9 and TIMP-1 with lung abnormalities (Brixia score, FiO2, SpO2/FiO2 ratio, severity and mortality). Chi-Square = 16.32, df = 12, p value = 0.177, RMSEA = 0.075. The path analysis was done using Lisrel 8.8.



FiO2, fraction Inspiration of oxygen; MMP, matrix metalloproteinases; SpO2, peripheral oxygen saturation; TIMP, tissue inhibitor matrix metalloproteinases.

Discussion

Characteristics of samples

COVID-19 can affect both male and female patients; and so significant difference in sample parameters was observed between genders. Previous studies have reported that COVID-19 has higher mortality in men [30], independent of age [31]. Other studies have shown no gender difference in patient mortality [34]. Studies have reported that more older people die of COVID-19 [32,33]. In this study, we did not observe any gender-based severity and mortality. No correlation existed between gender and age, and severity or mortality (Table 3).

The study found that almost 60% of severe and dead COVID-19 patients had more comorbidities than non-severe and survivor groups. This finding was consistent with previous studies, which found that 84% of

Table 5. Correlation between MMP-9, TIMP-1, and laboratory results.

	MMP-9	TIMP-1	MMP-9/TIMP-1
Leucocyte	r = 0.078 (p = 0.336)	r = 0.002 (p = 0.981)	r = -0.196 (p = 0.015)*
Lymphocyte	r = 0.058 (p = 0.475)	r = 0.016 (p = 0.846)	r = 0.179 (p = 0.026)*
Neutrophil	r = -0.19 (p = 0.812)	r = 0.010 (p = 0.899)	r = -0.133 (p = 0.098)
NLR	r = -0.058 (p = 0.475)	r = -0.019 (p = 0.815)	r = -0.180 (p = 0.025)*
Monocyte	r = -0.006 (p = 0.961)	r = -0.007 (p = 0.954)	r = -0.067 (p = 0.572)

p value at < 0.05 level was considered significant for the Spearman test. MMP, matrix metalloproteinases; NLR, neutrophil lymphocyte ratio; TIMP, tissue inhibitor matrix metalloproteinases.

COVID-19 patients have at least one comorbidity [34]. The study found no correlation between the length of stay and severity and morbidity. Meanwhile, Vishuddo *et al.* reported that severe patients died faster than non-severe patients [33]. This difference may be due to the different types of hospitals where COVID-19 patients were treated, such as tertiary or secondary referral hospitals.

Oxygen and imaging variables

Hypoxemia is very common in pneumonia and COVID-19. PaO_2/FiO_2 is the oxygenation index most frequently used to evaluate sepsis and ARDS [26]. PaO_2/FiO_2 < 300 is defined as acute lung injury (ALI) [35]. ARDS, characterized by diffuse alveolar cell damage, is often found in COVID-19 patients. SpO_2/FiO_2 can be a non-invasive prognostic for evaluating hospitalized COVID-19 patients [36]. In this study, FiO_2 positively correlated with severity and mortality in COVID-19 patients; $p < 0.05$. Severe patients needed higher oxygen supplementation (FiO_2). PaO_2/FiO_2 negatively correlated with severity. Additionally, there was a negative connection between SpO_2/FiO_2 and mortality and severity in COVID-19 patients who were hospitalized (Table 3). The SpO_2/FiO_2 ratio at hospital admission can be used for early identification of severe COVID-19 [36].

Chest X-rays are crucial in diagnosing and following hospitalized COVID-19 cases [37]. This imaging modality is the first line of detection in the emergency room for the initial triage of suspected COVID-19 patients [30]. Chest X-rays can be quick and effective diagnostic tools for tracking the progression of critically ill patients in the intensive care unit (ICU), even though they cannot detect lung involvement in the early stages of the disease. Chest X-rays can reflect lung abnormality and disease severity [37]. Scoring chest X-rays using Brixia can predict the severity and mortality of COVID-19 [38]. In this study, Brixia score in the severe group was higher than in the non-severe group. Assessment of six chest X-ray zones presented significant differences in the two severity groups with significantly different statistics. The Brixia score in the non-survivor group was higher than in those who survived. The infiltration areas in the severe and non-severe groups dominated in the lower third region (zones C and F) or basal area. Likewise, basal lung infiltration was more frequent in the survivors vs. non-survivors [39]. Thus, chest X-ray abnormality is related to clinical parameters [10].

COVID-19 inflammation and ECM

SARS-CoV-2 infiltrates the lungs, leading to an acute and severe inflammatory response induced by a cytokine storm [40]. When SARS-CoV2 binds to ACE2 receptors, it increases NG-II, activating MMP-9, causing lung injury. Severe COVID-19 has a unique immunological profile characterized by immune factors such as interferon-gamma (IFN-γ); cytokines such as IL-4, IL-5, IL-6, IL-10, IL -12, and IL-13; vascular endothelial growth factor (VEGF); thymic stroma-lymphopoietin (TSLP); MMP-1 and MMP-3; MMPs; and inflammatory cells that differ from other respiratory infections such as H1N1 [18]. MMPs were produced by parenchymal cells and inflammatory cells [41]. Growth factors (TGF, TNF), platelet-derived growth factor (PDGF), basal fibroblast growth factor (bFGF), cytokines (IL-1, IL-6), basal fibroblast growth factor (bFGF), and other hormones cause increased MMP production [18]. MMP-9 secretion increases in response to inflammation, with growth factors, cytokines, and other hormones [42]. When inflammatory mediators increase, MMP production at the site of inflammation can lead to pathological remodeling of the lung ECM [18]. MMP-9 accelerates ECM degradation processes during tissue remodeling, stimulating fibroblast migration and cytokine and collagen secretion [17]. The balancing act between MMP and TIMP causes the production and proteolysis of the ECM. A potent MMP inhibitor, TIMP-1, also inhibits MMP-9 by binding to pro- or latent MMP-9 [18].

Alteration of MMP-9 and TIMP-1 in COVID-19

MMPs are essential for remodeling physiological and pathological systems, acting at neutral pH and relying on zinc and calcium [19,20]. They are involved in wound healing, tissue repair, and injury response to

inflammation, infection, and neoplastic diseases [42]. MMPs are less expressed in tissues in normal conditions but are enhanced during injury, inflammation, ECM turnover, and repair. MMP expression is dysregulated in ARDS, suggesting MMPs may play a crucial role in disease development and resolution [18].

This study found that MMP-9 levels were elevated in COVID-19 patients of all severities. An increase in MMP-9 was found on average from 819.90 to 1430.09 ng/mL; normal range from 30 to 537 ng/mL (mean: 189 ng/mL). MMP-9 gene expression increased in COVID-19 patients [24]. In the severe and non-survivor groups, the increase in MMP-9 was higher (1430.09 ng/mL and 1264.43 ng/mL, respectively). This result was similar to another study that stated that MMP-9 was increased in COVID-19 cases and was not associated with comorbidities [24]. Other studies report that non-COVID-19 sepsis patients had MMP-9 higher than control (676 ng/mL) [43]. Thus, MMP-9 can be used as a biomarker to predict the severity of COVID-19.

Observations of MMP and TIMP levels may help explain the cause of severe sequelae after recovery [41]. Normal TIMP-1 serum levels are 48–211 ng/mL (mean 112 ± 115 ng/mL) [44]. In this study, a decrease in TIMP-1 levels was found on average from 4.88–8.61 ng/mL. TIMP-1 levels did not significantly decrease in severe and non-survivor COVID-19 patients compared to non-severe and survivors. TIMP-1 serum levels were higher in severe cases (8.61 vs. 4.88, $p = 0.106$) and non-survivors (7.32 vs. 5.24, $p = 0.950$). In this investigation, there was an inverse relationship between the rise in MMP-9 and the fall in TIMP-1 ($r = 0.928$, $p = 0.000$). This study differs from another study that mentions that an increase of TIMP-1 was higher in more severe patients (WHO ≥ 5) compared to non-severe patients (WHO < 5) [26]. Thus, TIMP-1 cannot be used as a biomarker of severity in COVID-19.

Correlation of MMP-9, TIMP-1, Brixia, and PaO_2/FiO_2 ratio

MMP-9 is significantly related to lung abnormalities (Brixia score) and oxygen demand (FiO_2). The Brixia score and PaO_2/FiO_2 ratio correlated with increase of MMP-9 and decrease of TIMP-1. MMP-9 and TIMP-1 can be used as biomarkers of lung imaging abnormalities. During the acute phase of ARDS, MMP-2 and MMP-9 mediate the repair of mechanical ventilation-induced alveolar epithelial endothelial space injury. This acute phase also promotes their expression due to hypoxia and the need for massive mechanical ventilation [18].

Our study revealed that patients with higher severity, higher oxygen demand, and more pulmonary abnormalities show a higher increase in MMP-9 levels and a decrease in TIMP-1 levels. Other studies have reported that an increase in MMP-9 was directly linked to the risk of respiratory failure [24]. This study found that MMP-9 expression in the moderate to severe Brixia score category was significantly higher than in the normal to mild Brixia score category. The ratio of MMP-9 to TIMP-1 in the moderate to severe Brixia score category was also considerably higher. The mean MMP-9 and TIMP-1 in the normal Brixia category were substantially higher than in the severe Brixia category. The study also found that a higher MMP-9 to TIM-1 ratio along with mild Brixia score resulted in less infiltrate in lung imaging, suggesting potential proteolytic activity in the lungs. Gelzo *et al.* reported that MMP-9 increased in COVID-19 patients but was unrelated to severity [22]. These results differ from our research where MMP-9 facilitates the migration of inflammatory cells and contributes to lung tissue deterioration by promoting inflammation and breaking down the protective alveolar-capillary barrier [45].

We found no correlation between MMP-9 and neutrophils or monocytes. Nevertheless, we have a low significant correlation between the MMP-9/TIMP-1 ratio to leucocyte, lymphocyte, and NLR (Table 5). Infected monocytes can also boost the production of MMP-9 [45]. The genetics of each sample may cause those differences. Ueland *et al.* found MMP-9 to be strongly correlated with neutrophil count [46].

Correlation between MMP-9, TIMP-1 with severity and mortality

MMP-9 was higher in severe COVID-19 and non-survivor patients, although this difference was not significant. MMP-9 can be used as a biomarker of severity. The MMP-9/brain-derived neurotrophic factor (BDNF) ratio can predict the severity of COVID-19 [25]. MMP-9 may help define the severity of COVID-19 [22]. TIMP-1 was decreased in this study but cannot be used as a severity biomarker in COVID-19. Mortality has a positive correlation to the severity of COVID-19. Although the MMP-9 level was higher in non-survivors of COVID-19, MMP-9 could not predict mortality. However, another study reported that MMP-9 was a better mortality predictor in COVID-19 than SOFA scores [24]. TIMP-1 levels were decreased in this study but cannot be used as a biomarker of mortality in COVID-19. Another study reports that TIMP-1 can be a promising mortality biomarker in women with ARDS [46].

MMP-9 and TIMP-1 pathway and clinical significance

The relationship between MMP-9 and TIMP-1 in the ECM is crucial to COVID-19. The hypothetical path is described in Figure 2. An increase in MMP-9 levels can lead to a decrease in TIMP-1, which affects lung abnormalities. The Brixia score, a measure of lung disorders [29,48], directly and indirectly, affects TIMP-1 levels. In this study TIMP-1 levels were lower than the normal limit, causing ECM proteolysis. MMP-9 is directly related to TIMP-1 (r = 0.97), directly and indirectly affecting lung disorders. Brixia was directly significant associated with FiO_2 (r = 0.547) and SpO_2/FiO_2 ratio (r = -0.476), and mortality was directly related to severity (r = 0.458). Lung abnormalities were related to oxygen consumption, and an increased infiltrate in the lung parenchyma increased oxygen demand. MMP-9 and TIMP-1 cannot be used as mortality biomarkers in COVID-19.

The correlation between MMP-9 and TIMP-1 levels and lung abnormalities is significant due to the body's balance in maintaining the lung ECM. MMP-9 can predict severity and lung injury in COVID-19, and there was a significant association between pulmonary anatomic abnormalities and oxygenation parameters. This could lead to research opportunities for exogenous anti-MMP-9 or TIMP-1 therapy to prevent lung injury in COVID-19 cases [18]. MMP-9 may also play a role in alveolar epithelial repair. Meanwhile, MMP inhibitors such as doxycycline, tetracycline, non-steroidal anti inflammation drugs (NSAID), glucocorticoid, and zinc inhibit this repair; potentially harming SARS-CoV-2 patients with ALI/ARDS [49].

Limitations

Our study has limitations, including the limited sample size, biases, and research methods. It also has limitations in chest radiography, as investigators could not perform chest CT scans on all patients. Blood sampling for MMP-9 and TIMP-1 examination was only done at the beginning of hospitalization, and Brixia scoring and oxygen requirement measurements were only done at the beginning. These limitations may affect the study's results. Regular examinations of these variables could help understand ECM's role in lung abnormalities.

Conclusions

COVID-19 patients exhibit an increase in MMP-9 and a decrease in TIMP-1, with higher levels in severe cases. MMP-9 can be a biomarker of COVID-19 severity and early respiratory failure. TIMP-1 decreases more in non-severe patients with lower Brixia scores and oxygen requirements. The severity of COVID-19 is related to comorbidities, mortality, Brixia score, and FiO_2; and PaO_2/FiO_2 and SpO_2/FiO_2 are inversely correlated. MMP-9 and TIMP-1 serum changes can be used as biomarkers for lung abnormalities.

This limited study shows that the balance of MMP-9 and TIMP-1 in maintaining the ECM was also found in cases of COVID-19. These results fulfil our research objectives and may be used as a basis for developing MMP-9 inhibitor therapies or administering exogenous TIMP-1. More extensive research is needed to increase insight into the role of MMP-9 and TIMP-1 in COVID-19.

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Ethics approval and consent to participate

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Universitas Airlangga Hospital, with approval number 206/KEP/2021. All participants received an explanation of the study and signed informed consent, prior to sample and data collection.

Authors' contributions

ANR, ADP: conceptualization, methodology, resources, data curation, formal analysis, writing original draft; ADS: methodology, data validation, data curation, writing original draft; JN, MA: data validation, data curation, review and editing the manuscript; supervision.

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Availability of data and materials

The datasets presented in this study are available from the corresponding author upon reasonable request.

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