Egyptian Journal of Medical Microbiology Volume 33 / No.4 / October 2024 1-9 Online ISSN: 2537-0979

ORIGINAL ARTICLE

CD177, S100A8 and S100A12 as Markers of Severity in COVID 19 Patients

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Key words:
SARS-CoV-2, COVID-19 severity, CD177, S100A8, S100A12

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ABSTRACT

Background: *Several important biomarkers associated with specific phases of the evolution of COVID-19 have been identified. CD177 is a specific neutrophil marker of activation, adhesion to the endothelium, and transmigration. The association of neutrophil activation signature with COVID-19 severity has also been described with CD177 gene being one of the most differentially expressed gene in advanced disease. S100 protein family are abundantly expressed by neutrophils. elevated expression levels of calprotectin (S100A8 and S100A12) was suggested to be associated with severe COVID-19 patients.* ***Objectives:*** *This study investigate the relation between gene expression of CD177, serum level of CD177, S100A8 and S100A12 and susceptibility to develop severe COVID-19.* ***Method:*** *This study included 60 COVID-19 patients who were hospitalized in the isolation hospital (30 severe and 30 non-severe) and 30 healthy volunteers as a control group. For all study groups CD177 expression level was measured by RT-PCR and CD177, S100A8 and S100A12 serum level was measured by ELISA technique.* ***Results:*** *The mean serum levels of CD177 gene expression were much higher in severe than non-severe group* ***(p = 0.001)****. Serum CD177 level was much higher in severe than non-severe group* ***(p = 0.0004)*** *and Serum S100A8 level was much higher in severe than non-severe group* ***(p = 0.01).*** *Our results show that combined serum S100A8 and serum CD177 levels could predict the severity of COVID-19 infections with a sensitivity 84%, Specificity 87.5%, cut off value>162.8 ng/ml (p=0.001), and >84.7ng/ml(p<0.0001) respectively.* ***Conclusion:*** *Our results suggest that gene expression of CD177, serum level of CD177 and S100A8 were positively correlated with severe COVID 19 patients while that of serum level of S100A12 was not. Also combined serum S100A8 and serum CD177 levels could predict the severity of COVID-19 infections.*

INTRODUCTION

The clinical signs of COVID-19 vary widely, with the majority of individuals presenting with mild upper respiratory tract illness and 15% of patients needing hospitalization due to severe disease. Due to severe lower respiratory tract illnesses, acute respiratory distress syndrome (ARDS), and extrapulmonary signs that might result in multiorgan failure and death, some of them need intensive care[1]

The etiology of severe diseases has been linked to dysregulated immunological responses, inflammation, and cytokine storms. Severe COVID-19 patients had high neutrophil and monocyte counts, lymphopenia, and a high neutrophil to lymphocyte ratio (N/L ratio), which is a predictor of critically ill patients mortality[2,3]

Cluster of differentiation 177 (CD177) is a specific neutrophil marker of activation, adhesion to the endothelium, and transmigration. The homing of activated neutrophils toward infected lung tissue during acute lung injury has been identified as the neutrophil activation signature. This is followed by the release of neutrophil extracellular traps (NETs), which causes an oxidative burst and the start of thrombus formation, as well as the initiation of aggressive responses. Clinically, lung autopsies from severe COVID-19 patients have shown neutrophil chemotaxis, endothelial cell infiltration, and extravasation into alveolar spaces.[4]

The neutrophil activation signature has also been linked to the severity of COVID-19, and one of the genes that is most differentially expressed in advanced illness is CD177. Clinical situations such severe infections or burns, which are linked to enhanced neutrophil production, are related with elevated levels of neutrophil CD177 mRNA. Additionally, it has been shown that there is a high correlation between the dynamics of serum CD177 levels and the severity of COVID-19 infection, ICU hospitalization, and patient survival [5].

Neutrophils exhibit extensive expression of the S100 protein family. The low molecular weight (10–14 kDa) S100 protein family comprises three distinct members: S100 calcium binding protein A8 (S100A8), S100 calcium binding protein A9 (S100A9), and S100 calcium binding protein A12 (S100A12). They share a conserved calcium binding motif known as the elongation factor hand and are all capable of binding calcium. The S100 protein designation came from their shared property of being soluble in 100% saturated ammonium sulfate at neutral pH[6].

The S100 protein family induce neutrophil recruitment, adhesion, and release from the bone marrow. They are crucial for neutrophil migration to inflammatory sites in response to bacterial infection, Lipopolysaccharide (LPS), and monosodium urate (MSU) crystals (the causative agent of gout). Silvin et al., 2020 described elevated expression levels of calprotectin (S100A8 and S100A12) in severe COVID-19 patients.[5]

Neutrophil activation is a defining characteristic of severe COVID-19 that happens prior to the development of critical disease. This study highlights relation between gene expression of CD177, serum level of CD177, S100A8 and S100A12 and susceptibility to develop severe COVID 19.

METHODOLOGY

Study design:

This case-control study was carried out at the Medical Microbiology and Immunology Department and Central Research Laboratory, Faculty of Medicine, Sohag University Hospital in collaboration with Elhelal Sohag Hospital in the period from May 2021 to May 2022. The study included 60 COVID-19 patients who were hospitalized to the isolation hospital (30 severe and 30 non-severe (mild and moderate cases)) and 30 normal subjects as controls.

The COVID-19 groups were subjected to complete clinical examinations, routine laboratory tests, and radiographic evaluations and their infection with SARS-CoV-2 was confirmed by RT-PCR. Depending on the severity of the disease, the COVID-19 patients were divided into two categories, according to the Egyptian protocol of COVID-19, which was based on symptoms, clinical examination, and chest radiography. Severe COVID-19 patients were presented with a respiratory rate of more than 30 times per minute, a room oxygen saturation of <92 at rest, and a chest CT showing at least 50% lung involvement or progressing lung lesion within 24 to 48h from the first CT on admission and non-severe COVID-19 patients were presented with a room oxygen saturation of ≥ 92 at rest, and a chest CT showing less lung involvement[7]. RT-PCR negative patients were excluded.

Ethical consideration

The study protocol was reviewed and approved by the Medical Research Ethics Committee of the Faculty of Medicine, Sohag University (No. Soh-Med-21-11-22, dated November 2021). An informed consent was taken from each participant before enrolled in the study.

Methods:

Study participants were subjected to full history taking and routine laboratory testing at the hospital laboratory, according to their routine protocols. Complete blood count (CBC) by (Genius, KT 6200, China), Random blood sugar by (Fia Biomed, Germany), Liver function tests: SGOT, SGPT and International Normalized Ratio (INR) by (autoanalyzer cobas c311 Roche⁄ Hitachi cobas system, Switzerland) , serum ferritin by (mini-VIDAS system kit Biomerieux, USA), D-dimer by (Wondfo Finecare FIA Meter, India), CRP by (Wondfo Finecare FIA Meter, India). Serum urea and creatinine by (diamond, Germany). Patients' data were obtained from hospital records. All such information for the study groups were collected and recorded in a data sheet.

I- Quantitative estimation of serum CD177 and S100A8, &S100A12 concentration by ELISA:

Approximately 15 ml of venous blood were obtained from all participants and divided into 2 aliquots in the appropriate sterile vacutainers. The first one was 10 ml blood transferred to a plain tube for estimation of serum level of CD177, S100A8, and S100A12 by ELISA. It was centrifuged at 5300 rpm for 20 minutes, serum was collected from the upper part of the tube, divided into three 1.5 ml microcentrifuge tubes and were stored at -20 till ELISA technique was done.

A- Quantitative estimation of serum CD177 concentration by ELSA assay (Sinogeneclon Biotech Co., Ltd, China) according to the manufacturer's :

Fifty µL standards were pipetted to the testing standard well. 40 µl sample dilution was pipetted to the testing sample well, then 10µl testing sample was added. The plate was covered with the adhesive strip and was incubated for 30 min at 37℃. The adhesive strip was uncovered, the liquid was discarded, and washing buffer 300 µL for each well was pipetted to every well for the 30s then drained, and then was repeated 5 times. 50 µL HRP-Conjugate reagent was pipetted to each well, except blank well. The plate was covered with the adhesive strip provided and was incubated for 30 min at 37℃. Second wash was done as the first was step. Fifty µL Chromogen Solution A and Chromogen Solution B were pipetted to each well and were preserved in absence of light for 15 min at 37℃. 50 µL Stop Solution was pipetted to each well to stop the reaction, the color of solution changed from blue to yellow. Blank well was taken as zero. Absorbance was read by Microplate reader (Stat Fax, USA) 2100 model at 450 nm after pipetting Stop Solution within 15 min.

B- Quantitative estimation of serum S100 calcium binding protein A8 & S100 calcium binding protein A12 concentrations by ELSA (Elabscience Biotech Inc, United States) according to the manufacturer's:

One hundred µL of standard or sample was added to each well. The plate was covered with the adhesive strip and was incubated for 90 min at 37.The adhesive strip was uncovered; liquid was discarded. 100 µL of biotinylated detection Ab/Ag was added. The plate was covered with the adhesive strip provided and was incubated for 1 hour at 37℃. The adhesive strip was uncovered, liquid was discarded, washing buffer was pipetted 300 µL for each well to every well for 30 second then was drained, then was repeated 3times. 100 µL HRP-Conjugate reagent was pipetted to each well, except blank well. The plate was covered with the adhesive strip provided and was incubated for 30 min at 37℃ **.** The adhesive strip was uncovered, liquid was discarded, washing buffer was pipetted 300 µL for each well to every well for 30 second then was drained, then was repeated 5 times. 90 µL for substrate reagent was added. Incubated for 15 min at 37℃. 50 µL for stop solution was added to each well to stop the reaction. Blank well was taken as zero. Absorbance was read by Microplate reader (Stat Fax, USA) 2100 model at 450 nm after pipetting stop solution immediately.

Calculation of result:

The standard concentration drawn as the horizontal line and the OD value for the vertical. The standard curve was drawn on graph paper. The corresponding concentration was calculated according to the sample OD value by the sample curve and was multiplied by the dilution multiple. The result was the sample actual concentration.

II- Detection of CD177 gene expression using Real time PCR:

RNA extraction from whole blood:

For extraction of RNA, 5 ml peripheral blood sample was collected in tubes containing an anticoagulant (ethylene-diamine-tetra-acetic acid). RNA was extracted using total RNA mini extraction kit (spin column) from (Applied Biotechnology, China.**)**

Complementary DNA (cDNA) synthesis, amplification and detection (reverse transcription):

RNA was converted by using H-minus cDNA synthesis kit from (Applied Biotechnology, China). All steps were done following the manufacture's protocol. For random hexamer primed synthesis incubate for 5 min at 25℃ followed by 60 min at 42℃. The reaction was terminated by heating at 70℃ for 5 min. The cDNAs were then immediately freezed and stored at – 20°C till its use.

RT-PCR assay for CD177 gene expression:

It was carried out by real time PCR (STEP 1 from Thermo fisher, America) using Maxima SYBER Green/Rox q PCR Master Mix(2X). The reaction volume (25µL) contained Maxima SYBR Green/ROX qPCR Master Mix (2X) (12.5 µl), Forward Primer (1.25 µl), Reverse Primer (1.25 µl), Template DNA (2 ng), Water, nuclease-free (8 µl).

The following amplification protocol was used according to the manufacturer's: 10 min at 95°C for Initial denaturation followed by 45 cycles each of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and primer extension at 72°C for 30 s. Melting curve was analysed after the end of cycling to ensure that the amplification was specific and yielded the target RNA. The temperature was gradually elevated (from 65°C to 95°C) with monitoring the intensity of fluorescence signal.

Calculation of PCR results:

The cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold in real-time PCR. Expression of miRNAs will be reported as delta (Δ) Ct value. The ΔCt will be calculated by subtracting the Ct values of miRNA SNORD68 from the Ct values of the target miRNAs. There is an inverse correlation between ΔCt and miRNA expression level, and the lower ΔCt values will be associated with increased miRNA. The resultant normalized ΔCt values were used in calculating relative expression values by using $2^{-\Delta(Ct)}$, and these values are directly related to the miRNA expression levels. The $2^{-\Delta\Delta(Ct)}$ method will be used to determine relative quantitative levels of individual miRNAs.

Statistical analysis

Data were analyzed using STATA version 14.2. Quantitative data were represented as mean, standard deviation, median and range. As the data was not normally distributed, Kruskal Wallis test was used for comparison of three or more groups and Mann-Whitney test was used to compare two groups. Qualitative data were presented as number and percentage and compared using either Chi square test or fisher exact test. Roc curve analysis was used to detect cutoff of different markers that predict COVID 19 severity. Sensitivity, specificity, positive predictive value and negative predictive value were also calculated. Odds ratios were obtained from logistic regression analysis. Spearman correlation analysis was used for correlation analysis. Univariate logistic regression analyses were used to find the predictor of ICU admission and predictors for COVID 19 severity. $P<0.05$ was considered as statistically significant.

RESULTS

Patient characteristics

This case-control study included 60 confirmed COVID-19 hospitalized patients who were categorized according to disease severity into 30 severe patients (13 males/17 females), (mean age 65.7±13.60 years), 30 non-severe patients (16 males/14 females), (mean age 59.07±9.96years) and 30 healthy controls (18males/12

females) with (mean age 56.5±3.99 years). There was statistically significant difference between severe and non-severe patients regarding age (p=0.04). **(table 1).**

Table 1: Age and gender of the participants:

Variable	Non-severe N=30	Severe N=30	Controls N=30	P value	P1	P2	P3
Age/year							
Mean ± SD	59.07±9.96	65.7±13.60	56.5±3.99	**0.002**	**0.04**	0.97	**0.002**
Median (range)	60 (40:79)	67.5 (22:87)	55 (50:68)				
Gender							
Male	16(53.33%)	13 (43.33%)	18(60.00%)	0.43	0.44	0.60	0.20
Female	14(46.67%)	17 (56.67%)	12(40.00%)				

P value compared the three group, P1 compared non-severe & severe, P2 compared non-severe and controls, P3 compared severe and controls.

There were significant differences between severe and non-severe infected patients according to the clinical features (myalgia, diarrhea and altered conscious level) and as regarding oxygen demand (CPAP and ventilation) , as regarding comorbidities (hypertension and diabetes and as regarding CT (Extensive Bilateral GGO plus pneumonia) as regarding the outcome **(table 2).**

Table 2: Clinical and radiological data and outcome of COVID patients:

Variable	Non-severe N=30	Severe N=30	P value
Clinical features			
Fever	30 (100%)	30 (100%)	1.00
Cough	30 (100%)	29 (96.67%)	1.00
Breathless	26 (86.67%)	30 (100%)	0.11
Fatigue	23 (76.67%)	27 (90.00%)	0.17
Myalgia	5 (16.67%)	25 (83.33%)	**<0.0001**
Vomiting	2 (6.67%)	2 (6.67%)	1.00
Diarrhea	9 (30.00%)	1 (3.33%)	**0.01**
Headache	17 (56.67%)	24 (80.00%)	0.052
Rigors	2 (6.67%)	3 (10.00%)	1.00
Nausea	0	4 (13.33%)	0.11
Altered conscious level	2 (6.67%)	20 (66.67%)	**<0.0001**
Oxygen demand			
Room air	4 (13.33%)	1 (3.33%)	0.35
Simple mask	19 (63.33%)	0	**<0.0001**
Mask reservoir	7 (23.33%)	6 (20.00%)	0.75
CPAP	0	6 (20.00%)	**0.02**
Ventilator	0	18 (60.00%)	**<0.0001**
Comorbidities			
Hypertension	11 (36.67%)	25 (83.33%)	**<0.0001**
Chronic heart disease	2 (6.67%)	5 (16.67%)	0.42
Diabetes	5 (16.67%)	25 (83.33%)	**<0.0001**
Chronic chest disease	1 (3.33%)	1 (3.33%)	1.00
Smoking	17 (56.67%)	12 (40.00%)	0.20
CT chest			
Free	2 (6.67%)	0	0.49
Unilateral GGO	23 (76.67%)	3 (10.00%)	**<0.0001**
Bilateral GGO	5 (16.67%)	10 (33.33%)	0.14
Extensive Bilateral GGO plus pneumonia	0 (%)	14 (46.67%)	**<0.0001**
Treatment			
Antiviral	30 (100%)	30 (100%)	1.00
Antibiotic	30 (100%)	30 (100%)	1.00
Corticosteroid	20 (66.67%)	30 (100%)	**0.001**
Hydrochloroquine	19 (63.33%)	30 (100%)	**<0.0001**
Antifungal	4 (13.33%)	22 (73.33%)	**<0.0001**
Outcome			
Dead	7 (23.33%)	23 (76.67%)	**<0.0001**
Live	23 (76.67%)	7 (23.33%)	

GGO ground-glass opacity

Among the biochemical parameters, WBC, absolute neutrophils, absolute lymphocytes, NLR, Platelet count, Hb, INR, SGOT, SGPT, urea, ferritin, D-dimer, CRP, serum S100A8, serum CD177 and CD177 gene expression were found to be significantly different in severe and non-severe patient groups (table 3).

Table 3: Laboratory data of the study groups:

Variable	Non-severe N=30	Severe N=30	Controls N=30	P value	P1	P2	P3
CBC							
WBC*10^9/L							
Mean ± SD	3.46±1.02	7.47±3.33	5.79±1.11	**0.0001**	**0.0001**	**0.0001**	0.14
Median (range)	3.3 (1.3:5.7)	6.6 (3.5:15)	5.5 (4.4:8.2)				
Absolute neutrophils *10^9/L							
Mean ± SD	4.97±1.52	0.78±0.45	7.01±0.83	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Median (range)	5.15 (2:8.5)	0.75 (0.3:2.3)	6.7 (5.5:8.7)				
Absolute lymphocytes*10^9/L							
Mean ± SD	1.54±0.97	0.44±0.15	4.04±0.93	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Median (range)	1.2 (0.4:3.3)	0.4 (0.2:0.8)	4 (2.3:5.6)				
Absolute monocytes *10^9/L							
Mean ± SD	0.37±0.10	0.35±0.09	0.46±0.22	0.31	0.73	0.24	0.14
Median (range)	0.3 (0.2:0.6)	0.3 (0.2:0.6)	0.4 (0.2:0.9)				
N/L ratio %							
Mean ± SD	2.08±0.49	4.17±1.07	1.81±0.58	**0.0001**	**0.051**	**0.0001**	**0.0001**
Median (range)	2.1 (1.2:2.9)	4.25 (2.3:6.5)	1.7 (1:3)				
Platelet count *10^9/L							
Mean ± SD	165.3±12.40	107.67±23.58	177.77±16.69	**<0.0001**	**<0.0001**	**0.03**	**<0.0001**
Median (range)	163.5 (150:190)	114.5 (50:140)	180 (149:199)				
Hemoglobin level g/dl							
Mean ± SD	13.17±2.26	7.34±2.45	13.97±0.99	**<0.0001**	**<0.0001**	0.38	**<0.0001**
Median (range)	13.4 (3:16)	8 (3.4:12)	14 (11.3:15.4)				
INR							
Mean ± SD	0.92±0.12	1.94±0.28	0.91±0.10	**<0.0001**	**<0.0001**	1.00	**<0.0001**
Median (range)	0.9 (0.8:1.1)	1.9 (1.6:3.0)	0.9 (0.8:1.1)				
Liver function							
SGOT units/liter							
Mean ± SD	24.63±9.38	72.13±11.61	21.7±8.73	**0.0001**	**0.0001**	0.22	**0.0001**
Median (range)	23 (10:44)	72 (46:90)	23 (11:44)				
SGPT units/liter							
Mean ± SD	72.7±9.79	104.33±7.31	42.7±9.79	**0.0001**	**0.0001**	1.0	**0.0001**
Median (range)	44 (23:56)	102 (95:120)	44 (23:56)				
Renal function test							
Urea mg/dl							
Mean ± SD	26.9±2.70	91.9±6.43	6.65±0.81	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Median (range)	27.5 (23:30)	89.5 (85:102)	6.7 (4:8)				
Creatinine mg/dl							
Mean ± SD	0.72±0.21	0.72±0.20	0.64±0.21	0.27	0.98	0.17	0.15
Median (range)	0.7 (0.4:1.2)	0.7 (0.5:1.2)	0.6 (0.3:1.2)				
Serum ferritin ng/ml							
Mean ± SD	171.9±84.65	763.6±1319.5	129.43±49.30	**0.0001**	**0.0001**	**0.048**	**0.0001**
Median (range)	150 (66:400)	506.5 (23.1:7498)	135 (20:220)				
Serum D-dimer ng/ml							
Mean ± SD	533.33±660.59	2665.3±3641.9	152.0±42.46	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Median (range)	400 (100:3640)	710 (330:16000)	140 (100:260)				
Serum CRP mg/l							
Mean ± SD	25.09±27.09	69.56±53.08	3.23±1.25	**0.0001**	**0.0002**	**0.0001**	**0.0001**
Median (range)	13.5 (2:96)	60 (2.9:234)	3 (2:6)				
Serum S100A8 level ng/ml							
Mean ± SD	272.85±364.46	312.46±203.97	224.68±219.08	**0.009**	**0.01**	0.83	**0.005**
Median (range)	168.5(38:1921.8)	274 (34:1087.4)	175.6 (9:1003)				
Serum S100A12 level ng/ml							
Mean ± SD	294.22±291.56	435.27±355.69	302.41±308.16	0.44	0.26	0.87	0.27
Median (range)	212.4(39.6:1465.2)	443.25(26.2:1340.1)	210(61.2:1287)				
Serum CD177 level ng/ml							
Mean ± SD	118.16±112.72	205.72±227.92	48.17±15.52	**0.0001**	**0.0004**	**0.0001**	**0.0001**
Median (range)	80.5 (35.5:522)	117.5 (70:1225)	44.25(30.5:112)				
CD177 gene expression							
Mean ± SD	0.90±0.63	2.25±2.66	0.86±0.72	**0.0003**	**0.001**	0.67	**0.0003**
Median (range)	0.77 (0.02:2.48)	1.79 (0.15:14.39)	0.78 (0.12:4.02)				

P value compared the three group, P1 compared non-severe & severe, P2 compared non-severe and controls, P3 compared severe and controls

CBC: complete blood picture; WBCs: white blood cells; N/L: neutrophil and lymphocyte ratio; INR: international normalized ratio; SGOT: glutamic-oxaloacetic transaminase; SGPT: glutamic-pyruvic transaminase; CRP: C-reactive protein

Reference interval for laboratory marker:

CRP: negative <6mg/L

Serum Ferritin: male 17-60 year equal 70-435 ng/ml Female cyclic 10-160 Menopausal 25-280ng/ml

D-Dimer: negative <500 ng/ml

Renal function: Urea 6-24 mg/dl; Creatinine male 0.7 -1.3 Female 0.6-1.1 mg/dl

Liver function: SGOT <35 IU/L, SGPT <45 IU/L

CBC: WBC: 4-11* 109/L; Absolute neutrophils: 1.8-8.25* 109/L; Absolute lymphocyte: 0.8-4.95* 109/L; Absolute monocytes: 0-0.9*109/L; N/L ratio: 2.3-3.0 %; Hb: male 14-16 g/dl Female 12-16 g/dl; INR 0.85 -1.15

The univariate binary logistic regression analysis, indicated that 15 factors including: age, Myalgia, diarrhea, altered conscious level, antifungal treatment, WBCs, absolute lymphocytes, N/L ratio, hemoglobin level, serum ferritin, serum D-dimer, serum CRP and CD177 gene expression were correlated with severe COVID-19 infection. The multivariate logistic regression analysis, revealed that altered conscious level, absolute lymphocytes, N/L ratio, hemoglobin level, Serum ferritin, Serum CRP and CD177 gene expression were independent predictors of the severity of COVID-19 infections. **(table 4)**

Table 4: Multivariate binary logistic regression analysis for factors predictive of COVID-19 severity:

Variable	Odds ratio (95% confidence interval)	P value
Age/year	1.04 (0.93:1.17)	0.50
Myalgia	3.97 (0.06:250.93)	0.51
Diarrhea	0.05 (0:70.25)	0.42
Altered conscious level	58.51 (1.53:2232.55)	**0.03**
Unilateral GGO	0.03 (0.001:0.67)	**0.03**
Antifungal	15.66 (0.89:275.89)	0.06
WBCs	278.64 (0.86:89767)	0.06
Absolute lymphocytes	0.00000001 (0.0:0.07)	**0.02**
N/L ratio %	306.36 (1.96:47753.7)	**0.03**
Hemoglobin level g/dl	0.49 (0.33:0.73)	**0.001**
Serum ferritin ng/ml	1.01 (1.003:1.02)	**0.002**
Serum D-dimer ng/ml	1.00 (0.999:1.001)	0.24
Serum CRP mg/l	1.04 (1.01:1.08)	**0.02**
CD177 gene expression	4.72 (1.27:17.53)	**0.02**

The Roc curve for different markers in predicting COVID severity is expressed in **(table 5).**

Table 5: Cut off point, Area under the curve, sensitivity, specificity, PPV, NPV of different markers in predicting COVID severity

Marker	Cut off point	AUC (95% CI)	Sensitivity %	Specificity %	P value
Serum S100A8	>157 ng/mg	0.689 (0.557:0.803)	90.0	46.7	**0.007**
Serum S100A12	>328.5 ng/mg	0.584 (0.450:0.710)	60.0	80.0	0.291
Serum CD177 level	>84.5 ng/mg	0.768 (0.641:0.867)	93.3	56.7	**<0.0001**
CD177 gene expression	>1.684	0.748 (0.619:0.851)	56.7	90.0	**0.0001**
Combined serum S100A8 and serum CD177 levels			84%	87.5%	

DISCUSSION

This study highlights the relation between gene expression of CD177, serum level of CD177, S100A8 and S100A12 and susceptibility to develop severe COVID 19. The study included 60 confirmed COVID-19 hospitalized cases (30 severe and 30 non-severe) along with 30 healthy controls.

In this study there was a significant difference between severe and non-severe COVID- 19 infection as regarding the comorbidities; hypertension and diabetes were the most common comorbidity (83.33%) (p<0.0001). Other studies reported comparable results. Xiang etal,2020 reported that the most common comorbidities were hypertension, followed by diabetes mellitus, cardiovascular diseases and neuropathy, with non-significant differences between mild and severe cases[8]. Levy et al, 2021 reported that the most common comorbidities included hypertension, obesity, and diabetes[9]**.** Sanyaolu et al, 2020 reported that the most common comorbidities identified in these patients were hypertension (15.8%), cardiovascular and cerebrovascular conditions (11.7%), diabetes (9.4%), the less common comorbidities were respiratory illnesses (1.4%) and renal disorders (0.8%)[10].

Angiotensin receptor blockers (ARBs) and angiotensin converting enzyme (ACE) inhibitors used to treat hypertension may be associated with hypertension as a risk factor for a severe COVID-19 course. The

invasion and replication of SARS-CoV-2 in cells may be affected by modifications made to the renin-angiotensin system (RAS). It is known that those therapies may raise ACE2 levels in a variety of tissues, which may strengthen SARS-CoV-2's ability to invade those tissues[11,12].

Diabetes was linked to a worse COVID-19 outcome as well. This is most likely the result of atherosclerosis-promoting chronic inflammation, which exacerbates the symptoms. Diabetes not only makes people more susceptible to infectious diseases due to general immune system weaknesses, but it may also make viruses easier to enter and replicate. Moreover, a rise in type-1 membrane-bound protease furin has been linked to diabetes. Since furin aids in coronavirus entrance into cells, it may make viral entry and replication easier[13,14].

Neutrophils were significantly higher among severe cases compared to non-severe and controls with **(*p<0.0001*).** Neutrophilia in COVID-19 patients may be also be used as a predictive marker for disease severity. Following SARS-CoV-2 infection, the increased emergence of immature neutrophils in the blood has been directly correlated with the severity of COVID-19. Most organs experience the recruitment of neutrophils from the circulation into tissues, particularly in highly vascularized regions like the kidneys and lungs. Neutrophils invade the surrounding parenchyma and blood vessels in some way. When neutrophils encounter danger signals, they are prompted to release neutrophil extracellular traps, or NETs. Pathogens are limited and immobilized by NETs, which makes it easier for antimicrobial medicines to kill them[15].

Lymphocytes were significantly lower among severe cases compared to non-severe and controls with **(*p<0.0001*).** This was similar to the result detected in other studies [16, 17]**.** Severe cases were not only associated with lower lymphocytes, but also with lower eosinophils and platelets. One important laboratory finding that may be used as a prognostic indicator for COVID-19 patients is lymphocytopenia. The degree of lymphopenia corresponds with the severity of the illness, and a bad prognosis is indicated by absolute lymphocyte counts (ALC) less than 1000/mm3[18].

The pathological mechanism of lymphopenia during severe COVID-19 disease has multiple hypothesized mechanisms: (1) SARS-CoV-2 may directly infect cells and cause lysis or apoptosis because it is known to impact tissues and increase ACE2 expression. Additionally, lymphocytes express the ACE2 receptor on their surface (2) The cytokine storm is characterized by a notable increase in interleukins and a rise in tumor necrosis factor (TNF-α), both of which cause lymphocyte death. (3) Coexisting lactic acidosis can inhibit the growth of lymphocytes. (4) increased cytokines may also contribute to lymphoid organ atrophy [15]

Neutrophil to lymphocyte ratio was significantly higher among severe cases compared to non-severe and controls with **(*p<0.0001*).** NLR has been recognized as essential independent predictive factors for identifying at-risk COVID-19 patients. Elevated NLR indicate that patient health is deteriorating [19]**.** *Ketenci etal.2022* also concluded that NLR can be served as independent prognostic markers of disease severity in COVID-19[16.]

Platelets level were significantly lower among severe cases compared to non-severe and controls with ***(p<0.0001).*** A meta-analysis reported that a significant association between thrombocytopenia at admission and severe disease. Platelets count was significantly lower in severely ill patients[20]. Mechanisms behind thrombocytopenia in SARS-CoV 2 infection could involve the virus directly affecting megakaryocyte maturation and hematopoiesis, as well as enhanced platelet adhesion, activation, and consumption in the microcirculation of injured lung tissue[21].

This study detected that serum level of *S100A8* was significantly higher among severe cases compared to non-severe cases (*P*-value= 0.01). Similar result was reported that *S100A8* expression level were significantly higher in patients with fatal outcome (p=0.004) suggesting that neutrophil activation is associated with mortality in COVID-19 patients [22]. Also other study from Michigan, USA reported that elevated calprotectin levels in the blood of COVID-19 patients with severe disease [23]. On the other hand, there was no significant difference for serum level of *S100A12* among severe cases compared to non-severe cases and controls. Similar result was reported that *S100A12* expression was not significantly different between the moderate cases and the healthy controls[24]

In severe COVID-19, neutrophils release the protein *S100A8/A9* in large quantities, which may function as a damage associated molecular pattern(DAMP). These released substances could be major pathogen recognition receptors (PRR) signaling initiators and have a role in immunopathology. Moreover, T cell-induced IFN-γ production is suppressed by granulocytic myeloid-derived suppressor cells produced from COVID-19 cells, obstructing a crucial antiviral effector mechanism[25,5].

S100B concentration was associated with inflammation markers (Ferritin, C-Reactive Protein, Procalcitonin), and organ damage markers (Alanine Aminotransferase, Creatinine). In individuals infected with SARS CoV-2, serum S100B can serve as a measure of clinical severity and plays a part in COVID-19[26].

CD177 gene expression was significantly higher among severe cases compared to non-severe cases and controls **(*P*-value =0.001).** Also, serum level of CD177 was significantly higher among severe cases compared to the non-severe cases and controls (***p -value=0.0004).*** This finding is consistent with a prior study that

discovered a strong correlation between the CD177 gene expression and the ELISA-measured CD177 protein levels[9]. Also in other study was reported that the gene that most significantly contributed to the grouping of seriously ill individuals was CD177, whose abundance was correlated with serum levels of the CD177 protein[27].

Since CD177 is a marker exclusive to neutrophils and is the gene that exhibits the greatest variation in expression in patients, concentrated on it as a representation of neutrophil activation. The correlation between serum protein levels and CD177 gene abundance in COVID-19 patient blood emphasizes the marker's importance[29].

Acute Kawasaki disease patients who are resistant to IV immunoglobulin therapy have also been found to have elevated CD177 mRNA expression; this phenomenon has been suggested as a potential side effect of pediatric SARS-CoV-2 infection. It implies that neutrophil activation has a significant influence in the severity of respiratory virus infection by their migration toward infected lungs and in influenza-infected people[29,30].

Using the multivariate binary logistic regression analysis for the factors predictive of COVID-19 severity (ICU admission), we found that the possible independent predictive factors included altered conscious level, absolute lymphocytes, N/L ratio, hemoglobin level, serum ferritin, serum CRP and CD177 gene expression. In other study only age group and WBC were found to be significantly associated with disease outcome in the multivariable binary logistic regression model at 5% level of significance[31].

Some limitations of the current study should be considered. The first limitation was the relatively small sample size. Secondly, limited studies assessing the factor of neutrophil activation (CD177, S100A8 and S100A12). Thirdly, the younger age of control group to diseased group (ICU and non-ICU). It would be interesting to perform the same analysis in different centers and other ethnic groups.

CONCLUSION

In conclusion, findings of the current study suggested that gene expression of CD177, serum level of CD177 and S100A8 were positively correlated with severe COVID 19 while that of serum level of S100A12 was not. Thus the genetic expression of CD177 and its serum level and estimation of serum level of S100A8 in patients with COVID-19 could help to predict those who are at high risk of developing severe course of COVID-19 and who needs ICU admission. Also combined serum S100A8 and serum CD177 levels could predict the severity of COVID-19 infections.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
This manuscript has not been previously published and is not under consideration in another journal.
Funding: Authors did not receive any grants from funding agencies.

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