The role of Sirtuin1 expression in predicting COVID-19 severity and survival

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Abstract

Cytokine storm is the fundamental disorder of the immune system in COVID-19 patients, which causes multiorgan dysfunction and systemic inflammation that might result in multiorgan failure if not adequately managed. **Aim:** The study aims to evaluate the levels of silent information regulator (Sirtuin1) (SIRT1), Nicotinamide adenine dinucleotide (NAD^+), A disintegrin and metalloprotease 17 (ADAM17), and poly (ADP-ribose) polymerases (PARP1) and their relation to the pathophysiology and severity of the cytokine storm in COVID-19 patients. This is a cross-sectional study. Sixty COVID-19 cases and 24 healthy controls were included in this investigation from Chest Disease Department and Intensive Care unit, Assiut University Hospital, during winter 2021. Cases were classified into - mild-moderate and severe. Blood samples were collected to measure Sirtuin1, NAD^+, ADAM17, and PARP1 concentrations by ELISA and gene expression fold changes - by qRT-PCR. Depletion of NAD^+, Sirtuin1, and elevation of ADAM17 and PARP1 were demonstrated in COVID-19 cases relative to control cases. Moreover, the changes were more significant in severe cases and non-survivors than in others. Sirtuin1 is a good predictor of COVID-19 mortality, with a sensitivity of 80.0%, a specificity of 73.3%, and a total accuracy of 75.0%. Drugs that boost Sirtuin1 levels and hence raise NAD+ levels may be used to alleviate the cytokine storm and maybe improve COVID-19 survival.

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1. Introduction

On February 11, 2020, the WHO designated the coronavirus disease 2019 (Covid19), which is distinguished by its high infectiousness, asymptomatic incubation period, and contagiousness. COVID-19 has caused 6 million deaths worldwide as of March 3, 2022, substantially impacting public health, society, and the economy [1]. The pathogenesis of COVID-19 is intricate; however, numerous hypothesis had been advanced. The prevailing theory is that SARS-CoV-2 assaults the angiotensin-converting enzyme (ACE2) receptors in the lung, resulting in intracellular viral replication until the cell bursts, leading to the release of multiple virus particles that infect more cells. Subsequently, this also results in the activation of innate and adaptive immunity, which causes harmful tissue injury both locally and systemically as a result of the cytokine storm [2]. NAD^+-consuming enzymes with antiviral properties, including silent information regulators (sirtuins), and poly (ADP-ribose) polymerases (PARPs), play prominent roles in the immune

response during SARS-CoV-2 infection, ranging from interferon (IFN) induction regulation, expression of interferon-stimulated (ISGs) genes, to proinflammatory response promotion. Clearly, NAD^+ depletion and metabolites generated during NAD^+-consuming enzyme activities profoundly affect the clinical prognosis of SARS-CoV-2 infection due to the dependence of these antiviral enzymes on NAD^+ availability [3]. Sirtuin1 upregulation directly inhibits A disintegrin and metalloprotease 17 (ADAM17) and decreases viral replication, thereby reducing tumor necrosis factor (TNF-α) and interleukins (IL-6 and IL-1β). Conversely, absence of Sirtuin1-mediated downregulation of ADAM17 will permit viral replication, and unregulated rise in IL-1β, IL-6, and TNF-α levels [4]. Eventually, uncontrolled hyperinflammatory response that might be elicited as in case of COVID-19 [5]. Several PARP-mediated mechanisms promote innate immune responses by regulating the expression of IL-6, TNF-α, and IL-1, as well as the nuclear factor NF-κB. Yet, the suppression of PARP

was suggested to provide additional lung protection [4]. In various preclinical animal models, PARP inhibitors (PARPi) were found to reduce lung fibrosis, a common consequence of SARS-CoV-2 lung inflammation [6]. Therefore, this work aimed to estimate Sirtuin1, NAD$^+$, ADAM17, and PARP1 levels in COVID-19 individuals and their relation to cytokine storm, severity, and patient outcome.

2. Subjects and methods

This is a cross-sectional study carried out at Chest Department and Intensive Care unit, Assiut University Hospital, from December 2020 to March 2021 (winter 2021-second wave), registered at ClinicalTrials.gov ID: T04907916. The Assiut Medical School Institutional Review Board (IRB No. 17300543) approved the protocol. Consent in writing with knowledge was obtained. Participants' ages, clinical, and laboratory information were collected from hospital records. Sixty adult patients were diagnosed COVID-19 based on CT findings and confirmed by PCR. Subsequently twenty-nine were designated as mild to moderate COVID-19, while thirty-one were designated as severe COVID-19 according to clinical and radiological criteria [7]. Twenty-four subjects served as controls. Blood sample collection and handling: Venous blood samples (5 ml) were collected from the participants. Where, 3 ml of blood were separated into serum and kept at −80°C for ELISA assay and 2 ml of blood on EDTA were allocated for RNA gene expression analysis, Enzyme-linked immunosorbent assay (ELISA) investigations: Sirtuin1, ADAM17, NAD$^+$, and PARP1 concentrations were measured using commercially available ELISA kits (Cat. No.: E-EL-H1546, Cat. No.: E-EL-H2305, Cat. No. ab65348 and Cat. No. 17532 respectively) based on the manufacturer's specifications. The kits were supplied by Elabscience, Abcam, and Glory Science Co. Ltd, correspondingly and RNA extraction and Q-PCR: Total RNA was extracted from the blood using the TRIzol Reagent (catalog No. T9424) based on the manufacturer's specifications. RNA was used for reverse transcription to complementary DNA (cDNA) with the cDNA Reverse Transcription Reagent (catalog No. RT5201, SinaClon) following quantification and purity check with a NanoDrop spectrophotometer. Subsequently, cDNA was used as a template for Sirtuin1, PARP1, and ADAM17and amplified using the SYBR Green qPCR Master Mix reagent (catalog no. Q03-01-01) and the primer sets listed in Table 1. Using the Applied Biosystems 7500 Fast Real-time PCR machine (Applied Biosystems, Germany), a two-step reaction protocol was carried out as follow: an initial denaturation cycle of 95°C for 10 min, followed by 40 amplification cycles of 95°C for 15 sec and 60°C for 1 min. Gene expression values were expressed relative to healthy control samples using the $2^{-\Delta\Delta CT}$ method. Where, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the reference gene. All statistical calculations were conducted using the statistical package for social science, SPSS Inc., Chicago, IL, USA, version 22. Data were statistically described as mean ± standard deviation (Mean ± SD) or median and range when they were not normally distributed. Moreover, relative frequencies (percentages) and frequencies (number of cases) were used when they were suitable. A comparison of quantitative variables was made using the Mann-Whitney U test for non-normally distributed data and the student t-test for normally distributed data. A chi-square (χ2) test was performed to compare categorical data. To discover correlation between quantitative variables, Spearman correlation was used. The sensitivity, specificity, positive and negative predictive values, and ROC curves were calculated using the Medcalc tool. When P 0.05, the Pvalue is considered statistically significant.

3. Results

The most common presenting symptoms was fever, which was documented in 24 cases (40.0%), followed by dry cough in 12 cases (20.0%), dyspnea in 11 cases (18.3%), anosmia in seven cases (11.7%), and chest pain in six cases (10.0%), while two cases (3.3%) presented with a sore throat and one case (1.7%) presented with diarrhea and shortness of breath. Nearly half (43.3%) of the studied COVID-19 patients had comorbidities; diabetes mellitus and hypertension were the most prevalent associated comorbidities reported in ten cases (16.7%) each. Chronic obstructive pulmonary disease (COPD), renal disease and ischemic heart disease were documented in 3 (5.0%), 2 (3.3%) and 1 (1.7%) respectively (Table 2). COVID-19 patients had significantly higher ADAM17 and PARP1 and lower Sirtuin1 and NAD$^+$ than controls (P< 0.001) as assessed by ELISA and PCR Table 3. By comparing the vital signs among the studied COVID-19 cases according to disease severity we observed that pulse rate, respiratory rate and ventilation mood were significantly higher among patients with severe COVID-19 infection (P<0.001) for both. Other vital signs show no significant relation with disease severity (P>0.05) for all, by comparing the arterial blood gases and laboratory data among the studied COVID-19 cases according to disease activity we observed that, patients with severe COVID-19 infection showed higher PH, and lower PaO2, and O2 saturation (P<0.001), CRP was significantly higher among patients with severe COVID-19 infection (P<0.001). Also, patients with severe COVID-19 infection showed lymphopenia compared to patients with mild to moderate infection (P<0.001). Regarding minerals; patients with severe COVID-19 infection showed lower sodium level, and higher potassium level compared to patients with mild to moderate infection (P<0.001). Also, patients with severe COVID-19 infection showed prolonged INR, and higher serum ferritin level compared to patients with mild to moderate infection (P<0.001, for both). Other laboratory data show no significant relation to COVID-19 disease activity (Table 4). The concentration and expression level of the studied biomarkers were examined in relation to COVID-19 severity. The results showed that ADAM17 was significantly higher (P = 0.006), while Sirtuin1 was significantly lower in severe COVID-19 cases than in mild-moderate infection cases (P = 0.003) (Table 5). Non-survivor COVID-19 cases were more likely to have generalized bone pain (P = 0.045). As expected, Non-survivor COVID-19 cases were presented with comorbidities (diabetes, hypertension, renal disease, COPD and ischemic heart disease) (P = 0.035), been more likely admitted to the red zone (P = 0.001), needed assisted ventilations (P < 0.001), and suffer from severe COVID-19 infection (P = 0.002). Other studied variables including age, sex, presenting symptoms (fever, dyspnea, anosmia, dry cough, sore thorat, chest pain and diarrhea) showed no significant relationship with disease outcome (P > 0.05). The non-survivor COVID-19 cases had significantly lower Sirtuin1 (0.16 ± 0.06) "assessed by PCR" than survivors (0.27 ± 0.10), with P <

0.001. Other examined biomarkers exhibited no significant differences between COVID-19 non-survivors and survivors (P > 0.05) (Table 6). Table 7 and Figure 1 show at a cutoff value of ≤ 0.22; the areas under the ROC curves were 82.4% (95%CI: 0.714 – 0.933, P<0.001) with a sensitivity of 80.0%, specificity of 73.3%, and accuracy of 75.0%. Sirtuin1 levels were positively correlated with O_2 saturation (r=0.424, p=0.001), NAD^+ levels (r=0.530, p<0.005) and negatively correlated with INR (r=-0.324, p=0.012), ventilation mood (r=-0.602, p<0.005), PARP1(r=-0.854, p<0.005) and ADAM17 levels (r=-0.692, p<0.005) (Table 8).

4. Discussion

Syndrome of severe acute respiratory distress Coronavirus-2 (SARSCo-V2) infection seriously threatens public health. COVID-19 penetrates the lungs via ACE2 receptors, modifies multiple pathways, and induces immune cells to generate excess cytokines, causing multiple organ failures, lung injury, and mucosal inflammation [8]. The COVID-19 outbreak has caused a significant burden on the health of infected patients, particularly those with underlying comorbidities [9]. In the current work, the associated comorbidities were documented in about half of the studied cases (43.3%), where hypertension and diabetes were the most prevalent associated comorbidities. These results agree with the recent meta-analysis of Ng et al. [9], who reported that the three most pervasive comorbidities among COVID-19 cases were hypertension, obesity, and diabetes. Additionally, this work demonstrated that severe COVID-19 individuals or non-survivors had a substantially higher INR than mild disease cases or survivors. However, this finding is corroborated by a recent meta-analysis by Zinellu et al. [10], which concluded that increased INR levels were substantially linked to COVID-19 mortality and severity [10]. This observation might be explained by the fact that COVID-19-associated coagulopathy is distinguished by specific characteristics that are at least in part driven by the close connection among endothelial cells, severe acute respiratory syndrome coronavirus 2, and the causative agent [11]. Moreover, lymphocyte count has been an indicator of interest since the first descriptive investigation of COVID-19 infection in China [12]. It has been linked to the virulent COVID-19 [13]. However, it was reported that non-survivors of COVID-19 had a substantially lower lymphocyte count than survivors [13]. This finding agrees with the current study, which indicates that the cases with severe COVID-19 infection or non-survivors suffered from lymphopenia compared to patients with mild disease or survivors. This study reported that Sirtuin1 levels were reduced in COVID-19 cases vs. controls, with a marked reduction in Sirtuin1 levels in severe cases and non-survivors. In line with our findings, reduced alveolar septal expression of Sirtuin1 was recently reported in COVID-19-infected pulmonary epithelial cells, which was also more prominent in severe cases. Multiple Sirtuin1 activities, such as inhibiting glycolysis , inhibiting fatty acid synthesis, and inducing fatty acid oxidation by deacetylation of the key enzymes, indicate that Sirtuin1 deficiency is advantageous for viral replication [14]. NAD is used as a substrate for poly (ADP-ribosyl) ation (PARylation) reactions mediated by poly (ADP-ribose) polymerases, Overactivity of PARP-1 targeted by metabolic stress leads to NAD depletion and bioenergetic failure and subsequent accumulation reactive oxygen species (ROS) and cellular death, NAD^+ depletion further diminishes Sirtuin's protective function [15]. On the other side SIRT1 downregulates nicotinamide phosphoribosyltransferase NAMPT, which leads to a decrease in the concentration of NAD, further diminishing the activity of SIRT1[16]. Interestingly, by comparing the levels of studied biomarkers "ADAM17, PARP1, Sirtuin1, and NAD^+" in relation to COVID-19 disease severity, we observed that cases with severe infection had significantly higher levels of ADAM17 "assessed either by ELISA and/or PCR" and significantly lower level of Sirtuin1 "assessed either by ELISA and/or PCR" than mild-moderate cases. Thus, serum ADAM17 and Sirtuin1 levels can function as a significant COVID-19 triage and management predictive biomarker. These finding could be explained by the mechanism that SIRT1 downregulates ADAM 17, thereby controlling TNF-α formation in a negative feedback loop that secondarily influences IL-1b and IL-6 production, which are dependent on TNF-α [17]. COVID-19 disease also activates the NAD^+-dependent enzyme, poly ADP ribose polymerase 1 (PARP1). Hyperactivity of PARP1 results in depletion of cellular NAD^+ pools, leading to ATP deficiency, energy loss, and subsequent cell death. These processes have the potential to enhance the pro-inflammatory cascade. This explains the positive correlation observed between ADAM17 and PARP1, and the negative correlation between ADAM17 and Sirtuin [17]. In the current study, COVID-19 non-survivors had significantly lower Sirtuin1 levels than survivors of COVID-19 infection. Other studied biomarkers showed no relation to the survival status of the studied participants. The predictive ability of Sirtuin1 was analyzed using the ROC curve. However, the results displayed that at a cutoff ≤ 0.22 (AUC = 0.824), Sirtuin1 is a good predictor of COVID-19 mortality with a sensitivity of 80.0%, a specificity of 73.3%, and a total accuracy of 75.0%.

5. Conclusions

In conclusion, Sirtuin1 is a good predictor of COVID-19 mortality. Depletion of NAD^+, Sirtuin1, and elevation of ADAM17 and PARP1 were demonstrated in COVID-19 cases relative to controls. However, the changes were more significant in severe cases and non-survivors. Consequently, maintaining NAD^+ levels via the SIRT system can reduce COVID-19 symptoms. Therefore, emerging therapeutic targets include regulating Sirtuin levels/activity and, thus, mitochondrial bioenergetics. To diminish the cytokine storm and possibly enhance survival, drugs that augment Sirtuin levels and thereby increase NAD^+ levels in hyper-inflammatory cases may be administered.

Table 1: The sequences of the PCR primers for different genes

Gene	5′-3′ primer sequence
Sirtuin1	**Forward** TGCTGGCCTAATAGAGTGGCA **Reverse** CTCAGCGCCATGGAAAATGT
PARP1	**Forward** AAGCCCTAAAGGCTCAGAACG **Reverse** ACCATGCCATCAGCTACTCGGT
ADAM17	**Forward:** CCGCTGTGTGCCCTATGT **Reverse** CCAGGACAGACCCAA
GAPDH	**Forward:** ACCCACTCCTCCACCTTTGAC **Reverse:** TGTTGCTGTAGCCAAATTCGTT

Table 2: Demographic and clinical data of the COVID-19 patients and controls (n = 84)

Parameters	Controls (n = 24)		Cases (n = 60)		P-value
Age (years)					
• Mean ± SD	46.88 ± 5.71		51.23 ± 12.34		NS
• Median (range)	46 (34 – 57)		52.5 (30 – 70)		
Sex					
• Male	17	(70.8%)	33	(55.0%)	NS
• Female	7	(29.2%)	27	(45.0%)	
Presenting symptoms					
• Fever			24	(40.0%)	
• Dry cough			12	(20.0%)	
• Dyspnea			11	(18.3%)	
• Anosmia			7	(11.7%)	
• Chest pain			6	(10.0%)	
• Generalized bone pain			4	(6.7%)	
• Sore throat			2	(3.3%)	
• Diarrhea			1	(1.7%)	
Associated comorbidities			26	(43.3%)	
• Diabetes			10	(16.7%)	
• Hypertension			10	(16.7%)	
• Ischemic Heart Disease			1	(1.7%)	
• Renal Disease			2	(3.3%)	
• COPD			3	(5.0%)	

Quantitative data are presented as mean ± SD and median (IQR); qualitative data are presented as numbers (percentage). NS = non-significant, Significance defined by P < 0.05, SD = standard deviation, Mann-Whitney U test was used to compare differences in means between groups. COPD = Chronic Obstructive Pulmonary Disease.

Table 3: Biomarker levels of the COVID-19 patients and controls (n = 84)

Biomarkers	Controls (n=24)	Cases (n=60)	P-value
• **ADAM17 concentration** (pg/ml)	967.0 (783.5-1260.8)	2822.4 (1466.7-3261.43)	**< 0.001**
• **ADAM17 expression**	0.86 (0.79 - 0.89)	4.2 (1.85 - 5.25)	**< 0.001**
• **PARP1 concentration** (ng/ml)	4.57 (3.26 - 5.74)	8.29 (6.45 - 9.57)	**< 0.001**
• **PARP1 expression**	0.48 (0.43 - 0.83)	1.82 (1.65 - 1.92)	**< 0.001**
• **Sirtuin1concentration** (ng/ml)	0.84 (0.69 - 1.12)	0.48 (0.42 - 0.56)	**< 0.001**
• **Sirtuin1 expression**	1.23 (0.87 - 1.28)	0.24 (0.14 - 0.28)	**< 0.001**
• **NAD$^+$ concentration** (ng/ml)	7.61 (6.73 - 8.24)	4.42 (2.41 - 6.94)	**< 0.001**

Quantitative data are presented as mean ± SD and median (IQR); qualitative data are presented as numbers (percentage). NS = non-significant, Significance defined by P < 0.05, SD = standard deviation, Mann-Whitney U test was used to compare differences in means between groups. COPD = Chronic Obstructive Pulmonary Disease, PCR (Polymerase Chain Reaction) expressed by ΔΔCt.

Table 4: Vital signs and laboratory data among the studied COVID-19 cases (n=60)

Vital signs	Mild to moderate cases (n=29)	Severe cases (n=31)	P value
Temperature (°C)	39 (39 – 40)	39 (39 – 40)	NS
Systolic BP (mmHg)	120 (120 – 120)	120 (120 – 120)	NS
Diastolic BP (mmHg)	80 (80 – 80)	80 (80 – 80)	NS
Pulse rate (beats/min)	98 (94 – 100)	110 (100 – 120)	**<0.001**
Respiratory rate (breath/min)	22 (20 – 22)	30 (28 – 33)	**<0.001**
GCS	12 (12 – 14)	14 (12 – 14)	NS
Ventilation Mode	Room Air: 8 (27.6%) Nasal Cannula: 7 (24.1%) HFNC: 2 (6.9%) Venturi Mask: 12 (41.4%)	Nasal Cannula: 4 (12.9%) Venturi Mask: 2 (6.5%) NRM: 8 (25.8%) NIV: 7 (22.6%) MV: 10 (32.3%)	**< 0.001**
Arterial Blood gases			
• Ph	7.4 (7.4 - 7.5)	7.5 (7.5 - 7.5)	**<0.001**
• PaCO2 (mmHg)	33 (27 – 40)	30 (30 – 32)	**0.025**
• PaO2 (mmHg)	72 (70 – 80)	59 (55 – 60)	**<0.001**
• O2 saturation%	85 (85 – 90)	70 (70 – 80)	**<0.001**
• HCO3	22.0 (19.5 - 28.5)	24.0 (20.0 - 24.0)	NS
CRP	55.0 (40.0 - 80.0)	110.0 (80.0-145.0)	**<0.001**
HCT %	44 (34 – 49)	42 (29 – 49)	NS
Lymphocytes (10^3/ul)	1.0 (0.9 - 1.3)	0.5 (0.42 - 0.87)	**<0.001**
Liver function			
• AST (IU/L)	35 (28 – 40)	30 (30 – 40)	NS
• ALT (IU/L)	48 (35 – 70)	48 (35 – 70)	NS
• ALB (g/l)	4.2 (3.7 - 4.5)	4.2 (3.7 - 4.5)	NS
Minerals			
• Na (mmol/l)	140 (135 – 144)	127 (120 – 130)	**<0.001**
• K (mmol/l)	3.7 (3.0 -4.1)	4.7 (4.7 - 5.5)	**<0.001**
• Ca (mmol/l)	8.5 (8.0 - 8.7)	7.3 (7.0 - 9.4)	NS
• Mg (mmol/l)	1.8 (1.8 - 2.7)	2.1 (1.8 - 3.3)	NS
Kidney function			
• Creatinine (mg/dl)	1.5 (1.2 - 2.0)	1.5 (1.3 - 1.7)	NS
• Urea (mg/dl)	20 (18 – 30)	20 (18 – 30)	NS
Coagulation profile			
• PT (s)	12.5 (12.0 - 14.4)	14.0 (12.0 - 14.4)	NS
• INR	1.08 (1.03 - 1.14)	1.8 (1.1 - 2.3)	**<0.001**
D. Dimer (ng/ml)	1.29 (0.79 - 1.65)	1.65 (0.79 - 3.65)	NS
Serum ferritin (mcg/l)	475 (340 – 590)	1290 (409 – 2115)	**0.001**

Quantitative data are presented as median (IQR), qualitative data are presented as number (percentage). Significance defined by p < 0.05, Mann Whitney U test was used to compare differences in means between groups,BP=Blood Pressure, GCS=Glasgow Coma Scale,HFNC=high flow nasal cannula,, NRM= Non-rebreather mask, NIV= Noninvasive Ventilation, MV= Mechanical Ventilation,PaO2 (partial pressure of oxygen), Paco2 (partial pressure carbon dioxide),CRP (C-reactive protein), HCT (hematocrit), ALT (alanine transaminase), AST (aspartate aminotransferase), ALB (albumin), Na (Sodium), K (Potassium), Ca (calcium), Mg (Magnesium), PT (Prothrombin Time), INR (international normalized ratio)

Table 5: Comparison of the levels of ADAM17, PARP1, Sirtuin1and NAD$^+$ according to COVID-19 disease severity (n = 60)

Biomarkers	Mild to moderate cases (n = 29)	Severe cases (n = 31)	P-value
ADAM17 concentration (pg/ml)	2145.0 (1245.0 - 2990.2)	3133.9 (2549.8 - 3456.0)	**0.006**
ADAM17 expression	3.42 (1.87 - 4.23)	5.24 (1.75 - 6.04)	**0.004**
PARP1 concentration (ng/ml)	8.21 (6.02 - 8.74)	8.92 (7.26 - 10.52)	NS
PARP1 expression	1.81 (1.62 - 1.92)	1.87 (1.73 - 1.93)	NS
Sirtuin1concentration (ng/ml)	0.52 (0.45 - 0.58)	0.45 (0.42 - 0.49)	**0.003**
Sirtuin1expression	0.26 (0.24 - 0.33)	0.15 (0.11 - 0.24)	**< 0.001**
NAD$^+$ concentration (ng/ml)	5.55 (3.25 - 6.72)	3.67 (2.31 - 7.0)	NS

Quantitative data are presented as median (IQR), Significance defined by P < 0.05, Mann-Whitney U test was used to compare differences in means between groups.

Table 6: Demographic, clinical data, and biomarker levels among survivor and non-survivors of COVID-19 cases (n = 60)

Parameters	Survivor (n = 45)		Non-survivors (n = 15)		P-value
Age (years)					NS
• Mean ± SD	50.36 ± 13.10		53.87 ± 9.58		
• Range	30 – 70		39 – 70		
Sex					NS
• Male	25	(55.6%)	8	(53.3%)	
• Female	20	(44.4%)	7	(46.7%)	
Presenting symptoms					
• Generalized bone pain	1	(2.2%)	3	(20.0%)	**0.045**
• Associated comorbidities	16	(35.6%)	10	(66.7%)	**0.035**
• Diabetes	7	(15.6%)	3	(20.0%)	NS
Zone of admission					**0.001**
• Green	5	(11.1%)	0	(0.0%)	
• Yellow	26	(57.8%)	2	(13.3%)	
• Red	14	(31.1%)	13	(86.7%)	
Ventilation mood					**<0.001**
• Non-assisted ventilation	33	(73.3%)	2	(13.3%)	
• Assisted ventilation	12	(26.7%)	13	(86.7%)	
Disease severity					**0.002**
• Mild to moderate	27	(60.0%)	2	(13.3%)	
• Severe	18	(40.0%)	13	(86.7%)	
Biomarkers • ADAM17 concentration (pg/ml)	2749.15 (1245.0 - 3254.47)		3069.03 (2456.0- 3356.46)		NS
• ADAM17 expression	3.80 (1.92 - 4.56)		5.28 (1.75 - 6.04)		NS
• PARP1 concentration (ng/ml)	8.22 (6.45 - 9.58)		8.74 (6.44 - 9.56)		NS
• PARP1 expression	1.82 (1.62 - 1.92)		1.87 (1.75 - 1.96)		NS
• Sirtuin1concentration (ng/ml)	0.49 (0.43 - 0.56)		0.48 (0.37 - 0.56)		NS
• Sirtuin1expression	0.25 (0.22 - 0.33)		0.14 (0.11 - 0.22)		**<0.001**
• NAD+ concentration (ng/ml)	5.36 (2.36 - 6.87)		3.27 (2.45 - 7.16)		NS

Quantitative data are presented as mean ± SD and range, and qualitative data are presented as number (percentage). Significance defined by $p < 0.05$. An Independent sample t-test was used to compare differences in means between groups. Chi-square or Fisher Exact tests were used to compare differences in frequency between groups.

Table 7: The best cut off, sensitivity and specificity for prediction of death among 60 studied patients with COVID-19 infection by studied biomarkers.

Markers	Cut off	95%CI	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	p-value
SIRTUIN1expression	≤ 0.22	0.714 – 0.933	80.0%	73.3%	50.0%	91.7%	75.0%	0.824	**<0.001***

PPV: positive predictive value; NPV: negative predictive value; AUC: Area under the curve; CI: confidence interval. *Significance defined by $p < 0.05$

Table 8: Correlation between biomarker levels and each other in COVID-19 patients

|		ADAM17 ELISA	ADAM17 PCR	PARP1 ELISA	PARP1 PCR	Sirtuin1 ELISA	Sirtuin1 PCR	NAD$^+$ ELISA
ADAM17 ELISA	Correlation	1	**.401**	**.808**	**.535**	**-.397**	**-.585**	**-.260**
	Sig.(2-tailed)		**< 0.005**	**< 0.005**	**< 0.005**	**< 0.005**	**< 0.005**	**.018**
ADAM17 PCR	Correlation	**.401**	1	**.390**	**.597**	**-.485**	**-.692**	**-.0458**
	Sig.(2-tailed)	**< 0.005**		**.002**	**< 0.005**	**< 0.005**	**< 0.005**	**< 0.005**
PARP1 ELISA	Correlation	**.808**	**.390**	1	**.505**	**-.357**	**-.479**	-.217
	Sig.(2-tailed)	**< 0.005**	**.002**		**< 0.005**	**.004**	**< 0.005**	.085
PARP1 PCR	Correlation	**.535**	**.597**	**.505**	1	**-.557**	**-.854**	**-.437**
	Sig.(2-tailed)	**< 0.005**	**< 0.005**	**< 0.005**		**< 0.005**	**< 0.005**	**< 0.005**
Sirtuin1 ELISA	Correlation	**-.397**	**-.485**	**-.357**	**-.557**	1	**.612**	**.313**
	Sig.(2-tailed)	**< 0.005**	**< 0.005**	**.004**	**< 0.005**		**< 0.005**	**.004**
Sirtuin1 PCR	Correlation	**-.585**	**-.692**	**-.479**	**-.854**	**.612**	1	**.530**
	Sig.(2-tailed)	**< 0.005**	**< 0.005**	**< 0.005**	**< 0.005**	**< 0.005**		**< 0.005**
NAD+ ELISA	Correlation	**-.260**	**-.458**	-.217	**-.437**	**.313**	**.530**	1
	Sig.(2-tailed)	**.018**	**< 0.005**	.085	**< 0.005**	**.004**	**< 0.005**	
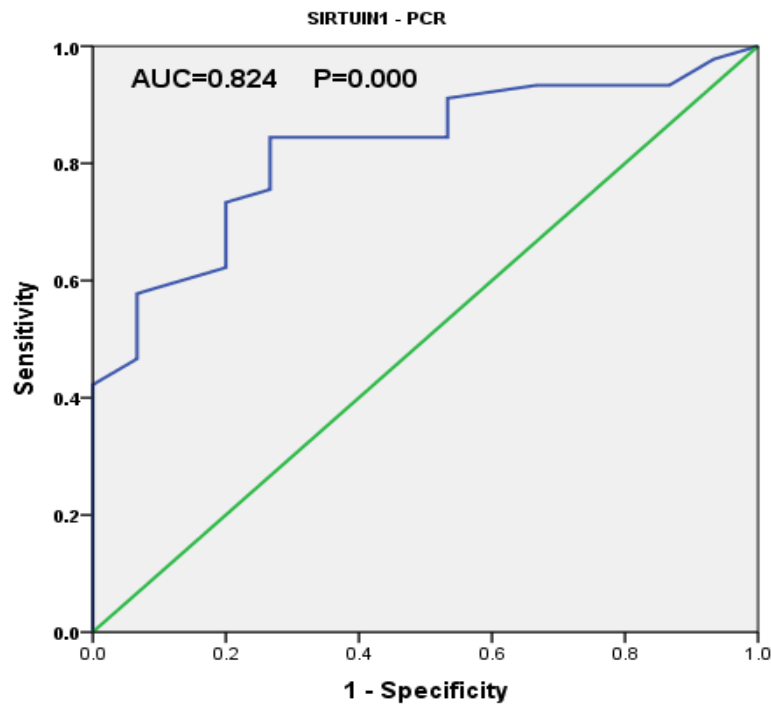
Figure 1: ROC curves for prediction of death among COVID-19 infected patients. SIRTUIN1 - PCR (blue) and reference line (green). Area under the curve = 0.824 (0.714 to 0.933), P value < 0.001.

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