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Research Article

Angiopoietin-2 levels in COVID-19 patients as a marker of endotheliopathy

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Abstract

Background: COVID-19 is described as endothelial disease, endothelial markers in COVID-19 patients could serve as an early indicator of severity of the disease, Angiopoietin -2 is an angiogenesis regulator it is released upon endothelial activation. This research seeks to assess serum level of angiopoietin-2 among COVID-19 patients requiring ICU admission compared to non-ICU patients with mild symptoms to assess the potential of angiopoietin-2 to predict ICU admission among patients with COVID-19 and investigated for the relation between angiopoietin-2 levels and COVID-19 severity markers. **Subjects and Methods:** The study included forty consecutive COVID-19 patients admitted through the emergency department who met established criteria for hospitalization. They were divided according to requirement to ICU admission in to two groups Group I included 20 patients admitted to chest ICU with severe COVID-19 symptoms and 20 patients that did not require ICU admission with mild to moderate symptoms, All COVID-19-suspected patients undergo Clinical examination, chest computed tomography (CT) scan, and laboratory assessment. **Results:** There was statistically significant increase in serum angiopoietin-2 of ICU patients when compared to non-ICU patients (P value = 0.001*) Angiopoietin-2 showed significant negative correlation with absolute lymphocytes among ICU patients (r = -0.454*, p value = 0.015*), ROC curve analysis showed higher sensitivity and specificity of Angiopoietin-2 over D dimer in predicting ICU admission for COVID 19 patients. **Conclusions:** Estimation of serum level of angiopoietin-2 could act as valuable predictor of the severity of COVID-19 patients, so It is a crucial predictive factor for direct ICU admission in COVID-19 patients and These data may be crucial for initiating treatment in the early stages of the disease.

Keywords: COVID-19; angiopoietin-2; intensive care unit

Introduction

COVID-19 is a disease caused by the novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the first human affection was reported in Wuhan china on November 17 2019, the clinical presentations of the disease are ranging from moderate upper respiratory symptoms to severe or critical states, COVID-19 can lead to respiratory distress, coagulopathy, and multiple organ dysfunction, which may result in death [(1)]. COVID-19 was described as an endothelial disease as the target receptor of SARS-CoV-2 (angiotensin converting enzyme) is strongly expressed in endothelial cells[(2)], Binding of SARS-CoV-2 to its target receptor induce endothelial damage which is rapidly followed by activation of the platelets, adhesion to subendothelial matrix and aggregation and finally the formation of platelet plug followed by the acute release of von willbrand factor and exposure of tissue factor with activation of of blood coagulation [(3)]. As a result investigating endothelial markers in COVID-19 patients could be an initial sign of intensity of the disease, several studies investigate different endothelial biomarkers including thrombo-modulin, von willbrand factor (vWF) and and PAI-1, these markers were commonly observed

to be elevated in COVID-19 patients compared to healthy individuals [4].

Angiopoietin-2 is a key regulator of angiogenesis stored within Weibel-Palade bodies. It is quickly released by activated endothelial cells by thrombin or inflammatory cytokine in response to thrombin or inflammatory cytokines. Angiopoietin-2 is a key component of the angiopoietin/Tie-2 pathway, which plays a vital role in regulating endothelial homeostasis, angiogenesis, and cell proliferation.[5]. Angiopoietin-2 induces inflammation and vascular hyperpermeability and considered as a marker of endothelial injury[6]. Circulating angiopoietin-2 levels have been shown to be elevated in patients with sepsis or ARDS and associated with mortality risk in ARDS patients. [7]. Some studies showed that angiogenesis markers show elevated levels in all hospitalized COVID-19 patients and markers of endotheliopathy were notably elevated in critically ill patients, particularly those who did not survive the illness.But there was no enough studies done indicating the importance of Angiopoietin-2 in COVID-19.Current study aims to evaluate serum level of angiopoietin-2 among patients diagnosed with COVID -19 and investigate its relation to markers of progression of COVID – 19 .

Subjects and Methods

Study design:

This prospective case-control study was performed in 40 subjects, patients were selected from chest Departments and ICU at Minia university faculty of medicine in the period from January 2022 to June 2022. All cases provided written informed consent. The study received approval from the Minia College of Medicine Ethical Committee, following the Helsinki Declaration and its revisions. Ethical approval number: (206:12/2021 Date of approval: 27 December 2021). All patients were positive for SARS-CoV-2 polymerase chain reaction (PCR) test result, and screened for hospitalization criteria based on local guidelines. All patients undergo clinical assessment, chest CT scanning, and laboratory evaluation. Our patients were divided according to requirement to ICU admission in to two groups Group I included 20 patients admitted to chest ICU with severe COVID-19 symptoms and 20 patients that did not require ICU admission with mild to moderate symptoms.

Blood Sampling Protocol:

A total of 8 mL of venous blood was drawn from each subject under sterile conditions via venipuncture. The sample was divided as follows: 1.8 mL was transferred into a tube containing 0.2 mL of trisodium citrate for the evaluation of prothrombin concentration, APTT, and D-dimer levels. Additionally, 2 mL of blood was collected in a sterile EDTA-containing tube for complete blood count (CBC) analysis. Four ml of whole blood was collected in a serum separator gel vacutainer tube for serum separation. The Serum was obtained by allowing whole blood to clot for 30 minutes at 37°C, followed by centrifugation at 3500 rpm for 15 minutes. The extracted serum was then used for renal function tests, liver enzymes, CRP, LDH, serum ferritin and the remaining serum was stored at -20°c for determination of serum Ang2.

Laboratory method:

Routine investigation: CBC was carried out using automated hematology analyzer (Celltac G, Nihon Kohden Corporation Automated Hematology Analyser, Japan). Differential leucocytic count was confirmed through microscopic examination of a Leishman-stained blood film. PC and INR were measured by (Stago analyzer, Behring diagnostic inc. USA), APTT was determined by turbodensitomertic method using (Labitec coadata 4004, Biochemical technology GmbH, Germany). D.dimer was determined by kinetic fluroscence immunoassay by using (TOSOH AIA 360 Automated Immunoassay analyzer, Japan). C-Reactive protein was measured by nephelometry by using (Genuri, biotech Inc, kinetic assay, China). Renal function tests and liver enzymes were measured by (auto-analyzer Selectra PRO XL, Elitech Group, clinical chemistry automation systems, Netherlands). Serum Ferritin was determined by kinetic fluroscence immunoassay by using (TOSOH AIA 360 Automated Immunoassay analyzer, Japan). LDH was determined by kinetic method, the kit was supplied by spectrum diagnostic Company, Germany using (Microlab 300 Ellitech, Holland). Angiopoietin- 2 was measured by ELISA, the kit was supplied by Bioassay Technology

Laboratory, China for quantative detection of human angiopoietin-2 in serum.

Statistical analysis:

Data analysis was conducted using SPSS software, version 25. The Shapiro-Wilk test was applied to assess the normality of the data. Parametric quantitative variables were reported as mean ± standard deviation (SD), along with their minimum and maximum values. Non-parametric quantitative data were presented as median and interquartile range (IQR). Qualitative data were described using frequencies and percentages.

Group comparisons were performed using the Independent Samples t-test for parametric data and the Mann-Whitney U test for non-parametric data. The Chi-square test was used to compare categorical variables. Pearson's correlation coefficient was employed to evaluate the relationship between continuous variables.

The Receiver Operating Characteristic (ROC) curve was used to determine the diagnostic performance of variables in predicting COVID-19 cases, including the calculation of the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. A p-value less than 0.05 was considered statistically significant.

Results

ICU patients group included 14 males and 16 females with mean age 63.2±14.4 years. While the non- ICU group included 16 males and 14 females with mean age 44.1±12.4 years. There was high statistically significant increase in age of ICU patients when compared to non-ICU patients **(P value = < 0.001*)** On the other hand, there was no statistically significant difference between two groups regarding to sex **(P value = 0.39).** Moreover, there was statistically significant increase in diabetic subjects in ICU patients when compared to non-ICU patients (**P value = 0.009***). Regarding hypertension. There was statistically significant increase in hypertensive subjects in ICU patients when compared to non ICU patients **(P value = 0.04*).**

For the presence of fever, There was no significantly difference when comparing between ICU patients and non-ICU patients (**P value = 0.236**).Also, for dyspnea there was no significantly difference when comparing between ICU patients and non-ICU patients (**P value = 0.06**).

For the presence of sore throat, there was no significant difference when comparing between ICU patients and non-ICU patients (**P value = 0.932**).Moreover, there were no statistical significance difference regarding loss of taste and smell and GIT manifestations when comparing ICU patients and non-ICU patients (**P value = 0.456, 0.950**) respectively.Also, there was statistical significant decrease in oxygen saturation in ICU patients when compared to non-ICU patients (**P value = <*0.001**. **(Table 1).**

Table (1): Comparison between ICU and Non-ICU patients regarding demographic data and clinical manifestations

Variables	ICU patients N=20	Non ICU N=20	P value
Age (years)			
Range	(30-89)	(23-63)	***<0.001****
Mean ± SD	63.2±14.4	44.1±12.4	
Sex n (%)			
Male	9 (46.4%)	12 (60%)	*0.39*
Female	11(53.6%)	8(40%)	
D.M. n (%)			
No	6 (28.6%)	14 (70%)	***0.009****
Yes	14(71.4%)	6(30%)	
HTN n (%)			
No	6 (28.6%)	12 (60%)	*0.04**
Yes	14(71.4%)	8(40%)	
Fever n (%)			
No	6 (28.6%)	2 (10%)	*0.236*
Yes	14(71.4%)	18(90%)	
Cough n (%)			
No	8(39.3%)	2 (10%)	*0.03**
Yes	12(60.7%)	18(90%)	
Dyspnea n (%)			
No	4 (21.4%)	10 (50%)	*0.06*
Yes	16 (78.6%)	10 (50%)	
Sore throat n (%)			
No	14 (71.4%)	14 (70%)	*0.932*
Yes	6 (28.6%)	6 (30%)	
Loss Taste & Smell n (%)			*0.456*
No	18 (89.3%)	16 (80%)	
Yes	2 (10.7%)	4 (20%)	
GIT manifestation			
No	18 (89.3%)	18 (90%)	*0.950*
Yes	2 (10.7%)	2 (10%)	
Oxygen (%)			
range	(60-96)	(97-100)	***<0.001****
Mean ± SD	89.9±6.7	98.8±0.9	

: Significant level at p value< 0.05

Renal function tests showed a highly statistically significant increase in serum urea levels in ICU patients compared to non-ICU patients (P = 0.010*). Serum creatinine levels were also significantly higher in ICU patients than in non-ICU patients (P = 0.020*). Regarding liver enzymes, there was a statistically significant increase in ALT levels in ICU patients compared to non-ICU patients (P = 0.021*). Similarly, AST levels were significantly higher in ICU patients compared to non-ICU patients (P = 0.013*). Additionally, LDH levels were significantly elevated in ICU patients compared to non-ICU patients (P = 0.026*).

As regard to hematological investigations there was no statistically significant difference in Hb level, TLC and platelets count of ICU patients when compared to non-ICU patients **(P value = 0.928) (P value = 0.136) (P value = 0.104)**

respectively. Also, there was statistically significant decrease in absolute lymphocytic count of ICU patients when compared to non-ICU patients **(P value = 0.024*).** Also, there were no statistical significances regarding monocytes %, eosinophils % and basophils when compared between ICU and non-ICU patients **(p=0.182, p=0.211, p=0.750)** respectively. No statistically significant difference was observed in prothrombin concentration between ICU and non-ICU patients (P = 0.650). Similarly, there was no significant difference in D-dimer levels between ICU and non-ICU patients (P = 0.062). However, serum angiopoietin-2 levels were significantly higher in ICU patients compared to non-ICU patients (P = 0.001*) (Figure 1).

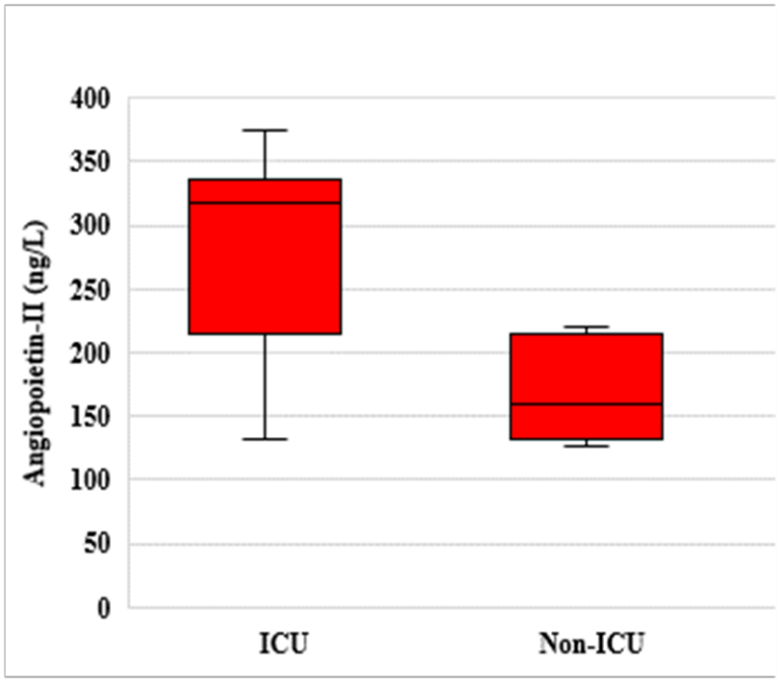

Figure (1:) shows Serum angiopoietin -2 level among studied groups

Table (2): Comparison between ICU and Non-ICU patients regarding laboratory investigation

Variables	ICU patients N=20	Non-ICU N=20	P value
Urea (mg/dl) *Median* *IQR*	52.5 (40-72.3)	35.5 (29.5-47.5)	***0.010****
Creatinine (mg/dl) *Median* *IQR*	1.3 (1-1.7)	1 (0.7-1.2)	***0.020****
ALT(U/L) *Median* *IQR*	43 (26.5-56.8)	24.5 (18.5-34)	***0.021****
AST (U/L) *Median* *IQR*	50.5 (33.3-65)	25 (20.8-38)	***0.013****
LDH (U/L) *Median* *IQR*	410.5 (249-561)	228.5 (188.3-289.3)	***0.026****
CRP (mg/l) **Median** **IQR**	24 (12-48)	18 (10.5-30)	***0.452***
S. ferritin (ng/ml) **Median** **IQR**	599.5 (310-915.5)	562.5 (431-854.3)	***0.895***
Hb (g/dl) **Range** **Mean ± SD**	(6.7-17) 12±2.6	(6.1-15.1) 11.9±3.3	***0.928***
TLC (x103 /µl) **Median** **IQR**	11.2 (6.2-15.2)	14.8 (8.6-18.3)	***0.136***
PLT (x103 /µl) **Median** **IQR**	202 (153-268.5)	308.5 (188.5-457.8)	***0.104***
Lymphocyte (%) **Median** **IQR**	7.5 (6-15.3)	10.5 (6-18.5)	***0.473***
Absolute lymphocytes (count/ µl) **Median** **IQR**	931 (661.5-1314)	1327.5 (949.5-3070)	***0.024****
Monocyte (%) **Median** **IQR**	5 (3.3-5.8)	4 (2-5.3)	***0.182***
Neutrophil (%) **Median** **IQR**	79 (70.8-84)	80 (69.5-84.8)	***0.908***
Basophil (%) **Median** **IQR**	0 (0-1)	0 (0-2)	*0.750*
Band (%) **Median** **IQR**	3 (3-6)	4 (3-5)	*0.516*

PC (%) **Range** **Mean ± SD**	 (36-100) 78.3±15.6	 (55-96) 80.5±14.8	*0.650*
INR **Range** **Mean ± SD**	 (1-2.2) 1.2±0.2	 (1-1.4) 1.2±0.1	*0.519*
APTT (seconds) **Range** **Mean ± SD**	 (27-71) 39.6±9.5	 (28-56) 38.8±8.7	*0.786*
D.D (µg/ml). **Median** **IQR**	 0.6 (0.4-1.7)	 0.4 (0.2-0.8)	*0.062*
Angiopoietin-2(ng/L) **Median** **IQR**	 317 (214.5-336.5)	 159.5 (132.3-215)	*0.001**

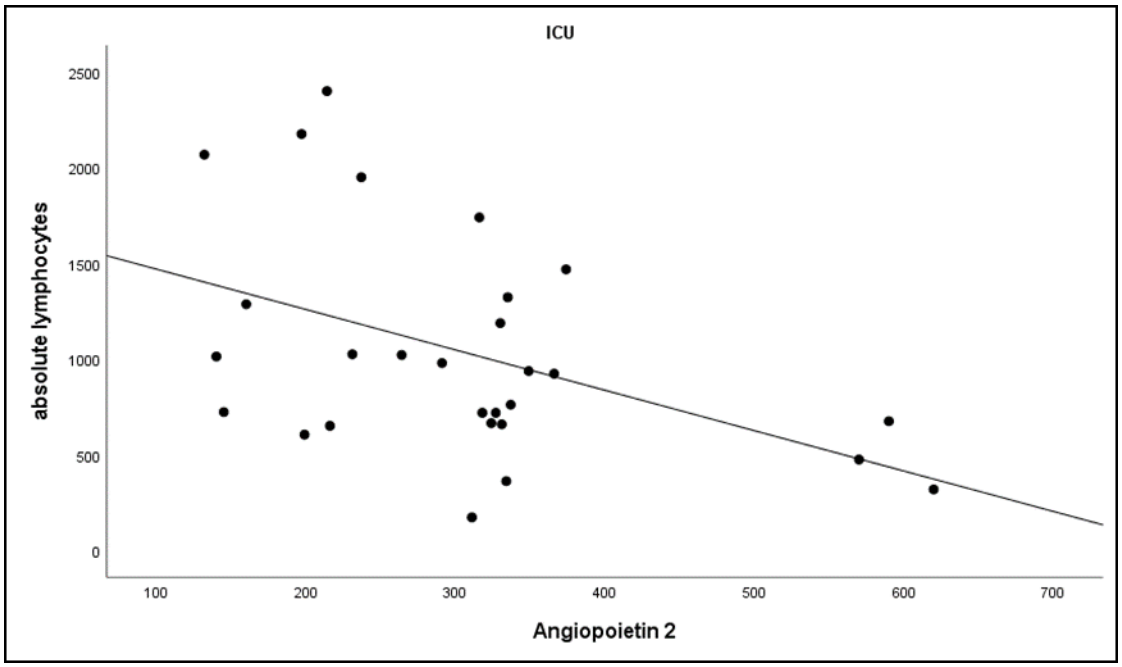

Figure (2): Negative correlation of absolute lymphocytes vs angiopoietin 2 among ICU patients

Correlations between angiopoietin-2 and different laboratory parameters in ICU patients:

Angiopoietin-2 showed significant negative correlation with absolute lymphocytes among ICU patients (r = **-0.454*,** p value = **0.015*) (Figure: 2).**

Diagnostic performance of D dimer and Angiopoietin -2 in predicting ICU admission

To determine the optimal cut-off levels that balance false-negative and false-positive rates with the best positive predictive value, a receiver operating characteristic (ROC) curve analysis was performed for D-dimer and angiopoietin-2. The area under the D-dimer ROC curve (AUC) was 0.7, with sensitivity and specificity of 67.86% and 60%, respectively, when the cut-off value was ≥ 0.4 µg/ml (P = 0.032*). The area under the angiopoietin-2 ROC curve (AUC) was 0.862, with sensitivity and specificity of 78.57% and 80%, respectively, when the cut-off value was ≥ 213 ng/L (P < 0.001*). Angiopoietin-2 demonstrated higher sensitivity and specificity than D-dimer in predicting ICU admission for COVID-19 patients (Figure 3).

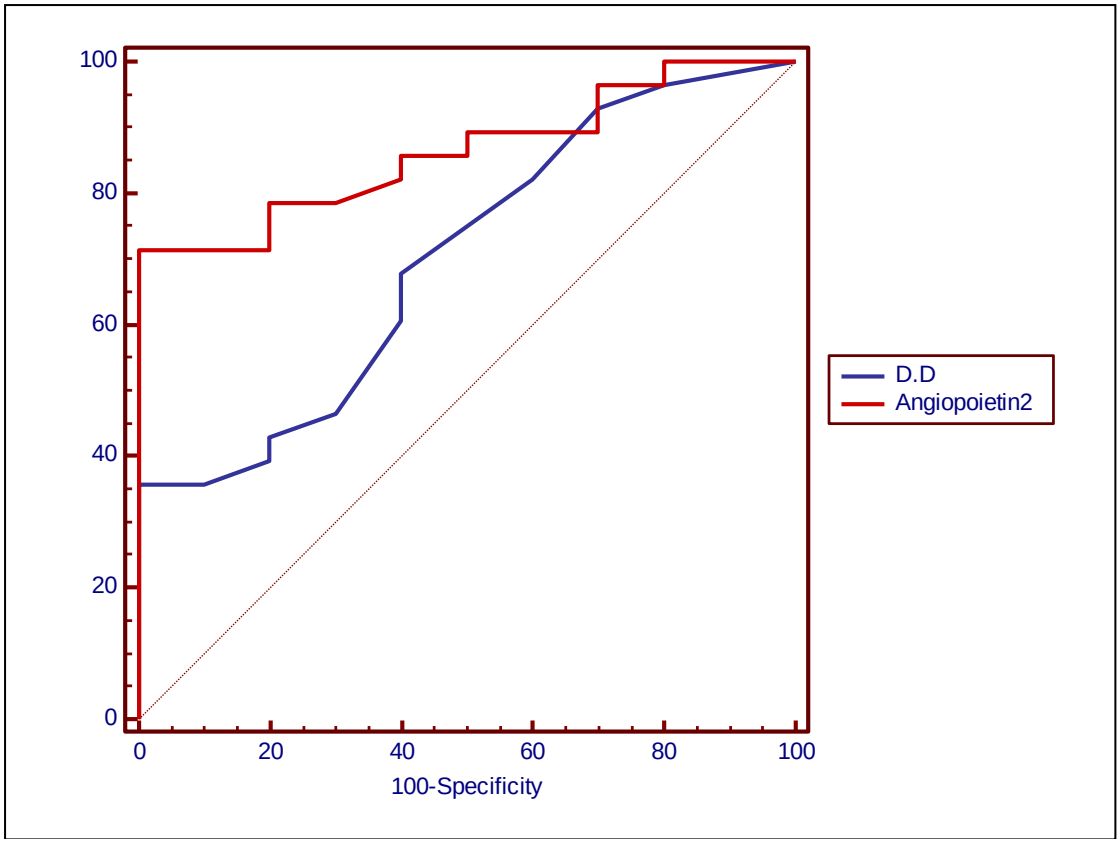

Figure (3): ROC curve analysis of D dimer and Angiopoietin -2 in predicting ICU admission for COVID-19 patients

Discussion

Endothelial dysfunction, or endotheliopathy, is a significant pathological hallmark of COVID-19 [8]. It is considered a major factor in the development of severe COVID-19 complications including induction of a profound hypercoagulable state, acute respiratory distress syndrome (ARDS) and multiple organ failure [9, 10]. In addition to COVID-19-associated coagulopathy biomarkers, novel biomarker was recognized during the initial phase of the pandemic, markers of endothelial cell activation or injury were identified. These biomarkers increase at distinct stages of the progression of COVID-19 [11]. It is important to find a marker that early anticipate the necessity of COVID-19 patients to be hospitalized in non-ICU or ICU at the time of their presentation to the emergency services and early medical interference before deterioration of the case and thus decreasing mortality rate. Angiopoietin-2 is an important biomarker of endothelial dysfunction that released from Weible palade bodies upon endothelial injury [12], It may act as an early and useful indicator of the clinical progression of COVID-19 and could be a key factor in disease pathogenesis [13]. Therefore, we conduct this study to evaluate serum level of Angiopoietin-2 in patients with COVID-19 for early detection of critically ill patients and predict the necessity of ICU hospitalization

In the current study, it was found that angiopoietin-2 serum level was markedly higher in ICU patients when compared to non-ICU patients, and this was in agreement with several studies as pine et al study[14] who Further confirmed that elevated angiopoietin-2 levels at the time of ICU admission were strongly associated with in-hospital mortality in patients with critical COVID-19.Moreover Smadja et al., [12], noted significantly elevated levels of Ang-2 in ICU-admitted COVID-19 patients. They noted that patients with extremely high Ang-2 levels had ninefold greater odds of ICU admission reinforcing the significance of the angiopoietin-2 pathway in

endothelial inflammation. Araújo et al., [15] found that elevated Ang-2 levels were significantly linked to an increased likelihood of severe AKI. Also supported by study of Li et al., [16] who reported Ang-2 levels have been observed to increase in patients with ARDS and sepsis, both linked to widespread endothelial damage, and to act as a marker of mortality risk in ARDS patients. However, our findings were contrary with study by done by Volleman et al., [17] who found that higher circulating level of angiopoietin-2 in non-ICU patients compared to initially hospitalized ICU, however they found that angiopoietin-2 level is elevated only in severely ill ICU. As They evaluated level of angiopoietin- 2 at time of admission, 1 week and 2 weeks. This discrepancy in results may be due to different time of sampling. as Regard correlation of angiopoietin-2 with other laboratory investigations ,there was significant negative correlation between andiopoietin-2 and absolute lymphocytes count (r=-0.454, p=0.015) and this was in agreement with (Ruhl et al., [18] who stated that endothelial dysfunction, along with impaired lymphocyte responses, plays a role in severe COVID-19. The current study reported higher diagnostic performance of Angiopoietin-2 (cut-off value >213 ng/L) over D-dimer (cut-off value >0.4 ng/L) with higher Ang-2 sensitivities (78.57% for Ang2 vs. 67.86% for D.D) in predicting ICU admission, to the best of our knowledge few studies[14] were done to evaluate role of angiopoietin-2 in predicting ICU admission, further studies are required validating this cutoff value in a separate group of patients, In the current study the mean age of ICU patients (63.2) years was significantly higher than Non ICU patients (51.5) years and this was in agreement with [19] who reported that there are strong indications of age dependence in clinical severity and fatality rate among COVID-19 patients. Moreover, the percentage of diabetic and hypertensive patients in ICU were significantly higher than non-ICU and this was in line with Gallo et al.,[20] who showed Hypertension may contribute to increased susceptibility to SARS-CoV-2 infection, more severe progression of COVID-19, and elevated COVID-19-related mortality. also, a study done by Qian et al., who reported that hypertensive COVID-19 patients had a significantly higher risk of admission to ICU and need for invasive ventilation. Regarding oxygen saturation, the study demonstrated a statistically significant decrease in ICU group when compared to non-ICU group and this agreed with Al-Hadrawi et al.,[21] who showed that the resulting lung injuries, often detected through abnormalities on chest CT scans, are associated with reduced oxygen saturation, which can exacerbate inflammatory responses and may persist even after apparent clinical recovery.

Moreover, there was a highly statistically significant increase in renal function test values among ICU patients compared to non-ICU patients. This finding aligns with Chen et al., [23], who reported that patients with severe COVID-19 exhibited elevated levels of serum creatinine and urea compared to those with non-severe COVID-19. Furthermore, since severe infection exacerbates kidney damage, patients with severe COVID-19 were more likely to develop acute kidney injury compared to those with non-severe disease.[24], Also there was statistically significant increase in ALT & AST enzymes of ICU patients when compared to non-ICU patients and this supported by phipps et al., study [25] who stated that COVID-19-associated sepsis can cause hypoxic injury and hepatic ischemia, leading to elevated liver enzymes. This explains the higher levels of AST and ALT observed in patients with severe COVID-19 compared to those with non-severe disease.As regarding LDH, in this study we found that serum LDH was elevated in ICU patients when compared to non-ICU patients, this was in agreement with (Huang et al., [10] who reported that elevated LDH levels were a significant marker of disease severity among COVID-19, caused by SARS-CoV-2 infection, has been shown to primarily affect the lungs, while also impacting other tissues and organs, resulting in hypoxia, thrombosis, inflammation, and multi-organ injury. that leading to elevation of serum LDH .However the current study found no significant difference when comparing serum ferritin between ICU patients and non-ICU patients, and this was in line with (Abulseoud et al., study [26] who found that **The rise in serum ferritin levels is less pronounced in critically ill covid-19 elderly patients with** concurrent mental health conditions and explained that This attenuated response may be attributed to reduced ferritin synthesis or increased ferritin degradation through ferritinophagy in these patients,

Moreover, the presence of comorbid diabetes and hypothyroidism may attenuate the ferritin response to inflammation, as the expression of the H-ferritin gene is regulated by thyrotropin, T4, T3, insulin, and IGF-1.[27] In contrary Kaushal et al., study[28] who reported that Ferritin levels were significantly elevated in patients with severe to critical COVID-19 compared to those with mild to moderate disease,Regarding absolute lymphocytic count, A statistically significant difference was observed between ICU and non-ICU patients this was in agreement with Wu et al., study[29] who stated that severe and critical COVID-19 patients exhibited significantly reduced total lymphocyte levels. Because lymphocytes express ACE2, this could be a direct result of the effects of SARS-CoV-2 infection.

Alternatively, lymphocyte depletion may stem from the exhaustion of these cells due to the aggrevated inflammatory immune response, which occurred in some patients (30). Furthermore, the production of elevated levels of pro-inflammatory cytokines may directly impair lymphocyte function, hinder lymphocyte proliferation, and trigger early apoptosis. Our study was constrained by a relatively small sample size, which could limit the ability to generalize the findings to a larger population. the study was conducted within a limited geographic area, which may affect the applicability of the results to other contexts or regions.Additional research is necessary to evaluate the relation of angiopoietin-2 to disease severity in patients with new COVID-19 variants. dynamic angiopoietin-2 assessment of COVID-19 patients admitted to hospital to assess the risk of in-hospital mortality. further studies are required to investigate the key role of angiopoitin-2 in endothelial cell disruption.

Conclusions

We concluded that the estimation of serum angiopoietin-2 levels can predict the severity of COVID-19 in patients, offering an early indication of disease progression. As such, it serves as a relevant predictive factor for direct ICU admission, and these findings may be crucial for initiating treatment at the early stages of the disease.

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