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Clinical significance of serum Krebs von den Lungen-6 levels as reliable effective marker in COVID-19 patients

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Abstract— Background: Coronavirus Disease 2019 (COVID-19) is a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) related illness that has spread globally and may worsen as pulmonary fibrosis develops. Krebs von den Lungen-6 (KL-6) has been recommended as a marker of COVID-19 and the severity of the illness. It is a chemical that is mostly expressed by injured alveolar type II cells. In order to confirm KL-6 potential as a predictive biomarker of severity in patients with COVID-19, this pilot investigation measured blood concentrations of KL-6. **Materials and Methods**: One hundred and forty patients with COVID-19 participated in the research. According to the severity of the disease, the COVID-19 patients were classified into three groups: critical disease (n=43), severe disease (n=52), and mild/moderate disease (n=45), with (n=60) healthy volunteers as the control group. Blood samples were taken as well as all of the patients' fundamental clinical and demographic information. **Results**: The blood KL-6 concentration on admission was considerably greater in each group as compared to that in all COVID-19 cases (critical, severe, and moderate) and normal control participants ($p<0.0001$), which was positively correlated with age, SBP,DBP,WBCs, neutrophil, CRP, D-dimer, ferritin, FBG, ALT, AST, and ALPwhereas, negative correlate with lymphocytes, SpO_2,albumin, and vitamin D.A cutoff value of 207.5 U/ml for KL-6 predicted severe COVID-19 with a sensitivity of 93.6% and a specificity of 88.3% (AUC: 0.965, 95%CI 0.943–0.988; $p<0.0001$). **Conclusion**: Critical and severe COVID-19 patients had considerably higher serum KL-6 concentrations, which might be a valuable indicator of the disease's severity. High blood KL-6 concentrations in the early stage of COVID-19 should be closely monitored in order to avoid the development of pulmonary fibrosis as soon as possible.

Keywords: COVID-19, Severe COVID-19, Krebs von den Lungen-6 (KL-6).

Introduction

The global spread of the coronavirus 2019 (COVID-19) pandemic has had a serious impact on millions of people's health. The scientific community is in desperate need of trustworthy COVID-19 biomarkers for screening, clinical treatment, and the avoidance of catastrophic consequences[1]. Numerous hematological, inflammatory, and biochemical variables, including as lymphocyte count, neutrophil count, ,C-reactive protein(CRP), ferritin,and D-dimer have previously been proven to be able to stratify individuals at high risk and can thus be utilized as predictive biomarkers. Krebs von den Lungen 6 (KL-6) is a glycoprotein with mucin-like properties that is primarily found on the surface of type II alveolar epithelial cells and respiratory bronchiolar epithelial cells in the normal lung[2]. It has been demonstrated that KL-6 has chemotactic and anti-apoptotic effects on fibroblast cells [3].High levels of KL-6 have been found in the epithelial lining fluid of patients with acute pulmonary infection (ALI) and lung damage when compared to controls[4, 5]. Significantly higher plasma levels of KL-6 have also been observed in non- survivors when compared to survivors of ALI. Elevated blood KL-6 levels are associated to idiopathic pulmonary fibrosis and interstitial lung disease (ILD) related to connective tissue disease severity[6–10], and they may also be a valuable indicator of early development in individuals with ILD linked to systemic sclerosis[7,9].Additionally, *Pneumocystis jivorecii* and viral respiratory infections have been linked to high levels of KL-6 in the blood[11, 12].

KL-6 has been suggested as a biomarker for infective pneumonia and acute respiratory distress syndrome (ARDS)[11–15]. KL-6 has also been suggested as a disease severity marker since the onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic[16–19].Although the exact pathogenetic mechanism of COVID-19, the lung disease caused by SARS-CoV-2, is unclear, it is assumed that increased blood levels of inflammatory cytokines and mediators of oxidative stress lead to lung damage and facilitate the development of acute respiratory syndrome (similar to ARDS).It is believed that the host's vulnerability and the virus's direct cytopathic effects against type I and type II pneumocytes are key factors in mediating and maintaining lung damage.Our research group reported elevated serum concentrations of KL-6 in critical and severe COVID-19 patients which was confirmed by other researchers[16,18, 19].

Materials and Methods

This case control study of 200 subjects was conducted age 40 to 65 years. Between January and March 2022, 140 confirmed COVID-19 patients were admitted to Al-Amal Specialized Hospital for infectious diseases in an Najaf governorate, Iraq. Patients with COVID-19 were diagnosed based on positive quantitative RT-PCR and chest x-ray or chest computed tomography (CT) scan findings, with 60 healthy participants providing as a control group with similar ages ranges to the patients. This study excluded participants with diabetes, liver illness, chronic renal disease, pulmonary disease, pregnant women, and smokers to prevent the impact of additional comorbidities. Before participating in this study, all controls and patients provided written informed permission.

The COVID-19 patients were divided into three groups upon admission to the hospital based on the clinical findings, respiration rates, oxygen saturation (SpO2) levels, and low-dose chest CT results. The classification's specifics are as follows:

Mild /Moderate illness:SpO2 of 94% on room air, minor clinical symptoms, minor respiratory symptoms, and positive low-dose CT pneumonial signals.

Severe illness: Those who meet any of the following criteria:

1. Respiratory rate >30 times per min;
2. SpO2 <94% at room air;
3. Lung infiltrates >50% on low-dose CT.

Critical disease: Patients with acute respiratory distress syndrome (ARDS) may have septic shock, multiple organ failure, and coagulation problems[20].

The research was carried out in accordance with Iraqi and international ethical and privacy rules, as well as the declaration of helsinki of the world medical association.

After an overnight fast, samples of venous blood were taken from both the patient and control groups, utilizing disposable needle and plastic syringes, between 8.00 and 9.00 a.m. We used two tubes to collect blood samples. Prior to centrifuging 3 ml at 3000 Xg for 10 minutes to extract serum, allow the sample to clot at room temperature for 10 to 15 minutes. Following that, the serum samples were separated into tubes and stored in the refrigerator at -20°C until they were ready for analysis. The rest of the blood (2 ml) was used to calculate the complete blood count. Using Biolabo® kits from Maizy, France, the levels of serum ALT, AST, ALP, albumin and FBG were measured spectrophotometrically. Fluorescence immunoassay was used to determine serum ferritin, C-reactive protein, vitamin D, and D-dimer levels were measured by (ichroma™). An autohematology analyzer was used to determine the whole blood count (linear, Spain).KL-6 levels (U/ml) were assessed in accordance with the manufacturer's instructions using the chemiluminescence assay "Lodicules1 G KL-6" (Fujirebio® Europe, UK). An automated immunoassay system was used to measure the results (LUMIPULSE G1200; Fujirebio, Inc., Tokyo, Japan).

Statistical Analyses

The statistical studies were conducted using IBM SPSS Statistics 26 software. The analyses' findings were presented as mean standard deviation. The cutoff point for statistical significance was $p<0.05$. Using the student's t-test, two independent samples were compared. The Pearson's correlation analysis was used to assess the parametric variables. Analysis of variance (ANOVA) was employed in the study to examine any variations in scale variables between categories. To establish the cutoff value for KL-6, the receiver operating characteristic (ROC) analysis approach was used. The area under curve (AUC) value was calculated using the ROC curve.

Results

The 140 patients enrolled in this study were categorized according to severity of COVID-19. Forty fivepatients had moderate disease, while remaining patients experienced either severe or critical illness (52 and 43 patients, respectively). The latter two groups of patients had a significantly higher mean age compared to patients with moderate disease (55.74±4.54, 50.71±2.36and 46.02±3.05 years, respectively; $p = 0.05$). Distributions of BMI subgroups in the three disease severity groups showed no significant differences. Patients were also defined by the laboratory parameters listed in Table 1. Means of these parameters showed significant differences between the three disease severity groups except Hb. The mean serum KL-6 levels of the patients with critical, severe and mild/moderate COVID-19 were 1198.34±169.67, 595.73±108.39 and 254.66±57.92, respectively ($p<0.0001$).Pearson's correlation analysis was performed tocalculate correlation coefficients (r) between KL-6 and laboratory parameters in COVID-19 patients.KL-6 showed significant negative correlation withSpO_2,Lymphocyte, albumin, and vitamin D(r = -0.883, -0.658, -0.742 and -0.677respectively; $p < 0.01$), while it was significant positively correlated with age, SBP, DBP, WBCs, neutrophil, CRP, D-Dimer, Ferritin, FBG, ALT, AST, and ALP(r = 0.495, 0.745, 0.292, 0.758, 0.620, 0.683, 0.844, 0.791, 0.699, 0.836, 0.819 and 0.654respectively; $p < 0.01$) (Table 2 and Figure 1).As it was shown in Figure 2, KL-6 significantly increase in the critical group in comparison with another groups(severe, moderate, and healthy control).The efficacy of KL-6 for the prediction of severe disease was evaluated by ROC analysis. AUC of KL-6 was found as 0.965(95%CI 0.943–0.988; $p<0.0001$). When the cutoff value for KL-6 in predicting of severe disease was determined to be 207.5, the sensitivity was determined as 93.6%, whereas the specificity was 88.3% (Figure 3).

Table 1. Comparison of the demographical and laboratory data of patients with COVID-19 and control groups.

Variables	COVID-19cases; n= 140			Healthy control (n=60)	p-value
	Critical (n=43)	Severe (n=52)	Mild/Moderate (n=45)		
Age (year)	55.74±4.54	50.71±2.36	46.02±3.05	50.45±5.88	0.05
Gender M/F	27/16	32/20	25/20	40/20	----
BMI (kg/m^2)	23.55±0.54	23.91±0.93	24.71±1.08	24.07±0.80	0.104
SBP (mmHg)	141.72±4.38	134.86±5.30	129.60±4.81	128.38±3.45	0.00
DBP (mmHg)	81.04±3.17	76.65±2.99	78.06±3.42	78.38±2.09	0.00
SpO_2	71.17±8.37	88.13±5.72	95.61±1.01	99.03±0.51	0.00
Hb (g/dl)	12.76±1.01	12.56±1.30	12.72±1.42	12.47±1.29	0.590

WBCs ×10^9/L	13.40±0.93	11.90±1.07	10.08±1.53	8.93±1.02	0.000
Lymphocyte ×10^9/L	2.44±0.54	2.98±0.94	4.03±0.94	4.37±0.57	0.000
Neutrophil ×10^9/L	10.14±1.53	8.36±2.06	6.93±2.05	6.18±1.30	0.000
CRP (mg/L)	40.74±6.99	33.78±9.11	29.73±7.70	3.72±1.28	0.000
D-Dimer (ng/ml)	3673.32±779.34	2735.78±879.89	1202.67±400.25	284.81±114.07	0.000
Ferritin (ng/ml)	668.58±108.65	498.25±71.65	432.42±68.99	127.90±41.72	0.000
FBG (mg/dl)	264.04±47.95	211.51±44.82	197.28±53.41	96.48±7.55	0.000
ALT (U/L)	44.23±4.07	33.94±6.42	21.21±7.50	17.01±5.37	0.000
AST (U/L)	57.23±6.24	47.01±5.21	29.22±10.42	19.66±6.11	0.000
ALP (U/L)	89.13±8.77	80.67±9.70	70.71±11.60	52.21±13.27	0.000
Albumin (g/dl)	1.78±0.38	2.68±0.75	3.30±0.84	4.18±0.51	0.000

Vitamin D (ng/ml)	16.40±5.69	23.02±7.51	30.03±8.74	70.81±11.49	0.000
KL-6 (U/ml)	1198.34±169.67	595.73±108.39	254.66±57.92	171.40±31.89	0.000

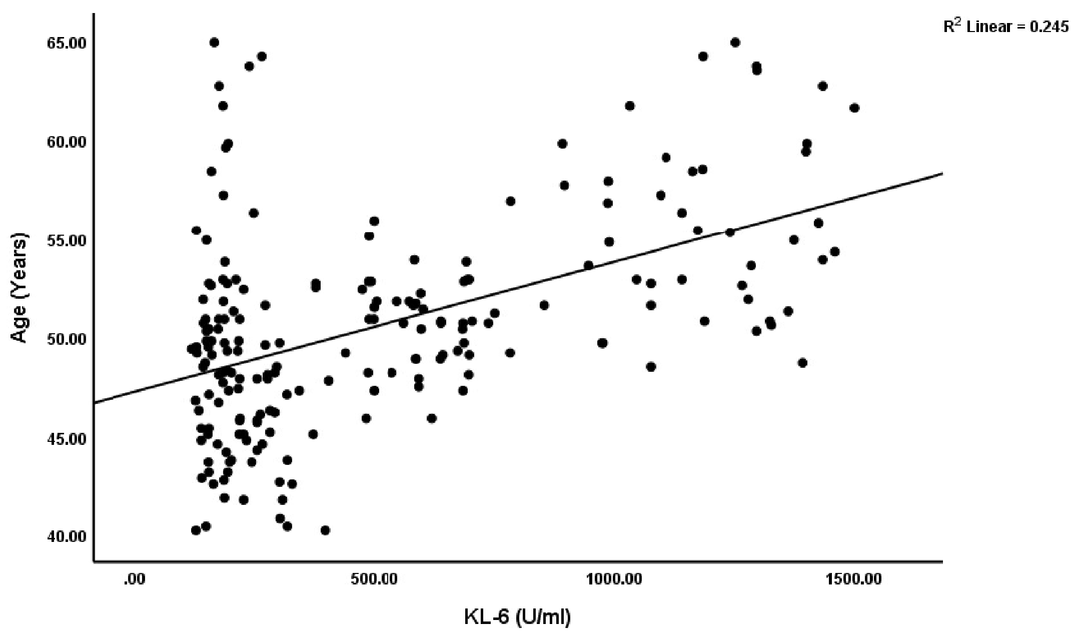
Abbreviations: M/F, Male/Female; BMI, body mass index; SBP, systolic blood pressure ; DBP, diastolic blood pressure ; SpO_2, saturation oxygen percentages ; Hb, hemoglobin; WBCs, white blood cells; CRP, C-reactive protein; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; KL-6, Krebs von den Lungen 6. Values are given as mean ± standard deviation.

Table2.The correlation between clinical parameters and serum KL-6 level in COVID-19 patients

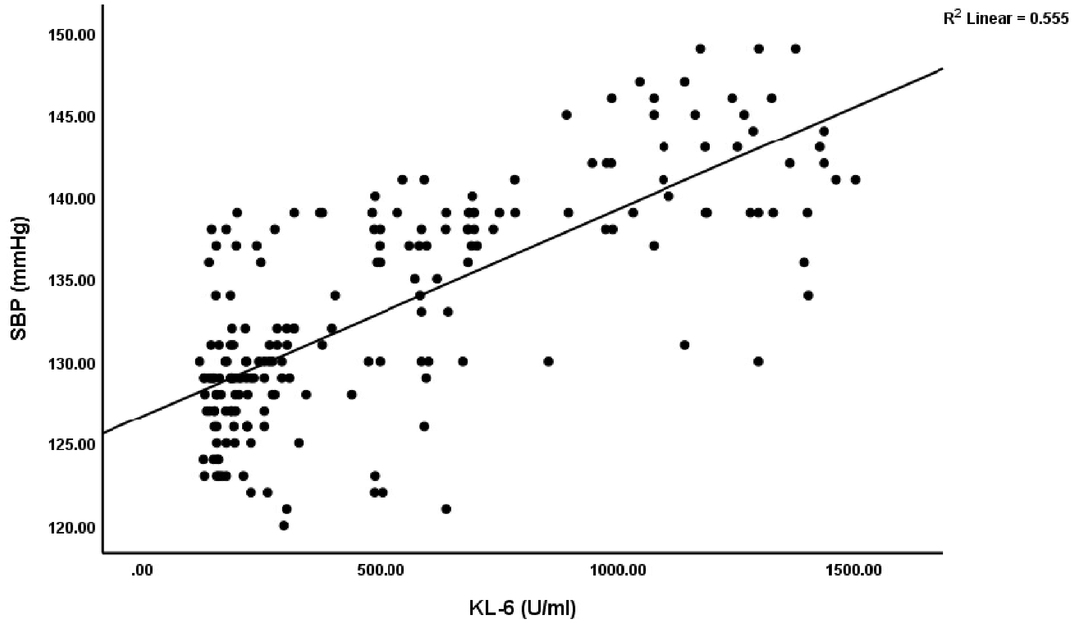
Variables	r
Age (year)	0.495*
SBP (mmHg)	0.745*
DBP (mmHg)	0.292*
SpO_2	-0.883*
Hb (g/dl)	0.06
WBCs $\times 10^9$/L	0.758*
Lymphocyte $\times 10^9$/L	-0.658*
Neutrophil $\times 10^9$/L	0.620*
CRP (mg/L)	0.683*
D-Dimer (ng/ml)	0.844*
Ferritin (ng/ml)	0.791*
FBG (mg/dl)	0.699*

ALT (U/L)	0.836*
AST (U/L)	0.819*
ALP (U/L)	0.654*
Albumin (g/dl)	-0.742*
Vitamin D (ng/ml)	-0.677*

Abbreviations:BMI, body mass index; SBP, systolic blood pressure ; DBP, diastolic blood pressure ; SpO_2, saturation oxygen percentages ; Hb, hemoglobin; WBCs, white blood cells; CRP, C-reactive protein; FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; r, Pearson's correlationcoefficient; *, p<0.01.



(A)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150.00
145.00
140.00
135.00
130.00
125.00
120.00
SBP (mmHg)
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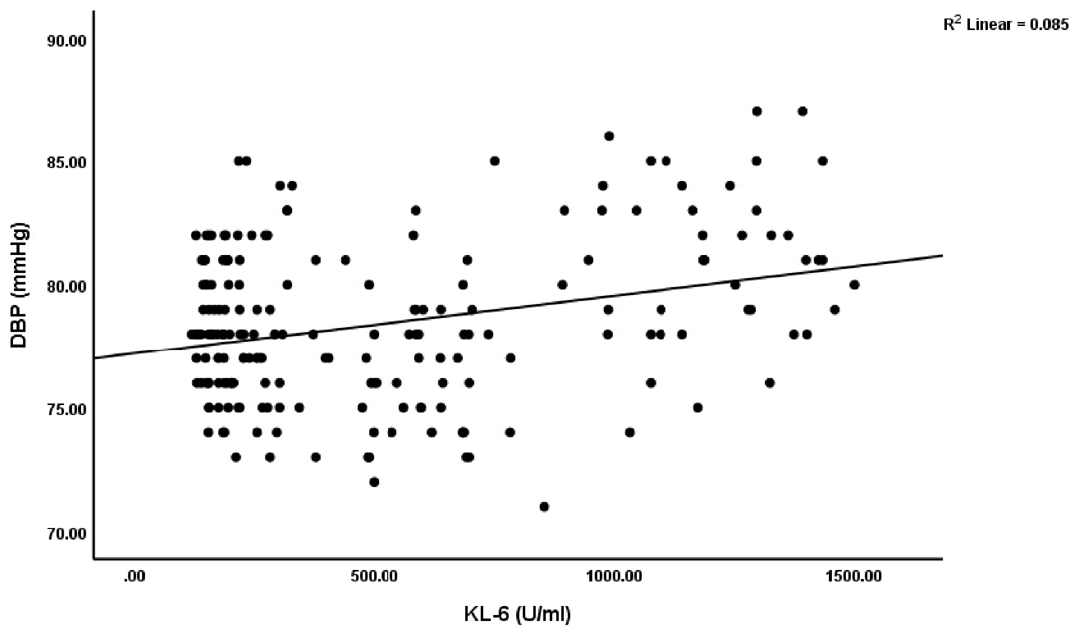
1000.00
1500.00
KL-6 (U/ml)

(B)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DBP (mmHg)
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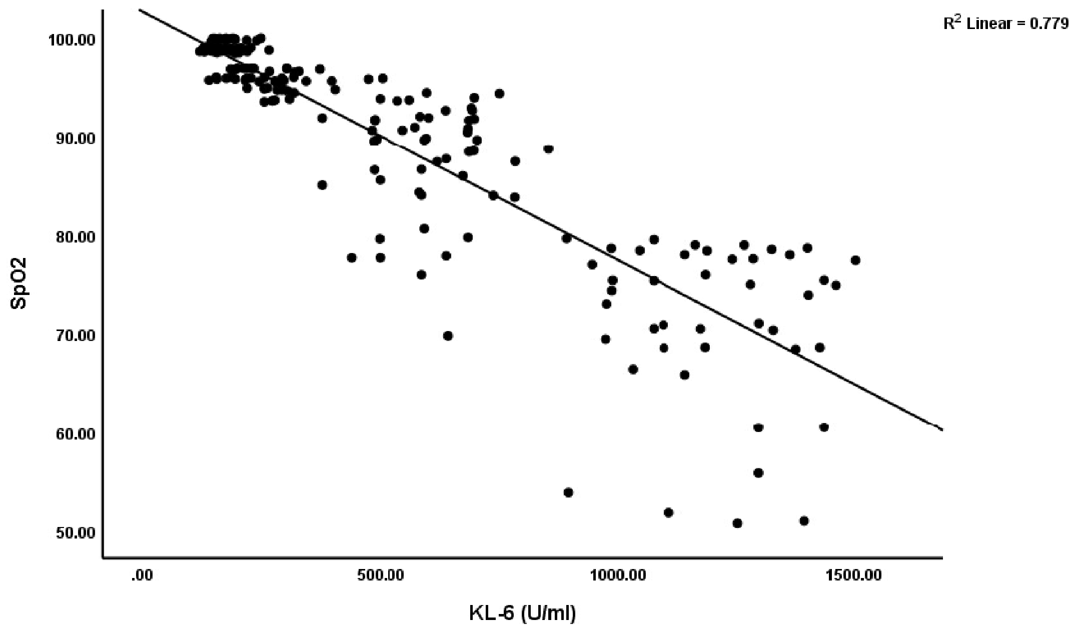
KL-6 (U/ml)

(C)


R² Linear = 0.779
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90.00
80.00
70.00
60.00
50.00
SpO2
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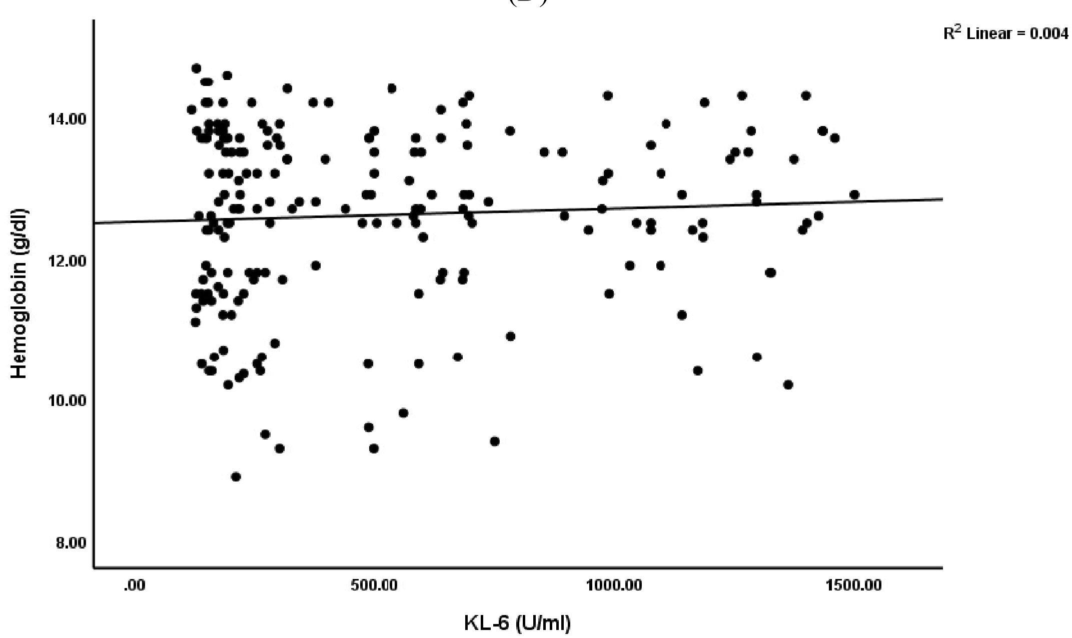
500.00
1000.00
1500.00
KL-6 (U/ml)

(D)


R² Linear = 0.004
14.00
12.00
10.00
8.00
Hemoglobin (g/dl)
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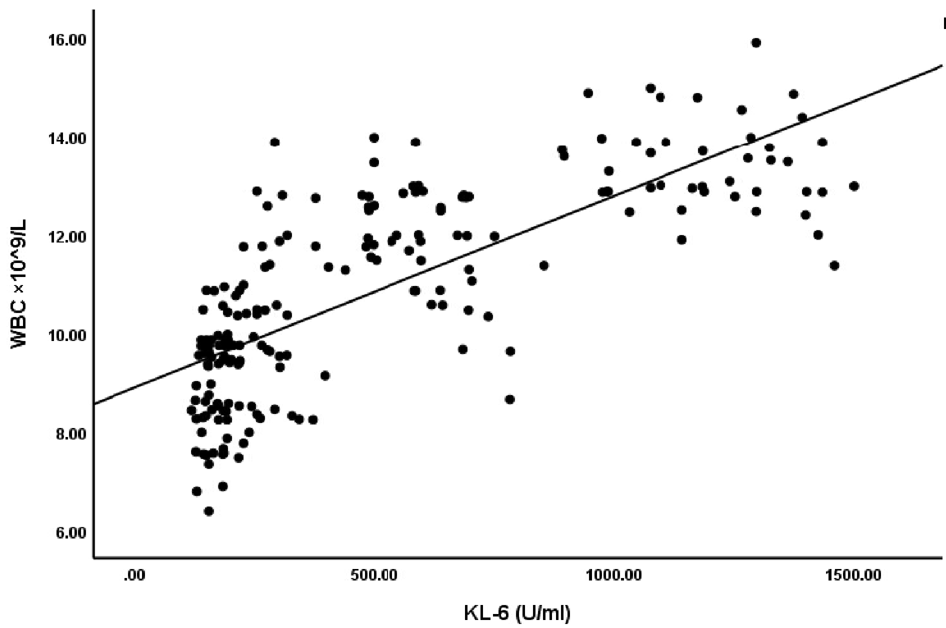
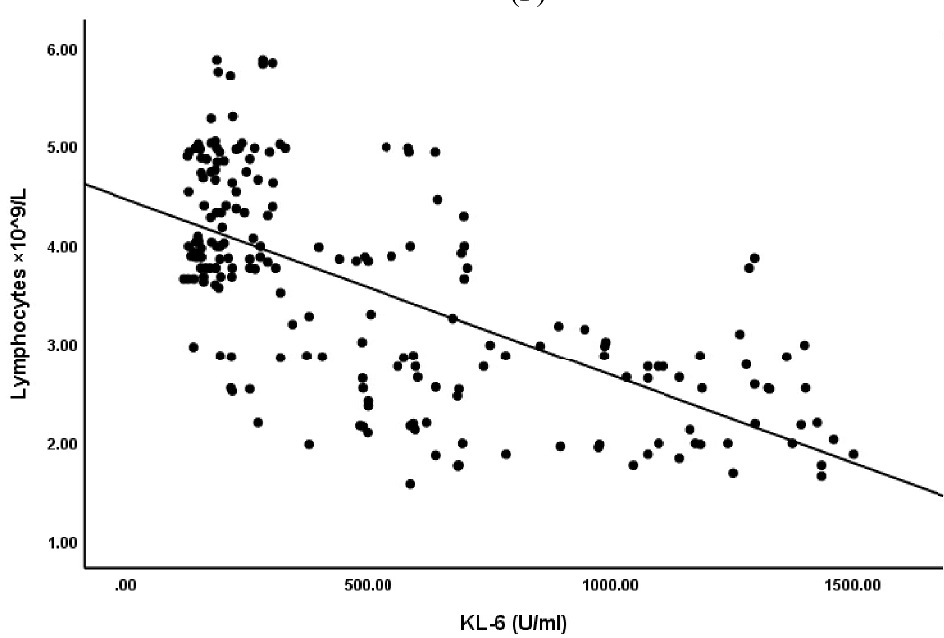
500.00
1000.00
1500.00
KL-6 (U/ml)

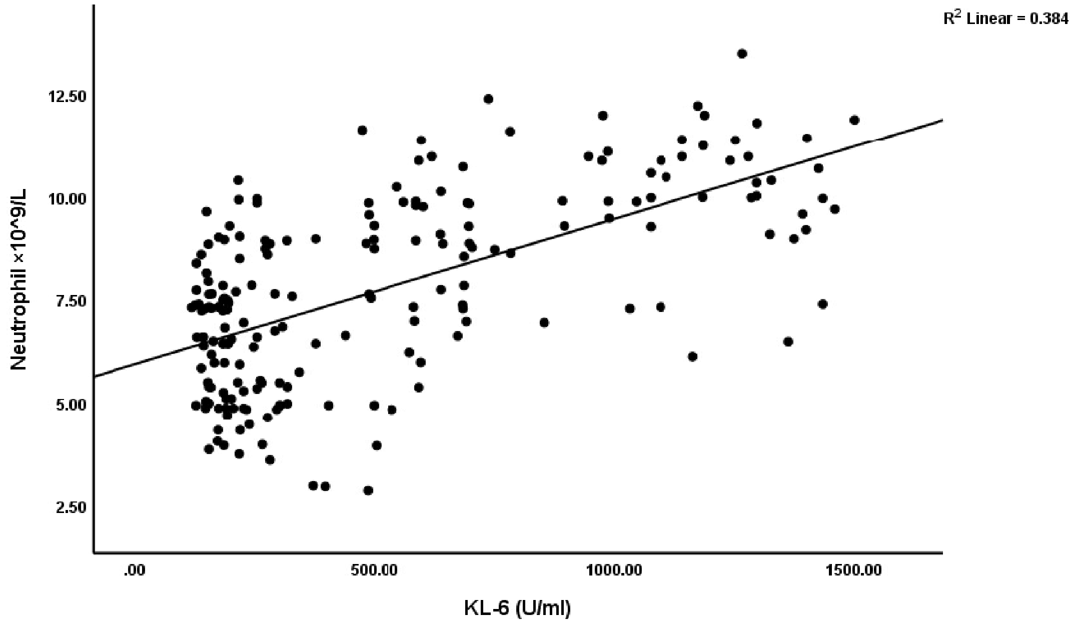
(E)


R² Linear = 0.574
16.00
14.00
12.00
10.00
8.00
6.00
WBC ×10^9/L
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(F)


R² Linear = 0.433
6.00
5.00
4.00
3.00
2.00
1.00
Lymphocytes ×10^9/L
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(G)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Neutrophil ×10^9/L
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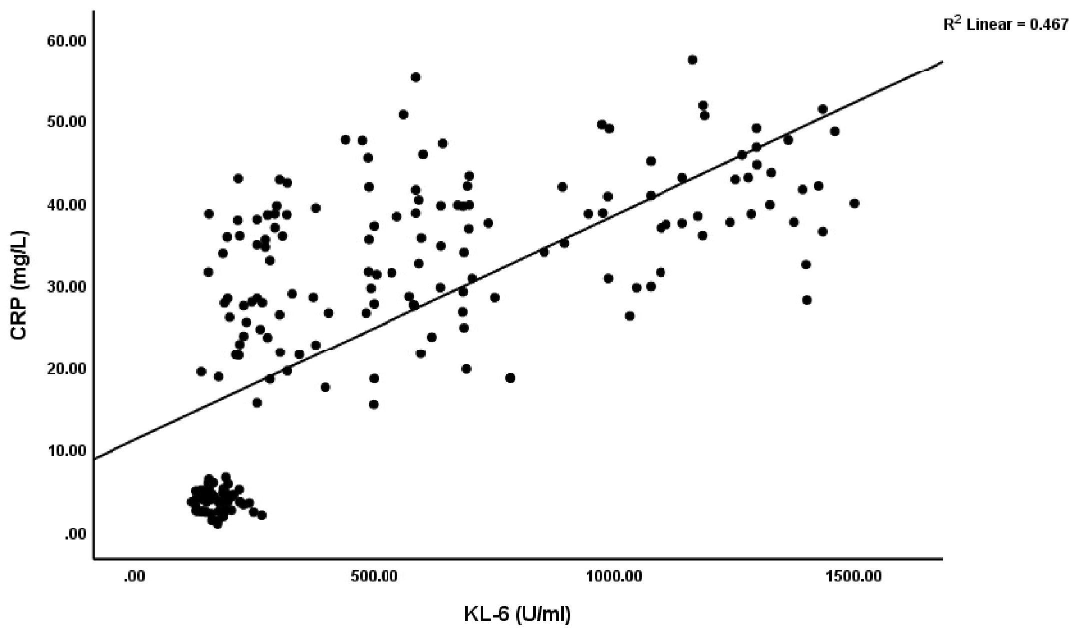
10.00
7.50
5.00
2.50
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(H)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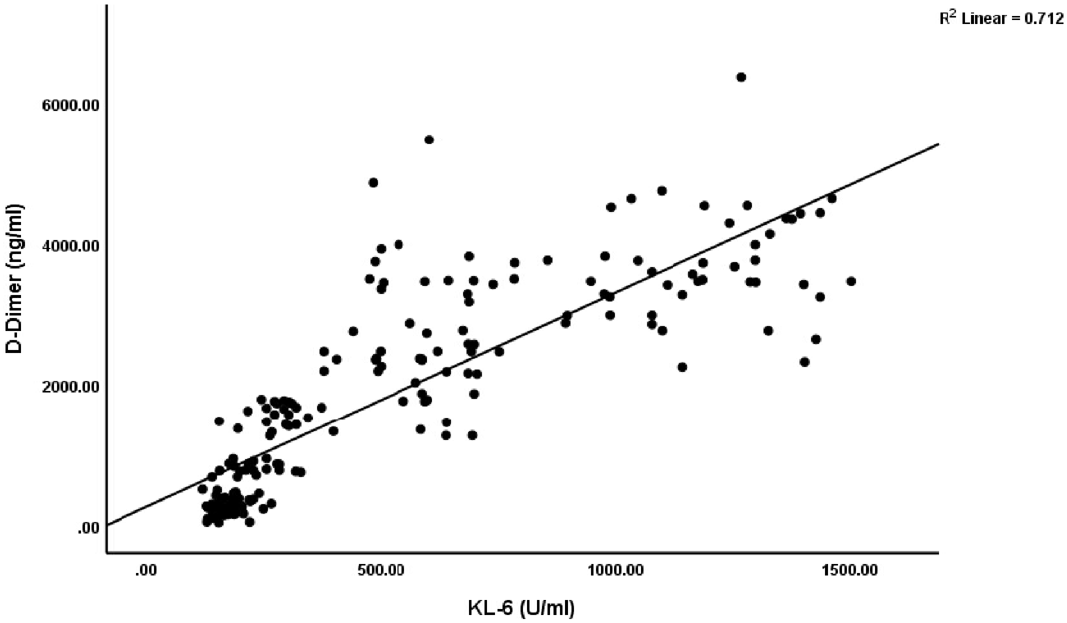
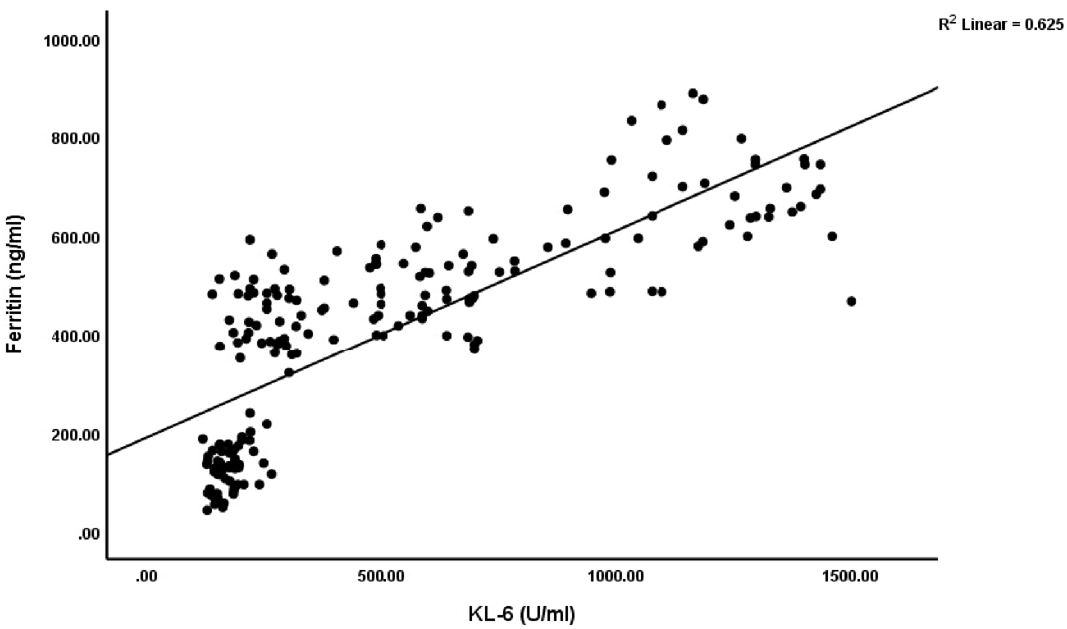
CRP (mg/L)
60.00
50.00
40.00
30.00
20.00
10.00
.00
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(I)


R² Linear = 0.712
6000.00
4000.00
2000.00
.00
D-Dimer (ng/ml)
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(J)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800.00
600.00
400.00
200.00
.00
Ferritin (ng/ml)
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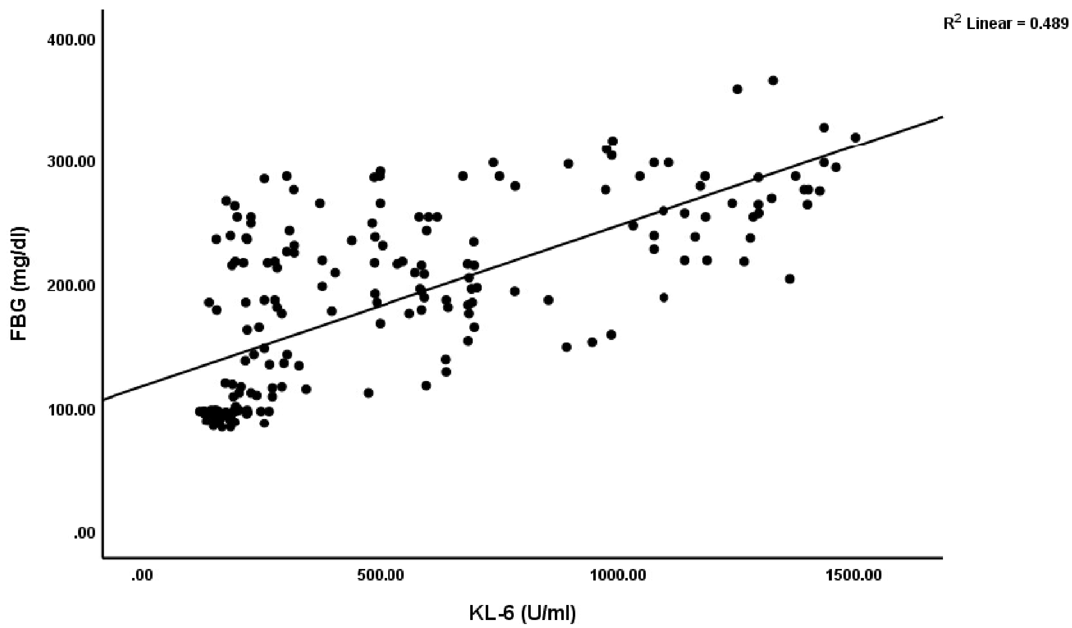
500.00
1000.00
1500.00
KL-6 (U/ml)

(K)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FBG (mg/dl)
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500.00
1000.00
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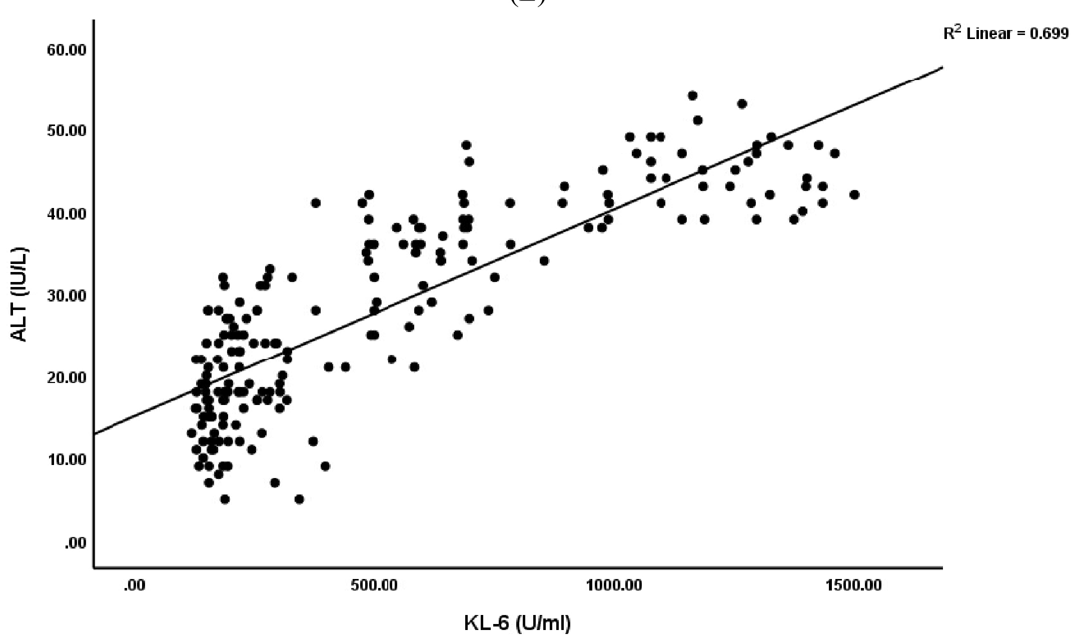
KL-6 (U/ml)

(L)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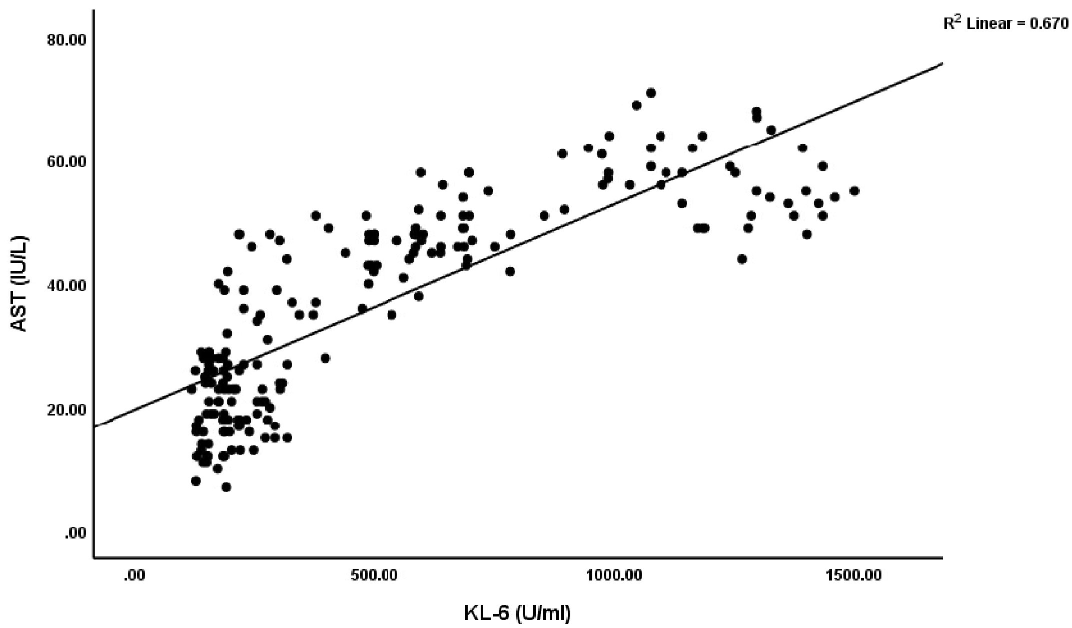
60.00
50.00
40.00
30.00
20.00
10.00
.00
ALT (IU/L)
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(M)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20.00
.00
AST (IU/L)
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1000.00
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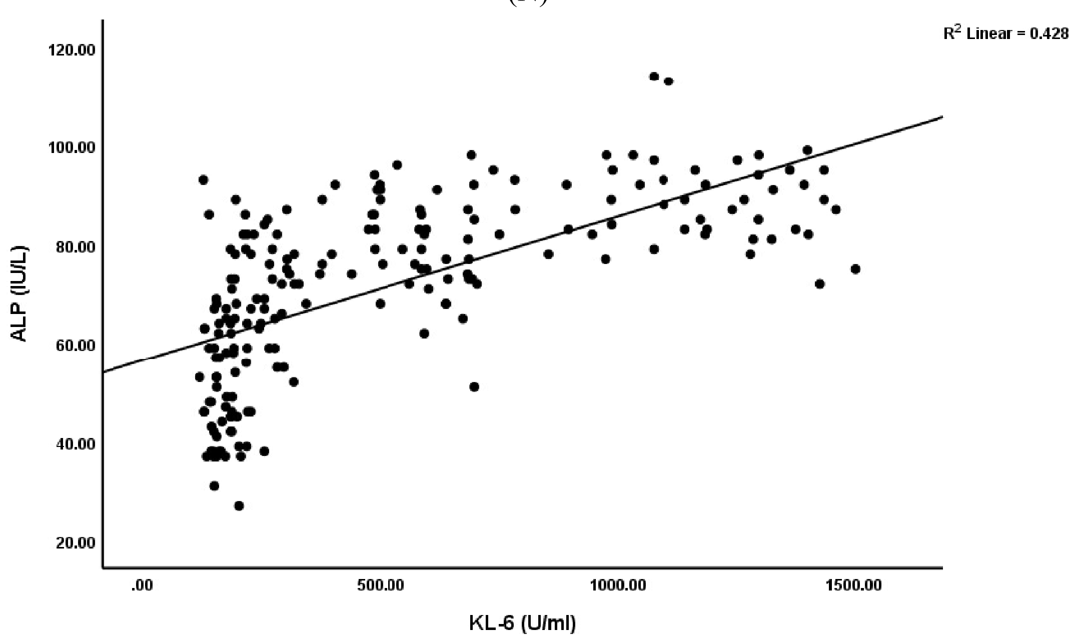
KL-6 (U/ml)

(N)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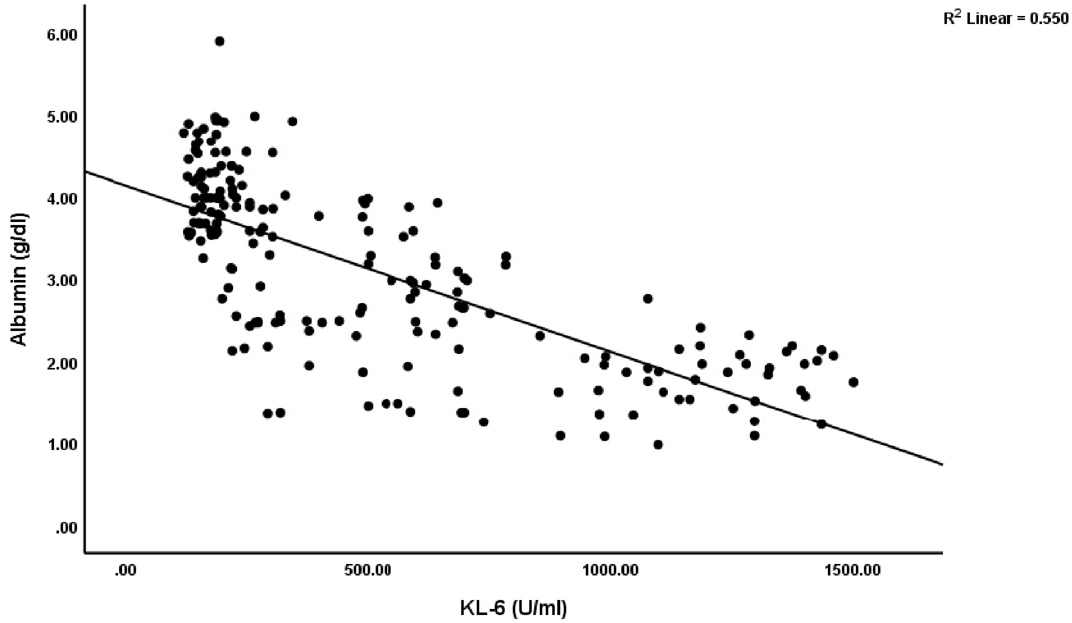
120.00
100.00
80.00
60.00
40.00
20.00
ALP (IU/L)
.00
500.00
1000.00
1500.00
KL-6 (U/ml)

(O)



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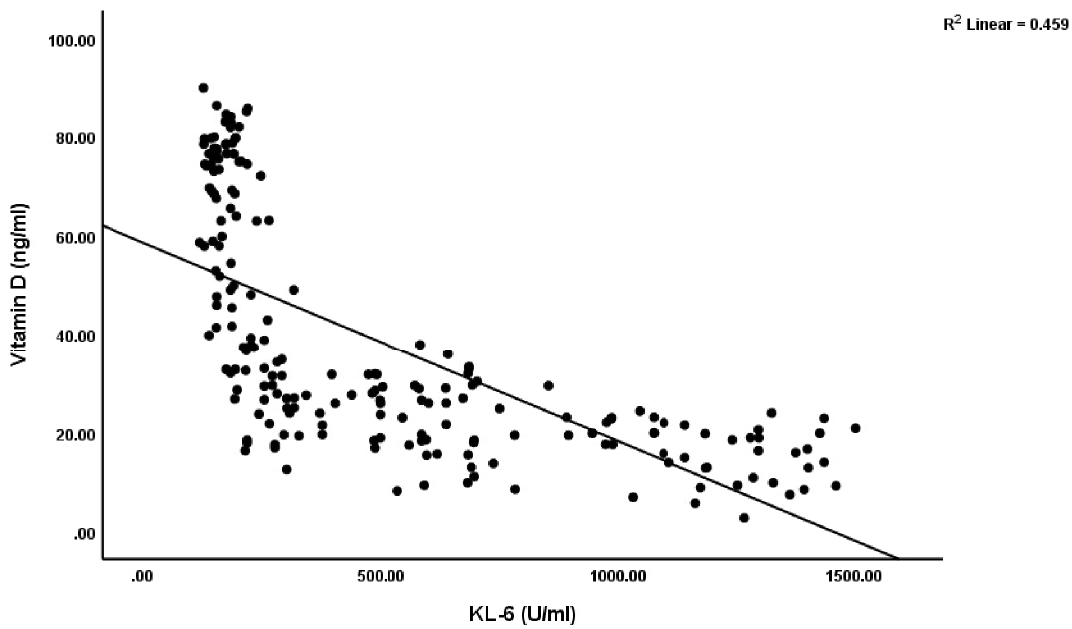

(Q)

Figure 1. Correlation between serum KL-6 levels and (A) Age, (B) SBP (C) DBP (D) SpO_2, (E) Hemoglobin, (F) WBC, (G) Lymphocytes, (H) Neutrophil, (I) CRP, (J)D-Dimer, (K) Ferritin, (L) FBG, (M) ALT, (N) AST, (O) ALP, (P) Albumin, and (Q) Vitamin D.

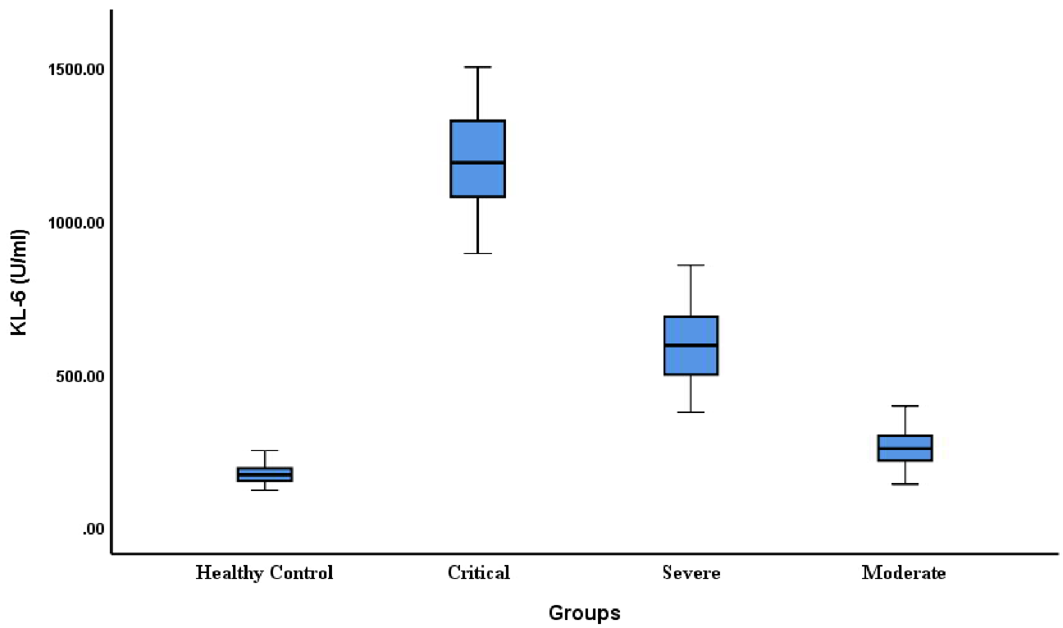

Figure 2. Comparison between groups (COVID-19 cases and healthy control) of KL-6 level.

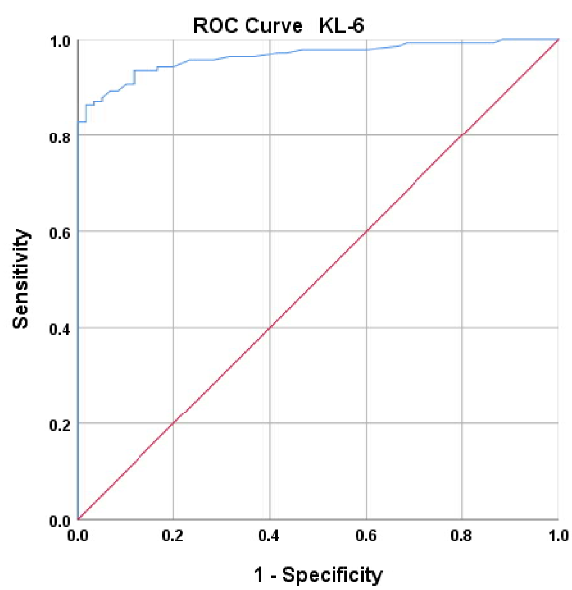

Figure 3. Receiver operating characteristic curve analysis of KL-6 for diagnosis of COVID-19.

Discussion

We focused at the relation between serum KL-6 concentrations and the severity of COVID-19. Assessing severity is crucial since patients with severe and critical COVID-19 have a significant mortality rate. Serum KL-6 was a good biomarker for COVID-19 severity, according to our study.

The serum KL-6 glycoprotein, which is mostly expressed in type II alveolar epithelial cells, may be a good indicator of the degree of lung damage in some illnesses or conditions[21]. Interstitial lung disease associated with rheumatoid arthritis, idiopathic interstitial pneumonia, acute aggravation of idiopathic pulmonary fibrosis, lung cancer, and interstitial lung disease associated with connective tissue have all been linked to it[22–25]. According to certain research, COVID-19 patients' severity is also indicated by their increased blood KL-6 levels, which are an indication of type II pneumocyte destruction and lung injury[26, 27].

For the assessment of pulmonary fibrosis for COVID-19, KL-6, which overexpresses in the injured or repaired type 2 alveolar epithelium and mostly represents the degree of interstitial lung impairment, may be useful [10]. The KL-6 level rises in COVID-19 patients, according to recent research, and it is connected with the disease's severity, making it a useful diagnostic evaluation indicator[18,28]. According to radiographic data, individuals with severe pulmonary fibrosis have bigger, irregular lesions that are challenging to completely absorb [29, 30]. As a result, the level of lung fibroblast activation may range for individuals with diverse degrees of severity[31].

The body's immune system would be activated by the SARS-CoV-2 infection. As a result, a number of immunoglobulins and chemical compounds are secreted. The integrity of the epithelial/endothelial barrier and the lung capillary endothelial cells, including pneumocytes, would also be compromised by this infection[32]. The injured pneumocytes, in particular the type II pneumocytes, produce a lot of KL-6 as a result of this destruction[33].The COVID-19 patients' virus loads will rise as they continue to deteriorate. The alveolar epithelium is damaged by the massive viral replication process. The KL-6 is therefore secreted. Alveolar epithelium rupture leads to basement membrane leakage, which in turn increases the permeability of the pulmonary vasculature and raises blood KL-6 levels. Therefore, the amount of lung injury in COVID-19 patients may be indicated by the blood KL-6 level[21, 26].

Since the blood KL-6 level was significantly greater in patients with severe COVID-19 compared to those with mild-to-moderate illness in prior studies, it is possible to use KL-6 to evaluate the severity and predict the prognosis of COVID-19[34]. The findings of the present study indicated that patients with greater KL-6 levels were older than the other groups. The advanced age and significant symptoms of COVID-19 patients have been shown to be risk factors for a poor outcome in a previous research[35,36].The study also revealed that patients with elevated KL-6 levels had a several of abnormal laboratory findings, such as lower SpO_2 lymphocyte counts, albumin levels, and vitamin D levels and higher neutrophil counts, liver enzyme levels, blood glucose levels, D-dimer levels, ferritin, and CRP, all of which have been suggested as risk factors for a more severe case and a poor prognosis for COVID-

19[37–39]. Additionally, it was shown that abnormalities in testing parameter values and KL-6 levels had a good correlation. As a result, using our data, KL-6 can be utilized as a clinical indicator to assess the severity and outlook of COVID-19.

Conclusions

In conclusion, our investigation showed that serum KL-6 levels were elevated in COVID-19 patients with critical and severe illness and were helpful in assessing disease severity. Patients with high serum KL-6 levels have severe diseases that need for vigorous therapies and thorough monitoring.

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Declaration of Interests

The authors declare no conflict of interests

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