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PHYSIOLOGY & MOLECULARBIOLOGY

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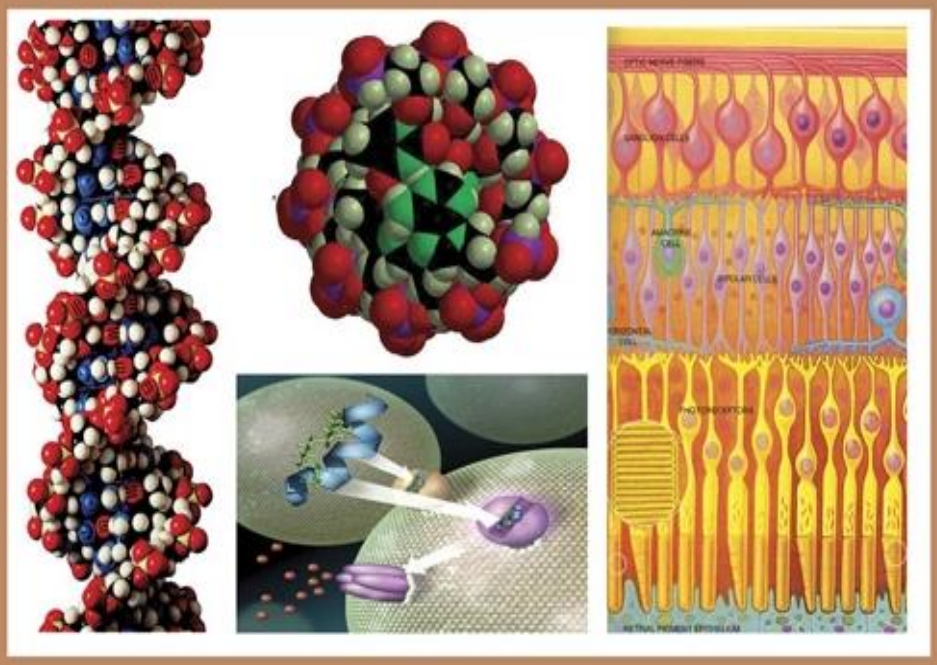

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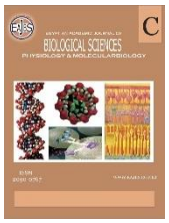

Assessment of Serum Lipocalin-2 Level as Early Predictor Marker to Severe COVID-19 Disease

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ABSTRACT

The coronavirus disease of 2019 (COVID-19) is an emerging coronavirus that affects people's respiratory systems (Severe acute respiratory syndrome coronavirus) and can the rapidly growing COVID-19 pandemic represents a significant global challenge, It can be considered that lipocalin-2 was highly associated with the severity of COVID-19. Therefore, it may be a useful biomarker for diagnosing disease severity in COVID-19 patients. lipocalin-2 was initially identified as a secreted protein from human neutrophils. Alveolar type II cells that have been damaged primarily express this substance. To verify lipocalin-2's potential as a diagnosing biomarker for COVID-19 patients, Lipocalin-2 levels in the blood were examined in this pilot investigation. To examine the relationship between serum lipocalin-2 levels and the severity of COVID-19 infection to see if this protein may be utilized as a disease indicator. This study was done in a case-control study with One hundred and twenty patients (79 males, 41 females) with COVID-19 who participated in the research. The COVID-19 patients were divided into three groups based on the severity of the illness: critical disease (n = 30), severe disease (n = 30), and mild/moderate disease (n = 60), with (n = 60) healthy volunteers serving as the control group (35 males, 25 females). Between January 2022 and May 2022, the patients were obtained from Al-Amal hospitals and the AL-Shefaa centre in AL-Najaf City, Iraq., All of the patients' fundamental clinical and demographic data were collected along with blood samples. Enzyme-linked immunosorbent tests were used to measure the blood's level of LCN2 (ELISA). The levels of total cholesterol, triglycerides, and High-Density lipoprotein were assessed using colorimetric methods. Ichroma was tested for serum ferritin, D-dimer, and CBC by Swelab. ran a statistical study to see if they were related to the severity of the disease.

Higher lipocalin-2 levels were observed in the patient group, particularly in cases of mild/moderate (1.32±0.30) (P. 0.001), severe(2.16±0.42) (P. 0.001), and critical(4.71±1.01) (P. 0.001) comparing cases to healthy controls (0.86±0.51) respectively, groupings. (SPO_2 %, Hb, TC, HDL, LDL, and lymphocyte) levels were found to significant negative correlation with one another in the COVID-19 patient group, with p-values=0.001 for each of these relationships. Moreover, a significant positive correlation between (TG, VLDL-C, WBCs, neutrophil, platelet, N/L ratio, D-dimer, Ferritin, and CRP, p.value=0.001 for each one of them) levels with lipocalin-2 in the COVID-19 patients group. a cut-off value of 1.215 (ng/mL) for lipocalin-2 predicted severe COVID-19 with a sensitivity of 81.7 % and a specificity of 80.2 % (AUC: 0.9, 95%CI 0.852-0.949 ; p<0.0001).

INTRODUCTION

The coronavirus disease of 2019 is brought on by the severe acute respiratory syndrome coronavirus-2 (SARSCoV-2) (COVID-19), a respiratory infection that first appeared in humans in Wuhan, China, in December 2019 and has since spread globally (Kumar *et al.,* 2021). SARS-CoV-2 is an infection caused by a virus that, as far as is known, transmits from an infected person to others by respiratory secretions and aerosols. Misdiagnosis and asymptomatic transmission are responses to the COVID-19 pandemic's quick global spread. (Syal 2021) .

Acute shortness of breath, fever, muscle soreness, and loss of taste and smell are just a few of the moderate symptoms that COVID-19 can produce (Cascella *et al.,* 2022). Therefore, accurate early diagnosis and thorough infectious illness prevention and control are necessary around the world before developing a COVID-19 treatment agent because elderly individuals, immunocompromised individuals, and patients with underlying disorders cause death in severe situations. (Rahier *et al.,* 2009). As they function as the structural underpinnings of cellular and viral membranes, lipids are essential in viral infection. (Lorizate & Kräusslich 2011and Hsu *et al.,* 2010). In to modify host cells and create lipids for their envelopes, viruses assault lipid production and signaling. (Murillo *et al.,* 2015 and Diamond *et al.,* 2010).

Since lipids play a crucial role in viral membranes, enveloping, and conversion during viral replication, chemicals that affect lipids like cholesterol and sphingolipids may be specifically targeted to prevent viral reproduction. (Gualdoni *et al.,* 2018). Viruses must enter and exit the host cell through the membrane because they reproduce inside the host cell. (Lorizate and Kräusslich 2011)(Thai et al. 2015). Lipids have several roles in viral invasion, including entry cofactors, fusion cofactors, and direct and indirect viral receptors. (Murillo *et al.,* 2015 and Thai *et al.,* 2014).

Lipocalin 2 (LCN2), commonly referred to as Neutrophil Gelatinase-Associated Lipocalin (NGAL), is a crucial immunological component with indicator properties. A tiny protein called NGAL, an iron claw carries tiny ligands like vitamins and pheromones. Neutrophils produce it in three isoforms, measuring 25 kDa, 45 kDa, and 145 kDa (Passov et al. 2019). It is almost always produced in the kidney's proximal and distal tubules of the nephron in the 25 kDa monomeric form. (Cai et al. 2010). NGAL is a somewhat accurate and sensitive urine marker of kidney damage in cases of acute kidney injury, post-contrast nephropathy, polycystic kidney disease (ADPKD), or glomerulonephritis. (Gala-Błądzińska & Kuzniewski 2013 and Hirsch *et al.,* 2007) .

Patients having cardiac surgery have employed urine and plasma NGAL as early indicators for AKI, which is always linked to neutrophil activation. (Passov *et al.,* 2019) . However, each time neutrophils are stimulated, the NGAL concentration changes considerably, which could make interpretation difficult. Several researchers have referred to plasma NGAL in renal patients as an "expensive [alternative to] creatinine" due to the tests' consistently demonstrated sensitivity. (Testani and Brisco 2016)(Villa *et al.,* 2005) .

The lipocalin family is made up of tiny proteins that control immunological function and serve as carriers for retinoids, fatty acids, and steroids, among other chemicals. This family includes lipocalin-2.(Makris and Kafkas 2012). It has antifungal and antibacterial effects and is secreted by numerous cells and tissues under pathological and normal situations. A cytokine that binds iron is called lipocalin-2. Its capacity to bind to iron limits the germs' access to iron, which prevents the growth of pathogens. Furthermore, lipocalin-2 protects against oxidative stress brought on by an excess of free iron. (Xiao, Yeoh, and Vijay-

Kumar 2017)(Makris and Kafkas 2012).

MATERIALS AND METHODS

This study was done in a case-control study conducted with One hundred and twenty patients (79 males, 41 females) with COVID-19 who participated in the research. The COVID-19 patients were divided into three groups based on the severity of the illness: critical disease (n = 30), severe disease (n = 30), and mild/moderate disease (n = 60), with (n = 60) healthy volunteers serving as the control group (35 males, 25 females). Between January 2022 and May 2022, the patients were obtained from Al-Amal hospitals and the AL-Shefaa centre in AL- Najaf City, Iraq., The patients' basic clinical and demographic data, as well as blood samples, were collected. They were diagnosed quantitatively by RT-PCR and chest X-ray or CT scan at 7-12 days from the onset of symptoms. They were all older than 20 years old Patients for the COVID-19 trial were collected at the time of admission., and Murray scores were used to evaluate the severity of the condition. (Murray 1989) Sample collection was conducted during the period from January 2022 to May 2022.

Patients with a history of vasculitis connective tissue disease, who were currently on long-term treatment with oral corticosteroids, IL-6 antagonists, or TNF antagonists, were not included in the study. Chronic diseases such as diabetes, cardiovascular disease, infection and inflammation were also excluded from the study. Patients with cancer, kidney disorders, smokers, and thyroid problems are not eligible for this study. In addition, any patient who received this vaccination in the past six months or less, as well as any patient under the age of 20, is also ineligible.

Five ml of venous Blood samples from patients and controls were collected. It was divided into two tubes to separate blood samples. The first 3 mL was allowed to clot at room temperature for 10–15 minutes before centrifugation at (3000 rpm) for 10 minutes to obtain serum. Serum samples were then divided into tubes and kept at −20 °C until the time of analysis. The second tube contains 2 mL of blood with the anticoagulant EDTA for complete blood count measurement. Obtain a complete blood count (CBC) using an automatic hematology analyzer (Swelab Alpha, Swedish in origin) with residual blood (2 ml).

To measure serum ferritin and dimer levels, a Fluorescent immunoassay (FIA) was used, as well as to measure lipoclin 2 by used Enzyme-linked immune sorbent assays (ELISA) were used to detect the concentration levels in the serum sample. ichroma TM technology was used to measure the levels of CRP in the serum sample.

Because of the patient's high temperature, the patient's respiratory symptoms, as well as radiological indicators of pneumonia, it was determined that the patient had mild to moderate COVID-19. Patients were classified as having severe COVID-19 if any of the following changes were found in their bodies: (Murray *et al.*, 1988).

1. Breathing more than 30 times per minute.
2. Oxygen saturation is less than 94 % SpO2 in room air.
3. Lung leakage greater than 50% on low-dose computed tomography.
4. Admission of the patient to the intensive care unit to use mechanical ventilation due to the collapse of the patient's respiratory system.

Critical Illness: Patients with acute respiratory distress syndrome (ARDS) may develop septic shock, multi-organ failure, and coagulation of problems.

In addition, a patient who has passed away is not counted as having survived their illness. This inquiry study was given its stamp of approval by the local medical ethics committee, as well as by each participant individually and collectively before the study ever began. The patients were asked to fill out registration forms, after which a list containing their names was given to them.

Statistical Analyses:

Software from IBM called SPSS 26 was used to conduct statistical studies. The

mean and standard deviation of the results of the analyses are displayed. The cut-off point for statistical significance was $p<0.05$. The two independent samples were contrasted using Student's t-test. Using Student's t-test, correlation coefficient approach to evaluate parametric variables, and analysis of variance (ANOVA) was performed in the study to look for differences in scale variables between groups. An approach known as receiver operating characteristic (ROC) analysis was utilized to establish the lipocalin-2 cut-off value. The ROC curve was used to determine the area under the curve (AUC) value.

RESULTS

The severity of COVID-19 was used to classify each of the 120 patients who participated in this study. As stated in Table (1), sixty healthy individuals served as control, and One hundred and twenty as a patient.

Table 1: Demographic and clinical characteristics of the patient's categories and control groups.

Parameters	Control Group Mean±S.D N=60	Patient Groups			P. value			
		mild/moderate Mean±S.D N=55	severe Mean±S.D N=35	Critical Mean±S.D N=30				
No .	60	60	30	30				
No . M/F	35/25	22/38	20/10	21/9				
Age (Years)	47.61±10.96	50.26±11.27	48.86±11.72	55.96±10.28	A0.014	B 0.023	C0.573	D0.036
Height (m)	1.70±0.10	1.68±0.09	1.67±0.07	1.71±0.08	A0.121	B0.086	C0.941	D0.185
Weight (kg)	73.36±10.45	75.81±9.57	78.16±11.46	73.86±12.03	A0.119	B0.413	C0.324	D0.128
BMI (kg/m2)	25.54±4.84	27.19±4.40	27.97±4.26	25.26±3.66	A0.091	B0.051	C 0.435	D0.068
SBP (mmHg	128.75± 5.51	129.50±14.11	135.12± 16.40	138.75 ± 12.20	A 0.03	B 0.01	C0.01	D0.001
DBP (mmHg)	79.86 ± 4.10	78.30± 7.40	77.53± 8.32	81.30± 15.43	A0.01	B0.02	C 0.11	D0.01
SPO2%	98.8±0.75	93±0.52	87.32±6.55	71±10.32	A0.001	B0.001	C 0.01	D0.001
Hb (g/dL)	12.77±1.36	12.86±1.33	12.13±1.38	12.56±1.03	A0.208	B0.304	C0.014	D0.431
T-WBC $\times10^9$/L	8.65±0.96	10.12±1.32	12.02±0.66	13.30±1.01	A0.0001	B0.0001	C0.0001	D0.0001
Neut. $\times10^9$/L	5.89±1.54	7.44±1.77	8.78±1.94	9.49±1.78	A0.114	B0.0001	C0.001	D0.0001
Lym. $\times10^9$/L	3.93±0.55	3.65±0.80	3.07±0.72	2.21±0.66	A0.0001	B0.0001	C0.0001	D0.0001
NLR	1.53±0.47	2.15±0.76	3.04±1.05	4.64±1.54	A0.0001	B0.0001	C0.0001	D0.0001
PLT $\times10^9$/L	297.25±35.55	241.±37.07	277.23±46.78	296.83±36.91	A0.049	B0.0001	C0.0001	D0.0001
D-dimer(ng/mL)	306.70±115.87	1121.28±404.43	2513.81±401.65	3883.63±891.92	A0.0001	B0.0001	C0.0001	D0.0001
Ferritin(ng/mL)	147.06±53.68	435.56±83.45	475.93±65.91	846.43±66.45	A0.0001	B0.0001	C0.514	D0.0001
CRP (ng/mL)	3.33±1.46	24.26±5.64	58.87±8.21	74.21±6.44	A0.0001	B0.0001	C0.0001	D0.0001
TG(mg/dL)	132.50±8.39	231.31±22.12	269.54±18.46	279.87±13.59	A0.017	B0.0001	C0.0001	D0.0001
TC(mg/dL)	171.1±12.897	169.35±14.29	161.95±9.79	157.86±27.64	A0.328	B0.002	C0.046	D0.0068
HDL.C(mg/dL)	46.95±6.41	34.60±6.15	34.26±9.51	29.13±4.96	A0.004	B0.0001	C0.825	D0.0001
VLDL.C(mg/dL)	26.96±1.67	46.26±4.42	53.91±3.69	55.01±6.42	A0.291	B0.0001	C0.0001	D0.0001
LDL.C(mg/dL)	97.64±12.46	88.48±16.11	73.77±13.25	67.54±12.76	A0.092	B0.0001	C0.0001	D0.0001
Lipocalin-2(pg/mL)	0.86±0.51	1.32±0.30	2.16±0.42	4.71±1.01	A0.0001	B0.0001	C0.0001	D0.0001

Data represented as Mean ± SD: standard deviation, SBP: Systolic blood, Pressure, DBP diastolic blood pressure SPO2%: Oxygen saturation percentage, Hb: hemoglobin, WBC: White blood cell, LYM: lymphocyte, Neut: neutrophil, NLR: neutrophil/ lymphocyte ratio, PLT: Platelet; CRP= C-reactive protein TG: triglyceride, HDL.C: High-density lipoprotein cholesterol, TC: total cholesterol, LDL.C: Low-Density lipoprotein cholesterol VLDL.C: Very Low-Density Lipoprotein cholesterol., A=p.value(Critical×Severe),) B= p.value (Critical×Moderate), C=p.value(Severe×Moderate), and D= (All COVID-19 patients×healthy control).

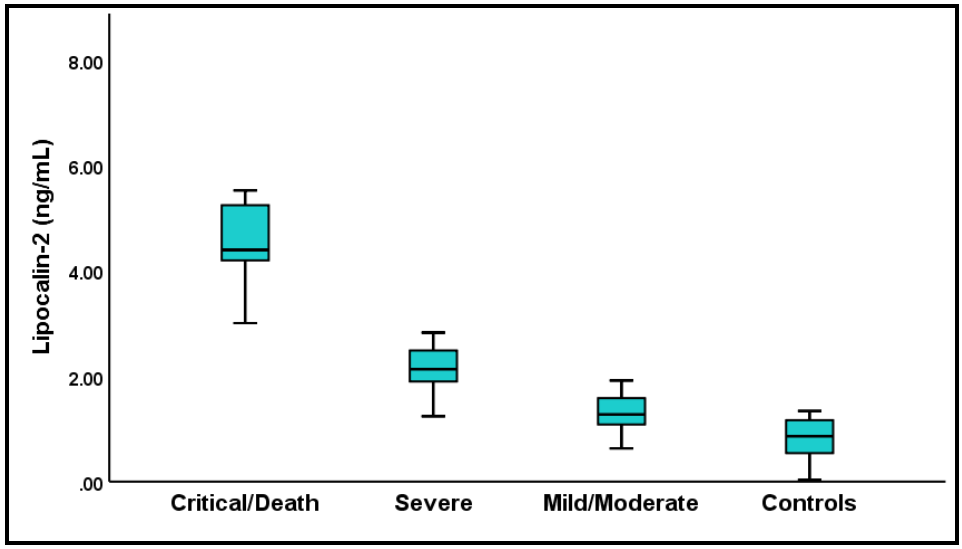


Fig. 1: Comparison of Lipocalin-2 level between categories of COVID-19 patients groups and control group.

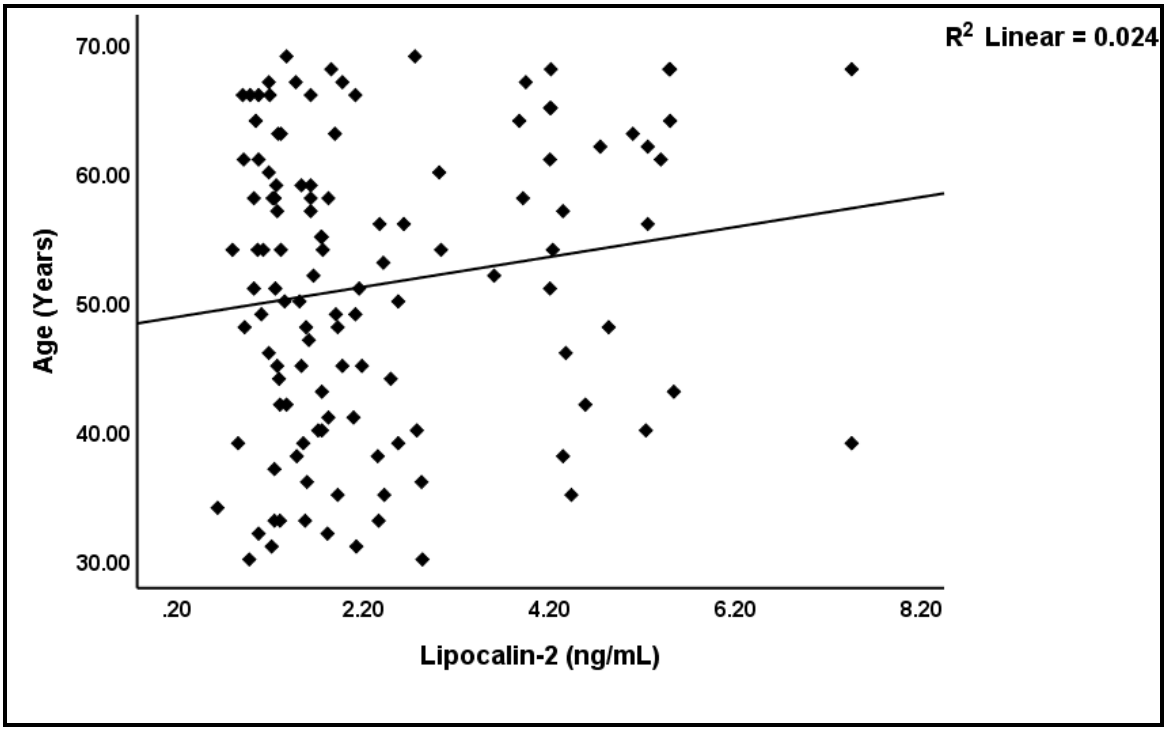
Table (1), the mean age in the group of patients (Critical) has a significant difference compared with the healthy group (55.96±10.28, 47.61±10.96 years, respectively; p = 0.05). There were no statistically significant variations in the BMI subgroup distributions among the three disease severity categories. Statistically significant changes in the means of the laboratory measures reported in Table (1) between the three groups of illness severity were seen for all save Hb. Comparison of patients with mild/moderate, severe, and critical test findings of COVID-19, were compared with controls in the data of serum Lipocalin-2 levels (0.86±0.51, 1.32±0.30, 2.16±0.42, 4.71±1.01), ferritin (147.06±53.68, 435.56±83.45, 475.93±65.91, 846.43±66.45), D-dimer (306.70±115.87 ,1121.28±404.43, 2513.81±401.65, 3883.63±891.92), CRP (3.33±1.46, 24.26±5.64, 58.87±8.21, 74.21±6.44) Control, mild/moderate, severe, and Critical values, ($p<0.001$) respectively.

Table 2 and Figure 2 show a significant negative correlation was obtained between (Weight, BMI, SBP, DBP, SPO_2 Hb., TC, HDL.C, and LDL.C) levels in the COVID-19 patients group. At the same time, a significant positive correlation was obtained between (Age, Height, T.WBCs, Neutrophil, D-dimer, Ferritin, CRP, TG and VLDL.C) levels with Interferon -λ1 in the COVID-19 patients group.

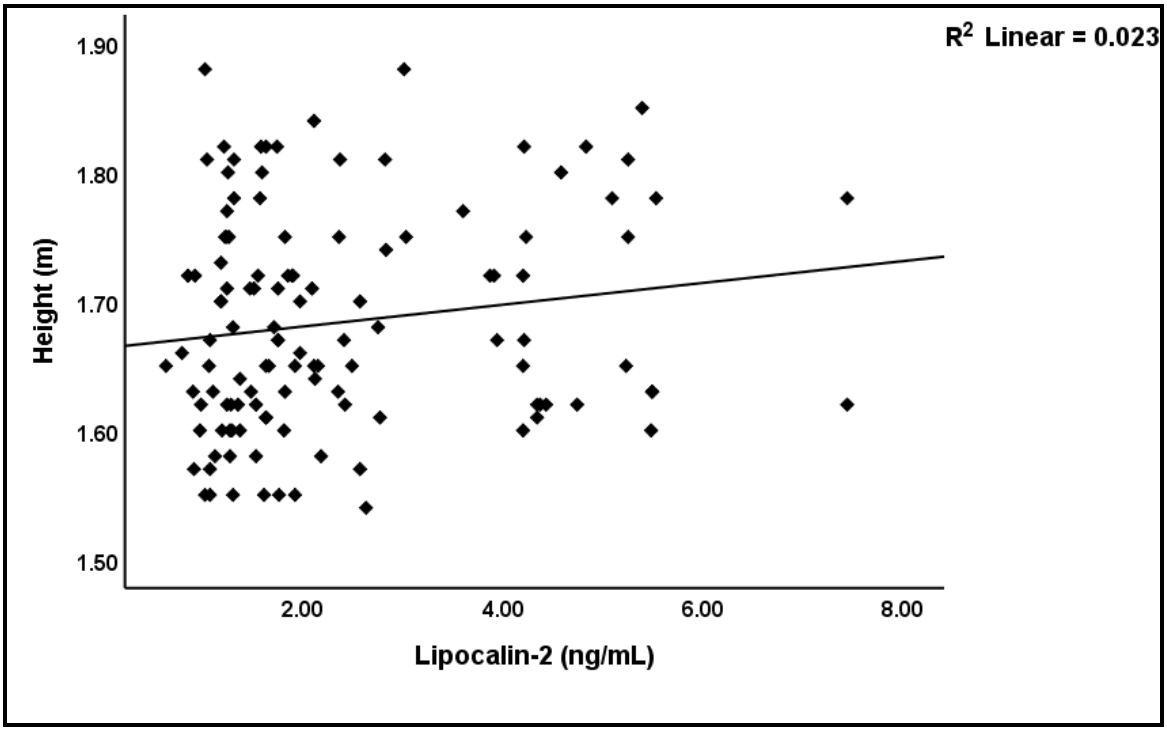
Table 2: Correlation analysis between serum Lipocalin-2 with biochemical parameters in COVID-19 patients.

Variables	ρ	Variables	ρ
Age (Years)	0.071	NLR	0.573**
Height (m)	0.147	Platelet $\times 10^9$/L	0.452**
Weight (kg)	-0.012	D-Dimer (ng/mL)	0.851**
BMI (kg/m^2)	-0.086	Ferritin (ng/mL)	0.626**
SBP(mmHg)	-0.16	CRP (mg/L)	0.951**
DBP (mmHg)	-0.079	TG (mg/dL)	0.645**
SPO_2 %	-0.62	TC (mg/dL)	-0.429**
Hb (g/dL)	-0.145	HDL-C (mg/dL)	-0.258**
T-WBCs $\times 10^9$/L	0.719**	LDL-C (mg/dL)	-0.473**
Neutrophil $\times 10^9$/L	0.385**	VLDL-C (mg/dL)	0.618**
Lymphocyte $\times 10^9$/L	-0.529**		

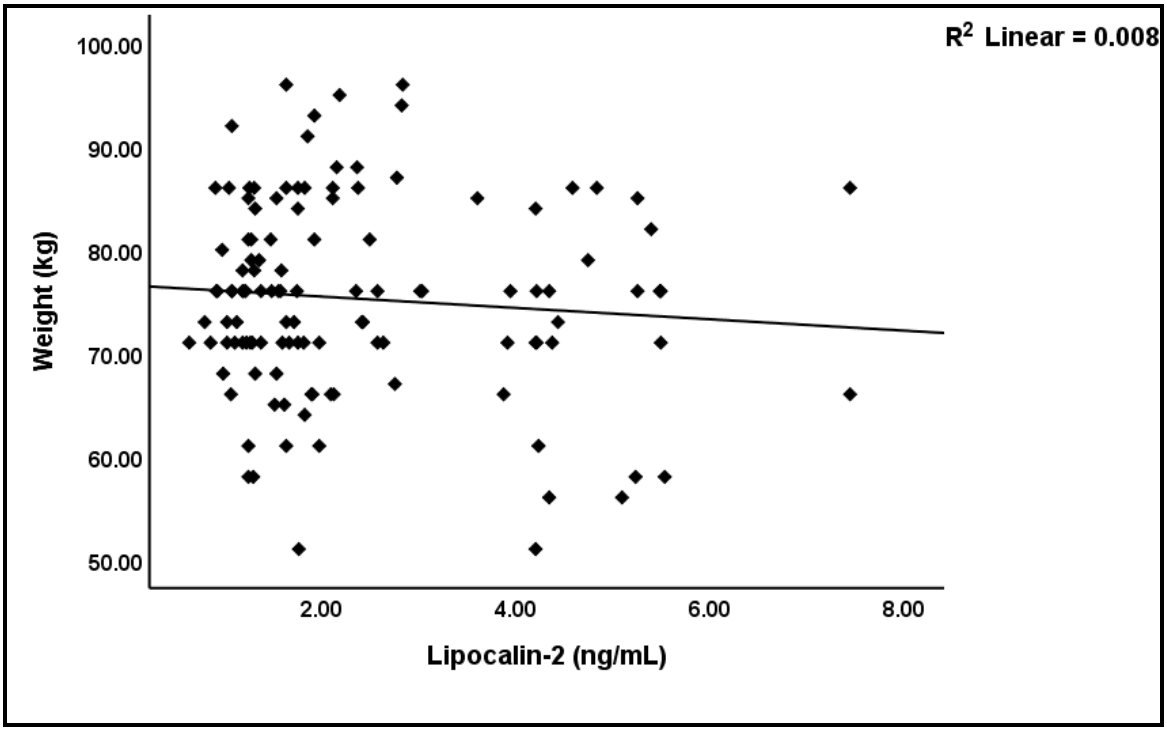
** Correlation is significant at the 0.01 level, ρ = Spearman's correlation coefficient


R² Linear = 0.024
70.00
60.00
50.00
40.00
30.00
Age (Years)
.20
2.20
4.20
6.20
8.20
Lipocalin-2 (ng/mL)

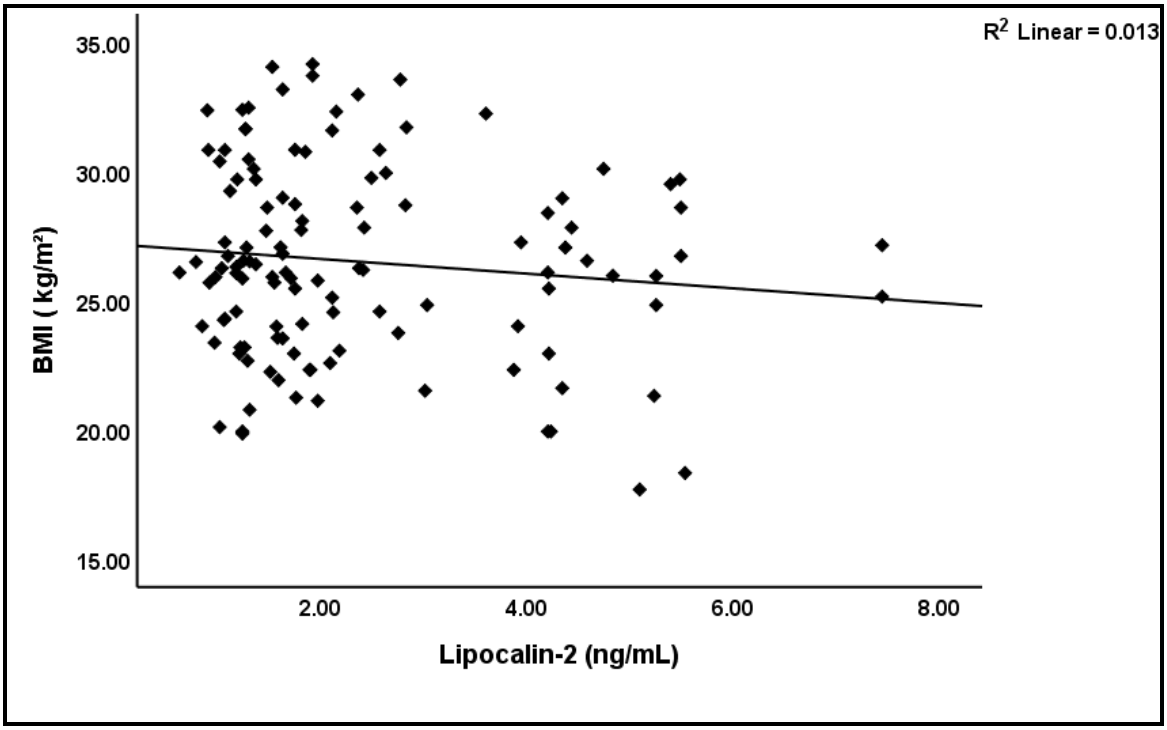
(A)


R² Linear = 0.023
1.90
1.80
1.70
1.60
1.50
Height (m)
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

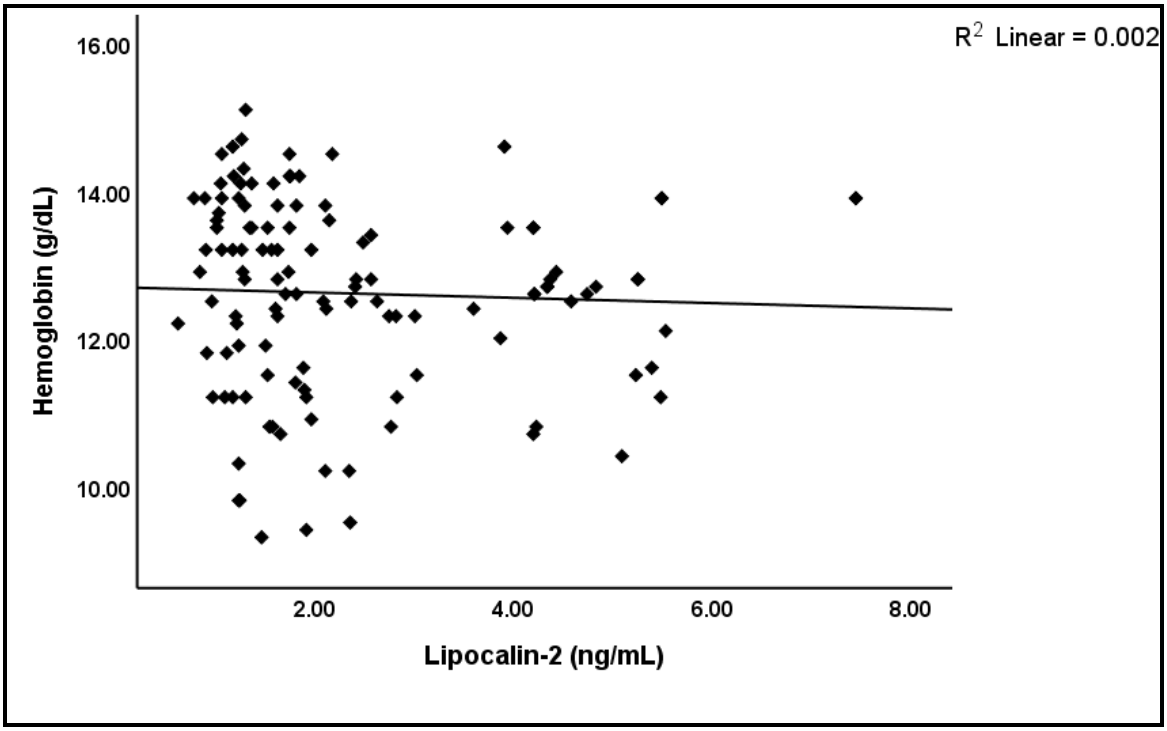
(B)


R² Linear = 0.008
100.00
90.00
80.00
70.00
60.00
50.00
Weight (kg)
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

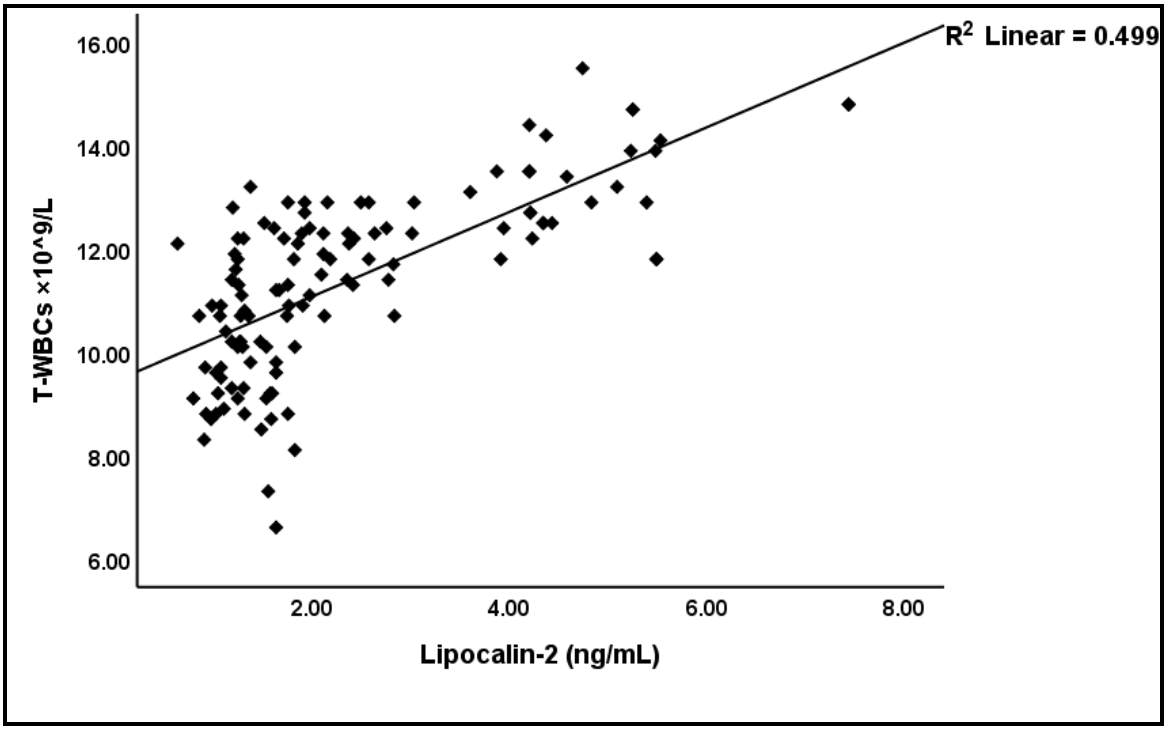
(C)


R² Linear = 0.013
35.00
30.00
25.00
20.00
15.00
BMI (kg/m²)
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

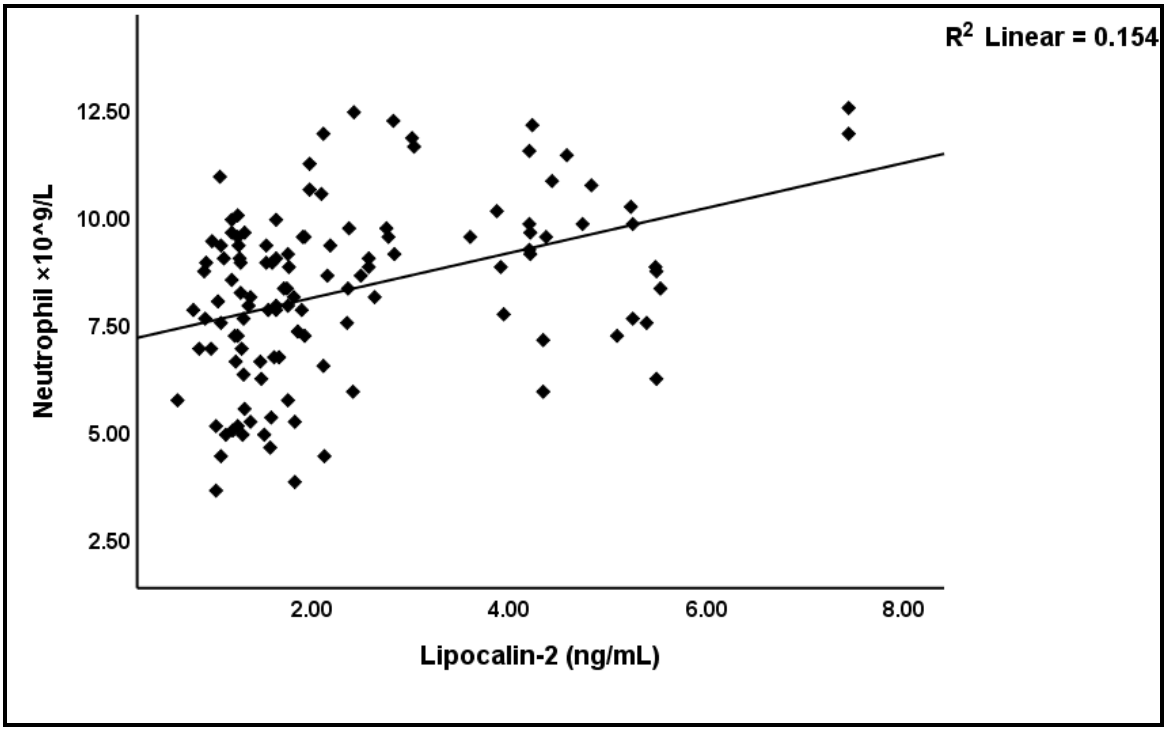
(D)


R2 Linear = 0.002
16.00
14.00
12.00
10.00
Hemoglobin (g/dL)
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

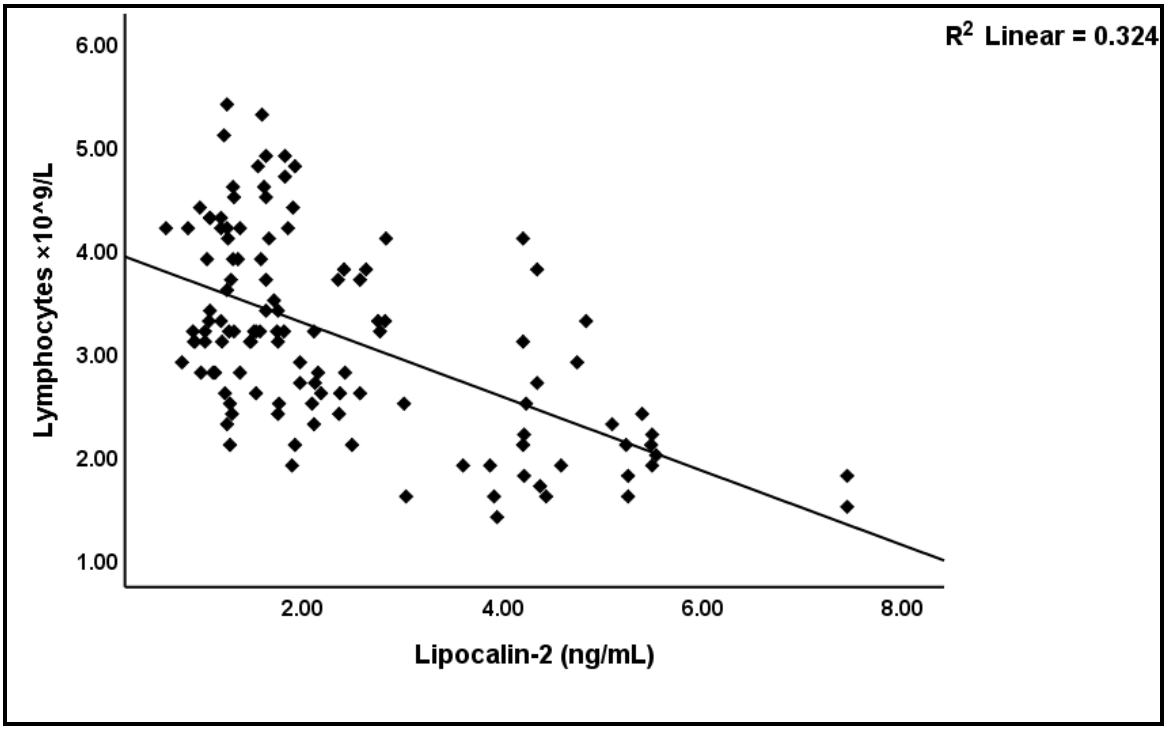
(E)


R2 Linear = 0.499
16.00
14.00
12.00
10.00
8.00
6.00
T-WBCs ×10^9/L
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

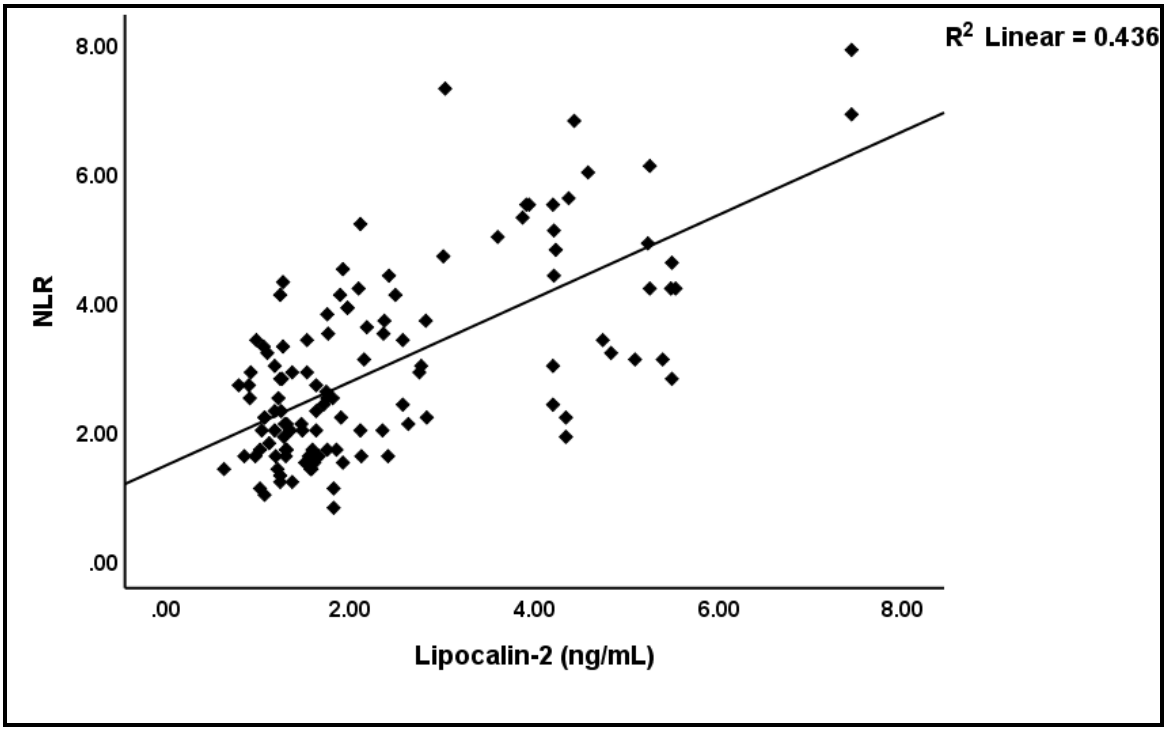
(F)


R² Linear = 0.154
Neutrophil ×10^9/L
12.50
10.00
7.50
5.00
2.50
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

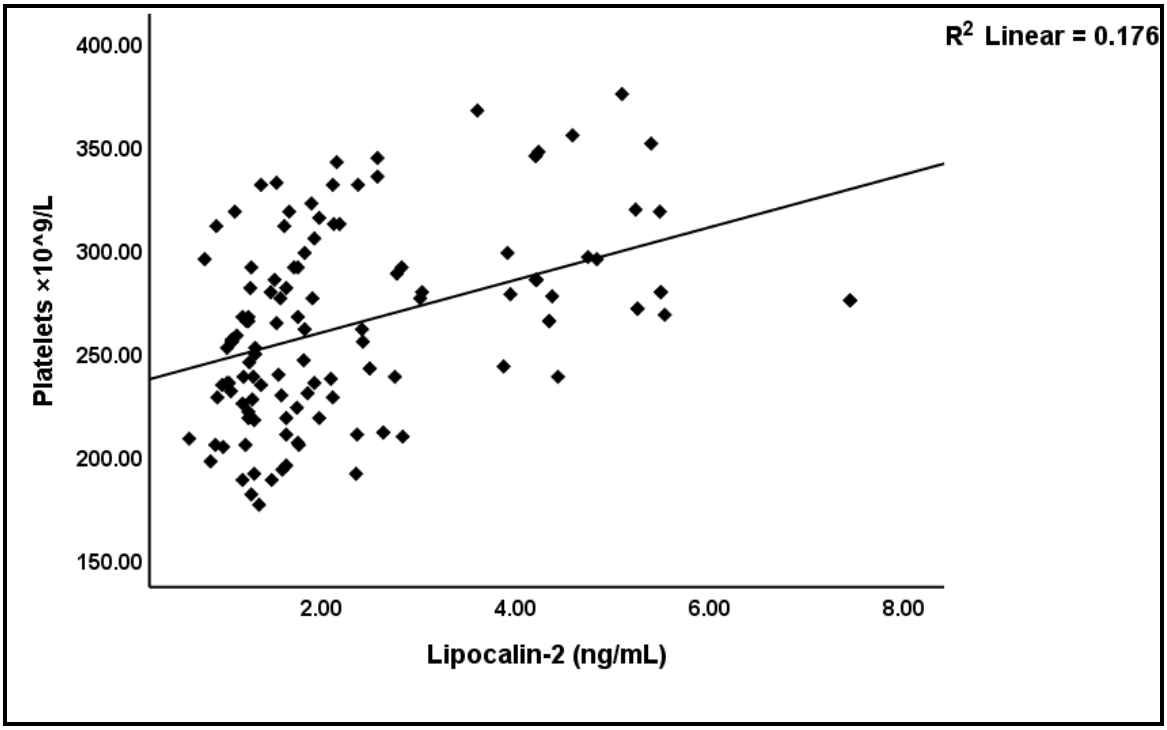
(G)


R² Linear = 0.324
Lymphocytes ×10^9/L
6.00
5.00
4.00
3.00
2.00
1.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

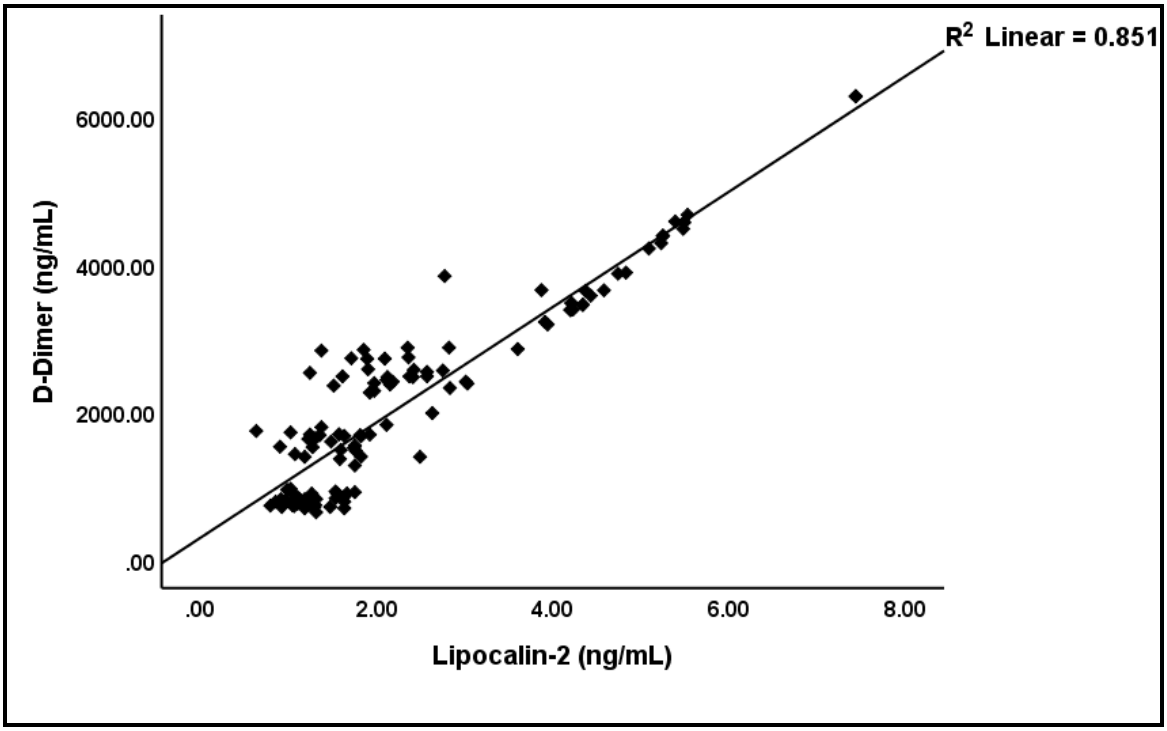
(H)


R² Linear = 0.436
NLR
8.00
6.00
4.00
2.00
.00
.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

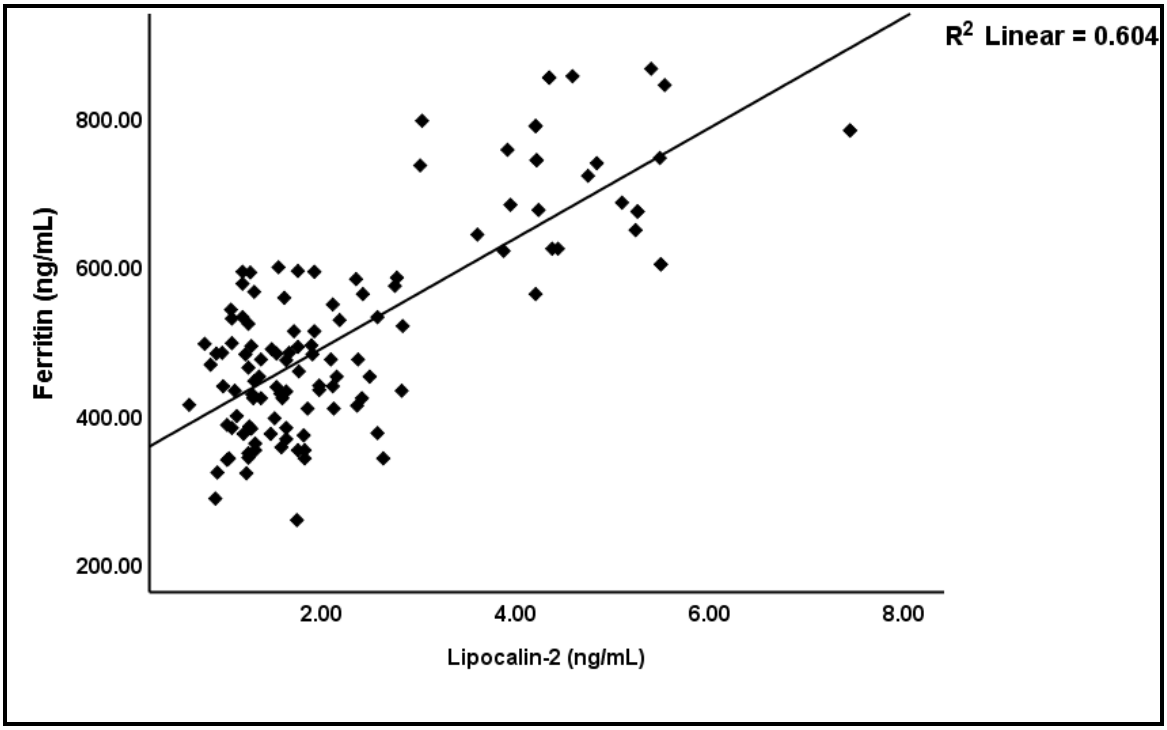
(I)


R² Linear = 0.176
Platelets ×10^9/L
400.00
350.00
300.00
250.00
200.00
150.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

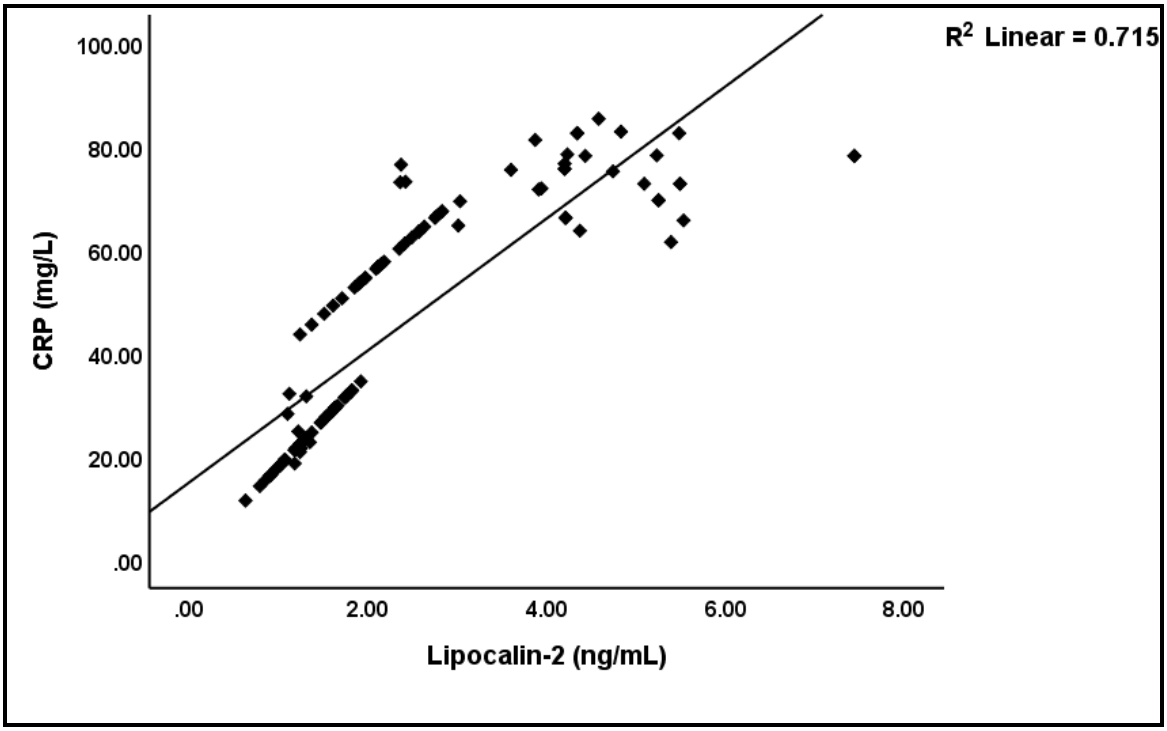
(J)


R² Linear = 0.851
D-Dimer (ng/mL)
6000.00
4000.00
2000.00
.00
.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

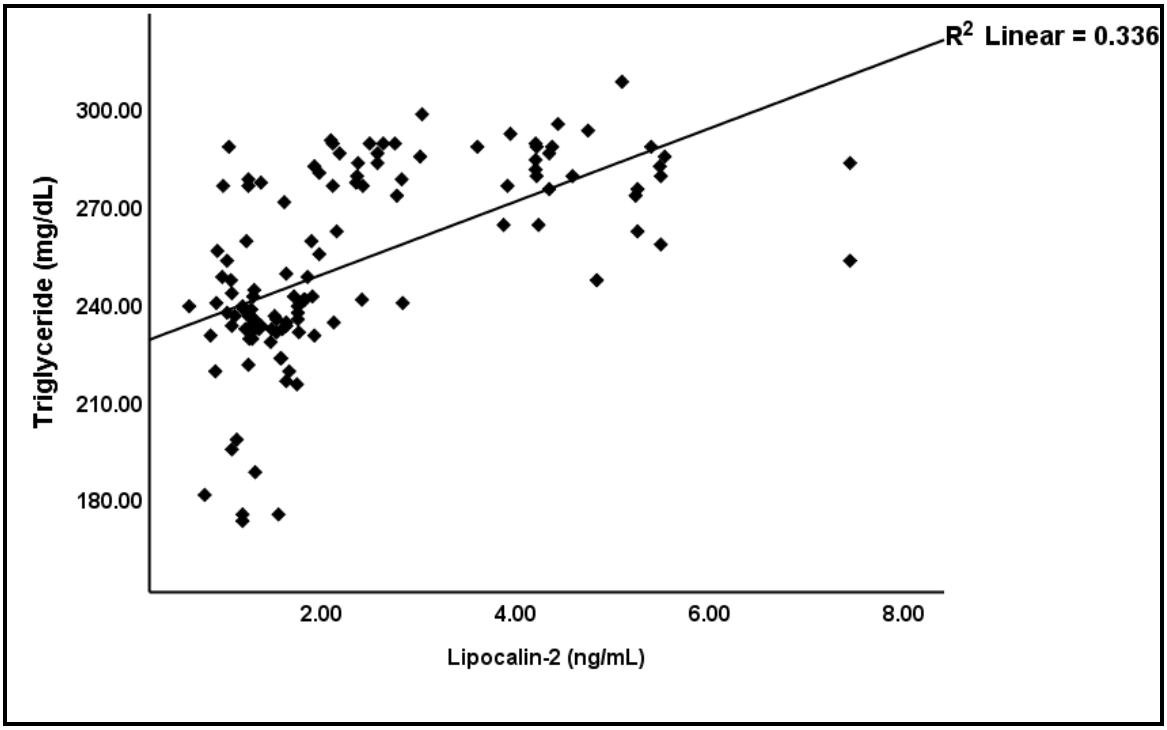
(K)


R² Linear = 0.604
Ferritin (ng/mL)
800.00
600.00
400.00
200.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

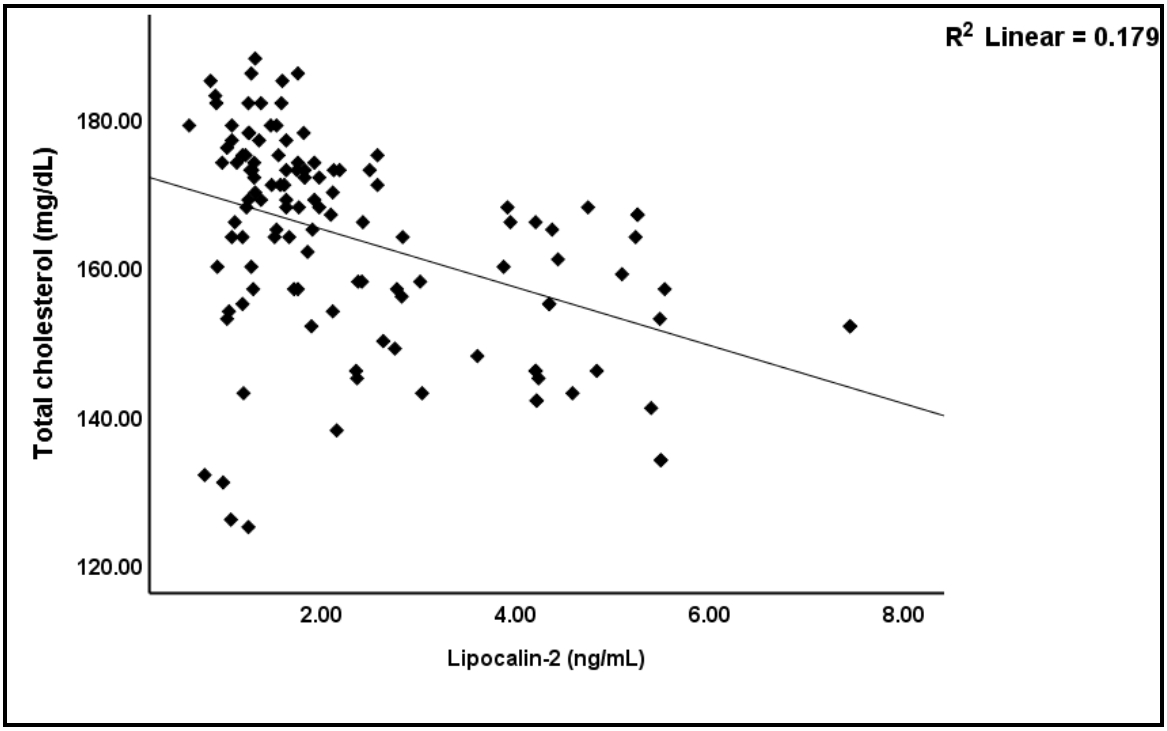
(L)


R² Linear = 0.715
CRP (mg/L)
100.00
80.00
60.00
40.00
20.00
.00
.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

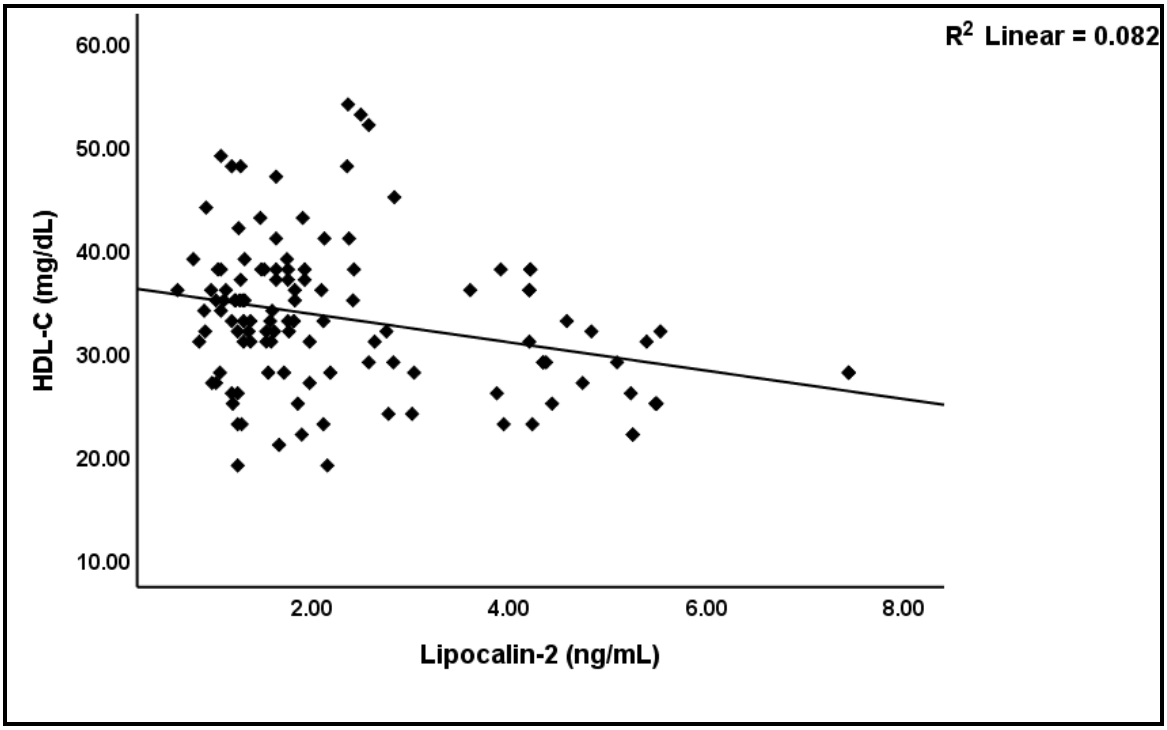
(M)


R² Linear = 0.336
Triglyceride (mg/dL)
300.00
270.00
240.00
210.00
180.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

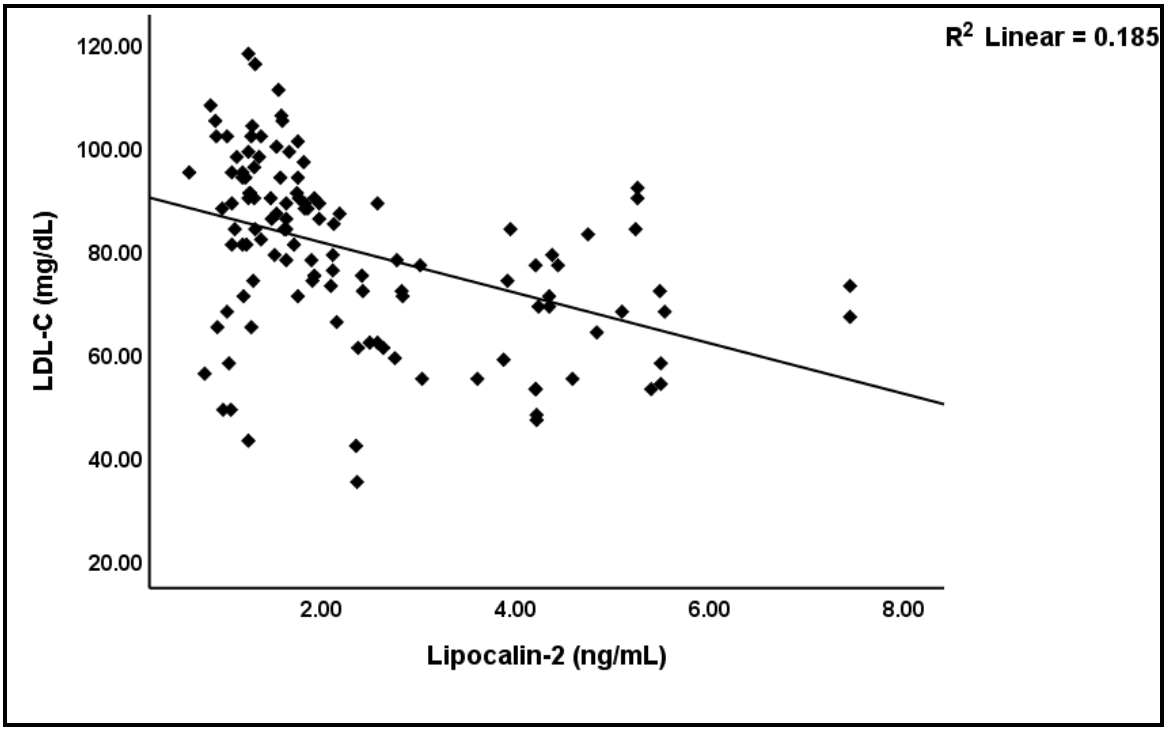
(N)


R² Linear = 0.179
Total cholesterol (mg/dL)
180.00
160.00
140.00
120.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

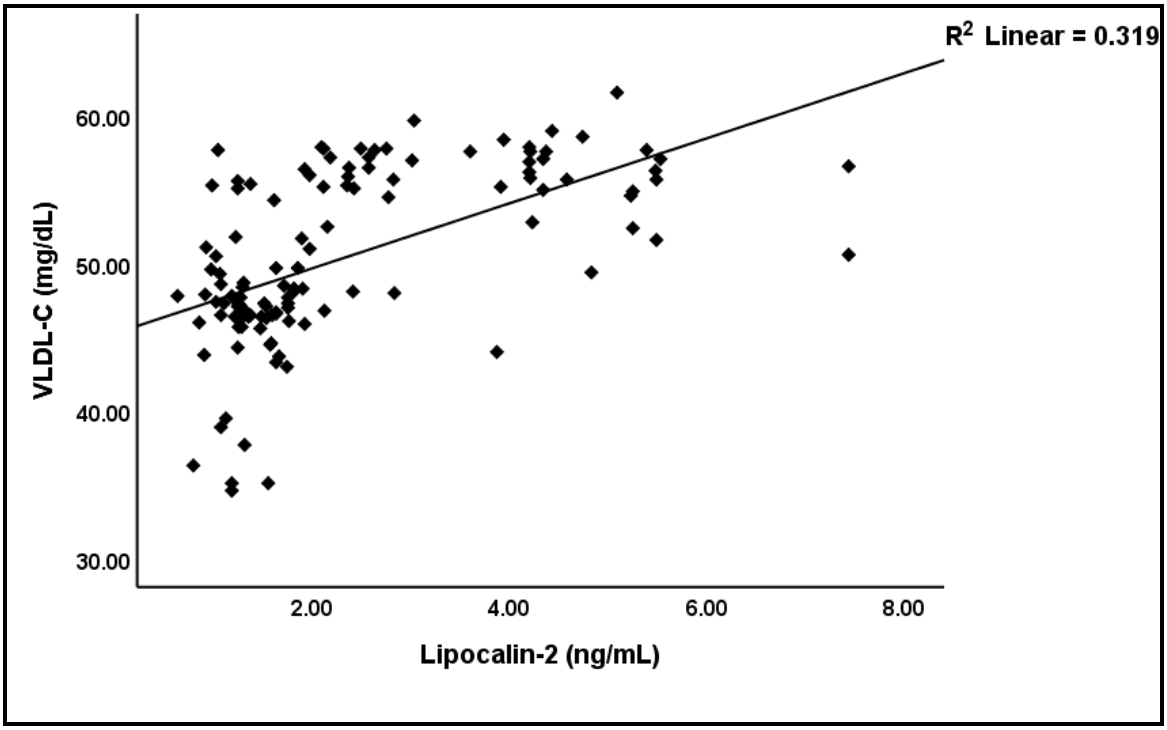
(O)


R² Linear = 0.082
HDL-C (mg/dL)
60.00
50.00
40.00
30.00
20.00
10.00
2.00
4.00
6.00
8.00
Lipocalin-2 (ng/mL)

(P)



(Q)



(R)

Fig. 2: Correlation between serum Lipocalin-2 levels and A: Age, B: Height, C: Weight, D: BMI, E: Hemoglobin, F: T-WBCs, G: Neutrophil, H: Lymphocytes, I: NLR, J: Platelet, K: D-Dimer, L: Ferritin, M: CRP, N: TG, O: TC, P: HDL-C, Q: LDL-C and R: VLDL-C.

Table 3: Receiver operating characteristic-area under curve analysis of the measured Interferon-λ1 for the diagnosis of COVID-19.

Variable	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	p-value
Lipocalin-2 (ng/mL)	1.215	81.7	80.2	0.900	0.852-0.949	<0.0001

AUC: Area Under the Curve, CI: Confidence interval.

The area under the curve (AUC) for Lipocalin-2 was 0.900 (95% CI, 0.852-0.949, p < 0.001). The sensitivity of Lipocalin-2 for predicting the severity of illness was calculated to be 81.7% when the cutoff value of 1.215 was established, while the specificity was calculated to be 80.2 %.

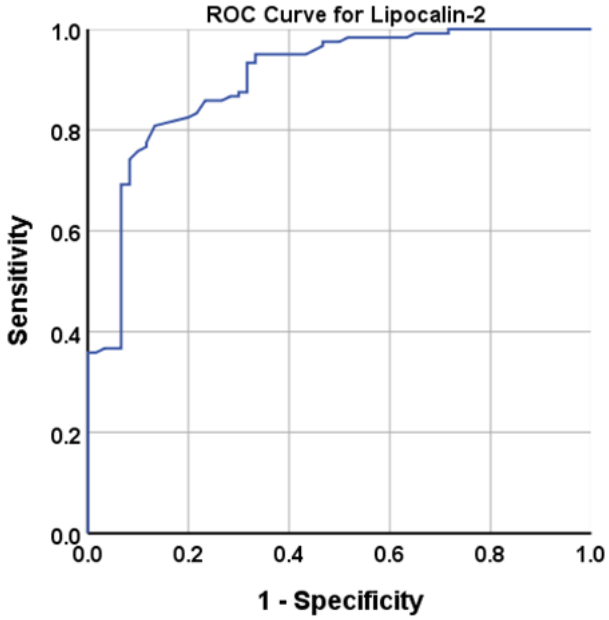


Fig. 3: Receiver operating characteristic (ROC) curve of Lipocalin-2 for diagnosis of COVID-19.

DISCUSSION

Symptomatic and asymptomatic COVID-19 patients showed elevated serum levels of lipocalin-2 compared to healthy controls/volunteers. (Serwin et al. 2022) . No notable differences in levels were found between patients of different severity, and no significant difference in levels was seen between critically ill patients in the ICU and patients recovering from COVID-19 after the ICU stay. Levels were raised in severe and critical cases (compared to healthy controls). (Abers et al. 2021). Moreover, levels were not significantly different between COVID-19 patients who required ICU admission and those who did not, indicating that serum lipocalin-2 was not a reliable indicator of ICU admission in COVID-19 patients. (Shakked et al. 2022). Levels were higher in severe and critical cases when compared to healthy controls, and elevated blood lipocalin-2 levels were associated with mortality. (Abers et al. 2021).

Coagulation activation in COVID-19 significantly overlaps with bacterial sepsis, because both inflammation and coagulation are important host responses to infection. (D'Alonzo, De Fenza, and Pavone 2020). The tissue factor reliant on the pathway is the major pathway by which coagulation activation begins. (Gando, Levi, and Toh 2016). Activated neutrophils, macrophages, monocytes, injured endothelium cells, and active neutrophils all express tissue factors. (Iba and Levy 2018). Moreover, the coagulation cascade is further activated by

phosphatidylserine expressed on numerous injured cells and microvesicles. (Iba and Ogura 2018).

Because late antibodies, the immunoglobulin G class, develop two weeks to about one month after infection, it is expected that high levels of NGAL persist for at least one to three months following the virus encounter., depending on the type of contamination. Blood was collected from hospital patients starting in January 2022. By employing the same product (plasma), technology (ELISA), and supplier, the raised mean plasma NGAL concentration following symptomatic infection (2.38±1.51 ng/mL) is comparable to the healthy control (0.86±0.51ng/mL).

The most recent information is crucial because various sets of materials (serum/plasma) and manufacturers can produce different results when used. Yet, it has been shown in numerous studies that there is a multiple-fold rise in NGAL concentration when related to healthy individuals or that some inflammatory disorders respond to both surgical and non-surgical treatment when compared to an appropriate reference group.

Blood- NGAL is primarily released by active neutrophils, the first line of the innate immune system, later replaced by specialized adaptive lymphocytes and immunoglobulins after an infection or reinfection. The majority of bacterial and fungal infections are the focus of this reaction, but little is known about how neutrophils react to intracellular viruses. During respiratory viral infections, neutrophils are a significant effector immune cell that is drawn to the lungs, but their function is much more complicated. In contrast to the ICU COVID-19 patients' lower lymphocyte counts, these patients have significantly higher neutrophil counts and G-CSF (granulocyte colony-stimulating factor) concentrations. (Velavan and Meyer 2020).

Due to their central role in the caused immunopathology of many respiratory disorders, elevated and activated neutrophils, particularly in the lungs, can lead to immunopathology and worsen disease severity. It must be determined if neutrophils are active participants in passive cells driven to the lungs and airways by virus-induced inflammation or the antiviral immune response. Since NGAL is linked to the development and worsening of renal and cardiac illness when it is released by neutrophils, it may be important in this situation. Furthermore, as the release of NGAL only occurs during the acute stage of an illness, it is still unclear how much of this molecule is left over after contracting COVID-19 or SARS-CoV-2.(Nystrom and Hammarstrom 2022).

According to reports, people with active inflammatory diseases had higher amounts of the acute-phase protein Lcn2 in their urine, serum, faeces, and epithelium. (Kang et al. 2018)(Guan et al. 2020) However, it is still unclear how Lcn2 reduces inflammation at the cellular level.

CONCLUSIONS

The demographic characteristics of severe and moderate COVID-19 patients in this study did not differ much from the epidemiological picture of Iraqi patients according to previous literature. The severity of the disease was significantly associated with the advanced age of the patient, Lipocalin-2 was a good forecaster of COVID-19 severity with good sensitivity, specificity, and accuracy.

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Declaration of Interests: The authors say they have no competing interests.

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