Evaluate Level of Serum Amyloid a As a Prognostic Marker for Predicting COVID-19 Severity in Iraqi Patients

Aiman Jawad Abbas[1], Muthana Saleh Mashkour[1], Hanaa Addai Ali[1], Sayed Omid Ranai Seyadat[2], Rawaa Adday Ali[3], Majid Jawad Al-Ghazali[4]
[1]Department of Chemistry, Faculty of Science, University of Kufa, Najaf 54001, Iraq.
[2]Protein Research Center of Shahid Beheshti University, Iran
[3]Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51001, Iraq
[4] Al-Hakim General Hospital / Najaf – Iraq.
E-mail: aymen.jwad2017@gmail.com

Abstract
Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes the unique infectious disease known as coronavirus disease 2019 (COVID-19) first appeared in Wuhan and spread fast around the globe. Patients with COVID-19 who are critically unwell have a significant fatality rate. In order to identify their predictive significance in predicting the severity of disease, this study was conducted to evaluate the levels of human serum amyloid A (SAA) and C-reactive protein (CRP) in patients with coronavirus disease 2019 (COVID-19). Methods: A total 120 COVID -19 patients serum samples were collected and healthy group (n=60) with age-matched, serum levels of cytokine and biomarkers were measured. Amyloid levels were measured by ELISA kit. Spectrophotometer techniques were used to monitor metabolic parameters. Age, BMI, elements, and electrolytes were analyzed along with the correlation coefficients between serum Amyloid A levels and these variables. Results: Serum Amyloid A, CRP, D-dimer and ferritin levels were significantly high in Patients with COVID-19 (110.25±3.99), (42.67±1.84), (4188.21±198.73) and (738±20.09) respectively, compared with healthy group (0.296±0.008), (3.36±0.25), (289.43±251) and (130.66±9.2) respectively, (P <0.001). The correlation of Amyloid A levels in COVID-19 patients was positively with age, CRP, D-dimer and ferritin levels but negatively with Iron, Ca and Na levels. The serum of Amyloid A levels in critical COVID-19 patients were significantly high compared with severe and moderate patients group. Conclusions: Serum Amyloid A levels as was creased in COVID-19 patients. Therefore, Amyloid A levels demonstrate a prognostic value for predicting the severity of COVID-19. Continuous SAA, results over the course of the study period revealed that the severe group's values were higher than those of the non-severe group.
Keywords: COVID-19 patients, Serum amyloid A, C-reactive protein, Ferritin and Electrolytes.

1. Introduction

A new coronavirus was discovered among pneumonia patients in Wuhan, Hubei Province, China, in December 2019, and the virus then quickly spread throughout the world (1). In patients with coronavirus disease, oxidative stress, irregular coagulation, and multi-organ failure are significantly correlated with a condition of excessive local and systemic inflammation and immunological activation (COVID-19) (2,3)
It has been demonstrated that an emergent infection is brought on by a novel form of coronavirus B with an enveloped ribonucleic acid, known as SARS coronavirus 2 (SARS-CoV-2) (4). Infection with COVID-19 linked to thrombotic problems due to platelet activation, endothelium damage from the virus, and aberrant blood flow dynamics (5).
Angiotensin signaling dysregulation, which results in inflammation and enhanced signaling through thrombin and purinergic receptors to promote platelet activation, is the main mechanism causing lung thrombosis (6). Type II alveolar epithelial cells of the human lungs include the ACE2 receptors, which are responsible for converting angiotensin to nitric oxide. Researchers noted that SARS-CoV-2 enters the human body through (ACE2) receptors on the membrane of cells (7). Since ACE2's discovery 20 years ago, knowledge of its underlying biology and physiology has grown, and this has significantly advanced our understanding of the renin-angiotensin system (RAS) (8-9). ACE2 performs a variety of functions, including as catalyzing reactions with diverse substrates, acting as a functional receptor for coronaviruses associated with the severe acute respiratory syndrome (SARS-CoV), and transporting amino acids. (7-10)
SARS-CoV-2 therefore focused its attention on the lungs in COVID-19 (10). Infection with SARS-CoV-2 is responsible for causing COVID-19, which has been classified as a pandemic by the WHO. Although SARS-CoV-2 has been shown to largely affect the respiratory system. (11) In this situation, the use of

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biomarkers for disease severity and clinical progression would make it easier to identify patients who need aggressive management and surveillance early on and would help ensure that healthcare resources are used wisely. At the first stage of 2019-novel coronavirus (nCoV) infection, primary inflammation takes place. This inflammation is fueled by rapid viral replication and the release of powerful pro-inflammatory cytokines. (16) The acute phase response, which includes a number of phenomena that happen in the presence of inflammation and infection, such as elevated temperature, hormonal and metabolic changes, greatly activates serum amyloid A (SAA) genes and proteins (12). Serum amyloid A (SAA) is also a critical mediator of disease pathogenesis and can stimulate cells via TLR2 to elicit a robust signaling cascade in human monocytes. (13) Circulating SAA concentrations, which are normally low under healthy conditions (20–50 mg/l), can rise up to 1,000-fold during the first 24–48 hours after an acute phase response. This results from increased synthesis in the liver, which is stimulated by a variety of factors, such as tumour necrosis factor (TNF), interleukin (IL)-1, IL-6, and interferon gamma (IFN-g) (14).

The role of this protein in cell-cell communication and feedback in inflammatory, immunologic, neoplastic, and protective pathways has come to be recognized. The primary acute phase reaction is likely controlled by SAA, who may also help the response spread. (15) Early research connected SAA to lipids, specifically HDL and cholesterol. Amphipathic properties predicted for SAA monomers involving lipid-binding areas (15, 17). Estimated that only 5-6% of circulating SAA is reactive at the top of a density gradient, and that 95% of circulating SAA is in the HDL fraction. Particularly, SAA participates in the metabolism of cholesterol under both physiologically healthy and inflammatory situations (15).

2. 2- Materials and methods

The case-control study comprised 90 patients (min.-max.ages:30-70 years) infected with COVID-19 who were admitted to AL- Amal Hospital, AL-Najaf province, for infectious disease in Iraq. After getting approval from the ethics committee of the Iraqi Ministry of Health and Environment, the study took place between January 2022 and September 2022 with informed consent from every participant. Within 7 to 12 days of the onset of symptoms, these individuals were confirmed and diagnosed using quantitative RT-PCR and a chest X-ray or CT scan. Three groups of patients were created: (50) mild / moderate case if they had a fever, respiratory symptoms, and radiological pneumonia evidence, of Severe case (41) and the (29) critically patients who after a later died. Patients with COVID-19 were gathered at admission, and the severity of the disease was determined using Murray scores (19). A patient were deemed to have severe COVID-19 if they satisfied any of the following criteria:

1. (≥ 30 /min) diversion from repetition.
2. Resting oxygen saturation of 90% or higher
3. Arterial oxygen (PaO2)/inspired oxygen fractions ≤ 300 mmHg. Or
4. Repertory failure needing intensive care unit and mechanical ventilation (ICU).

Additionally, critical patients who pass away are counted as non-survivors.

The patients' names, ages, sexes, weights, and heights were entered into a file along with their registration information. Ninety supposedly healthy persons were chosen as the control group. The distribution of their ages and sexes was similar to that of the patients.

Those who meet the exclusion criteria are smokers, pregnant women, those with systemic immunological illnesses, and volunteers who have thyroid gland disease or any other chronic disease were excluded such as diabetes, cardiovascular disease, or who are taking long-term oral corticosteroids.

Medical syringes that used to collect five milliliters of venoussblood from each patients and controls group. The leftover blood was placed in gel tubes and left at room temperature for 15 minutes to coagulate before being centrifuged for 10 minutes at (3000 Xg) to provide the serum. Two milliliters of the blood were deposited in EDTA tubes for complete blood count analysis. The sera were divided and kept at (-20 C0) in Eppendorf tubes until a biochemical assay could be done. complete blood count levels were assessed using an auto hematological analyzer (linear, Spain). Enzyme linked immune sorbent assay (ELISA) (Melsin, Chain) was used to measure the levels of Amyloid A tests in serum samples. This was typically done within the first 24-48 hours of admission. Also, the total cholesterol (TC), triglycerides (TG), High-density lipoprotein – Cholesterol (HDL-C). Electrolytes concentration (Na, K, Ca) were measured by using colorimetric methods kits (Agappe). Elements (Zn and Fe) were measured by using colorimetric methods kits (Agappe). Fluorescence immunoassay (or FIA) was used to measure the levels of serum ferritin and D-dimer (ichromaTM).

2-1-Statistical Analysis

The Statistical Package of Social Science (SPSS) version 26 and Graphpad Prism version 5 were used for the statistical analysis. The mean and standard deviation were used to express continuous variables (SD). For variables with equal and unequal frequencies, the significance of differences was determined using the paired t-test and the independent t-test, respectively. Standardized Pearson coefficients were used to evaluate bivariate correlations. P values less than 0.05 and less than 0.01 were regarded as statistically significant and highly statistically significant, respectively. To establish the cutoff value for Amyloid A, the receiver operating characteristic (ROC) analysis approach was used. The area under curve (AUC) value was

calculated using the ROC curve.

3. 3- Results and Discussion

3.1 Demographic Characteristics of Patients and Control group

As shown in Table (1), 120 confirmed SARS-CoV-2 cases (59 males and 61 females) were included (50) cases (23 males and 27 females) in mild/moderate category, (41) cases (21 males and 20 females) in the severe category, and 29 cases (15 males and 14 females) in the critical category.

Table (1) Comparison of general biochemical parameters studied patients between COVID-19 patient's categories and the healthy group

Parameters	COVID-19 patients group Mean ± SD				P-value
	Critical (29)	Sever (41)	Mild/moderate (50)	Healthy (60) Mean ± SD	
Sex, F/M	11/18	17/24	32/18	30/30	0.37
Age (years)	67.31±3.59	70.79±2.89	46.12±6.41	58.26 ± 5.76	A 0.00 B 0.00 C 0.00 D 0.06
BMI (kg/m^2)	29.11±5.31	29.13.04	25.51±1.99	24.81 ± 3.43	A 0.61 B 0.85 C 0.31 D 0.85
SBP (mmHg)	169.0±5.91	149.17±4.83	153.01±2.16	132.83±6.52	A0.06 B 0.00 C 0.08 D 0.00
DBP (mmHg)	82.93±0.6	80.47±0.4	77.93±0.8	80.76 ± 4.89	A 0.00 B 0.01 C 0.07 D 0.60

Data represented as Mean ± SD: standard deviation, BMI: body mass index, SBP: Systolic blood pressure, DBS diastolic blood presser F: females, M: male A= P.value (critical+ sever), B= P.value (critical + moderate) C P.value (sever+ moderate) D = (covid + healthy)

The general characteristics of the study groups are presented in Table (1) which consists the data of the 120 patients of Covid-19, this group was divided in to three groups (mild/moderate, severe and critical) compared with group of 60 healthy subject. The baseline characteristics Are non-significant in age between healthy and covid.19 group. Systolic Blood pressure are significant between the between healthy and covid.19 group. In the current study, severe covid-19 patient group has higher age than critical and mild groups.

The patients' average age in relation to COVID-19 severity (67.31±3.59 years, 70.79±2.89 years and 46.12±6.41) was none significantly when compared with control group's age (58.26 ± 5.76 years). Patients with severe infections of COVID-19 had a noticeably higher mean BMI. (29.13.04) than patients with critical and moderate disease (29.11±5.31and 24.81 ± 3.43 kg/m2, respectively). In actuality, severe and critical cases were recorded as being above (50), while more than half of mild/moderate cases were reported as being under (50). Except in the moderate category, sex distributions in the two illness severity groups of critical and severe (males more than females) (females more than males) respectively.

Table 2. Comparison of serum level of biochemical and laboratory test results of patients with COVID-19 parameters in covid-19 patients groups with healthy group.

Parameters	Critical Group (29) Mean ± SD	Sever Group (41) Mean ± SD	Moderate group (50) Mean ± SD	healthy group (60) Mean ± SD	P-value	T-test Value
TG (mg\dL)	283.41±2.66	282.34±1.23	237.03±0.62	135.83±1.52	a- 0.00 b- 0.00 c- 0.00	a-51.63 b-68.97 c-52.21
TC (mg\dL)	156.45±2.26	167.19±1.99	178.01±1.69	177.64±1.87	a- 0.00 b- 0.000 c- 0.891	a-7.18 b-3.705 c--0.138
LDL-C (mg\dL)	70.63±2.32	94.97±1.79	77.51±2.46	102.68±1.92	a- 0.00 b- 0.00 c- 0.00	a-10.58 b-2.77 c-8.11
VLDL-C (mg\dL)	56.68±0.532	56.46±0.247	47.4±0.124	27.16±0.304	a- 0.00 b- 0.00 c- 0.00	a--51.639 b--68.97 c--52.216

HDL-C (mg\dL)	29.13±0.816	33.208±1.63	35.62±0.323	47.79±0.93	a- 0.00 b- 0.00 c- 0.00	a-14.02 b-8.293 c-10.285
CRI-1 (TC/HDL)	5.093±1.23	4.47±0.732	5.141±0.98	2.30 ± 0.68	a-0.06 b-0.215 c-0.00	a-1.88 b- -1.262 c-4.137
CRI-11 (LDL/HDL)	2.291±0.83	2.478±0.424	2.566±0.506	1.119 ± 0.50	a-0.388 b-0.55 c-0.334	a- -0.874 b- -0.589 c-0.978
AIP(log TG/HDL)	0.079±0.02	0.05±0.011	0.073±0.018	0.401 ± 0.19	a-0.00 b-0.00 c-0.00	a-4.137 b- -0.664 c- -3.193
Iron conc (µg/dL)	40.95±3.63	41±3.57	48.15±3.95	100.19±5.99	a-0.00 b-0.00 c-0.00	a-7.37 b-7.23 c- 6.39
zinc conc (µg/mL)	127.3±7.55	111.05±10.55	122.75±10.03	84.22±3.32	a-0.00 b-0.00 c-0.00	a- -5.87 b- -2.90 c- 4.26
Ca (mg/dl)	9.11±0.135	8.51±0.23	8.7±0.134	9.56±0.133	a-0.02 b-0.00 c-0.00	a- 2.24 b- 4.17 c- 4.33
Na(mmol/L)	134.2±1.18	140.52±2.136	136.35±1.94	139.48±0.66	a-0.00 b-0.579 c- 0.08	a- 4.19 b- -0.558 c- 1.78
K (mmol/L)	3.95±0.122	6.42±1.87	4.21±0.211	3.9±0.23	a- 0.78 b- 0.097 c- 0.352	a- -0.163 b- -1.695 c- -0.939
Hb % (g/dL)	12.66±0.29	12.5±0.27	13.07±0.273	12.78±0.22	a- 0.74 b- 0.423 c- 0.408	a- 0.329 b- 0.808 c- -0.834
T-WBC %	13.6±0.25	11.61±0.29	10.65±0.37	9.05±0.194	a- 0.00 b- 0.00 c- 0.00	a- -14.51 b- -7.58 c- -4.13
Neut %	9.8±0.47	8.67±0.4	6.15±0.43	5.86±0.3	a- 0.00 b- 0.00 c- 0.00	a- -7.35 b- -5.63 c- -0.575
Lymph. %	2.49±0.13	2.8±0.181	4.22±0.192	4.2±0.08	a- 0.00 b- 0.00 c- 0.911	a- 11.77 b-7.85 c- -0.113
Plt. (109/L)	309.07±8.59	251.6±11.25	221.82±13.9	294.4±5.35	a- 0.133 b- 0.00 c- 0.00	a- -1.52 b- 3.799 c- 5.539
CRP(mg/L)	42.67±1.84	30.53±2.13	29.06±1.763	3.36±0.25	a- 0.00 b- 0.00 c- 0.00	a- -25.63 b- -15.42 c- -17.53
D-Dimer (ng/ml)	4188.21±198.73	2796.15±14862	1438.33±80.107	289.43±251	a- 0.00 b- 0.00 c- 0.00	a- -23.72 b- -20.116 c- -15.951
Ferritin (ng/ml)	738±20.09	503.75±19.46	453.8±13.87	130.66±9.2	a- 0.00 b- 0.00 c- 0.00	a- -30.34 b- -19.04 c- -19.99
Amyloid A (µg/mL)	96.8±3.27	110.25±3.99	82.82±11.57	0.296±0.008	a- 0.00 b- 0.00 c- 0.00	a- -36.28 b- -33.91 c- -8.78

Data represented as mean ± SD, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein-cholesterol VLDL; C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol; CRI.1: (TC/HDL) Castelli Risk Index; CRI-11: (LDL/HDL) Coronary risk index; AIP: (log TG/HDL-C) atherogenenic index. Data represented as Mean ± SD: standard deviation, Hb: hemoglobin, WBC: Wight blood cell, LYM: lymphocyte, NEUT: neutrophil, N/L: neutrophil/ lymphocyte, PLT: Platelet; a= p-value (critical + control); b = p-value (severe +control); c= p-value (mild + control).

The data of lipids profile consist of TG, VLDL-C levels were significantly higher (283.41±2.66), (56.68±0.532) respectively and HDL-C, LDL-C levels were significantly lower (29.13±0.816), (70.63±2.32) respectively in compared with healthy group. The data of Iron conc, zinc conc and Ca conc were significantly in compare with healthy group. The data of serum ferritin, D-Dimer levels were significantly higher (738±20.09) and (4188.21±198.73) respectively.

The level of serum Amyloid A compared with healthy group was significantly higher.

In the severe patients serum Amyloid A was higher than that of critical and moderate patients groups (110.25±3.99),(96.8±3.27) and (82.82±11.57) respectively based on laboratory findings from 120 patients. Additionally, major coagulation indicators, including D-dimer, were significantly raised in covid-19 patients, especially in circumstances when the condition was severe.

Table (3) comparison between general data and laboratory test results between COVID-19 patient's categories groups.

Parameters	Critical Group (29) Mean ± SD	Sever Group (41) Mean ± SD	Moderate Group (50)Mean ± SD	P-Value	T-test Value
TG (mg\dL)	283.41±2.66	282.34±1.23	237.03±0.62	a- 0.717 b- 0.00 c- 0.00	a- 0.365 b- 16.97 c- 32.67
TC (mg\dL)	156.45±2.26	167.19±1.99	178.01±1.69	a- 0.001 b- 0.00 c- 0.00	a- -3.55 b- -7.62 c- -4.13
LDL-C (mg\dL)	70.63±2.32	94.97±1.79	77.51±2.46	a- 0.04 b- 0.00 c- 0.00	a- -2.03 b- -8.29 c- -5.72
VLDL-C (mg\dL)	56.68±0.532	56.46±0.247	47.4±0.124	a- 0.717 b- 0.00 c- 0.00	a- 0.365 b- 16.97 c- 32.67
HDL-C (mg\dL)	29.13±0.816	33.208±1.63	35.62±0.323	a- 0.03 b- 0.00 c- 0.155	a- -2.22 b- -7.39 c- -1.45
Iron conc (µg/dL)	40.95±3.63	41±3.57	48.15±3.95	a- 0.78 b- 0.45 c-0.53	a- -0.282 b- -0.765 c- -0.635
zinc conc (µg/mL)	127.3±7.55	111.05±10.55	122.75±10.03	a- 0.30 b- 0.76 c- 0.42	a- 1.075 b- 0.306 c- -0.825
Ca (mg/dl)	9.11±0.135	8.51±0.23	8.7±0.134	a- 0.049 b- 0.048 c-0.535	a- 2.104 b- 2.115 c- -0.632
Na (mmol/L)	134.2±1.18	140.52±2.136	136.35±1.94	a- 0.01 b- 0.37 c- 0.19	a- -3.077 b- 2.115 c- -0.632
K (mmol/L)	3.95±0.122	6.42±1.87	4.21±0.211	a- 0.19 b- 0.51 c- 0.23	a- -1.350 b- -0.665 c- 1.244
Hb % (g/dL)	12.66±0.29	12.5±0.27	13.07±0.273	a- 0.68 b- 0.30 c- 0.14	a- 0.412 b- -1.026 c- -1.48
T-WBC %	13.6±0.25	11.61±0.29	10.65±0.37	a- 0.00 b-0.052 c- 0.03	a- 5.15 b- 6.53 c- 2.006
Neut %	9.8±0.47	8.67±0.4	6.15±0.43	a- 0.07 b- 0.00 c- 0.00	a- 1.18 b- 5.67 c- 4.32
Lymph. %	2.49±0.13	2.8±0.181	4.22±0.192	a- 0.16 b- 0.00 c- 0.00	a- -1.42 b- -7.47 c- -5.36
Plt. (10^9/L)	309.07±8.59	251.6±11.25	221.82±13.9	a- 0.00 b- 0.00 c- 0.00	a- 4.05 b- 5.32 c- 1.66
CRP (mg/L)	42.67±1.84	30.53±2.13	29.06±1.763	a- 0.00 b- 0.00 c- 0.60	a- 4.29 b- 5.32 c- 0.528
D-Dimer (ng/ml)	4188.21±198.73	2796.15±14862	1438.33±80.107	a- 0.00 b- 0.00 c- 0.00	a- 5.6 b- 12.83 c- 8.04
Ferritin (ng/ml)	738±20.09	503.75±19.46	453.8±13.87	a- 0.00 b- 0.00 c- 0.04	a- 8.37 b- 11.63 c- 2.09
Amyloid A (µg/mL)	96.8±3.27	110.25±3.99	82.82±11.57	a- 0.03 b- 0.26 c- 0.03	a- -2.419 b- 1.166 c- 2.278

Data represented as mean ± SD, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C:

Low-density lipoprotein-cholesterol VLDL; C: Very Low-Density Lipoprotein- Cholesterol, TC: Total cholesterol; AIP: (log TG/HDL-C) atherogenenic index. Data represented as Mean ± SD: standard deviation, Hb: hemoglobin, WBC: Wight blood cell, LYM: lymphocyte, NEUT: neutrophil, N/L: neutrophil/ lymphocyte, PLT: Platelet; a= p-value (critical + control); b = p-value (severe +control); c= p-value (mild + control).

In table (3) show there is no significant differences in serums Amyloid A levels when compare between COVID-19 patients categories groups.

Table (4) Comparison of serum amyloid level between males and females groups of patients and healthy

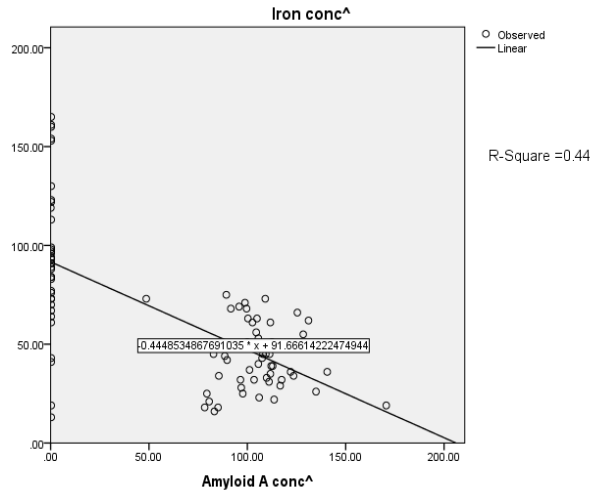
P-Value	T-test Value	Mean±S.D	Groups	Parameters
0.00	-16.52	0.28±0.05	Male (control)	Amyloid A conc. (µg/mL)
		102.08±23.85	Male (patient)	
0.00	-34.48	0.3±0.0	Female (control)	
		101.15±11.32	Female (patient)	

Table (4) demonstrated there is a high significant differences in serums Amyloid A level when compare between male, female patients and control (102.08±23.85, 101.15±11.32 and 0.28±0.05, 0.3±0.0) respectively (p< o.o5).

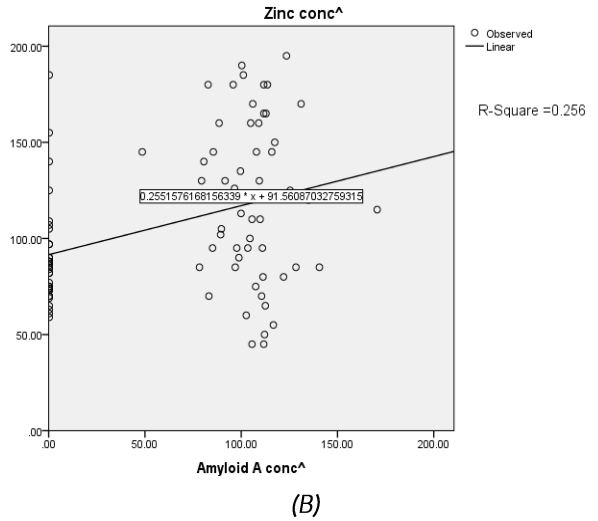
Table (5): Correlation between serums Amyloid A Level with clinical Parameters in COVID-19 patients group

Parameters	r-	P. value
Age	0.207	0.050
BMI (kg/m2)	0.044	0.0001
SBP (mmHg)	0.695	0.001
DBP (mmHg)	0.282	0.001
SpO2	0.897	0.001
TG (mg\dL)	0.177	0.095
TC (mg\dL)	-0.259	0.014
HDL-C (mg\dL)	-0.188	0.076
VLDL-C (mg\dL)	0.177	0.095
LDL-C (mg\dL)	-0.238	0.024
CRP-I (TC/HDL)	0.267	0.011
CRP-II (LDL/HDL)	0.173	0.032
Iron (µg/dL)	-0.661	0.000
Zinc (µg/mL)	0.364	0.001
Ca (mg/dl)	-0.431	0.001
Na (mmol/L)	-0.209	0.049
K (mmol/L)	-0.138	0.195
Hb % (g/dL)	-0.037	0.726
T-WBC %	0.230	0.029
Neut. %	0.214	0.043
Lym. %	-0.169	0.110
Plt. (10^9/L)	0.162	0.127
CRP (mg/L)	0.253	0.001
D-Dimer (ng/ml)	0.218	0.039
Ferritin (ng/ml)	0.250	0.017

Table (5) shown a significant correlation between serum Amyloid A levels with Iron, Zinc, Ca, Na, TC, LDL-C, T-WBC, Neut., CRP, Age, D-dimer and ferritin levels in COVID-19 patients group, as in figure (1).



(A)



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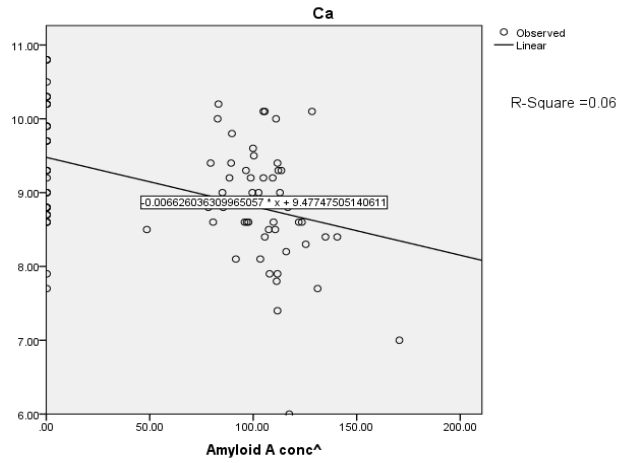
Ca
Observed
Linear
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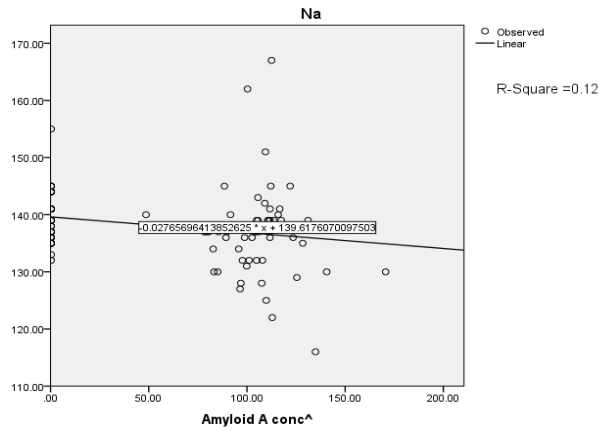
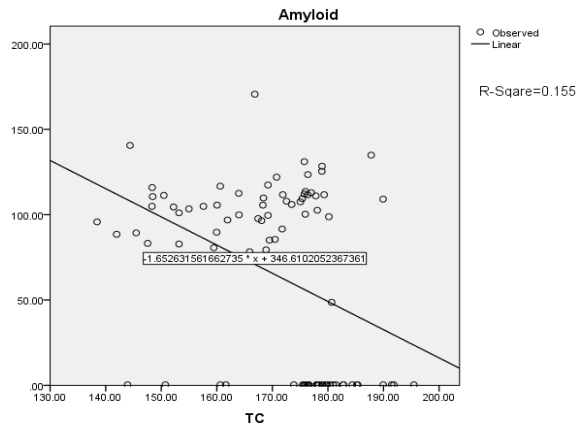
Amyloid A conc^

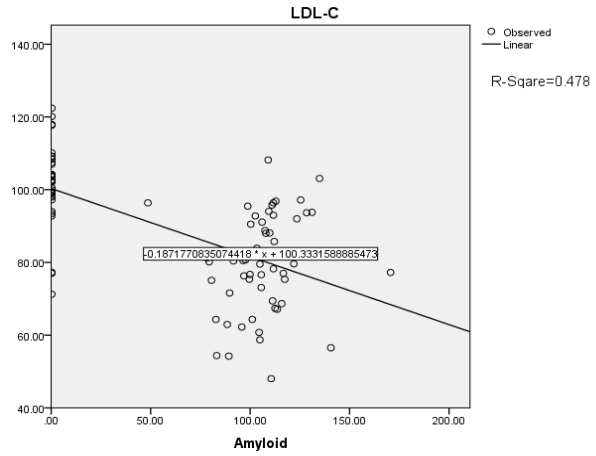
(C)


Na
Observed
Linear
R-Square =0.12
-0.027656964138526 25 * x + 139.6176070097503
Amyloid A conc^

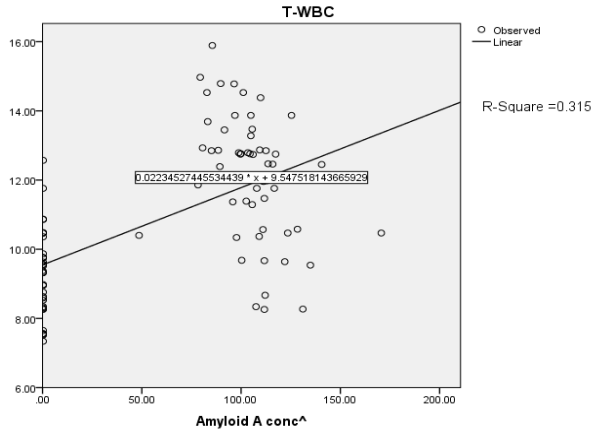
(D)


Amyloid
Observed
Linear
R-Sqare=0.155
-1.652631561662735 * x + 346.6102052367361
TC

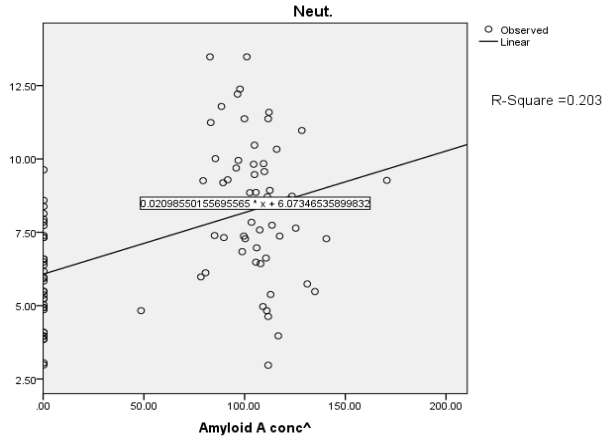
(E)


LDL-C
Observed
Linear
R-Sqare=0.478
-0.1871770835074418 * x + 100.3331588885473
Amyloid

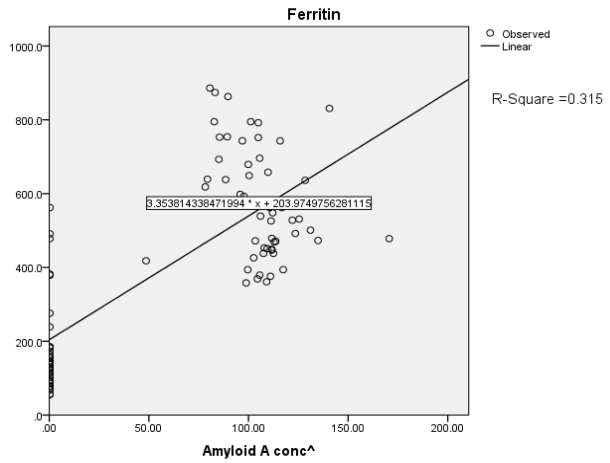
(F)


T-WBC
Observed
Linear
R-Square =0.315
0.02234527445534439 * x + 9.547518143665929
Amyloid A conc^

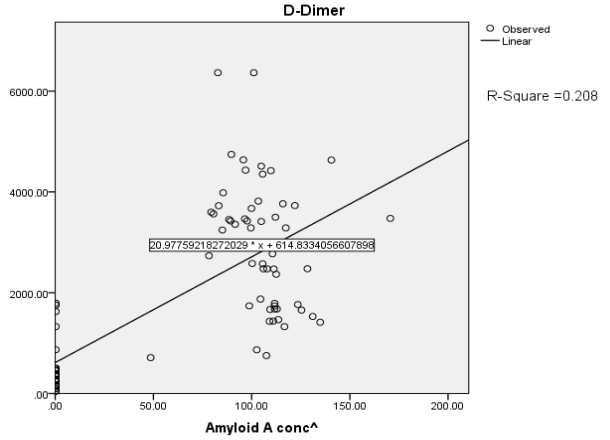
(G)


Neut.
Observed
Linear
R-Square =0.203
0.020985501556955 65 * x + 6.073465358998 32
Amyloid A conc^

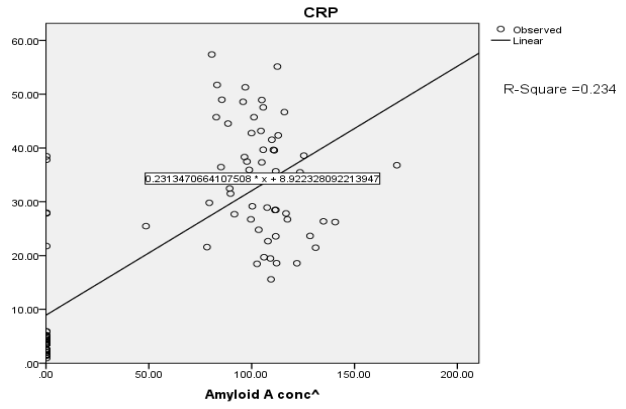
(H)


Ferritin
Observed
Linear
R-Square =0.315
3.353814338471994 * x + 203.9749756281115
Amyloid A conc^

(I)


D-Dimer
Observed
Linear
R-Square =0.208
20.97759218272029 * x + 614.8334056607898
Amyloid A conc^

(J)



(K)

Figure (1). Correlation between serum Amyloid A levels with (A) Iron, (B) Zinc, (C) Ca,(D) Na, (E) TC, (F) LDL-C, (G) T-WBC, (H) Neut., (I) ferritin, (J) D-dimer and (K) CRP in COVID-19 patients

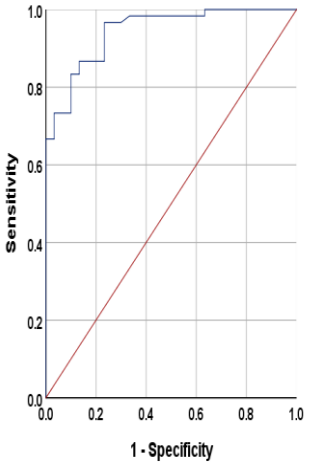


Figure (2) ROC curve of SAA levels

Table (6) sensitivity and specificity of serum Amyloid A levels in COVID-19 patients						
Variable	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	p-value
Amyloid A (µg/mL)	84.50	86.7	83.3	0.944	0.900-0.988	<0.0001

SARS-CoV-2 has recently spread over the world, hurting individuals and testing many nations' medical systems. Because SARS-CoV-2 is so contagious, hospitals are taking on a lot of patients quickly, putting many health systems in danger of failing. Additionally, the presence of asymptomatic carriers makes it more challenging to contain the present pandemic (20). Inflammatory markers and immune cell counts were examined in this study as potential indicators of COVID-19 severity. Due to the hyperactive immune response that culminates in lung inflammation and/or a cytokine storm, SARS-CoV-2 infections are fatal (21). Patients with ARDS run the risk of dying or suffering from severe lung fibrosis. Hospitalized COVID-19 patients over 60 years of age have a greater mortality rate than other patients (22).

The gold standard for diagnosing SARS-CoV2 infection to date is molecular testing that uses nasopharyngeal samples to identify virus particles by RT-PCR (23). The inflammatory polypeptide biomarkers indicated above can be useful in determining the severity of an illness. Increased levels of SAA, ferritin, CRP, D-Dimer, and iron were frequently seen in non-survivors as well as when circumstances progressed from mild to severe and critical. The non-specific acute phase reactants of these biomarkers. Clinicians must thus emphasize the clinical symptoms of patients who have SARS-CoV-2 infection and work to improve their capacity to differentiate between SARS-CoV-2 infection and other illnesses. To enable prompt action and stop the spread of SARS-CoV-2, it is important to diagnose patients as soon as feasible. This is especially important when the clinical manifestations include non-respiratory symptoms (24)

Clinical and radiological evaluation, serial trends of the biomarkers rather than single evaluations, are thought to be the greatest indicators when choosing treatment methods. Routine blood tests have detection rates comparable to those of molecular testing, therefore they can be used as early predictors of molecular tests along with CRP and LDH levels (25).

Recent research has shown that severe or critically ill cases were the cause of the majority of deaths (26). A portion of asymptomatic cases have a chance of developing into severe or critically ill ones, increasing the risk of death. In this aspect, stopping the disease from progressing from an early stage to a more severe one could be a viable tactic to lower disease mortality. In order to achieve this, it is crucial to find biomarkers that can quickly predict the likelihood of disease progression in COVID-19 patients (27, 28)

In reaction to inflammation and tissue damage, serum amyloid A (SAA), a significant conserved and sensitive acute phase protein, is highly produced. With regard to this broad role, healthy individuals typically have fairly low levels of SAA in their blood, but during the acute-phase response (APR), SAA hepatic synthesis causes a notable increase in h serum values within the first 24 hours, followed by very low levels beyond the acute phase (29). SAA also has immunological effects by stimulating immune cells and cytokines and chemokines production (30).

Acute phase reactants such as C-reactive protein and SAA were linked to an excessive inflammatory response. In patients hospitalized with community-acquired pneumonia, C-reactive protein was connected to 28-day mortality and had predictive relevance (31). This study's findings are consistent with a prior study in that critically sick patients' levels of D-dimer, ferritin, CRP, SAA, and LDH were significantly higher than those of mildly and severely ill patients (32).

As was seen above, patients with non-severe disease

and healthy individuals had CRP levels that were significantly different from those of patients with critical and severe cases compared to non-severe instances. Additionally, patients with COVID-19 had considerably greater serum SAA levels than healthy individuals, and both of these levels were higher in cases of severe disease.

According to a study by Zhang et al. and colleagues (29), all COVID-19 patients had elevated SAA levels, and there were statistically significant differences between those with severe and mild instances. Additionally, another study found that SAA changes were more significant than changes in CRP, lymphocyte count, and neutrophil count and were related to the severity of COVID-19 (30). The data presented above supported our study's findings and demonstrated that the severity of COVID-19 was correlated with a higher level of SAA.

According to the results of the current investigation, serum potassium and HDL-C levels were inversely associated to how severe COVID-19 was. In accordance with Dong C's (32) work, hypokalemia predominated in COVID-19 patients, and its treatment was difficult due to ongoing renal K+ loss brought on by the breakdown of ACE2. In cases of community-acquired pneumonia, serum HDL-C levels may fall and serum total cholesterol/HDL-C ratios may rise proportionately. In Wei C's investigation, the spike protein of SARS-CoV-2 linked to HDL and suppressed SARS-CoV-2 infection through antagonists of HDL receptor-Scavenger receptor class B type I. (33)

The limitations of our study should be taken into account when interpreting the findings. The sample size was firstly somewhat small. Second, because this was a study with a single measurement and no patient follow-up over time or observation of how Amyloid A behavior evolved, we are planning a cohort study in which Amyloid A will serve as the baseline measurement and include following-up on the variables of interest, giving the findings of this study more significance.

4. Conclusions

The management of problems, the prognosis, and the discharge of patients from hospital settings are all significantly influenced by biomarkers. Biomarkers must be substantially included into clinical processes and treatment decision-making in addition to clinical assessment. The aforementioned protein biomarkers, which are recognized markers of inflammation include SAA, can be used to detect and track the progression of COVID-19. A mix of biomarkers as opposed to a single biomarker provides more information that is useful to physicians. In order to stratify patients into high-risk groups and extract relevant information about their health state, it is also crucial to take the time of biomarker testing into account.

5. Acknowledgements

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Declaration of Interests

The authors declare no conflict of interests.

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