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Measurement Serum Level of Leucine-rich Alpha-2-glycoprotein-1 in Iraqi Hospitalized COVID-19 Patients

Maha H. Gadhi*, Eman S. Saleh

Department of Clinical Laboratory Science, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
*Correspondence to: Maha H. Gadhi (E-mail:maha.hasan1200m@copharm.uobaghdad.edu.iq)
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Abstract

Objective: The study aimed to assess Leucine-rich alpha-2-glycoprotein-1 biomarker serum level in hospitalized COVID-19 patients.

Methods: The case control study from multi-center in Baghdad included 45 adult patients (19 females and 26 males) with COVID-19, diagnosed with a positive real-time reverse transcription polymerase chain reaction and excluded negative RT-PCR for COVID-19 and comorbidity conditions. Second group, 43 control (20 females and 23 males).

Results: This study found decrease Leucine-rich alpha-2-glycoprotein-1 biomarker serum level in these patients and a significant difference in D. dimer, neutrophil count, lymphocyte count, and neutrophil lymphocyte ratio between the patients and controls at a *P* value equal to 0.000.

Conclusion: The concentration of Leucine-rich alpha-2-glycoprotein-1 in the patients after taking Tocilizumab was greatly decreased in many studies. Most of the patients in the study were treated with Tocilizumab which agent that directly blocks the effect of IL-6 by blocking the IL-6 receptors and greatly decreased the expression of LRG1 to impair production of the angiopathogenic constituent.

Keywords: COVID-19, interleukin-6, Leucine-rich alpha-2-glycoprotein-1

Introduction

Coronavirus Disease 2019 (COVID-19) pandemic issued through the World Health Organization (WHO)[1] at 11/3/2020 after announcement that popular of health emergency at 30/1/2020.[2] The coronavirus detected according to genomic sequence of virus.[3] The viral detection through the real-time or reverse transcription polymerase chain reaction (RT-PCR) testing.[4] Coronavirus is an enveloped RNA virus from Beta coronavirus genus,[5] Nidovirale order, and Coronaviridae family.[6] COVID-19 infection extended from minor and severe infection and very common to cause to insult of different body tissues like heart, kidneys, gastrointestinal tract, and brain,[7] COVID-19 characterized by abnormal laboratory testing such as leukopenia, lymphopenia, elevated levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), D-Dimer, serum ferritin, aminotransferase, and the abnormal finding further seen in computed tomography (CT) and chest x-ray.[8] COVID-19 enter the host cells through angiotensin-converting enzyme2 (ACE-2) protein[7] that present on type II pulmonary epithelial cells by a spike protein of the virus. The process of virus attachment is succeeded by host trans membrane serine protease 2 (TMPRSS2) that prim S2 subunit of spike protein to facilitate entry into host cell and caused an early phase through viral-related tissue injury directly and continued after the second phase after the infected host cells cause the activation of immune reaction by releasing the cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), interferon (IFN)-γ, tumor necrosis factor-α (TNF α), and other pro inflammatory mediators. Over activation of immune response caused liberations greater amounts of cytokines mainly TNF-α and IL-6 to the circulations as a cytokine storm and producing locally and extensively of the inflammatory reactions.[9] This inflammation caused accumulation of fluid lead to Acute respiratory distress syndrome (ARDS) that a major contributed factor for mortality within patients of COVID-19.[10] The pro-inflammatory mediator like IL-6 and TNF-α can induce level of Leucine-rich alpha-2-glycoprotein-1 (LRG1) that is about 50 kDa plasma glycoprotein contain 312 amino acid residues and weighing 23% carbohydrate of it. Its expressed within macrophages, neutrophils, the endothelial cells, and liver cells.[9] LRG1 transcription was activated by IL-6 by a phosphorylation and linking STAT3 to a consensus sequence in promoter site of the LRG1. The inflammatory cytokines disrupted the vasculatures by many factorial, such as disruption the vascular effects that mediated by cytokine inducing LRG1,[11] causing pulmonary edema and increase the vascular permeability.[12] The circulating LRG1 levels have been presented to be raised in severe COVID-19 patients[13] where vascular damage is a primary caused. Blocking of IL-6 signal at pulmonary endothelium, by anti-IL6 receptor antibody, Tocilizumab is an immunomodulating agent and highly specific monoclonal antibody directly block effect of the IL-6 by blocking the IL-6 receptors and greatly decreased the expression of LRG1. LRG1 has been defined an acute phase protein released into the circulation and as biomarker the possible pathological role, prognostic biomarker through determination of severity of infection, and as the subsequent therapeutic targets in COVID-19.[11] The study aimed to assess the serum level of Leucine-rich alpha-2-glycoprotein-1 biomarker in Iraqi hospitalized COVID-19 patients. Measurement of correlation between the serum levels of Leucine-rich alpha-2-glycoprotein-1 biomarker, D.Dimer. lymphocyte count, and neutrophil count.

Materials and Methods

Study Design

This study was approved by the University of Baghdad/College of Pharmacy and the Iraqi Ministry of Health/Rusafa Health Department. The study was involved a case control from the multi-center, the samples were collected from AL-Kindy teaching hospital, Al-Ataa Hospital, and Sheikh Dhari Al-Fayadh Hospital, in Baghdad-Iraq from September/2021 to January/2022. Hospitalized patients with COVID-19 tested.

The inclusion criteria were involved adult patients from age (20 to 60) years (median age 47) with COVID-19 who were diagnosed clinically, patients who showed the positively result to COVID-19 infection through the real-time reverse transcription polymerase chain reaction (RT-PCR) of respiratory samples from nasal/oropharyngeal swabs,[14] had a fever and pulmonary symptoms (cough, shortness of breath, chest tightness, and pain), and patients with radiological findings of consolidation either on chest X-ray or computed tomography (CT). Exclusion criteria include the negatively result of RT-PCR to COVID-19 infection, and comorbidities conditions (liver, renal, cardiovascular diseases, hypertension, diabetes, and autoimmune disease). The participants were divided into two groups:

Group 1: 45 (20 females and 25 males) COVID-19 patients with ages that range between 20 and 60 years old.

Group 2: 43 (20 females and 23 males) control subjects with ages that range between 20 and 60 years old.

Laboratory Analysis

The three milliliters of blood samples that have been drawn from patients with COVID-19 and healthy control were (1 ml in a gel tube) and left to coagulate for 15 minutes, then the samples were centrifuged at 5,000 rounds per minute (RPM) for 5 minutes to obtain the serum was collected by using the micropipette in a plain tube and stored about –20°C to measure the Human Leucine-rich alpha-2-glycoprotein-1 (µg/ml) sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit.[15] Other blood samples 2 ml put in:

- Sodium citrate tube for measurement of D-dimer level in COVID-19 patients. The sample of blood in was mixed gently for one minute to mix the sample with an anticoagulant reagent, then centrifuged at 4000 RPM for 6–10 minutes, collected plasma, and was used immediately for measurement level D-dimer by using fluorescence immunoassay.[16]
- EDTA test tube to prevent coagulation of blood sample. Sysmex/XN-350 analyzer was used for evaluating the White Blood Cell differential count.[17]

Statistical Analysis

The data were performed by using IBM SPSS software (version 26.0; IBM) and Microsoft excel 2010. Continuous variables were presented as median (interquartile range, IQR) because the variables not normally distributed. The number and percentage for the categorical variables of patients and healthy individuals were compared by using a Chi Square. The Mann-Whitney U test used for comparing the continuous variables between both groups. The two-tailed Spearman correlation coefficient (non-parametric) for showing the correlation between the Leucine-rich alpha-2-glycoprotein-1 biomarker serum level with D.Dimer, lymphocyte count, neutrophil count, and neutrophil lymphocyte ratio (NLR). The analysis of Receiver Operating Characteristic (ROC) curve for assessing a test's diagnostic performance or accuracy in distinguishing diseased from normal instances. The tests were two-tailed, and the statistically significant differences were considered at *P*-values of <0.05.

Results

The study consisted from 45 hospitalized COVID-19 patients, there were 20 females and 25 males and apparent Healthy subjects 43 were 20 females and 23 males. The result of present study expressed as no significant difference (*P* value = 0.467) between the gender of patients and the control. This relationship between categorical data, percentages, and numbers are calculated by a chi-square test. The descriptive data were presented as median (interquartile range) due to these variables not normally distributed and *P*-values were calculated using the Mann-Whitney U test, a non-parametrical test and the significant differences were considered statistically at *P*-values of <0.05. The categorical data was represented as numbers and percentages and the relationship between both groups was compared and calculated *P*-values by the Chi Square and the statistically significant differences were considered at *P*-values of <0.05. The result of present study expressed as no significant difference between the age and gender of patients and the control at *P* value equal to 0.514, 0.467 respectively and significant difference between the Leucine-rich alpha-2-glycoprotien-1, D. dimer, neutrophil count, lymphocyte count, and neutrophil lymphocyte ratio between the patients and control at *P* value equal to 0.000 for these variables (Table 1).

Table 1. **General characteristics of the variables between COVID-19 patients and control**

Variables	Patients	Controls	*P* value
Age (20–60) year median (IQR)	47 (14)	49 (9)	0.514
Gender male (female) Number "percent"	25 "28.4%" (20 "22.7%")	23" 26.1%" (20 "22.7%")	0.846
LRG1 µg/mL median (IQR)	7.477 (7.963)	12.196 (37.449)	0.000
D. dimer µg/ml median (IQR)	1.06 (1.735)	0.1 (0.048)	0.000
NEU × 10^3/µL median (IQR)	9.8 (4.250)	6.65 (0.99)	0.000
LYM × 10^3/µL median (IQR)	1.2 (0.43)	1.88 (0.42)	0.000
NLR median (IQR)	7.6613 (4.59)	3.075 (1)	0.000

*$P < 0.05$ statistically significant differences. LRG1: Leucine-rich alpha-2-glycoprotien-1 µg/mL, NEU × 10^3/µL: neutrophil × 10^3/µL, and LYM × 10^3/µL: lymphocyte × 10^3/µL, NLR: neutrophil lymphocyte ratio.

Table 2. **Correlation between leucine rich alpha2 glycoprotien1 and D.Dimer with D.Dimer neutrophil count, lymphocyte count**

Parameter	LRG1		D.Dimer	
LRG1 µg/mL	*P* value	*r.*	*P* value	*r.*
	–	–	0.007	–0.284**
D.Dimer µg/ml	*P* value	*r.*	*P* value	*r.*
	0.007	–0.284**	–	–
NEU × 10^3/µL	*P* value	*r.*	*P* value	*r.*
	0.008	-0.28**	0.000	0.759**
LYM × 10^3/µL	*P* value	*r.*	*P* value	*r.*
	0.002	0.331**	0.000	–0.54**
NLR	*P* value	*r.*	*P* value	*r.*
	0.000	–0.412**	0.000	0.733**

**Correlation is significant at the 0.01 level (2-tailed). $P < 0.05$ statistically significance. *r.*: correlation coefficient, LRG1: Leucine-rich alpha-2-glycoprotien-1 (µg/mL), NEU × 10^3/µL: neutrophil × 10^3/µL, and LYM × 10^3/µL: lymphocyte × 10^3/µL, NLR: neutrophil lymphocyte ratio.

Table 3. **Receiver Operating Characteristic Curve for study of COVID-19 Patients for D.Dimer, lymphocyte count, neutrophil count and neutrophil lymphocyte ratio**

Variable(s)	Accuracy	Area (AUC)	Significance Asymptomatic	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
D.D (µg/ml)	Excellent	0.929	0.000	0.864	0.995
NEU × 10^3/µL	Excellent	0.981	0.000	0.961	1.000
NLR	Excellent	0.992	0.000	0.981	1.000
LYM × 10^3/µL	Very good	0.839	0.000	0.754	0.924

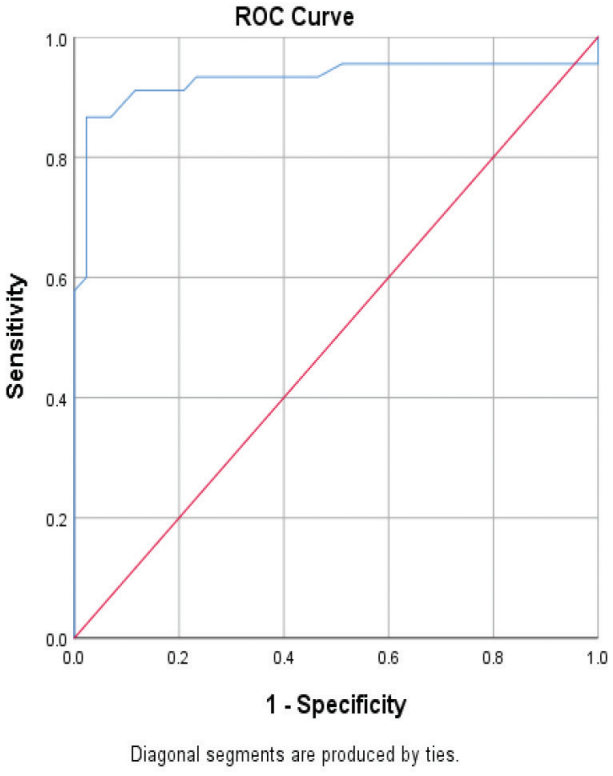

Fig. 1 **Receiver Operating Characteristic Curve for D. Dimer µg/ml studied groups.**

In the Table 2 LRG1 µg/ml and D.Dimer µg/ml expressed a significant correlation with NEU × 10^3/µL, LYM × 10^3/µL, NLR and the significant correlation between LRG1 µg/ml and D.Dimer µg/ml Sperman correlation revealed a strong correlation between D.Dimer µg/ml and NEU × 10^3/µL and NLR and correlation coefficient (*r.*) values (0.759, 0.733) respectively.

The Table 3 is showing use of receiver operating characteristic (ROC) for measurement the accuracy and area under the curve of the variables (D.Dimer, neutrophil count, lymphocyte count and NLR). The accuracy of D-dimer for both groups. Area under the curve (AUC) of D.D was 0.929, the cutoff value of D-dimer in the s (0.2155 µg/ml), sensitivity 86.7% while the specificity is 97.7% as represented in Figure 1. Area under the curve (AUC) of lymphocyte count was 0.839, the cutoff value of lymphocyte count (0.78 × 10^3/µL), sensitivity 2.2% while the specificity is 100% as represented in Figure 2A while Area under the curve (AUC) of neutrophil count was 0.981, the cutoff value of neutrophil count (10 × 7.55^3/µL), sensitivity 88.9% while the specificity is 90.7% and area under the curve (AUC) of neutrophil lymphocyte ratio was 0.992, the cutoff value of neutrophil lymphocyte ratio (4.3282), sensitivity 95.6% while the specificity is 97.7% as represented in Figure 2B.

Discussion

The study showed the male patients are more susceptible to COVID-19 disease than women, and no significant differences in age between both genders, the study agreed with other

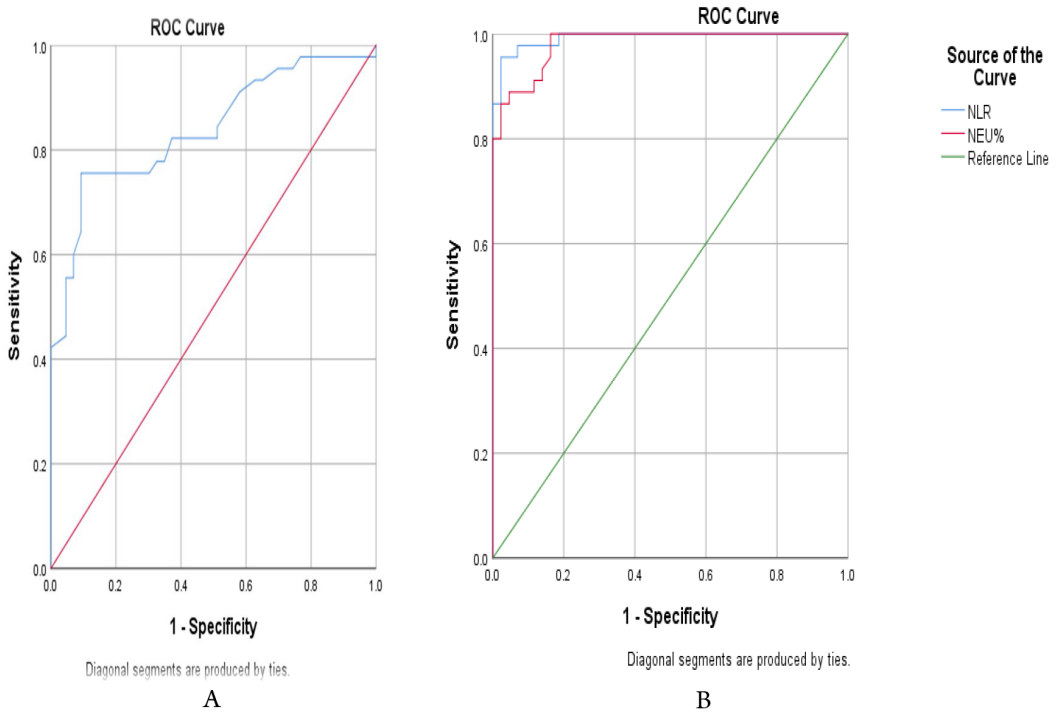

Fig. 2 **Receiver Operating Characteristic Curve for Studied groups. (A) Lymphocyte count (B) Neutrophil count and Neutrophil Lymphocyte ratio.**

studies that the males (72%) highly affected than females (28%) and no significance difference in both gender of patients, (P = 0.750) in severe COVID-19 infection.[18] This study also a significant differences in lymphocytes count and neutrophils count and NLR in Covid-19 patients and agreed within the previous studied such as Khartabil et al. Complete blood count is widely used routine laboratories analysis, the detection of neutrophil count, NLR were extremely increase in COVID-19 patients with comparison with healthy subjects and decrease level of lymphocyte count in those patients.[17] The data of complete blood count that represented in the neutrophil count and lymphocyte count, and related to NLR. An increase the level of neutrophil count related to the systemic inflammation intensity while decrease the level of lymphocyte count related to sequestration of lymphocytes at site of inflammation and their apoptosis. The combination of these two biomarkers will be better indicator for detection the severe infection in COVID-19.[19] Huang et al. that described same determinations, the patients of ICU accomplished by increase leukocyte count, neutrophil count, with decrease lymphocyte count compared without ICU.[20] When decrease lymphocyte counts below 0.8×10^9/L can related to the severe COVID-19 infection and increase neutrophil count higher than 3.5×10^9/L considered as bad medical outcomes.[21] The COVID-19 prognosis predicted when increase neutrophil-to-lymphocyte ratio (NLR), reported by Yang et al. study.[22] The meta-analysis for six studies showed the elevation of NLR might propose the poor prognostic within COVID-19 patients.[21] ROC analysis curving appeared NLR is greatest of the accuracy over markers of complete blood count for measuring the severity of COVID-19 within cut off value 4.3282 with acceptance to previous studies Ciccullo, A. et al. that show the significant increase NLR with patients of severe COVID-19 in the cohort study for 452 hospitalized patients.[23] In this study appear the significant differences in D.Dimer of severe form COVID-19 patients and agreed within the previous study Ye et al. showed increase D-dimer level in severe forms of COVID-19 infection, an increased D-Dimer values due to increase the activity of coagulation[19] and Contributed mechanism due to inflammatory mediator activation and contributed with rupturing of plaque by inflammatory response directly, induce of pro-coagulatory factors, and hemodynamically changing causing ischemia and thrombosis also the angiotensin converting enzyme 2 (ACE-2) that SARS-CoV-2 receptor express with vascular endothelium, least ways for the possible direct viral invasion into myocardium.[24] Level of Leucine-rich alpha-2-glycoprotien-1 biomarker in COVID-19 patients in this study in compared with other studied as Demichev et al. that reported of a cohort study that increased levels of the inflammatory and acute phase protein within the time such as LRP1 and related to possibility the death due to COVID-19 infection.[25] Among the patients that collected the data of them were presenting in severe infection of COVID-19 and presented in the hospitals that collect the data from it in long periods more that 25 days and greater. Those patients had been taken Tocilizumab as the humanized monoclonal antibody that its a interleukin-6 receptors blocker. In COVID-19, first uses within 21 patients from Chinese have serious states and notable enhancements. At the initial appearance of IL-6 blocker strategies for applying the treatment another patients of COVID-19 that include Italian patients with different results that leading to the clinical trial of phase II multicenter.[26] In scientific reports of Dritsoula et al., showing the block signal of IL-6 receptors within Tocilizumab to vascular endothelium of the lung causing reduce level of LRG1 led to impair production the angiopathogenic constituent. the meta-analysis and other studies reported Tocilizumab for treatment the severly and critical illness of COVID-19 have benefit results.[11] The limitation in this study, timing of the sample that collected form the

patients, the sample should collect within the time of them admission to the hospital for measuring differences in the serum of biomarkers after treatment.

Conclusion

The direct participation pathogenesis of immune system in COVID-19 infection due to the pro-inflammatory cytokine causing induce systemic inflammation and pulmonary insult. The previous studies were showing the inflammatory mediators, as IL-6 cause induce damage of epithelium. The LRG1 biomarker is the one of significant biomarkers producing angiopathogenesis causing disruption in the usual vascular physiology.

Abbreviation

COVID-19: Coronavirus Disease 2019, WHO: World Health Organization, RT-PCR: real-time reverse transcription polymerase chain reaction, CRP: C-reactive protein, LDH: lactate dehydrogenase, CT: computed tomography, ACE-2: angiotensin-converting enzyme2, TMPRSS2: trans membrane serine protease 2, IL-1: interleukin-1, IL-6: interleukin-6, IFN-γ: interferon-γ, TNF-α: tumor necrosis factor-α, ARDS: acute respiratory distress syndrome, LRG1: Leucine-rich alpha-2-glycoprotein-1, NLR: neutrophil lymphocyte ratio, ROC: receiver operating characteristic, NEU × 10^3/μL: neutrophil × 10^3/μL, LYM × 10^3/μL: lymphocyte × 10^3/μL, AUC: area under the curve, ICU: intensive care unit.

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Conflict of Interest

No conflicts of interest.

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