Measurement of the serum level of Elabela for the early detection of acute kidney injury in hospitalized Iraqi COVID-19 patients

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Abstract:
Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is caused coronavirus disease 2019 (COVID-19) affecting people worldwide. The angiotensin converting enzyme 2 (ACE2) represents a receptor of SARS-CoV-2 on the infected host cell. Apelin or its receptor agonists suppress the production of angiotensin-converting enzyme (ACE) and angiotensin II (Ang-II) and is characterized by a protective effect against SARS-CoV-2.

Objective: The study aims to assess the serum level of Elabela biomarker as an early detector for Acute Kidney Injury (AKI) in patients with COVID-19.

Cases and Methods: This is a case-control study which included 45 hospitalized adult patients in multiple centers (public hospitals) receiving COVID-19 cases in Baghdad. These cases had a positive real-time or reverse transcription polymerase chain reaction (RT-PCR) of nasal/oropharyngeal swabs. Excluded from the study were those with a negative PCR and comorbidities and 43 apparently healthy adult subjects as controls. The age range of the cases and controls was (20 to 60) years.

Result: There are no statistically significant differences between the two groups in terms of age and gender distribution. Statistically significant differences were found in terms of eGFR, S. Creatinine, D. dimer, NEU×10³/µL, LYM×10³/µL and ELA biomarker. Significant negative correlations were found between Elabela with D. dimer and NEU×10³/µL, and between eGFR with S. creatinine, D. Dimer, and NUT×10³/µL.

Conclusion: The Elabela biomarker can be used for the early detection of acute kidney injury in COVID-19 patients.

Keywords: COVID-19, ACE2, Acute kidney injury, Elabela.
COVD-19-associated AKI is caused by the extensive release of pro-inflammatory mediators, hypoperfusion, renal congestion caused by the increased amount of positive end-expiratory pressure, and viral invasion directly into the epithelial cells of the kidney tissue and podocytes (8). The enzyme responsible for the conversion of Angiotensin-I (Ang-I) to Angiotensin-II (Ang-II) is the Angiotensin-converting enzyme (ACE) that accumulates in the pneumocytes, endothelial cells (9), heart, and kidney (10). The virus enters the host cell by tissue spike (S) protein of the virus and binds with transmembrane ACE2 of the host cell (9)(11), accompanied by a reduced response to expressing ACE2 and causing a rise in Ang-II production (12) leading to vasoconstriction, activation of the inflammatory response, endothelial damage, and collagen synthesis within fibroblasts, ending in fibrosis in these organs. ACE2 is a counter-regulatory enzyme that decomposes Ang-II forming angiotensin 1–7 causing vasodilation and enhancing Ang-II interfered inflammation (13). ACE2 is presented with an excess amount of renal than pulmonary tissues (14) arranged in the brush border membrane of the proximal tubule and present in small amounts in the podocytes (8). The virus penetrates renal tissues by attacking the podocyte, entering tubular secretions, and attaching within ACE2 at the proximal tubule (15). The apelinergic system act as an essential signaling pathway by the modulation of cardiovascular homeostasis. Its G protein-coupled receptor which shares the same tendency of tissue deposition coupled to apelin peptide jejunum (APJ) is defined as the angiotensin II type 1 receptor (AT1R) and its endogenous ligand (16), Elabela (ELA) is a 32-residue peptide hormone, an endogenous ligand for (APJ) (17). It adjusts fluid homeostasis (18), reduces blood pressure (19), increases the formation of new blood vessels (20), and is well preserved in case of renal insult (21). Studies demonstrate that these peptides may diminish the intensity of Acute Lung Injury (ALI) by reducing the accumulation of fluid in the alveoli, secretion of cytokines, and hypoxemia, thus developing with COVID-19-linked ARDS causing a subsequent insult to the cardiac, renal, and other tissues (22). So that those peptides would inhibit the severe effects of COVID-19, and their favorable effects expand to protecting different body tissues from increased levels of inflammatory cytokines and reducing fatality (23).

Patients and Methods:
The data for this multi-center study was collected from several public hospitals including Al-Kindy teaching hospital, Al-Ataa hospital, and Sheikh Dhari Al-Fayadh hospital, in Baghdad-Iraq from September 2021 to January 2022. This study was approved by the University of Baghdad / College of Pharmacy and the Iraqi Ministry of Health / Rusafa Health Directorate. Hospitalized patients with COVID-19 and with a positive real-time reverse transcription polymerase chain reaction (RT-PCR) of respiratory samples taken from nasal/oropharyngeal swabs (24) were included in this study as the cases. Inclusion criteria were: Adult patients between 20 to 60 years of age with COVID-19 diagnosed clinically with fever and pulmonary symptoms (cough, shortness of breath, chest tightness, and pain), a positive RT-PCR for COVID-19, and radiological findings of consolidation either on chest X-ray or computerized tomography (CT). Exclusion criteria include Negative RT-PCR for COVID-19, pregnant women, and comorbidities (liver, renal, cardiovascular diseases, hypertension, diabetes, and autoimmune diseases). Two groups were included in the study: Group 1: 45 (20 females and 25 males) severe COVID-19 cases with respiratory distress (dyspnea). Group 2: 43 (20 females and 23 males) were healthy age-matched as control.

Laborotory Analysis
- Five milliliters of blood were drawn from COVID-19 patients. Three ml was placed in a gel tube and left to coagulate for 15 minutes. The samples were then centrifuged at 5,000 rounds per minute (RPM) for 5 minutes and the serum was collected by a micropipette in a plain tube and stored at (−20° C) to measure Human Elabela (pg/ml) sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit (25). The remaining serum was used for measuring S. creatinine (mg/dL) by using a multiple wavelength spectrophotometer (26).
- The remaining 2 ml of blood was used as follows: Sodium citrate tube for measurement of D-dimer level in COVID-19 patients. The sample of blood was mixed gently for one minute with an anticoagulant reagent, then centrifuged at 4000 RPM for 6-10 minutes. The collected plasma was used immediately for measurement level D-dimer by using fluorescence immunoassay (27).
- EDTA test tube to prevent coagulation of blood sample. Sysmex XN-350 analyzer was used to measure the White Blood Cell differential count (28). According to modification of Diet in Renal Disease [MDRD] Study Equation, GFR (mL/min/1.73 m²) = 175 × (Scr)-1.154 × (Age)-0.203 × (0.742 if female) is used for calculating the estimated glomerular filtration rate (eGFR) (ml/min) (29).
Statistical Analysis:
Descriptive data were presented as numbers and percentages for categorical variables, mean ± standard deviation for normally distributed variables, and median for variables that are not normally distributed. The Chi-square test was used to test associations between variables, the Mann–Whitney U test was used for the group’s analysis of non-normally distributed variables and the Student t-test was used to study differences between means of the groups for normally distributed data. Non-parametrical correlation (spearman correlation) was used for assessing the correlation between Elabela biomarker and eGFR with different laboratory parameters including S. Creatinine µmol/L, D. Dimer µg/ml, NEU×10³/µL, and LYM×10³/µL. Assessing a test’s diagnostic performance or accuracy in distinguishing diseased from normal cases was done by using Receiver Operating Characteristic (ROC) curve analysis. All tests were two-tailed, and differences were considered statistically significant at (P-values < 0.05).

Results
This study was conducted on 45 hospitalized COVID-19 patients, 20 (22.7%) females and 25 (28.4%) males, and 43 apparently healthy subjects, 20 (22.7%) females and 23 (26.1%) males. The distribution of the cases and controls by gender did not show any significant associations (p-value =0.467).

Table 2: Correlations between Elabela and eGFR with other variables

| Parameter          | Elabela (pg/mL) | P r. value | eGFR (µmol/L) | P r. value |
|--------------------|-----------------|------------|---------------|------------|
| Elabela (pg/mL)    | P r. value      | 0.386      | 0.094         |            |
| D. Dimer (µg/mL)   | P r. value      | 0.000      | -0.407**      | -0.374**   |
| S. Creatinine (µmol/L) | P r. value | 0.160      | -0.151       | 0.000      |
| NEU×10³/µL         | P r. value      | 0.000      | -0.513**      | -0.385**   |
| LYM×10³/µL         | P r. value      | 0.000      | 0.380**       | 0.033      |

r: correlation coefficient.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 2 shows the correlation between Elabela biomarker and eGFR with different laboratory parameters. It shows that significant correlations were found between Elabela and D. dimer (r = -0.407; p = 0.000), Elabela with NEU×10³/µL (r = -0.514; p = 0.000), between eGFR with S. creatinine (r = -0.753; p = 0.000), D. Dimer (r = -0.374; p = 0.000), and NUT×10³/µL (r = -0.385; p = 0.000). Significant positive correlations were found between Elabela and eGFR with LYM×10³/µL (r = 0.380; p = 0.000) and (r = 0.227; p = 0.033) respectively.

Figure 1: Receiver Operating Characteristic Curve of D-dimer for COVID-19 Patients

Figure 2: Receiver Operating Characteristic Curve of Elabela for COVID-19 Patients
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Discussion:

This study shows that there were more male COVID-19 cases than females. This agrees with other studies that showed males to be more affected by Covid-19 (72%) than females (28%) (30). A significant difference was found between controls and patients for eGFR and S. creatinine. The patients are admitted to the intensive care unit when their eGFR falls below 60 mL/min/1.73 m² which is a prognostic parameter for mortality in patients with COVID-19. eGFR decreases with age, even in people without kidney disease, older patients are known to have a higher risk. An elevated level of S. creatinine associated with COVID-19 disease causes admissions of patients to ICU, who should be monitored more carefully for early intervention on admission(31)(32). There was a significant difference in D-dimer between the patients and controls. Elevated level of D-dimer contributed to the severity of the disease(36). The impaired renal function causes the activation of an inflammatory process even if the patient does not develop severe respiratory failure. Therefore, measurement of renal function should be checked. The mild respiratory signs due to dehydration and hypoperfusion, are mostly attributed to AKI following intravenous fluids replacement therapy to control hemodynamics and fluids balance(33). The inflammatory process can cause multiorgan failure in COVID-19 patients(35), the inflammatory process can stimulate the production of platelets and damage the endothelium, causing clotting, coagulation problems, vascular injury, and the elongation of international normalized ratio in patients of AKI(31). Many drugs such as the antivirals which were tested for their effectivity against COVID-19 and the antibiotics which are used for the treatment of secondary bacterial infections in COVID-19 patients have adverse effects on the kidneys and may have a co-adjuvant effect with SARS-CoV-2 itself in increasing the incidence of AKI incidence. Testing kidney functions aid in accomplishing the most appropriate drug therapeutic concentrations and diminishing the risk of adverse drug reaction of most drugs that are prescribed to treat COVID-19(34). This study shows significant differences in lymphocyte count and neutrophil count in Covid-19 patients in comparison with healthy subjects.

Table 4: ROC Curve Analysis of D-dimer, ELA, eGFR, and S. creatinine in the study groups

| Test Variables | Accuracy | Area (AUC) | Significance | Asymptomatic | 95% Confidence Interval |
|---------------|----------|------------|--------------|--------------|-------------------------|
| D.D (µg/ml)   | Excellent| 0.929      | 0.000        | 0.864        | 0.995                   |
| ELA (pg/ml)   | Good     | 0.764      | 0.000        | 0.664        | 0.864                   |
| eGFR (mL/min/1.73 m²) | Good | 0.717      | 0.000        | 0.604        | 0.829                   |
| S. creatinine (µmol/L) | Good | 0.725      | 0.000        | 0.618        | 0.832                   |

Table 4 shows the use of receiver operating characteristic (ROC) in measuring the accuracy of D-dimer, ELA, eGFR, and S. creatinine in the studied groups. The area under the curve (AUC) of D.D was 0.929, and the cutoff value of D-dimer is (0.054 µg/ml), as represented in figure (1), area under the curve of ELA is 0.764 and the cutoff value (376.11 pg/ml), as represented in figure (2), area under the curve of eGFR 0.717, and cutoff value (161 mL/min/1.73 m²) as represented in figure (3), and area under the curve of S. creatinine 0.725, and cutoff value (42.88µmol/L) as represented in figure (4).

Figure 4: Receiver Operating Characteristic Curve of S. creatinine for COVID-19 Patients
Lymphopenia is an important feature of COVID-19 that is more marked in critically ill patients(35). The correlation between eGFR and S. creatinine in the presence of other parameters that include D-dimer, lymphocytes, and neutrophils count is similar to other the findings of other studies, as well as S. creatinine and eGFR in the association of disturbance hematological parameters(37). The serum level of the Elabela biomarker is lowered in COVID-19 patients compared with control group with a significant correlation between Elabela, D-dimer, and lymphocytes and neutrophils count. Apelin and agonists of its receptor increase the activity of the ACE-2 leading to inhibition of the effect of the ACE-Ang-II system that produces acute pulmonary insult, coagulation problem, and acute or chronic cardiac insult in COVID-19 patients so that apelin may act as a predictive parameter for the lung and extra-pulmonary insults (38). Interleukin-6 is a pro-inflammatory mediator elevated in COVID-19 patients and plays a role in the severity of COVID-19 infection that complicated AKI.

This study had some limitations including the small number of patients.

Conclusion:
The Elabela biomarker can be used for the early detection of acute kidney injury in COVID-19 patients.

Authors’ contributions:
Pharmacist Maha H. Gadhi: conception and design of study, Investigation, Methodology, interpretation and analysis of data, Writing, preparation of original manuscript, Writing Review and Editing.
Prof. Dr. Eman S. Saleh: Supervision, preparation of original manuscript, conception and design of study, Investigation, Methodology, Writing Review and Editing.

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Measurement of the serum level of Elabela for the early detection of acute kidney injury in hospitalized Iraqi COVID-19 patients

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Cites a study by Gadhi measuring the serum level of Elabela in hospitalized Iraqi COVID-19 patients.

The objective was to evaluate the serum level of Elabela in Iraqi COVID-19 patients for early detection of acute kidney injury.

The study included 43 healthy individuals as a control group and 45 COVID-19 patients who were transferred to the hospital in Baghdad and tested positive for COVID-19.

Results showed significant differences between the patients and the control group in the following parameters:

- eGFR mL/min/1.73 m² (P = 0.000)
- ELA pg/mL (P = 0.000)
- S. creatinine μg/mL (P = 0.000)
- Dimer µg/mL (P = 0.000)
- NEU × 10³/L (P = 0.000)
- LYM × 10³/L (P = 0.000)

Conclusion:

The level of Elabela in the serum of COVID-19 patients is lower than that in healthy individuals, indicating that Elabela or its receptors increase the activity of ACE2 or activate it, leading to the inhibition of ACE-Ang-II, which may lead to acute respiratory distress syndrome, whether it occurs inside or outside the lungs.

Keywords: COVID-19, angiotensin-converting enzyme 2, acute kidney injury, Elabela.