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 enzyme2 (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.

Result: There are no a 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, NEUX10^{3}/µL, LYMx10^{3}/µL and ELA biomarker. Significant negative correlations were found between Elabela with D. dimer and NEUX10^{3}/µL, and between eGFR with S. creatinine, D. Dimer, and NUTx10^{3}/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.

Introduction:
The term COVID-19 was introduced by the World Health Organization (WHO) in January/2020(1) to name the condition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. It may be a minor disease or progress to a multi-organ failure causing death(2). The virus is an enveloped RNA virus referred to Betacoronavirus genus(3). The first manifested case of (Covid-19) infection in Iraq was diagnosed on February 24, 2020 in Al-Najaf governorate, in a young man coming from Iran(4). WHO recorded 519 729 804 reported cases, and 6 268 281 reported deaths worldwide update 17 May 2022(5). Symptoms of COVID-19 are fatigue, headache, fever, dyspnea, cough, myalgias, and also can caused diarrhea. In addition to symptoms, the imaging abnormalities could be recognized as COVID-19 infection through chest x-ray and computed tomography (1).

COVID-19 causes atypical pneumonia and severe respiratory failure which it's leading to may cause Acute Kidney Injury (AKI)(6), with significantly poor outcomes(7). The patients that are more likely to develop AKI are required to be admitted to the intensive care unit (ICU), therefore they required renal replacement therapy. COVID-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 need to be admitted to the intensive care unit (ICU) and are These patients need to be admitted to the intensive care unit (ICU) and are more likely to develop AKI, therefore they require renal replacement therapy. COVID-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, therefore they require renal replacement therapy.
COVID-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 acts 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. Laboratory 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).
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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 1: Distribution of the study groups by demographic and lab data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median or mean±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.0</td>
<td>0.923</td>
</tr>
<tr>
<td>Gender n. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (28.4%)</td>
<td>0.467</td>
</tr>
<tr>
<td>Female</td>
<td>20 (22.7%)</td>
<td></td>
</tr>
<tr>
<td>S. creatinine (µmol/L)</td>
<td>57.8±21.09</td>
<td>0.000</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m2)</td>
<td>136</td>
<td>0.000</td>
</tr>
<tr>
<td>ELA (pg/ml)</td>
<td>270.9</td>
<td>0.000</td>
</tr>
<tr>
<td>NEU×10³/µL</td>
<td>9.8</td>
<td>0.000</td>
</tr>
<tr>
<td>LYM×10³/µL</td>
<td>1.2</td>
<td>0.000</td>
</tr>
<tr>
<td>D.D (µg/ml)</td>
<td>1.1</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Descriptive data were presented as median for non-parametrical variables and mean±SD for parametrical variables.

eGFR: estimated glomerular filtration rate, ELA: elabela, D.D: D. dimer, NEU×10³/µL: neutrophile cell×10³/µL, and LYM×10³/µL: lymphocyte cell×10³/µL.

Table 2: Correlations between Elabela and eGFR with other variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Elabela (pg/mL)</th>
<th>Elabela (µg/ml)</th>
<th>eGFR (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>P r.</td>
<td>Value</td>
<td>P r.</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELA (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEU×10³/µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LYM×10³/µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.D (µg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
Elevated serum creatinine is a prognostic parameter for mortality in patients admitted to the ICU when their eGFR falls below 60 mL/min/1.73 m2. 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 (28%) 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 effectiveness 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**

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

**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
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 Elabella biomarker is lowered in COVID-19 patients compared with control group with a significant correlation between Elabella, D-dimer, and lymphocytes and neutrophils count. Apelin and agonists of its receptor increase the activity of 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 Elabella 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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How to Cite this Article:

قياس مستوى إيلابيلا في مصل الدم للكشف المبكر عن إصابة الكلى الحاد في مرضى كوفيد-19 العراقيين الراقيين في المستشفى الصيدلانية بها حسن غاضي
أ.د. إيمان سعدي صالح

الخلفية: بسبب فيروس كورونا 2 في جائحة كوفيد-19 التي انتشرت على العالم، تسبب متلازمة التهاب الجهاز التنفسي الشديد الحادة المستفيرة باستخدام الإنزيم المحول للأنجيوتنسين 2 كمستقبل له. يعتبر الابلين أو مستقبلات تأثيرًا وقائيًا في كوفيد-19 من خلال قمع انزيم الإنزيم المحول للأنجيوتنسين وإنتاج الأنجيوتنسين 2 (Ang-II).

المات: هدفت الدراسة إلى قياس مستوى إيلابيلا في مصل الدم ككأشف مبكر لأصابة الكلى الحاد مع كوفيد-19.

الطريقة: ضمت دراسة الأشخاص السالمين 43 شخصًا بالغاً كمجموعة تحكم ومريضًا بالغاً تم نقله إلى المستشفى في بغداد، يُلاحظ أن إيلابيلا في الدم كأشف مبكر لأصابة الكلى الحاد مع كوفيد-19. اظهرت دراسة التفاعلات الإيجابية في إطار الدراسة والعمل على تطبيق القياسات الإيجابية في مراكز متعددة في بغداد والمناطق المجاورة.

نتائج: أظهرت وجود فروق جزئية إيجابية بين المرضى والمجموعة الضابطة تم أخذها في الاعتبار إحصائيًا عند قيمة P = 0.05. في eGFR mL / min / 1.73 m2، Dimer µg / mL، NEU × 103/L، LYM × 103/L، ELA pg / mL، S، P = 0.000، P = 0.000، P = 0.000، P = 0.000، P = 0.000، P = 0.000، P = 0.000.

الاستنتاج: ينخفض مستوى إيلابيلا في مصل الدم من المرضى مقارنة مع الأشخاص السالمين. يشير مرض كوفيد-19 إلى أن الأبلين أو مستقبلاته تزيد من تنظيم EZ-Ang-II الذي يسبب في إصابة الرئة الحادة في مرضى كوفيد-19 بحيث يمكن أن تكون إيلابيلا علامة بديعية لـ إصابات الرئة وخارج الرئة. الكلمات المفتاحية: كوفيد-19، الإنجيوتنسين 2، إصابة الكلى الحاد، إيلابيلا.