DOI: https://doi.org/10.31351/vol32issSuppl.pp91-98

Serum Soluble Angiotensin-Converting Enzyme-2 Level and Its Potential Association With The Renin-Angiotensin-Aldosterone System in Non-Hypertensive Iraqi COVID-19 Patients: An Observational Study

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[#] 2nd Scientific Conference for Postgraduate Students Researches.
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Abstract

The novel coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-Cov2) which utilizes angiotensin converting enzyme-2 (ACE2) to invade the host cells. This membrane-bound peptidase is widely distributed in the body; its activity antagonizes the renin-angiotensin-aldosterone system (RAAS). Once SARS-Cov2 enters the cell, it causes downregulation of ACE2, resulting in the unopposed activation of RAAS. The unregulated activity of the RAAS system can deteriorate the prognosis in COVID-19 patients. A soluble form of ACE2 (sACE2) was reported to have a role in the SARS-Cov2 invasion of the susceptible cells.

This study aims to investigate the potential association of serum levels of sACE2 and RAAS components in severe COVID-19 patients compared to healthy individuals.

Eighty-five participants enrolled in the study were grouped into 45 non-hypertensive severe COVID-19 patients and 40 apparently healthy individuals with comparable age and gender. Serum levels of sACE2, renin, angiotensin-2, and aldosterone were measured by ELISA, whereas serum potassium level was determind by turbidimetric method.

The results showed significantly lower serum levels of sACE2, and elevated levels of renin, angiotensin-2 and aldosterone in COVID-19 patients as compared to the control subjects (p-value <0.001; for all measured parameters.Non-hypertensive severe COVID-19 patients have lower serum sACE2 and higher RAAS peptide levels, hence these can serve as diagnostic markers for severe COVID-19 cases.

Keywords: Aldosterone, Angiotensin, COVID-19, Renin, Soluble angiotensin converting enzyme-2.

مستوى الإنزيم المحول للأنجيوتنسين القابل للذوبان الثاني في المصل و ارتباطه المحتمل بنظام الرنين-أنجيوتنسين-الألدوستيرون في مرضى كوفيد -١٩ غير المصابين بارتفاع ضغط الدم: دراسة مشاهدة#

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#المؤتمر العلمي الثاني لطلبة الدراسات العليا
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الخلاصة

بسبب الراشح المتلازمة التنفسيه الحاده الوخيمه الثاني (فيروس سارس ٢), ينتج مرض كورونا الجديد, الذي يستخدم الإنزيم المحول للأنجيوتنسين الثاني لغزو الخلايا المضيفة. يتم توزيع هذا الببتيد المرتبط بالغشاء على نطاق واسع في الجسم. نشاطه يعادي نظام الرينين - أنجيوتنسين – الألدوستيرون. بمجرد دخول الراشح الى الخلية, فإنه يتسبب في تقليل تنظيم الإنزيم المحول للأنجيوتنسين الثاني، مما يؤدي إلى تنشيط نظام الرينين - أنجيوتنسين – الألدوستيرون بدون معارضة. يمكن أن يؤدي هذا النشاط غير المنظم إلى تدهور ناتج العدوى لدى مرضى كوفيد ١٩. لوحظ شكل قابل للذوبان من الإنزيم المحول للأنجيوتنسين الثاني قد يكون له دور في غزو الخلايا المعرضه لذلك الراشح.

تهدف هذه الدراسة إلى التحقق من الارتباط المحتمل لمستويات المصل لمكونات من الإنزيم المحول للأنجيوتنسين الثاني المذاب و نظام الرينين - أنجيوتنسين – الألدوستيرون في مرضى كورونا الحاد مقارنة بالأفراد الأصحاء.

تم تجميع ٨٥ مشاركًا مسجلين في الدراسة بشكل ٤٥ مريضًا غير مصابين بارتفاع ضغط الدم و مصابين إصابة شديده بكوفيد ١٩ و ٤٠ فردًا يتمتعون بصحة جيدة من نفس العمر والجنس. تم قياس مستويات المصل من الإنزيم المحول للأنجيوتنسين الثاني القابل للذوبان، الرنين، الأنجيوتنسين ٢، والألدوستيرون بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم ومستوى البوتاسيوم في الدم بطريقة العكر.

أظهرت النتائج فرقا معنويا في مستويات المصل من الإنزيم المحول للأنجيوتنسين الثاني المذاب (أقل) و الرينين ، أنجيوتنسين ٢ ، والألدوستيرون (أعلى) في مرضى كوفيد ١٩ شديدي الإصابة مقارنة مع الأشخاص الأصحاء و كانت القيمه المعنوية أقل من ٠,٠٠١ لجميع القراءات. مرضى كوفيد ١٩ شديدي الاصابه الغير المصابين بارتفاع ضغط الدم لديهم مستويات أقل من الإنزيم المحول للأنجيوتنسين الثاني المذاب ومستويات أعلى من نظام الرينين - أنجيوتنسين – الألدوستيرون ويمكن أن يكونوا بمثابة علامات تشخيص لمرضى كورونا الحاد.

الكلمات المفتاحية: الألدوستيرون ، الأنجيوتنسين ، كوفيد ١٩ ، الرنين ، الإنزيم المحول للأنجيوتنسين القابل للذوبان الثاني

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Received: 9/4 /2023
Accepted: 8/ 5/2023

Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by the new coronavirus, SARS-Cov2. The World Health Organization (WHO) declared COVID-19 a pandemic in March 2020 [1-4]. Most COVID-19 cases displayed mild upper respiratory tract symptoms, diarrhea, shortness of breath, and fever [5-7]. Some cases remained asymptomatic [8]. However, severe cases can progress to pneumonia, multi-organ failure, and death [9-13]. SARS-Cov2, as well as other coronaviruses, utilizes angiotensin converting enzyme-2 (ACE2), a membrane-bound peptidase, as a gate to access the host cells [14, 15]; ACE2 expression was shown to be correlated with elevated viral load in human cell lines [16, 17] and rodents [18]. ACE2, on the other hand, has anti-inflammatory effects by downregulating the proinflammatory peptides mediating the Renin-angiotensin system [19] and the Kinin-kallikrein system [20].

The Renin-angiotensin-aldosterone system (RAAS) is made up of harmonized hormones and receptors derived from different organ systems in the body. Its main activity is concerned with maintaining optimum levels of electrolytes and fluids as well as controlling systemic vascular resistance to establish optimum blood pressure [21].

Angiotensin converting enzyme-2 transforms the vasoconstrictor peptide angiotensin-2 into the vasodilator, anti-inflammatory, and antifibrotic form angiotensin 1-7. Moreover, ACE2 converts angiotensin-1, the precursor of angiotensin-2, into angiotensin 1-9, which eventually transforms into angiotensin 1-7 [21-23]. SARS-Cov2 binding with ACE2 was reported to downregulate ACE2 [24], contributing to an increase in angiotensin-2 and decreasing angiotensin 1-7. The downregulation of ACE2 can also result from the cytokine storm mediating the pathogenesis of COVID-19 [24].

Recently, the role of a soluble form of ACE2 (sACE2) in mediating SARS-CoV2 entry into host cells was demonstrated. Yeung *et al.* show that SARS-CoV2 links sACE2 and subsequently utilizes type 1 angiotensin receptors to invade the susceptible cells [25]. On the other hand, Krishnamurthy *et al.* proposed that sACE2 might be targeted as a treatment option in COVID-19; acting as a decoy receptor for SARS-CoV2 makes the membrane-bound ACE2 available to maintain its regulatory effect on RAAS[26]. sACE2 is produced by shedding from the membrane-bound ACE by the action of a disintegrin and metalloproteinase-17 (ADAM17) (also known as tumor necrosis factor-alpha (TNF-α) converting enzyme (TACE)) [27].

This study aims to investigate the possible association between the serum level of sACE2 and the components of RAAS, as well as potassium, as a simple, easily measured marker affected by RAAS alterations.

Materials and Methods

Ethical consideration

The ethics committee granted formal approval for the research protocol in the College of Pharmacy, University of Baghdad (ethics board approval code: 112021A) on the 5th of November 2021. In addition, participants were informed about the purpose of the study, and their written consent was obtained.

Study design

In an observational case-control study, adult non-hypertensive severe COVID-19 patients were compared with apparently healthy control group.

Setting

This multi-center study was conducted on hospitalized adult non-hypertensive severe COVID-19 patients in Al-Khadymia, Dar-Alsalam, and Al-Ataa hospitals in Baghdad/ Iraq during the period extending from November 2021 to June 2022

Variables

This research measures the serum levels of sACE2, renin, angiotensin-2, aldosterone, and potassium and compare the results between the non-hypertensive severe COVID-19 patients and the healthy control.

Sample size

G*Power (RRID: SCR_013726) version 3.1.9.7 software was utilized to calculate the required number for participants. A two-tailed alpha of 0.05 with a confidence interval of 95%, a power of 90%, and an effect size of 0.80 was used. The sample size was shown to be at least 80 participants (f). In this study, a total of 85 participants were enrolled, 45 in the COVID-19 group and 40 in the control group.

Eligibility criteria

Adult normotensive patients aged between 20-60 years old who were diagnosed with severe COVID-19 infection were enrolled in the study. The diagnosis depended on clinical examination, positive RT-PCR for SARS-Cov2, and radiological findings namely computed tomography (CT) scan and chest X-ray [28, 29]. Clinically, patients had shortness of breath with oxygen saturation of less than 94% on room air at sea level, cough, fever, chest tightness, and pain categorized as severe COVID-19 illness, according to the National Institute of health and Centers for Disease Control and Prevention [30, 31]. The control group involved healthy individuals of comparable age and gender to the patients, all participants had their blood pressure checked to ensure normal blood pressure before enrollment.

Exclusion criteria

Individuals who had the following conditions were excluded from the study: hypertension or other cardiovascular diseases, diabetes mellitus, malignant tumors, chronic renal or hepatic diseases, smoking, and those who were on any medication that may interfere with the measured variables.

Bias

During the selection of the study sample, bias can occur. Such occurrence is particularly true when exposure and outcomes have occurred before the recruitment of the participants. However, as the results in this study are unknown at the time of enrolment, sampling errors are less probable to occur. The ideal study population will likely produce the intended outcome, well-defined, reliable, and conveniently accessible. To deter any source of bias, participants were enrolled in a method that didn't favor individuals with abnormally low or high levels of exposure to COVID-19. In addition, control participants were asked if they felt they had been infected to detect volunteer bias in the sample.

Study procedure

Blood samples were collected under similar circumstances from all participants after being interviewed by the researcher. Data were taken directly from the patients' or the patients' case files after verbal approval. Three milliliters (ml) of blood samples were taken from each participant. After collection, the blood sample was placed into a gel tube and left for 10-20 minutes to coagulate, followed by centrifugation at 2000-3000 rpm to obtain serum. Serum was collected into Eppendorf tubes and stored at -20 ºC until all samples were collected. They were utilized for the quantitative measurement of sACE2, renin, angiotensin-2, and aldosterone using sandwich-type ELISA kits [32], as well as the measure of potassium level using the turbidimetric method [33].

Materials and instruments

The analysis utilized materials of the maximum possible purity. A list of the kits used in this study is presented in Table 1.

Table 1. Summary of the chemical kits used

Diagnostic kits	Supplier
Renin ELISA kit	MyBioSource; USA
Angiotensin-2 ELISA kit	MyBioSource; USA
Aldosterone ELISA kit	MyBioSource; USA
sACE2 ELISA kit	MyBioSource; USA
Potassium kit	Agappe; India

Statistical analysis

IBM SPSS Statistics (RRID: SCR_016479) have been utilized during the statistical analysis process. Data distribution uniformity was checked with the Shapiro-Wilk test. The median and interquartile range (IQR) was used to present the data of continuous variables; the Mann-Whitney U test was used to determine the significance of the difference of these variables between the two study groups. Categorical variables were presented as frequency and percentages and analyzed using the chi-square test. A *P*-value below 0.05 was considered significant. The receiver operating characteristic (ROC) curve was also used to measure the area under the curve (AUC), as well as the optimal cut-off value specificity and sensitivity of serum levels of sACE2, renin, angiotensin-2, aldosterone, and potassium as diagnostic criteria to discriminate between the non-diseased and the severe COVID-19 patients.

Results

This research included 85 subjects, 45 of whom had severe COVID-19, and 40 were appearantly healthy. Both of the severe COVID-19 patients and the control subjects were chosen to be of comparable age and gender (p=0.06 and 0.25, respectively). A summary of the demographic characteristics is shown in Table-2.

Table 2. Demographic characteristics of participants

Variable		Covid-19 patients (n=45)	Control (n=40)	p-value
Age (Median)		54 (IQR=14)	49.50 (IQR=15)	0.058
Gender	Male	26 (57.8%)	21 (52.5%)	0.253
	Female	19 (42.2%)	19 (47.5%)	

Where: n=number.

As shown in Table-3, the sACE2 level was significantly lowered in the COVID-19 group with a median of 33.17 (IQR=8.85) ng/ml compared to the control group with a median of 78.78 (IQR=14.82) ng/ml; (p<0.001). Additinaly, serum renin level was significantly elevated in the COVID-19 group with a median of 187.12 (IQR=66.5) pg/ml, while the median was 103.82 (IQR=38.3) pg/ml in the control group; (p<0.001). Furthermore, serum angiotensin-2 level was significantly higher in the COVID-19 group with a median of 581.08 (IQR=125.39) pg/ml; while the median was 361.22 (IQR=156.13) pg/ml in the control group; (p<0.001). Similarly, serum aldosterone level was significantly elevated in the COVID-19 group with a median of 3.29 (IQR=1.04) pg/ml; while the median was 0.52 (1.04) pg/ml in the control group; (p<0.001). Finally, there was no significant difference in serum potassium level between the COVID-19 group, with a median of 4.20 (IQR=1.2) mmol/L, and the control group, with a median of 3.9 (IQR=0.7) mmol/L; (p=0.08).

Table 3. Biochemical characteristics of participants

Variable	Group	Median	IQR	*p*-value
sACE2 (ng/ml)	COVID-19	33.17	8.85	<0.001*
	Control	78.78	14.82	
Renin (pg/ml)	COVID-19	187.12	66.50	<0.001*
	Control	103.82	38.30	
Angiotensin-2 (pg/ml)	COVID-19	581.08	125.39	<0.001*
	Control	361.22	156.13	
Aldosterone (pg/ml)	COVID-19	3.29	1.04	<0.001*
	Control	0.52	1.04	
Potassium (mmol/L)	COVID-19	4.20	1.20	0.08
	Control	3.90	0.70	

Where: *P<0.05= statistically significant; IQR= interquartile range; sACE2= Soluble angiotensin converting enzyme-2.

Table-4 displays the distribution of participants according to their serum potassium levels into normokalemic (3.5-5 mmol/L), hyperkalemic (>5 mmol/L), and hypokalemic (<3.5 mmol/L).

Table 4. Distribution of participants according to their serum potassium levels.

Groups	Normokalemic No. (%)	Hyperkalemic No. (%)	Hypokalemic No. (%)	*p*-value
COVID-19	28 (62.2%)	9 (20%)	8 (17.8%)	0.423
Control	29 (72.5%)	4 (10%)	7 (17.5%)	

The ROC curves showed that sACE2, renin, angiotensin-2, and aldosterone have excellent diagnostic value in discriminating between severe COVID-19 patients and non-infected subjects, as shown in Table-5 and Figures 1 and 2

Table 5. Receiver operating characteristic curve of the studied markers

Variables	AUC	95% CI	*p*-value	Cut-off	SN	SP
sACE2 ng/ml	1.000	1.00-1.00	<0.001*	≤52.72	100%	100%
Renin pg/ml	0.96	0.91-1.00	<0.001*	≥155.4	90.9%	97.5%
Angiotensin-2 pg/ml	0.96	0.91-0.999	<0.001*	≥478.89	88.6%	92.5%
Aldosterone pg/ml	0.94	0.89-0.99	<0.001*	≥2.31	86.4%	87.5%
Potassium mmol/L	0.61	0.49-0.73	0.091	≥4.05	59.1%	65%

Where: *p<0.05= statistically significant. AUC, area under the curve; CI, confidence interval; SN, sensitivity; SP, specificity; sACE2= Soluble angiotensin converting enzyme-2.

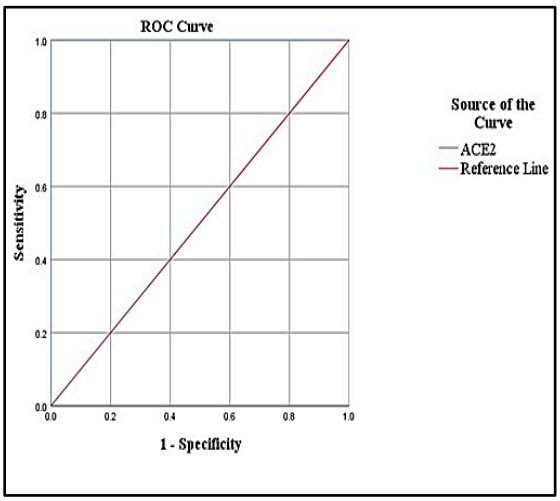

Figure 1. Receiver operating characteristics curve of serumsACE 2 in severe COVID-19.

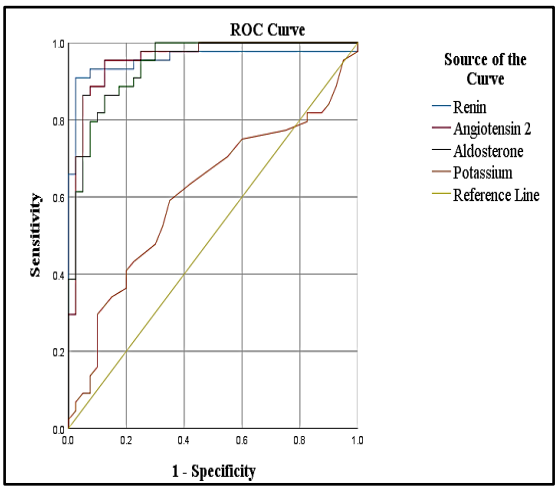

Figure 2. Receiver operating characteristics curve of serum renin, angiotensin 2, aldosterone, and potassium in severe COVID-19.

Discussion

Angiotensin converting enzyme-2 counterbalances the effect of RAAS by transforming the vasoconstrictor peptide angiotensin-2 and its immediate precursor, angiotensin-1, into the vasodilator, anti-inflammatory, and antifibrotic form angiotensin 1-7 [21-23]. The unopposed activation of RAAS leads to elevated production of angiotensin-2, which acts through angiotensin-1 receptor leading to vasoconstriction and elevation of blood pressure, as well as cellular differentiation and growth, endothelial dysfunction and coagulability, inflammation and production of reactive oxidative species [22, 23, 34]. During COVID-19, SARS-Cov2 results in ACE2 internalization and downregulation, thus, abolishing its regulatory role in RAAS [35].

In the present study, the sACE2 level was significantly lower in the COVID-19 group compared to the control group (Table-3). sACE2 is the product of proteolytic shedding of the membrane-bound ACE2 by ADAM17 [27]. The lower sACE2 level reflects the membrane-bound ACE2 downregulation. Rieder *et al.* have reported no significant difference in the baseline sACE2 level, as well as other components of RAAS, between SARS-Cov2 positive and SARS-Cov2 negative patients [36]. COVID-19 patients were included in the study regardless of the severity of the infection, and SARS-Cov2-negative patients had similar symptoms to COVID-19 patients. Moreover, in addition to the small sample size of COVID-19 patients [21], Rieder *et al.* did not exclude patients with conditions or medications that affect sACE2 levels.

Furthermore, in the present study, serum renin, angiotensin-2, and aldosterone levels were significantly higher in the COVID-19 group compared to the control group (Table-3). The available data about the alterations in serum levels of the RAAS peptides during SARS-Cov2 infection are scarce and conflicting. Rysz *et al.* proposed that RAAS peptide imbalance could partially mediate severe COVID-19 [37]. In agreement with the present study's findings, Kutz *et al.* reported higher serum angiotensin-2 level and sACE2 activity in COVID-19 patients than in control subjects [38]. Elevated serum angiotensin level in hospitalized COVID-19 patients was also reported by Liu et al.; however, the study did not involve SARS-CoV-2 negative control subjects to establish the independent association of the infection and serum angiotensin-2 levels [39]. On the contrary, Kintscher *et al.* found no significant difference in RAAS peptide levels and sACE2 activity between COVID-19 patients and the control subjects [40]. However, the latter study enrolled hypertensive patients on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Furthermore, the enrolled COVID-19 patients were not suffering from severe infection; serum RAAS peptides were measured using LC-MS/MS technology,which may contribute to the difference in findings from that of the present study.

As discussed earlier, the downregulation of ACE2 during COVID-19 abolishes its regulatory role in RAAS, resulting in higher serum levels of RAAS peptides [35]. In ACE2 knock-out mice, the exposure to SARS-Cov2 spike protein led to an elevation of angiotensin-2 level and was associated with a worse prognosis. By blocking of angiotensin-1 receptor, the effect was partially reversed [41]. Activation of angiotensin-2/angiotensin-1 receptors stimulates the release of aldosterone and upregulated protein-C receptors in the vascular endothelium [39], which is heavily linked to the prothrombotic state [42]. Away from RAAS, activation of angiotensin receptor 1 by elevated angiotensin-2 level results in elevated reactive oxygen species production, hypercoagulability, overwhelming systemic inflammation, and thus poor COVID-19 prognosis [43].

Abnormalities in serum potassium levels are frequent in COVID-19 patients; hypokalemia and hyperkalemia have been reported [44-48]. The alterations in circulatory potassium levels were associated with the infection prognosis [45, 49]. In a retrospective cohort study involving 21,676 hospitalized COVID-19 patients, Mallow et al. showed that serum potassium imbalance was a common finding; 15.8% of patients had hypokalemia, and 13.2% of them had hyperkalemia [50].

In hypokalemia cases, elevated aldosterone levels result in increased urinary potassium excretion [51]. Hyperactivation of RAAS in COVID-19 patients may upregulate and enhance the epithelial sodium channel (ENaC) activity in the distal convoluted tubules resulting in kaliuresis [52-54]. In addition to RAAS hyperactivation, other potential causes of potassium loss in COVID-19 patients include vomiting, anorexia, and tubular damage by the invading virus [55, 56]. While hyperkalemia is less frequent in COVID-19 patients than hypokalemia [52], the elevated serum potassium level has been attributed mainly to the development of acute kidney injury that results in potassium retention [57].

In the present study, there was no significant difference in potassium levels between COVID-19 patients and the control subjects; (Table-3). About 20% of COVID-19 patients showed hyperkalemia, 17.8% showed hypokalemia, and the remaining had normal serum potassium levels. On the other hand, 10% of the control subjects showed hyperkalemia, and 17.5% showed hypokalemia. Therefore, alteration in potassium levels in the control subjects could be due to alcohol consumption or their dietary habits.

This study also found that sACE2 can serve as a diagnostic marker differentiating severe COVID-19 patients from non-infected subjects, with a cut-off ≤52.72 ng/ml has 100% sensitivity and specificity. Moreover, serum renin, angiotensin-2, and aldosterone displayed promising results in this regard; (Table-5 and Figures 1 and 2). Fagyas *et al.* reported that sACE2 cut-off value ≤45.4 mU/L at admission has a sensitivity of 60% and specificity of 71.2% to estimate disease severity; the same cut-off value of sACE2 can predict the disease outcome with a sensitivity of 61.8% and specificity of 65.5% [58].

Conclusions

Non-hypertensive severe COVID-19 patients have lower serum sACE2 and higher RAAS peptide levels than control subjects do.

Limitations

This study had many exclusion criteria to reduce the confounding impact. However, the effects of the excluded conditions on sACE2 and RAAS peptides must be further studied. In addition, the number of participants was only modest

Acknowledgments

The authors would like to thank the participating subjects and the team of COVID-19 care units in Al-Khadymia, Dar-Alsalam, and Al-Ataa hospitals for their support during sample collection.

Funding

We hereby confirm that no external funding or financial contributions were received or utilized in the preparation, research, writing, or publication of this article.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Ethics Statements

This article was approved by the ethical committee of the College of Pharmacy/University of Baghdad .

Author Contribution

Authors have contributed equally to this study.

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