Determining ACE-2 Level and Some Relevant Biochemical Parameters and studying the effect of Gender in Iraqi Diabetic Patients with Glomeruli and Renal Tubules Fibrosis as Early Prediction Marker

Reham Khuldon Ibrahim* 1
Kadhim K. Ghudhaib1
Ali Abdulmajid Dyab Allawi2

1Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq
2Department of Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq
*Corresponding author: raham.khaldoun1105a@csw.uobaghdad.edu.iq
E-mail addresses: kadhimkg_chem@csw.uobaghdad.edu.iq, aliallawi@comed.uobaghdad.edu.iq

Received 16/9/2022, Revised 16/12/2022, Accepted 18/12/2022, Published Online First 20/4/2023

Abstract:
Diabetic kidney disease is an illness of the glomerulus that interferes with the glomerular filtration barrier (GFB), which is worked to enable kidney to selective purification of water and solutes in addition to limiting the movement of large macromolecules such as albumin. In the glomerular endothelium, mesangial cells, foot cells, and the brush border of the proximal tubules, ACE-2 is expressed and that the kidneys represent the highest-expressing region of this enzyme. Thus, the current study aimed to evaluate ACE-2 level in this case compared to healthy condition. The study Conducted with 120 male and female ranging in age (30-65) years old. Ninety patients with type 2 diabetes subdivided into three groups on the basis of ACR criteria including normoalbuminuria, microalbuminuria, macroalbuminuria (30 patients for each group) and 30 healthy people served as the control group, all visited Baghdad Teaching Hospital / Medical City and Al-Yarmouk Teaching Hospital, at the period between December 2021 and May 2022. ACE-2 levels were determined using the ELISA technique. Urea results showed significant differences between diabetic nephropathy in patient and control group in female cases but no significant differences in male patients with diabetic nephropathy and control group. Similar results were obtained in K ion. Also the results revealed significant differences in Na ion, ACR, eGFR, Urea, FBS, creatinine between diabetic nephropathy groups and healthy group. ACE-2 represents a good marker for early prediction in diabetic nephropathy case. ROC data analysis support the importance of ACE-2 in diagnosis of the studied disease case.

Keywords: ACE-2, DKD, Diabetes Mellitus, Diabetic Nephropathy, Fibrosis, Gender effect.

Introduction:
Recent years have witnessed great interest in diabetes and its complications. More recently, the association between diabetes and periodontitis has been investigated1-3, as studies have shown that patients with high blood sugar suffer from persistent inflammation, and many studies were conducted previously, including diabetic kidney disorders. A phenomenon known as diabetic kidney disease (DKD) is characterized by alterations in the glomerular filtration rate, leakage of protein (particularly albumin), metabolites, and ions into the urine (GFR)47, diabetic neuropathies previous studies found that about half of diabetic patients have neuropathy, which consists of weakness in the peripheral nerves and may occur in various ways, including sensory, focal/multifocal, and autonomic neuropathy8-11, and the relationship between diabetes and osteoporosis increases blood sugar and decreases insulin secretion leading to an increase in susceptibility to osteoporosis12-14, in addition to a number of other studies in the field of diabetes treatments in which blood sugar was naturally controlled in patients with type 2 diabetes15,16.

Diabetic kidney disease is an illness of the glomerulus that interferes with the glomerular filtration barrier (GFB). It works in tandem to enable water and solutes to be selectively purified, while limiting the movement of large
macromolecules such as albumin\textsuperscript{17}. The glomerular filtration membrane consists of 3 membranes, the glomerular basement membrane (GBM), the podocyte, and the fenestrated endothelium. Cellular holes that can take up to 40% of the cell surface are what distinguish glomerular endothelial cells from other cell types. This modification makes the glomerular endothelium highly permeable to water, resulting in effective filtration function\textsuperscript{18}. The glycocalyx, a layer of proteoglycans and glycoproteins covered the glomerular endothelial cells, with particular molecular and charge properties that controls endothelial permeability and glomerular filtration\textsuperscript{19}.

The glomerular basement membrane GBM, which is made up of a layer of extracellular matrix (ECM) proteins positioned between the glomerular endothelium and the podocyte, divides the urine area from the vasculature. Renal hypertrophy and increased glomerular blood flow are the primary pathogenic changes in DN, which are then followed by mesangial cell enlargement and the onset of glomerular fibrosis \textsuperscript{20}. In both experimental animal models and human kidney diseases, glomerular fibrosis is caused by an excessive buildup of extracellular matrix (ECM), such as collagen I and fibronectin (FN), in which transforming growth factor-\textbeta\textsuperscript{b} (TGF-\textbeta\textsuperscript{b}) is a major stimulus factor that activates the downstream \textit{Smad} signaling pathway\textsuperscript{21}. Because the angiotensin-converting enzyme produces more angiotensin II, the renin-angiotensin system, and particularly local renal RAS activation, is crucial to the development of DN \textsuperscript{22,23}, AngII exerts a variety of effects via its type 1 receptor (AT1R) overt albuminuria, glomerular fibrosis, and increased glomerular permeability. Although the currently recommended treatment for DN is RAS inhibition with an ACEI receptor inhibitor and/or Ang II \textsuperscript{24}. Considering the reduction in proteinuria, kidney histology, and the avoidance of disease development, there is only a partial response, emphasizing the need for additional treatment options for the downregulation of the RAS. Angiotensin-Converting Enzyme 2 (ACE-2), a catalytically active homolog of ACE, converts Ang II into Ang1-7 and counteracts the negative effects of Ang II in DN Human models \textsuperscript{25}.  

Materials and Methods:  
One hundred and twenty men and women, aged 30-65 participated in the study, 90 patients with diabetes who visited Baghdad Teaching Hospital / Medical City and Al-Yarmouk Teaching Hospital between December-2021 and May-2022 and 30 healthy individuals as control group.

Groups of Analysis Included:  
1) Control group: included 30 apparently healthy persons without any diseases.
2) Patients groups: included 90 patients were divided into three groups according to ACR criterion:  
   - Normoalbuminuria group: Included 30 patients the range of ACR <30 mg/g
   - Microalbuminuria group: Included 30 patients the range of ACR 30-300 mg/g
   - Macroalbuminuria group: Included 30 patients the range of ACR >300 mg/g

Inclusion Criteria:  
- Patients ranging in age from 30 to 65 years.
- Type 2 diabetes medical history.
- The duration of the disease 5-25 years

The Control Group of Volunteers Was Formed Using the Following Criteria:  
- Clinically healthy.
- Negative for clinical indicators of systemic illnesses.
- Negative for diabetes

Exclusion Criteria  
- Patients over the age of 65.
- Behaviors such as smoking, drinking, and chewing tobacco.
- Patients with diabetic neuropathy.
- Patients with diabetic retinopathy.
- Patients with systemic lupus erythematosus (SLE).

Collection and Analysis of Samples  
Seven ml of blood from antecubital vein were withdrawn and divided into two parts. Part 1 5ml in gel tubes coagulated at room temperature for 30 minutes. After 10 minutes of centrifugation, the serum was separated and kept in Eppendorf tubes. The first part was utilized to rapidly identifying FBS, Urea, Creatinine, Na, K in serum using an Auto Spectrophotometer; a clinical chemistry analyzer performed the diagnostic tests. Also, it was utilized after maintaining at -20°C to assess ACE2, which were evaluated using a My BioSource manufactures an enzyme–linked immunosorbent test ELISA kit, USA. Part 2 test tube contained anticoagulant for HbA1c measurement by I-chroma a device 2 ml.

Statistical Analysis  
SPSS software version 22 was used to statistically analyzing the data. The variables’ means and standard deviations were reported. To ascertain whether there are statistically significant variations in the means of the four independent studied groups, one-way analysis of variance(ANOVA) was utilized (control, DM with normoalbuminuria, DM with
microalbuminuria, and DM with macroalbuminuria).

Results:

Results of female groups are 190.23 ± 34.20 for normoalbuminuria, 199.66±34.38 for microalbumin, 203.06±50.05 for macroalbuminuria and 87.75 ± 5.15 for control group. The results revealed a highly significant difference p=0.0001 between control and patient groups. Similarly the levels of FBS in male patient groups, normoalbuminuria 215.0±47.74, microalbuminuria 222.66±46.83, macroalbuminuria 232.0 ± 84.21 and control group 87.0 ± 6.60 showed a highly significant difference p=0.0001 between control and patient groups. That means gender has no effect on the obtained results for FBS. Table 1 shows significant difference p=0.0001 in HbA1C levels between patient female groups that including normal albuminuria, micro and macroalbuminuria 9.08 ± 1.76, 9.40 ± 1.18 and 10.79 ± 3.94 respectively compared with 4.5-5.6 for female control group. Table 1 shows mean ± SD of HbA1C among different male patient groups [normalalbuminuria 10.28 ± 1.97, microalbuminuria 10.76 ± 2.35 and macroalbuminuria 10.12 ± 1.63, in addition to control group 5.23 ± 0.33. The results showed a highly significant difference between the studied patient groups and control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=30</th>
<th>Normoalbuminuria N=30</th>
<th>Microalbuminuria N=30</th>
<th>Macroalbuminuria N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dL)</td>
<td>87.75 ± 5.15 (^b)</td>
<td>190.23 ± 34.20 (^a)</td>
<td>199.66 ± 34.38 (^a)</td>
<td>203.06 ± 50.05 (^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>87.0 ± 6.60 (^b)</td>
<td>215.0±47.74 (^a)</td>
<td>222.66 ± 46.83 (^a)</td>
<td>232.0 ± 50.05 (^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1C Female</td>
<td>5.13 ±0.32 (^b)</td>
<td>9.08 ± 1.76 (^a)</td>
<td>9.40 ± 1.18 (^a)</td>
<td>10.79 ± 3.94 (^a)</td>
<td>0.0001**</td>
</tr>
<tr>
<td>HbA1C male</td>
<td>5.23 ±0.33 (^b)</td>
<td>10.28 ± 1.97 (^a)</td>
<td>10.76 ± 2.35 (^a)</td>
<td>10.12 ± 1.63 (^a)</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

Table 1. FBS, HbA1C level in the studied groups (female and male).

**Significant difference between means using ANOVA -test at 0.01 level.
- Significant variants are denoted by different small letters.
- Non-significant variations are denoted by identical small letters.

Creatinine and urea results of both female and male in patients and control groups were recorded in Table 2. The results of creatinine in both genders showed a significant difference p=0.0001 between macro groups 3.50 ± 2.85 \(^a\)f, 3.36 ± 2.99 \(^a\)m group and other studied groups that included micro, normo and control groups for both genders 1.50 ± 0.67 \(^b\), 0.91 ±0.20 \(^b\), 0.70 ±0.12 \(^b\) for female groups and 1.30 ± 0.74 \(^b\), 0.95 ± 0.27 \(^b\), 0.80 ±0.06 \(^b\) for male groups, respectively.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=30</th>
<th>Normoalbuminuria N=30</th>
<th>Microalbuminuria N=30</th>
<th>Macroalbuminuria N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>0.70 ± 0.12 (^b)</td>
<td>0.91 ±0.20 (^b)</td>
<td>1.50 ± 0.67 (^b)</td>
<td>3.50 ± 2.85 (^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.80 ±0.06 (^b)</td>
<td>0.95 ± 0.27 (^b)</td>
<td>1.30 ± 0.74 (^b)</td>
<td>3.36 ± 2.99 (^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>27.50 ± 4.77 (^c)</td>
<td>35.93 ± 7.83 (^c)</td>
<td>61.01 ± 22.12 (^b)</td>
<td>88.83 ± 40.61 (^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>34.15 ± 2.79 (^b)</td>
<td>32.93 ± 10.27 (^b)</td>
<td>59.24 ± 25.92 (^a)</td>
<td>81.82 ± 39.67 (^a)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 2. Levels of urea, creatinine in the studied groups (female and male).

**Significant difference between means using ANOVA -test at 0.01 level.
- Significant variants are denoted by different small letters.
- Non-significant variations are denoted by identical small letters.

In contrast, results of urea level in of female revealed significant differences p=0.0001 among macro 88.83 ± 40.61 \(^b\), micro 61.01 ± 22.12 \(^b\) and normoalbuminuria 35.93 ± 7.83 \(^c\) with control 27.50 ± 4.77 \(^c\) groups. At the same time, findings of urea for male referred to significant differences 0.0001 between both macro 81.82 ± 39.67 \(^a\) and micro 59.24 ± 25.92 \(^a\) groups compared to both normo 32.93 ± 10.27 \(^b\) and control 34.15 ± 2.79 \(^b\) groups, as reported in Table 2. Subsequently, there is slightly effect of gender in the case of urea but no effect in creatinine factor levels. Values of ACR and eGFR of the studied groups for both genders are recorded in Table 3. The
results of eGFR in female revealed significant P=0.0001 decreased in all patient groups (normo, micro and macro 15.86 ±5.37 a, 91.53 ± 59.70 b, 629.04 ± 248.6 c), respectively, compared with control group 105.81 ± 13.30 a. Similar results were found in male, where their eGFR values found to be decreased significantly P=0.0001 and gradually in patient groups that include normo, micro and macroalbuminuria 98.40 ± 21.10 a, 81.93 ± 34.72 b, 44.17 ± 27.29 c compared with control group 113.30 ± 6.21 a. That means gender has no effect on eGFR.

Table 3. Values of eGFR rand ACR in the studied groups (Female and Male).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=30</th>
<th>Normo-albuminuria N=30</th>
<th>Micro-albuminuria N=30</th>
<th>Macro-albuminuria N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR(mL/min/1.73m)</td>
<td>105.81 ± 13.30 a</td>
<td>80.26 ± 22.31 b</td>
<td>50.73 ± 30.22 c</td>
<td>29.46 ± 21.10 d</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Female n=15</td>
<td>(82-128)</td>
<td>(48-116)</td>
<td>(0.00-111.0)</td>
<td>(5.73)</td>
<td></td>
</tr>
<tr>
<td>Male (n=15)</td>
<td>(103-125)</td>
<td>(48-126)</td>
<td>(27-121)</td>
<td>(7-87)</td>
<td></td>
</tr>
<tr>
<td>ACR(mg/g)</td>
<td>15.86 ± 5.37 a</td>
<td>91.53 ± 59.70 b</td>
<td>629.04 ± 248.6 c</td>
<td>504.64 ± 212.92 a</td>
<td>0.0001**</td>
</tr>
<tr>
<td>Female (n=15)</td>
<td>(10.6-22)</td>
<td>(34.0-181.0)</td>
<td>(7.5-903.0)</td>
<td>(4.02-847.0)</td>
<td></td>
</tr>
<tr>
<td>Male (n=15)</td>
<td>(16.06 ± 5.75 c)</td>
<td>(130.80 ± 56.37 a)</td>
<td>(504.64 ± 212.92 a)</td>
<td>(4.02-847.0)</td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference between means using ANOVA -test at 0.01 level.
- Significant variants are denoted by different small letters.
- Non-significant variations are denoted by identical small letters.

In similar manner, ACR values for female revealed significantly increased in patient groups (normo, micro and macro 15.86 ±5.37 , 91.53 ± 59.70 , 629.04 ± 248.6). Also, same results for male were obtained, thus gender has no effect on ACR in the case of patient groups, as shown in Table 3.

Results of in ACE-2, Na and K were recorded for both male and female in Table 4. However, the results of ACE2 for female revealed significant increase P=0.001** in both micro, and macroalbuminuria patient groups in comparison with control group. Whereas, there is no significant differences P=0.077 among the studied groups in the male case. Therefore, according to these results, the level of the ACE-2 enzyme is affected by gender, as there are significant differences in female but not in male. The same results were found in the case of K⁺, which referred to that no significant differences p=0.271 among the studied groups in the case of female. While, there was a high significant decrease p= 0.006** recorded for both micro and macro groups than normo and control groups in male cases as shown in Table (4). On the other hand, results of Na ion showed significant increase for both female p= 0.001** and male p= 0.006** in macro and micro groups related to normo and control groups, that means gender has no effect on Na ion in this case, as noticed in Table 4.

Table 4 Level of ACE-2 enzyme, Na, and K in the studied groups (male and female).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=30</th>
<th>Normo-albuminuria N=30</th>
<th>Micro-albuminuria N=30</th>
<th>Macro-albuminuria N=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE-2 (pg/ml)</td>
<td>1305.73 ± 27.691 b</td>
<td>2122.11 ± 800.21 b</td>
<td>2754.25 ± 623.61 a</td>
<td>2778.54±1885.34 a</td>
<td>0.001**</td>
</tr>
<tr>
<td>Female</td>
<td>(500.3-2414.3)</td>
<td>(780.46-3786.14)</td>
<td>(1914.86-3667.00)</td>
<td>(1680.2-3734.1)</td>
<td></td>
</tr>
<tr>
<td>ACE-2 (pg/ml)</td>
<td>1795.29 ± 803.14 a</td>
<td>2357.60 ± 776.27 a</td>
<td>2365.36 ± 844.9 a</td>
<td>2498.9 ± 580.50 a</td>
<td>0.077</td>
</tr>
<tr>
<td>Male</td>
<td>(614.5-2679.6)</td>
<td>(973.3-3529.0)</td>
<td>(767.1-3707.6)</td>
<td>(1631.4-144.6)</td>
<td></td>
</tr>
<tr>
<td>Na (mg/dL)</td>
<td>135.53 ± 5.75 b</td>
<td>138.36 ± 3.97 ab</td>
<td>140.20 ± 4.16 b</td>
<td>140.41 ± 2.54 a</td>
<td>0.011*</td>
</tr>
<tr>
<td>Female</td>
<td>(127.50-143.10)</td>
<td>(132.60-145.60)</td>
<td>(131.10-146.70)</td>
<td>(163.1-144.6)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(137.72 ± 4.62 ab)</td>
<td>(135.4-145.3)</td>
<td>(135.4-145.3)</td>
<td>(135.1-147.2)</td>
<td></td>
</tr>
<tr>
<td>K (mg/dL)</td>
<td>4.59 ± 1.06 a</td>
<td>4.38 ± 0.55 a</td>
<td>4.28 ± 0.47 a</td>
<td>4.09 ± 0.50 a</td>
<td>0.271</td>
</tr>
<tr>
<td>Female</td>
<td>(2.50-5.90)</td>
<td>(3.20-5.30)</td>
<td>(3.6-4.0)</td>
<td>(3.30-4.90)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4.77 ± 0.87 a</td>
<td>4.40 ± 0.43 a</td>
<td>4.07 ± 0.43 b</td>
<td>4.033 ± 0.627 b</td>
<td>0.006**</td>
</tr>
</tbody>
</table>

**Significant difference between means using ANOVA -test at 0.01 level.
- Significant variants are denoted by different small letters.
- Non-significant variations are denoted by identical small letters.
ROC Analysis: Fig. 1 shows ROC curve of ACE2 for microalbuminuria group of diabetic nephropathy. The results of ROC analysis revealed that ACE-2 represents a good diagnostic marker with (AUC equal to 0.8) at 95% CI to predict diabetic patients with microalbuminuria related to normal control, as shown in Fig. 1.

Discussion:

Long-term high blood sugar often leads to cause kidney disorders. It was found that the renal dysfunction can occur during long duration of the most patients with type 2 diabetes mellitus. Diabetic nephropathy is considered as a silent disease during a long period without any symptoms. Subsequently, chronic hyperglycemia affects different types of kidney cells which finally leads to progressive fibrosis glomerular and tubular damage resulting kidney failure. Thus, the difficulty in identifying risks with preclinical of kidney disease reflects the challenge in prevention of diabetic nephropathy in patients with type 2 diabetes mellitus. Results of the current study showed that there are no any effect of gender on the rate of hyperglycemia, this is in agreement with many other studies. An increase in fasting blood sugar was found in patients with micro and macro groups compared to normal albuminuria in both male and female.

Current study did not find any effect of gender on the rate of hyperglycemia. Results in this study are in agreement with many other studies. An increase in fasting blood sugar was found in patients with micro and macro groups compared to normal albuminuria in both male and female. Hyperglycemia brought on by insulin secretion, insulin action, or a combination of the two is what distinguishes diabetes as a metabolic disorder. Where it differs from another study which showed that compared to female patients, male patients had worse glycemic control. According to reports, women with normal glucose tolerance are more insulin-sensitive and have better -cell activity than men. Lack of testosterone in men has been found to raise insulin resistance and blood sugar levels.

Results of current study showed that there was no effect of gender on urea and creatinine levels among groupings of men and women. This contrasts with other studies’ findings that female kidneys appear to be better protected than male kidneys, despite estrogen shortage following menopause. An increase in female NO levels may be responsible for renal protection. In the kidney, NO is crucial for tubular transport autoregulation and modulation. It has been found that the overall production of nitric oxide (NO) declines when chronic kidney disease (CKD) worsens. Men's renal vasculature also gets more and more dependent on nitric oxide as they age. Lack of testosterone may contribute to endothelial dysfunction by lowering NO levels by regulating NO synthase expression and activity. ACR evaluation in urine was recommended for estimation of albuminuria in diagnosis of diabetic nephropathy in patients with T2DM develop of fibrosis in glomerular and tubular is attributed to the endothelial damage which forms microalbuminuria in diabetic patients. So, the prevention of kidney disease progression depends on early diagnosis, hyperglycemia leads to severe kidney damage that cause decline eGFR, therefore eGFR can be helpful as a marker for early renal impairment diagnosis in patients with type 2 diabetes in addition to albuminuria.

Both albuminuria and eGFR are significantly better indicators of end-stage renal disease, but urine ACR was found to have a greater impact on clinical practice than eGFR. As a result, the presence of albuminuria indicates damage to both the glomeruli and the tubules, whereas an eGFR disorder only indicates glomerular damage. According to the findings of this study, assessment of ACR and GFR could serve as helpful markers in this setting for the early detection of diabetic nephropathy, the prevention of overt nephropathy, and the progression to end stage renal disease. Gender has no impact on ACR or GFR in this study.

Because it is linked to renal salt retention, diabetes mellitus type 2 is of particular concern. Although the underlying causes of increased renal sodium absorption are not fully understood, there is an evidence that epithelial sodium channel (ENaC)-mediated salt absorption is involved. Aldosterone's cumulative effects and the combined effects of hyperinsulinemia and hyperglycemia appear to enhance this transport. It has been proposed that...
enhanced glomerular filtration of glucose may boost the activity of the proximal tubular Na+-glucose co-transporter and may contribute to sodium retention in addition to hyperinsulinemia-mediated renal tubular sodium transporters. The kidney produces renin enzyme when renal perfusion is reduced. This enzyme can be converting angiotensinogen to angiotensin I. Renal endothelium product angiotensin converting enzyme, this enzyme converting angiotensin I to angiotensin II. Angiotensin II acts on tubular Na reabsorption and K excretion. In the current study, there was no effect of gender on the proportion of sodium, but we found a difference in the proportion of potassium in the blood between male and female.

A significant consequence of chronic kidney disease (CKD) is hypertension, which accelerates the illness's course and increases cardiovascular morbidity and death. For CKD stage G5, the incidence of hypertension rises to >90% with declining glomerular filtration rate (GFR). It is not fully known how CKD imparts Na, Cl sensitivity or how the many mechanisms that control Na+ balance and blood pressure interact in CKD. Hypertension in CKD is a salt-sensitive variant of hypertension. The ability to eliminate salt and water will be limited by a decrease in nephron number, and the pressure natriuresis that results will cause hypertension in CKD. To further exacerbate salt-sensitive hypertension, a number of antinatriuretic variables may also be at work in CKD. For instance, in CKD, plasma aldosterone is frequently increased without an increase in plasma renin, which may be brought on by metabolic acidosis due to hypokalemia. Additionally, local angiotensin II (ANG II) synthesis in the kidneys may cause CKD to activate the intrarenal renin-angiotensin system (RAS). Additionally, it has been noted that a high salt diet can activate the mineralocorticoid receptor without the help of aldosterone. ANG II, aldosterone, and MR activate Na+ transport proteins in the kidney, including Na+, Cl−.

The liver appears as the main source of this protein in the kidney. However, angiotensinogen can also be synthesized in the proximal tubule and can be secreted to the tubular lumen, playing a potential role in intratubular Ang II synthesis. The glomerular endothelium, mesangial cells, podocytes, and the brush border of the proximal tubule, which is its highest region of expression in the kidney, are all extensively expressed in the nephron as well. Increased levels of ACE in the kidneys have been associated with elevated levels of renal Ang II. Other studies have shown that if inflammation occurs for three days, it leads to the production of monocytes and microphages. These cells are a result of cytokines IL-6, and monocyte chemoattractant protein-1 (MCP1) inflammation induced produced CTGF not related with fibrosis. After 1 week of infusion of Ang II increase product extra culler matrix protein collagen and fibronectin with the continued rise CTGF and formation of fibrosis. After 2 weeks of infusion with Ang II increase level of TGF-β1 and CTGF by epithelial cells, mesangial cells and endothelial cells. TGF-β causes mesangial enlargement due to mesangial cell hypertrophy, proliferation (and eventually apoptosis), and the production of extracellular matrix (ECM). It also causes endothelium to mesenchymal transition, which results in glomerular myofibroblasts, a significant source of ECM, to occur. Current study showed a significant difference between male and female in ACE-2 levels. The reason of this difference is that male patients are more likely to have high blood pressure due to drinking alcohol and smoking compared to women so males need treatment, and this treatment is considered an enzyme inhibitor and thus reduces kidney problems.

Conclusion:
On the basis of the obtained results in this study, gender affects ACE-2 level in kidney disease caused by type 2 diabetes. Furthermore, ROC analysis data revealed that ACE-2 is found to be a good marker. Also could be used in diagnosis of diabetic nephropathy (DN) disease.

Acknowledgement:
Authors thanks and appreciation to the staff of the Medical City/College of Medicine-University of Baghdad and Al-Yarmouk Hospital-Baghdad for their assistance in collecting and analyzing samples and for their facilities that assisted in the achievement of this study.

Authors' Declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration’s approval
- Ethical Clearance: The project was approved by the local ethical committee in Medical City.

Ethics Approval
Approval was obtained from Baghdad Teaching Hospital No : 46741
Authors’ Contributions Statement
This work was carried out in collaboration between all authors. R. K. I. diagnosed the cases and conducted the collection of samples and the test. K. K. G. wrote and edited the manuscript. A. A. D. did the analysis of the data. All authors read and approved the final manuscript.

References:


