Protective Effect of Angiotensin II Type 1-Receptor-Blocker on Diabetic Nephropathy in Rats: Role of Nephrin

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Abstract

Background: Angiotensin II-Receptor Blockers (ARBs) has a questionable nephroprotective effect especially in diabetic nephropathy. Nephrin protein molecules exist in the slit diaphragm of the glomerular filtration membrane and control the passage of plasma protein.

Aim of Study: To examine the possible role of Nephrin in the pathogenesis of diabetic nephropathy and the protective effect of ATII-RB (candesartan) on diabetic nephropathy.

Material and Methods: Thirty-two male rats were used in this experiment. Half of the rats were fed a high-fat diet (HFD) and the other half were fed a standard diet for 4 weeks. A model of diabetic nephropathy (DN) was created by a 35mg/kg intraperitoneal dose of Streptozotocin (STZ) in the HFD-fed rats. Then the rats were divided into four groups (8 rats/group). (1) Normal control (NC) group; (2) Diabetic nephropathy (DN) group; (3) ARB-treated NC group: Normal rats were treated with the ARB (Candesartan Cilexetil), in a dose 0.1mg/kg; (4) ARB-treated DN group: The rats with DN and received the above dose of candesartan for 4 weeks.

The urine was collected to measure the urine flow rate, urinary concentrations of Nephrin, creatinine, and protein. Blood samples were used to measure urea, creatinine, glucose, insulin, and the homeostatic model assessment (HOMA-IR). Then renal tissue samples were used to measure Superoxide dismutase (SOD) activity and Malondialdehyde (MDA) in addition to histopathological examination. The mean arterial blood pressure (MABP) was also recorded.

Results: Candesartan treatment in the DN group showed significant reductions of glucose, HOMA-IR, amelioration of renal antioxidant system, reductions in creatinine, reduction of proteinuria, reduction of urinary Nephrin, and MABP. Histological improvement was also detected in the form of mild glomerulosclerosis and interstitial fibrosis. The urinary Nephrin was 90 times high in the condition of DN. This huge rise of urinary Nephrin was significantly reduced by Candesartan treatment. Moreover, in the DN group the urinary Nephrin level showed a significant correlation with glucose level, serum creatinine, urinary protein-creatinine ratio, and MABP.

Conclusion: ATII-RB could reduce urinary Nephrin excretion and possibly alters its expression through its inhibitory effect on the reactive oxygen species, the Renin-Angiotensin-Aldosterone system and subsequently maintains the normal physiological activity of podocytes in rats with STZ-induced diabetic nephropathy.

Key Words: Angiotensin II-Receptor Blockers – Nephrin – Diabetic nephropathy.

Introduction

DIABETES mellitus (D.M) is the commonest metabolic disorder [1], that can be considered as a microangiopathic disease affecting different organs mainly kidneys, heart, eyes, and nervous system [2].

Diabetic Nephropathy represents a chronic renal disorder by non- or badly-controlled diabetics in the form of excessive proteinuria with subsequent hypoalbuminemia and conceivably generalized edema [3].

The clinical manifestations of diabetic nephropathy reflect the cellular and functional pathological changes in the glomeruli which represent the end-result of different pathophysiological pathways in which hyperglycemia plays a pivotal role [4].

Nephrin is a recently discovered protein that controls the passage of plasma protein through the glomerular barrier. Nephrin molecules exist in the slit diaphragm between the adjacent podocyte foot processes which represent the essential structure in the glomerular filtration membrane [8].

It has been reported, that the expression of Nephrin is markedly reduced in different proteinuric renal diseases including diabetic nephropathy, moreover the urinary content of Nephrin is significantly increased in diabetic kidney diseases [6].
The correlation between the level of Nephrin expression and certain renal diseases characterized by excessive proteinuria is not completely understood [7].

Angiotensin II-Receptor Blockers (ATII-RBs) are frequently used as first-line therapy against hypertension. The effects of ARBs based on their ability to block the Renin-Angiotensin-Aldosterone system with subsequent reduction of blood pressure [8].

Candesartan is a well-known anti-hypertensive drug that belongs to ATII-RBs, and many studies showed that candesartan has a nephroprotective effect as it could be used in diabetic nephropathy [9].

Regardless of its anti-hypertensive effect, candesartan therapy showed a significant reduction in urinary albumin excretion and improvement in kidney function tests, indicating that different mediators play an essential role in this nephroprotective effect of candesartan [10].

The present study aims to examine the possible role of Nephrin in the pathogenesis of diabetic nephropathy and the protective effect of ATII-RB (candesartan) on diabetic nephropathy.

Material and Methods

Animals and study protocol:
A total of 32 male (180-200g) Wistar rats were purchased from the Faculty of Veterinary Medicine, Zagazig University, and used for this study during the period between June and September 2019. The animals were housed in the animal facility in the Faculty of Medicine, Zagazig University. Rats were left for one week before the start of the experimental protocol to be acclimatized to the new environment. Rats were fed the standard rodents' diet (Carbohydrate 62.8%, fat 11.4%, and protein 25.8%) with water ad libitum. The room temperature was around 25°C with a light/dark cycle at 12:12 [11]. The study protocols were approved by the Institutional Research Board, and Ethics Committee, Faculty of Medicine, Zagazig University.

Later on, the feeding of rats was changed in half of them to be a High-fat diet [12] for 4 weeks (carbohydrate 21.3%, fat 60.3%, protein 18.4%), while other rats were kept on the standard diet. Then HFD-fed rats were injected with a single intraperitoneal dose of Streptozotocin (Sigma-Aldrich, St. Louis, MO, USA), equal 35mg/kg in citrate buffer (pH 4.5) [13] to induce a model of diabetic nephropathy. Normally fed rats were injected with the same volume of normal saline. Two weeks later, rats were housed individually in the metabolic cages and divided into four groups (8 rats/group). (1) Normal control (NC) group: Which received only 1ml of 0.9 normal Saline every day by a rigid oral gavage. (2) Diabetic nephropathy (DN) group: In which blood glucose level was >200mg/dl by a Glucometer from rat tail blood samples and urinary protein became higher than that of the NC group [14] and received a vehicle by oral gavage. (3) ARB-treated NC group: Normal rats which were treated with the Angiotensin II Type 1-Receptor Blocker, candesartan (Candesartan Cilexetil, Astra Zeneca, Sweden), in a dose 0.1mg/kg, suspended in 0.9% normal saline solution at the volume of 1 ml/rat and administered by oral gavage [15]. (4) ARB-treated DN group: The animals with DN and received the same dose of candesartan described above. This treatment protocol was continued for 4 weeks.

Renal function assessment:

At the end of the treatment protocol, a blood sample was collected after overnight fasting via cardiac puncture under ether anesthesia, followed immediately by the collection of the right kidney for histopathological examination. Blood samples were collected in tubes, centrifuged, and stored at -20°C until the time of measurement.

Biochemical analysis:

The urinary Nephrin concentration measured by ELISA kits (LifeSpanBioSciences, Inc, USA-Catalogue no LS-F7323) [18]. The concentration of urinary protein was measured with a calorimetric technique using Sensiprot Kit (Labtest, Brazil - Ref: no: 36). Diluted samples (1:50 dilution ratio) were used to quantify urinary creatinine by a colorimetric enzymatic reaction (Labtest, Brazil -
Ref. no: 27) [16]. The level of glucose was measured by the glucose oxidase method using a Beckman Analyzer (Beckman, USA). Serum insulin levels were measured by a commercial ELISA kit according to the described protocol (MyBioSourceUSA Catalogue no MBS724709) [19]. The homeostatic model assessment (HOMA-IR) as an indicator of insulin insensitivity was calculated based on the following equation: Fasting insulin (U/l) x fasting glucose (mg/dl)/405 [20]. Serum urea and creatinine levels were measured by commercial colorimetrickits (Spinreact, S.A.U. Spain).

Assessment of renal antioxidant system:

Tissue samples (200mg) of right kidney were harvested and weighed, then washed with phosphate-buffered saline to be homogenized at 4°C by a DY89- homogenizer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China), the homogenates were centrifuged and stored at –20ºC until time of analysis. Superoxide dismutase (SOD) activity and Malondialdehyde (MDA) content were measured using commercially available kits, according to manufacturer’s protocol [21]. The SOD activity was expressed in units of enzyme/mg of tissue protein while MDA in nmol/gram of tissue sample.

Measurement of mean arterial blood pressure:

A non-invasive blood pressure monitoring device (NIBP 250, serial No: 21202-108 BIOPAC system Inc; USA) was used to record the tail blood pressure. Rats were held in a restrainer on with an occlusion cuff applied on the base of the tail. The values of the systolic (SBP), diastolic (DBP) blood pressure and heart rate were demonstrated on the screen then the Mean arterial blood pressure (MABP) was calculated based on this equation MAP = DBP + 1/3 (SBP- DBP) [22].

Histopathology:

Immediately after decapitation, the right kidney was excised, washed with saline then fixed in 10% formalin solution and embedded in paraffin, sectioned. The sections were stained with Hematoxylin-Eosin. Stained sections were examined under a light microscope (200x) equipped with a digital camera. The pathologist was blind regarding the treatment protocol and requested to search for stigmata of glomerulosclerosis, interstitial cellular infiltration, or presence of fibrosis.

Statistical analysis:

Study parameters were presented in the form of means ± SD and SPSS 23 was used for analysis (SPSS Inc. Chicago, IL, USA). One way Analysis of variance (ANOVA) with LSD-post hoc test was used to compare means of different groups. Based on the small sample used in this experiment it was recommended to use the Spearman correlation coefficient to detect associations urinary Nephrin and other study parameters in different groups. p-values <0.05 was the cutoff of significance.

Results

Characteristics of diabetic nephropathy:

As shown in Tables (1,2), glucose level was significantly higher in DN groups in comparison to NC groups, with insignificant changes in Insulin indicating marked rise of HOAM-IR value (5.58±0.81 vs 1.73±0.58, p<0.001). The SOD activity was significantly lower, and MDA was significantly higher in the DN group. The manifestations of disturbed renal functions were also evident in form of significant rise of serum creatinine, serum urea, urinary protein, urinary protein-urinary creatinine ratio, urinary Nephrin, and mean arterial blood pressure, together with a significant reduction of urine flow, urinary creatinine, and GFR. Microscopic manifestations included marked interstitial inflammatory infiltrate, scattered fibrosis, and evident glomerulosclerosis in form of with diffuse and nodular mesangial expansion (Fig. 1, B vs A).

Effects of ARB treatment:

Candesartan treatment in rats with DN showed a significant reduction of glycemia, reduction of HOMA-IR value, amelioration of renal antioxidant system. Furthermore, candesartan improved the indicators of renal functions in the form of significant reductions in serum creatinine, reduction of proteinuria, reduction of the ration of urinary protein to creatinine, reduction of urinary Nephrin, and MABP. Histological improvement was also detected in the form of mild glomerulosclerosis and interstitial fibrosis (Fig. 1, D vs B). Interestingly these ren-protective roles were not significant in the physiological condition when comparing ARB-treated NC with the NC group.

Role of nephrin:

The urinary Nephrin was 90 times high in the condition of DN. This huge rise of urinary Nephrin was significantly reduced by Candesartan treatment. Moreover, correlation studies (Table 3) indicated that in the physiological conditions (group NC), the urinary Nephrin level showed a significant correlation with serum urea, urinary creatinine, and renal SOD activity. Besides, in diabetic nephropathy, Nephrin also showed a significant correlation with glucose level, serum creatinine, urinary protein-creatinine ratio, and MABP.
Table (1): Studied parameters in all groups (represented as Mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control (n=8)</th>
<th>Diabetic Nephropathy (n=8)</th>
<th>ARB-treated Normal Control (n=8)</th>
<th>ARB-treated Diabetic Nephropathy (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td></td>
<td>80.17±9.64</td>
<td>284.50±83.50***a</td>
<td>83.50±11.02</td>
<td>227.83±40.19***a,***b</td>
</tr>
<tr>
<td>Insulin (uIU/l)</td>
<td></td>
<td>8.83±0.94</td>
<td>8.02±0.69</td>
<td>7.98±0.70</td>
<td>8.12±1.16</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td>1.73±0.58</td>
<td>5.58±0.81***a</td>
<td>1.63±0.15</td>
<td>4.48±0.33***a,b</td>
</tr>
<tr>
<td>Superoxide Dismutase (U/mg tissue protein)</td>
<td></td>
<td>11.46±0.95</td>
<td>5.17±0.65***a</td>
<td>10.94±1.16</td>
<td>6.78±1.11***a,b</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/g tissue)</td>
<td></td>
<td>35.65±2.71</td>
<td>61.82±6.94***a</td>
<td>34.61±2.67</td>
<td>47.02±2.27***a,b</td>
</tr>
</tbody>
</table>

*** = p<0.001. ** = p<0.01. * = p<0.05.  
 a: Versus NC Group.  
 b: Versus DN Group.

Table (2): Renal function parameters in all groups (represented as Mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control (n=8)</th>
<th>Diabetic Nephropathy (n=8)</th>
<th>ARB-treated Normal Control (n=8)</th>
<th>ARB-treated Diabetic Nephropathy (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td></td>
<td>0.63±0.11</td>
<td>2.72±0.53***a</td>
<td>0.65±0.16</td>
<td>2.12±0.22***a,***b</td>
</tr>
<tr>
<td>Serum Urea (mg/dl)</td>
<td></td>
<td>25.15±3.32</td>
<td>43.17±7.28***a</td>
<td>23.67±5.43</td>
<td>39.83±3.66***a</td>
</tr>
<tr>
<td>Urinary creatinine (mg/dl)</td>
<td></td>
<td>48.83±3.43</td>
<td>22.33±4.55***a</td>
<td>49.67±4.32</td>
<td>32.67±6.71***a,a,b</td>
</tr>
<tr>
<td>Urinary protein (mg/dl)</td>
<td></td>
<td>4.38±1.28</td>
<td>36.17±6.24***a</td>
<td>4.77±0.37</td>
<td>25.08±5.30***a,b</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td></td>
<td>0.0076±0.0007</td>
<td>0.0038±0.0008***a</td>
<td>0.0071±0.0012</td>
<td>0.0029±0.0009***a</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td></td>
<td>0.61±0.16</td>
<td>0.03±0.01***a</td>
<td>0.59±0.22</td>
<td>0.04±0.01***a</td>
</tr>
<tr>
<td>Urinary Protein-Urinary Creatinine Ratio</td>
<td></td>
<td>0.09±0.2</td>
<td>1.64±0.19***a</td>
<td>0.10±0.01</td>
<td>0.80±0.23***a,b</td>
</tr>
<tr>
<td>Urinary Nephrin (µg/24h)</td>
<td></td>
<td>0.049±0.005</td>
<td>4.47±0.78***a</td>
<td>0.047±0.005</td>
<td>2.04±0.892***a,b</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td></td>
<td>84.92±5.43</td>
<td>144.20±9.35***a</td>
<td>77.33±14.69</td>
<td>90.50±15.33***b</td>
</tr>
</tbody>
</table>

*** = p<0.001. ** = p<0.01. * = p<0.05.  
 a: Versus NC Group.  
 b: Versus DN Group.

Table (3): Correlation coefficient of urinary Nephrin with other studied parameters in all groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control (n=8)</th>
<th>Diabetic Nephropathy (n=8)</th>
<th>ARB-treated Normal Control (n=8)</th>
<th>ARB-treated Diabetic Nephropathy (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>0.54±0.46</td>
<td>0.714*</td>
<td>0.406</td>
<td>0.314</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>−0.48±0.14</td>
<td>0.486</td>
<td>0.143</td>
<td>−0.200</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td>0.48±0.46</td>
<td>0.543</td>
<td>−0.256</td>
<td>−0.200</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td></td>
<td>0.257</td>
<td>0.667*</td>
<td>−0.145</td>
<td>0.543</td>
</tr>
<tr>
<td>Serum Urea</td>
<td></td>
<td>0.657*</td>
<td>0.601</td>
<td>0.319</td>
<td>0.754*</td>
</tr>
<tr>
<td>Urinary Creatinine</td>
<td></td>
<td>0.899*</td>
<td>0.543</td>
<td>0.257</td>
<td>−0.086</td>
</tr>
<tr>
<td>Urinary Protein</td>
<td></td>
<td>0.468</td>
<td>0.429</td>
<td>0.518</td>
<td>0.467</td>
</tr>
<tr>
<td>Urine flow</td>
<td></td>
<td>0.413</td>
<td>−0.543</td>
<td>0.029</td>
<td>−0.174</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td></td>
<td>0.200</td>
<td>−0.257</td>
<td>0.143</td>
<td>0.429</td>
</tr>
<tr>
<td>Urinary Protein-Urinary Creatinine Ratio</td>
<td></td>
<td>0.257</td>
<td>0.771*</td>
<td>−0.029</td>
<td>0.725*</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td>−0.943**</td>
<td>−0.314</td>
<td>−0.371</td>
<td>−0.429</td>
</tr>
<tr>
<td>MDA</td>
<td></td>
<td>0.429</td>
<td>−0.029</td>
<td>0.371</td>
<td>0.316</td>
</tr>
<tr>
<td>MABP</td>
<td></td>
<td>−0.029</td>
<td>0.765*</td>
<td>−0.212</td>
<td>0.429</td>
</tr>
</tbody>
</table>

** = p<0.01. * = p<0.05.
Discussion

Renal impairment is one of the most common pathological changes that is closely related to diabetes mellitus [23]. Shaw JE, et al. (2010) [24] reported that most cases of end-stage renal diseases in the United States evoked by DM.

For a better understanding of pathological changes in DN, the Renal Pathology Society provide a new pathological classification of the histopathological changes in DN depending mainly on the glomerular changes and dividing it into 4 classes: Class I: Shows mainly thickening of the glomerular basement membrane; Class IIa: Shows mild mesangial expansion; Class IIb: Shows severe mesangial expansion; Class III: Shows nodular sclerosis and finally Class IV: Shows global glomerulosclerosis in more than 50% of glomeruli [25].

Owing to the great resemblance of the histopathological changes associated with diabetic nephropathy in rats and humans [26], in the present study we have produced a model of diabetic nephropathy by intraperitoneal injection of STZ.

The results of this study showed the presence of histopathological changes in STZ-induced diabetic groups in the form of marked interstitial inflammatory infiltrate with scattered fibrosis and evident glomerulosclerosis with diffuse and nodular mesangial expansion. These findings according to the new classification of the Renal Pathology Society could be considered as class IV DN.

In addition, there was a marked elevation in serum glucose level and HOMA-IR value accompanied with marked disturbance in renal functions in the form of a significant rise in serum creatinine, serum urea, urinary protein, urinary protein-urinary creatinine ratio, together with a significant reduction of urine flow, urinary creatinine, and GFR in STZ-induced diabetic groups when compared to NC groups.

Various factors involved in the pathogenesis of DN besides the genetic predisposition as the activation of the Renin-Angiotensin-Aldosterone system (RAAS), reactive oxygen species (ROS), and the increase of advanced glycation end-products [27].

It has been shown that the oxidative stress induced by chronic hyperglycemia may play a role in the development and pathogenesis of DN [28]. Furthermore, Jia J et al. (2008) [29] reported that chronic hyperglycemia could produce renal oxidative injury by stimulating oxidative stress with over production of ROS.

The renal cells are more vulnerable to oxidative injury due to their high rate of oxygen consumption;
therefore this oxidative injury is usually associated with enzymatic inactivation in the form of impaired lipid peroxidation with the enhancement of MDA production [30].

On the other hand, renal cells characterized by a normal physiological defense mechanism, as they contain endogenous cellular antioxidant system which counteracts to a certain limit the harmful effects of over production of ROS [31].

These reports are consistent with the results of this study. As in the present study, the DN group showed a significant elevation of MDA which is an indicator of ROS over-activity and a marked reduction in SOD that indicates the depletion of the endogenous antioxidant activity of renal tissues.

Interestingly, the candesartan-treated group in this study showed significant improvement of the antioxidant capability of renal cells in the form of a significant elevation of serum SOD level with a significant reduction of serum MDA indicating one of the different mechanisms of candesartan in treatment and prevention of DN. This was contrary to the finding of Sugimoto et al. (2005) [32] which proves that both candesartan and enalapril insignificantly change the antioxidant levels in kidney tissue of the 5/6 nephrectomized rats. The different rat models may put the interpretation of this discrepancy.

Furthermore, the ROS overproduction could induce mitochondrial-dependent apoptotic pathway through the overexpression of different apoptotic protein e.g. Bax and cleaved caspase-3 with subsequent injury and apoptosis of renal cells mainly podocytes, that play also an important role in the pathogenesis of DN [33].

Although the role of RAAS in the pathogenesis of DN is extensively studied, the exact mechanisms still need to be completely elucidated [34]. Giacchetti et al. (2005) [35] reported that hyperglycemia induces over production of Angiotensin II (Ang II) which induces through different local mediators e.g. oxygen free radicals and inflammatory mediators endothelial cell damage and renal vascular remodeling. Also, Magee et al. (2017) [36] showed that the progression of the DN is strongly correlated to insulin resistance, which could be deteriorated through the overactivity of RAAS in renal tissues.

These results are consistent with the result of the current study as the STZ-induced diabetic rats hav ingelevated HOMA-IR value showed a significant reduction in HOMA-IR value under candesartan therapy.

Several previous studies assumed that the RAAS and oxidative stress could mutually control each other through diverse mechanisms and participate in the development of DN [37]. Ang II together with ROS may be responsible for organ damage through activation of proinflammatory, profibrotic, and mitogenic signaling pathways [38].

Different studies showed increased activity of Mitogen-activated protein kinases (MAPKs) which is the main signaling pathway in fibrosis, inflammation, and mitosis in renal tissues in diabetic subjects [39]. So, in this study, the activity of MAPKs could be suppressed by candesartan therapies ATII-RB indicating that the role of ATII-RBs in diabetic nephropathy is independent of its antihypertensive effect.

The present study observed that candesartan treatment in rats with DN significantly improved the indicators of renal functions in the form of a significant reduction in serum creatinine and reduction of proteinuria. Also, the histological improvement was detected in the form of mild glomerulosclerosis and interstitial fibrosis. These findings are consistent with those of Mimran and Alfaro (2003) [40] who reported the beneficial effects of candesartan in blocking angiotensin receptors and decreasing renal vascular resistance & urinary albumin excretion.

The occurrence of albuminuria indicates either structural or functional changes in the glomerular filtration barrier (GFB) [41]. Maezawa et al. (2015) [42] found that in DN podocytes which are the most important element of GFB wasexposed to marked apoptosis through ROS which activates NADPH oxidase with subsequent micro-and macroalbuminuria, which agrees with our assumption about the pathogenesis of DN.

Nephrin is a recently discovered protein that is considered as the slit diaphragm of the podocytes. Besides, the integrity of the podocyte filtration barrier depends mainly on the vitality of Nephrin expression [5]. Recent research essuggested a significant decline in Nephrin expression and marked increased inurinary Nephrin in diabetic kidney diseases [43]. Moreover, Pan et al. (2018) [44] proved that some compounds like catalpol possess a renoprotective effect that may improve the glomerular filtration barrier in DN through enhancement of Nephrin expression.

In the present study, we examined the role of the RAAS and its blocker candesartan on the podocytes and Nephrin activity. The results of this
study revealed marked elevation of urinary Nephrin (90 times high) in the condition of DN. This huge rise of urinary Nephrin was significantly reduced by Candesartan treatment. The latter finding is consistent with the suppressive effect of candesartan on Nephrin gene expression in hypertensive nephropathy [45].

Moreover, correlation studies indicated that in the physiological conditions (group NC), the urinary Nephrin level showed a significant correlation with serum urea, urinary creatinine, and renal SOD activity. Also, in diabetic nephropathy, Nephrin showed significant correlations with glucose level, serum creatinine, urinary protein-creatinine ratio, and MABP.

The present study supposes that the RAAS in combination with ROS through MAPKs regulates the apoptotic activity of different renal cells including podocytes. So, this assumption coincides with some recent studies that reported significant disturbance in the podocytes of GFB with subsequent disturbance in Nephrin expression in DN [46].

Finally, this study concludes that candesartan as ATII-RB could reduce urinary Nephrin excretion and possibly alters its expression through its inhibitory effect on the ROS and RAAS and subsequently maintains the normal physiological activity of podocytes in rats with STZ-induced diabetic nephropathy.

Acknowledgment: To Dr. Hanan Lotfy, Lecturer of Pathology, Zagazig University, Faculty of Medicine, for assistance in preparation, staining, and reporting of the pathology sections.

References
Protective Effect of Angiotensin II Type 1-Receptor-Blocker on Diabetic Nephropathy


التأثير الواقعي لحصصات مستقبلات الأنجيوتنسين II على الكلى في حالات الاعتلال الكلوي السكري دوري النفرين

خلفية: البحث حصر مستقبلات الأنجيوتنسين II لها تأثير أو أقابل للنفرينات الكلوية وخاصة في حالات اعتلال الكلى السكري وهذا التأثير يحتاج إلى مزيد من الدراسة. توجد جزيئات نفرين في المحاجر الجناح الشقي للفشة الترشيع الكلوي وتمكنهم من مور بروتين البليزما من خلاة

المهدف من العمل: دراسة دور المحتمل لبروتين النفرين في التسبب في اعتلال الكلى السكري ودوره في التأثير الوقائي لحصصات مستقبلات الأنجيوتنسين II مثل الكانيدنسيراتان على اعتلال الكلى السكري

المواد والأساليب: تم استخدام ثلاثين من الفئران الذكور في هذه التجربة. تم تقسيم نصف الفئران بنظام غذائي غني بالدهون والنصف الآخر تم إطعامهم بنظام غذائي قاسي لمدة 4 أسابيع. تم إنشاء نموذج من اعتلال الكلى السكري عن طريق جرعة داخل الفضاء البروتينية (5 ملغ/كم²) من النوريتين في الفئران التي تتغذى على الغذاء علالي الدهن. تم قسمت الجرارات إلى أربعة مجموعات (4 فئران/مجموعة):

- المجموعة الأولى: المجموعة الضابطة الطبيعية.
- المجموعة الثانية: مجموعة اعتلال الكلى السكري.
- المجموعة الثالثة: مجموعة الفئران الطبيعية والمعالجة بالكاديندراتان حيث تم علاج الفئران العاليا بناءً على مستقبلات الأنجيوتنسين II وهو الكاديندراتان سيليستين، جرعة 1 ملغ/كم² لمدة 4 أسابيع.
- المجموعة الرابعة: مجموعة اعتلال الكلى السكري والمعالجة بالكاديندراتان الفئران التي أصيبت باعتلال الكلى السكري وتمت جرعة مسبقة

من زيت نبات الزيتون من نباتيات القدسيس.

تم جمع البول لياسس معدل الفرو والتركيزات البولية من النفرين والكولاجينات والبروتينات. تم استخدام بعض إنترنشيب الكولاجين، ما هو الإفراز العلالي عند الفئران (HOMA-IR) في المجموعة الأفراز الكولاجين (MDA) وباستخدام نشاط انزيم سيرير بالإضافة إلى فص الأنسجة ميكروسكوبيا. كما تم أيضاً تسجيل مستوى ضغط الدم المرئي من ديل الفئران.

نتائج: HOMA-IR يظهر أن الكاديندراتان يقلل من مستوى الكلي النسيجي ونفرينات الكليات. تقليل مستوى النفرين في البول، حيال مستويات النفرين في البول، وكذلك انخفاض مستوى حمض الدم المرئي من الدل في الكليات. كما أن هذه التأثيرات تكون نتيجة لتفص البول في حالة مستويات الكليات السكرية. والجدل بشكل أسرع في ForeignKey الكلاسيك السكريات. علاج على ذلك، في مجموعة اعتلال الكليات السكري، أظهر مستوى النفرين البولي ارتباطاً كبيراً مع مستويات النوريتين والكولاجينات في الدم مرئي وفي الوضعية البولي ومستويات الدم المرئي. 

الخلاصة: يمكن لحصصات مستقبلات الأنجيوتنسين II أن تقلل إفراز النفرين في البول وأيضاً كما تغير مستويات النفرين من خلال تأثيرها المشتبه على نظائر الكولاجين النتيجة البولي البولي، ونظام الإرتجاع الجليكوزي الثبطي، ويضايق هذه العلاج يحافظ على النشاط الفسيولوجي العضلي النسيجي للفشة الترشيع الكلوي في الجرائد المصابа باعتلال الكليات السكري