Comparison of Urinary Ace 2 Levels in Individuals with Hypertension and Type 2 Diabetes with Individuals who have Hypertension but not Diabetes

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ABSTRACT
Background: In this investigation, the levels of urine Angiotensin Converting Enzyme(ACE2)in patients with Type 2 diabetes and high blood pressure were evaluated and their results compared in individuals having raised blood pressure but they had normal blood sugar control. Given the growing body of evidence linking ACE 2 insufficiency to the etiology of hypertension in diabetic patients, we hoped to find higher Angiotensin Converting Enzyme2 levels in the urine of hypertensive diabetic patients than in those without diabetes.

Aim: As a result, new pathways for the development of antihypertensive medicines aimed at protecting Angiotensin Converting Enzyme2, particularly in diabetic patients, may open up.

Methods: Two groups, each with 49 subjects, were created from a population of chosen subjects. Patients with diabetes and hypertension were chosen from the Services Institute of Medical Sciences diabetic clinic and medical wards in Lahore. In the clinics, anthropometric characteristics and blood sugar levels were recorded. In the Physiology Laboratory at University of Health Sciences, blood samples were obtained and maintained in order to evaluate biochemical characteristics.

Results: We calculated the median value in each group because the data for urine Angiotensin Converting Enzyme 2 readings was not dispersed evenly. Non-diabetic hypertensive participants had a median of 26.47mg/dl, while hypertensive-diabetic subjects had a median of 22.86mg/dl. This difference in ACE 2 levels in the urine was statistically significant (p=0.05). Non-diabetic, hypertensive patients had greater urinary Angiotensin Converting Enzyme 2 levels than diabetic, hypertensive patients.

Conclusion: Contrary to our expectations, we were unable to confirm that urine Angiotensin 2 readings were higher in people with high arterial pressure and type 2 diabetes mellitus. This is despite the fact that the current study confirmed that both type 2 diabetes mellitus and hypertension are risk factors for chronic kidney disease.

Key words: Renin Aldosterone-Angiotensin System, urinary Angiotensin Converting Enzyme 2 levels, Chronic Kidney Disease

INTRODUCTION
Insulin resistance, which has been linked to the renin-angiotensin system (RAS), is the leading cause of type 2 diabetes1-3. In general, the traditional pressor limb, which includes the last generation of angiotensin II (Ang II), and an opposing depressor limb, which includes angiotensins 1-7, can be divided into two advantageously antagonistic arms. In a healthy being, both branches operate in tandem, perfectly harmonizing both RAS activity that is out of balance causes a variety of diseases. Both ACE and ACE2 are peptides with a high amino acid difference at the C terminus. Both function through G-protein linked receptors, AT1R and Mas R, in turn, but with contrasting outcomes4. Angiotensin II is degraded by ACE 2, a homologue of the angiotensin converting enzyme (ACE), into Angiotensin 1-7, a 7 amino-acid peptidpeptide. Human urine contains soluble ACE2, which is thought to be produced through detachment from cells in the nephrons, not by plasma filtration5. Angiotensin II may raise the levels of ADAM 17 protein in the kidney, implying that Angiotensin II activates and enhances ADAM176,7. The RAS is thought to play a role in the progression of hypertension. The ACE-Ang II /ACE2-Ang-(1-7) axes have discovered to have an important role in cardiovascular homeostasis8. Essential hypertension is caused by augmented flowing amounts of vasoconstrictors and hormones such Ang II9. Angiotensin II causes ROS (reactive oxygen species) generation, which up-regulate ADAM 17, causing ACE2 detachment and favoring hypertension feed-forward process10, ACE2 overexpression, on the other hand, decreases generation of ROS down-regulating TACE. ACE2 action was discovered enhanced in humans and animal models in pathological situations (ischemic heart disease, heart failure), most likely as a shielding contrivance to restore normal amount of Angiotensin II11.

Hepatic insulin resistance12, which is assumed to be caused by elevated Angiotensin II levels, has been proposed as the primary aetiology of type 2 diabetes mellitus13,14. The anti-oxidant effect of the ACE2/Ang-(1-7)/Mas axis, as well as the decrease in PEPCK transcription15, may help to reduce insulin resistance16. Hyperglycemia promotes angiotensin-11 and activates ADAM17, resulting in greater ACE2 shedding17. Low podocyte ACE2 concentration initiates Ang-II build up, aggravating glomerular damage & albuminuria, as discerned studies on diabetic mice18. Elevated levels of ACE2 in podocytes cause Ang-II to be converted to Ang-1-7, preventing the effects of increased Ang-II on the kidney19. Urinary ACE2, a tubular damage indicator, had a high connection with albuminuria, suggesting that it could identify imminent DN20. Diabetes increases the press or limb of RAS11 while suppressing the protective arm, the probable cause of systemic hypertension.

MATERIALS AND METHODS
Over the course of a year, researchers in the Physiology Unit of University of Health Sciences (Lahore) conducted a cross-sectional, comparative study after getting permission from ethical review board. We selected 96 hypertension patients from SIMS medical OPD and diabetic facilities, ranging in age from 30 to 60. They were split into two groups: Group B, which had 49 diabetic and hypertensive subjects, and Group A, which contained 47 non-diabetic, hypertensive patients. General and systemic examinations were performed after each subject gave written informed consent to rule out any underlying disease. A sphygmomanometer was used to measure blood pressure. The body mass index (BMI) was computed using the formula: BMI=body weight (kg)/height (m)². On-the-spot blood sugar readings were taken. Under aseptic conditions, five milliliters of blood were extracted from the ante-cubital vein. It was then put into serum tubes. To get serum, the tubes were centrifuged for 10 minutes at 3000 revolutions per minute (rpm). Serum was obtained and kept at -40°C in aliquots using disposable blue tips.
Urinary Ace 2 Levels in Individuals with Hypertension and Type 2 Diabetes

Elabscience’s ELISA kit was used to verify the amount of ACE 2 in the urine (USA).

**Statistical analysis:** IBM SPSS version 20 was used to input and evaluate the data.

The mean standard deviation (SD) for quantitative variables with normal distribution and the median interquartile range (IQR) for those with non-normal distribution were provided. Shapiro-Wilk statistics were used to assess the data distribution. The data were non-normally distributed if the p-value was less than 0.05. The Mann-Whitney U test (non-parametric statistics) was used to compare group means for non-normally distributed quantitative variables, while the student “t” test was used to compare group means for normally distributed quantitative data. Pearson correlation (r) was used to identify correlation between quantitative variables with a regular distribution, whereas Spearman’s rho correlation (r) was used to identify correlation between quantitative variables with a non-normal distribution. The p-value was less than 0.05 as taken as significant.

**RESULTS**

Because the data for the urine ACE2 measurements was not distributed normally (as shown by the Shapiro-Wilk test), we estimated the median value in each group. 26.47 (19.5-34.3) mg/dl was the median for non-diabetic hypertensive subjects, while diabetic hypertensive subjects had a median (IQR) of 22.86 (16-28.2) mg/dl. This difference in ACE2 levels in the urine was statistically significant (p<0.05). The comparison of urine ACE2 levels in cohort A and B participants is shown in Table 1. In group A, the median of uACE2 was higher than in group B. With a p-value of 0.007, we found a significant difference between the medians in both groups, as previously stated.

**DISCUSSION**

Hypertension and Diabetes are two protracted diseases whose prevalence is increasing by the day. Diabetes incidence among adults over 18 years has climbed from 4.7% in 1980 to 8.5% in 2014, according to W.H.O. Diabetes was directly responsible for an estimated 1.6 million fatalities in 2015. World Health Organization (WHO), predicts diabetes will be the 7th largest reason of death by 2030.

According to the world health statistics, 2012, one in every three persons worldwide has high blood pressure with suggestion of a rapid rise in the disorders that cause cardiac disorder, as well as, other protracted ailments, particularly in low and middle-income nations. Bearing in mind the increased prevalence of hypertension and diabetes, as well as its associated ailments and death rate, a lot of research is being done aiming to diagnose and prevent complications.

In group A, the mean uACE-II concentration was substantially higher than expected. Based on multiple research findings that emphasized the involvement of ADAM-17 as a denuber of ACE-II from the nephrons, we assumed that people with both hypertension and diabetes would have higher levels of ACE-II in their urine than those who simply had hypertension. ACE-II protects the kidneys while also lowering systemic blood pressure. An increase in uACE-II concentration of hypertensive, diabetic people was seen in a recent investigation, supporting our theory but contradicting our findings. Although a few researches back up our findings, our sample error could be to blame for the outcomes. The current study’s sampling was executed at Services Institute of Medical Sciences (SIMS) in Lahore. Despite the fact that patients from both congregates were as demographically matched as probable, we identified a gap in the class of therapy and patient management. This was due to the fact that group B individuals were picked from diabetic SIMS Centre, while group A individuals were enrolled from the OPD and ER of medicine. Regrettably, we did not account for the differences in medical treatment provided by both departments at the time of sampling. The diabetes Centre is a well-ordered, meticulous, and proficiently run unit with excellent patient advising/training and a strong follow-up system. Despite the fact that they welcome new, uncontrolled diabetes patients, the vast majority of their patients are diabetics with good control who visit for routine checks. As a result, they fared better and had fewer difficulties than other department patients. The medical OPD/emergency, on the other hand, is ill-organized, with a high patient turnover. Mostly patients are illiterate, misled, and destitute, with little knowledge of their medical problem, let alone their treatment options. When compared to patients who visit the diabetic Centre, compliance is substantially lower. As a result, patients selected from this context were often in worse health than those recruited from the diabetic

![Image](Image 69x193 to 271x364)

**Table 1:** Comparison of uACE 2 in Group A and Group B.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>p-value</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>uACE 2</td>
<td>26.5 (19.5-34.3)</td>
<td>22.9 (16.0-28.2)</td>
<td>0.007*</td>
<td>Non-Gaussian</td>
</tr>
</tbody>
</table>

*p-value generated by Independent Sample t-Test for normally distributed data

**Table 2:** Comparison of data for other parameters in the two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>p-value</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary ACE 2 (mg/dl)</td>
<td>82.0±38.8</td>
<td>88.3±50.0</td>
<td>0.310</td>
<td>Gaussian</td>
</tr>
<tr>
<td>sCreatinine (mg/dl)</td>
<td>1.0 (0.8-1.3)</td>
<td>1.1 (0.8-1.4)</td>
<td>0.306</td>
<td>Non-Gaussian</td>
</tr>
<tr>
<td>Blood Sugar Random (mg/dl)</td>
<td>102.5 (98.0-133.5)</td>
<td>200.0 (144.0-267.5)</td>
<td>0.000</td>
<td>Non-Gaussian</td>
</tr>
<tr>
<td>sUrea (mg/dl)</td>
<td>55.4±71.56</td>
<td>78.0±200.0</td>
<td>0.786</td>
<td>Non-Gaussian</td>
</tr>
<tr>
<td>Glomerular Filtration Rate (ml/min)</td>
<td>64.5±92.77</td>
<td>55.0±71.56</td>
<td>0.000</td>
<td>Non-Gaussian</td>
</tr>
</tbody>
</table>

*p-value generated by Mann-Whitney U Test for non-normally distributed data

To further highlight the significant difference in uACE2 median (IQR) values of both groups, we created a box plot, taking the groups on the X-axis and the medians of uACE2 on the Y-axis. We were thus, able to appreciate the significantly higher value in group A (Fig. 1).

**Fig 1:** Relationship of means of uACE2 in Cohort A & B.

![Image](Image 69x193 to 271x364)
Centre. We were not aware of this limitation at the time of sampling and testing. We only recognized our accidental patient selection flaw during retrograde analysis. Whilst analyzing the data, we have to take into account the difference in overall patient management of both cohorts. The majority of group B subjects received proper treatment and were aware of their medication. The majority of patients in group A, on the other hand, were either on no regular treatment, or were oblivious of their medicine. As a result, a key confounder in our study could be the disparity in therapeutic care and management obtained by both cohorts. Preferably, we should have chosen patients who were not taking any medications and were new to the clinics.

Patients of cohort B received better treatment and had more effective follow-up than those in group A. As a result, they were in a better state of health and had fewer issues. Urinary ACE-2 levels were lower in this group due to better renal function, which may have contributed to this difference. Our theory states that group B individuals with uncontrolled DM should lose more ACE-2 from their kidneys. Nonetheless, the renal shedding of ACE-2 should be lower if the renal status is healthier than in group A patients. Checking if uACE2 is a more accurate biochemical biomarker of early kidney injury was one of our research's goals. Our findings did not favor that.

CONCLUSION

All of our assumptions were disproved by the results. Although differences in CKD staging and overall management of the two cohorts may be to blame for theabstruseness, an ACE2 link to DM and hypertension could not be established. Similarly, we were unable to determine whether uACE2 levels are a stronger predictor of renal injury than microalbuminuria.

Conflict of interest: The authors have no conflict of interest to declare.

REFERENCES