DOI: https://doi.org/10.53350/pjmhs2216415

**ORIGINAL ARTICLE**

# Correlation between Urinary Angiotensin Converting Enzyme-2 & Micro Albuminuria in Non-Diabetic-Hypertensive and Type 2 Diabetic-Hypertensive patients

MAH-E-JABEEN SEAR[1], SAIMA MUKHTAR[2], IRAM QAMAR[3], SHAISTA HUSSAIN[4], SARA NAEEM[5], HAFEESA NAZ[6]
[1,5]*Assistant Professor, Rahbar Medical & Dental College, Lahore, Pakistan;*
[2,3,4]*Associate Professor, Rahbar Medical & Dental College, Lahore, Pakistan.*
[6]*PGR, Department of Endocrinology & Metabolism, Services Hospital, Lahore.*
*Correspondence to Dr. Mah-e-Jabeen Sear, Email: jabeenraza@live.co.uk Mobile No. 03324003169*

## ABSTRACT

**Background:** The Renin Angiotensin System (RAS) is one of the main contributors to insulin resistance which leads to T2DM and its complications. The ACE2/Angiotensin-(1-7)/Mas axis has lately been anticipated to function as a negative regulator of the RAS, consequently, rendering a shielding task against progress of type 2 diabetes, as well as, lowering blood pressure.
**Aim:** To correlate urinary angiotensin 2 levels and microalbuminuria in Type 2 Diabetic and non-Diabetic-hypertensive patients.
**Methods:** A particular population of selected subjects was categorized into 2 groups, with 49 subjects in each group. Diabetic and hypertensive patients were selected from the SIMS diabetic Clinic and medical wards, Lahore. Anthrometric parameters, as well as, BSR were recorded in the clinics. Blood and urine samples were collected and stored for evaluating the biochemical parameters in the Physiology Laboratory, UHS.
**Results:** Microalbuminuria, as well as urinary ACE2 levels in group A (non-diabetic-hypertensive subjects), were greater than in group B (diabetic-hypertensive subjects). There was a significant positive correlation between uACE2 levels and microalbuminuria in group A patients.
**Conclusion:** Although the current study reinforced the fact that both T2DM and hypertension are risk factors for CKD, contrary to our prediction based on evidence gained from various research studies, we were unable to prove that urinary ACE 2 levels are higher in patients with hypertension compounded with diabetes mellitus.
**Keywords:** The Renin Angiotensin System (RAS), angiotensin converting enzyme 2, microalbuminuria levels.

## INTRODUCTION

The RAS plays a vital position in BP management and fluid homeostasis. It can be broadly divided into two, beneficially antagonistic arms; the classical, pressor arm, with the ultimate production of angiotensin II (Ang II) and a counterbalancing, depressor arm, including angiotensin 1-7.

The main cause of type 2 diabetes mellitus is insulin resistance, proposed to be associated with the renin-angiotensin system (RAS)[1]. Both ACE and ACE 2 are peptides differing only in single amino acid at C end. Both act via G-protein coupled receptors angiotensin II type 1 receptor (AT1R) and Mas receptor respectively but with opposing effects. ACE 2, an angiotensin-converting enzyme (ACE) homologue, degrades angiotensin II to angiotensin-(1-7), a seven amino acid peptide fragment with vasodilator effects, thereby conferring a reno-protective role to ACE 2. ACE 2 has been found to be shed in cultured human embryonic kidney cells by a disintegrin and metalloprotease-17 (ADAM-17), a type 1 transmembrane protein that belongs to a superfamily of Zn-dependent metalloproteases. Soluble ACE 2 detected in human urine is hypothesized to be derived by shedding from cells along the nephrons rather than a result of filtration from plasma. Angiotensin II may increase ADAM-17 protein levels in the kidney resulting in angiotensin II activation and enhancement of ADAM-17. The RAS is postulated to contribute towards the development of hypertension. The discrepancy in the ACE-Ang II /ACE 2-Ang-(1-7) axes has been found to play a vital part in cardiovascular homeostasis. Increased circulating levels of vasoconstrictors and hormones like Ang II can cause essential hypertension.[10,11] Angiotensin II induces production of reactive oxygen species (ROS) which in turn upregulate ADAM-17 inducing ACE 2 shedding and favoring a feed forward mechanism of hypertension[12] On the other hand, ACE 2 overexpression reduces ROS formation identifying it as a new antioxidant[10]. In pathological states (ischemic heart disease, heart failure), however, ACE 2 activity has been found to be elevated in human subjects as well as rodent models, probably as a compensatory/protective mechanism to bring the increased Angiotensin II level back to normal[11].

The basic etiology of type 2 diabetes mellitus has been cited as hepatic insulin resistance which in turn is thought to be due to raised angiotensin II levels[1,2,12]. The ACE 2/Ang-(1-7)/Mas axis may diminish insulin resistance by its antioxidant influence as well as decreasing phosphoenolpyruvate carboxykinase (PEPCK) transcription[13]. Hyperglycemia stimulates angiotensin II, as well as, activates ADAM-17, leading to increased shedding of ACE 2[14]. Low ACE 2 level in the podocytes causes Ang II accumulation, worsening albuminuria and glomerular lesions as observed in studies on transgenic and non-transgenic diabetic mice[15]. High ACE 2 concentration within the podocytes degrades Ang II to Ang-(1-7), thereby shielding the kidney from the effects of increased Ang II, as well as generating a reno-protective end-product i.e. Ang-(1-7)[16]. Urinary ACE 2, which is an indicator of tubular damage, showed a strong correlation with albuminuria, suggesting its use as a biomarker to detect impending diabetic nephropathy[17]. Diabetes suppresses the protective arm of RAS besides enhancing the pressor arm which may be responsible for systemic hypertension[4,13].

A key micro-vascular complication of type 2 diabetes is diabetic nephropathy, heading to end-stage renal disease, which occurs despite utilization of various present day therapeutic regimes. A generally deemed sign of renal dysfunction and an initial forecaster of diabetic nephropathy, is microalbuminuria, outlined as a urinary albumin excretion between 30 and 300mg/day[1]. In spite of therapeutic intercession, kidney performance gradually deteriorates in numerous diabetics, highlighting the magnitude of innovative and disease-specific biomarkers. One possible candidate is ACE2, localized in the proximal tubule and glomerulus, which performs a fundamental task in minimizing renal impairment by counteracting the RAS.

The study aimed to demonstrate that ACE2 may be a valuable and reliable biomarker for renal dysfunction, especially associated with glucose intolerance in T2DM patients. As there is no current study available from Pakistan, pertaining to ACE2 and its role in diabetic nephropathy, the present study helps to highlight its importance and possible application in our setting. We hoped to highlight what part ACE2 deficiency performs in the pathogenesis of hypertension in diabetic patients. This, in turn, may open up new avenues for the development of anti-hypertensive drugs.

We used this study to measure and compare urine ACE 2 levels along with microalbuminuria in type 2 diabetic, hypertensive and non-diabetic, hypertensive patients. By obtaining higher urinary ACE2 levels ahead of microalbuminuria in diabetic-hypertensive subjects, as compared to non-diabetic-hypertensive patients, we hoped to prove that ACE 2 deficiency contributes to the development of hypertension in diabetic patients and can be used as an earlier marker of kidney damage. This in turn may open up new avenues for the development of antihypertensive drugs.

Received on 10-10-2021
Accepted on 21-03-2022

## MATERIALS AND METHODS

This Cross Sectional, Comparative study was conducted in the Department of Physiology, University of Health Sciences, Lahore, over a period of 1 year after permission from Ethical Review Committee. A total of 96 hypertensive subjects, between the ages of 30-60 were selected from the medical OPD and diabetic centers of SIMS. They were categorized into 2 groups; Group A including 46 non- diabetic and Group B comprising 49 diabetic, hypertensive patients. After obtaining written, informed consent from each participant, general and systemic examinations were conducted to rule out any underlying disease. Blood pressure was estimated by using sphygmomanometer. Body mass index was calculated with the help of formula; BMI=body weight (Kg)/ height($m^2$). Random blood sugar was determined on the spot. The levels of urinary ACE 2 were verified using enzyme-linked immunosorbent assay (ELISA) kit manufactured by Elabscience (USA). Micro-albumin levels in urine were determined using albumin RIA Kit manufactured by Beckman Coulter and Geiger counter.

**Statistical analysis:** The data was analyzed using the SPSS version 20. Mean±standard deviation (SD) is given for normally distributed quantitative variables and median±Interquartile range (IQR) is given for quantitative variables which were not distributed normally. Distribution of the data was checked by Shapiro-Wilk's statistics. A p-value <0.05 meant the data was not normally distributed. For normally distributed quantitative variables, student "t" test was applied to compare group means whereas for non-normally distributed quantitative variables, Mann-Whitney U test (non-parametric statistics) was used to compare means of variables of two groups. Pearson correlation (r) was employed to detect the correlation between quantitative variables which were normally distributed and Spearman's rho correlation (rho) to spot the correlation between quantitative variables not distributed normally. Lesser than 0.05 p-value was deemed statistically meaningful for all intents.

## RESULTS

As the data obtained for urinary ACE 2 levels was not distributed normally (as per Shapiro-Wilk's test), we determined the median value in each group. Median (IQR) was 26.47 (19.5-34.3) mg/dl for non-diabetic, hypertensive patients and 22.86 (16-28.2) mg/dl for diabetic, hypertensive patients. This difference of urinary ACE 2 levels was significant (Figure 1). Mean±SD microalbuminuria of non-diabetic, hypertensive subjects was 64.5±92.77 mg/dl and diabetic, hypertensive subjects was 55.4±71.56mg/dl (Tables 1, Figure 2). This difference of microalbuminuria was non-significant (Figure 3).

Figure 1: Comparison of Means of Urinary ACE 2 in Group A & Group B

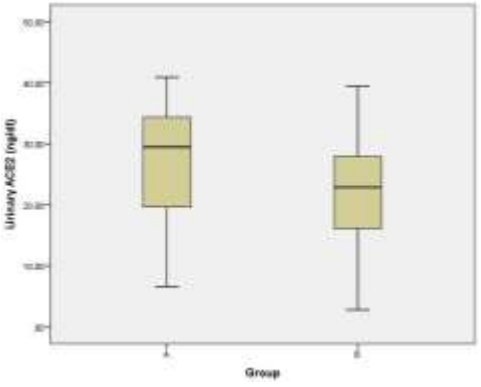


Figure 2: Comparison of means of microalbuminuria in Group A & Group B.

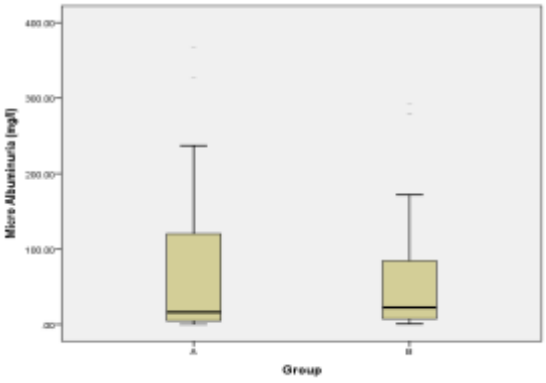


Figure3: Correlation between uACE2 and microalbuminuria.

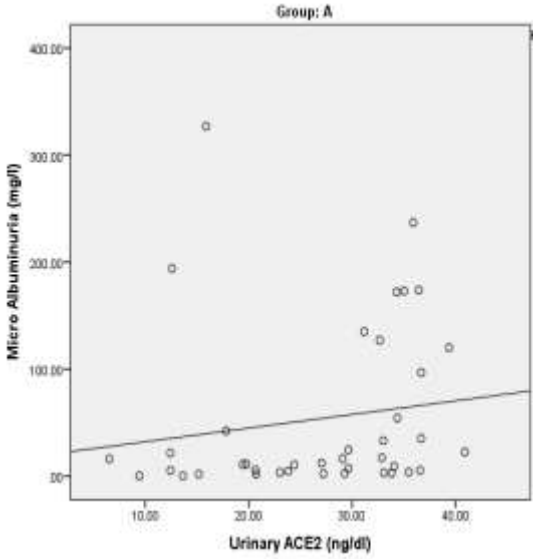


Table1: Data Distribution & Comparison of All Parameters in Group A & Group B.

| Parameters | Group A | Group B | p-value | *distribution |
|---|---|---|---|---|
| | Descriptive statistics** | Descriptive statistics** | | |
| Age (years) | 54.0 (45.0-60.0) | 55.0 (48.0-60.0) | 0.843 | Non-normal |
| Height (m) | 1.6±0.1 | 1.6±0.1 | 0.203 | Normal |
| Weight (kg) | 68.5 (62.0-74.0) | 75 (68-88.5) | 0.001 | Non-normal |
| Waist (cm) | 89.2±11.7 | 107.9±11.9 | 0.000 | Normal |
| Hip (cm) | 100.3±6.8 | 111.0±13.7 | 0.000 | Normal |
| BMI | 26.0 (24.9-28.1) | 30.4 (26.2-34.8) | 0.000 | Non-normal |

Biochemical Parameters

| | | | | |
|---|---|---|---|---|
| BSR (mg/dl) | 102.5 (98.0-133.5) | 200.0 (144.0-267.5) | 0.000 | Non-normal |
| sCr (mg/dl) | 1.0 (0.8-1.3) | 1.1 (0.8-1.4) | 0.306 | Non-normal |
| sVit. D (ng/dl) | 14.4 (10.2-20.9) | 15.8 (11.1-23.6) | 0.415 | Non-normal |
| uGlucose (mg/dl) | 0.0 | 50.0 (0.0-150.0) | **0.000** | Non-normal |
| uACE (ng/dl) | 0.7 (0.5-0.9) | 0.6 (0.5-0.9) | 0.440 | Non-normal |
| uACE2 (ng/dl) | 26.5 (19.5-34.3) | 22.9 (16.0-28.2) | **0.007** | Non-normal |
| uCr (mg/dl) | 4.0 (2.2-5.9) | 4.3 (2.9-7.1) | 0.440 | Non-normal |
| Microalbuminuria (mg/l) | 64.5±92.77 | 55.4±71.56 | 0.788 | Non-normal |
| GFR (ml/min) | 82.0±38.8 | 88.5±50.00 | 0.310 | Normal |

*value generated according to Shapiro Wilk Test
p-value ≤ 0.05 is considered statistically significant (Bold)
** Mean±SD for normally distributed, Median(IQR) for non-normally distributed Data.

Table 2: Correlation of urinary biochemical parameters with each other in group A.

| Parameters | | Urinary glucose | Urinary creatinine | Urinary ACE2 | Urinary ACE | Micro albuminuria |
|---|---|---|---|---|---|---|
| Urinary glucose | Rho | 1.000 | 0.035 | -0.201 | -0.161 | -0.255 |
| | p-value | | 0.820 | 0.213 | 0.308 | 0.09 |
| | N | 46 | 45 | 40 | 42 | 45 |
| Urinary creatinine | Rho | 0.035 | 1.000 | **0.398*** | -0.012 | 0.227 |
| | p-value | 0.820 | | **0.011** | 0.938 | 0.138 |
| | N | 45 | 45 | 40 | 42 | 44 |
| Urinary ACE2 | Rho | -0.201 | **0.398*** | 1.000 | 0.154 | **0.354*** |
| | p-value | 0.213 | **0.011** | | 0.342 | **0.025** |
| | N | 40 | 40 | 40 | 40 | 40 |
| Urinary ACE | Rho | -0.161 | -0.012 | 0.154 | 1.000 | 0.021 |
| | p-value | 0.308 | 0.938 | 0.342 | | 0.897 |
| | N | 42 | 42 | 40 | 42 | 42 |
| Micro albuminuria | Rho | -0.255 | 0.227 | 0.354* | 0.021 | 1.000 |
| | p-value | 0.09 | 0.138 | 0.025 | 0.897 | |
| | N | 45 | 44 | 40 | 42 | 43 |

*p less than 0.05 is significant (Bold)
Spearman rho correlation was applied to non-normally distributed data
Pearson's correlation was applied to normally distributed data

Table 1: Correlation of urinary biochemical parameters with each other in group B.

| Parameters | | Urinary glucose | Urinary creatinine | Urinary ACE2 | Urinary ACE | Micro albuminuria |
|---|---|---|---|---|---|---|
| Urinary glucose | Rho | 1.000 | -0.136 | -0.049 | **0.468*** | 0.071 |
| | p-value | | 0.362 | 0.768 | **0.002** | 0.641 |
| | N | 48 | 47 | 39 | 40 | 45 |
| Urinary creatinine | Rho | -0.136 | 1.000 | 0.028 | -0.292 | 0.166 |
| | p-value | 0.364 | | 0.866 | 0.071 | 0.282 |
| | N | 47 | 47 | 39 | 39 | 44 |
| Urinary ACE2 | Rho | -0.049 | 0.028 | 1.000 | -0.226 | 0.065 |
| | p-value | 0.768 | 0.866 | | 0.172 | 0.700 |
| | N | 39 | 39 | 39 | 38 | 38 |
| Urinary ACE | Rho | **0.468*** | -0.292 | -0.226 | 1.000 | 0.041 |
| | p-value | **0.002** | 0.071 | 0.172 | | 0.808 |
| | N | 40 | 39 | 38 | 40 | 38 |
| Micro albuminuria | Rho | 0.071 | 0.166 | 0.065 | 0.041 | 1.000 |
| | p-value | 0.641 | 0.282 | 0.700 | 0.808 | |
| | N | 45 | 44 | 38 | 38 | 38 |

*p less than 0.05 is significant (Bold)
Spearman rho correlation was applied to non-normally distributed data
Pearson's correlation was applied to normally distributed data

## DISCUSSION

Diabetes and hypertension are two of the chronic ailments whose incident rate is rising every day. According to WHO[19] , global prevalence of diabetes among adults over 18 years has risen from 4.7% in 1980 to 8.5% in 2014. In 2015, an estimated 1.6 million deaths were directly caused by diabetes. WHO (2016) expects diabetes will be the seventh leading cause of death in 2030.

One in three adults worldwide has raised blood pressure[20] (responsible for over half the deaths due to stroke and heart attack) was reported in the world health statistics, 2012[21], with evidence of dramatic increase in the conditions that trigger heart disease and other chronic illnesses[22], particularly in low and middle-income countries. Given the ever-rising incidence of diabetes and hypertension, along with their related morbidity and mortality, a lot of research is aimed at understanding the pathology of their onset, as well as, development of complications, identifying predisposing factors, discovering new biochemical markers of complications so as to prevent, diagnose and treat them more effectively[23].

As evident from the abundance of research, ACE 2 shifts the balance of the RAS towards the depressor limb, consequently preventing hypertension and playing a reno-protective role[1]. Hyperglycemia activates ADAM17 (which cleaves ACE 2 from renal tubules) directly, as well as indirectly by stimulating angiotensin-11[2],[3], leading to increased shedding of ACE2. Therefore, diabetes suppresses the protective limb of RAS, gearing towards development of hypertension. Keeping this in mind, we had proposed that the level of ACE 2 shed in the urine would be greater in patients with both diabetes and hypertension compared to those who only had hypertension based on various research studies which highlighted the role of ADAM-17 as a shedder of ACE from the nephron[14]. Contrary to our supposition, the mean urinary ACE II concentration in group A was significantly higher than in group B. Supporting our hypothesis but opposing our results, increasing ACE II concentration in the urine of diabetic, hypertensive individuals were observed in a recent study.[17] Although a few studies support our findings, our error in sampling may be responsible for these results[24]. The sampling for the present study was done in services Institute of Medical Sciences (SIMS), Lahore.

Although patients of both groups were demographically paired as possible as we could, we discovered a disparity in the quality of treatment and control of the patients. This was because group B subjects were selected from the diabetic center of SIMS whereas group A subjects were recruited from the medical OPD and emergency of SIMS. Unfortunately, at the time of sampling, we did not take into account the difference in medical care that both departments dispense. The diabetic center is a very well-organized, methodical, efficiently run department with a superb patients' counseling/education facility, as well as, an effective follow-up system. Although they entertain new, uncontrolled diabetic patients but predominantly, the patients are regular, well-controlled diabetics who come for follow-up also. Consequently, they fair well and had lesser complications than patients who visit the other departments of SIMS. In contrast, the medical OPD/emergency is not as well-organized and well-equipped and they handle a larger number of patients. Majority of patients are uneducated, misguided and poor patients who have hardly any knowledge regarding their medical condition let alone their treatment. Compliance is much less as compared to the patients coming to the diabetic center, as well as, follow-up visitation. Therefore, patients recruited from this setting were predominantly in a worse medical condition than those from the diabetic center. At the time of sampling and running the tests, we were not aware of this limitation. It was only during compilation of the results and retrograde analysis that we realized our unintentional fault in patient selection. Another fact that must be taken into account whilst interpreting the results is the disparity in treatment received by both patients. Most of the patients of group B were adequately treated, as well as mindful of their medication. On the other hand, most of the patients in group A were either receiving no treatment, non-medical treatment or unaware of their medication. Therefore, the difference in medical care and treatment received by both groups could be a major confounder in our study. Ideally, we should have recruited patients who were not under any medication and were fresh and visiting the clinics for the first time.

The patients in group B were well-treated and with an efficient follow-up, as compared to those in group A. Therefore, they were in lesser advanced stage of the disease with fewer complications. Most importantly, their renal health was better which could explain the lower levels of urinary ACE-2 in this group. According to our hypothesis, uncontrolled DM in group B patients should cause a greater loss of ACE-2 from the kidneys[1] but if the renal status is better than those in group A, this factor may over shadow the former. One of the aims of our study was to see whether uACE2 is a better biochemical marker for early renal damage. Our results showed no correlation between uACE2 and CKD staging in either of the groups. Nor was there any correlation between uACE2 levels and serum creatinine levels in either of the groups. Although we were unable to show that diabetes contributes towards the pathogenesis of hypertension by increasing the shedding of ACE 2 in urine, we did highlight the fact that there is a correlation between the levels of urinary ACE 2 and deteriorating renal status.

Microalbuminuria in group A was higher than in Group B (Table 1, Fig. 2). Yet again, the results were contradictory to our hypothesis. Diabetes mellitus is a chronic disease which damages various target organs especially the kidneys[25] . Microalbuminuria is one of the biomarkers used to identify early onset of diabetic nephropathy and plan intervention therapy. Besides diabetes, hypertension is a major risk factor for CKD[2]. Hypertension, in DM, significantly increases the incidence of renal damage, with resultant microalbuminuria. Therefore, we expected a higher level of micro-albuminuria in group B than in group A. True to this, majority research results demonstrate both DM and hypertension, to be major risk factors of renal damage, with a high level of microalbuminuria[2]. But the results obtained were contrary to our expectations. As for ACE-2, the most likely reason for this outcome is the error in our sampling. As previously explained patients of group B were well-treated and cared for, as compared to, those of group A. Consequently, they had less complications and a better renal status, as evidenced by CKD grading. The mean value of CKD in group A was 2.1 whereas it was 1.92 in group B, indicating a significantly better kidney health in group B. Although the patients in group B were compounded by two renal risk factors (hypertension and DM), they were well looked after in the diabetic center, with a strict medical control and therefore, delayed and less severe complications. Furthermore, the mean of sCreatinine in group A (1.5mg/dl) was higher than that in group B (1.2mg/dl), indicating a better renal profile in the latter. Both hypoglycemic and anti-hypertensive drugs have been found to have a beneficial effect on renal well-being[2]. As patients in group B were on a strict, regular treatment regime, targeting both hypertension and diabetes, they presented with lesser derangement of renal function, as evidenced by lower levels of serum creatinine and microalbuminuria (Table 1). On the other hand, although the hypertensive patients in group A were not diabetic, most of them were on no regular treatment and with hardly any follow-up plan. Many of them were oblivious of the regular need for anti-hypertensive therapy, with a very low compliance level, and those taking treatment were ignorant of what drugs they were taking. Progression of CKD in these patients was greater with resultant higher levels of proteinuria and serum creatinine. Keeping this in mind, it is easy to explain why our results did not fall in line with our predicted hypothesis. Microalbuminuria showed a positive correlation with serum creatinine in both groups. This was in accordance with majority of research studies according to which both serum creatinine and microalbuminuria are biomarkers for renal health. Serum creatinine showed a negative correlation with GFR in cases of group B[2], which falls in line with most of the research conducted[2]. There was a positive correlation of microalbuminuria with uACE-II as discussed earlier.

One of the aims of our study was to see whether uACE2 is a better biochemical marker for early renal damage. Our results showed no correlation between uACE2 and CKD staging in either of the groups. Nor was there any correlation between uACE2 levels and serum creatinine levels in either of the groups. On the other hand, there was a positive correlation of microalbuminuria with serum creatinine in both groups, as well as, with CKD staging in group B. In the light of our findings, it seems that microalbuminuria is a better indicator of CKD than uACE2. Although a significant variation in urinary ACE2 levels amongst subjects with CRD and healthy subject has been recorded earlier[3], it has not been proved to be a better indicator of CKD than microalbuminuria so far. Further research may do so in the future but unfortunately we were unable to in our work.

## CONCLUSION

We obtained results contradicting all of our assumptions. Although we may argue that difference in CKD staging and the difference in treatment and care received by the two groups may be responsible for this ambiguity, we were unable to demonstrate that ACE 2 may be a link between DM and hypertension. Similarly, we could not ascertain that urinary ACE 2 levels are a better indicator of renal damage as compared to microalbuminuria. However, we were able to show a direct relationship between the levels of urinary ACE 2 and deteriorating renal status.

**Conflict of interest**: Nil

## REFERENCES


1. Henriksen EJ. Improvement of insulin sensitivity by antagonism of the renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol. 2007; 293(3):R974-80.
2. Perkins JM, Davis SN. The renin-angiotensin-aldosterone system: a pivotal role in insulin sensitivity and glycemic control. Curr Opin Endocrinol Diabetes Obes. 2008; 15(2):147-52.
3. Sarzani R, Salvi F, Dessi-Fulgheri P, Rappelli A. Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: an integrated view in humans. J Hypertens. 2008; 26(5):831-43.
4. Coppo M, Bandinelli M, Chiostri M, Modesti PA, Poggesi L, Boddi M. T Cell-Based RAS Activity and Insulin Levels in Obese Subjects with Low Grade Inflammation. Am J Med Sci. 2021 Sep 24:S0002-9629(21)00327-X. doi: 10.1016/j.amjms.2021.09.003. Epub ahead of print. PMID: 34571038.
5. Padda RS, Shi Y, Lo CS, Zhang SL, Chan JS. Angiotensin-(1-7): A Novel Peptide to Treat Hypertension and Nephropathy in Diabetes? J Diabetes Metab. 2015; 6(10).
6. Nomura H, Kuruppu S, Rajapakse NW. Stimulation of Angiotensin Converting Enzyme 2: A Novel Treatment Strategy for Diabetic Nephropathy. Front Physiol. 2022 Jan 11;12:813012. doi: 10.3389/fphys.2021.813012. PMID: 35087423; PMCID: PMC8787214.
7. Xiao F, Hiremath S, Knoll G, Zimpelmann J, Srivaratharajah K, Jadhav D, et al. Increased urinary angiotensin-converting enzyme 2 in renal transplant patients with diabetes. PLoS One. 2012; 7(5):e37649.
8. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-α convertase (ADAM-17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE 2). J Biol Chem. 2005; 280(34):30113-9.
9. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, et al. Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. Nat Med. 2005; 11(8):867-74.

10. Zheng J, Li G, Chen S, Bihl J, Buck J, Zhu Y, et al. Activation of the ACE 2/Ang-(1-7)/Mas pathway reduces oxygen-glucose deprivation-induced tissue swelling, ROS production, and cell death in mouse brain with angiotensin II overproduction. Neuroscience. 2014; 273:39-51.
11. Xu H, Du S, Fang B, Li C, Jia X, Zheng S, Wang S, Li Q, Su W, Wang N, Zheng F, Chen L, Zhang X, Gustafsson JÅ, Guan Y. VSMC-specific EP4 deletion exacerbates angiotensin II-induced aortic dissection by increasing vascular inflammation and blood pressure. Proc Natl Acad Sci U S A. 2019 Apr 23;116(17):8457-8462. doi: 10.1073/pnas.1902119116. Epub 2019 Apr 4. PMID: 30948641; PMCID: PMC6486760.
12. de Queiroz TM, Xia H, Filipeanu CM, Braga VA, Lazartigues E. alpha-Lipoic acid reduces neurogenic hypertension by blunting oxidative stress-mediated increase in ADAM-17. Am J Physiol Heart Circ Physiol. 2015; 309(5):H926-34.
13. Wu M, Wang X, Duan Q, Lu T. Arachidonic acid can significantly prevent early insulin resistance induced by a high-fat diet. Ann Nutr Metab. 2007;51(3):270-6. doi: 10.1159/000105448. Epub 2007 Jul 9. PMID: 17622786.
14. Reddy AB, Ramana KV, Srivastava S, Bhatnagar A, Srivastava SK. Aldose reductase regulates high glucose-induced ectodomain shedding of tumor necrosis factor (TNF)-alpha via protein kinase C-delta and TNF-alpha converting enzyme in vascular smooth muscle cells. Endocrinology. 2009 Jan;150(1):63-74. doi: 10.1210/en.2008-0677. Epub 2008 Sep 4. PMID: 18772236; PMCID: PMC2630901.
15. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. 2006 Feb;116(2):288-96. doi: 10.1172/JCI27699. PMID: 16453013; PMCID: PMC1359063.
16. Ortiz-Melo DI, Gurley SB. Angiotensin converting enzyme 2 and the kidney. Curr Opin Nephrol Hypertens. 2016 Jan;25(1):59-66. doi: 10.1097/MNH.0000000000000182. PMID: 26575394.
17. Park SE, Kim WJ, Park SW, Park JW, Lee N, Park CY, Youn BS. High urinary ACE2 concentrations are associated with severity of glucose intolerance and microalbuminuria. Eur J Endocrinol. 2013 Jan 17;168(2):203-10. doi: 10.1530/EJE-12-0782. PMID: 23144053.
18. Gall MA, Hougaard P, Borch-Johnsen K, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. BMJ. 1997 Mar 15;314(7083):783-8. doi: 10.1136/bmj.314.7083.783. PMID: 9080995; PMCID: PMC2126209.
19. Organization, W. H. 2016. Global report on diabetes, World Health Organization.
20. van de Vijver S, Oti S, Addo J, de Graft-Aikins A, Agyemang C. Review of community-based interventions for prevention of cardiovascular diseases in low- and middle-income countries. Ethn Health. 2012;17(6):651-76. doi: 10.1080/13557858.2012.754409. Epub 2013 Jan 9. PMID: 23297746.
21. Organization, W. H. 2012. World health statistics: a snapshot of global health.
22. Martin, J. 2008. Hypertension guidelines: revisiting the JNC 7 recommendations. The Journal of Lancaster General Hospital, 3(3).
23. Liu D, Chen Y, Zhang P, Zhong J, Jin L, Zhang C, Lin S, Wu S, Yu H. Association between circulating levels of ACE2-Ang-(1-7)-MAS axis and ACE2 gene polymorphisms in hypertensive patients. Medicine (Baltimore). 2016 Jun;95(24):e3876. doi: 10.1097/MD.0000000000003876. Erratum in: Medicine (Baltimore). 2016 Aug 07;95(31):e5074. PMID: 27310975; PMCID: PMC4998461.
24. Patel SK, Velkoska E, Freeman M, Wai B, Lancefield TF, Burrell LM. From gene to protein-experimental and clinical studies of ACE2 in blood pressure control and arterial hypertension. Front Physiol. 2014 Jun 24;5:227. doi: 10.3389/fphys.2014.00227. PMID: 25009501; PMCID: PMC4067757.
25. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. 2006 Feb;116(2):288-96. doi: 10.1172/JCI27699. PMID: 16453013; PMCID: PMC1359063.
26. Fox CS, Larson MG, Leip EP, Culleton B, Wilson PW, Levy D. Predictors of new-onset kidney disease in a community-based population. JAMA. 2004 Feb 18;291(7):844-50. doi: 10.1001/jama.291.7.844. PMID: 14970063.
27. Nowak N, Skupien J, Smiles AM, Yamanouchi M, Niewczas MA, Galecki AT, Duffin KL, Breyer MD, Pullen N, Bonventre JV, Krolewski AS. Markers of early progressive renal decline in type 2 diabetes suggest different implications for etiological studies and prognostic tests development. Kidney Int. 2018 May;93(5):1198-1206. doi: 10.1016/j.kint.2017.11.024. Epub 2018 Feb 2. PMID: 29398132; PMCID: PMC5911430.
28. Yoo TH, Li JJ, Kim JJ, Jung DS, Kwak SJ, Ryu DR, Choi HY, Kim JS, Kim HJ, Han SH, Lee JE, Han DS, Kang SW. Activation of the renin-angiotensin system within podocytes in diabetes. Kidney Int. 2007 May;71(10):1019-27. doi: 10.1038/sj.ki.5002195. Epub 2007 Mar 14. PMID: 17361112.
29. Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. Am J Kidney Dis. 2014 May;63(5):820-34. doi: 10.1053/j.ajkd.2013.12.006. Epub 2014 Jan 28. PMID: 24485147; PMCID: PMC4001724.
30. Mizuiri S, Ohashi Y. ACE and ACE2 in kidney disease. World J Nephrol. 2015 Feb 6;4(1):74-82. doi: 10.5527/wjn.v4.i1.74. PMID: 25664248; PMCID: PMC4317630.