Genetic Polymorphism of Angiotensin Converting Enzyme and the Angiotensin II Type1 Receptor as Risk Factors in Patients with Chronic Kidney Disease

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Abstract: **Background:** The rennin angiotensin system (RAS) is central to the pathogenesis of hypertension, cardiovascular disease and kidney disease. Angiotensin converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R) are two of the main components of the RAS. The genetic polymorphisms of these key components provide a basis for studying the relationship between genetic variants and the development of vascular or renal damage in individual subject. This work aimed to study the distribution of I/D polymorphism of ACE and AT1R polymorphism in chronic kidney disease (CKD) patients and their association with other clinical and laboratory variables in these patients. **Methods:** Gene polymorphisms were studied in 64 CKD patients (40 on hemodialysis "HD" & 24 on conservative treatment "CT") and 20 healthy controls using PCR amplification for ACE gene and PCR-RFLP technique for AT1R gene. Serum ACE activity, kidney functions and lipid profile were measured in all the studied groups. **Results:** Higher frequency of D allele of ACE gene was observed in CKD (both on HD or CT) patients than healthy controls (p<0.0001 & p<0.01 respectively) with higher distribution in HD patients than those on CT (p <0.05). HD group had higher frequency of DD genotype compared to controls (p<0.01). The I allele and II genotype showed higher distribution in healthy controls than CKD patients (HD and CT) (p<0.0001 and p<0.01 respectively). Patients on CT had higher frequency of I allele and II genotype when compared to those on HD (p<0.05 for both). The C allele of AT1R showed higher frequency in HD group in comparison with controls (p<0.05) but the A allele showed lower frequency in the HD patients compared to controls (p<0.05). No significant difference was found in comparing the frequency of AA, AC or CC genotypes between the studied groups. The number of hypertensive patients was higher in patients carrying DD genotype than those carrying II and ID genotype (p<0.05). Estimated glomerular filtration rate (e-GFR) was lower in AC and CC genotype carriers than AA genotype carriers (p<0.05). Study of the frequency of combined genotypes revealed that ACE-ID+AT1R-AA is the most frequent genotype combination in CKD patients (40.6%) and ACE-II+AT1R-AA is the most frequent combination in controls (60%). **Conclusion:** The D allele of ACE gene and the C allele of AT1R are important genetic determinants in CKD. They are more frequent in HD patients than those on CT. Patients carrying these alleles have higher ACE activity and more prone to hypertension. They also have more decline in kidney function as evidenced by lower values of e-GFR. They can be considered as risk alleles.

[Amal M Abdel-Aal, Elham O. Hamed , Sahar Abou el- Fetouh, Abeer Fakher El-Deen , Nayel A Zaky , Medhat M Ali and Yasser M Kamal **Genetic Polymorphism of Angiotensin Converting Enzyme and the Angiotensin II Type1 Receptor as Risk Factors in Patients with Chronic Kidney Disease** Life Sci J 2013; 10(2):874-881]. (ISSN: 1097-8135). http://www.lifesciencesite.com 123

Keywords: Chronic renal failure, angiotensin converting enzyme, angiotensin II type one receptor gene

1. Introduction:

Chronic kidney disease is a complex disorder encompassing a large variety of phenotypes. The complexity of the phenotypic makeup of renal diseases makes it difficult to diagnose and predict their progression and to decide on the optimal treatment for each patient (Elshamaa *et al.*, 2011). End stage renal disease (ESRD) is one of the most important causes of premature death among patients and a major health concern. Any kind of kidney disease tends to progress to ESRD rapidly over time (Anbazhagan *et al.*, 2009). The last ten years have witnessed an increase in the number of patients entering renal replacement therapy due to end stage renal disease. ESRD is a multifactorial disease, clinically; it is an advanced form of chronic renal failure where renal functions decline to 10% of the normal prior to the initiation of dialysis or transplantation time (Tripathi *et al.*, 2008). The impact of genetic variability on the development of renal failure is becoming clearer and emphasizes the need to elucidate the genetic basis for renal diseases and its complications (Elshamaa *et al.*, 2011).

Hypertension is the main contributor in the progression of renal failure and a major risk factor for the development of ESRD (Anbazhagan *et al.*, 2009). It is a common, polygenic and complex disorder which may be due to the interaction of several genes

with each other and also with the environmental factors (Basset *et al.*, 2002). Hypertension is about twice as common in subjects who have one or two hypertensive parents, and many epidemiological studies suggested that genetic factors account for approximately 30% of the variation in blood pressure in various populations (Levey, 2002). The renin angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and may play a role in their interaction (Saggar *et al.*, 2000; Tripathi *et al.*, 2006). The circulating RAS is an important pathway that regulates blood pressure and electrolyte balance (Grill *et al.*, 2009). In low blood pressure and low circulating sodium chloride, the renin enzyme synthesized by juxtaglomerular cells of the afferent renal arterioles of kidney and released in blood. Renin cleaves angiotensinogen, to angiotensin-1 which is cleaved by angiotensin converting enzyme to the biologically active angiotensin-II. Two isoforms of angiotensin receptors exist in different tissues: AT1 and AT2. They are from seven transmembrane G-protein coupled receptor family. Angiotensin-II exerts most of its effects via the activation of AT1 receptors which are expressed in vascular smooth muscle cells and adrenal glands, among others. This receptor is coupled to the Gq protein to increase intracellular calcium (Irani and Xia, 2008).

Among the candidate genes of the RAS, the angiotensin converting enzyme (ACE) and angiotensin II type I receptor (AT1RA^{1166}C) genes seem to be particularly biologically and clinically relevant to renal disease. The angiotensin converting enzyme is a dipeptidyl that is encoded by the ACE gene. This gene is located on chromosome 17q23 and contains 26 exons and 25 introns (Sayed *et al.*, 2006). ACE gene is subjected to an insertion/deletion (I/D) polymorphism that is a common determinant of plasma and tissue ACE levels (Elshamaa *et al.*, 2011).

Studying the polymorphism in these genes will give a wide knowledge about a patient's disease progression and this will help in designing better treatment options (Anbazhagan *et al.*, 2009). So, this work aimed to study the distribution of the I/D polymorphism of ACE and AT1RA^{1166}C polymorphism of ACE receptor genes in CKD patients on hemodialysis and on conservative treatment. Also, the association between these gene polymorphisms and other clinical and laboratory variables in CKD patients was studied.

2. Subjects and methods:

Subjects and study design:

The study included 64 (42 males and 22 females) CKD patients, who consecutively were selected from hemodialysis unit and nephrology clinic at Sohag University Hospital from March 2012 to July 2012. Their ages ranged from 40-70 years (55±12 years). Also, 20 apparently healthy subjects were included as a control group (12 males and 8 females); they were selected to be matched for age and gender to the patient groups, as well as within the same body mass index limits. They had no clinical signs of vascular or renal disease and no family history of renal disease as assessed by medical history and clinical examination. Their ages ranged from 45-68 years (49±15years). The study conducted through cooperation between Clinical Pathology department, Sohag University and Clinical Pathology department, Assiut University. It was approved by the faculty committee for research ethics. Patients who participated in this study gave informed consent.

Patients were assigned to 2 groups based on estimated glomerular filtration rate according to the National Kidney Foundation classification:

1-Patients with advanced CKD (stage IV) (n=40): They were treated with hemodialysis for 3-4 hrs three times weekly with a polysulfone membrane using bicarbonate buffered dialysate. The Duration of hemodialysis was 4.37±3.13 years. The causes of renal failure were: diabetic nephropathy (n=13), hypertensive nephropathy (n=12), glomerulopathy (n=6), renal hypoplasia (n=2), and unknown causes (n=7). They were diagnosed on the basis of ultrasound, CT scan and renal biopsy.

2- Patients with CKD (stages II &III) (n=24): They undergo conservative treatment and were not recommended for hemodialysis. The causes of CKD were renal hypertensive nephropathy (n=14), glomerulopathy (n=4), renal cyst (n=2) and unknown (n=4).

Methods:

Venous blood sample and urine sample were collected in the morning after an overnight fast on a midweek dialysis day, before the dialysis session. Five ml of venous blood sample was collected; one ml on EDTA coated tubes used for the extraction of genomic DNA, and four ml were centrifuged for serum separation which was stored in aliquots at -20◦C. Serum glucose, kidney function tests (including s.urea, s.creatinine, s.uric acid and albumin/creatinine ratio) and complete lipid profile estimations were determined by autoanalyzer Cobas c 311 (Roche/Hitachi cobas c systems). Estimated glomerular filtration rate were done by Modification of Diet in Renal Disease (MDRD) equation: MDRD = 186× s.creatinine (mg/dL) $^{-1.154}$ × age (years)$^{-0.203}$ ×0.742 (if female). Body mass index (BMI, kg/m^2) was calculated. The detection of ACE activity in serum was done by a kinetic colorimetric determination via FAPGG (N-[3-(2-furyl) acryloyl]-

L-phenyl alanyl glycyl glycine) method. The ACE presented in the serum catalyzes the hydrolysis of the FAPGG; forming furyl acryloyl phenylalanine (FAP). The decrease of the absorbance in the unit time at 340 nm is proportional to the activity of the ACE in the serum.

Determination of ACE I/D and AT1RA^{1166}C polymorphism genotypes:

Genomic DNA was extracted from 200 µl whole blood by protease digestion using QIAamp blood mini kit (QIAGEN, Germany). The D and I alleles of ACE gene were identified on the basis of polymerase chain reaction (PCR) amplification of a fragment of intron 16 of the ACE gene (Lindpaintner *et al.,* 1995). DNA was amplified with specific primers: 5′ GCCCTGCAGGTGTCTGCAGCATGT 3′ (forward) and 5′ GGATGGCTCTCCCCGCCTTGTCTC 3′ (reverse). The 25 µl reaction mixture contained PuRe Taq Ready to go PCR beads supplied by GE health Care Kit, 50 pmol of each primer and 200 ng of genomic DNA. The thermal cycling conditions included 30 amplification cycles with the following temperature profile: 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. Amplification products corresponding to D and I alleles (319 bp and 597 bp, respectively) were visualized under UV light after electrophoresis on ethidium-bromide stained 2% agarose gel. Hence, single bands of 319 or 597 bp revealed homozygous DD and II genotypic state respectively, whereas two bands of 319 and 597 bp confirmed heterozygous ID genotype. All samples yielding amplification of the D allele (and therefore potentially typed as DD) were subjected to a second independent amplification using primers specific for the sequence inserted in intron 16 of the I allele. The samples were amplified under the same conditions as described previously and a temperature profile of 94°C, 67°C, and 72°C for one minute each. The primer sequences are forward 5′-TGGGACCACAGCGCCCGCCACTAC-3′, and reverse, 5′-TCGCCAGCCCTCCCATGCCCATAA-3′, which result in an amplification product of 335 bp only in the presence of an I allele and no product in homozygous samples for DD.

- For detection of A^{1166}C polymorphism of the angiotensin II receptor AT1, genotyping was done using PCR-RFLP assay. Two oligonucleotide primers (forward) 5′-AAT GCT TGT AGC CAA AGT CAC CT-3′ and (reverse) 5′-GGC TTT GCT TTG TCT TGT TG-3′ were used for amplification the corresponding DNA fragment. The reaction was performed in a 25-μL final volume and contained 50 pmol of each primer and 250 ng of genomic DNA in addition to PuRe Taq Ready to go PCR beads supplied by GE health Care Kit. Amplification was done according to the following protocol: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 1 min and 30 sec; and final extension at 72°C for 5 min. PCR products (856 bp) were digested with 2 µ BstDEI restriction enzyme (Sib Enzyme) in a total 15 µl volume for 2 hrs at 60°C. The wild-type allele (A allele) has one cleavage site and digested to 600 and 256 bp fragments, whereas the mutant allele (C allele) has two cleavage sites and 256 bp fragment is cleaved to 146 and 110 bp fragments too. Thus, the homozygote CC produces three bands (600, 146 and 110 bp), the homozygote AA produces two bands (600 and 256 bp), and the heterozygote produces all four bands. Digested samples were separated by electrophoresis on a 2% agarose gel and visualized under UV light by ethidium bromide staining.

Statistical analysis:

Statistical analyses were performed using SPSS version 13. Data were summarized as mean ± SD, range or percentage. Allele and genotypic frequency was calculated by direct gene counting method. Comparison of the different genotypes was done by using Chi square test. Odd's ratios were calculated with a 95% confidence interval limit. Clinical characteristics of CKD patients with different ACE and AT1R genotypes were compared using independent t test. *P*-value <0.05 was considered significant.

3. Results:

ACE I/D polymorphism was assessed and is represented in Fig. 1, with the 597-bp band indicating II genotype, 597 and 319 bp indicating ID genotype, and 319 bp indicating DD genotype. AT1R polymorphism is represented in Fig. 2, with the 600 and 256 bp band indicating AA genotype, and 600, 256, 146 and 110 bp indicating AC genotype, and 600, 140 and 110 bp indicating CC genotype.

Clinical characteristics and laboratory variables of the studied groups were demonstrated in Table 1. Comparison of systolic and diastolic blood pressure revealed a highly significant difference between patients and controls (p<0.01). Serum urea, creatinine, uric acid and glucose levels showed statistically significant elevation in patient group compared to control group (p<0.01). Albumin/creatinine ratio was higher in the patient group than the control group. In contrast, estimated glomerular filtration rate (e-GFR) was significantly lower in the patient group when compared to controls. Lipid profile showed significant elevation of serum triglycerides and reduction of HDL levels in patients in comparison with controls, but no statistically significant difference was found between

the two groups as regarding cholesterol and LDL levels. ACE activity showed significant elevation in CKD patients in comparison to controls ($p<0.05$).

Distribution of genotype and allele frequency of ACE and AT1R gene polymorphisms in both groups of patients (HD &CT) and control group is shown in table 2. We found higher frequency of the D allele of ACE gene in both HD and CT patients compared to controls (70% and 52.1% versus 25%, $p<0.0001$ & $p<0.01$ respectively). In comparing patient groups, significant higher frequency of the D allele was found in patients on HD than patients on CT ($p<0.05$). In contrast, we found lower frequency of the I allele of ACE gene in both patient groups when compared to controls (30% and 47.9% vs 75%, $p<0.0001$ & $p<0.01$ respectively). Moreover, HD patients showed lower frequency of the I allele compared to patients on CT ($P<0.05$). As regarding genotypes of ACE, we found higher frequency of DD genotype in both patient groups compared to controls but this difference was statistically significant only in HD group (42.5% vs 10%, $p<0.01$). In comparing patient groups we didn't find significant difference. The heterozygous DI genotype frequency didn't show significant difference between the studied groups. But, the homozygous II genotype frequency was lower in both patient groups (HD and CT) compared to control group (2.5%, 16.7% vs 60% & $p<0.0001$, $p<0.01$). Patient on HD had lower frequency of II genotype than those on CT ($P<0.05$).

Study of the polymorphism of angiotensin type 1 receptor polymorphism revealed high frequency of the C allele in both patient groups when compared to controls (22.5% and 16.6% versus 7.5%), but this difference was statistically significant only in HD group ($p<0.05$). The A allele was present in higher frequency in control group than both patient groups (92.5% versus 77.5% and 83.4%), but this difference was statistically significant only in HD group ($p<0.05$). the frequency of both the C and I alleles didn't show significant difference when comparing patients on HD and on CT. However, the frequency of genotypes (CC, AC & AA) didn't show significant difference in comparing the studied groups.

We studied the effect of gene polymorphism on different clinical and laboratory parameters in patients with CKD. In ACE I/D polymorphism, due to the small number of patients with II genotype, ID and II genotypes were pooled as one group for comparison with DD genotype carriers. Similarly, in AT1A^{1166}C polymorphism, AC and CC genotypes were pooled due to the small number of patient with the CC genotype for comparison with AA genotype carriers. We found that; DD genotype carriers had higher frequency of hypertensive patients than II &ID genotype carriers (72.7% versus 45.2%, $p<0.05$). In contrast, the percent of hypertensive patients didn't show significant diffirence when comparing AA genotype carriers with those carrying AC or CC genotype (53.4% versus 57.1%). Estimated glomerular filtration rate show significant reduction in patients carrying the AC and CC genotype when compared with those carrying AA genotype (11.3±2.1 versus 16±2 ml/min, $p<0.05$). but, no statistical significant difference was found in e-GFR between DD genotype carriers and patients carrying II and ID genotype (13.3±3.5 versus 15.2±1.6 ml/min). Determination of the activity of ACE revealed significant elevation in DD genotype carriers in comparison with II and ID genotype carriers (60±35.2 versus 39±22.1 IU/l), $P<0.05$). Moreover, ACE activity was higher in AC and CC genotype carriers than AA genotype carriers (67±35.9 versus 47±31.1 IU/l, $p<0.05$).

We also studied the frequency of combination between genotypes of both ACE I/D polymorphism and AT1RA^{1166}C polymorphism in both patients and controls (Table 3). We found that the combined genotype ACE-ID + AT1R-AA had the highest frequency in the studied CKD patients (40.6%). In addition, the combined genotype ACE-II + AT1R-AA had the highest frequency in the control group (60%).

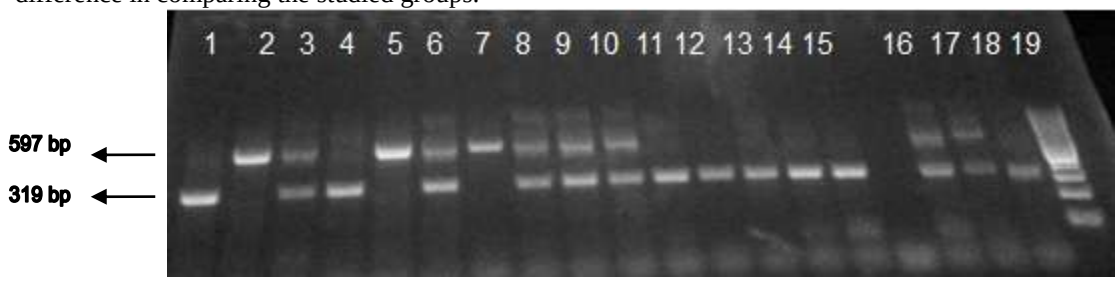

Figure (1): Determination of ACE I/D polymorphism by PCR Amplification. Lanes 1, 4, 11, 12, 13, 14, 15 and 18 - DD (319 bp), Lanes 2, 5 and 7- II (597 bp), Lanes 3, 6, 8, 9, 10, 16 and 17- ID (319 bp and 597 bp), 19-DNA ladder.

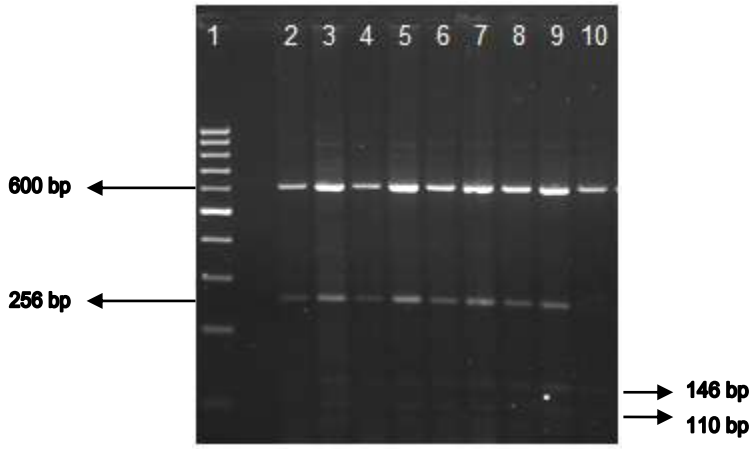

Figure (2): Determination of AT1RA[1166]C polymorphism by PCR-RFLP. Lane 1- 100 bp ladder, Lane 2 -AA (600 and 256 bp), Lanes 3, 4, 5, 6, 7, 8 and 9- AC (600, 256, 146 and 110 bp), Lane 10- CC (600, 146 and 110 bp).

Table 1: Clinical and laboratory variables of studied groups

	All patients (n=64)	Controls (n=20)	*p*-values
Age (range)	40-70 (55±12)	45-68 (49±15)	-
Males %	65.6	60	-
DM %	20.3	-	-
Hypertension %	54.6	-	-
Systolic BP (mm Hg)	159±28.3	120.1±6.1	< 0.01
Diastolic BP (mm Hg)	91±6.3	78.7±4.9	< 0.01
BMI (kg/m^2)	23.3± 4.4	22.9±4.1	Ns
Cholesterol (mg/dl)	160.4±34.3	160±22	Ns
HDL-C (mg/dl)	36.9±10.3	45.8±5.1	< 0.05
LDL-C (mg/dl)	95.3±19	95.7±24	Ns
Triglyceride (mg/dl)	166.3±74.2	150.9±35.9	< 0.05
S.glucose (mg/dl)	106±35	84.9±12.1	< 0.01
S.creatinine (mg/dl)	7.6±4.4	0.67±0.18	< 0.01
S.urea (mg/dl)	149.2±50.6	20.5±5.9	< 0.01
S.uric acid (mg/dl)	7.1±1.6	4.6±1.3	< 0.01
Alb/creat. ratio	1.6±2.3	0.006±0.005	< 0.01
GFR, mL/min/1.73 m^2	18.7±3.16	82±5.9	< 0.01
ACE activity (IU/L)	50.2±33.1	35.1±10.5	< 0.05

NS: non significant p >0.05

Table 2: Distribution of genotype and allele frequency between patients group

Genotype	Hemodialysis n=40	Conservative n=24	Controls n=20	*p*-value	OR (95% CI)*	*p*-value	OR (95% CI)**
ACE-I/D polymorphism Allele frequency							
I	24 (30%)	23 (47.9%)	30 (75%)	<0.0001	0.14 (0.06-0.34)	<0.01	0.31 (0.12-0.76)
D	56 (70%)	25 (52.1%)	10 (25%)	<0.0001	7 (2.95-16.55)	<0.01	3.26 (1.30-8.12)
Genotype frequency							
II	1 (2.5%)	4 (16.7%)	12 (60%)	<0.0001	0.02 (0.00-0.15)	<0.01	0.13 (0.03-0.54)
ID	22 (55%)	15 (62.5%)	6 (30%)	NS	2.85 (0.91-8.93)	NS	3.89 (1.10-13.76)
DD	17 (42.5%)	5 (20.8%)	2 (10%)	<0.01	6.65 (1.36-32.61)	NS	2.37 (0.41-13.79)
Angiotensin II type one receptor Allele frequency							
A	62 (77.5%)	40 (83.4%)	37 (92.5%)	<0.05	0.28 (0.08-1.01)	NS	0.41 (0.10-1.64)
C	18 (22.5%)	8 (16.6%)	3 (7.5%)	<0.05	3.58 (0.99-12.98)	NS	2.47 (0.61-10.00)
Genotype frequency							
AA	26 (65%)	17 (70.9%)	17 (85%)	NS	0.33 (0.08-1.31)	NS	0.43 (0.09-1.94)
AC	10 (25%)	6 (25%)	3 (15%)	NS	1.89 (0.46-7.82)	NS	1.89 (0.41-8.78)
CC	4 (10%)	1 (4.1%)	0 (0%)	NS	Can't calculated	NS	Can't calculated

* Comparison between Hemodialysis & control groups **Comparison between Conservative & control groups

Table 3: Distribution of combined genotypes

Genotypes	Patients (n=64)	Controls (n=20)
ACE-DD+ AT1R-CC	2 (3.1%)	0%
ACE-DD+ AT1R-AC	5 (7.8%)	0%
ACE-DD+ AT1R-AA	15 (23.4%)	2 (10%)
ACE-DI+ AT1R-CC	3 (4.7%)	0%
ACE-DI+ AT1R-AC	8 (12.5%)	3 (15%)
ACE-DI+ AT1R-AA	26 (40.6%)	3 (15%)
ACE-II+ AT1R-CC	0%	0%
ACE-II+ AT1R-AC	3 (4.7%)	0%
ACE-II+ AT1R-AA	2 (3.1%)	12 (60%)

4. Discussion:

The impact of genetic variability on the development of renal failure is becoming clearer and emphasizes the need to elucidate the genetic basis for renal disease and its complications (Elshamaa *et al.,* 2011). Progression of renal disease results from the interaction of multiple environmental and genetic factors. Several studies had shown a relationship between genetic variants of the renin-angiotensin system genes and renal diseases as well as the rate of progression of renal damage (Nakayama *et al.,* 2009). Angiotensin-converting enzyme, a major enzyme of the renin-angiotensin-aldosterone system, exhibits genetic polymorphisms affecting its concentration in blood (Rigat et al. *et al.,* 1990). Accordingly, the insertion/deletion (I/D) polymorphism has been intensively studied in cardiovascular, nephrologic, and renal transplantation populations (Mallamaci *et al.,* 2000; Samuelsson *et al.,* 2000; Tripathi *et al.,* 2008; Akman *et al.,* 2009). It is associated with progression of glomerulonephritis, polycystic kidney disease, diabetic nephropathy, coronary heart disease, arterial hypertension, and renal transplant dysfunction (Siekierka *et al.,* 2009).

In this work, we studied the association between polymorphisms of the angiotensin converting enzyme-1 and angiotensin II type 1 receptor genes, and chronic kidney disease at different stages. We also studied the effect of these polymorphisms on risk parameters in these patients.

This study revealed higher frequency of the D allele and DD genotype of ACE gene in patients with CKD both on hemodialysis and on conservative treatment in comparison with the control group. This difference suggest that ACE gene polymorphism is an important genetic determinant of CKD in Egyptian patients and that the D allele of ACE gene might confer a high risk of developing renal diseases and this association was highly validated when D allele was present in homozygous state.. However, the homozygous II genotype frequency was higher in controls than CKD patients suggesting a protective role for this genotype. Moreover, the present study found higher frequency of the D allele in HD patients when compared to those taking CT. Study of AT1RA^{1166}C polymorphism revealed higher frequency of the C allele in HD patients in comparison with control group. These findings suggest an association between the D allele of ACE gene and the C allele of AT1R gene, and advanced stage of CKD. Previous studies also found higher frequency of the D allele and C allele in CKD patients especially those on HD so, they can be considered as risk alleles (Tripathi *et al.,* 2008; Elshamaa *et al.,* 2011). It was previously reported that DD genotype of ACE gene may be associated with development of diabetic nephropathy among Egyptian patients (El-Bazz *et al.,* 2011; Badr *et al.,* 2012). Another study reported that idiopathic nephrotic syndrome is associated with a higher incidence of DD genotype, especially in non-steroid sensitive patients and DD genotype may play a role in the clinical response to steroid (Fahmy *et al.,* 2008).

RAS is known for its regulation and maintenance of salt balance, systemic and glomerular blood pressure. The hyperactivation of this system leads to a increase in systemic and glomerular blood pressure followed by fibrosis and progressive loss of renal function. Various factors are involved in deregulation of RAS. Polymorphisms in the RAS genes are the most common factor that is responsible for increased RAS activity resulting in hypertension (Anbazhagan *et al.,* 2009). In this study, patients having the homozygous DD genotype had higher frequency of hypertension than those having DI or II genotypes. Tripathi *et al.* (2008) postulated that ACE-DD genotype confers a greater role in hyperactive state and this phenomenon could be one of the major causes behind the association of ACE-DD genotypes with impaired renal function. While an association between hypertension and ACE gene polymorphism has not been found in the general population, in some particular conditions, such as malignant hypertension, the D allele has been shown to be a significant risk factor (Stefansson *et al.,*

2000). In dialysis patients, blood pressure can be controlled by sodium and fluid removal. Carriers of the D allele seem to be less sensitive to sodium state than the I allele carriers and could therefore be less responsive to sodium removal by ultrafiltration in dialysis (Giner *et al.,* 2000).

As regarding ACE activity, DD genotype patients was found to have higher ACE activity than DI and II genotype carriers which suggest the role of gene polymorphism in regulation of enzyme activity. There is evidence that DD genotype is associated with higher plasma ACE levels whereas II genotype is associated with lower ACE levels and ID genotype with middle levels. The reason for the higher ACE activity in the D allele has assumed that the I allele has a sequence like to a silencer sequence (Salimi *et al.,* 2011). Moreover, ACE activity in the studied patients carrying the C allele of AT1R gene was higher than those having the homozygous AA genotype. Inhibition of the RAS either through reducing the production of angiotensin II with ACE or by blocking the action of angiotensin II at the AT1R receptor level with angiotensin II-type 1 receptor blockers, is particularly effective at preventing renal injury (Elshamaa *et al.,* 2011).

Renal functions and blood pressure are lightly linked. Physiologically, kidneys provide a key mechanism of chronic blood pressure control (Guyton, 1991), whereas elevated blood pressure affects renal function via pressure naturesis mechanism (Elshamaa *et al.,* 2011).

Our study revealed lower e-GFR in patients AC and CC genotypes of AT1R receptor when compared to those having AA genotype. However, patients with DD genotype had lower e-GFR than II and DI genotypes but this difference was statistically non significant. Elshamaa *et al.* (2011) reported that patients carrying the C allele showed more rapid deterioration of renal function than those with AA genotype. The mechanism by which the AT1RA^{1166}C polymorphism affects the development of renal disease and its progression to ESRD remains to be elucidated. It is possible that predisposition to renal disease is related to genetic variability in the sensitivity of target tissues to angiotensin II whose actions are mediated by the AT1R receptor. The studied polymprphism is located in the 3'untranslated region of the gene and is apparently a non-functional mutation (Bonnardeaux *et al.,* 1994). It may be linked, however, to an unidentified functional mutation in the AT1R gene or in another closely linked gene possibly located in regulatory regions and involved in the development and progression of renal damage (Elshamaa *et al.,* 2011).

Study of the frequency of combination between genotypes of both ACE I/D polymorphism and AT1RA^{1166}C polymorphism in both patients and controls revealed that the combined genotype ACE-ID + AT1R-AA had the highest frequency in the studied CKD patients (40.6%). In addition, the combined genotype ACE-II + AT1R-AA had the highest frequency in the control group (60%) which suggest that this combination is protective from CKD .

There were some limitations in our study. The small sample size of the patients leads to low statistical power of combined two locus genotypic analyses. Further large study on the Egyptian population from different renal centers should be done for better interpretation for the role of ACE and AT1R gene polymorphisms on the progression of renal failure.

Conclusion:

The D allele of ACE gene and the C allele of AT1R are important genetic determinants in CKD. They are more frequent in HD patients than those on CT. Patients carrying these alleles have higher ACE activity and more prone to hypertension. They also have more decline in kidney function as evidenced by lower values of e-GFR. They can be considered as risk alleles.

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References:

1. Akman B, Tarhan C, Arat Z, Sezer S, Ozdemir FN (2009) Renin-angiotensin system polymorphisms: a risk factor for progression to end-stage renal disease in vesicoureteral reflux patients. Ren Fail 31:196-200.
2. AnbazhaganK, SampathkumarK, Ramakrishna n M, Gomathi P, Gomathi S, Selvam GS (2009) Analysis of polymorphism in renin angiotensin system and other related genes in South Indian chronic kidney disease patients. Clin Chim Acta 406(1-2):108-12
3. Badr S, Abd Raboh N, R and Ali S (2012) Angiotensinogen M235T and angiotensin-converting enzyme I/D gene polymorphism and their association with type 2 diabetes in Egypt. Life Science Journal 9(2):1226-33
4. Basset el-EA, Berthoux P, Cécillon S, *et al.* (2002) Hypertension after renal transplantation and polymorphism of genes involved in essential hypertension: ACE, AGT, AT1R and ecNOS. Clin Nephrol 57:192-200.

5. Bonnardeaux A, Davies E, Jeunemaitre X, Féry I, Charru A, Clauser E, Tiret L, Cambien F, Corvol P, Soubrier F (1994) Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension 24(1):63-9.
6. El-Bazz W, Mohamed K , Mansour BH, Mohamed R, 1Wagida M.F. *et al.* (2011) Detection Of Angiotensin Converting Enzyme Gene Polymorphism In Egyptian Patients With Diabetic Nephropathy. Australian Journal of Basic and Applied Sciences 5(11): 244-251,
7. Elshamaa M, amar Sabry S, Hafez M Bazaraa M, Koura H *et al.* (2011) Genetic polymorphism of ACE and the angiotensin II type1 receptor genes in children with chronic kidney disease. Journal of Inflammation 8:20
8. Fahmy ME, Fattouh AM, Hegazy RA, Essawi ML (2008) ACE gene polymorphism in Egyptian children with idiopathic nephrotic syndrome. Bratisl Lek Listy 109:298-301.
9. Giner V, Poch E, Bragulat E, Oriola J, González D, Coca A, De La Sierra A (2000) Renin-angiotensin system genetic polymorphisms and salt sensitivity in essential hypertension. Hypertension 35(1 Pt 2):512-7.
10. Grill S, Rusterholz C, Zanetti-Dällenbach R, Tercanli S, Holzgreve W, Hahn S, Lapaire O (2009) Potential markers of preeclampsia a review. Reprod Biol Endoc 14:7-70
11. Guyton AC (1991) Blood pressure control special role of the kidneys and body fluids. Science 28; 252(5014):1813-6.
12. Irani RA, and Xia Y (2008) The functional role of the rennin angiotensin system in pregnancy and preeclampsia. Placenta vol. 29, no. 9, pp. 763-771.
13. Levey AS (2002) Nondiabetic kidney diseases. N Engl J Med 347:1505-11.
14. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH (1995) A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 16; 332(11):706-11.
15. Mallamaci F, Zuccala A, Zoccali C, *et al.* (2000) The deletion polymorphism of the angiotensin-converting enzyme is associated with nephroangiosclerosis. Am J Hypertens 13:433-437.
16. Nakayama Y, Nonoguchi H, Kohda Y, Inoue H, Memetimin H, Izumi Y,Tomita K (2009) Different Mechanisms for the Progression of CKD with ACE Gene Polymorphisms. Nephron Clin Pract 111:c240-246.
17. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F ET AL (1990) An Insertion/Deletion Polymorphism in the Angiotensin I-converting Enzyme Gene Accounting for Half the Variance of Serum Enzyme Levels J. Clin. Invest 86:1343-1346
18. Saggar-Malik AK, Afzal AR, Swissman JS, Bland M, Sagnella GA, Eastwood JB (2000) Lack of association of ACE/angiotensinogen association genotype with renal function in autosomal dominant polycystic kidney disease. Genet Test 4:299-303.
19. SalimiS, MokhtariM, YaghmaeiM, Jamshidi M, Naghavi A (2011) Association of angiotensin-converting enzyme intron 16 insertion/deletion and angiotensin II type 1 receptor A1166C gene polymorphisms with preeclampsia in South East of Iran. J Biomed Biotechnol 941515.
20. Samuelsson O, Attman PO, Larsson R, *et al.* (2000) Angiotensin I converting enzyme gene polymorphism in non-diabetic renal disease. Nephrol Dial Transplant 5:81-86.
21. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JC (2006) ACE polymorphisms. Circulation Research vol. 98, no. 9, pp. 1123-1133.
22. Siekierka-Harreis M, Kuhr N, Willers R, *et al.* (2009) Impact of genetic polymorphisms of the renin-angiotensin system and of non-genetic factors on kidney transplant function: a single-center experience. Clin Transplant 23(5):606-15
23. Stefansson B, Ricksten A, Rymo L *et al.* (2000) Angiotensin-converting enzyme gene I/D polymorphism in malignant hypertension. Blood Press 9: 1054-1059
24. Tripathi G, Dharmani P, Khan F, Sharma RK, Pandirikkal V, Agrawal S (2006) High prevalence of ACE DD genotype among North Indian end stage renal disease patients. BMC Nephrology 17:7-15.
25. Tripathi G, Sharma RK, Baburaj VP, Sankhwar SN, Jafar T, Agrawal S (2008) Genetic risk factors for renal failure among north Indian ESRD patients. Clin Biochem 41(7-8):525-31