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Association Between Genetic Polymorphism of Rennin-angiotensin System and Development of End Stage Renal Disease Relation with Disease Progression

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End-stage Renal Disease (ESRD) is the terminal state of the kidney when its function has been permanently and irreversibly damaged. Renin-angiotensin System (RAS) has been considered to be responsible for the pathogenesis and progression of kidney diseases. This study aimed to determine the association between genetic polymorphism of the three members of RAS system, Angiotensin-converting Enzyme (ACE), Angiotensinogen (AGT) and Angiotensin II type 1 receptor (AT1R) and the development of ESRD and their relation with disease progression. The study included 330 subjects who were classified into 2 groups. GroupI:180 patients with ESRD on maintenance haemodialysis and Group II:150 healthy controls. All individuals underwent genotyping for ACE I/D polymorphism, AGT (M235T) polymorphism and AT1R (A1166C) polymorphism using (RFLP).Genotype distribution of both ACE I/D and AGT (M235T) polymorphisms showed significant statistical difference between ESRD patients and controls. DD genotype of ACE gene polymorphism was associated with 3.3 fold increased risk for ESRD (95% CI: 1.9-5.8) and TT genotype of AGT (M235T)polymorphism was associated with 2.5 fold increased risk for ESRD (95% CI: 1.5-4.1). However CC genotype of AT1R had 2.6 fold increased risk for development of ESRD (95% CI: 1.3-5.2).TT genotype of AGT, DD genotype of ACE and CC genotype of AT1R polymorphism had a strong positive relation with disease progression with the shortest time needed for the start of dialysis compared to other genotypes. A significant association between ACE I/D polymorphism, AGT (M235T) polymorphism and both the development of ESRD and rate of disease progression.

Key words: Genetic polymorphism, renin–angiotensin system, end-stage renal disease, angiotensin-converting enzyme

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INTRODUCTION

Chronic Kidney Disease (CKD) is a multifactorial disorder; its risk factors may include diabetes, hypertension, obesity and inherited factors (Su *et al*., 2012). It remains unclear why only minority of patients with CKD are susceptible to the complication of progressive renal failure and ultimately End Stage Renal Disease (ESRD) (Hammady *et al*., 2012). Genetic factors linked to early signs of renal disease, particularly increased albumin excretion and hypertension, which contribute to the pathophysiological processes of the deterioration of renal function, might be related to the progression of CKD (Luttropp *et al*., 2009). CKD may run in families that can be explained by the combined effects of genetic susceptibility and environmental exposure (Su *et al*., 2012).

The Renin-Angiotensin system (RAS) is the major regulator of blood pressure and kidney functions. It also has a role in their interactions (Tripathi *et al*., 2006). Its role in the pathogenesis of hypertension and its contribution to chronic renal failure and its progression is well documented. Angiotensin Converting Enzyme (ACE) inhibitors and angiotensin receptor blockers upon acting on RAS lead to lowering of blood pressure and attenuation or even prevention of renal damage (Norris and Vaughan, 2003). However, response to similar drugs differ according to the underlying cause (Michel *et al*., 2004).

ACE is an important component of RAS. Its main function is catalyzing the conversion of Angiotensin I to Angiotensin II (Naresh *et al*., 2009). ANG-II is considered a vasoactive peptide which has different effects on the kidney like vasoconstriction, release of aldosterone, control of glomerular filtration rate, adjustment of tubular transport, enhancement of collagen synthesis and control of nitric oxide release (Wolf, 1999).

Angiotensin II receptors, which mediate the vasoconstrictive and salt-conserving actions of the Rennin-Angiotensin System, include two subtypes: type 1 (AT1R) receptor and types 2 receptor. The AT1R receptor is a member of the G- protein-coupled receptor superfamily. Activation of this receptor leads to vasoconstriction, vascular smooth muscle cell proliferation, water retention and hypertrophy (Wu *et al*., 2009).

It was proved by genetic studies that the genes of RAS can take different shapes, which increase the probability that the genetic frame of RAS affects the status of RAS individuals (Naresh *et al*., 2009).

ACE gene has many insertion-deletion polymorphism which characterized by the occurrence or absence of a 278bp Alu repetitive sequence in intron 16 (Buraczynska *et al*., 2006). A point mutation of AGT gene, resulting in an amino acid substitution of thereonine for methionine at position 235 (M235T) has been associated with essential hypertension and renal diseases (Nordfors *et al*., 2002).The A1166C polymorphism of AT1R gene, occurs due to exchange of cytosine for adenine at the position 1166 in 3 untranslated region, which has been considered as a risk factor for hypertension and cardiovascular diseases (Kim and Iwao, 2000).

The aim of this study was to examine the association between ACE (I/D), AGT (M235T) and AT1R (A1166C) gene polymorphisms and the risk for ESRD development and whether these polymorphisms have relation with disease progression.

MATERIALS AND METHODS

All the subjects participating in the study gave informed written consent before blood sampling. Approval was obtained from the Research Ethics Committee of Menoufiay Faculty of Medicine. The study included 330 unrelated participants who were divided into 2 groups: Group I (ESRD group): included 180 patients with ESRD, who were on maintaince hemodialysis, they were recruited from dialysis center of nephrology unit in Internal Medicine Department, Menoufiya University Hospital. Group II (control group):- included 150 healthy subjects of matched age and sex with neither clinical signs of renal disease nor past or family history of renal disease. They were 84 male and 66 female.

Group I (ESRD group) were 63 patients (35%) had glomerulonephritis, 51 (28.3%) had diabetic nephropathy, 30 patients (16.7%) had hypertensive nephrosclerosis, 24 patients (13.3%) had obstructive uropathy and 12 patients (6.7%) had adult polycystic kidney diseases as the underlying chronic kidney disease. They were 102 males and 78 females with age ranging from 45 to 67 years. Chronic Kidney Disease (CKD) was defined as kidney dysfunction (Glomerular Filtration Rate (GDFR) <60 mL min^{-1} per 1.73 m^2) or kidney damage (usually reflected by albuminuria) that persist for at least 3 months (Fink *et al*., 2012).

A full medical history was taken from each subject which included the underlying chronic renal disease, time elapsed till the start of dialysis (Renal Replacement Therapy), Dialysis duration, history of diabetes, hypertension and treatment protocols.

The time elapsed from diagnosis of the renal disease till the onset of ESRD was used as a measure for the rate of progression of the disease. Its means duration was 6.15± 2.7 years (R: 2.5-14) with duration of dialysis ranged from 0.5-6 years.

Serum cholesterol (Meiattini *et al*., 1978), triglycerides (Fossati and Prenciphe, 1982), HDL-c (Gordon *et al*., 1977) and LDL-c (Friedewald *et al*., 1972) were done (by enzymatic colorimetric test). Serum urea was estimated by enzymatic end point method (Taylor and Vadgama, 1992). Serum creatinine is estimated by enzymatic fixed rate colorimetric test (Perrone *et al*., 1992). Sodium and Potassium were estimated by ion selective electrode method (Maas *et al*., 1985). All the subjects included in the study underwent genotyping for ACE I/D polymorphism, AGT (M235T) polymorphism and AT1R (A1166C) polymorphism using restriction fragment length polymorphism (RFLP) technique.

Blood sampling: Ten milliliter of venous blood were withdrawn after 12-14 h fasting from the cubital vein. Five milliliter was transferred slowly into EDTA tube for isolation of White Blood Cells for genotyping. Five milliliter was transferred into a plain tube, left for 30 min for clotting and centrifuged for 10 min at 4000 r.p.m. The serum obtained frozen at - 20 °C till used for determination of serum urea, creatinine, total cholesterol, triglycerides and HDL-C.

Peripheral Blood Mononuclear Cells Isolation (PBMCs): For separation of PBMCs, Lymphoflot solution was used (Bio test AG, Dreieich, Germany).

DNA extraction: Using QIA Amp® DNA Blood Mini kits (QIAGEN Hilden, Germany).

Genotyping of ACE gene I/D polymorphism: The amplification reaction was done in 25 µL final volume (10 µL DNA template±15 µL master mix containing 2.5 uL 10×PCR buffer, 1.5 µL MgCl 25 mM, 0.5 µL dNTPs 10 mM, 0.5 µL Taq polymerase 5U μL^{-1}, 1.0 µL forward primer 50 mM {F 5' CTGGAGACCACTCCCATCCTTTCT 3'}, 1.0 µL reverse primer 50 mM {R 5' GATGTGGCCATCACATTCGTCAGAT 3'} and 8 uL distilled water.

PCR amplification was done using Perkin Elmer thermal cycler 2400 (USA). PCR conditions were as follow: 96°C for 5 min, then 35 cycles were as follow 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min. Then a final extension step of 72°C for 5 min.

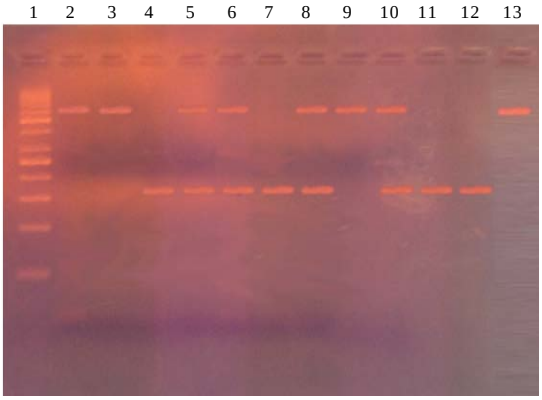

Fig. 1: Electrophoresis of digested ACE PCR products, Lane 1: 50 bp ladder, Lane 2, 3, 9, 13: II genotype, Lane 4, 7, 11, 12: DD genotype, Lane 5, 6, 8,10: ID genotype

Recognition Site Hind III:

5'... A▾AGCTT... 3'

3'... TTCGA▴A... 5'

Amplified DNA was digested with Hind III restriction enzyme at 37°C for 16 h (2 µL 10x buffer, 1.0 µL BgI II, 7 µL DW and 10 µL PCR product).

The Hind III digestive products were separated by electrophoresis on 3% agarose gel stained with ethidium bromide for 30 min. The bands were visualized under ultraviolet light. Digested PCR products yielded visible fragments of 490 bp for I allele and 190 bp for D allele. So, single bands of 190 and 490 bp confirmed homozygous DD and II genotypic state whereas two bands of 190 and 490 bp confirmed heterozygous ID genotype (Fig. 1)

Genotyping of Angiotensionogen gene M235T polymorphism: The amplification reaction was done in 25 uL final volume (10 µL DNA template±15 µL master mix containing 2.5 µL 10×PCR buffer, 1.5 µL MgCl 25 mM, 0.5 µL dNTPs 10 mM, 0.5 µL *Taq* polymerase 5U μL^{-1}, 1.0 µL forward primer 20 mM {F 5`-CCGTTTGTGCAGGGCCTGGCTCTCT-3`}, 1.0 µL reverse primer 20 mM {R 5`-CAGGGTGCTGTCCACACTGGACCCC -3`} and 8 uL distilled water.

PCR amplification was done using Perkin Elmer thermal cycler 2400 (USA). PCR conditions were as follow:96°C for 5 min, (94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1 min) for 35 cycles. Then a final extension step of 72°C for 7 min.

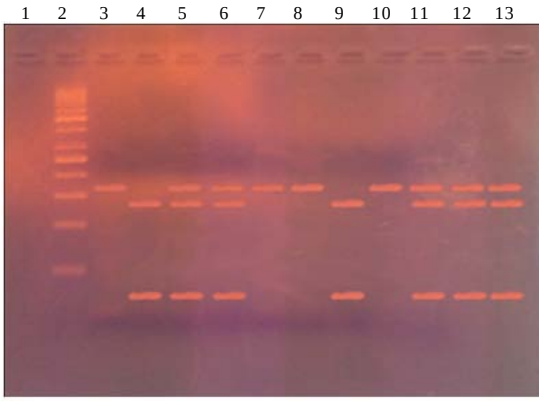

Fig. 2: Electrophoresis of digested Angiotensinogen gene M235T PCR products, Lane 1: 50 bp ladder, Lane 2, 6, 7, 9: MM genotype, Lane 3, 8: TT genotype, Lane 4, 5, 10, 11, 12: MT genotype

Recognition site psyI:

5'... GACN▾NNGTC... 3'

3'... CTGNN▴NCAG... 5'

The PCR product was incubated with PsyI restriction enzyme at 37°C for 4 h (2 μL 10×buffer, 2.5 μL PsyI, 10 μL PCR product and 5.5 μL DW). DNA fragments were separated by electrophoresis on 2% agarose gel stained with ethidium bromide. The M allele was detected as a band of 165 bp, whereas the mutated T allele showed two fragments, 141 and 24 bp (Fig. 2).

Genotyping for AT1R (A1166) gene polymorphism: The amplification reaction was done in 25 μL final volume (10 μL DNA template±15 μL master mix containing 2.5 μL 10×PCR buffer, 1.5 μL MgCl 25 mM, 0.5 μL dNTPs 10 mM, 0.5 μL *Taq* polymerase 5 U μL^{-1}, 1.0 μL forward primer 50 mM {F5`-GCAGCACTTCACTACCAAATGGGC-3`}, 1.0 μL reverse primer 50 mM {R5`-CAGGACAAAAGCAGGCTAGGGAGA -3`} and 8 μL distilled water. PCR amplification was done using Perkin Elmer thermal cycler 2400 (USA). PCR conditions were as follow; 95°C for 5 min, (94°C for 1 min, 55°C for 1 min, 72°C for 1 min) for 35 cycles. A final cycle of 72°C for 10 min.

Recognition site for BsuRI:

5'... GG▾CC... 3'

3'... CC▴GG... 5'

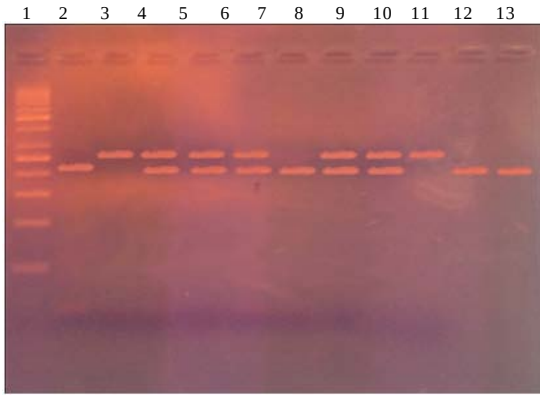

Fig. 3: Electrophoresis of digested AT1R (A1166) gene PCR products, Lane 1: 50 bp ladder, Lane 2, 7, 11, 12: CC genotype, Lane 3, 10: AA genotype, Lane 4, 5, 6, 8, 9: AC genotype

The PCR product (262 bp) was digested 4 h with 2 U BsuRI restriction enzyme at 37°C. The BsuRI digestive products were separated on 3% agarose gel electrophoresis stained with ethidium bromide for 30 min. The bands were visualized under ultraviolet light. Fragment size of 231 bp corresponds to CC allele, whereas 255 bp AA allele Whereas 231 bp and 255 bp correspond to AC allele (Fig. 3).

RESULTS

In this study, concerning the baseline characteristics, there was no significant statistical difference between ESRD group and controls regarding age and sex ($p>0.05$). While there was significant statistical difference between the two groups regarding the presence of diabetes, hypertension and family history of renal diseases ($p<0.001$)(Table 1).

Renal function tests showed that serum levels of urea and creatinine were significantly higher in ESRD group ($p<0.001$). Lipid profile showed that serum cholesterol, triglycerides and LDL were significantly higher in ESRD group while serum HDL level were higher in controls ($p<0.001$). $Na^{\pm}$ level were higher in control group compared to ESRD group while $K^{\pm}$ levels were higher in ESRD group ($p<0.001$) (Table 2).

For the M235T polymorphism of AGT gene, there was significant statistical difference between patients and controls regarding genotype distribution with predominance of TT genotype in ESRD group. Increased risk for ESRD was observed in TT genotype (OR: 2.5 95% CI: 1.5-4.1). The frequency of T- allele carriers was significantly higher in ESRD group ($p<0.05$) with 3.3 fold increased risk for ESRD (95% CI: 2.01-5.6) (Table 3).

Table 1: Distribution of the studied groups regarding age, sex, risk factors, cause of kidney disease and Time for ESRD

	Groups				
	Patients (n =180) Mean±SD		Controls (n =150) Mean±SD		p-value
Age (year)	57.5±5.7		56.9±5.6		
	R = 45-67		R = 45-67		>0.05
	Patients (n = 180)				
	Mean±SD 6.15±2.7		Range 2.5-14.0		
Time for ESRD (y)	No	%	No	%	p-value OR (95%)
Sex					
Male	102	56.7	84	56.0	>0.05 1.03
Female	78	43.3	66	44.0	(0.5-2.19)
Family history					
Positive	54	30.0	0	0.0	<0.001 -
Negative	126	71.7	150	100	
Diabetes					
Yes	51	28.3	0	0.0	<0.001
No	129	71.7	150	100	
Hypertension					
Yes	120	66.7	0	0.0	<0.001
No	60	33.3	150	100	
Cause of kidney disease					
Hypertensive nephrosclerosis	30	16.7			
Ch. Glomerulonephritis	63	35.0			
Diabetic nephropathy	51	28.3			
Obstructive uropathy	24	13.3			
PCO	12	6.7			

Table 2: Distribution of the studied groups regarding investigations

	Groups (Mean±SD)			
Parameters	Patients (n=180)	Controls (n =150)	t-test	p-value
Urea mg dL^{-1}	113.9±12.2 R=59-143	29.9±2.6 R = 24-36	47.89	<0.001
Creatinine mg dL^{-1}	9.37±0.9 R=7.8-11.4	0.9±0.1 R=0.8-1.2	59.33	<0.001
Cholesterol mg dL^{-1}	224.0±34.4 R=180-300	191.7±9.1 R=176-210	6.44	<0.001
TG mg dL^{-1}	149.5±24.8 R=120-200	117.6±14.4 R=96-180	8.02	<0.001
HDL.c mg dL^{-1}	31.8±1.2 R=30-34	42.8±2.3 R=39-48	32.58	<0.001
LDL.c mg dL^{-1}	162.9±33.7 R=115-252	125.4±10.2 R=103-144	7.59	<0.001
Na^+ mEq/L	136.7±1.9 R=134-141	139.7±2.1 R=130-144	8.12	<0.001
K^+ mEq/L	5.7±0.4 R=4.9-6.6	3.9±0.2 R=3.7-4.3	27.28	<0.001

For the I/D polymorphism of ACE gene, there was significant statistical difference between patients and controls regarding genotype distribution (p<0.05) with predominance of DD genotype which carried 3.3 fold increased risk for ESRD (95% CI: 1.9-5.8). The D-allele frequency didn't show statistical difference between the two group (p>0.05). However D- allele carriers had 2.1 fold increased risk for development of ESRD (Table 3). For the

Table 3: Distribution of the studied groups regarding their genotype

	Groups					
	Patients (n = 180)		Controls (n = 150)			
	No	%	No	%	p-value	OR (95% CI)
AGT						
TT	63	35.0	27	18.0	<0.05	2.5 (1.5-4.1)
MT	87	48.3	63	42.0		0.3 (0.2-0.5)
MM	30	16.7	60	40.0		-
ACE						
DD	63	35.0	21	14.0	<0.05	3.3 (1.9-5.8)
ID	84	46.7	81	54.0		0.5 (0.3)
II	33	18.3	48	32.0		-
AT1R						
CC	33	18.3	12	8.0	>0.05	2.6 (1.3-5.2)
AC	102	56.7	81	54.0		0.5 (0.3-0.9)
AA	45	25.0	57	38.0		-
T	213	62.6	117	39.0	<0.05	3.3 (2.0-5.6)
D	210	58.3	123	41.0	>0.05	2.1 (1.3-3.5)
C	168	50.9	105	35.0	>0.05	1.8 (1.2-3.0)

A1166C polymorphism of AT1R gene, there was no significant statistical difference between patients and controls regarding genotype distribution (p>0.05) However, CC genotype had 2.6 fold increased risk for ESRD (95% CI: 1.3-5.2). Also, the allele frequency didn't show significant statistical difference between the two groups (p>0.05) (Table 3).

Regarding M235T AGT polymorphism, ESRD patients were further classified into 3 groups: TT, MT and MM groups. Form the 180 patients of this group, 63 patients (35%) were of TT genotype, 87 patients (48.3%) were of MT genotype and 30 patients (16.7%) were of MM genotype. The mean age of TT group was 52.8±4.3 Of the MT group was 58.4±4.4 while of the MM group was 64.6±2.1. The mean age of the MM group was significantly higher than those of MT and TT group. (Table 4). Of the TT group, there were 42 males (66.7%) and 21 females (33.3%). Of the MT group, there were 51 males (68.6%) and 36 females (41.4%) while of the MM group, there were 9 males (30%) and 21 females (70%). There was no significant difference between the 3 groups regarding sex distribution (p>0.05) (Table 4).

The mean time passed till development of ESRD in the TT group was 3.9± 0.9, for the MT group was 6.1±1.2 while for the MM group was 11.1±1.6. There was significant difference between the 3 groups (p<0.001) with the shortest time needed till ESRD developed in the TT group.

Concerning the underlying kidney disease, there was significant difference between the 3 groups (p<0.05) with predominance of chronic glomerulonephritis in the TT group (42.9%) and MT group (41.4%) while diabetic nephropathy predominate in the MM group (80%).

There was significant statistical difference between the 3 groups regarding the presence of family history

Table 4: Distribution of the AGT genotype among the patients regarding age, sex, risk factors and cause of kidney disease

	AGT						
	TT (n=63)		MT (n=87)		MM (n=30)		
	Mean ±SD		Mean ±SD		Mean ±SD		p-value
Age (year)	52.8 ± 4.3		58.4 ± 4.4		64.6 ± 2.1		<0.001 (P1-P2-P3)
Time for ESRD	3.9 ± 0.9		6.1 ± 1.2		11.1 ± 1.6		<0.001 (P1-P2-P3)
	No	%	No	%	No	%	
Sex							
Male	42	66.7	51	58.6	9	30	>0.05
Female	21	33.3	36	41.4	21	70	
Cause							
H.nephrosclerosis	15	23.8	6	13.8	3	10	<0.05
Ch.GN	27	42.9	36	41.4	0	0	
D.nephropathy	6	9.5	21	24.1	24	80	
O. uropathy	6	9.5	15	17.2	3	10	
PCO	9	14.3	3	3.4	0	0	
Family history							
Positive	33	52.4	18	20.7	3	10	<0.05
Negative	30	47.6	69	79.3	27	90	
Diabetes							
Yes	6	9.5	21	24.1	24	100	<0.001
No	57	90.5	66	75.9	6	0	
Hypertension							
Yes	48	76.2	54	62.1	18	60	>0.05
No	15	23.8	33	37.9	12	40	

P1: Between TT and MT, P2: Between TT and MM, p3: Between MT and MM

Table 5: Distribution of the AGT genotype among the patients regarding investigations

	AGT (Mean±SD)				
Parameter mg dL^{-1}	TT (n=63)	MT (n=87)	MM (n=30)	F Test	p-value
Urea	113.6 ± 13.6	115.2 ± 10.9	111.1 ±13.2	0.43	>0.05
Creatinine	9.3 ± 1.1	9.5 ± 0.9	9.2± 0.9	0.5	>0.05
Cholesterol	225.5 ± 35.5	217.5 ± 31.6	239.8± 38.1	1.62	>0.05
TG	142.5± 21.3	147.6 ± 25.6	169.5± 20.3	4.71	<0.05(P1-P3) >0.05 (P2)
HDLc	31.9 ± 1.2	31.8 ± 1.1	31.6± 1.3	0.23	>0.05
LDL	164.6 ± 33.4	158.2 ± 33.7	173.3± 35.4	0.77	>0.05
Na^+ mEq L^{-1}	136.6 ± 1.90	136.6 ± 1.97	136.8± 1.5	0.03	>0.05
K^+ mEq L^{-1}	5.6 ± 0.4	5.8 ± 0.5	5.7± 0.5	0.71	>0.05

P1 = between TT and MT, P2 =between TT and MM, P3 = between MT and MM, F= ANOVA

(p<0.05) with strongest familial background in TT group (Table 4). There was significant statistical difference between the 3 groups regarding the presence of diabetes (p<0.001) while there was no significant difference between the 3 groups regarding the presence of hypertension (p>0.05) (Table 4).

The biochemical profile showed no statistical difference between the 3 genotype groups regarding serum levels of urea, creatinine, $Na^{\pm}$, $K^{\pm}$, Cholesterol , HDLc and LDLc (p>0.05) while triglycerides showed significant higher levels in T-allele Carriers (p<0.05) (Table 5).

Regarding I/D ACE polymorphism, ESRD patients were classified into 3 groups, DD, ID and II groups, 63 patients (35%) were of DD genotype, 84 patients (46.7%) were of ID genotype, and 33 patients (18.3%) were of II genotype. The mean age of DD group was 55.3±4.7, of the ID group was 57.1±5.5 while of the II group was 62.6±5.4. There was significant statistical difference regarding age with the highest mean age in II group.

Of the DD group, there were 48 males (76.2%) and 15 females (23.8%),in the ID group , there were 48 males (57.1%) and 36 females (42.9%) while the II groups, there were 6 males(18.2%) and 27 females (81.8%). There was significant statistical difference between the 3 groups regarding sex distribution. The number of females was significantly higher in II groups compared to other two groups (Table 6).

Table 6: Distribution of the ACE genotype among the patients regarding age, sex, risk factors and cause of kidney disease

	ACE						
	DD (n = 63)		ID (n = 84)		II (n = 33)		
	Mean±SD		Mean±SD		Mean±SD		p-value
Age (year)	55.3±4.7		57.1±5.5		62.6±5.4		<0.0001(P2)
Time for ESRD	4.9±1.7		5.6±1.7		10.0±2.8		<0.05(P3)
	No.	%	No.	%	No.	%	p-value
Sex							
Male	48	76.2	48	57.1	6	18.2	<0.05
Female	15	23.8	36	42.9	27	81.8	
Cause							
H.nephrosclerosis	6	9.5	18	21.4	6	18.2	<0.05
Ch.GN	21	33.3	36	42.9	6	18.2	
D. nephropathy	24	38.1	6	7.1	0	0	
O. uropathy	6	9.5	18	21.4	21	63.6	
PCO	6	9.5	6	7.1	0	0	
Family history							
Positive	24	38.1	30	35.7	0	0	<0.05
Negative	39	61.9	54	64.3	33	100	
Diabetes							
Yes	24	38.1	6	7.1	21	63.6	<0.001
No	39	61.9	78	92.9	12	36.4	
Hypertension							
Yes	36	57.1	63	75.0	21	63.6	>0.05
No	27	42.9	21	25.0	12	36.4	

P1: Between DD and ID, P2: Between DD and II, P3: Between ID and II

Table 7: Distribution of the ACE genotype among the patients regarding investigations

	ACE (Mean±SD)				
Parameters	DD (n = 63)	ID (n = 84)	II (n = 33)	F-test	p-value
Urea (mg dL^{-1})	113.1±10.2	115.1±14.1	112.7±11.2	0.24	>0.05
Creatinine (mg dL^{-1})	8.9±0.8	9.7±1.0	9.4±0.9	3.16	<0.05
Cholesterol (mg dL^{-1})	227.7±33.3	216.4±33.3	236.3±37.8	1.52	>0.05
TG (mg dL^{-1})	154.5±25.8	139.5±21.1	165.0±22.2	5.06	<0.05 (P_1-P_3)
HDLc (mg dL^{-1})	31.8±1.1	31.9±1.2	31.6±1.2	0.20	>0.05
LDLc (mg dL^{-1})	166.3±36.2	157.5±31.6	170.7±34.8	0.76	>0.05
Na^+ (mEq L^{-1})	136.2±1.47	136.9±2.1	137.0±1.8	1.01	>0.05
K^+ (mEq L^{-1})	5.7±0.3	5.7±0.4	5.6±0.5	0.06	>0.05

P1: between DD and ID, P2: Between DD and II, P3: Between ID and II, F: ANOVA

The mean time passed till development of ESRD in DD group was 4.9±1.7, for. ID group was 5.6±1.7 while for II group was 10.0± 2.8. There was significant statistical difference between the 3 group ($p<0.001$) with the shortest time in the DD group.

For the underlying kidney disease, there was significant statistical difference between the 3 groups with predominance of diabetic nephropathy in the DD group (38.1%), chronic glomerulonephritis in the ID group (42.9%) and obstructive uropathy (63.6%) in the II group (Table 6).

There was significant inference between the 3 groups regarding the presence of family history of renal disease ($p<0.05$) with the strongest familial background in the DD group.

There was significant statistical difference between the 3 groups regarding the presence of diabetes ($p<0.001$) while there was no statistical difference regarding the presence of hypertension ($p>0.05$)(Table 6).

Regarding the biochemical profile, there was no significant statistical difference between the 3 groups regarding the levels of urea, $Na^{\pm}$, $K^{\pm}$, cholesterol, HDLc and LDLc while serum creatinine was higher in ID group and triglycerides level was higher in II group ($p<0.05$). (Table 7).

For the A1166 C polymorphism of AT1R gene, ESRD patients were classified into CC, AC and AA groups, 33 patients (18.3%) were of CC genotype , 102 patients (56.7%) were of AC genotype and 45 patients (25%) were of AA genotype. the mean age of CC group was 58.1±5.9,

of the AC group was 57.2±5.8 while of the AA group was 57.7±6.0. there was no significant difference between the 3 groups regarding age (p>0.05)(Table 8).

Of the CC groups there were 21 males (63.6%) and 12 females (36.4%), of the AC group, there were 60 males (58.8%) and 42 females (41.2%) while of the AA group, there were 21 males (46.7%) and 24 females (53.3%). There was no statistical difference between the three groups regarding sex distribution (p>0.05) (Table 8).

The mean time passed till development of ESRD in the CC group was 6.0± 1.9, in the AC group was 5.9±2.7 and in the AA group was 6.7±3.2 there was no significant difference between the 3 groups (p>0.05).

There was significant statistical difference between the 3 groups regarding the presence of family history of renal disease (p<0.05), while there was no significant statistical difference between the 3 groups regarding the underlying cause, the presence of diabetes and the presence of hypertension (p>0.05).

Regarding the biochemical profile, there was no statistical difference between the 3 groups regarding serum levels of urea, creatinine, $Na^{\pm}$, $K^{\pm}$, Cholesterol, triglycerides, HDLc and LDLc (p>0.05) (Table 9).

Table 8: Distribution of the AT1R genotype among the patients regarding age, sex, risk factors and cause of kidney disease

	AT1R						
	CC (n = 33)		AC (n = 102)		AA (n = 45)		
	Mean±SD		Mean±SD		Mean±SD		p-value
Age (year)	58.1±5.9		57.2±5.8		57.7±6.0		>0.05
Time for ESRD	6.0±1.9		5.9±2.7		6.7±3.2		>0.05
	No.	%	No.	%	No.	%	p-value
Sex							
Male	21	63.6	60	58.8	21	46.7	>0.05
Female	12	36.4	42	41.2	24	53.3	
Cause							
H.nephrosclerosis	12	36.4	9	8.8	9	20	>0.05
Ch.GN	6	27.3	48	47.1	9	20	
D.nephropathy	9	38.1	24	23.5	18	40	
O. uropathy	6	18.2	12	11.8	6	13.3	
PCO	0	0	9	8.8	3	6.7	
Family history							
Positive	9	27.3	42	41.2	3	6.7	<0.05
Negative	24	72.7	60	58.8	42	93.3	
Diabetes							
Yes	9	27.3	24	23.5	18	40	>0.05
No	24	72.7	78	76.5	27	60	
Hypertension							
Yes	24	72.7	66	64.7	30	66.7	>0.05
No	9	27.3	36	35.3	15	33.3	

Statistical analysis: Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 16. Two types of statistics were done: descriptive e.g., percentage (%), range, mean and standard deviation SD, and analytical as Student's t-test: used to collectively indicate the presence of any significant difference between two groups for a normally distributed quantitative variable. One a way ANOVA (F test): used to collectively indicate the presence of any significant difference between several groups for a normally distributed quantitative variable. Kruskal -Wallis test: used to collectively indicate the presence of any significant difference between several groups for a not normally distributed quantitative variable. Post hoc test: used to the least significant difference between groups after ANOVA (F test) and Kruskal-Wallis test.

Chi-squared (χ^2): Used to compare between two groups or more regarding one qualitative variable.

DISCUSSION

ESED is an advanced form of chronic renal failure when renal function has decreased to approximately 10% of normal prior to initiation of dialysis (Agrawal *et al.*, 2010). Many causes of renal disease lead to Chronic Kidney Disease (CKD) and ESRD. Genetic Factors have a less direct power on the development of CKD. However

Table 9: Distribution of the AT1R genotype among the patients regarding investigations

	AT1R (Mean±SD)				
Parameters	CC (n = 33)	AC (n = 102)	AA (n = 45)	F-test	p-value
Urea (mg dL^{-1})	117.9±9.2	114.7±13.5	109.3±9.8	1.78	>0.05
Creatinine (mg dL^{-1})	9.6±0.8	9.4±1.1	9.1±0.9	0.82	>0.05
Cholesterol (mg dL^{-1})	226.9±37.8	217.7±33.7	236.3±32.2	1.60	>0.05
TG (mg dL^{-1})	155.5±26.2	145.0±23.9	155.1±25.3	1.26	>0.05
HDLc (mg dL^{-1})	31.7±1.1	31.8±1.1	31.9±1.3	0.13	>0.05
LDLc (mg dL^{-1})	164.1±35.4	158.4±34.6	172.5±30.3	0.92	>0.05
Na^+ (mEqL^{-1})	137.4±2.2	136.4±1.6	136.6±2.1	1.03	>0.05
K^+ (mEqL^{-1})	5.7±0.3	5.7±0.4	5.7±0.5	0.05	>0.05

F: ANOVA

genetic frame, racial differences, socioeconomic status, environmental factors increase the hazard for complication of progressive renal failure and occurrence of ESRD (Wattanakit *et al*., 2006). Recently many studies concentrate on the role of polymorphisms in the genes encoding for components of RAS in the occurrence and /or progression of renal disease (Kujawa-Szewieczek *et al*., 2010). Angiotensin I Converting Enzyme (ACE), Angiotensinogen (AGT) and Angiotensin II Type Receptor (ATIR) genes have long been considered factors that lead to cardiovascular diseases especially to essential hypertension (Gumprecht *et al*., 2000).These observations are now very important in renal diseases, since proteins encoded by these genes are involved not only in the regulation of systemic blood pressure, but also in the regulation of internal hemodynamics (Lovati *et al*., 2001).

This study aimed to examine the association between polymorphisms in the genes encoding for components of RAS and the development of ESRD and the rate of disease progression.

In this study, the distribution of genotypes and allele frequencies were compared between patient and controls. For the AGT (M235T) polymorphism, there was a significant statistical difference between patients and control regarding genotype distribution with predominance of TT genotype in ESRD group. TT genotype had 2.5 fold increased risk for ESRD (95% CI:1.5-4.1) Also, the frequency of T-allele carriers was significantly higher in ESRD group with 3.3 fold increased risk (95% CI:2.0-5.6).

There was significant difference between M235T genotypes (TT, MT and TT groups)regarding age, primary cause of renal disease, family history and the presence of diabetes while there was no significant difference regarding sex distribution and presence of hypertension. Regarding laboratory profile, only triglycerides level was higher in T-allele carriers.

These results were in line with (Gumprecht *et al*., 2000) who stated that T-allele was more frequent in patient with CKD and that interstitial nephritis predominate in the TT genotype as the primary cause for CKD, similar results were obtained by Lin *et al*. (2009), Buraczynska *et al*. (2006) and Mtiraoui *et al*. (2011) while no association was documented by Marre *et al*. (1997), Lovati *et al*. (2001), Su *et al*. (2012) and Kujawa-Szewieczek *et al*. (2010).

Study done by Lin *et al*. (2009) proved that higher concentrations of plasma angiotensinogen are associated with the AGT 235T allele and may be directly related to the number of allele present thereby supporting the theory, that components in the RAS are physiologically activated and may promote glomerulosclerosis and interstitial fibrosis via accumulation of extracellular matrix. Increased levels of ANG II will in turn affect renal hemodynamics and tubular transport, stimulate cytokine formation and release, stimulate growth and proto-oncogens in various renal cell types (Gumprecht *et al*., 2000). It may also enhance ammoniogenesis in renal tubular cells which in turn stimulate tubular hypertrophy. Increased ammonia concentration in the renal cortex, by interacting with complement C3 can induce tubulointerstitial disease (Ibrahim *et al*., 1997).

Thus increased production of angistens in II inside nephrons and their vasculature could have a role in local renal injury through both hemodynamic and non- hemodynamic actions (Remuzzi *et al*., 2005).

A DNA sequence analysis of both I and D alleles of the ACE gene in laboratory discovered a pair of identical 14-base DNA sequences, the first on one end of the insert and a repeat which flanks the opposite end of the insert. The arrangement of theses two repeats suggest a possible origin of the D-allele. During meiosis, one of these repeats may have aligned with the complement of the other, producing a ?loop-out' of the intervening 287bp fragment. This polymorphic model suggests that the nature of the mutational event may have been deletion, not insertion. Continued DNA synthesis would have then taken place on the shorter template strand (Yoshida *et al*., 1996).

The results of this study revealed significant association between DD genotype and the development of ESRD (OR:3.3 95% CI:1.9-5.8). While the D-allele frequency didn't show significant association. When the ESRD group was subdivided for their ACE genotype, there was significant difference between the genotype groups regarding age, sex, primary cause of renal disease, family history of renal disease and presence of diabetes while there was no significant difference regarding the presence of hypertension. These results were similar to results obtained by Yoshida *et al*. (1996), Gumprecht *et al*. (2000), Dehwah *et al*. (2008) and Naresh *et al*. (2009). Another study done by Samuelson *et al*. (2000) who divided ESRD patients according to their ACE I/D genotypes found no significant statistical difference between the 3 genotype groups regarding age, sex, presence of hypertension and serum levels of cholesterol and triglycerides. Study done by Tripathi *et al*. (2006) found no significant difference between the genotype groups regarding age, serum urea and lipid profile while these was significant difference regarding the presence of hypertension. Another studies failed to show any significant association between the polymorphism and risk for ESRD (Marre *et al*., 1997; Buraczynska *et al*., 2006).

A model for the genetic control of plasma ACE level based on the result of family study was proposed by (Cambien *et al*., 1988). In this study, the genetic analysis of familial phenotypes suggested that these levels are affected by a major gene, which was estimated to account for 29% of the total phenotypic variance of plasma ACE in adults. This major gene's effect was confirmed by Rigat *et al*. (1990) who also found that serum ACE levels showed a significant relationship with I/D polymorphism and that DD genotype is associated with higher levels of ACE and increasing D-alleles had an additive effect. Another study proved the same result and had considered the I/D polymorphism as major determinant of plasma and tissue levels (Van der Kleij *et al*., 2002).

Results of this study showed no significant statistical difference between patients and controls regarding neither genotype distribution nor allele frequency of A1166C polymorphism on ATIR gene. However, CC genotype had 2.6 fold increased risk for ESRD (95%CI: 1.3-5.2). This was in agreement with (Yu *et al*., 1996) who reported absence of linkage between this polymorphism and ESRD in African Americans and (Frimat *et al*., 2000) whose results showed no association with the development nor the progression of the disease. Several studies also failed to show association between this polymorphism and ESRD (Table *et al*., 2005; Kujawa-Szewieczek *et al*., 2010; Hammady *et al*., 2012). While others reported a significant difference in the frequency of C-allele and CC homozygous between ESRD patients and controls and that the risk of renal failure associated while the C-allele seems to be more apparent in dialyzed patients with a positive family history (Buraczynska *et al*., 2002; Buraczynska *et al*., 2006; Lin *et al*., 2009).

In a previous study, it was reported that methyl gonovin induces more coronary artery vasoconstriction in patients with at least one copy of the C-allele compared with patients homozygous for the A-allele (Amant *et al*., 1997). So it was hypothesized that there would be an association between the ATIR polymorphism and renal and systemic hemodynamic function. This hypothesis was tested by comparing baseline function in normal healthy male and premenopausal female subject ingesting a controlled Na and protein diet and grouped according to A1166C polymorphism and results showed that base line for GFR, renal plasma flow and renal blood flow were significantly lower in the C-allele carriers compared with AA group (Miller *et al*., 1999).

In an attempt to explain relationship between ATIR A1166C polymorphism and ESRD, it was postulated that its predisposition to renal disease is related to differences in the sensitivity of target tissues to angiotensin II whose actions are done by the ATIR receptor. However, the studied polymorphism is located in the 3u ntranslated region of the gene and is apparently a non-functional mutation but it may be linked to an undetermined functional mutation in the ATIR gene or closely linked gene found in the regulatory region involved in the development of renal damage (Buraczynska *et al*., 2006). Concerning the rate of progression to ESRD, results of this study showed rapid progression linked with the TT genotype of M 235 T polymorphism rapid of AGT gene and DD genotype of I/D polymorphism of ACE gene with the shortest time passed till the development of ESRD while there was no significant association between A 1166 C polymorphism of ATIR gene and rate of progression of the disease.

It was stated that male patients with DD genotypes had a significantly higher rate of progression than male patients with ID and II genotypes, whereas in the female subgroups, there was no association (Samuelson *et al*., 2000). Many studies proved an association between ACE DD genotypes and faster rate of progression of renal disease (Hunley *et al*., 1996; Stratta *et al*., 1999; Dixit *et al*., 2002; Woo *et al*., 2011).

Two studies investigated the role of angiotensinogen M235T genotype in the progression of renal disease (Hamaguchi *et al*., 1995; Hunley *et al*., 1996). In neither of them, the distribution pattern of genotypes was significantly different. An association was found, between ACE DD genotype and faster rate of progression while there was no association with the M235Tg enotypes (Lovati *et al*., 2001). Another study failed to show any association between A1166 C polymorphism and the rate of progression into renal failure (Yoshida *et al*., 1995). In contrast to (Buraczynska *et al*., 2006) who stated that progression of renal disease was only influenced by the AT1R polymorphism with more rapid progression in the C-allele carriers. However, another study failed to show any association between the 3 polymorphism and progression of the disease (Frimat *et al*., 2000).

CONCLUSION

In conclusion, the present study confirmed the association between AGT M235 T polymorphism and ACE I/D polymorphism and the development and progression of ESRD while it failed to show any association between A1166 C polymorphism with neither the development nor the progression of ESRD.

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