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Angiotensin converting enzyme genotype prevalence among Egyptian primary nephrotic and end stage renal diseases patients

F.M.Zahran[1]*, S.Hamed[2], A.T.Keshta[1], M.Hussien[1]

[1]Biochemistry Division, Dept. of Chemistry, Faculty of Science, Zagazig University, (EGYPT)

[2]Urology& Nephrology Center, Mansoura University, (EGYPT)

E-mail : fatenzahran55@yahoo.com

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ABSTRACT

The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and their interaction. In such a situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin converting enzyme-1 (ACE-1) is an important component of RAS which determines the vasoactive peptide Angiotensin-II. In the present study, we have investigated 103 end stage renal diseases (ESRD), 104 primary nephrotic (P.N) patients and 102 normal healthy controls from Mansoura city in Egypt to deduce the association between ACE gene polymorphism and ESRD, P.N. The selected samples were assayed for genotyping of ACE I/D by (PCR) based DNA amplification using specific flanking primers. The results revealed that there was a significance distribution in DD genotype between ESRD and control group ($p<0.05$), with risk value ($OR>1$) which resulting in increasing the risk for ESRD. There was significance distribution in ID genotype between ESRD and control group ($p<0.05$), without disease risk ($OR <1$). Based on these observations we conclude that ACE DD genotype implicate a strong possible role in the in renal damage among Egyptians. The study will help in predetermining the timing, type and doses of therapy for ESRD patients.

KEYWORDS

Renin-Angiotensin system;
Angiotensin converting enzyme-1;
End stage renal diseases;
Primary nephrotic;
Genotyping;
Gene polymorphism.

INTRODUCTION

Angiotensin-converting enzyme (ACE) is one of the limiting enzymes in the renin-angiotensin-aldosterone system (RAAS). An elevated angio-tensin II level causes deleterious effects on renal haemodynamics and induces the expression of different growth factors, leading to glomerulo-sclerosis[1]. ACE inhibitors and angiotensin receptor blockers reduce proteinuria in patients with nephrotic syn-drome[2], stressing the role of the RAAS inthe pathogenesis of nephritic syndrome. A polymor-phism of the ACE gene, consisting of a 287-bp fragment within intron 16 defined by inser-tion (I) or deletion (D), has been shown toinfluence the circulating and cellular ACE concen-tration[3,4].

The ACE gene I/D polymorphism is reportedly associated with the progression ofseveral renal diseases, including diabetic nephro-pathy, IgA nephropathy, au-

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tosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels [5]. The ACE DD genotype is associated with increased circulating ACE levels, which are gen-erally two times as high as those found for the II genotype; ID heterozygotes are associated with intermediate ACE levels [6]. The ACE gene I/D polymorphism are reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels.

Our study aims to determine the ACE I/D genotype distribution in adult primary nephrotic syndrome and end stage renal disease patients and evaluate its effect on clinical parameters.

SUBJECTS & METHODS

Patients and controls

Our subjects divided in to three different groups,104 primary nephrotic patients, 58 males (55.8%) and 46 females (44.2%), and their age ranged from 19-46 years with a mean of 30.63±6.99 years. 103 ESRD patients 63 males (61.2%) and 40 females (38.8%), and their age ranged from 19-55 years with a mean of 38.99±8.2 years. And, 102 unrelated healthy adults with no renal diseases as a control group; 59 males (57.8%) and 43 females (42.2%), and their age ranged from 20-45 years with a mean of 27.89±5.39 years.

Genotyping of the ACE gene I/D polymorphism

Genomic DNA was extracted from 300 µl of whole blood, DNA extraction kit (promega company, USA). I/D polymor-phism of the ACE gene was determined accord-ing to the method of Rigat*et al*[3]. The sequences of the sense and antisense primers were (5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'), respec-tively. PCR was per-formed in a final volume of 50 µl that contained 25 µl master mixes, H"500 ng of genomic DNA, 12.5 pmol of each primer and 5% dimethylsulphoxide (DMSO). Amplification was performed using a Gene Amp PCR systemG-storm. Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 min-ute at 72°C. PCR products were electrophoresed in 1.6% agarose gel and visualised directly withethidium bromide staining. I allele was detected as a 490-bp band, and the D allele was detected as a 190-bp band (figure 1). 1st PCR was confirmed by using the second PCR[9]. A second PCR amplification was performed for each DD type with a primer pair that recognizes an insertion-specific sequence (5'- TGG GAC CAC AGC GCC CGC CAC TAC-3'; 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3'), with identical PCR conditions except for an annealing temperature of 67°C and the absence of 5% DMSO[6].

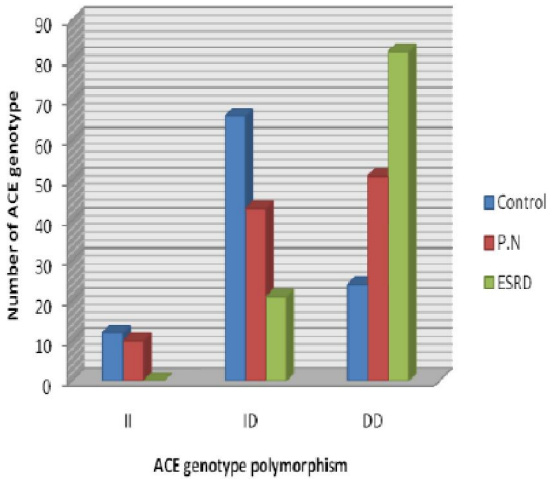

Figure 1 : Shows1st PCR genotyping

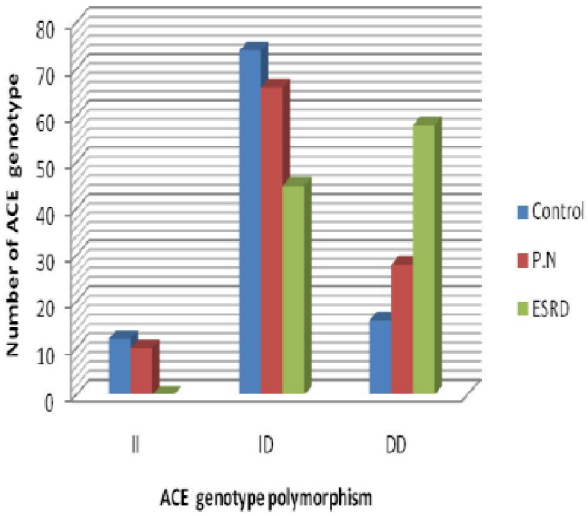

Figure 2 : Shows2nd PCR genotyping

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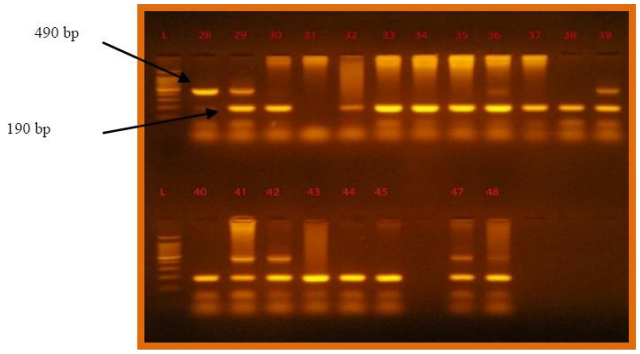

Figure 3 : Agarose gel electrophoresis of 1st PCR products of ACE gene. Lines 29, 39, 41, 42, 47 heterozygous ID, 30, 32-38, 40, 43, 44, 45, 48 homozygous DD cases, 28 homozygous II cases.(I allele 490 bp, D allele 190bp).

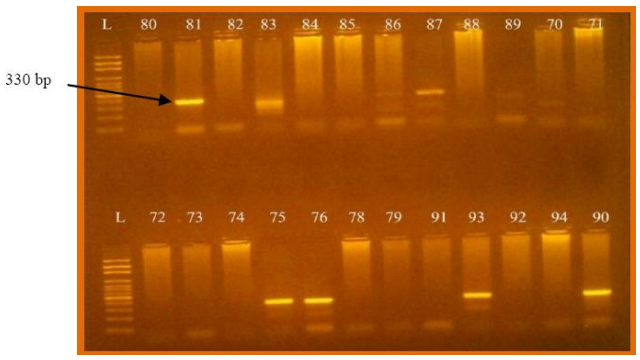

Figure 4 : Agarose gel electrophoresis of 2nd PCR products of ACE gene. Lines 81, 87, 75, 76, 93, 90 heterozygous ID, the other Lines represent DD homozygous. (ID 330 bp)

TABLE 1 : Chi-Square Test I/D allelein ESRD, P.N and control groups

genotype groups	II	ID	DD	I allele	D allele	p-value
Control	12	74	16	98	106	----
P.N	10	66	28	86	122	0.172
ESRD	0	45	58	45	161	0.000

P value Chi-Square Test <0.05

Biochemical parameters

Total cholesterol, Triglyceride & LDL, Serum creatinine &albumine were measured for all study population according tomethods of;Young[7]; Stein,[8]; Henry[9]; Doumas et al.[10]; respectively.

Statistical analysis:

The allele distribution of the ACE gene I/D poly-morphism was tested for Hardy-Weinberg equi-librium in patient and control groups. A computer software package (SPSS), version 14[11], was used in the analysis. For quantitative variables, mean and median (as a measure of central tendency), stan-dard deviation. Frequency and percentage are presented for qualitative vari-ables. Chi square test used to estimate differences in qualitative variables. P value < 0.05 was considered to be statisti-cally significant.

RESULTS

The genotype frequencies 1st PCR (II, ID and DD-genotypes) in nephrotic group were 10, 43 and 51; respectively. In addition the incidences of II, ID and DD in ESRD group were 0, 21 and 82; respectively. Where, in control group the incidences of II, ID and DD were 12, 66 and 24; respectively. The genotype frequencies 2nd PCR (II, ID and DD-genotypes) in nephrotic group were10, 66 and 28;respectively. In addition the incidences of II, ID and DD in ESRD were 0, 45 and 58; respectively. Where, in control group the incidences of II, ID and DD were 12, 74and 16respectively.

TABLE 2 : Represents Odds Ratio (OR) for ESRD, P.N and control groups

Alleles	Control	Primary Nephrotic	OR	(95% CI)	Risk	P-value for chi square test
D	106	122	1.312	(0.889-1.93)	+	P >0.05
I	98	86	0.762	(0.517-1.125)	-	P >0.05
Alleles	**Control**	**ESRD**	**OR**	**(95% CI)**	**Risk**	**P-value for chi square test**
D	106	161	3.30	(2.15-5.08)	+	P<0.05
I	98	45	0.302	(0.19-0.46)	-	P<0.05

P Chi-square Test <0.05 significant; P OR <1 Risk disease; P OR >1 Non Risk disease

There was a significance in distribution in DD genotype between primary nephrotic and control group (p=0.049), with risk value equal 1.98>1 which resulting in increasing the risk for disease. While there was no significance distribution in both ID & II genotype between both P.N and control group (p>0.05), without disease risk(OR <1).

There was a significance in distribution in DD geno-

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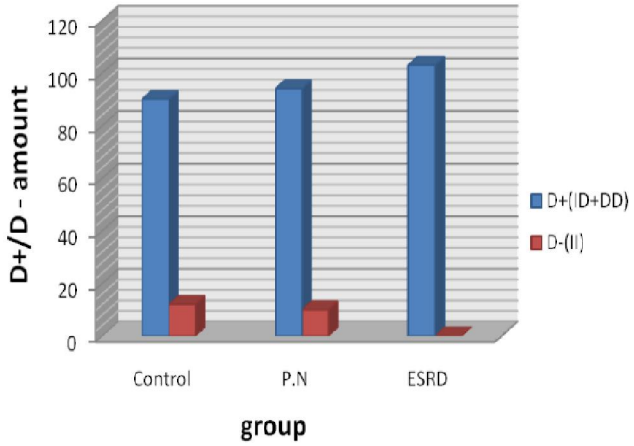

Figure 5 : D^+/D^- in P.N, ESRD & control groups

TABLE 3 : Prevalence of ACE I and D polymorphism genotype 1st PCR in patients and control groups

genotype groups	II	ID	DD	p-value
Control	12	74	16	----
P.N	10	66	28	0.143
ESRD	0	45	58	0.000

P Chi-square Test <0.05 significant

TABLE 4 : Prevalence of ACEI and D polymorphism genotype 2nd PCR in patients and control groups

Genotype groups	II	ID	DD	p-value
Control	12	66	24	0.001
P.N	10	43	51	
Control	12	66	24	0.000
ESRD	0	21	82	

P Chi-square Test <0.05 significant

type between ESRD and control group (p=0.000), with risk value equal 6.92>1 which resulting in increasing the risk for ESRD. There was significance distribution in ID genotype between ESRD and control group (p=0.000), without disease risk (OR <1).

DISCUSSION

The genetic origin of kidney diseases has been a focus of research in the past few years. There is significant evidence showing that the RAAS is involved in the pathogenesis of progressive renal disorders. As a matter of fact, in recent studies the association between disease progression and the ID/DD genotype of the ACE gene has been well described[2]. Interestingly, it has been found that patients with the DD genotype experienced a more severe clinical course [12]. Angiotensin

TABLE 5 : Comparison of lipid profile,creatinine& albumin in Control,P.N &ESRD if ACE ID/DD

Group	Cholesterol mg/dL mean±S.d	T. glyceride mg/dL mean±S.d	LDL mg/dL mean±S.d	Creatinine mg/dL mean±S.d	Albumine g/dL mean±S.d
Control	170.75	70.09	73.3	0.95	4.19
ID	±18.3	±16.7	±10.9	±0.97	±0.44
Control	171.3	73.25	70.8	1.3	3.99
DD	± 17.95	±18.7	± 12.6	± 2.06	± 0.83
P.Nephrotic	220.12	176.2	99.64	2.6	3.13
ID	±89.7	±9.8	±34.1	±2.2	±0.99
P.Nephrotic	228.4	174.5	112.0	3.10	3.1
DD	± 93.7	± 10.6	±0.80	± 2.4	± 0.89
ESRD	234.76	248.9	150.5	10.49	3.02
ID	±35.1	±42.3	±12.3	±2.76	±0.44
ESRD	236.45	256.4	149.7	10.71	3.03
DD	± 42.1	± 43.1	±12.7	±2.99	±0.42

II is the most active product of the RAAS and it has a strong influence on local and systemic haemodynamic regulation.

Angiotensin II is also a renal growth factor that modulates key elements of renal disease progression, including renal mesangial cell growth, extracellular matrix synthesis, and degradation and inflammatory processes. The effects of angiotensin II are medi-ated by the release of several factors, including transforming growth factor-B (TGF-B), plasminogen acti-vator inhibitor-1, monocyte chemoattractant pro-tein-1, and the activation of various nuclear transcription factors, including activator protein-1 and nuclear factor kappa B (NF-κB)[13]. The ACE DD genotype is associated with increased circulating ACE levels, which are gen-erally two times as high as those found for the II genotype; ID heterozygotes are associated with intermediate ACE levels[6]. A polymor-phism of the ACE gene, consisting of a 287-base pair fragment within intron 16 defined by inser-tion (I) or deletion (D), has been shown to influence the circulating and cellular ACE concen-tration. The ACE gene I/D polymorphism are reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels.

In the current study, we tried to increase the specificity of ACE I/D genotyping by using DMSO in the first PCR. In addition, a second PCR was performed for the samples that showed DD genotype in the first step. Out of 51 patients with P.N syndrome showed

initially the DD genotype, 23 patients were proven to have the ID. This was confirmed by the second PCR. Out of 82 patients with ESRD showed initially the DD genotype 24 patients were proven to have the ID genotype,also out of 24 control showed initially the DD genotype 8 control were proven to have the ID this was confirmed by using the second PCR.

The frequencies of the II, ID, and DD genotypes in controls were found to be 12, 74, and 16, respec-tively, whichstatistically nonsignificant compared to those of P.Nand significant compared to ESRDpatients. As a matter of fact, in recent studies the association between disease progression and the ID/DD genotype of the ACE gene has been well describedbyBagga and Srivastava[14].

This agreeswith the study of Sasongko*et al.*[15]who reported that the differences in the distribution of the ACE gene polymorphism between INS patients and controls were not statistically sig-nificant. This is, however, disagree withmost other studies in which the DD genotype was sig-nificantly more common in nephrotic patients compared to normal individuals[2,4,16].

Our study revealed a highly significant difference in the presence of DD genotype and D allele of ACE gene among ESRD patients and normal controls validating that the ACE gene polymorphism is an important genetic determinant of non-diabetic nephropathies. Overall findings were demarcating that D allele of ACE gene confers a high risk of developing renal diseases (OR = 3.30) and this association was highly compounded when D allele was present in homozygous state (OR = 6.92). Even inclusion of the heterozygous ID state known to have low levels of ACE production along with the DD genotype depicted a high risk of renal failures (OR = 0.29). It has been found that patients with the DD genotype experienced a more severe clinical course Maha*et al.*,[17]. We confirmed our results by measuring different clinical parameters and their relation to polymorphism. ID polymorphism showed increase in cholesterol, triglyceride, LDL, and creatinin by (29%, 151%, 35%, 188%) in P.N patients;while (37%, 254%, 105%, 105%) in ESRD compared to control. On the other hand; DD showed increase in cholesterol, triglyceride, LDL, and creatinin by (33%,275%,60%,20%)in P.N patients;while (38%, 250%, 112%, 723%) in ESRD compared to control. Moreover, ID and DD polymorphism showed decrease in albumin by 25% and 23% in P.N and ESRD patients; respectively compared to control. Kidney disease is clinically characterized by increasing rates of urinary albumin excretion, starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria/overt nephropathy, and eventually to ESRDChoudhry*et al.*, [18].Therefore;ACE genepolymorphism appears to be an important genetic determinant in causation and progression of renal diseases and ACE DD genotype was found to be strongly associated with ESRD.

CONCULSION

Conclusively, ACE gene polymorphism appears to be animportant genetic determinant in progressionof renal diseases and ACE DD genotype was found tobe strongly associated with ESRD.

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