British Journal of Medicine & Medical Research
4(8): 1763-1771, 2014

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Association of Angiotensin-I-Converting Enzyme (ACE) Insertion/Deletion Gene Polymorphism with End Stage Renal Disease in Egyptian Patients

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 8th April 2013
Accepted 5th December 2013
Published 2nd January 2014

ABSTRACT

Angiotensin-I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has been associated with the genetic susceptibility to end stage renal disease (ESRD) in different populations. ACE DD genotype and D allele are associated with ESRD as risk factors in several studies. In this study, we investigated the association between ACE I/D gene polymorphism and ESRD in the Egyptian patients. Frequencies of D allele and DD genotype were significantly increased, while frequencies of I allele and ID genotype were significantly decreased in the ESRD patients when compared with the control subjects (P = .012, OR = 1.82, 95% CI = 1.139-2.915 for DD) and (P = .018, OR = 1.6, 95% CI = 1.082-2.33 for D). In conclusion, ACE DD genotype and D allele are associated with ESRD as risk factors for ESRD in the Egyptian population.

Keywords: Angiotensin-I-converting enzyme (I/D) polymorphism; end stage renal disease.

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1. INTRODUCTION

End stage renal disease (ESRD) represents a clinical condition in which there has been an irreversible loss of endogenous renal function. ESRD is an increasing problem worldwide and is a major health problem associated with very high morbidity and mortality [1]. ESRD is an advanced form of chronic renal failure where renal function has declined to approximately 10% of normal prior to initiation of dialysis or transplantation [2]. The main causes of ESRD in Egypt, other than diabetic nephropathy, included hypertensive kidney disease, chronic glomerulonephritis, unknown etiology, chronic pyelonephritis, schistosomal obstrctive uropathy and schistosomal nephropathy [3]. Abnormal calcium (Ca), phosphorus (P) and vitamin D metabolism are very common in patients with ESRD [4].

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. The Renin-angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and may play a role in their interaction [2]. Renin is a proteolyitic enzyme that is produced in the juxtaglomerular cells of kidney. When renin is released to the circulation it metabolizes its only substrate, angiotensinogen (AGT), which is primarily synthesized in and released from the liver to the blood stream. The action of renin on AGT results in the production of a decapeptide, angiotensin I (Ang I) [5]. Angiotensin-I-converting enzyme (ACE) gene is one of the most intensely studied genes because of the key role it plays in the renin-angiotensin system (RAS) [6]. Mostly in pulmonary circulation, Ang I is converted into the octapeptide Ang II by the angiotensin converting enzyme type 1 (ACE1 or ACE) which is nearly ubiquitously bound to vascular endothelial cells. ACE also inactivates the potent vasodilators bradykinin and kallidin [5]. The gene encoding ACE is located on the long arm of chromosome 17 (17q23) in humans and contains 26 exons and 25 introns. A common insertion/deletion (I/D) polymorphism in the non-coding region of the ACE gene was identified in 1990. The I allele results from the presence of a 287bp DNA fragment in intron 16 and the D allele results from the absence of this DNA sequence [7]. The ACE gene consists of either an insertion (I) allele or a deletion (D) allele that form three possible genotypes: II, ID or DD [1]. Numerous studies have been done to evaluate the relationship between development and progression of ESRD, and ACE I/D gene polymorphism [1,8,9]. This work aims to study the effect of ACE I/D gene polymorphism in ESRD in the Egyptian population.

2. SUBJECTS AND METHODS

2.1 Subjects

The present study was carried out on the ESRD patients (n = 147; 86 men and 61 women), with a mean age of 44 ± 14.8 years. All the patients were undergoing dialysis treatment following diagnosis of ESRD by nephrologists. The control subjects (n = 140; 84 men and 56 women), with a mean age of 39.7 ± 14.1 years. The control subjects were collected from donor blood bank; the serum creatinine, uric acid, BUN, routine urine test and other biochemical parameters were tested. The control subjects had normal kidney functions and they were free from any Kidney diseases, also they were free from any other diseases. This study was approved by Ethical Board of the Mansoura University. Informed written consent was obtained from all participants, (patients and control subjects), in this study.

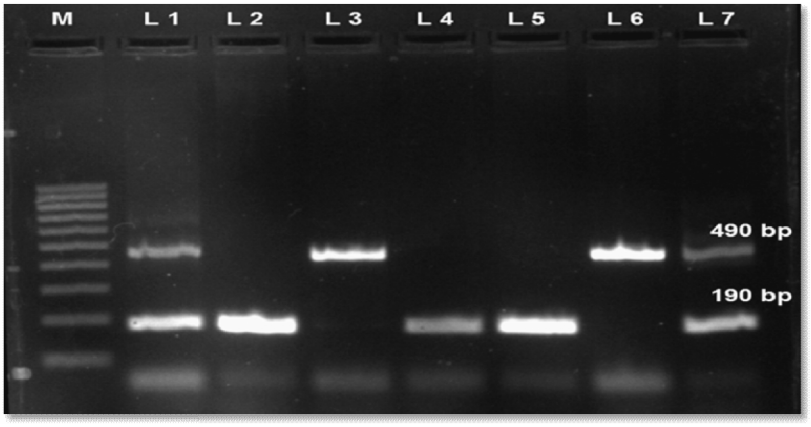
Four milliliters (4ml) of peripheral blood samples were collected and divided into (1ml) in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for DNA extraction and then the PCR technique on these DNA samples was applied and (3ml) in tubes without EDTA allowed to clot for 10-15 minutes, centrifuged and the separated serum is used to analyze the biochemical parameters in the ESRD patients as well as in the control subjects.

2.2 Determination of Genotypes

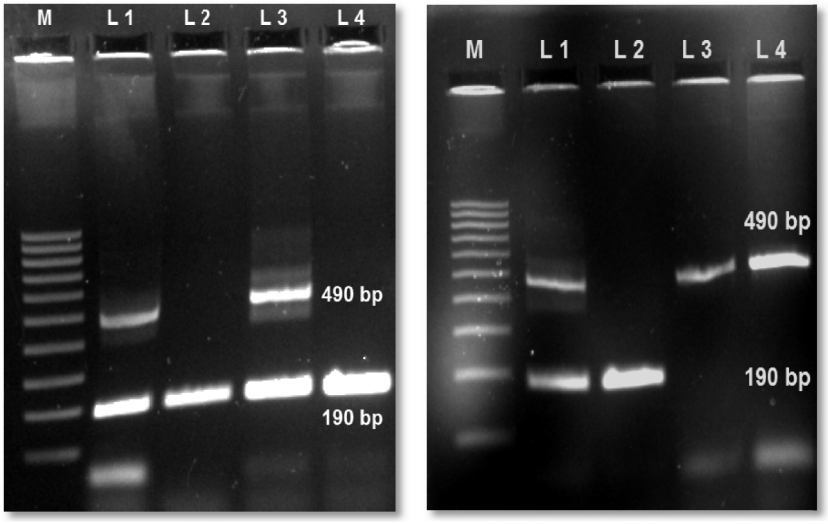
Genomic DNA was isolated and purified from whole blood using a DNA purification kit (Fermentas spin columns, Canada). The ACE genotypes (DD, ID and II) were determined by polymerase chain reaction (PCR) method [10]. The sense primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and anti-sense primer: 5'-GATGTGGCCATCACATTCGTCAGAT-3'. The PCR was carried out in 10 µl of 10 mmol/l Tris-HCl, 50 mmol/l KCl, 2 mmol/l $MgCl_2$, 200 µmol/l of each of the four deoxynucleotides, 1 µmol/l each of the primers, and 0.4 units *Taq* polymerase. After an initial denaturation at 94ºC for 3 min, the DNA was amplified by 30 PCR cycles of denaturation at 94ºC for 30 s, annealing at 58ºC for 45 s, and extension at 68ºC for 45 s, followed by a final extension at 68ºC for 7 min. The amplified PCR products were separated by electrophoresis on 2% agarose gel and the DNA was visualized under ultra violet (UV) transilluminator after staining with ethidium bromide. The insertion (I) allele was detected as band of 490 bp fragment and deletion (D) allele was identified as a band of 190 bp fragment. Therefore, there were three genotypes after electrophoresis: A 490 bp band (genotype II), 190 bp band (genotype DD), or both 490 and 190 bp band (genotype ID). All of the samples were genotyped twice independently in the Genetic Unit of Faculty of Medicine in Mansoura University. Genotyping was conducted twice, and the concordance rate of the two independent genotyping assays was 99%, see (Fig. 1).

2.3 Statistical Analysis

Data were obtained using SPSS version 19. Data were expressed as means ± standard deviation (SD). Results of ESRD patients and control subjects were performed using Hardy-Weinberg equilibrium, chi-square 2x2 analysis and independent t-test. Chi square and odds ratio were calculated with 95% confidence interval. A *p* value less than .05 was considered statistically significant.



(A)



(B) (C)

Fig. 1. Ethidium bromide-stained 2% agarose gel of representative PCR products of ACE gene I/D polymorphism. Lane (M) is 100 bp DNA Ladder. (A): The DD genotype (190 bp, Lanes 2, 4 and 5), the ID genotype (490 bp, 190 bp, Lanes 1 and 7), the II genotype (490 bp, Lane 3 and 6). (B): The DD genotype (190 bp, Lanes 2 and 4), the ID genotype (490 bp, 190 bp, Lanes 1 and 3). (C): The DD genotype (190 bp, Lane 2), the ID genotype (490 bp, 190 bp, Lane 1), the II genotype (490 bp, Lanes 3 and 4).

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the clinical characteristics of ESRD patients (n = 147) and control subjects (n = 140). The renal functional parameters showed highly significant differences, ($P < .0001$), between two groups.

Table 2 shows the distribution of angiotensin converting enzyme (ACE) insertion/deletion (I/D) genotypes and alleles frequency in the ESRD patients (n = 147) and in the control subjects (n = 140).

The frequencies of ACE DD genotype and D allele were significantly higher in the ESRD patients, (61.9% and 79.6%, respectively), as compared with the control subjects (47.1% and 71.1%, respectively), ($P = .012$, OR = 1.82, 95% CI = 1.139-2.915 for DD) and ($P = .018$, OR = 1.6, 95% CI = 1.082-2.33 for D). In contrast, the frequencies of ACE ID genotype and I allele were significantly lower in the ESRD patients as compared with the control subjects. The frequency of II genotype was no significantly decreased in the ESRD patients as compared with the control subjects.

Table 1. Clinical characteristics of ESRD patients and control subjects

Parameters	ESRD patients (Mean ± SD)	Control subjects (Mean ± SD)	*P*-value
Hemoglobin (g/dL)	7.8 ± 1.2	14.1 ± .8	< .0001**
Creatinine (mg/dL)	9.5 ± .3	.5 ± .1	< .0001**
BUN (mg/dL)	88.8 ± 8.2	7.2 ± .5	< .0001**
Uric Acid (mg/dL)	6.6 ± .2	4.3 ± .3	< .0001**
Sodium (mmol/L)	131.9 ± 3.3	144.9 ± 2.2	< .0001**
Potassium (mmol/L)	5.3 ± .2	4.1 ± .1	< .0001**
Calcium (mg/dL)	9 ± .2	10.1 ± .1	< .0001**
Phosphorus (mg/dL)	5.5 ± .2	3.2 ± .2	< .0001**
Total Protein (g/dL)	7.2 ± .1	8.4 ± .1	< .0001**
Albumin (g/dL)	3.2 ± .1	4.5 ± .1	< .0001**
Total Bilirubin (mg/dL)	.7 ± .1	.6 ± .1	< .0001**
ALT (U/L)	20.7 ± 6.4	14 ± .7	< .0001**
AST (U/L)	22.2 ± 3.6	15.6 ± .7	< .0001**
Alkaline Phosphatase (U/L)	139.7 ± 63.1	80 ± 4.4	< .0001**
Total Cholesterol (mg/dL)	186.5 ± 46.5	143.2 ± 11.4	< .0001**
Triglyceride (mg/dL)	205 ± 62.2	88.9 ± 12.2	< .0001**
HDL (mg/dL)	27.9 ± 1.6	49.2 ± 4	< .0001**
LDL (mg/dL)	126.9 ± 36.2	82.9 ± 16.4	< .0001**
Systolic BP (mm Hg)	144.6 ± 10.5	116.2 ± 2.3	< .0001**
Diastolic BP (mm Hg)	90 ± 3.2	72.9 ± 4	< .0001**
Dialysis Duration (years)	7.94 ± 4.05	0	< .0001**
Age (years)	44 ± 14.8	39.7 ± 14.1	.01*
Gender (Men / Women)	86 / 61	84 / 56	.79

**significant value (P < .05). Results were expressed as mean ± SD. SD: standard deviation. ESRD: end stage renal disease.*

Table 2. ACE genotypes and alleles frequency in ESRD patients and control subjects

ESRD Patients Hardy–Weinberg Equilibrium (HWE)				
	Observed Frequency N(%)	**Expected Frequency N(%)**	**X^2**	***P*-value**
Genotypes				
DD	91 (61.9 %)	93.1 (63.3 %)		
ID	52 (35.4 %)	47.8 (32.5 %)		
II	4 (2.7 %)	6.1 (4.2 %)		
Alleles				
D	234 (79.6 %)	-	**1.2**	**.28**
I	60 (20.4 %)	-		
Control Subjects Hardy–Weinberg Equilibrium (HWE)				
	Observed Frequency N (%)	**Expected Frequency N(%)**	**X^2**	***P*-value**
Genotypes				
DD	66 (47.1 %)	70.7 (50.5 %)		
ID	67 (47.9 %)	57.6 (41.1 %)		
II	7 (5 %)	11.7 (8.4 %)		
Alleles				
D	199 (71.1 %)	**-**	**3.76**	**.1**
I	81 (28.9 %)	**-**		

Comparison of different genotypic and allelic states			
	X^2	***P*-value**	**OR (95% CI)**
D v/s I	5.6	.018*	1.6 (1.082-2.33)
DD v/s ID+II	6.3	.012*	1.82 (1.139-2.915)
ID v/s DD+II	4.6	.032*	1.67 (1.044-2.692)
II v/s DD+ID	1.01	.32	1.88 (.539-6.574)

**Significant value (P < .05). Results obtained using Hardy-Weinberg equilibrium and chi-square 2x2 analysis. I: insertion polymorphism. D: deletion polymorphism. ID: insertion-deletion polymorphism. OR: odds ratio. CI: confidence interval.*

3.2 Discussion

End stage renal disease (ESRD) is a multifactorial disease. Clinically, ESRD is an advanced form of chronic renal failure where renal function has declined to ~10% of the normal prior to initiation of dialysis or transplantation. The genetic variability in the number of genes affecting the pathogenesis of ESRD is postulated to contribute in a variable manner [11]. Renin-angiotensin-aldosterone system (RAAS) is an important circulation endocrine system in human body, which can adjust blood pressure by action on vascular tension, kidney blood flow dynamics and electrolyte balance and is closely related with vascular endothelial proliferation and interactions of many cytokines [12].

Angiotensin-I-converting enzyme (ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. By stimulation of renin, angiotensinogen is converted to angiotensin I. ACE then converts angiotensin I to angiotensin II, the main active product of the RAAS [13]. Angiotensin II has several actions, including vasoconstriction, stimulation of the central nervous system, release of vasopressin and aldosterone, stimulation of protein synthesis, growth and cellular migration, pro-inflammatory genes and oxidative stress [14]. The ACE is as a part of RAAS has a key role in both cardiovascular and renal pathophysiology [13]. The ACE gene spans 21 kilo bases is located on the 17th chromosome q23 and consists of 26 exons and 25 introns. The polymorphism exists in

intron 16. The genotype is classified into three types: deletion homozygotes, DD; insertion homozygotes II and heterozygotes, ID [15].

In the present study, we have observed that there were highly significant differences in the biochemical parameters between ESRD patients and control subjects ($P < .0001$).

The present study showed that there were highly association between ACE I/D gene polymorphism and ESRD. The distribution of ACE DD genotype and D allele frequency were significantly increased in the ESRD patients as compared with the control subjects. They were considered as risk factors for ESRD; while the distribution of ACE ID genotype and I allele frequency were significantly decreased in the ESRD patients as compared with the control subjects ($P < .05$).

These results were in agreement with studies [1,9,11] who observed that there was a strong association of ACE DD genotype with ESRD as a risk factor for ESRD. Our results were in agreement with a study [16] who found that the allelic frequency of D allele of ACE I/D gene polymorphism was higher in the Malaysian ESRD patients than in the control subjects. In contrast to those studies, there was a study [17] failed to support the hypothesis that ACE I/D gene polymorphism plays a major role in the risk of ESRD. Also, the study [8] showed that there was no association between ACE genotypes and/or D allele and ESRD.

The current investigation could provide new evidence regarding the role of the ACE gene in the pathogenesis of ESRD, which may have significant clinical implications. The ACE (DD) genotype and D allele can be used as predicting and prognostic factors to avoid any complications lead to ESRD in the Egyptian population.

4. CONCLUSION

This study investigates that there is an association between ACE DD genotype and D allele, and ESRD as risk factors, while I allele are highly associated with ESRD, as protective factor, in the Egyptian population.

CONSENT

Informed written consent was obtained from all participants, (patients and control subjects), in this study.

ETHICAL APPROVAL

This study was approved by Ethical Board of the Mansoura University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhou T, Yin S, Qin Y. Association between angiotensin converting enzyme insertion/deletion gene polymorphism and end-stage renal disease susceptibility. Journal of the Renin-Angiotensin-Aldosterone System. 2012;1-10.

2. Elshamaa MF, Sabry SM, Bazaraa HM, Koura HM, Elghoroury EA, Kantoush NA, et al. Genetic polymorphism of ACE and the angiotensin II type1 receptor genes in children with chronic kidney disease. Journal of Inflammation. 2011;8:20.
3. El-Minshawy O. End stage renal disease in El-Minia governorate, Egypt: data of year. Nephrol-Urol Mon. 2011;3(2):118-121.
4. Sisman Y, Gokce C, Sipahioglu M, Ertas ET, Unal A, Oymak O, et al. Torus palatinus in end-stage renal disease patients receiving peritoneal dialysis: does renal osteodystrophy play a role. Journal of Dental Sciences. 2012;154-158.
5. Marcus Y, Shefer G, Stern N. Adipose tissue renin–angiotensin–aldosterone system (RAAS) and progression of insulin resistance. Molecular and Cellular Endocrinology. 2012;1-14.
6. Eleni S, Dimitrios K, Vaya P, Areti M, Norma V, Magdalini G. Angiotensin-I converting enzyme gene and I/D polymorphism distribution in the Greek population and a comparison with other European populations. Journal of Genetics. 2008;87(1):91-93.
7. Li Z, Pan X, Han B, Han H, Zhang Z, Gao L. No association between ACE polymorphism and risk of nasopharyngeal carcinoma. Journal of the Renin-Angiotensin-Aldosterone System. 2011;13(1):210-215.
8. Buraczynska M, Ksiazek P, Drop A, Zaluska W, Spasiewicz D, Ksiazek A. Genetic polymorphisms of the renin-angiotensin system in end-stage renal disease. Nephrol Dial Transplant. 2006;21(4):979-983.
9. Tripathi G, Dharmani P, Khan F, Sharma RK, Pandirikkal V, Agrawal S. High prevalence of ACE DD genotype among north Indian end stage renal disease patients. BMC Nephrology. 2006;7:15.
10. Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, et al. Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. Diabetes. 2002;51:1986-1988.
11. Tripathi G, Sharma RK, Baburaj VP, Sankhwar SN, Jafar T, Agrawal S. Genetic risk factors for renal failure among North Indian ESRD patients. Clinical Biochemistry. 2008;41:525-531.
12. Han JG, Jin H, Gao LB, Zhang J, Deng XK, Li LJ, et al. Relationship between polymorphisms of angiotensin-converting enzyme gene insertion/deletion, endothelial nitric oxide synthase gene intron 4 VNTR and risk for cervical cancer. Life Science Journal. 2012;9(2):100-104.
13. Kumar PS, Jaganmohan P, Subbarao M. Studies on association of ACE insertion/deletion polymorphism in smokeless tobaccoInduced renal failures in costal belt of Andhra Pradesh, India. Am-Euras. J. Sci. Res. 2012;7(5):220-225.
14. Fyhrquist F, Eriksson A, Saijonmaa O, Nordestgaard BG, Kontula K, De Faire U, et al. Telomere length is associated with ACE I/D polymorphism in hypertensive patients with left ventricular hypertrophy. Journal of the Renin-Angiotensin-Aldosterone System. 2012;1-8.
15. Nikzamir A, Nakhjavani M, Golmohammadi T, Dibai L, Saffary R. Polymorphism in the angiotensin-converting enzyme (ACE) gene and ACE activity in type 2 diabetic patients. Acta Medica Iranica. 2008;46(4):277-282.
16. Ali A, Vasudevan R, Ismail P, Seong CLT, Chakravarthi S. Analysis of insertion/deletion polymorphisms of the angiotensin converting enzyme gene in Malaysian end stage renal disease patients. Journal of the Renin-Angiotensin-Aldosterone System. 2011;1-7.

17. Pereira TV, Nunes CF, Rudnicki M, Magistroni R, Albertazzi A, Pereira AC, et al. Influence of ACE I/D gene polymorphism in the progression of renal failure in autosomal dominant polycystic kidney disease: a meta-analysis. 2006;21(11):3155-63.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=380&id=12&aid=2935