Original Article

Allelic frequency and genotype distributions of angiotensin converting enzyme gene among three essential Iraqi ethnic group population

Salwa J. Al-Awadi[1], Abdulameer M. Ghareeb[2], Wisam H. Salo[3]

Abstract

Allelic frequency and genotype distributions of angiotensin converting enzyme gene among three essential Iraqi ethnic group population

Salwa J. Al-Awadi[1], Abdulameer M. Ghareeb[2], Wisam H. Salo[3]

IJPS, 2012; 8(1) : 27-33

Received : 27 September 2011 **Accepted :** 20 January 2012

Human angiotensin-converting enzyme (ACE) gene has an insertion-deletion (I/D) polymorphic which produce three allelic variants: II, ID & DD that associated with many genetic disease. ACE gene polymorphisms have been studied in different ethnic populations. The polymorphisms of ACE gene were investigated using polymerase chain reaction to detect the I/D mutation in a total of 191 healthy individual's sample selected randomly from northern and central Iraqi ethnics groups: Arab, Kurd and Turkman. The results showed a high D allele frequency in Arab population (D 65.5%, I 34.5%), high I allele frequency in Kurd population (D 42.3%, I 57.7%), while an equal value of D and I (50%) in Turkman population. Allele frequency D and I was calculated according to the Hardy-Weinberg equilibrium. The DD, ID and II genotypes distribution were observed in Iraqi ethnic groups as: 50 (50%), 31 (31%) and 19 (19%) in Arab population and 10 (20%), 24 (46%) and 18 (34%) in Kurd population while the result showed 12 (30.7%), 15 (38.4%) and 12 (30.7%) in Turkman population, respectively. We conclude from this study that the frequency of ACE gene polymorphic are different in the main populations of Iraq.

Key words: ACE (angiotensin-converting enzyme), ACE polymorphism. Iraqi Ethnics group

[1] Assist. Prof. Ph.D. in Medical Molecular genetics, Genetic Engineering and Biotechnology Institute, Baghdad University, Baghdad, Jaderyah, Iraq.

[2] MSc. In Biotechnology, Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq, Baghdad, Jaderyah, Iraq

[3] MSc. In Biotechnology, Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq, Baghdad, Jaderyah, Iraq Correspondence to Dr. Salwa J. AL-Awadi, Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq, Baghdad, Jaderyah (GEBI)-Baghdad University , Email: fsalwaj@yahoo.com

Introduction:

Angiotensin-converting enzyme (ACE) is a key enzyme of the rennin-angiotensin system. It is localized in the kidney (Braam *et al.,* 1993). The ACE catalyzes the conversion of angiotensin I to the biologically active peptide, angiotensin II which is involved in the control of fluid-electrolyte balance and systemic blood pressure (Wang *et al.,* 2000). The ACE is encoded by a 21 Kb gene that consists of 26 exons and is located on chromosome 17q23. A polymorphism of the ACE gene involves the insertion (I) or deletion (D) of a 287 bp AluYa5 repeat sequence inside intron. Although I/D polymorphism is located in a non-coding region of the ACE gene it is not silent and that the D allele is associated with increased activity of ACE in serum (the highest serum ACE activity was seen in the DD genotype while the lowest seen in II genotype) (Rigat *et al.,* 1990, Sakuma *et al.,* 2004).

Moreover, various reports were published suggesting inter-ethnic variations in the frequency of allelic forms of the ACE genes. It has been shown that plasma level of ACE varies significantly in different populations but it is similar between the members of a family. Also, extensive interethnic variations in the frequency of the I and D alleles have been reported worldwide for various populations. The frequencies of the I and D alleles of the ACE gene among Sudanese, Somalis and Arab nationals of the United Arab Emirates and Oman indicate a preponderance of the D allele among the Arab and African populations studied (Bayoumi *et al.,* 2006). Identification of specific individual genetic backgrounds has a critical role in understanding of the genetic variations among a population and in determining the susceptibility to different human diseases (Abdi-rad *et al.,* 2011).

Several investigators suggested that the genetic predisposition of the ACE I/D polymorphism was associated with several diseases including coronary heart diseases, stroke, hypertension and diabetes mellitus (Hubert *et al.,* 1992, Tarnow *et al.,* 1998, Gesang *et al.,* 2002, Moleda *et al.,* 2007).

Gender may be an important factor as recent studies have demonstrated an association between the D allele and hypertension in white American men (O'Donnel *et al.,* 1998) as well as Japanese men (Johanning *et al.,* 1995) but not in women. The objective of the present study is to compare the frequency distribution of the ACE I/D polymorphism in three Iraqi ethnic group population-based unrelated samples (b) to study the potential modifying effect of gender.

Materials and Methods:

Samples collection and DNA extraction: Blood samples were obtained from 191 healthy individuals selected randomly of northern and central Iraqi ethnics groups presented as 100 Iraqi Arab populations (53 male and 47 female) 52 Iraqi Kurd population(34 male 18 female) and 39 Iraqi Turkman population (21 male 18 female). Blood was collected in tube containing EDTA. DNA was extracted from the samples by wizard genomic DNA purification kit(Promega-Canada) according to the "Isolating Genomic DNA from whole blood protocol".

PCR Amplification and Genotyping: The specific segment of ACE gene was amplified by polymerase chain reaction (PCR) using the following primers using the specific primers ACE-F (5-TGGA-GACCACTCCCATCCTT TC-3) and ACE-R (5-GATGT-GGCCACACATTCGTCAGA T-3) (Alpha DNA, Monitorial, Canada). The PCR amplification was performed in a total volume of 25 µl containing 5 µl DNA (conc. 20 ng), 12.5 µl Go Taq green master mix 2X (green maschuiter mix, promega) is a premixed ready to use solution contains Taq DNA polymerase (Bayoumi *et al.,* 2006). Two icroliters of each primer (10 pmol/1µL) and up to 25µl with nucleases free water. The thermal cycling was as follows: initial denaturation at 94 °C for 5 min, followed by 25 cycles of 94 °C for 1, 57°C for 1 min, and 72 °C for 2 min, with final incubation at 72 °C for 5min (Bayoumi *et al.,* 2006) using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). Two-percent agar gel with ethidium bromide was used for electrophoresis of

Results

At baseline Iraqi blood samples were obtained from 191 healthy individual's sample selected randomly of northern and central Iraqi ethnics group: Arab, Kurd and Turkman according to the numbers 100, 52, 39 respectively. The PCR result showed (Figure 1) the homozygous individuals for the D allele (DD genotype) were identified by the presence of a single 190 bp PCR product. The homozygous for I allele (II genotype) were identified by the presence of a single 490 bp PCR product. The heterozygous individuals (ID genotype) were identified by the presence of both 190 and 490 bp PCR products.

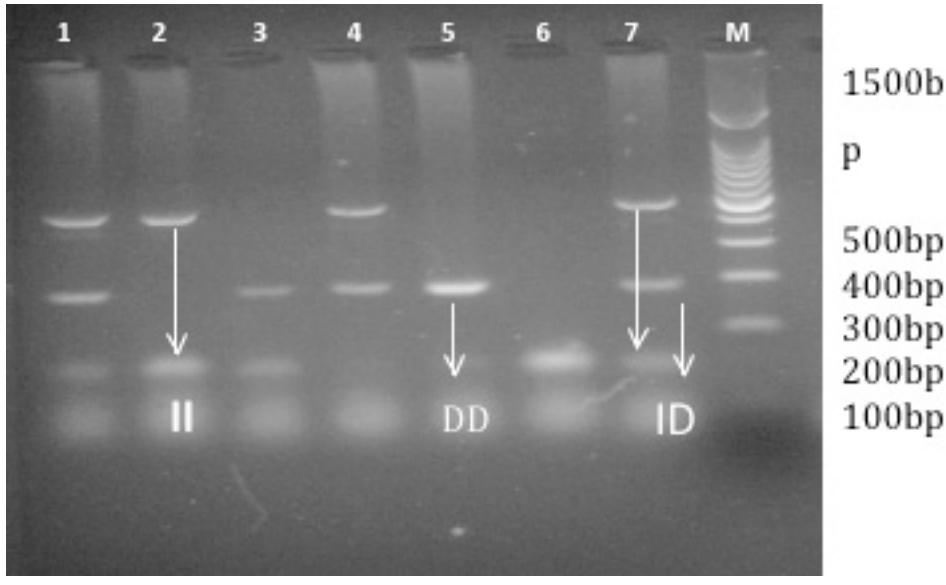

Figure. (1): PCR products of ACE gene I/D polymorphism shows the D allele (190 bp, lane 3, 5), the I allele (490 bp, lane 2), and the ID genotype (190 bp, and 490 bp, lane 1, 4, 7); lane 6 (Negative control), electrophoresis carried on 2% agarose gel, 5V/cm at 1hr. Lane M is a size marker (100 bp DNA Ladder Promega).

The *ACE* allele frequency D and I of Iraqi populations distributed to three ethnic groups calculated according to the Hardy-Weinberg equilibrium. The results showed a high D allele frequency in Arab population (*D* 65.5%, I 34.5%), high I allele frequency in Kurd population (*D* 42.3%, I 57.7%) and an equal value of D and I (50%) in Turkman population as shown in table 1.

The DD, ID, and II genotypes distribution of three ethnic groups showed as: 50 (50%), 31 (31%), and 19 (19%) within Arab group participants, respectively. While Kurd group showed genotypes as, 10 (20%), 24 (46%), and 18 (34%), respectively. Also Turkmen group observed as 12 (30.7%), 15 (38.4%), and 12 (30.7%), respectively. As a result, the genotype distribution of the alleles in Iraqi population was: DD: 66 (34.5%), ID: 75(39%) and II: 50 (27%), as shown in table 2. The dominant genotype was the DD in Iraqi population.

Table 1. Angiotensin-Converting Enzyme Alleles frequency among a population of three ethnics groups in Iraq.

Ethnic group	ACE Allele	Allele frequency (%)*		
		All	Men	Women
Arab group	D	65.5	62.3	61.6
No.100 (Men 53,Women 47)	I	34.5	37.7	38.4
Kurd group	D	42.3	36.8	52.8
No.52 (Men 34,Women 18)	I	57.7	63.2	47.2
Turkman group	D	50	45.2	55.6
No.39 (Men 21,Women 18)	I	50	54.8	44.4

* calculations are according to the Hardy-Weinberg equilibrium

Table 2. Angiotensin-Converting Enzyme genotype distribution among three ethnics groups in Iraq.

ACE Genotype	Arab(n=100)	Kurd(n=52)	Turkman(n=39)
DD	50%	20%	30.7%
I/D	31%	46%	38.4%
II	19%	34%	30.7%

More important fact was that genotype distributions between male and female were different hereditarily in Iraqi population. According to table 3, the distribution of ACE genotype of DD, ID and II subjected in Arab: (n= 100, 53 male & 47 female) showed that 25 (47.17%), 16 (30.19%) and 12 (22.6 %) men, 19 (40.43%), 20 (42.55%) and 8 (17.02%) women. While Kurd group showed genotypes as follow: (n=52, 34 male & 18 female) 6 (17.6%), 13 (38.2%) and 15 (44.2 %) men, 4 (22.2%), 11 (61.1%) and 3 (13.7%) women. Also Turkmen group (n= 39, 21 male & 18 female) observed in 5 (23.8%), 9 (42.8%) and 7 (33.3 %) men, 7 (33.6%), 6 (27.7%) and 5 (27.7%) women as shown in table 3.

Table 3. Angiotensin-Converting Enzyme genotype distribution within male and female of three ethnics groups in Iraq.

Ethnic group		DD	ID	II
Arab group n=100	Male n=53	25 (47.17%)	16 (30.19%)	12 (22.6 %)
	Female n=47	19 (40.43%)	20 (42.55%)	8 (17.02%)
Kurd group n=52	Male n=34	6 (17.6%)	13(38.2%)	15 (44.2 %)
	Female n=18	4 (22.2%)	11 (61.1%)	3 (13.7%)
Turkmen group n=39	Male n=21	5 (23.8%)	9 (42.8%)	7 (33.3 %)
	Female n=18	7 (33.6%)	6 (27.7%)	5 (27.7%)

Discussion and Conclusion

Several investigations have provided a substantial database on genotype distribution in a number of population groups (Katsuya *et al.,* 1998). The ethnic background appears to influence the ACE gene I/D polymorphism globally. It demonstrates the importance of using a homogeneous population in the selection of the study samples, making possible the identification of more exact distributions of the ACE genotypes among racial populations.

In accordance with previous studies performed on Arab populations, such as the Tunisians, Algerians and Moroccans (SAS, 2004), the Somalis, the Omanis, the Emiratis, and the Sudanese (Comas *et al.,* 2000), the genotype DD were the dominant gene in the study population too. These results were expected because most of Iraqi Arab population came from tribes that migrate from Arabian Peninsula as most other Arab populations (Frossard *et al.,* 1997). Compared to other geographic groups the frequency of the D allele in the Arab populations is among the highest reported. The frequency of the D allele is highest among sub-Saharan Africans (Salem *et al.,* 2009) and Arabs (Barley *et al.,* 1994, SAS, 2004), moderate for Caucasians (Ulu et al., 2006) and low among various Asian populations (Cambien et al., 1992, Barley *et al.,* 1994, Saha *et al.,* 1996).

The Yanomami Indians, Samoans and Australian Aborigines seem to have the lowest frequencies (Lee *et al.,* 1994, Salem *et al.,* 2009). The worldwide distribution of the D allele suggests that the ancestral state present in the human population was the D allele and that an AluYa5 (the youngest AluY subfamily in the human genome) element later inserted at the locus (Lester *et al.,* 1999),

The higher frequency of I allele in the Kurd groups is in agreement with Asiatic, Mongoloid and Indian populations (pasha et al 2002) but differs from the Americans, Caucasians and Europeans, who had a greater frequency of D allele and were reported to have a higher in Arab population. Turkman have the same result that obtained from previous studies on central Asian population showed high frequency of I allele and high abundance of ID genotype distribution in Kyrgyz, Kazakhs and Uzbeks (Polupanov *et al.,* 2007).

With regard to our data, at present there is no explanation for the higher frequency of the D allele in the Arab and the I allele in Kurd and Turkmen populations. The reasons for this difference could be the genetic drift as is found in many other polymorphisms such as that of blood groups. The influence of some unknown sampling bias such as community bias cannot be excluded.

The difference was significant between the male and female groups; it showed that the dominant genotype in Iraqi Arab male group was the DD while dominant genotype in Iraqi Arab female group was the ID. Where in Kurd group the genotype ID was the dominant in both female and male. In Turkmen group the ID genotype was the dominant in male while the DD genotype was the dominant in female. It became clear that the distribution of ACE gene is different between male and female, it is of our interest the ACE genes is related so with the presence of X chromosome due to the obvious difference between male and female group (Yang *et al.,* 1997)

Conclusion:

The results of the distribution of the ACE I/D gene polymorphism obtained for the D allele among Iraqi population are comparable to those obtained from previous studies in other population, add to the data indicating the wide variations observed in the frequency of the ACE alleles among the peoples of the world and highlights that great care needs to be taken when interpreting clinical data on the association of the ACE alleles with different diseases especially for hypertension and diabetes. The result of this study supports the hypothesis that the DD genotype is in linkage disequilibrium with a functional variant of the ACE gene.

Acknowledgements:

We would like to thank all volunteers 'that agreed to give us the blood samples, and Dr.Ismael / Karkuk Hospital for provide us with Kurd and Turkman blood samples.

References:

Abdi-rad, I, Bagheri, M. Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism in General Population of West Azarbaijan, *Iran*. *Iranian Journal of Kidney Diseases* 2011; 5(2):86-92

Bayoumi RA, Simsek M, Yahya TM. Insertion-Deletion Polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene among Sudanese, Somalis, Emiratis, and Omanis. *Human Biology* 2006; 78: 103-108.

Barley J, Blackwood A, Carter ND, Crews DE, Cruickshank JK, Jeffery S, Ogunlesi AO, Sagnella GA. Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. *J Hypertens* 1994; 12:955-957.

Braam B, Mitchell KD, Fox J, Navar LG. Proximal tubular secretion of angiotensin II in rats. *Am J Physiol* 1993; 264: 891-898.

Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; 359: 641-644.

Comas D, Calafell F, Benchemsi N, Helal A, Lefranc G, Stoneking M, Batzer MA, Bertranpetit J, Sajantila A. Alu insertion polymorphisms in NW Africa and the Iberian Peninsula: evidence for a strong genetic boundary through the Gibraltar Straits. *Hum Genet* 2002; 107:312-319.

Frossard PM, Obineche EN, Elshahat YI, Lestringant GG. Deletion polymorphism in the angiotensin-converting enzyme gene is not associated with hypertension in a Gulf Arab population. *Clin Genet* 1997; 51:211-213.

Gesang L, Liu G, Cen W, Qiu C, Zhuoma C, Zhuang L, Ren D, PincuoZ, Chan Y. Angiotensin-converting enzyme gene polymorphism and its association with essential hypertension in a Tibetan population. *Hypertens Res* 2002; 25:481-485.

Johanning C L, Johnston K E, Tamura T, Goldenberg R L. Ethnic differences in angiotensin converting enzyme gene polymorphism; *J Hypertens* 1995; 13 710–711

Katsuya T, Baba S, Higaki J, Mannami T, Sato N, Ogihara T. The deletion polymorphism of the angiotensin converting enzyme gene increases the risk of hypertension in a large Japanese general population: the Suita study. *Circulation* 1998; 98 :I- 859

Lee EJ. Population genetics of the angiotensin-converting enzyme in Chinese. *Br J Clin Pharmacology* 1994; 37: 212-214.

Lester S, Heatley S, Bardy P, Bahnisch J, Bannister K, Faull R, Clarkson A. The DD genotype of the angiotensin-converting enzyme gene occurs in very low frequency in Australian Aboriginals. *Nephrol Dial Transplant* 1999; 14: 887-890.

Moleda P, Majkowska L, Safranow K, Adler G, Goracy I. Relationship between I/D polymorphism of angiotensin I converting enzyme gene and microvascular complications in type 2 diabeticpatients. *Przegl Lek* 2007; 64:134-139.

O'Donnel CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ. Evidence for association and genetic linkage of the angiotensinconverting enzyme locus with hypertension and blood pressure in men but

not in women in the Framingham heart study. *Circulation* 1998; 97:1766-1772.

Pasha M, Kumar R. *et.al.* Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in Indian populations of different ethnic origins.*J. Biosci* 2002; 27(1):67-70.

Polupanov, A. Halmatov, A.; Pak, O.; Romanova, T. The I/D polymorphism of the angiotensin converting enzyme gene as a risk factor for ischemic stroke in patients with essential hypertension in Kyrgyz population Tork Kardiyol Dern Ar 2007; 35:347-353

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86:1343-1346.

Rigat B, Hubert P, Corvol P, *et al.* PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidy 1 carboxypeptidase 1).*Nucleic Acids Res* 1992; 20:1433-1439.

Saha N, Talmud PJ, Tay JS, Humphries SE, Basair J. Lack of association of angiotensin-converting enzyme (ACE). Gene insertion/ deletion polymorphism with CAD in two Asian populations. *Clin Genet* 1996; 50:121-125

Sakuma T, Hirata R D, Hirata M H. Five polymorphisms in gene candidates for cardiovascular disease in Afro-Brazilian individuals. *J Clin Lab Anal* 2004; 18: 309–316.

Salem A, Batzer MA. High frequency of the D allele of the angiotensin-converting enzyme gene in Arabic population's .*BMC Research Notes* 2009; 2: 99-102.

SAS. SAS / STAT Users Guide for Personal Computers. Release 7.0. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).2004.

Tarnow L, Gluud C, Parving H-H. Diabetic nephropathy and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. *Nephrol Dial Transplant* 1998; 13:1125-1130

Ulu A, Elsobky E, Elsayed M, Yldz Z, Tekin M, Akar N. Frequency of five thrombophilic polymorphisms in the Egyptian population. *Turkish Journal of Hematology* 2006; 23: 100-103.

Wang JG, Staessen JA. Genetic polymorphisms in the reninangiotensin system: relevance for susceptibility to cardiovascular disease. *Eur J Pharmacol* 2000; 410:289-302.

Yang YM, Park JH, Moon E S. Genotype distribution and gene frequency of angiotensin I- converting enzyme in Korean population. *J Gene Med* 1991; 1: 17-22.