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Genotype Distribution of Angiotensin I- Converting Enzyme in Iraqi Arab Population

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Abstract :

The angiotensin-converting enzyme (ACE) gene in humans has an insertion-deletion (I/D) polymorphic which produce three allelic variants: II, ID & DD that associated with population relativity and relation with many genetic disease. The polymorphisms of ACE gene were investigated using polymerase chain reaction. The results showed a high frequency of the DD allele among the Iraqi Arab populations (50%), which is similar to those obtained from previous studies for Arab populations. More ever the distribution of ACE genotype among male and female (n= 100, 53male & 47 female) showed DD: 47.17%, II: 22.6% & ID: 30.19% in the male group and DD: 40.43%, II: 17.02% & ID42.55:% in the female group. Significant association of hypertension & diabetic with D allele of the ACE gene has been in Iraqi Arab population.

Key words: *ACE (angiotensin-converting enzyme) genotype, ACE polymorphism.*

Introduction:

Angiotensin-converting enzyme (ACE), a key enzyme of the rennin-angiotensin system, is localized in the kidney (1). 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 (2).

The ACE is encoded by a 21 Kb gene that consists of 26 exons and 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)) (3,4).

Moreover, reports were published suggesting inter-ethnic variations in the frequency of allelic forms of the ACE genes. 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 (5)

Several investigations suggested that genetic predisposition of the ACE I/D polymorphism with several diseases including coronary heart diseases, stroke (6), hypertension and diabetes mellitus (7).

However, conflicting results have been reported regarding the association between ACE polymorphism and disease (8, 9).

Gender may be an important factor as recent studies have demonstrated an association between the D allele and hypertension in white American men (10) as well as Japanese men (11) but not in women.

The objectives of the present study for using: (a) on compare the frequency distribution of the ACE I/D polymorphism in Iraqi Arab population-based unrelated samples (b) examine the association of I/D polymorphism with family history of chronic disease (c) study the potential modifying effect of gender.

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Materials and Methods:

Samples collection & DNA extraction: Blood samples were obtained from unrelated and were randomly selected of 100 Iraqi Arab populations (53 male and 47 female,).

Blood was collected in tube containing EDTA; DNA was extracted from the samples by wizard genomic DNA purification kit, (Promega) according to the "Isolating Genomic DNA from whole blood protocol". The volume of the extracted DNA solution was usually 100 µl were stored at -20 °C.

PCR Amplification & 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-TGGAGACCACTCCC ATCCTTTC-3) and ACE-R (5-GATGTGGCCATCACATTCGTCAGAT-3). (Alpha DNA, Canada). The PCR amplification was performed in a total volume of 25 µl containing 5µl DNA (conc. 20 ng), 12.5 µl of 2X Go Taq green master mix. The reaction were performed in GeneAmp 7900 (ABI, USA), with 4 min of initial denaturation at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s at 57°C and 1 min at 72°C and one cycle of 10 min at 72°C as a final extention.

Statistical Methods: The statistical analysis system – SAS 2004 program (12) was used to investigate the effect of different factors (Gender, Family history & Age) on distribution of the gene polymorphism in this study (ID, D & II). The Chi-square ($\chi 2$) test was used to the significant compare between percentage on 0.05 & 0.01 level of probability.

Results and Discussion:

At baseline 100 Iraqi Arab blood samples were obtained from unrelated randomly selected of populations (53 male and 47 female). According to questioner list that contain the: gender, medical family history with chronic disease (hypertension& diabetes).

The PCR result showed that, 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 while the heterozygous individuals (ID genotype) were identified by the presence of both 190 and 490 bp PCR products as showed in figure: (1).

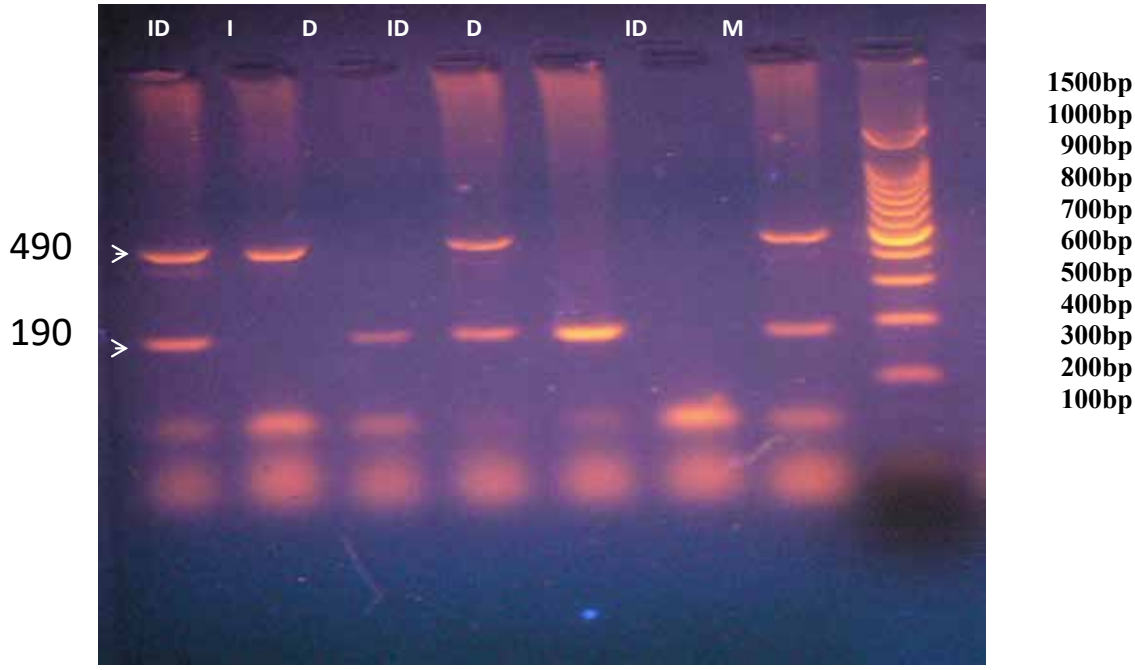

Figure. (1): PCR products of ACE gene I/D polymorphism shows the D allele (190 bp, lane 3, 5), the I allele (490 bp, lane 2,5), and the ID genotype (190 bp, and 490 bp, lane1, 4,6); lane 7, electrophoresis carried on 1.5% agarose gel,5V/cm for 1hr. is a size marker (100bp DNA Ladder Promega).

The distribution of I, D alleles in Iraqi Arab population that we studied showed in table (1). In accordance with previous studies performed on Arab populations, such as Tunisians, Algerians , Moroccans (13), Somalis, Omanis, Emiratis, and the Sudanese (5, 14), the result showed that the genotype DD were the dominated in Iraqi Arab populations.

These results were expected because most of Iraqi Arab population came from tribe that migrate from Arabian Peninsula as most other Arab populations were lived (15, 5). 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 (16), Arabs (13, 17), moderate for Caucasians (18) and low among various Asian populations (19, 17, 20).

The Yanomami Indians, Samoans and Australian Aborigines seem to have the lowest frequencies (16, 21). 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 (22),

Table (1): the distribution I, D alleles in Iraqi Arab population.

genotype	No. of 100 sample	percentage
II	**19**	**19%**
ID	**31**	**31%**
DD	**50**	**50%**

More important fact was that genotype distributions between male and female were different hereditarily in Iraqi Arab population.

According to table: 2, the distribution of ACE genotype of the subjects (n= 100, 53male & 47 female) showed DD: 47.17%, II: 22.6% & ID: 30.19% in the male group and DD: 40.43%, II: 17.02% & ID42.55:% in the female group. The difference was significant between the male and female groups (* (P<0.05), ** (P<0.05), ns: non-significant.

It showed that the Iraqi Arab male group had more DD genotype II genotype than the Iraqi Arab female group, 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 (23)

Table (2). Effect of gender in distribution of gene (ID, D & II)

Gene	Gender				χ2 value	Total
	Male		Female			
	No.	%	No.	%		
ID	**16**	**30.19**	**20**	**42.55**	**3.44 ***	**36**
DD	**25**	**47.17**	**19**	**40.43**	**4.71 ****	**44**
II	**12**	**22.6**	**8**	**17.02**	**5.00 ****	**20**
Total	**53**	**53 %**	**47**	**47 %**	**1.096 ns**	**100 (100%)**

** (P<0.05), ** (P<0.05), ns: non-significant. χ2: chi-square.*

The genotypic and allelic distribution of the ACE I/D polymorphism according to medical family history subjects are given in Table (3).

Twenty eight sample of 100 sample, most of them divided between hypertension and diabetes, for the hypertension the ACE I/D polymorphism distribution was ID: DD: II: as 2.78%: 18.18%: 8.335%, and for diabetes ID: DD: II: as 2.78%: 4.55%: 2.78%. It is obviously the DD genotype is the most related to disease than the other genotypes.

Table (3). Effect of Family history in distribution of gene (ID, D & II).

Gene	Family history												$\chi 2$ value	Total
	healthy		hypertension		Diabetes		hearts		diabetes + hypertension		asthma			
	No.	%	No.	%	No	%	No.	%	No	%	No	%		
ID	27	75	1	2.78	1	2.78	1	2.78	6	16.67	0	0	4.06 *	36
DD	32	72.73	8	18.18	2	4.55	0	0	2	4.55	0	0	5.39 **	44
II	13	65	3	8.335	1	2.78	0	0	2	10	1	5	5.18 **	20
Total	72	72%	12	12%	4	4%	1	1%	10	10%	1	1%	5.72 **	100 (100%)

** ($P<0.05$), ** ($P<0.01$). $\chi 2$: chi-square.*

The angiotensin I-converting enzyme (ACE) gene major function is the conversion of angiotensin I to vaso-active, natriuretic octapeptide angiotensin II.

Angiotensin II binds to plasma membrane receptors and produces arteriolar constriction and a rise in systolic and diastolic blood pressure, hence its significance in hypertension and cardio-vascular diseases.

Significant association of hypertension with D allele of the ACE gene has been documented in the African- American (24), Chinese (25), and Japanese populations (26). However, other studies have failed to detect any such association (27).

High ACE activity is associated with high angiotensin II level. These findings seem to indicate that low ACE activity is advantageous to glucose metabolism. The ACE gene I/D polymorphism were identified in 1990 by Rigat and coworkers (4). Subjects with the genotype DD displayed the highest mean activity of serum ACE, those with the genotype II displayed the lowest, and those with the genotype ID displayed the intermediate.

In Conclusion, The results of the distribution of the ACE I/D gene polymorphism obtained for the D allele among Iraqi Arab population are comparable to those obtained from previous studies in other Arabs, 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.

In this study, a significant association of ACE I/D polymorphism with hypertension and was diabetes was observed. The result of this study supports the hypothesis that the DD genotype is in linkage disequilibrium with a functional variant of the ACE gene.

The sample size in this study was relatively small which may raise some questions, but a clear interrelation between this allele and the tow disease is established, and should be taken in mind.

References:

1. Braam B.; Mitchell K.D.; Fox J .and Navar L.G. (1993). Proximal tubular secretion of angiotensin II in rats. Am J Physiol, 264: 891-898.
2. Wang J.G. and Staessen J.A. (2000). Genetic polymorphisms in the reninangiotensin system: relevance for susceptibility to cardiovascular disease. Eur J Pharmacol, 410:289-302.
3. Sakuma T.; Hirata R. D. and Hirata M. H. (2004). Five polymorphisms in gene candidates for cardiovascular disease in Afro-Brazilian individuals. J. Clin. Lab. Anal. 18: 309–316.
4. Rigat B.; Hubert C.; Alhenc-Gelas F.; Cambien F.; Corvol P. and Soubrier F. (1990). An insertion/deletion polymorphism in the angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest, 86:1343-1346.
5. Bayoumi R.A.; Simsek M. and Yahya T.M. (2006). Insertion-Deletion Polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene among Sudanese, Somalis, Emiratis, and Omanis. Human Biology. 78: 103-108.
6. 6- Rigat B.; Hubert P. and Corvol P. (1992). PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidy 1 carboxypeptidase 1).Nucleic Acids Res. 20:1433-1439.
7. Gesang L.; Liu G.; Cen W.; Qiu C.; Zhuoma C.; Zhuang L.; Ren D.; PincuoZ.and Chan Y. (2002). Angiotensin-converting enzyme gene polymorphism and its association with essential hypertension in a Tibetan population. Hypertens Res. 25:481-485.
8. Moleda P;, Majkowska L;, Safranow K.; Adler G. and Goracy I. (2007). Relationship between I/D polymorphism of angiotensin I converting enzyme gene and microvascular complications in type 2 diabeticpatients. Przegl Lek .64:134-139.
9. Tarnow L.; Gluud C. and Parving H.H. (1998). Diabetic nephropathy and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. Nephrol Dial Transplant. 13:1125-1130
10. O'Donnel C.J.; Lindpaintner K.; Larson M.G.; Rao V.S.; Ordovas J.M. and Schaefer E.J.(1998). 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, 97:1766-1772.
11. Katsuya T.; Baba S.; Higaki J; Mannami T; Sato N. and Ogihara T. (1998) The deletion polymorphism of the angiotensin converting enzyme gene increases the risk of hypertension in a large Japanese general population: the Suita study. Circulation, 98 :I- 859
12. SAS. 2004. SAS / STAT Users Guide for Personal Computers. Release 7.0. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).
13. Comas D.; Calafell F.; Benchemsi N.; Helal A.; Lefranc G.; Stoneking M.; Batzer M.A.; Bertranpetit J.; and Sajantila A. (2000). Alu insertion polymorphisms in NW Africa and the Iberian Peninsula: evidence for a strong genetic boundary through the Gibraltar Straits. Hum Genet, 107:312-319.
14. Frossard P.M.; Obineche E.N.; Elshahat Y.I. and Lestringant G.G. (1997). Deletion polymorphism in the angiotensin-converting enzyme gene is not associated with hypertension in a Gulf Arab population. Clin Genet, 51:211-213.
15. Salem A. and Batzer M. A. (2009). High frequency of the D allele of the angiotensin-converting enzyme gene in Arabic population's .BMC Research Notes. 2: 99-102.
16. Barley J.; Blackwood A.; Carter N. D.; Crews D. E.; Cruickshank J.K.; Jeffery S.; Ogunlesi A.O.; and Sagnella G. A. (1994). Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. J Hypertens, 12:955-957.
17. Ulu A, Elsobky E, Elsayed M, Yldz Z, Tekin M, Akar N (2006) Frequency of five thrombophilic polymorphisms in the Egyptian population. Turkish Journal of Hematology. 23: 100-103.
18. Cambien F.; Poirier O.; Lecerf L.; Evans A.; Cambou J.P.; Arveiler D.; Luc G.; Bard J.M.; Bara L. and Ricard S.(1992). Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature. 359: 641-644.
19. Saha N.; Talmud P.J.; Tay J.S.; Humphries S.E. and Basair J. (1996). Lack of association of angiotensin-converting enzyme (ACE). Gene insertion/ deletion polymorphism with CAD in two Asian populations. Clin Genet. 50: 121-125
20. Lee E.J. (1994) Population genetics of the angiotensin-converting enzyme in Chinese. Br J Clin Pharmacology. 37: 212-214.
21. Lester S.; Heatley S.; Bardy P.; Bahnisch J.; Bannister K.; Faull R.; and Clarkson A. (1999). The DD genotype of the angiotensin-converting enzyme gene occurs in very low frequency in Australian Aboriginals. Nephrol Dial Transplant. 14: 887-890.
22. Cavalli-Sforza L.L.; Piazza A.; Menozzi P. and, Mountain J. (1988). Reconstruction of human evolution: bringing together genetic, archaeological, and linguistic data. Proc Natl Acad Sci USA. 85:6002-6006.
23. Yang Y.M.; Park J.H. and Moon E. S. (1997). Genotype distribution and gene frequency of angiotensin I- converting enzyme in Korean population. J.Gene. Med. 1: 17-22.
24. Duru K.; Farrow S.; Wang J.; Lockbetteb W. and Kurtz T. (1994). Frequency of a deletion polymorphism in the gene for angiotensin converting enzyme is increased in African-Americans with hypertension. Am. J. Hypertens. 7: 759-762.
25. Chiang F.T.; Chern T.H.; Lai Z.P.; Tseng C.D.; Hsu K.L.; Lo H.M. and Tseng Y.Z. (1996.) Age- and gender-dependent association of the angiotensin-converting enzyme gene with essential hypertension in a Chinese population. J. Hum. Hypertens. 10: 823-826.
26. Nakano Y.; Oshima T.; Hiraga H.; Matsuura H.; Kajiyama G. and Kambe M. (1998.) DD genotype of the angiotensin Iconverting enzyme gene is a risk factor for early onset of essential hypertension in Japanese patients. J. Lab. Clin. Med. 131: 502-506.
27. Kamdar S.; Daniel H.; Fogarty P.; Lawson M.; Munroe P. and Caulfield M. (1994). ACE insertion/deletion (I/D) polymorphism in Vincentian African Caribbean with essential hypertension. J. Hum. Hypertens. 8: 611-614.

توزيع التغايرات الاليلية لجين الانزيم المحول للانجيوتينسين للعراقيين العرب

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الخلاصة:

يحتوي جين الانزيم المحول للانجيوتينسين (ACE) في الانسان على نوعين من التغايرات الوراثية: الادراج والحذف (I/D) مما ينتج ثلاث انواع من الطرز الجينية:(II, ID & DD) والتي لها علاقة باصول المجتمعات وارتباطها ببعض الامراض الوراثية مثل ارتفاع ضغط الدم والسكر. في هذه الدراسة تم التحري عن التغايرات الوراثية لجين (ACE) باستعمال تقنية التفاعل المتسلسل لانزيم بلمرة الـ DNA (PCR)) واظهرت النتائج انتشار الطرز الاليلي (DD) اذ وجد بنسبة 50% بين العراقين العرب وهي نتيجة مشابه لبقية الدراسات التي اجريت على الشعوب العربية. كما كشفت الدراسة عن التوزيع الاليلي في الذكور والاناث حيث كانت: (DD:47.17%, II:22.6% & ID:30.19%) للرجال و(DD:40.43%, II:17.02% & ID42.55%) للنساء. كما اظهرت الدراسة وجود ارتباط معنوي بين شكل التغاير الاليلي DD ووجود الامراض الوراثية (ارتفاع ضغط الدم والسكر) في العراقين العرب.