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Insertion/Deletion Gene Polymorphism and Serum Level of Angiotensin Converting Enzyme

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ABSTRACT

Background: Angiotensin converting enzyme (ACE) plays an important role in cardiovascular regulation. Since the initial report regarding the association of insertion/deletion gene polymorphism and serum concentration of ACE, there have been investigations in different populations with contradicting results. The aim of this study was to assess the association of the ACE polymorphism and serum level in Iranians.

Materials and Methods: The study recruited 88 healthy individuals (70 males and 18 females; mean age: 30.07 yrs.) who were candidates for kidney donation. To investigate the association of ACE serum level and polymorphism, the serum concentration of ACE was measured spectrophotometrically and ACE genotyping was determined by polymerase chain reaction.

Results: The genotype distribution of DD, ID and II was 31, 24 and 33 respectively. The mean ACE serum concentration for DD, ID and II genotypes was 50.68, 36.65 and 32.06 IU/L, respectively. There was a significant difference in ACE serum level among the three genotypes ($p < 0.05$). While the highest ACE serum concentration was seen in DD group (nearly 1.5 times of that of ID and II genotypes, respectively), it was the lowest in the II group.

Conclusion: Our study showed that insertion/deletion polymorphism of ACE gene was strongly associated with plasma ACE levels in the Iranian population. (Tanaffos 2008; 7(2): 18-22)

Key words: Angiotensin Converting Enzyme (ACE), Insertion/Deletion Polymorphism

INTRODUCTION

Angiotensin-converting enzyme (ACE), a peptidylpeptide hydrolase that is a key component of the renin-angiotensin system (RAS), catalyses the

conversion of angiotensin I into active octapeptide angiotensin II, which has an important role in the maintenance of cardiovascular homeostasis (1). ACE gene is a candidate for cardiovascular system diseases (2). It has a different role in inactivating vasodilator bradykinin K.² Gene encoding ACE located at 17q23, (3) weighs 21 KD, consisting of 26 exons, (4) and contains a polymorphism due to the presence (insertion, I) or absence (deletion, D) of a

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287-bp *alu* sequence in intron 16 resulting in three genotypes of insertion/insertion (II), insertion/deletion (I/D), and deletion/deletion (D/D) (5, 6). Although the plasma level of ACE is considerably variable among different people, it remains consistent during one's lifespan (7, 8). It has been shown that ACE plasma concentration is under precise control of genetic and environmental factors. Since the initial report regarding the association of insertion/deletion gene polymorphism and serum concentration of ACE, (9) there have been investigations in different populations with contradicting results (8-12). Moreover, while some studies believe that insertion/deletion polymorphism of ACE affects both serum level and the amount of the enzyme linked to cell wall (which indicates the effect of polymorphism on the translation stage of the gene, and not the process of enzyme secretion), others on the basis of accurate investigation of genetic pedigrees have shown that the polymorphism may not be directly responsible for serum level variations. But, it is in strong linkage disequilibrium with a major gene effect at the ACE locus (like 4656 CT polymorphism), which controls up to 44% of the variability in ACE levels (13).

A further role of the insertion/deletion polymorphism of ACE gene has been reported in pathophysiology of sarcoidosis. In sarcoidosis however, it has been shown that ACE is produced by granuloma epitheloid cells and secreted in the plasma. Thereby its serum concentration may be an informative marker revealing the severity of granuloma formation. Although plasma level of ACE is widely used for the diagnosis of sarcoidosis, its sensitivity is low, and in different studies increased ACE level has been reported in no more than 33% to 88% of patients with established sarcoidosis (14). Results of a study performed by Sharma et al. (15) have shown that by defining the normal range of ACE plasma level on the basis of three different

genotypes of II, ID and DD, the role of increased ACE serum level as a diagnostic marker for sarcoidosis, will improve from 51.7% to 69%, which indicates increasable potential capacity of ACE plasma level to diagnose sarcoidosis (15).

In this study, we attempted to determine not only the association between insertion/deletion polymorphism and plasma level of ACE in Iranians, but also the normal ranges of ACE levels on the basis of three different ACE polymorphism genotypes.

MATERIALS AND METHODS

Design and participants. We conducted a prospective study, consisting of 88 Iranian unrelated healthy individuals (70 males, 18 females, mean age 30.7 years), who were suitable candidates for kidney donation. The participants were excluded if they had an acute or chronic disease, were taking medication, had abnormal radiographic images, had systolic/diastolic blood pressure more than 140/90 mm Hg, and/or had any abnormality of biochemical laboratory tests including blood sugar, blood urea, serum creatinin, sedimentation rate and liver function tests. All participants gave their written informed consent. The study was also approved by the Ethics Committee of National Research Institute of Tuberculosis and Lung Disease (NRITLD).

Two 5-mL samples of peripheral blood were collected into two separate tubes (with and without EDTA). The former sample was used for DNA extraction and the latter was utilized for enzyme measurement.

DNA extraction. Genomic DNA was isolated from leukocyte buffy coat using Fycole (Lymphocyte separator/Baharafshan. Iran) and buffering solution (PBS) as previously described (5).

Genotyping. The I/D polymorphism was typed as previously described (5, 16). Temporarily, the

genomic DNA was subjected to PCR using the forward primer 5'-CATCCTTTCCATTTC TC -3' and the reverse primer 5'-ATTCAGAGCAGGAATAAAAT T-3' in a solution containing 5 µl of isolated DNA, 16.6 mM (NH₄)₂ SO₄, 66.7 mM Tris-HCl, pH: 8.8, 2.0 mM MgCl₂, 200 µM each dNTP, 10 pMol of each forward and reverse primers, and 1 u Taq DNA polymerase (Roche, Germany). PCR was performed on thermal cycler with initial denaturation at 94°C for 5 min followed by 40 cycles of 95°C for 1 min, 53°C for 1 min and then by a final extension period at 72°C for 3 min. The amplified PCR product was separated on 2% agarose gel in the presence of ethidium bromide and was visualized with UV light. The length of amplified products was 371 bp and 47 bp for allele I and D respectively. According to the presence or absence of insertion allele, the genotype of the subjects could be classified as II (homozygote for the insertion allele), DD (homozygote for deletion allele), or ID (heterozygote).

Measurement of plasma ACE level. The plasma ACE activity levels of all subjects were measured by using standard methodologies based on the hydrolysis of furanacryloyl-l-phenylalanyl-glycerine (FAPGG) by ACE, with the subsequent decrease in absorbance at 340 nm being the measurement of ACE activity (17, 18). Each unit of the enzyme is the amount of that which could hydrolyze 1 mM of FAPGG in 1 min.

RESULTS

A total of 88 Iranian healthy individuals were recruited. Out of them, 18(20%) participants were females and 70(80%) were males. The mean age of the participants was 30.0 years. There was no significant difference among the mean ages of the participants of different genotypes (p= 0.3, Table 1).

Table 1. Mean ages and standard deviation of participants according to genotype.

Genotypes	Mean Age (Year)	Standard Deviation
II	29.88	8.81
ID	31.04	7.06
DD	29.29	6.82

There was not significant difference among the mean ages of the participants of different genotypes (P = 0.3)

Genotype distribution of DD, ID and II was 31(35%), 24(27%), and 33(37.5%) respectively. Moreover, derived allele frequencies for the I and D allele were 51.15 and 48.85 respectively.

The ACE serum levels of DD, ID and II genotypes were 50.68 ± 24.59, 36.35 ± 20.87, and 32.06 ± 24.2 IU/L, respectively. There was a significant difference in ACE plasma concentration among the three genotype groups (P < 0.005%). While the highest ACE serum concentration was seen in DD group (nearly 1.5 times of that seen in the ID and II genotypes, respectively), it was the lowest in group II (Table 2).

Table 2. Genotype distribution and ACE serum levels according to the genotype.

Genotypes	ACE Serum Level [*]	95% Confidence Interval	Frequency
II	32.06 ± 24.2	23.46, 40.46	33
ID	36.35 ± 20.87	27.43, 45.06	24
DD	50.68 ± 24.59	41.66, 59.7	31

* The ACE serum level was higher in DD genotype than other groups (P < 0.005)

Furthermore, after stratification by sex, the serum enzyme concentrations in men were significantly higher than in women in each genotype group (p < 0.005). The genotype distribution and average serum ACE levels of each genotype according to sex are summarized in Table 3.

Table 3. The genotype distribution and average serum ACE levels according to the sex

Genotypes	Gender	ACE Serum Level	95% Confidence Interval	Frequency
II	Female	28.60 ± 18.87	5.16, 52.03	5
II	Male	41.1 ± 25.63	34.99, 47.21	28
ID	Female	33.83 ± 14.49	18.62, 49.05	5
ID	Male	37.05 ± 22.91	25.66, 48.45	18
DD	Female	39.43 ± 26.10	15.29, 63.57	7
DD	Male	53.96 ± 23.69	43.95 ± 63.96	24

*The ACE serum levels in men were significantly higher than in women in each genotype group ($P < 0.005$)

DISCUSSION

This study is the first to evaluate the association between insertion/deletion gene polymorphism and serum level of angiotensin converting enzyme (ACE) in Iranians. The main finding of this study is that while plasma ACE concentration of DD polymorphism is significantly higher than the other two genotypes (II and ID), no meaningful difference between serum ACE levels of II and ID polymorphisms was seen. These results are consistent with the original study of the association between insertion/deletion gene polymorphism and serum concentration of ACE by Rigat et al.(9) which reported that this polymorphism accounts for half of the variance in serum ACE. Moreover, other studies were performed on Chinese people by Lee et al. (10) and in Japanese population by Tsutaya et al. (11) and Yamamoto et al. (12) showing that there was an association between insertion/deletion polymorphism and plasma ACE levels in these populations, findings that are similar to ours. These findings are slightly different from those of Bloem et al. (8) that failed to confirm the relationship of ACE polymorphism and serum levels in African Americans.

We also found a wide range of enzyme levels in each genotype group, a finding that is in agreement

with similar studies mentioned above. Therefore, the wide variation of circulating ACE levels between individuals appears to be universal across populations. The genetic factors account for such a wide range may be maintained in populations because of an unidentified protective effect of higher ACE levels. This hypothesis is supported by a study performed by Schachter et al (19).

Our second finding regarding the derived allele's frequency which was 51.15 for I and 48.85 for D, indicated that allele of I is more frequent than D in Iranians. This findings is in consistent with the Chinese (I: 0.7, D: 0.3) and Japanese (I: 0.625, D: 0.375) studies that reported excess frequency of I allele than D in Chinese and Japanese populations. It was in disagreement with Rigat's study in France (I: 0.4, D: 0.6) which showed that allele of D is more frequent than I, in French people, and Bloem's investigation that demonstrated higher frequency of D allele than I, in both whites and African Americans (I: 0.46, D: 0.54 for whites, and I: 0.36, D: 0.64 for African Americans).

Our observation was that the ACE serum levels in Iranian men were significantly higher than Iranian women in each genotype group. Although some studies revealed that serum ACE levels of infants were higher than adolescents, (20) however, no association was found between serum ACE levels and age or sex in different studies. This discrepancy in our finding and other studies may be due to small female sample size in our study, and it seems that reasonable interpretation requires further investigation with a larger sample size. Furthermore, although we attempted to identify normal range of serum ACE levels according to three different ACE genotypes in Iranians, to achieve more reliable standard varieties in Iranian individuals, supplementary studies with larger sample sizes are necessary.

CONCLUSION

Our results demonstrated that the DD genotype was associated with higher plasma ACE concentration in Iranians. In addition, our data suggested that the allele of I is more frequent than D in Iranian people. We also found that serum ACE levels were higher in Iranian males than females. Further studies in a large population are recommended.

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