Association of Angiotensin Converting Enzyme Gene Polymorphism and Possible High Risk Factors with Essential Arterial Hypertension in Egyptian Patients

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Abstract

Background: Hypertension (HT) is a public health challenge due to its high prevalence, and being a major risk factor for cardiovascular diseases. HT is a multifactorial disorder with genetic and environmental interactive factors.

Aim: This study aims to evaluate association of angiotensin converting enzyme gene polymorphism (I/D) with blood pressure among Egyptian patients with essential hypertension and the interrelationship with other clinical parameters.

Subjects and methods: Eighty four patients with essential hypertension were included in this cross sectional descriptive study. Venous blood samples were withdrawn for DNA extraction, determination of different genotypes of ACE gene (I/D) polymorphism by polymerase chain reaction (PCR) and measuring serum ACE levels, lipid profile, blood sugar and creatinin.

Results: The frequency of different ACE genotypes were; 41.7% homozygous (DD), 45.2% heterozygous (ID) and 13.1% homozygous (II) indicating that (D) allele is significantly associated with essential hypertension. Also serum ACE level was significantly correlated with ACE (I/D) polymorphism (p=0.001). Patients with DD genotype had the highest serum ACE level followed by patients with ID genotype and patients with II genotype had the lowest serum ACE level.

Conclusion: ACE (I/D) polymorphism is associated with increased serum ACE activity and consequently with increased risk for essential hypertension and its complications.

Keywords: Hypertension; Polymorphism; Multifactorial; Angiotensin converting enzyme; Polymerase chain reaction (PCR)

Abbreviations: ACE: Angiotensin Converting Enzyme; Ang II: Angiotensin II; ANOVA: One Way Analysis of Variance; BMI: Body Mass Index; bp: base pair

Introduction

Hypertension is a global public health issue as it contributes to the burden of heart diseases, stroke, kidney failure and premature mortality and disability. It disproportionately, affects populations in low-and middle-income countries where health systems are weak [1].

One billion of the world’s population has hypertension, resulting in four million deaths per year [2,3]. Almost three-quarters of people with hypertension live in developing countries [4,5]. Furthermore, the prevalence of hypertension is increasing and is predicted to grow by more than 500 million by 2025 [3,6].

The Egyptian National Hypertension Project (NHP, 1991) provided estimates of hypertension prevalence, awareness, treatment and control among Egyptians. Data from the NHP showed that, hypertension is common among Egyptians and appeared to be slightly more common in Egyptian women (28.9%) than men (25.7%) [7-10].

Approximately 30% of the inter-individual variability in blood pressure is genetically determined [11]. Numerous studies have focused on the role of genetic variation in genes implicated in the renin-angiotensin system (RAS), particularly the angiotensin-converting enzyme (ACE) gene [12]. ACE is the core enzyme in the RAS catalyzing the production of angiotensin II (Ang II) which is the key effector in RAS due to its role in blood pressure regulation, salt-water balance and angiotasis [13,14].

Concentrations of plasma and tissue ACE are determined by the ACE gene located on chromosome 17q23 [12]. This gene is polymorphic and shows inconsistent association with arterial blood pressure. An insertion/deletion (I/D) polymorphism in intron 16 (287 bp) of the ACE gene gives rise to 3 genotypes; the homozygotes II and DD, and the heterozygote ID. It has been suggested that this polymorphism could explain up to 47% of total phenotypic variation in ACE serum levels and determines ACE enzyme activity [12].

This study aims to evaluate association of angiotensin converting enzyme gene polymorphism (I/D) with blood pressure among Egyptian patients with essential hypertension and the interrelationship with other clinical parameters such as ACE serum activity, fasting blood sugar, lipid profile, creatinine and body mass index (BMI).

Methods

Study participants

The study was a cross sectional descriptive study and included 84 patients (20 male and 64 female) selected by a convenient sampling. Their mean age was 55 years old and diagnosed with essential arterial hypertension according to JNC7, 2003 [15]. They were recruited from the Cardiology Clinic, Suez Canal University Hospital, Ismailia, Egypt. All participants had no history of cardiac diseases, renal problems or diabetes mellitus. Arterial blood pressure was measured and body mass index was calculated.

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index (BMI) was calculated for each patient. The study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Suez Canal University and all patients were given written informed consent.

**Sample collection and processing**

A 5 ml venous blood sample was withdrawn from each patient after 8-12 h fasting. 2 ml of whole blood were collected into potassium EDTA tubes for DNA extraction and determination of different genotypes of ACE gene (I/D) polymorphism by polymerase chain reaction (PCR). The remaining 3 ml were collected into plain tubes and used for serum separation for measurement of fasting blood glucose (FBG), lipid profile (triglycerides (TG), total cholesterol, HDL-C and LDL-C), creatinine and serum ACE levels.

**Molecular analysis of blood samples**

**DNA extraction**: Genomic DNA was extracted from 300 μl of whole blood collected in potassium EDTA tubes using the Wizard genomic DNA purification kit (Promega, Madison, USA, Cat.# A1120) and DNA concentration and purity were measured by NanoDrop ND-1000 spectrophotometer (NanoDrop Tech., Inc. Wilmington, DE, USA).

**ACE (I/D) genotyping by thermal cycler PCR**: A fragment of 287 base pairs (bp) in intron 16 of ACE gene was amplified with forward primer 5'-CTG GAG ACC CCT CCC ATC TCT TCT -3' and reverse primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' [16]. PCR was carried out in a Eppendorf Mastercycler machine (Eppendorf AG, Hamburg, Germany) using GoTaq G2 Green Master Mix (Promega, Madison, USA, cat.# M7822) which is a premixed ready-to-use solution containing GoTaq G2 DNA Polymerase, 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP, reaction Buffer (pH 8.5) and 3 mM MgCl₂. The PCR reaction was carried out in a final volume of 25 μl including: 12.5 μl GoTaq G2 Green Master Mix, 1 μl forward primer, 1 μl reverse primer, 1 μl DNA template and 9.5 μl nuclease-free water. Thermal cycling conditions of PCR reaction were 30 cycles of denaturation at 94°C, annealing at 58°C and extension at 72°C, each for 1 min. PCR products were analyzed by electrophoresis in 2% agarose gel (w/v) and the gel was visualized by ultraviolet trans-illumination and photographed after staining with ethidium bromide. A 100 base pair (bp) DNA marker was loaded into the gel to determine the length of PCR products which were of 490 bp for I allele and 190 bp for D allele (Figure 1).

**Measurement of serum ACE level**: Serum ACE level was measured using human ELAb ACE ELISA kit (Wuhan ElAb Science Co.,Ltd, China) Catalogue No: E0004h.

**Biochemical tests**

FBG [17], Triglycerides (TG) [18], Total cholesterol [19], High density lipoprotein-cholesterol (HDL-C) [20] were all measured by enzymatic colorimetric method using Biodiagnostic kit. Creatinine was measured by Colorimetric kinetic method using Biodiagnostic kit [21].

**Statistical analysis**

Data were managed using the “Statistical Package for Social Sciences (SPSS)”, version 17.0 software. Descriptive statistics were represented as mean ± standard error (SE) for quantitative variables. Associations of genotypes with the biochemical tests and serum ACE level were analyzed by one way analysis of variance (ANOVA) test followed by Tukey’s test for multiple comparisons. Relationships of serum ACE level with the component of the biochemical tests in the study population were assessed using Pearson’s correlation coefficient analyzed by the bivariate correlations method. Confidence interval (CI) and odds ratio (OD) were determined to assess the strength of association and the reliability of the estimated results. A value of p<0.05 was considered statistically significant.

**Results**

**Characteristics of the study patients**

The mean age of the patients was 55 years old. Most of them were females (76.2%); the others (23.8%) were males. There was a slight difference between males and females in their body mass index, Systolic and diastolic pressures are slightly higher in females than males; however these differences are not statistically significant (Table 1).

Lipid profile, fasting blood glucose and serum creatinine levels were measured for all patients. Male hypertensive patients showed higher levels of total cholesterol, low density lipoprotein, triglycerides and fasting blood glucose, whereas female patients showed higher levels of high density lipoprotein and serum creatinine. However, the observed sex differences were not statistically significant except for a significant difference between males and females in their fasting blood sugar level (p=0.033).

The mean serum ACE level of patients was 4.6 mg/dL and also there was no significant difference in serum ACE level between males and females.

**Association of ACE gene (I/D) polymorphism with essential hypertension**

The frequency of different ACE genotypes is showed in (Figure 2 and Table 2). The (DD) homozygous were 41.7% [11.9% male and 29.8% female], the heterozygous (ID) were 45.2% [10.7% male and 34.5% female] and the (II) homozygous were 13.1% [7.4% male and 5.7% female].

**Table 1**: Clinical characteristics of the hypertensive patients (n=84).

| Variable                  | Male (%)       | Female (%)      | Total (%)       | p       |
|---------------------------|----------------|-----------------|-----------------|---------|
| Age (years)               | 55.70 ± 2.43   | 55.92 ± 1.33    | 55.87 ± 1.16    | 0.741   |
| BMI (kg/m²)               | 28.43 ± 0.48   | 29.25 ± 0.76    | 28.60 ± 0.40    | 0.001   |
| Systolic blood pressure   | 140.50 ± 3.51  | 142.97 ± 2.53   | 142.38 ± 2.09   | 0.254   |
| Diastolic blood pressure  | 90.00 ± 1.92   | 91.25 ± 0.95    | 90.95 ± 0.86    | 0.669   |

Data are represented as mean ± SE. BMI=Body Mass Index
The level of serum creatinine was similar in the different genotypes which suggest no contribution or association between the angiotensin gene ACE and serum creatinine (Table 5).

Correlation between serum ACE levels, ACE I/D polymorphism and clinical data of the patients

Serum ACE level was significantly associated with ACE I/D polymorphism. Patients with DD genotype had the highest serum ACE level (6.03 ± 0.08 ng/ml), followed by patients with ID genotype (4.19 ± 0.14 ng/ml), and patients with II genotype had the lowest serum ACE level (1.48 ± 0.26 ng/ml) (Figure 3).

Serum ACE level showed significant positive correlation with BMI, TG, total cholesterol, LDL-C and FBG and showed significant negative correlation with HDL-C (Figure 4). On the other hand, hypertensive patients of the II genotype show positive association with HDL-C. This indicates that the high level of HDL represents a high risk for II homozygotes.

Discussion

The distribution of different genotypes of the ACE gene within

Figure 2: Genotypes of angiotensin converting enzyme (ACE) gene polymorphism (I/D) in patients of different sexes.

Table 2: Genotypes of ACE gene polymorphism (I/D) in patients.

| Genotype | Total (n=84) | Male (n=20) | Female (n=64) | P value |
|----------|-------------|------------|---------------|---------|
| DD       | 35 (41.7%)  | 10 (11.9%) | 25 (30.8%)    | 0.582   |
| ID       | 38 (45.2%)  | 9 (10.7%)  | 29 (36.5%)    |         |
| II       | 11 (13.1%)  | 1 (1.2%)   | 10 (11.9%)    |         |
| Total    | 84 (100%)   | 20 (23.8%) | 64 (76.2%)    |         |

Data are represented by number (n) and percentage (%). Student T-test was used.

Association of ACE gene (I/D) polymorphism with clinical data & lab findings of the patients

The high risk DD genotype groups showed a significant increase in BMI than ID and II genotype carriers (p<0.001), whereas the II genotype showed the least BMI in both sexes. Males of the II genotype are at a higher risk of a higher systolic blood pressure and nearly so with regard to diastolic blood pressure compared with the other genotypes. Heterozygotes for the DI alleles seem to be at much lower risk concerning systolic and diastolic blood pressure compared with the homozygote types (Table 3).

Homozygotes for DD alleles had the highest level of total cholesterol, triglycerides and low density lipoprotein compared to D/I and II genotypes. The heterozygote D/I had intermediate values between the DD and II homozygotes (Table 4). The situation is the reverse for high density lipoprotein where II homozygote genotype showed the highest level compared with D/I and DD genotypes. All observed differences between the different genotypes: DD, DI and II, are statistically significant. Male and female hypertensive patients in this group show the same trend of changes in the measured levels. There seem to be no differences between male and female in the biochemical estimates of lipid profile and triglycerides.

Homoygous of the DD genotype had significantly higher blood glucose levels than carriers of the ID genotype (p=0.003 in total FBS and p=0.034 in females only) (Table 5), the data suggest that the similarity of blood glucose level between II and D/I genotypes points to dominance of the I allele over the D allele in that respect.
the studied hypertensive group was in Hardy-Weinberg equilibrium, indicating that the selected sample was representative. The frequency of homozygous for (DD) was 41.7% whereas the frequency of homozygous for (II) was 13.1%. Heterozygous (ID), were constituting the relatively larger group 45.2%. These results suggest that allele (D) with a frequency of 64.3% is the high risk allele and allele (I) with a frequency of 35.7% is the low risk allele significantly associated with essential hypertension. These findings are in agreement with the results reported by Li et al. [22]; Ali et al. [23]; Zarouk et al. [24], who noted that hypertensive cases showed a significantly higher frequency of the ACE mutant D allele carriage than the I allele carriage among Chinese, Saudi Arabia and Egyptian populations, respectively. Also a significant association of the ACE gene D allele with essential hypertension was documented in an African-American [25], Chinese [26], and Japanese populations [27,28].

However, the findings of Rasyid et al. [29] and Kabadou et al. [30], are inconsistent with the above mentioned reports as no significant association between ACE (D) allele and hypertension in a South Sulawesi Indonesian and a Tunisian populations, respectively was observed. Also, ACE I/D polymorphism was reported as negatively associated with hypertension in Emirati Arabs, Dutch, Spanish, and Cuban populations [31-35].

On the other hand, allele (I) was found to be associated with high blood pressure in an Australian population with strong evidence of familial hypertension [36].

The noted population variability in the association of ACE I/D polymorphism with essential hypertension has been suggested to be due to significant variations of population backgrounds such as different sample size, different criteria specifically clinical presentation, age and age onset of the disease, ethnic background, geographical area and concomitant environmental and social risk factors [37].

The serum ACE level in the studied group was significantly correlated with ACE I/D polymorphism. Patients with DD genotype had the highest serum ACE level (6.03 ± 0.48 ng/ml), and patients with II genotype had the lowest serum ACE level (1.48 ± 0.86 ng/ml), whereas patients with ID genotype had intermediate values (4.19 ± 0.84 ng/ml). These results are in agreement with the findings of Rigat et al. [38]; Park et al. [39]; Camós et al. [40], in Caucasian, Korean and Spanish populations, respectively. In contrast to these findings, Ljungberg et al. [41]; He et al. [42], found that serum ACE activities were highest in II carriers, than in DD carriers, and lowest in ID carriers among South East Sweden and Chinese populations, respectively.

No significant differences of ACE I/D genotype distribution or Serum ACE level between males and females were noted in subjects of this study. These results are in agreement with the findings of Kruit et al. [43]; Zhang et al. [44], but differ from those reported by Sagnella et al. [45], who observed a significant association between the D allele and hypertension in women of African descent. Also, Renée et al. [46], found significant genotype–sex interactions among Mexican American females, but not males. Katsuya et al. [47]; O’Donnell et al. [48], found that D allele has been associated with hypertension in white American and Japanese men but not in women.

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### Table 5: Other laboratory investigations of carriers of the different genotypes of ACE gene in the study population.

| Variables | DD Homozygots (n=35) | Carriers of ID (n=38) | II Homozygots (n=11) |
|-----------|----------------------|----------------------|----------------------|
| FBG (mg/dL) | 116.26 ± 5.92 | 82.50 ± 4.93 | 87.04 ± 6.58 |
| Male | 126.22 ± 18.26 | 78.78 ± 4.99 | 112.69 ± 28.98 |
| Female | 111.07 ± 11.08 | 83.19 ± 5.80 | 81.34 ± 4.53 |
| Creatinine (mg/dL) | 1.10 ± 0.12 | 1.02 ± 0.09 | 1.27 ± 0.21 |
| Male | 1.04 ± 0.25 | 1.05 ± 0.20 | 1.05 ± 0.35 |
| Female | 1.13 ± 0.13 | 1.01 ± 0.09 | 1.32 ± 0.25 |

Data are presented as mean ± SE. Comparisons were performed with one way ANOVA test followed by the Tukey’s test for multiple comparison. FBG=Fasting Blood Glucose Level; *indicates significant difference from homozygous of DD.
The potential role of ACE gene (I/D) polymorphism and thus of differential ACE plasma activity in the development of hypertension is still a matter of controversy [49,50]. Other environmental risk factors seem to be good candidates for causing the observed elevation in the arterial blood pressure in different I/D genotypes.

The serum ACE level showed a significant positive correlation with BMI, TG, total cholesterol, LDL-C and FBG, but it showed a significant negative correlation with HDL-C which is consistent with the findings of He et al. [42]; Ajala et al. [51]. On the other hand, Overlack et al. [52]; Persu et al. [53], suggested that the level of serum ACE activity is not of pathophysiological or clinical significance in essential hypertension as they confirmed the hypothesis that the membrane-bound ACE, instead of circulating ACE, was responsible for Angiotensin II generation and its cardiovascular consequences. Our results are in agreement and support of the contention that ACE serum level cannot be taken as an immediate risk factor in hypertension as both the I/I homozygotes [54]. The two Egyptian populations showed a high frequency of the ACE D allele (0.67). Another study by Zarouk et al. [24] reported significant association between DD genotype and D allele with hypertension in Egyptian patients. The study sample in the above two studies on Egyptians failed to describe data concerning their age, gender and inclusion or exclusion criteria.

These findings can be of great value in the management of hypertension whether the determining risk factor is the high level of serum ACE or is due to high level of HDL and proper treatment will be described according to these findings.

Conclusion

ACE gene polymorphism could be a molecular marker for hypertension as ACE gene (I/D) polymorphism is strongly associated with an increased risk for hypertension and its complications. The genetic predisposition of the DD, DI and II genotypes seems to be associated with specific environmental risk factors particularly serum ACE level, and high HDL, which play a significant role in the elevation of blood pressure leading to the clinical manifestations of hypertension. Knowledge of the ACE gene polymorphism and the two risk factors; ACE serum level and HDL will improve the treatment and management of essential hypertension.

Conflict of Interest

All authors (ER, EM, AZ and FM) declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest. Also no one of the authors have any non-financial competing interests.

Authors’ Contribution

ER, EM, FM Conceived and designed the experiments: ER, AZ. Collected samples and data: ER. Performed the experiments: ER, EM, FM. Analyzed the data: ER & FM wrote the paper: ER, EM, FM. Critical revision: NR, EM, FM. Final approval: EM, AZ, FM.

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