ORIGINAL ARTICLE

# ANALYSIS OF SERUM ANGIOTENSIN CONVERTING ENZYME (ACE) ACTIVITY ACROSS ACE GENOTPYE IN NEWLY DIAGNOSED HYPERTENSIVE PATIENTS

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## ABSTRACT

**Introduction:** Essential hypertension is a multifactorial disease in which genetic and environmental factors play an important role. Angiotensin-I converting enzyme is a core enzyme of renin-angiotensin system and is known to play a critical role in the homeostasis of blood pressure. **Aims:** To determine the association of the serum ACE activity with regard to ACE insertion/deletion polymorphism with essential hypertension in the adult Gujarati hypertensive patient.

**Research design and methods:** A cross-sectional study was conducted with 173 patients with essential hypertension and 186 controls were recruited for the study. DNA samples were isolated from peripheral blood. Polymerase chain reaction was used for genotyping and ACE activity and other biochemical variables were measured.

**Result:** Genotype distribution in patients and controls were significantly different. Albeit the genotype frequency resulted in a higher frequency of the D allele in the group of cases than controls, the difference in allele frequency did not reach statistical significance and a trend of increase in SBP, DBP and serum ACE activity with increasing number of D alleles was observed..

**Conclusion:** Serum ACE activity and ACE I/D polymorphism plays a significant role in the pathogenesis of hypertension.

**Keywords:** Angiotensin converting enzyme, ACE activity, Essential hypertension

## INTRODUCTION

Essential hypertension affects approximately 20% of the adult population and is one of the contributing factors for the onset of macrovascular complications.[1] Its pathogenesis involves interactions between genetic and environmental factors, with approximately 30% of the inter-individual variability in blood pressure being genetically determined.[2] The exact etiology of this disease is still unknown, but special attention has been given to renin–angiotensin system (RAS), due of its physiological magnitude in cardiovascular homeostasis and the genes that regulate the system may contribute to the development of hypertension and end-organ damage.[3] Angiotensin converting enzyme (ACE) is a core enzyme of the RAS, and a possible candidate gene.

ACE converts inactive angiotensin I into active angiotensin II, which is a peptide with multiple functions, including vasoconstriction, aldosterone production, and noradrenaline release from sympathetic nerve endings. It has hypertropic and hyperplastic effects on vascular smooth muscle and cardiomyocytes, synthesis of the extracellular collagen matrix, and is tightly intertwined with the cascade of inflammatory, thrombotic, and fibrotic factors.[4]

The ACE gene is located at 17q23 and contains a polymorphism distinguished by either an insertion (I) or deletion (D) of a 287 base pair segment in intron 16.[5] The ACE DD genotype has been associated with higher levels of ACE[6], BP[7], and increased cardiovascular risk.[6] Despite the degree of interest shown in the subject, relatively few studies have examined the ACE enzyme activity in essential hypertensive patients with respect to I/D polymorphism, particularly in Gujarati population. Thus, in present study an attempt has been made to explore the association of the serum ACE activity with regard to ACE insertion/deletion polymorphism with essential hypertension in the adult Gujarati hypertensive patient.

## METHODOLOGY

This study was designed to determine the serum ACE activity with regard to ACE I/D polymorphism in newly diagnosed hypertensive patients. 359 participants were enrolled from November 2015 to April 2017 on the basis of consecutive sampling technique and were categorized into two main groups which include:

**Group I :** Healthy controls i.e. subjects not suffering from diabetes, nor having any family history of diabetes, not suffering from hypertension or from any acute or chronic disease , nor taking any drugs believed to alter blood pressure; n = 186

**Group II :** Hypertensive patients [Based on the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VII)[8]; n = 173

**Exclusion criteria were:**

- Patients who needed continuous or periodic use of corticosteroids.
- Patients who visited the pregnancy clinic or who had given birth within the preceding six weeks.
- Patients taking blood pressure and or lipid lowering drugs.
- Lack of approval by physician for any reason(s).
- Subjects showing disinterest.

All subjects were studied as outpatient. Participant's examination included interviews for medical and nutritional history. Present and past history of each case was recorded in detail regarding their general information i.e. name, age, sex, address, religion, occupation, economic status, nutritional and personal habits, education, medication and history suggestive of any systemic illness. Each subject was then examined for various anthropometric parameters: Weight (Kg) and height (meters) were measured. Body Mass Index (BMI) was calculated by Weight (Kg) / height squared ($m^2$). Waist circumference was assessed in the standing position, midway between the highest point of the iliac crest and the lowest point of the costal margin in the mid-axillary line. Hip circumference was measured at the level of the femoral greater trochanter. All anthropomorphic measures reflect the average of 2 measurements. Blood pressure (BP) was measured in the seated position after 10 minutes of rest with a standard manual mercury sphygmomanometer. Subjects were diagnosed by physician with hypertension on the basis of JNC-7. Age was defined as the age at the time of interview.

After an overnight fast of 12 hours, venous sampling was done for biochemical determinations and for isolation of DNA. Serum and plasma was separated by centrifugation of blood sample and were subjected for analytical procedures. Glucose (Glucose oxidase method, CV %: 3.4), cholesterol (Cholesterol oxidase method, CV %: 3.9), triglycerides (Enzymatic method, CV %: 3.6), HDL-C (Phosphpotungstic method, CV %: 4.7), creatinine (modified Jaffe's method, CV % 4.1) and angiotensin converting enzyme activity by Cushman and Cheung method , modified by Letreut et al (1979) were measured. LDL and VLDL cholesterol were calculated. Genomic DNA was extracted from peripheral blood leukocytes using commercially available DNA extraction and purification kit based on standard proteinase K technique.

Two oligonucleotide primers, forward: 5'-CTG GAG ACC ACT CCC ATC CTT TCT T-3' and reverse: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'based on the flanking sequences of the I/D region on the intron 16 of ACE gene were used to amplify the corresponding DNA fragments by polymerase chain reaction (PCR). Amplification was carried out in a DNA Thermal Cycler in a final reaction volume of 50 µl containing 50ng genomic DNA, 20pM each primer, 2.5mM each deoxyribonucleotides triphosphate, 1U thermus aquaticus DNA polymerase, 1.5mM magnesium chloride, amplification buffer contained 20mM Tris-hydrocholric acid and 50mM KCl. The thermocycling profile consisted of one minutes of initial denaturation at 94°C followed by 30 cycles of amplification of denaturation at 95°C for 30 sec, annealing at 58°C for 1 min and extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min. Using agarose gel (1%) electrophoresis, amplified products were separated and distinguished using ethidium bromide under UV light as a 190 bp fragment in the absence of an Alu repeat insertion and a 490 bp fragment in the presence of the insertion (genotypes described as II-490 bp, ID-490+190 bp, and DD-190 bp). These experiments were approved by Institutional Ethical Committee.

**Statistical analysis**: Data analyses were performed with the SPSS 15.0 statistical software. The results for continuous variables are mean $\pm$ SD and are well within the normal curve (i.e. normality is maintained). The two tailed (unpaired) student's test for independent samples, analysis of variance (ANOVA) was used, in assessment of the significance of difference between group means. The distribution of alleles in studied groups was tested for fitting to the Hardy–Weinberg equilibrium (HWE) (using web base program: http://www.oege.org/software/hwe-mr-calc.shtml) through testing the difference between observed and expected frequencies of genetic variants using the $\chi 2$ goodness-of-fit test. For all analyses, the nominal level of statistical significance was<0.05.

## RESULTS

Table 1 shows the subgroup, anthropometric and clinical characteristics of the study participants.

Among the study participants there was a predominance of male individuals as compared with females ($p<0.05$) but mean age of hypertensive participants and controls were similar. A comparison of different variables revealed that the difference were statically significant between cases and controls with respect to glucose, BMI, lipid profile (higher levels of total cholesterol, TG and lower levels of HDL-C) along with higher systolic blood pressure (SBP) readings and diastolic pressure ($p<0.05$; Table 1).

Genotype distribution in patients and controls were significantly different. The difference was due to significantly higher frequency of ACE DD homozygotes, and lower frequency of heterozygote ACE ID (Table 2).

Albeit the genotype frequency resulted in a higher frequency of the D allele in the group of cases than controls, the difference in allele frequency did not reach statistical significance with this sample size. Testing genetic equilibrium between the observed and expected genotypes using HWE showed ACE genetic variants were confirming to the law in all groups (Table 2).

**Table 1: Characteristics of study population**

| Parameters | Group I; Control (n:186) | Group II; Hypertensive (N:173) |
|---|---|---|
| | Mean ± SD | Mean ± SD |
| **Age** | 49.3 ± 10.3 | 50.7 ± 11.4 |
| **Males [n (%)]** | 102(54.83%) | 108 (62.42%)* |
| **BMI (kg/m²)** | 22.7 ± 4.3 | 26.4 ± 5.7* |
| **Waist/hip ratio** | 0.91 ± 0.05 | 0.95 ± 0.06* |
| **Systolic BP (mmHg)** | 126 ± 10 | 144 ± 22* |
| **Diastolic BP (mmHg)** | 80 ± 8 | 88 ± 10* |
| **Fasting plasma glucose (mg/dl)** | 77.8 ± 11.3 | 91.4 ± 9.8* |
| **Triglycerides (mg/dl)** | 119.6 ± 21.8 | 161.7 ± 29.2* |
| **Total cholesterol (mg/dl)** | 162.5 ± 25.6 | 198.5 ± 44.3* |
| **HDL- cholesterol (mg/dl)** | 48.5 ± 6.4 | 43.5 ± 4.4* |
| **LDL- cholesterol (mg/dl)** | 87.9 ± 20.4 | 123.2 ± 31.2* |
| **VLDL- cholesterol (mg/dl)** | 25.4 ± 8.6 | 35.6 ± 9.8 * |
| **Serum ACE (SACE) (U/L)** | 17.46 ± 2.41 | 40.80 ± 3.96* |

*p-Value<0.05 (Group II Vs Group I)

**Table 2: Genotype distribution and allele frequencies of ACE I/D polymorphism in Hypertensive patients and in controls.**

| Genotypes | Control (n=186) | Cases (n=173) | p-Value | OR (CI) |
|---|---|---|---|---|
| **ID** | 109 (58.6%) | 63 (36.41%) | 0.008 | 0.42 (0.22 – 0.80) |
| **DD** | 40 (21.5%) | 75 (43.35%) | 0.007 | 2.60 (1.31 – 5.18) |
| **HWE: $x^2$ ; p-Value** | 2.30; 0.13 | 3.63; 0.06 | | |
| **p allele (I) frequency** | 0.49 | 0.39 | | |
| **q allele (D) frequency** | 0.51 | 0.61 | | |

**Table 3: Clinical characteristics and biochemical variables of hypertensive patients across ACE genotype**

| Characteristics | II (n=35) | ID (n=63) | DD (n=75) |
|---|---|---|---|
| **Age** | 51.4 ± 9.2 | 48.6 ± 10.2 | 51.2 ± 8.6 |
| **Males [n (%)]** | 22(62.85%) | 41 (65.07%) | 45 (60.0%) |
| **BMI (kg/m²)** | 26.6 ± 5.8 | 26.8 ± 4.8 | 25.9 ± 5.6 |
| **Waist/hip ratio** | 0.96 ± 0.04 | 0.94 ± 0.08 | 0.95 ± 0.04 |
| **Systolic BP (mmHg)** | 140 ± 18* | 146 ± 24* | 148 ± 20* |
| **Diastolic BP (mmHg)** | 84 ± 12* | 88 ± 8* | 90 ± 8* |
| **Fasting plasma glucose (mg/dl)** | 90.2 ± 10.4 | 94.6 ± 8.8 | 92.0 ± 6.6 |
| **Triglycerides (mg/dl)** | 158.7 ± 25.6 | 160.7 ± 30.4 | 164.8 ± 28.6 |
| **Total cholesterol (mg/dl)** | 196.5 ± 48.2 | 201.6 ± 42.6 | 197.3 ± 40.5 |
| **HDL- cholesterol (mg/dl)** | 43.6 ± 4.8 | 42.9 ± 4.7 | 43.8 ± 4.1 |
| **LDL- cholesterol (mg/dl)** | 121.2 ± 28.4 | 125.4 ± 32.1 | 123.7 ± 29.8 |
| **VLDL- cholesterol (mg/dl)** | 33.9 ± 9.1 | 36.1 ± 10.2 | 36.6 ± 8.4 |
| **Serum ACE(SACE) (U/L)** | 34.4 ± 4.1* | 42.8 ± 3.4* | 48.6 ± 3.2* |

*p-Value<0.05

**Table 4: Multivariate logistic regression analysis**

| Genotype | Control | Cases | OR (CI) |
|---|---|---|---|
| **Serum ACE activity (U/L)** | | | |
| **II** | 14.2 ± 2.2 | 34.4 ± 4.1 | 1.0 |
| **ID** | 16.1 ± 2.6 | 42.8 ± 3.4 | 1.2 (1.04-1.97) |
| **DD** | 22.1 ± 2.1 | 48.6 ± 3.2 | 1.8 (1.08-3.74) |

The comparison of blood pressure levels and other variables across ACE genotype showed that there was a trend of increase in SBP, DBP and serum ACE activity with increasing number of D alleles (Table 3). However, neither serum ACE concentrations nor BP values differ significantly between men and women and were not correlated to age and BMI. Table 4 provides the serum ACE activity according to variant form of ACE polymorphism, with activity of 34.4 ± 4.1, 42.8 ± 3.4 and 48.6 ± 3.2 U/L in II, ID and DD genotyped hypertensive patients respectively.

## DISCUSSION

Association between ACE levels, ACE polymorphism and essential hypertension, is controversial. In different studies[9,10] no strong correlation has been found between plasma ACE levels and hypertension or between ACE polymorphisms and hypertension. Even in experimental study carried out by Kessler et al[11], with transgenic mice, suggest that serum ACE level is not enough to maintain blood pressure normal and that endothelial cell-bound ACE is critically important for maintaining blood pressure. On the contrary, other studies have suggested that the plasma ACE activity is higher in adults with essential hypertension.[12,13] In children, ACE has been found to be positively associated with blood pressure levels and contribute to the development of hypertension.[14] We therefore considered it worthwhile to unravel the serum ACE activity in relation to I/D polymorphism in newly diagnosed adult hypertensive patients with respect to disease and indeed found a significant interaction with the disease phenotype.

Result presented here supports to the hypothesis that ACE activity and hypertension are associated. The primary finding from this study using a case control approach, involving individuals with newly diagnosed EHT is that ACE activities were highest in DD carriers, then in II carriers, and intermediate in ID carriers. Multivariate meta-analysis after adjustment for the covariates, did not change the significance of the results. ($p<0.05$, 95% CI 1.08 – 3.74, Table 4), implying a risk of approximately 1.8 times higher than for those homozygous for the I allele. This result was consistent with the studies that reported that ACE activities were significantly higher in patients with DD genotype versus the two other groups having ID and II genotype.[6,15,16] However, Ljungberg's[17] reported that individuals with the II group had considerably higher plasma ACE level than most DD carriers.

We also analyzed the genotypic and the allelic distributions of the ACE I/D polymorphism between controls and hypertensive patients. Genotype data were available for all participants, frequency of DD genotype was found to be significantly higher in cases than in control participants ($p<0.05$). Therefore, we suggest that the genetic variation at the ACE locus as DD variant in intron 16, contribute to an increased risk of hypertension. Our findings were in conformity with other studies[18,19] but not all.[20] This difference may possibly be due to different races, methods of quantitation and patient selection.

In conclusion, serum ACE activity and ACE I/D polymorphism revealed significant influence on hypertension. However, limited information on potential risk factors may restrict the ability to make a detailed confounding adjustment for all factors essential for the study of hypertension. Therefore, the relationship between the ACE I/D polymorphism and serum ACE activity, with EH is still inconclusive, large scale studies with different ethnicities are warranted.

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