A Study of Angiotensin Converting Enzyme (ACE) Gene Polymorphism in Essential Hypertension among a Business Community in Punjab

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ABSTRACT The present study was carried out to investigate the association of the angiotensin -converting enzyme deletion/insertion polymorphism with hypertension and its role in increasing the susceptibility to hypertension among Bania population in Punjab. The study blood samples consisted of 50 normal (28 males and 22 females) healthy individuals and 50 hypertensive, age and sex matched (28 males and 22 females) individuals. The genotype frequencies were found to be 0.22, 0.32 and 0.46 for II, DD, ID genotype in hypertensives. The same has been found for normotensives to be 0.26, 0.18 and 0.56 for II, DD and ID respectively. The observed overall genotype distributions were consistent with Hardy-Weinberg equilibrium. The present analysis suggested that the genetic variation at the ACE gene may be associated with some determinants for blood pressure.

INTRODUCTION

Many studies have demonstrated the genetic linkage between ACE gene and blood pressure variations (Jeunemaitre et al. 1992; Chiang et al. 1996; Nakano et al. 1998; Ashavaid et al. 2000; Cox et al. 2002; Morshed et al. 2002). Despite of the fact that significant positive association of ACE gene with hypertension has been reported by many authors. However, many other studies (Dura et al. 1994; Barley et al. 1996; O'Donnell 1998) have failed to identify such associations. It has been suggested (Barley et al. 1994; Stassen et al. 1997) that this inconsistent association may be due to the fact of different ethnicity of the population groups and environmental heterogeneity. Therefore, it is of interest to study regarding the deletion/ insertion (D/I) polymorphism of ACE gene and blood pressure regulation. Hence, the present study was carried out to investigate the association of I/D polymorphism of ACE gene with hypertension and its role of increasing the susceptibility of hypertension among Bania population in Punjab.

MATERIALS AND METHODS

The study included a total of 100 individuals

of both sexes, consisting 50 each age and sex matched controls and patients with hypertension. These individuals were selected from the Bania community in Punjab. This community is mostly engaged in business and trade. They are primarily vegetarians and non-alcoholic. However, many male members of the family prefer alcoholic drink time to time. They are living in patrilocal society with by and large nuclear family system in single household sharing both genes and environment. The age ranges of selected subjects are between 40 to 70 years.

Blood pressure was measured using a mercury sphygmomanometer in a sitting position on with the right forearm placed horizontal on the table, as recommended by the American Heart Association (1981). All efforts were made to minimize the factors which may influence blood pressure. Hypertension was defined as systolic blood pressure >140 mm Hg accompanied by diastolic blood pressure >90 mm Hg. None of the subjects had evidence of cardiac or renal diseases. About 5ml of peripheral blood samples were collected in a pre-labeled screw cap vial containing 20% EDTA. The specimens were transported from the place of collection to the laboratory on dry ice and were then stored at -20⁰ C till further analysis. DNA was isolated from whole blood using standard protocol of Gill et al. (1987). To determine the ACE genotype, genomic DNA samples were amplified by a polymerase chain reaction (PCR) using primer pair such as 5'-CTG GAG AGC CAC TCC CAT CCT TTC T-3'

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(sense) and 5' GAC GTC GCC ATC ACA TTC GTC AGA T 3' (antisense). The PCR reaction mixture contained 100 ng genomic DNA, 0.2 μ M of each primer, 200 μ M dNTP, 10 mM of Tris-HCL buffer (PH 8.3), 1.5 mM MgCl₂ (PH 8.3) and 0.024 units of Taq polymerase enzyme in a final reaction volume of 15.0 μ l. After initial denaturation at 95^o C for 5 minutes, the DNA was amplified by 30 cycles of denaturation at 94^oC for 45 seconds, annealing at 56^o C for 45 seconds and primer extension at 72^o C for 45 seconds. Final extension was performed at 72^o C for 10 minutes. The amplified PCR products were separated by electrophoresis on 2% agarose gel and the DNA was visualized under UV transilluminator after staining with ethidium bromide. The insertion (I) allele was detected as band of 490 bp fragment and deletion (D) allele was identified as a band of 190 bp fragment. All the data were analyzed by SPSS 10.0. Two- tailed probability levels for statistical significance are reported. The relevant null hypothesis on odds ratio (Matched Pairs) in the present case is tested H₀: OR_M=1.

RESULTS

Anthropometric and physiometric characteristics of the control and hypertensive subjects are presented in table I. The two study groups were well matched for sex, age and sample size. The mean systolic (SBP) and diastolic (DBP) blood pressures, BMI and WHR were significantly higher (P<0.001) among hypertensive

subjects than in control subjects. However, there is no significant mean age difference between both groups. The ACE genotype and allele frequencies distribution of control and hypertensive subjects are shown in table 2. The frequencies of II, ID and DD genotype among the control group were 26% (n=13), 54% (n=27) and 20% (n=10) respectively, whereas, in hypertensive group the same were found to be 22% (n=11), 32% (n=16) and 46% (n=23) respectively. There is significant difference (p<0.05) observed in the distribution of ACE genotype polymorphism between the two groups. It has been observed that the ACE DD genotype was significantly (p<0.05) higher in hypertensive subjects, whereas, ID genotype was significantly (p<0.05) higher in control subjects. The frequency of the D allele is also more frequent but not significant in hypertensive subjects than in control. The distribution of genotype frequencies associations of ACE gene polymorphisms between male and female among control and hypertensive subjects are given in table 3. The results showed that among three genotypes within control group, ID genotype was significantly more prevalent in male as compared to other two genotypes (odds ratio:3.0; CI: 0.152-5.84; χ^2 =14.0, P<0.001). Whereas, among female, II genotype is comparatively more prevalent but not significantly differ (odds ratio: 0.692; χ^2 =1.45, ns). In the hypertensive group, both male and female are more associated with DD genotype as compared to other two genotypes. However, these

Table 1: Anthropometric and physiometric characteristics of the control and hypertensive subjects

Variables	Controls (n=50) Mean $\pm$ SD	Hypertensive (n=50) Mean $\pm$ SD	p
Age (years)	54.29 $\pm$ 8.42	54.25 $\pm$ 9.03	ns
BMI (Kg/m ²)	26.56 $\pm$ 3.19	29.32 $\pm$ 3.25	<0.001
WHR	0.851 $\pm$ 0.005	0.971 $\pm$ 0.006	<0.001
SBP (mm Hg)	129.31 $\pm$ 10.13	148.23 $\pm$ 9.11	< 0.001
DBP (mm Hg)	85.19 $\pm$ 8.79	92.14 $\pm$ 7.37	<0.001

Table 2: Distributions of genotype and allele frequencies of ACE gene polymorphism in control and hypertensive subjects

Genotypes	Controls (n=50) Number (%)	Hypertensive (n=50) Number (%)	p
II	13 (26%)	11 (22%)	χ^2 = 8.11, <0.001
ID	27 (54%)	16 (32%)	
DD	10 (20%)	23 (46%)	
Alleles			χ^2 = 0.0032, ns
I	0.51	0.47	
D	0.49	0.53	

Table 3: Distributions of ACE genotypes between men and women

ACE genotypes	Control		Hypertensive	
	Male (n=28) Number (%)	Female (n=28) Number (%)	Male (n=28) Number (%)	Female (n=28) Number (%)
II	4 (14.28%)	9 (40.91%) ^b	6 (21.43%)	5 (22.73%)
ID	21 (75%) ^a	6 (27.27%)	9 (32.14%)	7 (31.82%)
DD	3 (10.72%)	7 (31.82%)	13 (46.43%) ^c	10 (45.45%) ^d
χ^2	11.30, P < 0.005		0.0124, ns	

ns= not significant

^aID against II + DD $\chi^2 = 14.0$, P < 0.001, OR=3.0 (0.152 - 5.84)^bII against ID + DD $\chi^2 = 1.45$, ns, OR=0.692^cDD against II + DD $\chi^2 = 0.286$, ns, OR=0.867^dDD against II + DD $\chi^2 = 0.364$, ns, OR=0.833

associations are not statistically significant (odds ratio (male)=0.867; $\chi^2=0.286$, ns; (female)=0.833, $\chi^2=0.364$, ns).

DISCUSSION

In the present study, it has been investigated the association of ACE insertion/ deletion (I/D) polymorphism with hypertension among selected individuals from Bania (a business community) population in Punjab. The study was carried out between two groups (control and hypertensive) which were perfectly matched for age and sex. However, they were significantly different with respect to BMI, WHR, SBP and DBP. The study suggested a possible positive association of DD polymorphism with hypertension in Bania Population in Punjab. Many studies (Cambien et al. 1992; Tiret et al. 1993; Duru et al. 1994; Morise et al. 1994; Barley et al. 1996; Samani et al. 1996; Zaid et al. 1998; Turgay et al. 1999; Higaki et al. 2000) have reported positive ACE DD genotype association with hypertension. The frequency of D allele (53%) in hypertensive group is more prevalent as compared to other allele between both the groups. Although, the difference is not significant ($\chi^2=0.0032$, ns). In the present study, sex specific association of DD genotype with hypertension has not been seen. The possible reason for negative association of DD genotype with hypertension between both sexes may be relatively smaller sample size. However, it is interesting to note that in control group ID genotype was more significantly (P<0.001) associated with male, whereas, II genotype is more prevalent (41%) in female though it is not statistically significant. Therefore, the present data suggest DD genotype have some association with hypertensive male and female individuals,

although, the data did not show any statistical significance.

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