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Association between the Angiotensin-converting Enzyme Gene Insertion/Deletion Polymorphism and Essential Hypertension in Young Pakistani Patients

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Several studies have demonstrated the importance of angiotensin-converting enzyme (ACE) insertion (I)/ deletion (D) polymorphisms in the pathogenesis of hypertension. This study sought to determine the association between the ACE I/D polymorphism and essential hypertension in young Pakistanis. The frequency of the ACE I/D polymorphism was established by a comparative cross-sectional survey of Pakistani patients suffering from essential hypertension and ethnically matched normotensive controls. Samples were collected from tertiary care hospitals in northern Pakistan. Hypertensive individuals were defined as those with a systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg on three separate occasions, or those currently receiving one, or more, anti-hypertensive agents. DNA samples obtained from hypertensive (n = 211) and normotensive (n = 108) individuals were typed by PCR. The frequency of the ACE I/I genotype was significantly higher in hypertensive patients, aged 20-40 years, than in normotensive controls of the same age group (χ^2 = 4.0, P = 0.041). Whereas no overall significant differences were observed between the I/I, I/D and D/D ACE genotypes (One way ANOVA, F = 0.672; P = 0.413). The association between the ACE I/I genotype and essential hypertension in individuals aged ≤ 40 years suggests that ACE has a role in early onset essential hypertension in Pakistan.

Keywords: ACE I/D polymorphism, Angiotensin-converting enzyme, Cross-sectional survey, Essential hypertension, Pakistani population

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Introduction

Cardiovascular diseases are fast emerging as a major health burden for developing economies like Pakistan (Nistar, 2002). Hypertension is a major modifiable risk factor of morbidity and mortality from cardiovascular causes. It is a multifactorial and polygenic disorder in which the interaction between several candidate genes and environmental factors play a role. The rennin angiotensin system is an important regulatory mechanism for maintaining normal blood pressure and fluid and electrolyte balance, and angiotensin-converting enzyme (ACE) is a key enzyme in this system, which catalyzes the conversion of angiotensin I to angiotensin II, a potent vasopressor (Erdos, and Skidgel, 1987).

ACE plasma level variability has been reported to be associated with the insertion (I)/ deletion (D) polymorphism of an *Alu* repeat sequences in intron 16 of the ACE gene (Rigat *et al*., 1990). Various studies have shown association between this polymorphism and several cardiovascular diseases like myocardial infarction (Ludwig *et al*., 1995), left ventricular hypertrophy (Schunkert *et al*., 1994), cardiomyopathy (Raynolds *et al*., 1993) and hypertension (Duru *et al*., 1994; Barley *et al*., 1996; Jeng *et al*., 1997). Moreover, studies have been carried out on the association between the ACE I/D polymorphism and hypertension in various populations, and both positive (Duru *et al*., 1994; Barley *et al*., 1996; Jeng *et al*., 1997) and negative (Higashimori *et al*., 1993; Vassilikioti *et al*., 1996; Chiang *et al*., 1997) associations have been reported. It has been postulated that the association between the ACE I/D polymorphism and hypertension might be related to gender and ethnicity (Barley *et al*., 1996; Sagnella *et al*., 1999).

However, to date no study of this type has been conducted in Pakistan. The present study was initiated to determine whether the presence or absence of the ACE I-allele polymorphism is associated with essential hypertension in the Pakistani population.

Materials and Methods

Peripheral blood samples of hypertensive (n = 211) and normotensive (n = 108) individuals were collected with informed consent from a tertiary care hospitals in the vicinity of Islamabad, Pakistan. The hypertensive patients and normotensive controls analysed were mainly from northern Pakistan. Essential hypertension was diagnosed in individuals with a systolic blood pressure (SBP) > 140 mmHg and/or a diastolic blood pressure (DBP) > 90 mmHg or in those currently receiving anti-hypertensive therapy. Secondary forms of hypertension were ruled out based on clinical history and laboratory investigations. The normotensive controls were healthy individuals with a negative history of hypertension and with a SBP < 140 mmHg and DBP < 90 mmHg measured on three separate occasions.

DNA samples were isolated from peripheral blood lymphocytes by the standard phenol extraction method (Sambrook *et al*., 1989). The ACE I/D polymorphism was detected by PCR as described by Batzer *et al*., (1996). Briefly, amplification was carried out in a final volume of 20 ml containing 40 ng genomic DNA, 1 mM of each primer, 200 mM dNTPs, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4), and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, USA).

The primers used were: 5'-CTGGAGACCACTCCCATCCTTTCT-3' as forward primer and 5'-GATGTGGCCATCACATTCGTCAGAT-3' as reverse primer. Amplification resulted in a combination of a 490-bp product and/or a 190-bp product depending on the presence or absence of the ACE I-allele fragment, respectively. The amplified products were analysed by electrophoresis on a 2% (w/v) agarose gel containing 0.5 μg/ml ethidium bromide (Sigma-Aldrich, St. Louis, USA) and were photographed under UV transillumination (Syngene, Cambridge, UK). Statistical Analysis was carried out using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, USA).

Results

The genotypic and allelic frequencies of the ACE I/D polymorphism in hypertensive and normotensive subjects are given in Table 1. The frequency of the ACE I-allele was similar in the hypertensive patients and normotensive controls (0.55). Overall no significant differences were observed between the I/I, I/D and D/D ACE genotypes in the patients and controls (One way ANOVA, F = 0.672; P = 0.413).

The distributions of the ACE I/D genotypes among sexes and in individuals below and above 40 years are shown in Fig. 1 and Table 2. It is noteworthy that the frequency of the ACE I/I genotype was significantly higher in hypertensive patients aged 20-40 years than in age-matched normotensive controls (Fig. 1A) ($\chi^2 = 4.0$; P = 0.041). The frequency of the ACE I/D genotype also tended to differ between these groups (Fig. 1A), but this was not statistically significant ($\chi^2 = 3.2$; P = 0.055). The I/I genotype also showed a tendency to be higher in hypertensive females (Fig. 1D), but again this was not statistically significant ($\chi^2 = 0.488$; P = 0.323).

Discussion

Cardiovascular diseases are rapidly emerging as a major health concern in most developing countries, including Pakistan, and hypertension is one of the major preventable causes of morbidity and mortality from cardiovascular disease. Preliminary surveys carried out by the Pakistan Medical Research Council (1998) estimated that 5.5 million men and 5.3 million women in Pakistan suffer from hypertension, which constitutes approximately 8% of the Pakistani population. The prevalence of hypertension also varies significantly among the different ethnic groups in Pakistan. It has been reported that the prevalence of hypertension is approximately 17% in Punjabies and 26% in Pathans (Jafar *et al*., 2003).

The association between hypertension and the ACE I/D polymorphism has not been studied in the Pakistani population. In the present study, we examined the association of the ACE I/D polymorphism in Pakistani patients suffering from essential hypertension.

In this study a significant association (P < 0.05) was found between the ACE I/I genotype and young hypertensive individuals (male and female; 20-40 years). Although in a pervious study, a much higher frequency (0.40) of this genotype (I/I) was reported in expatriate south Asians in the UK (Sagnella *et al*., 1999) than was observed in this study (0.33). However, the association between the ACE I/I genotype and young hypertensive individuals has not been reported to date.

No detectable association was found between the ACE I/D and D/D genotypes and essential hypertension in the northern Pakistani population. Age and gender dependent associations between the ACE gene deletion polymorphism (D/D genotype) and hypertension have been demonstrated in the Chinese (Chiang *et al*., 1996) and in Africans (Duru *et al*., 1994; Barley *et al*., 1996; Sagnella *et al*., 1999). This correlates with the finding that subjects with the D/D genotype have enhanced ACE expression, which results in higher levels of angiotensin II,

Table 1. The allelic and genotypic frequencies of the ACE insertion (I) and deletion (D) polymorphisms in hypertensive and normotensive Pakistani subjects

Affection Status	n	I-allele frequency	Genotype Frequency		
			I/I	I/D	D/D
Hypertensive	211	0.55	0.327	0.455	0.218
Normotensive	108	0.55	0.296	0.509	0.194

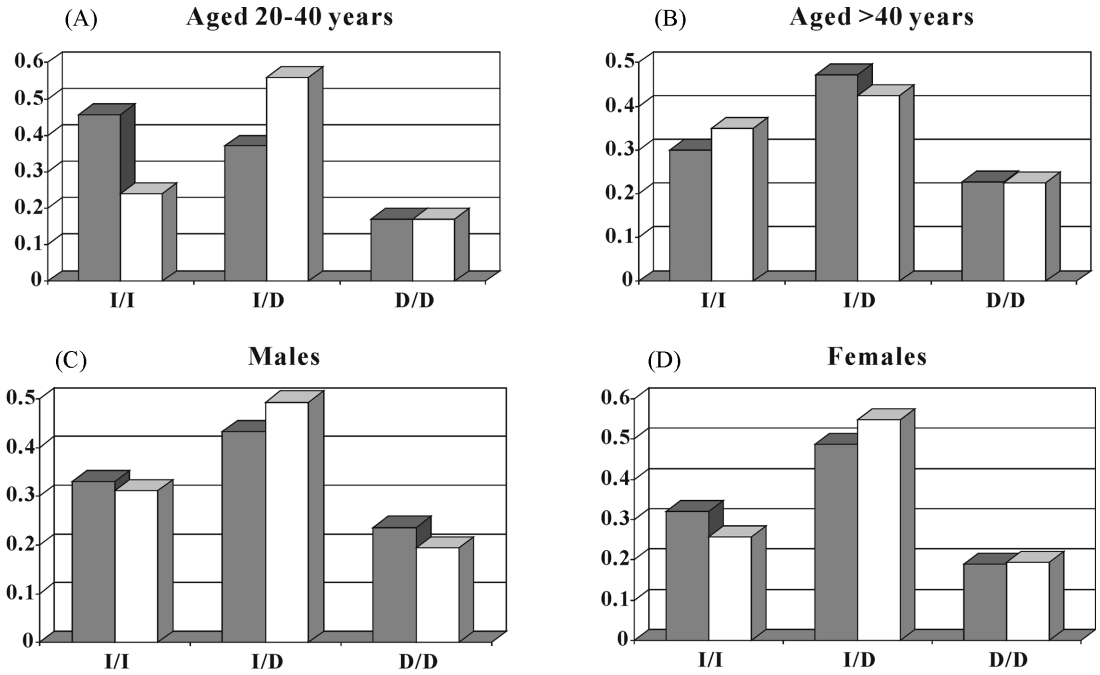

Fig. 1. The frequencies of angiotensin converting enzyme gene insertion/deletion polymorphisms in hypertensive patients (black bars) and normotensive controls (white bars): (A) aged 20-40 years; (B) aged >40 years; (C) males and (D) females.

Table 2. The frequencies of the ACE genotypes by age, sex and affection status

Age group	Sex	Affection status	n	ACE Genotype (frequencies)		
				I/I	I/D	D/D
≤ 40 years	Female	Hypertensive	19	0.42	0.47	0.11
		Normotensive	22	0.27	0.55	0.18
	Male	Hypertensive	16	0.50	0.25	0.25
		Normotensive	46	0.26	0.57	0.17
	Total	Hypertensive	35	0.46	0.37	0.17
		Normotensive	68	0.24	0.56	0.17
> 40 years	Female	Hypertensive	67	0.30	0.49	0.21
		Normotensive	9	0.22	0.55	0.22
	Male	Hypertensive	109	0.30	0.46	0.24
		Normotensive	31	0.39	0.39	0.23
	Total	Hypertensive	176	0.30	0.47	0.23
		Normotensive	40	0.35	0.42	0.22

and thus increases susceptibility to hypertension. In the Pakistani population, the frequency of the ACE D/D genotype did not differ significantly between hypertensive patients and normotensive controls (0.218 verses 0.194, respectively). Similar results have also been reported for the Greek (Vassilikioti *et al.*) and white European populations (Barley *et al*., 1996; Sagnella *et al*., 1999), which are genetically close to the Pakistani population as demonstrated by neutral microsatellite marker analysis (Ayub *et al*., 2003).

The association of the ACE I/I genotype with early onset essential hypertension in the Pakistani population is based on a limited number of young hypertensive individuals (n = 35) and a more extensive survey is required to confirm this finding, which may have possible diagnostic and therapeutic implications (Niu *et al*., 2002).

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