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The Insertion I/ Deletion D polymorphism of Angiotensin-Converting Enzyme (ACE) Gene Increase the Susceptibility to Hypertension and / or Diabetes

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ABSTRACT The causes of hypertension and type II diabetes (NIDDM) are mainly unknown, but they arise from interplay between several genetic and environmental factors. Hence the present study was aimed to investigate whether the Insertion I/ Deletion D polymorphism of angiotensin-converting enzyme (ACE) gene increase the susceptibility to hypertension and / or diabetes. ACE gene was genotyped in 200 hypertension patients, 100 type II diabetic patients and 200 age and sex matched controls. From the present data it was observed that in hypertension patients genotypic and allelic frequencies were significantly deviated from Hardy-Weinberg equilibrium ($p<0.05$). The DD genotype was strongly associated with hypertension [odds ratio (OR) = 2.02, confidence interval (CI) = 1.14-3.58, $p<0.05$] and remained so when patients with type II diabetes were excluded from the analysis (OR = 2.07, CI = 1.10-3.93, $p<0.05$) and significant association was not obtained in diabetic patients without hypertension. From the present results, it was concluded that *D* allele of ACE gene protects against diabetes, however it increases susceptibility to hypertension particularly when associated with type II diabetes.

INTRODUCTION

Patients with hypertension have a high risk of developing severe complications, such as diabetic nephropathy, retinopathy and cardiovascular disease. Type 2 diabetes is an important complication of hypertension and is observed in more than 30% of patients with hypertension (Dodson.1990). Studies demonstrating familial clustering of diabetic nephropathy, cardiovascular disease and hypertension (Seaquist et al. 1989; Earle et al. 1992; Freire et al. 1994) suggest that, in addition to poor blood pressure and glycemic control, genetic factors may affect susceptibility to the development of hypertensive micro-and macroangiopathy. In this context, genetic polymorphisms of the renin-angiotensin system (*RAS)* are attractive candidates to be studied, since inhibition of the activity of this system has shown to retard the development of diabetic complications, such as nephropathy and retinopathy (Lewis et al. 1993; Chaturvedi et al. 1998).

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Renin angiotensin system may play an important role in blood pressure regulation and acts as a key regulator of Sodium homeostasis. The gene coding for Angiotensin converting Enzyme *(ACE)* regulates vascular tone through the activation of angiotensin II, a potent vasoconstrictor (Timmermans et al. 1993), and inactivation of bradykinin (Atlas. 1998), a non-apeptide belonging to a class of active peptides (kinins) that are released from tissue to produce a variety of effects, including arterial vasodilatation and venoconstriction. The insertion/ deletion (I/D) polymorphism of the ACE gene is characterized by the presence (I) or absence (D) of a 287-bp alu repeat sequence within intron 16 of the *ACE* gene. *ACE* polymorphism appears to have a significant impact on narrowing of blood vessels that offer protection against type II diabetes but if these carriers do eventually develop the disease, they would face more serious complications.

The *ACE* I/D polymorphism is also associated with overall plasma ACE levels (Rigat et al. 1990). Patients homozygous for the D allele are characterized by elevated plasma levels of ACE compared with patients homozygous for the I allele, which might explain a diversity in the response to *ACE* inhibition (Marre et al. 1997).

Furthermore, the ACE I/D polymorphism has been suggested to play an important role in the individual antiproteinuric response to ACE inhibition (Jacobsen et al. 1998). Therefore, because the I/D polymorphism affects ACE and angiotensin II levels, it may also affect an individual's sensitivity to insulin and hence ACE might be a good candidate gene for determining insulin resistance.

The ACE insertion (I)/ deletion (D) polymorphism is not only effective in playing a role in hypertension and diabetes (Ruiz et al. 1994; Vijay et al. 2001) but also participates in cardiac complications (Cambien et al. 1992; Marian et al.1993; Schunkert et al. 1994, Lindpaintner et al. 1995). However, these studies yielded conflicting results: some claiming positive associations with *ACE* genotype, and others contradicting. Several case-control studies of hypertensive diabetes have demonstrated a positive association of the ACE I/D polymorphism (Bengtsson et al. 1999; Staessen et al. 1997). However the influence of ethnic background on the link between hypertension and/or diabetes and ACE polymorphism still remains unresolved. It is important to look for the gene association in the Asian Indian population in view of high prevalence of hypertension and diabetes.

Hence the present study was carried out to investigate the association of I/D polymorphism with hypertension and / or diabetes and its role in increasing the susceptibility to hypertension and / or diabetes.

MATERIAL AND METHODS

The study was carried out in 200 hypertensive patients (HTN), 100 normotensive type II diabetic patients (NDM), reported at CARE hospital aged 30-65 years and 200 age and sex matched random controls. Further, hypertensive group was sub-divided into 2 groups: Non-diabetic individuals with hypertension (NHTN, n=125), and type II diabetic individuals with hypertension (HDM, n=75).

Data were collected from each patient on clinical variables including age, height, weight, body mass index, cigarette smoking, alcohol consumption and family history etc. Diagnosis of hypertension and diabetes was based on the physical and clinical examination of patients by the doctors followed by appropriate laboratory and other investigations.

Hypertension and diabetes were defined according to WHO criteria (EC report 1998, VIIth JNC report 2003). Patients with secondary hypertension, nephropathies and Cardiac abnormalities were excluded from the present study.

Determination of ACE Genotype: DNA was isolated from whole blood using standard protocols (Miller et al. 1988) and ACE gene sequence was amplified by polymerase chain reaction (PCR-Thermo cycler, Biometra, U.S.A) initially using a flanking primer pair (Rigat et al. 1992) and subsequently when necessary with a primer pair that recognizes the insertion specific sequences for confirmation of specificity of the amplification reactions (Shanmugan et al. 1993). The amplicons were separated on 2% agarose gel electrophoresis (Bangalore Genei Ltd, Bangalore) and the bands were visualized under U.V light.

Statistical Analysis: Statistical comparisons between group means were done by ANOVA, while proportions were compared by means of χ^2 test. Deviations from the Hardy-Weinberg equilibrium were tested with χ^2 analysis. Multiple logistic regressions were used to test the effect of ACE genotypes on the likelihood of hypertension and / or diabetes while controlling for other confounding factors. The odd's ratios (OR) together with the 95% confidence interval (CI), comparing the allelic distribution in the four study groups were also calculated. Two-tailed p values less than 0.05 were considered significant. The SPSS package (10.0 version) was used to perform statistical analysis.

RESULTS

Table 1 describes baseline characteristics of patients with hypertension and /or diabetes and control subjects. It was observed that patient groups significantly differed from control subjects with respect to age, BMI, SBP ($p<0.05$). Significant difference for DBP was observed only in HTN patients as compared to controls ($p<0.05$). When comparisons were made with in the patient groups, it was observed that HTN patients were slightly older, with overweights than NDM group. HDM patients were observed to have elevated systolic and diastolic blood pressure (Table 1) as compared to NDM patients and controls. A greater proportion of males were observed to have hypertension in complication with diabetes. Significantly greater proportions of smokers and alcoholics were observed among NHTN patients as compared to other patient groups and controls

Table 1: Base line characteristics observed in patients with hypertension and/or diabetics and control subjects

Patients	*Variable*			*Controls (n=200)*
	NHTN (n=125)	*HDM (n=75)*	*NDM (n=100)*	
Mean age (Yrs)	50.9±0.94	[1]51.3±1.16	49.14±1.03	45.06±0.53
Mean BMI (Kg/m^2)	[1]26.7±0.43	26.1±0.41	26.5±0.48	25.4±0.29
Mean SBP (mm/Hg)	151.5±2.28	[1]152.8±2.81	125.6±0.70	119.9±0.50
Mean DBP (mm/Hg)	92.3±0.90	93.1±1.01	81.4±0.31	80.0±0.24
Male Sex (%)	67.9	75.4	66.8	73.5
Smokers (%)	26.9	24.6	26	9
Alcoholics (%)	[2]43.6	36.1	40	16.5
SLS (%)	29.5	[2]32.8	20	16
Familial (%)	[2]42.3	37.7	36	18.5

1. ANOVA, P<0.05
2. χ^2 test, P<0.05
NHTN- non-diabetic hypertension, HDM- hypertension with diabetes, NDM- normotensive diabetes, BMI=Body mass Index, SLS = Sedentary life style.

(p<0.05). Significantly greater proportion of physically inactive individuals was observed with hypertension in combination with diabetes. A strong genetic component was observed in NHTN patients as compared to other groups.

Table 2 describes the distribution of *ACE* genotypes in the four observational groups. It was observed that the prevalence of DD homozygotes was high (47.8%) in NHTN patients as compared to *NDM* patients (24.0%) and controls (22.5%). ID heterozygote frequency was high in HDM patients (37.4%) as compared to NDM patients (24%). In contrast prevalence of II homozygotes was high in NDM patients (52.0%) as compared to controls (40.0%).

Table 2: Distribution of ACE genotypes in the four observed groups

ACE genotype	*Patients*						*Controls*	
	NHTN		*HDM*		*NDM*			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
DD	59*	47.2	25	33.3	24	24.0	45	22.5
ID	35	28.0	28	37.3*	24	24.0	75	37.5
II	31	24.8	22	29.4	52	52.0	80	40.0

* p <0.05

The genotypic and allele frequencies differed significantly in HTN patients from the control group (p<0.05). No significant differences were observed in the distribution of the ACE I/D genotypes between *NDM* patients and control subjects (Table 2). The distributions of *ACE* genotypes observed were in agreement with the Hardy-Weinberg proportion except for NHTN group (p<0.05).

The adjusted odds of prevalence of hypertension and/or diabetes (Table 3a) revealed a strong association of DD genotype with hypertension taking II genotype as reference (odds ratio OR-2.02, 95% confidence interval CI- 1.14-3.58, p<0.05). And remained so in NHTN patients (OR-2.08, 95% CI- 1.10-3.93, p<0.05) and HDM patients (OR-1.98, 95% CI- 0.95-4.12, p<0.05). However, when ID genotype was taken as reference (Table 3b), HDM patients had 2.5 (95% CI- 1.73- 3.47, P<0.05) times higher odds of hypertension as compared to NDM patients and nearly 1.2 times risk (95% CI-1.09-2.18) as compared to NHTN patients. There was no association with NDM patients.

Table 3a: Odd's of prevalence of hypertension and/ or diabetes

Genotype	*Odd's ratio*[a]	*95% CI*	
		Lower limit	*Upper limit*
HTN			
DD vs. II	2.02*	1.14	3.58
ID vs. II	1.73	1.03	2.90
NHTN			
DD vs. II	2.08*	1.10	3.93
ID vs. II	2.06	1.01	4.21
HDM			
DD vs II	1.98*	0.95	4.12
ID vs. II	1.36	0.68	2.72
NDM			
DD vs II	0.82	0.38	1.78
ID vs. II	0.49	0.23	1.05

[a] Multivariate adjusted odd's ratio
* p<0.05

DISCUSSION

We examined ACE gene polymorphism, one of the important genes in rennin-angiotensin system in hypertension, type 2 diabetes (NIDDM) and healthy control groups. Genetic factors, life style modification, obesity are potential risk factors to improve the complications in hypertension (Reaven.1988; VIIth JNC report. 2003). Renin angiotensin system should modulate a risk for hypertension and its complications.

Table 3b: Odd's of prevalence of hypertension and/ or diabetes

Genotype	*Odd's ratio*[a]	*95% CI*	
		Lower limit	*Upper limit*
NHTN			
DD vs. ID	2.15*	1.09	3.18
HDM			
DD vs ID	3.35*	1.73	3.47
NDM			
DD vs ID	0.85	0.49	1.46

[a] Multivariate adjusted odd's ratio
* $p<0.05$

Previous studies revealed a strong association between ACE I/D polymorphism and hypertension (Katsuya etal.1995; Mastana and Nunn. 1997). There are also previous reports showing negative association between ACE genotypes and hypertension (Sagnella et al. 1999). Vijay et al (2001) did not find any association between ACE genotypes type 2 diabetes. We also did not find any association between ACE genotypes and type 2 diabetes. The findings of our study are in agreement with some previous studies (Mastana and Nunn. 1997; Vijay et al. 2001; and Pasha et al. 2002).

Our data also showed an association between ACE DD genotype and hypertensive type 2 diabetes. We found that carrying DD genotype in these patients 2.5 times increased than normotensive diabetic patients, and 1.2 times increased than non-diabetic hypertensive patients, suggesting that the DD genotype is associated with an increased susceptibility to diabetic complications in patients with hypertension. Our study appears to be similar to previous reports (Staessen et al. 1997; Bengtsson et al.1999). Bengtsson et al. (1999) reported a significant association of D allele with hypertension but not in diabetes and also suggested that D allele might increase the susceptibility to hypertension, particularly in hypertensive type 2 diabetic patients. Results obtained from the present study could be of prognostic value in identifying individuals at risk for diabetic nephropathy in HDM patients as suggested by earlier studies (Doi et al. 1996; Ohno et al. 1996; Fujisawa et al. 1998; Yoshida et al. 1999;Vijay et al. 2001).

The observation made in the present study revealed the protective role of *D allele* in NDM patients offering insulin sensitivity as suggested by Katsuya et al. (1995). In a different context Lee et al. (2002) have reported a strong association of II genotype with insulin resistance in NIDDM patients providing genetic evidence for the clustering of the metabolic syndrome or insulin resistance syndrome.

In conclusion, observations from the present study clearly indicate the strong association of *D* allele with hypertension and its protective role in diabetes. The *D* allele increases the susceptibility to hypertension particularly when associated with type II diabetes leading to the progression of complications like diabetic nephropathy. At present the indications are that ACE may have a central position in energy metabolism and primarily acts as an enzyme of importance to the vascular and inflammatory systems.

Future studies will show whether all these associations and pathophysiological aspects of *ACE* and its basic geno- and pheno-types will lead not only to a better understanding of hypertension and/ or diabetes, as well as its many complications, but may also lead to identification of at-risk patients and/or improved pharmacological or non-pharmacological interventions.

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