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Full Length Research Paper

Association of insertion/deletion polymorphism of *Alu* angiotensin converting enzyme insertion/deletion genotype with type 2 diabetes mellitus and hypertension in J&K population: A case control study

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Abstract

The objective of the study is to know the frequencies of insertion/deletion (I/D) allele and association of angiotensin converting enzyme (ACE), I/D polymorphism in Jammu and Kashmir (J&K) populations in relation to type 2 diabetes mellitus (T2DM) and hypertension (HTN). A total of 500 individuals were recruited for the present study. Out of these 500 individuals, 250 individuals had T2DM and HTN and 250 were healthy controls. Genotyping was performed using polymerase chain reaction (PCR) using allele specific oligonucleotide primers. The allele frequency for I allele and D allele was found to be 63% and 37% in patients with T2DM and HTN and 48% and 52% for healthy controls. Genotype frequency for homozygote insertion (II), heterozygote (ID) and homozygote deletion (DD) allele was in range of 99.23, 116.55 and 34.23 for patients with T2DM and HTN and 56.64, 124.71 and 68.64 for healthy controls. ID versus II+ID model for odds showed a significant association of ACE I/D polymorphism with T2DM and HTN. The I allele and ID genotype of ACE gene is associated with T2DM and HTN.

Key words: ACE, insertion, deletion, type 2 diabetes mellitus (T2DM) and hypertension (HTN).

INTRODUCTION

Diabetes and hypertension are the two most common and potent diseases that rarely exists in isolation. Type 2 diabetes mellitus (T2DM) and hypertension (HTN) are major health problems worldwide, associated with increased prevalence of obesity and excess morbidity and mortality. Furthermore, hypertensive patients with diabetes or obesity are more predisposed to target organ damage (Chobanian et al., 2003; Whiteworth, 2003; Olsen et al., 2010; Ogihara et al., 2009). In India, more than 41 million people are suffering from diabetes. Altered ways of living in most instances has changed the lifestyles of inhabitants which put them at risk of developing non communicable diseases such as obesity, diabetes mellitus, hypertension, cardiovascular disease (CVD) (Popkin, 1998; Vorster et al., 2000). T2DM is characterized either by decrease production of insulin or insulin resistance (Wild et al., 2004). HTN is a chronic medical condition in which the blood pressure is elevated (Anthea et al., 1993). The prevalence of HTN in India varies from 30 to 61% (Kutty et al., 2002; Hazarika et al., 2002). Besides conventional risk factors, genetic factors may also play a key role in the pathogenesis of T2DM and HTN. Both T2DM and HTN are multifactorial and polygenic disorders including several candidate genes such as ESR, KCNJ11, PPARG, ENPP1, angiotensin converting enzyme (ACE), CAPN 10 and TCF7L2 that have been suggested to influence the risk of T2DM and HTN (Zhou et al., 2012). HTN in patients with type 2 diabetes results from complex interplay that includes rennin angiotensin-aldosterone system (RAAS). Study on ACE in recent

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years showed it as a candidate gene for a variety of diseases. It is a key zinc metalloenzyme of a rennin angiotensin system widely distributed in kidney (Salem et al., 2009). The gene for ACE is located on long arm of chromosome 17 (17q23.3). This enzyme is one of the key components of the rennin angiotensin system (RAS) that generates the vasoconstrictor angiotensin II and degrades the vasodilator kinins (Costerousse et al., 1997). The ACE catalyzes the conversion of angiotensin I to angiotensin II which controls the electrolytic balance and systemic blood pressure (Yang et al., 2006). Several studies suggested that the high level of angiotensin II plays an important role in glucose and insulin regulation and systemic blood pressure. ACE insertion/deletion (ID) allele is a large insertion/deletion allele in intron 16 of the ACE gene. The homozygote deletion (DD) genotype is associated with higher levels of circulating ACE than heterozygote (ID) and homozygote insertion (II) genotype (Rigat et al., 1990). Many studies have assessed the association between ACE I/D polymorphism with the risk of T2DM and HTN. However, the results have been conflicting in all the previous studies. In the present stud, a case-control association study was carried out in Jammu population of J&K State to determine the role of ACE I/D polymorphism with increased risk of HTN and T2DM.

MATERIALS AND METHODS

Sample collection

The study sample size comprised of total of 500 individuals (250 diabetic and 250 non-diabetic, healthy controls). Individuals whose blood sugar level is normal and they do not have any family history of diabetes were considered as controls. T2DM was diagnosed using the criteria of the American Diabetes Association (ADA, 2004), that is, a medical record indicating either fasting plasma glucose level (126 mg/dl) or post glucose level (≥ 200 mg/dl). The diagnosis of T2DM is based on clinical records and medication. The individuals having normal blood sugar level, blood pressure level and with no family history of diabetes were considered as controls. The blood samples of patients and controls were collected from different hospitals and private clinics with their prior consent and they were appraised about the nature of work. The study was approved by ethical committee of the participating hospitals and private clinics.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using Phenol-Chloroform (Sambrook and Russel, 2001) with slight modifications. To determine the ACE gene I/D polymorphism, a genomic DNA fragment on intron 16 of the ACE gene was amplified using polymerase chain reaction (PCR) method with a pair of oligonucleotide primers. The upstream of primer sequence was 5 CTG GAG ACC ACT CCC ATC CTT TCC 3 and downstream of primer sequence was 5 GAT GTG GCC ATC ACA TTC GTC AGA T 3. The PCR amplification products were obtained using 25 µl reaction system containing 50 cng μL^{-1} genomic DNA, 15.7 µl ddH_20, 5X buffer, dNTP, 0.5 µl of each primer, $MgCl_2$ and Taq Polymerase on Lab Net thermal cycler.

After an initial denaturation at 95°C for 5 min, DNA was amplified by 35 cycles for denaturation at 94°C for 1 min, annealing at 55°C for 1 min, followed by final elongation at 72°C for 10 min. PCR products were separated and sized by electrophoresis on 1.5% agarose gel stained with containing ethidium bromide, at 100 V for 1 h as standard. After electrophoresis, the products were visualized under ultraviolet (UV) transilluminator.

Human ACE gene I/D polymorphism is characterized by the presence (insertion) or absence (deletion) of 287 bp *Alu* repeat sequence in intron 16, the homozygous individuals for insertion allele (II) were identified by the presence of a single 490 bp product, the homozygous for the deletion allele (DD) were identified by the presence of single 190 bp product and the heterozygous individuals (ID) were identified by the presence of both alleles. All the human interventions necessary for the present work was done after taking approval from Ethical Committee of University of Jammu.

Statistical analysis

Hardy Weinberg equilibrium calculation was carried out to find out the significant difference between observed and expected genotype in patients and controls. Furthermore, the differences between the two groups were analysed using t- test and data was presented as mean ± standard deviation (SD). Statistical analysis between patients and controls were performed using Statistical Package for Social Sciences (SPSS) software version 20. Means were compared using t-test and P value less than 0.05 were considered as statistically significant. Multiple logistic regression models were used to calculate the odds ratio (OR) with 95% confidence intervals. Models were adjusted for gender and body mass index (BMI). Population attributable risk and Chi square values were also calculated.

RESULTS

A total of 500 subjects were enrolled in the present study. Out of these 500 patients, 250 were positive cases of hypertension and type 2 diabetes mellitus and 250 were healthy controls. The mean age of patients with T2DM and HTN was found to be 63.44±13.83 and controls was found to be 62.22±14.20, respectively; however, P value was found to be 0.77, that is, it was found to be insignificant between cases and controls. Apart from the fact that these patients showed significantly higher level of random sugar, that is, 186.74±59.96, whereas sugar level for the controls was found to be 95.44±11.19, P value was found to be significant between cases and controls. Higher incidence of systolic blood pressure (132.41±8.38 for cases and 127.01±11.00 for controls, P=0.01) and diastolic blood pressure (98.43±11.67 for cases and 92.75±11.12 for controls, P=0.04), BMI of patients was observed to be 25.70±4.49 and that of control was 23.72±2.81, suggesting the significant difference in BMI of patients and controls (P=0.03).

Apart from the aforementioned characters, the demographic and the socio economic characteristics of the patients showed that about 73% of the patients consumed non vegetarian diet as compared to controls (32%). Physical activity was found to be significantly lower in diabetic patients than controls (43% for diabetic cases and 68% for controls) (Table 1).

Table 1. Anthropometric and demographic characteristics of the study sample.

S/N	Parameter	Patient	Control	P value
1	Age	63.44± 13.83	62.22±14.20	0.077
2	Sugar level	186.74±59.96	95.44±11.19	0.02
3	BMI	25.70±4.49	23.72±2.81	0.03
4	Systolic blood pressure (SBP)	132.41±8.38	127.01±11.00	0.01
5	Diastolic blood pressure (DBP)	98.43±11.67	92.75±11.12	0.04
6	**Physical exercise**			
	Performers (%)	43	68	-
	Sedentary (%)	57	32	-
7	**Dietary pattern**			
	Vegetarian (%)	27	68	-
	Non vegetarian (%)	73	32	-

Table 2. Percentage of different diseases associated with the patients of HTN and T2DM.

Associated diseases	Percentage
Cardiovascular diseases (CVD)	38.8
Eye sight	22.8
Thermo genesis/Numbness	16.8
Joints pain	16

The data on T2DM and HTN related complications were also collected from our patients and it was discovered that about 38.8% of the patients had clinically diagnosed coronary heart disease (CHD), 22.8% had ocular complications (for example cataracts, retinal problems and vision loss),16.8% had thermo genesis/ numbness and 16% were suffering from joints pain (Table 2).

In our study, among 250 cases with T2DM and HTN, the frequencies of insertion (I) and deletion (D) allele were found to be 0.63 and .037, respectively, whereas in case of controls, the frequency of I allele was found to be 0.48 and D allele was found to be 0.52. However, a significant difference was also observed between positive cases and healthy controls (genotype frequency for cases is II=99.23, ID=116.55, and DD=34.23 whereas for controls it is II=56.64, ID= 124.71 and DD=68.64). As per the calculations from Hardy Weinberg calculator, significant association of ACE I/D polymorphism was found for patients (P=0.0.01) and chi-square value was found to be 4.98, however, in case of controls, no significant association was found for controls (P=0.006) and chi-square value was found to be 2.6. However, the ID versus DD+II model of odds ratio showed a significant association with patients of T2DM and HTN. Population attributable risk (PAR) was found to be 20.58 (Table 3).

DISCUSSION

Proteins of the rennin angiotensin system are involved in the regulation of arterial blood pressure and local hemodynamic in tissues (Corvol et al., 1997). The activity of this system is regulated by the rate of angiotensin production and the activities of rennin and angiotensin converting enzyme. ACE is the key element of rennin angiotensin system; it not only converts angiotensin I into angiotensin II, but also inactivates the vasodilator peptide bradykinin. Studies on the use of ACE inhibitors to lower coronary heart disease risk showed a reduction in progression of impaired glucose tolerance to type 2 diabetes by 25 to 30% (Stephens et al., 2005). The I/D polymorphism of ACE gene was detected in 1990 (Rigat et al., 19992). However, in previous studies, the results of ACE I/D polymorphism remained conflicting.

The insertion of this sequence is represented as homozygosity II, ID as heterozygosity; while DD represents the absence/deletion of a 287 bp *Alu* repeated sequence. DD genotypes and the D allele as compared to II genotypes and the I allele have a strong association with increased risk of myocardial infarction (Samani et al., 1996), HTN and diabetes (Zee et al., 1992) and its complications (Marre et al., 1994). Conversely, many studies have pointed out that DD genotypes failed to show an association in HTN and diabetes (Chiang et al., 1997).

In our study, we found that II genotype and I allele of the ACE gene was strongly associated with the HTN and T2DM as compared to healthy controls (P=0.001) in Jammu population. These findings are in agreement with some previous studies done in other population by Ismail et al. (2004), in their study, they reported the association of insertion allele with hypertension in Pakistani population. Similar findings were reported by Sinorita et al. (2010) in Indonesian population, where the frequency of D allele was less frequent than I allele (32.61 versus

Table 3. ACE gene I/D polymorphism genotypes and allelic frequency distribution in T2DM Patients and Controls.

Group	Genotype frequency (%)			Allelic frequency (%)		Chi square	P value
	II	ID	DD	I	D		
Cases	99.23	116.55	34.23	0.63	0.37	4.98	0.01
Controls	56.64	124.71	68.64	0.48	0.52	2.6	0.05

67.39%) and the DD genotype was less frequent than II genotype (23.19 versus 57.97%). Jeng et al. (1997) and Daimon et al. (2003) in their study reported the association of ACE I/D polymorphism with type 2 diabetes and HTN. Ramachandran et al. (2008) also reported the association of D allele of ACE insertion/deletion polymorphism with T2DM and HTN. Jayapalan et al. (2010) reported the null association of ACE I/D polymorphism. Zhou et al. (2012) in their Chinese population found the null association of ACE I/D polymorphism with T2DM. However, in the present study, the association of ACE I/D polymorphism with T2DM and HTN was carried out for the first time in Jammu population of J&K State, as per the statistical analysis of the results, I allele and ID genotype showed significant association with T2DM and HTN. The results of the previous studies remained conflicting. Therefore, further studies are needed on a larger sample size to confirm the association of ACE I/D polymorphism with HTN and T2DM.

Conclusion

This study declares that the I allele and ID genotype were significantly associated with the risk for T2DM and HTN, so that it is reasonable to expect that I allele and ID genotype of I/D polymorphism of ACE gene may be associated with T2DM and HTN in Jammu population. Our study also shows that the BMI, physical exercise and diet pattern are independent risk factors for T2DM and HTN. The present study was a preliminary work to generate data on genetic association of ACE I/D polymorphism with T2DM and HTN. Further studies will be required to confirm and elucidate these findings.

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REFERENCES

Anthea M, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD (1993). Human Biology and Health. Prentice Hall, Englewood Cliffs, New Jersey, USA.

Chiang FT, Lai ZP, Chern TH, Tseng CD, Hsu KL, Lo HM, Tseng YZ (1997). Lack of association of the angiotensin converting enzyme polymorphism with essential hypertension in a Chinese population. Am. J. Hypertens. 10(2):197-201.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ (2003). Joint National Committee in Prevention, Detection, Ecaluation, Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 42(6):1208-1252.

Corvol P, Soubrier, F, Jeunemaitre X (1997). Molecular genetics of the rennin-angiotensin-aldosterone system in human hypertension. Pathol. Biol. 45(3):229-239.

Costerousse O, Danilov S, Alhenc-Gelas F (1997). Genetics of angiotensin I-converting enzyme. Clin. Exp. Hypertens. 19(5-6):659–669.

Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M, Kato T (2003). The D allele of the angiotensin-converting enzyme insertion/deletion (I/D) polymorphism is a risk factor for type 2 diabetes in a population based Japanese sample. Endocr. J. 50(4):393-398.

Hazarika NC, Biswas D, Narain K, Kalita HC, Mahanta J (2002). Hypertension and its risk factors in tea garden workers of Assam. Natl. Med. J. India 15(2):63-68.

Ismail M, Akhtar N, Nasir M, Firasat S, Ayub Q, Khaliq S (2004). Association between the angiotensin-converting enzyme gene insertion/deletion polymorphism and essential hypertension in young Pakistani patients. J. Biochem. Mol. Biol. 37(5):552-555.

Jayapalan JJ, Muniandy S, Chan PS (2010). Null association between *ACE* gene I/D polymorphism and diabetic nephropathy among multiethnic Malaysian subjects. Indian J. Hum. Genet. 16(2):78-86.

Jeng JR, Harn HJ, Jeng CY, Yueh KC, Shieh SM (1997). Angiotensin I converting enzyme gene polymorphism in Chinese patients with hypertension. Am. J. Hypertens. 10(5 Pt 1):558-561.

Kutty VR, Soman CR, Joseph A, Kumar KV, Pisharody R (2002). Random capillary blood sugar and coronary risk factors in a South Kerala population. J. Cardiovasc. Risk 9(6):361-367.

Marre M, Bernadet P, Gallois Y, Savagner F, Guyene TT, Hallab M, Cambien F, Passa P, Alhenc-Gelas F (1994). Relationship between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complication. Diabetes 43(3):384-388.

Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Matsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H (2009). The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). Hypertens. Res. 32(1):3-107.

Olsen MH, Mallion JM, Rahn KM, Erdine S, Viigimae M, Laurent S, Agabiti-Rosei E, Mancia G, Schmieder RE, Cifkova R, Dominiczak A, Kjeldsen SE, Redon J, Zanchetti A, Nilsson P, Narkiewicz K (2010). ESH Council. Agreement within Europe about antihypertensive treatment and education: Results from the European Society of Hypertension questionnaire. J. Hypertens 28(7):1593-1594.

Popkin, BM (1998). The nutrition transition and health implication in lower income countries. Public Health Nutr. 1(1):5-21.

Ramachandran V, Ismail P, Stanslas J, Shamsudin N, Moin S, Mohd Jas R (2008). Association of insertion/deletion polymorphism of angiotensin-converting enzyme gene with essential hypertension and type 2 diabetes mellitus in Malaysian subjects. J. Renin Angiotensin Aldosterone Syst. 9(4):208-214.

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin. Invest. 86(4):1343-1346.

Rigat B, Hubert C, Corvol P, Soubrier F (1992). PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1).. Nucleic Acids Res. 20(6):1433.

Salem AH, Batzer MA (2009). High frequency of the D allele of the angiotensin converting enzyme gene in Arabic populations. BMC . Res. Notes 2:99.

Samani N, Thompson JR, O' Toole L, Channer K, Woods KL (1996). A Meta analysis of the association of the deletion allele of the ACE gene with MI. Circulation 94:708- 712.

Sambrook J, Russell DW (2001). Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

Sinorita H, Madiyan M, Pramono RB, Purnama LB, Ikhsan RB, Asdie AH (2010). ACE Gene Insertion/Deletion Polymorphism among Patients with Type 2 Diabetes, and Its Relationship with Metabolic Syndrome at Sardjito Hospital Yogyakarta, Indonesia. Acta Med. Indones-Indones J. Intern. Med. 1(42):12-16.

Stephens JW, Dhamrait SS, Cooper JA, Acharya J, Miller GJ, Hurel SJ, Humphries SE (2005). The D allele of the ACE I/D common gene variant is associated with type 2 diabetes mellitus in Caucasian subjects. Mol. Genet. Metab. 84:83-89.

Vorster HH, Wissing MP, Venter CS, Kruger HS, Malan NT, Ridder JH, Veldman FJ, Steyn HS, Margetts BM, MacIntyre UE (2000). The impact of urbanization on physical, physiological and mental health of Africans in the North West Province of South Africa: The THUSA Study. S. Afr. J. Sci. 96:505-514.

Whiteworth JA, World Health Organization, International Society of Hypertension Writing Group (2003). 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J. Hypertens. 21(11):1983-1992.

Wild R, Roglic G, Green A, Sicree R, King H (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care *27:1047*–1053.

Yang M, Qiu CC, Xu Q, Xiang HD (2006). Association of Angiotensin Converting Enzyme Gene I/D Polymorphism with Type 2 Diabetes Mellitus1. Biomed. Environ. Sci. 19:323-327.

Zee RY, Lou YK, Griffiths LR, Morris BJ (1992). Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. Biochem. Biophys. Res. Commun. 184:9-15.

Zhou D, Ruiter R, Zhang J, Zhou M, Liu H, Liu W , Wang S (2012). Angiotensin-converting enzyme I/D polymorphism is not associated with type 2 diabetes in a Chinese population. J. Renin Angiotensin Aldosterone Syst. 13(3):372-378.

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