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Exploration of Insertion/Deletion Polymorphism of Angiotensin Converting Enzyme in Post-Transplant Diabetes Mellitus Individuals from an Asian Indian Population

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Abstract

This work intended to test the association of Angiotensin Converting Enzyme (ACE) gene insertion (I) and Deletion (D) polymorphism in the End Stage Renal Disease (ESRD) subjects who developed post-transplant diabetes mellitus (PTDM) on using immunosuppressive drugs from an Asian Indian population. ACE is known to play an important role in the adaptation and regulation of systemic and renal circulations through angiogenesis II formation and kinins metabolism. A total of 240 non-diabetic ESRD individuals were prospectively screened for PTDM after renal transplant and 42 (30%) patients developed PTDM, whereas remaining 98 (70%) patients were non-PTDM. Genomic DNA was isolated from all the subjects and genotyping was performed for I28005D polymorphism using PCR-based assay. Individuals with PTDM had a higher DD (33.3%) genotypes and D (0.51) allele compared to controls. There was a significant difference between the genotype and allele frequencies of the PTDM cases and controls [for D Vs I, χ^2=0.0244; p=0.02, odds ratio=1.98 (95% CI: 1.09-3.63; DD+ID Vs II, χ^2=0.0316; p=0.03, odds ratio=2.9 (95% CI: 1.07-7.8)]. From our results we conclude that ACE gene polymorphism has role and a conventional risk factor in developing the disease in PTDM individuals from Asian Indian population.

Keywords: End-stage renal disease; Post-transplant diabetes mellitus; Renal transplantation; Angiotensin converting enzyme gene

Introduction

Post-transplant diabetes mellitus (PTDM) or new-onset diabetes after transplantation (NODAT) is a serious and mild complication of end stage renal disease (ESRD), patients who undergo transplant and are on the calcineurin inhibitors [1,2]. Transplantation associated with hyperglycemia encompasses NODAT, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), all of which are associated with increased morbidity and mortality in Renal Transplant (RT) recipients [3]. Based on American Diabetes Association (ADA) criteria, we have identified that 30% of ESRD or RT patients develop PTDM between three- four months post-transplant with abnormal glucose metabolism. The term 'NODAT' has replaced the older term 'PTDM' to differentiate new-onset diabetes from diabetes developed prior to transplantation [4]. Presently, there are no precise risk factors for recognizing individuals who are likely to develop PTDM and the pathophysiology also seems to be indistinct, but it may mimic that of T2DM. Both PTDM and T2DM pathologies are characterized by a combination of deficiency of insulin secretion and insulin resistance [5,6]. Earlier studies have reported some risk factors for PTDM which could be categorized as non-modifiable, potentially modifiable and modifiable risk factors [2,7].

Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a carboxyl terminal dipeptidyl exopeptidase that converts angiotensin I to angiotensin II in the renin-angiotensin system. Angiotensin II is active in the circulating blood and local tissues [8]. The ACE gene is located on chromosome 17q23 and is encoded by a 21kb gene that consist of 26 exons and manifests a 287-bp repeated Alu sequence Insertion (I) or Deletion (D) polymorphism in intron 16 [9,10]. This polymorphism does not affect the protein structure but apparently influences expression of this gene, because of the enzyme's central role in the renin-angiotensin aldosterone system, numerous association studies have been carried out [11,12]. ACE gene plays an important role in blood pressure regulation and it is not surprising that the genes coding for this system are being investigated in relation with ESRD PTDM.

Genotyping diabetes-related polymorphisms could be a possible method of predicting a patients risk for developing PTDM, and this information could be a valuable asset in the selection of appropriate immunosuppressive regimens [13]. In this study, we have carried out the ACE gene I/D polymorphism in the ESRD patients i.e., (RT=PTDM+non-PTDM) several former studies have shown an association of PTDM with other genes but there are no such studies with ACE gene. In our earlier studies ACE I/D polymorphism were shown to be associated with T2DM, Diabetic Nephropathy (DN) and Gestational Diabetes Mellitus (GDM) [14,15]. The aim of the present study was to examine the association of common I/D polymorphism in the ACE gene with PTDM in an Asian Indian population.

Materials and Methods

Patients and controls

This is a cross sectional study carried out in 140 renal transplant (RT)

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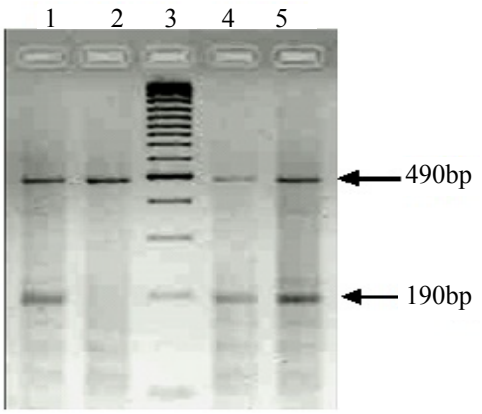

Lane 1, 4 and 5 represents 490/190 bp (I/D)
Lane 2 represents 190 bp (II)
Lane 3 represents 100bp DNA Ladder

Figure 1: Absence of the 287 bp intronic region in the ACE gene gives a 190bp fragment and 490 bp bands PCR product indicates heterozygous Insertion and deletion alleles are seen as 490/190 bp.

S.No	Demographic details	No. of individuals (Range in years)
1	ESRD	140
2	Males/Females	105/35
3	Mean age : a) Males (Mean ± SD) b) Females (Mean ± SD)	40.09 ± 10.7 (18 – 63) 40.52 ± 9.59 (21- 63)
4	Weight : a) Males (Mean ± SD) b) Females (Mean ± SD)	65.1 ± 13.01 (36 – 108) 67.06 ± 12.09 (38 – 90)
5	Sample collected after Transplant (months) (Mean ± SD)	20.4 ± 15.3 (03 – 36)

Table 1: Demographic details of the study population.

Genotypes	Controls (*n*=100)	PTDM Cases (*n*=42)	Non-PTDM (*n*=98)	ESRD (*n*=140)
II	31 (31%)	13 (31%)	45 (45.9%)	58 (41.4%)
ID	48 (48%)	15 (35.7%)	30 (30.6%)	45 (32.1%)
DD	21 (21%)	14 (33.3%)	23 (23.5%)	37 (26.5%)
ID+DD	69 (69%)	29 (69%)	53 (54.1%)	82 (58.6%)
I	110 (0.55)	41 (0.49)	120 (0.61)	161 (0.575)
D	90 (0.45)	43 (0.51)	76 (0.39)	119 (0.425)

Table 2: Distribution of ACE I/D genotypes and allelic frequencies of study population.

subjects, who were non-obese and non-diabetic prior to transplant. All the subjects were on routine follow up and were monitored periodically for renal function and PTDM at the department of Nephrology outpatient clinic. The selected subjects were on immunosuppressive drugs for more than 3 months 80 (57.1%) among them subjects were on Cyclosporine (CsA) while 60 (42.9%) subjects were on Tacrolimus (Tac) [16]. All the patients were recruited from Department of Nephrology, Kamineni Hospitals, Hyderabad, India. The age match controls (*n*=100) were selected from the general population without any clinical history of T2DM or any renal problems. Written informed consent was obtained from each participant and the hospital ethics committee's approval was granted before patient enrollment.

DNA and genotyping

Two mL of the venous blood was drawn from each patient in an EDTA vacutainer. Total genomic DNA was isolated from venous blood sample collected in an EDTA vacutainer by the salting out technique (Vattam et al. 2013). Appropriate fragments of ACE gene were amplified by Polymerase Chain Reaction (PCR), using one set of primers selected from our earlier publication [17]. The DNA was denatured at 95˚C for 5 min, and temperature cycling was set at 95˚C for 30 sec, 58˚C for 30 sec and 72˚C for 30 sec for 35 cycles followed by a final extension at 72˚C for 5 min. PCR products were visualized on 2% agarose gel with ethidium bromide. Absence of the 287 bp intronic region in the ACE gene gives a 190bp fragment and 490 bp bands PCR product indicates heterozygous Insertion and deletion alleles are seen as 490/190 bp (Figure 1).

Statistical analysis

Clinical data are expressed as mean ± standard deviation (M ± SD). The association between genotypes and ESRD/PTDM was examined by odds ratio with 95% Confidence Interval (CI) and chi-square analysis using Openepi software analysis. Yates correction was also performed. Hardy-Weinberg Equilibrium (HWE) was tested for ACE gene polymorphism. Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. The association of males and females with genotype was examined by Odds ratio with 95% CI and Chi-square analysis using the above mentioned soft-ware. Statistical significance was defined as $p<0.05$. Independent sample t-test was used to test the cases and controls.

Results

Clinical characteristics

Genotyping of ACE ID polymorphism was carried out in 240 individuals, who included 140 ESRD patients; their clinical characteristics are given in Table 1. The mean age of ESRD male patients were 40.09 ± 10.7 years (age group 18-63 years) and 40.52 ± 9.59 years for female ESRD patients are in the age group between 21-63 years. Patients with ESRD received the RT and were on immunosuppressive drugs, 80 (57.1%) of them were on cyclosporine (CsA) medication and 60 (42.9%) of them were on Tacrolimus (Tac). The mean weight of the males at the time of transplant was 65.1 ± 13.01 kgs and females were 67.06 ± 12.09 kgs. Samples were collected between 3-36 months after transplant i.e. at a mean time of 20.4 ± 15.3 months. Subsequently some of the patients developed Post Transplant Diabetes Mellitus (PTDM), these were categorized based on ADA criteria.

Insertion/deletion polymorphism risk

Distribution of genotype frequencies of the ACE gene polymorphism in controls was in accordance with the HWE. The genotype distribution of ACE I28005D polymorphism and allele frequency of I and D alleles in ESRD patients and controls is given in Table 2. When we compare the genotype and allele frequencies of ESRD and control subjects there was no significant difference [for D Vs I, χ^2=0.5864; p=0.58, odds ratio=0.90 (95% CI: 0.62-1.32)]. Based on the ADA criteria the ESRD patients were placed into two groups as PTDM and non-PTDM. 30% of the ESRD patients develop PTDM (*n*=42) and rest of them were considered as non-PTDM (*n*=98). We further evaluated the association of PTDM and non-PTDM subjects with ACE genotypes (Table 3). We found significant difference in the allele and genotypic distribution in males with PTDM and non-PTDM [for D Vs I, χ^2=0.0244; p=0.02, odds ratio=1.98 (95% CI: 1.09-3.63; DD+ID Vs II, χ^2=0.0316; p=0.03, odds ratio=2.9 (95% CI: 1.07-7.8)]. There was no significant difference in both allelic and genotypic distribution in females between the PTDM and non-PTDM subjects [D Vs I, χ^2=0.5107; p=0.50, odds ratio=0.67

PTDM (*n*=42)		Non-PTDM (*n*=98)	
Male (*n*=31)	Female (*n*=11)	Male (*n*=75)	Female (*n*=23)
6 (19.4%)	8 (72.7%)	31 (41.3%)	13 (56.5%)
13 (41.9%)	1 (9.1%)	24 (32%)	6 (26.1%)
12 (38.7%)	2 (18.2)	20 (26.7%)	4 (17.4%)
25 (0.40)	17 (0.77)	86 (0.57)	32 (0.70)
37 (0.60)	5 (0.23)	64 (0.43)	14 (0.30)

Table 3: Genotype and allele frequencies of ACE gene in PTDM and non-PTDM.

	CsA /PTDM (*n*=22)		CsA/ non-PTDM (*n*=58)	
	Male (*n*=7)	Female (*n*=15)	Male (*n*=43)	Female (*n*=15)
II	5 (71.4%)	4 (26.7%)	22 (51.2%)	9 (60%)
ID	1 (14.3%)	6 (40%)	13 (30.2%)	4 (26.7%)
DD	1 (14.3%)	5 (33.3%)	8 (18.6%)	2 (13.3%)
I	11 (0.79)	14 (0.47)	57 (0.66)	22 (0.73)
D	3 (0.21)	16 (0.53)	29 (0. 34)	8 (0.27)

Table 4: Distribution of genotype and allelic frequencies of ACE gene in PTDM and non-PTDM using cyclosporine (CsA) drug.

	Tac /PTDM (*n*=20)		Tac/ non-PTDM (*n*=40)	
	Male (*n*=16)	Female (*n*=4)	Male (*n*=32)	Female (*n*=8)
II	2 (12.5%)	3 (75%)	9 (28.1%)	4 (50%)
ID	7 (43.75%)	0 (0%)	11 (34.4%)	2 (25%)
DD	7 (43.75%)	1 (25%)	12 (37.5%)	2 (25%)
I	11 (0.34)	6 (0.75)	29 (0.45)	10 (0.625)
D	21 (0.66)	2 (0.25)	35 (0. 55)	6 (0.375)

Table 5: Distribution of genotype and allelic frequencies of ACE gene in PTDM and non-PTDM subjects using Tacrolimus (Tac) drug.

(95% CI: 0.20-2.18); DD+ID Vs II, χ^2=0.3702; p=0.36, odds ratio=0.48 (95% CI: 0.10-2.32)].

The genotype and allelic frequencies of CsA in PTDM and non-PTDM subjects have been shown in Table 4, respectively. The risk of CsA was significantly higher in PTDM females with D allele in comparison to non-PTDM females [D Vs I, χ^2=0.0365; p=0.03, odds ratio=3.14 (95% CI: 1.06-9.26)].

No significant difference was observed in the frequency of D and I alleles in males and females with PTDM and non-PTDM subjects who were on Tac regimen in [males; D Vs I, χ^2=0.3100; p=0. 30, odds ratio=1.58 (95% CI: 0.65-3.81); and females; D Vs I, χ^2=0.5489; p=0.54, odds ratio=0.55 (95% CI: 0.08-3.69)]. The genotype and allele distribution has been given in Table 5.

Discussion

The insertion/deletion polymorphism of the gene encoding angiotensin converting enzyme is a controversial risk factor and has been previously associated with T2DM, DN and GDM [14,15]. In this study, we have looked at (I28005D) polymorphism in the ACE with the development of PTDM in RT subjects. The patients were on immunosuppressive drugs like CsA and Tac which require to be absorbed and metabolized to give specific blood levels essential for graft maintenance [16,17].

PTDM is a common and serious metabolic complication after RT as transplant recipients are at a particularly high risk of developing PTDM. The renin-angiotensin system has attracted interest with regard to the pathogenesis of insulin resistance and Diabetes in the general population. Numerous studies have recommended that angiotensin II may affect glucose metabolism through insulin signaling pathways, blood flow, oxidative stress and adipogenesis [18].

In the present study it was demonstrated that the ACE I28005D polymorphism was significantly different between PTDM Vs non-PTDM (p>0.05) and individuals exhibit a dominant mode of inheritance (DD+ID vs II p=0.0078). To the best of our knowledge this is the first contemporary study to carry ACE I28005D gene polymorphism with PTDM. This hospital-based study from Hyderabad, which is a cosmopolitan city in south India, shows that the DD genotype and D allele of ACE gene polymorphism is associated with PTDM.

Zhou et al. [19] showed that DD genotype or D allele was associated with ESRD individuals after carrying out Meta-analysis in Caucasians. We have reported previously an association between ACE gene polymorphism with DN and GDM [14,15]. Kalita et al. [20] and Borah et al. [21] also showed a significant association between ACE DD genotype with stroke and hypertension in the Indian population. Alternatively Kothari et al. [22] showed no association with ACE gene polymorphism and this was in agreement with a publication from Pandey et al. [23] in Indian population. The D allele was associated with males who are having PTDM when we compare with females who are having PTDM. Irrespective of the immunosuppressant used individuals with D allele are at higher risk of developing PTDM.

In conclusion, our study showed association between I28005D polymorphism of ACE gene and PTDM in Asian Indian population. Based on small sample sizes of our study, a stable conclusion cannot be made and larger studies in more well-characterized subjects should be conducted in the future with different ethnicities.

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