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ACE GENE INSERTION /DELETION GENOTYPES BASED EVALUATION OF RESPONSE TO ANTI-HYPERTENSIVE'S IN CASES OF ESSENTIAL HYPERTENSION AND T2DM WITH HYPERTENSION – A PHARMACOGENOMIC STUDY

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ABSTRACT

The objective of present study was to investigate the pattern of response to anti-hypertensive in T2DM patients with hypertension and cases of essential hypertension having different ACE gene Insertion/ Deletion genotype. Blood samples from 45 cases of T2DM + hypertension and 40 cases belonging to essential hypertension (ET) were analyzed for ACE Insertion/deletion genotypes and correlated with response to anti-hypertensive's being taken by these patients. Blood samples from 50 healthy individuals served as controls, for comparing frequencies of the three different genotypes. The frequencies of DD genotype were found to be significantly ($P<0.05$) increased in both the clinical groups (31% in T2DM + HTN and 35% in E.T cases) compared to control. Genotype based analysis of responses to anti-hypertensive's revealed that 33.3% T2DM patients with hypertension having DD genotype responded to ACE inhibitors, while none in the essential hypertension group responded to ACE inhibitors. However, 77.7% ET patients with DD genotype responded to ARBS (either alone or in combination with calcium channel blockers/ Beta blockers). Interestingly the II genotype patients of both clinical groups responded in a comparable manner to ARBs (75% in ET and 80% in T2DM + HTN cases). In case of T2DM patients with ID genotype 72.2% responded to ARBs while only 57.14% cases in ET group Responded to ARBs either alone or in combination ($P<0.05$). Higher percentage of T2DM + HTN cases (II and ID genotypes) responded to ARBs than those in the essential hypertension group which may be attributed to higher rate of oxidative stress in T2DM + HTN cases.

KEYWORDS: ACE Gene I/D Polymorphism, Essential hypertension, T2DM with Hypertension, Response to Anti-Hypertensives.

INTRODUCTION

Hypertension (HTN) is the most common chronic condition affecting about 20-30%of the adult population.[1] There are certain evidences that suggest that genes may contribute up to 30% of variation in blood pressure (BP). Hypertension is defined as a mean systolic blood pressure of ≥140mmHg, and mean diastolic pressure of ≥ 90mm Hg or those who are on antihypertensive medications.[2] HTN is a risk factor for coronary heart disease, stroke and renal failure resulting from interaction of several genes with each other and with environmental factors.[3] The Rennin Angiotensin aldosteron system (RAAS) is believed to play an important role in the regulation of BP. Hence, Angiotensin converting enzyme (ACE) I/D gene polymorphism (which results in 3 genotypes 'DD', 'ID' and 'II') has been selected in the present study to evaluate response to anti- hypertensive's in patients with essential hypertension as well as T2DM patients with hypertension. The ACE 'DD' genotype is reported to have significantly increased serum concentration of Angiotensin I converting enzyme compared to 'ID' and 'II' genotypes.[4] Hence it is hypothesized that there may be significant differences in response to anti-hypertensives between patients with different ACE I/D genotypes.

THE ACE GENE I/D POLYMORPHISM

The ACE gene codes for a membrane bound dipeptidyl carboxypeptidase coenzyme located in the endothelial lining of blood vessels throughout the body. It was first discovered in the equine plasma.[5] Located on the long arm of human chromosome 17 the ACE gene consists of 26 exons. The Insertion/ Deletion polymorphism of ACE gene is characterized by the presence or absence of a 287 bp Alu repeat sequence in intron 16 giving rise to three genotypes (DD, ID and II).[6] ACE converts Angiotensin I to vasoactive Angiotensin II and inactivates bradykinin. The ACE I/D polymorphism is thought to be a marker for functional polymorphism which regulates the ACE activity. Certain studies have inferred that the DD genotype increases the incidence of hypertension because of significantly increased levels of Angiotensin II. It has also been reported that the ACE I/D gene polymorphism has been suggested for decision making regarding anti-hypertensive treatment regimen.[2] The physiological importance of the I/D polymorphism is due the fact that the 'DD' genotype is associated with increased circulating ACE levels. ACE is an enzyme of the RAAS catalyzing the conversion of Angiotensin I to Angiotensin II which is involved in fluid and electrolyte balance, as mentioned above and thus has an important role in regulating blood pressure.

The Need for the present study

Previous studies on the effectiveness of ACE inhibitors and Angiotensin receptor blockers (ARBs) in patients with diabetes with different ACE I/D gene polymorphism have largely been restricted to diabetic Nephropathy. In a recent critical review it has been suggested that blocking AT2T1 receptor proved more beneficial in terms of renoprotective effects like improving GFR, reducing proteinuria and above all accomplishing the Task of normalisation of glomerucular hypertension. Such studies revealed that The II genotype was protective whereas the DD genotype as well as D allele carriers was predisposed to this microvascular complication.

However, there are no reports available in diabetic cases with hypertension in view of a considerable proportion of such cases ending up in diabetic nephropathy / macro vascular complications.

As a first step towards accomplishing this objective the present study was planned to investigate if a specific relationship is observed between the ACE I/D genotype of the T2DM patients with HTN and the hypertensives they have been receiving. Once genotypes are known this additional information provides opportunity to critically asses on to what anti-hypertensives were being given to patients with II, DD and ID genotypes. So that Modifications may be made in anti- hypertensives dose/ combination particularly in patients with DD genotype. Essential hypertension cases were employed for comparison with T2DM patients. The Questions that are addressed in the present study are

i. Whether the anti-hypertensive being given to II genotype patients are different from those with DD genotype.
ii. What combinations of anti-hypertensive are proving more effective in which genotype?

METHODOLOGY

The study protocol for this study was approved by Institutional Review Board (Ethical Committee Deccan College of Medical Science and Owaisi group of Hospitals Hyderabad). A total of 85 cases suffering from hypertension were included. Of these 40 were suffering from essential hypertension (ET) and the remaining 45 were cases of T2DM + HTN. The cases were consecutively attending the clinic, (Department of General Medicine Princess Esra Hospital (Deccan college of Medical science, Hyderabad). All the cases selected were clinically confirmed as suffering from hypertension. A case is defined as suffering from hypertension when the systolic blood pressures was greater than or equal to 140 mm Hg and a diastolic blood pressure greater than or equal to 90 mm Hg or those who were currently receiving anti hypertensive therapy. All the patients were on anti hypertensive therapy at the time of inclusion in the study. Systolic blood pressure and Diastolic blood pressure was determined twice with a gap of 5 minutes and the average of two reading was considered as the final reading. For ACE Insertion/Deletion genotyping 2ml of Intravenous blood was drawn aseptically in tris-EDTA vacutainers and stored at -20 degrees centigrade till further use. Genomic DNA was extracted from the stored blood samples according to rapid salting out method as described by Lahiri at.al.[7] PCR amplification was performed in a 25 micro liter of Taq DNA master mix (2X Takara, Japan). The sequences of forward and reverse primers were respectively as follows:

(F) 5-CTGGAGACCACTCCATCCTTTCT-3 and
(B) 5-GATGTGGCCATCACATTGTCAGAT-3

PCR was performed in T100 model thermal cycler (BIORAD, USA) according to the method described by Seckin et.al.[8] The thermal programming was as follows: an initial denaturation step of five minutes at 94 degrees centigrade followed by 30 cycles of denaturation at 94 degrees centigrade for one minute, annealing at 58 degrees centigrade for one minute and extension at 72 degrees centigrade for 2 minutes and a final extension for 15 minutes at 72 degrees centigrade.

PCR products were separated by electrophoresis on a 3% agarose gel. Amplified DNA fragments, were then visualized under UV light in a GelDoc-Bio Rad (USA).The PCR fragment corresponded to 3 genotypes were, a 490 bp band (II),a 190 bp band (DD)and 490 and 190 bp bands(ID).

The endocrinologists who were treating the patients were not aware of the ACE I/D genotypes of the patients. The basic aim of the study was to carry out ACE I/D

genotypes of these patients and to identify the responders to various anti-hypertensives in each genotype group. These patients were on different anti-hypertensive treatment like ACE inhibitors, Angiotensin receptor blockers, Calcium channel blockers, Beta blockers and Diuretics.

The present study is likely to provide interesting information on personalized medicine based on ACE I/D genotypes because prior information about the genotype assists the clinician in prescribing appropriate anti-hypertensive treatment.

RESULTS

The mean age of T2DM + HTN patients 54.8 ± 8.87 years while that E.T cases was 51.56 ±10.79 years. Male to female ratio was as follows: T2DM + HTN 22 males: 23 females and ET group 18 Males: 21 females.

TABLE1: Genotype and allelic frequencies in T2DM +HTN and Essential Hypertension cases

Category	No	Genotype			Allelic frequencies	
		II	ID	DD	I allele	D allele
T2DM+HTN	45	5 (11%)	26 (58%)	14* (31%)	36 (40%)	54 (60%)
Essential Hypertension	40	4 (10%)	22 (55%)	14* (35%)	30 (37.5%)	50 (62.5%)
Control	50	14 (28%)	30 (60%)	6 (12%)	58 (58%)	42 (42%)

*P<0.05.

In essential hypertension and T2DM + HTN groups the frequency of ID patients was 58% and 55% respectively. However, the frequency of DD was 35% in essential hypertension group and 31% in T2DM + HTN group. While the frequencies of ID genotype are comparable between hypertension patients and controls there appears to be nearly threefold increase in frequency of DD genotype in two hypertension groups of patients compared to controls. This indicates that the individuals with DD genotypes have a high risk of developing hypertension. (Table-1) It is inferred that individuals with DD genotype are at a high risk of developing HTN with or without T2DM. Similar findings were also reported by other authors.[9] The frequencies of D allele in both groups (T2DM + HTN and E.T) were also significantly higher than in the control group (P<0.05). In view of this we evaluated anti-hypertensive response in relation to ACE I/D genotypes.

Table 2: Details of the proportions of the cases (in the two groups) responding to various anti-hypertensive (either alone or in combination)

Genotype	T2DM (N=45)	ACE	ARBs	Ca CH block	Beta Block	Diuretics (D.U)
ID	**26**	**1**	**19**	**4**	**2**	**8**
			ARB alone=9 ARB+D.U=8 ARB+CaB=3 ARB+B.B=1 ARB+CaB=1 ARB+B.B+D.U=1	ARB+CaB=3 ARB+CaB+B.B =1	ARB+B.B=1 ARB+B.B+CC =1	ARB+D.U =8
DD	**14**	**5**	**8**	**2**	**2**	**3**
		ACEi=5	ARB alone=5 ARB+D.U=2 ARB+CaB+B.B+ D.U=1	CaB+D.U=1 ARB+CaB+B.B +D.U=1	B.B=2	D.U=3
II	**5**	**0**	**4**	**1**	**1**	**1**
ESSENTIAL HYPERTENSION						
	Essential Hypertension (N=40)	ACE	ARBs (N=12)	CaB(N=5)	Beta block (N=4)	
DD	**14**	**0**	**8**	**4**	**2**	**4**
			ARBs alone=2 ARBs D.U=3 ARB+CaB=3 ARB+B.B=2	ARB+CC+D.U= 1 ARB+CaB=3 CaB=1	B.B=2 ARB+B.B=2	ARBs+D.U=3 ARB+CaB+D .U=1
			ARB+CaB+D.U=1			
ID	**22**	**2**	**13**	**5**	**2**	**9**
			ARBs alone=5 ARBs+D.U=8	CaB=3 CaB+B.B=2	BBalone=1 B.B+D.U=1 CaB+B.B=2	ARBs+D.U=8 B.B+D.U=1
II	**4**	**0**	**3**	**1**	**1**	**0**
			ARBs=3	CaB+B.B=1	CaB+B.B=1	

ARB= Angiotensin receptor blockers; D.U=Diuretics; Ca.B=Ca channel blockers; B.B=Beta blockers

*Since combination of different hypertensives were prescribed the total number of cases shown under columns of each

hypertensive drug for each genotype exceeds the no.of patients shown in column2 of table.

Difference between T2DM +HTN and Essential Cases with respect to responses to Anti-hypertensives

With regards to ID genotype 72.2% of cases in T2DM group responded to ARBs compared to 13/22 (57.14%) in essential HTN. The proportion of ID genotype patients responding to ARBs was significantly higher in T2DM group compared to that in Essential hypertension cases ($P<0.05$). It is likely that binding of ARBs to AT2 T1 (Angiotensin I T1 receptor) receptor proved to be more effective in not only regulating sodium retention but also may prevent Ang II induced ROS production by NADPH oxidase. The process of ROS production is expected to be at a much higher rate in T2DM patients due to hyperglycemia induced oxidative process, which may explain the effectiveness of ARBs in this group.

In T2DM patients with DD genotype 33.3% responded to ACEi (ACE inhibitors) whereas none in the essential hypertension group responded. So far as ARBs are concerned 77.7% in essential hypertension responded to ARBs (alone or in combination with CCB and BB) while 55.5% in T2DM group responded to ARBs.

DISCUSSION

The objective of the present study was to investigate whether response to anti-hypertensive differs in cases of T2DM + HTN cases and those of essential hypertension patients having different ACE gene I/D genotypes. The study was based on the reports that individuals with different ACE gene I/D genotypes (DD, ID and II genotypes) differ significantly with respect to serum Angiotensin –II concentration and hence are likely to respond differently to different anti- hypertensive's.[4] Moreover a study conducted on the role of various risk factors in predisposition to hypertension in T2DM cases reported that the DD genotype (of ACE gene I/D polymorphism) is the strongest predictor of HTN in T2DM patients by multiple logistic regression analysis.[9] The basis for selection of cases of T2DM + hypertension was due to the fact that the oxidative stress plays an important role in the pathogenesis of T2DM[10,11] while the role of this mechanism is believed to be relatively lesser in essential HTN. This tempted us to compare these two clinical groups. The high risk of ACE 'DD' genotype to T2DM also underscores the importance and relevance of such studies.[9]

Therefore it was thought clinically important to compare these two groups with hypertension in relation to ACE gene I/D polymorphism.

In the present study the effectiveness of ACE inhibitors in 33.3% T2DM patients with hypertension (Table2) having DD genotype can be explained on the basis of pleiotropic effects of Angiotensin II; since conversion of Angiotensin I to Angiotensin II is inhibited to a great extent by ACE inhibitors. In contrast to this 77.70% of the essential hypertension cases (with DD genotype) responded to ARBs (either alone or in combination with calcium channels blockers/ beta blockers) (Table2). As far the II genotype is concerned patients of both the groups having II genotype responded to ARBs (75% in ET and 80% in T2DM +HTN) in a comparable manner (Table 2). The effects of Angiotensin II are no more restricted to RAAS system but have been reported to be pleiotropic impacting other enzymes and metabolic pathways.[12] Angiotensin II is a potent inducer of NADPH oxidase a prooxidant enzyme present in the Endothelial cells of blood vessels and is regarded as one of the important sources of reactive oxygen production. The ROS so produced is reported to interact with nitric oxide (NO) and convert it into peroxynitrite which has a plethora of effects on various cellular structures and even may cause cell death.[13] Another major difference between cases of T2DM +HTN and Essential HTN cases is the hyperglycemia in diabetic patients which increases the process of oxidative stress via the polyol pathway and other mechanisms.[14] It inferred that prior knowledge of ACE gene I/D genotypes Appears to be useful in deciding anti-hypertensive therapy for hypertension in the two clinical groups study.

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