European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 08, Issue 3, 2021

A STUDY TO EVALUATE THE EFFECTIVENESS OF ANTIHYPERTENSIVE DRUGS ON NEWLY DIAGNOSED HYPERTENSIVE PATIENTS IN ASSOCIATION WITH ACE GENE POLYMORPHISM

Madhu Chaitanya Thota[1], Dr. Nilam Nigam[2*], Dr. Shravan Kumar[3], Dr. Anil Kumar[4]

[1]PhD Research Scholar, Department of Pharmacology, Rama Medical College, Rama University, Kanpur, UP
[2*]Prof. and HOD, Department of Pharmacology, Rama Medical College, Rama University, Kanpur, UP
[3]Prof. and HOD, Department of General Medicine, Rama Medical College, Rama University, Kanpur, UP
[4]Assistant Professor, Department of Biotechnology, Rama Institute of Engineering and Technology, Rama University, Kanpur, UP

Abstract

Background: Essential hypertension is among such lifestyle diseases which are the leading causes of premature deaths around the globe, due to their cardiovascular and kidney disease complications, if remains untreated. Angiotensin converting enzyme gene I/D polymorphism has been found to affect hypertension and the response of antihypertensive therapies.

Materials and Methods: This is prospective and cross-sectional study.

In Group – A: ACE gene polymorphism in hypertension

a) Azilsartan (40-80mg)
b) Atenolol (25-100mg)
c) Chlorthalidone (12.5-25mg)

Result: In our study baseline, systolic blood pressure was 172.31 mmHg Atenolol group, 179.94 mmHg Azilsartan group and in Chlorthalidone was 177.48 mmHg. Baseline Diastolic Blood pressure was 101.43 mmHg in Atenolol group, 103.78 mmHg in Azilsartan group and in Chlorthalidone was 109.59 mmHg. While comparing among 3 groups baseline blood pressure was statistically not significant. After 3 months of treatment with Atenolol group, 163.42 mmHg, in Azilsartan group 167.47 mmHg and in Chlorthalidone was 169.47 mmHg systolic blood pressure. Baseline Diastolic Blood pressure was 98.48 mmHg in Atenolol group, 97.58 mmHg in Azilsartan group and in Chlorthalidone was 98.37 mmHg. While comparing among 3 groups after 3 months blood pressure was statistically significant.

Conclusion: Angiotensin Converting Enzyme (ACE) gene polymorphism is linked to isolated systolic hypertension (ISH). Renin-Angiotensin-Aldosterone-System (RAAS) is one of the regulatory systems governing circulation, systemic vascular resistance, and kidney function.

Keywords: Antihypertensive drugs, Hypertensive patients, Ace gene polymorphism.

Introduction:

Essential hypertension is among such lifestyle diseases which are the leading causes of premature deaths around the globe, due to their cardiovascular and kidney disease complications, if remains untreated. [1] This disease is spreading at a fast pace with the change in the lifestyle pattern. One disease increases the probability of the other disease in same patient. Hypertension is an independent risk factor for cardiovascular complications including coronary heart disease, angina pectoris, stroke, ischemia, and atherosclerosis and it is linked with cardiovascular morbidity and mortality. [2] Hypertension are associated comorbid diseases which share common metabolic pathways such as obesity, insulin resistance, oxidative stress, inflammation, and genetics. [3] An estimate of World Health Organization (WHO) revealed that around 9.4 millions of deaths occur per year because of hypertension alone. [5]

The Joint National Committee VIII guidelines suggested the first line drug therapy for the treatment of hypertension as angiotensin converting enzyme inhibitors (ACE inhibitors), diuretics, beta blockers (BBs), calcium channel blockers (CCBs), and angiotensin receptor blockers (ARBs). [6] But these first line antihypertensive drug therapies were reported, by different studies, as the risk factor of new onset of type 2 diabetes mellitus in some hypertensive patients on prolonged use. [7] It was found that the use of antihypertensive medications for 3 to 6 years or more either as a monotherapy or as combination therapy may induce new onset of diabetes in 18% to 25% of patients. [8] Antihypertensive drugs mainly diuretics, BBs, and ACE inhibitors have been found to be associated with glycemic dysregulation via increasing insulin insensitivity or insulin resistance. [9]

Angiotensin converting enzyme is an enzyme of RAAS (renin-angiotensin-aldosterone) which is the key factor in regulating blood pressure and volume homeostasis. Angiotensin converting enzyme gene I/D polymorphism has been found to affect hypertension and the response of antihypertensive therapies. The II allele of ACE gene was found to be linked with higher reduction in mean arterial pressure as compared with DD genotype when patient was treated with diuretics. [10] The diabetic patients with DD genotype was found to be glucose intolerant as compared with other genotypes. [11] The metabolic disturbances have been found linked with elevated ACE level in the blood. The D genotype of ACE I/D polymorphism was found to be linked with higher ACE level which further leads to increased angiotensin II level and metabolic disturbance. These metabolic disturbances may be responsible for disturbed glucose homeostasis after antihypertensive treatment [12] Also D allele of ACE gene I/D polymorphism is associated with insulin resistance in hypertensive families which may be associated with glucose dysregulation and NOD. [13]

MATERIALS AND METHODS:

STUDY DESIGN: -This is prospective study and cross-sectional study
Total Number of Patients = 300
Each group contain 100 Patients

ACE gene polymorphism in hypertension

a) Azilsartan (40-80mg)
b) Atenolol (25-100mg)
c) Chlorthalidone (12.5-25mg)

Inclusion Criteria:

Both Male and female newly diagnosed hypertensive patients age between 25-60 years (SBP≥140mmHg and DBP ≥90mmHg) was included.

- Newly diagnosed Hypertensive patients.
- Patients who are ready to give informed consent to participate in the study.

Exclusion Criteria:

- Smokers/ alcoholics
- Pregnant & lactating women.
- Patients on steroid therapy, history of chronic infections like TB, leprosy, recent trauma, surgery.
- Patients with any co-morbid conditions like Liver, Kidney, Cardiac problems and psychiatric illness.
- Unwilling to participate or mental incapacity to take the drugs.
- Persons not willing to give informed consent.

RESULT

In our study baseline, systolic blood pressure was 172.31 mmHg Atenolol group, 179.94 mmHg Azilsartan group and in Chlorthalidone was 177.48 mmHg. Baseline Diastolic Blood pressure was 101.43 mmHg in Atenolol group, 103.78 mmHg in Azilsartan group and in Chlorthalidone was 109.59 mmHg. While comparing among 3 groups baseline blood pressure was statistically not significant in table 1.

Table 1: Baseline Systolic and Diastolic blood pressure

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Systolic	172.31±17.32	170.48±16.47	173.94±16.48	179.48±16.48	173.41±17.48	174.41±17.42	177.48±16.32	179.48±16.48	177.41±17.48	p=0.321 ns [95% CI -0.37 to 1.36]
Diastolic	101.43±10.38	103.78±9.41	109.59±10.59	102.45±10.36	108.58±10.54	104.78±9.43	103.63±9.51	103.63±9.39	101.21±10.26	p=0.432 ns [95% CI 0.32 to 1.43]

Table 2: Systolic and Diastolic blood pressure after 3 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Systolic	163.43±12.58	161.47±13.55	160.33±14.47	167.47±13.39	164.45±12.94	166.43±12.58	169.47±13.42	166.47±13.39	164.32±12.32	p=0.002s [95% CI 0.65 to 1.74]
Diastolic	98.48±8.48	99.49±8.52	93.57±9.83	97.58±8.58	94.51±9.73	99.49±8.62	98.37±8.43	99.31±8.43	98.32±8.73	p=0.001s [95% CI 0.39 to 1.64]

In table 2, after 3 months of treatment with Atenolol group, 163.42 mmHg, in Azilsartan group 167.47 mmHg and in Chlorthalidone was 169.47 mmHg systolic blood pressure. Baseline Diastolic Blood pressure was 98.48 mmHg in Atenolol group, 97.58 mmHg in Azilsartan group and in Chlorthalidone was 98.37 mmHg. While comparing among 3 groups after 3 months blood pressure was statistically significant in table 2.

Table 3: Systolic and Diastolic blood pressure after 6 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Systolic	130.46±9.38	131.32±9.47	133.38±9.37	133.32±9.20	134.36±9.44	137.46±9.38	133.32±9.31	132.32±9.20	134.12±9.12	p=0.003s [95% CI 0.43 to 1.13]
Diastolic	81.46±7.58	83.48±6.59	82.59±6.49	89.47±7.54	84.52±6.46	84.48±6.56	84.48±6.38	83.22±6.44	86.31±7.43	p=0.003s [95% CI 0.08 to 0.21]

Table 4: Baseline of HbA1c, FBS and PPBS

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value

HbA1c	5.89±0.79	5.92±0.66	5.64±0.82	5.83±0.53	5.71±0.63	5.49±0.88	5.64±0.82	5.92±0.66	5.73±0.63	P=0.532 ns[95% CI 1.94 to 3.95]
FBS	60.71±9.71	61.81±9.43	60.43±10.66	61.73±9.54	63.63±9.64	60.48±10.47	69.48±16.48	63.41±17.48	64.41±17.42	P=0.215 ns [95% CI 1.43 to 2.73]
PPBS	121.87±14.45	129.64±13.32	123.43±14.32	122.31±17.32	123.94±16.48	120.48±16.47	123.43±14.32	129.64±13.32	122.22±17.29	P=0.432 ns [95% CI-0.43 to 0.94]

Table 5: HbA1c, FBS and PPBS after 3 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
HbA1c	5.48±0.63	5.01±0.51	5.93±0.64	5.43±0.42	5.23±0.34	5.57±0.74	5.93±0.57	6.01±0.52	5.39±0.53	P=0.461 ns [95% CI 0.65 to 1.87]
FBS	63.34±7.48	67.32±7.43	61.39±7.48	67.41±7.39	63.21±7.39	79.47±7.55	67.47±13.39	64.45±12.94	66.43±12.58	P=0.543 ns [95% CI 0.32 to 1.36]
PPBS	123.38±11.58	124.54±11.42	129.74±12.64	118.43±12.58	121.33±14.47	119.47±13.55	129.74±12.64	124.54±11.42	128.32±12.43	P=0.373 ns [95% CI 0.23 to 1.25]

Table 6: HbA1c, FBS and PPBS after 6 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
HbA1c	5.01±0.48	5.21±0.34	5.42±0.48	5.03±0.21	5.21±0.21	5.22±0.53	5.42±0.38	5.21±0.31	5.21±0.39	P=0.221 ns [95% CI-1.43 to 2.43]
FBS	61.39±6.34	68.23±7.01	66.91±6.93	68.13±7.11	66.22±6.21	63.32±6.47	63.32±9.20	64.36±9.44	67.46±9.38	P=0.212 ns [95% CI-0.12 to 0.85]
PPBS	126.43±9.43	128.74±9.31	126.43±10.45	129.46±9.38	117.38±9.37	123.32±9.47	116.43±10.45	118.74±9.31	119.39±9.21	P=0.123 ns [95% CI 0.32 to 1.76]

Table 7: Baseline value of Lipid Profile

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Total Cholesterol	223.34±27.26	221.64±23.54	224.47±24.52	213.71±27.71	201.81±25.43	210.43±24.66	210.53±23.54	214.35±24.52	211.63±26.63	P=0.278 ns [95% CI 0.23 to 0.87]
Triglyce	185.52	173.	176.	172.3	170.48	173.9	175.43	172.3	170.31	P=0.443

rides	±17.37	59±16.42	55±17.55	1±17.32	±16.47	4±16.48	±17.49	1±17.32	±16.39	ns [95% CI 0.09 to 0.43]
HDL	42.28±7.27	42.43±7.48	41.28±8.27	43.49±7.88	41.83±7.74	42.53±7.72	41.42±7.53	41.19±8.27	41.29±7.21	P=0.584 ns [95% CI-0.03 to 0.31]
LDL	137.10±14.47	137.45±13.65	137.10±14.47	136.41±14.48	136.48±14.48	136.94±14.55	136.45±13.65	131.41±13.58	130.37±14.49	P=0.314 ns [95% CI 1.94 to 3.95]
VLDL	47.49±4.47	47.45±3.65	47.10±4.47	41.31±4.32	39.48±4.47	41.94±4.48	41.45±3.64	39.31±4.32	36.48±4.47	P=0.432 ns [95% CI 0.13 to 1.54]

Table 8: Lipid Profile after 3 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Total Cholesterol	209.54±21.85	204.44±19.69	201.58±20.48	203.34±21.48	197.32±19.43	191.39±19.48	204.39±19.69	201.43±20.48	203.21±19.52	P=0.112 ns [95% CI 0.18 to 2.87]
Triglycerides	163.38±14.58	164.54±12.42	179.74±12.64	168.43±12.58	169.47±13.55	161.33±14.47	158.69±12.53	168.43±12.58	169.29±13.43	P=0.213 ns [95% CI 0.65 to 2.34]
HDL	42.34±6.48	42.32±6.43	43.39±7.48	43.57±6.74	43.21±6.62	43.83±6.63	42.43±6.32	43.42±7.53	43.28±6.37	P=0.142 ns [95% CI 1.43 to 2.73]
LDL	133.34±13.48	132.32±14.43	134.39±13.48	134.45±13.94	134.47±13.39	133.33±14.22	132.32±14.43	134.44±13.83	134.53±13.27	P=0.104 ns [95% CI-0.36 to 1.46]
VLDL	41.34±3.48	32.32±4.43	34.39±3.48	38.43±4.58	39.47±3.55	38.33±4.47	32.43±4.42	38.43±4.58	39.47±3.55	P=0.013 ns [95% CI-0.12 to 0.85]

Table 9: Lipid Profile after 6 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Total Cholesterol	216.33±19.45	218.44±17.41	215.83±16.39	206.39±18.34	208.23±17.01	206.91±16.93	218.32±17.41	214.73±16.39	196.23±18.21	P=0.319 ns [95% CI 0.43 to 1.13]
Triglycerides	173.33±10.43	168.74±9.31	176.43±10.45	179.46±9.38	173.32±9.47	177.38±9.37	156.37±10.36	149.46±9.38	153.18±9.39	P=0.092 ns [95% CI 2.21 to 3.32]
HDL	43.39±5.34	43.23±5.01	42.91±6.93	44.22±5.53	44.33±5.44	45.54±0.33	44.32±5.23	43.83±6.84	42.38±5.42	P=0.442 ns [95% CI 0.28

										to 2.43]
LDL	136.39 ±13.34	133. 23±1 3.01	136. 91± 13.9 3	134.3 6±13. 44	132.32 ±13.20	132.3 8±13. 42	130.23 ±13.01	130.3 3±13. 54	131.21 ±13.32	P=0.197 ns [95% CI 0.08 to 0.21]
VLDL	40.39± 3.34	41.2 3±3. 01	40.9 1±3 .93	37.46 ±3.38	37.32± 3.47	37.38 ±3.37	33.39± 3.11	37.46 ±3.38	33.32± 3.47	P=0.093 ns[95% CI 0.28 to 2.23]

Table 10: Baseline value of Renal Profile

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Urea	39.73± 4.63	38.9 1±4. 03	37.3 3±4 .43	37.31 ±4.32	37.48± 4.47	37.94 ±4.48	37.32± 4.56	35.21 ±4.43	36.53± 4.42	P=0.363 ns [95% CI 0.36 to 3.13]
Creatinine	0.83±0. 79	0.81 ±0.6 6	0.83 ±0. 82	0.92± 0.68	0.93±0. 75	0.7±0. 88	0.93±0 .73	0.94± 0.68	0.88±0. 74	P=0.394 ns [95% CI 1.43 to 2.73]

Table 11: Renal Profile after 3 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/ D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Urea	37.34± 3.48	37.3 2±3. 43	36. 39 ±3. 48	38.43 ±3.5 8	39.47± 4.55	36.33 ±4.47	36.31 ±3.52	38.38 ±3.6 5	39.65± 4.56	P=0.323 ns [95% CI 2.21 to 3.32]
Creatinine	0.73±0 .63	0.92 ±0.5 1	0.7 3± 0.6 4	0.84 ±0.5 5	0.87±0 .44	0.84± 0.54	0.83± 0.54	0.81 ±0.5 5	0.90±0 .61	P=0.312 ns [95% CI-0.12 to 0.85]

Table 12: Renal Profile after 6 months

	Atenolol			Azilsartan			Chlorthalidone			
	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Urea	39.39± 3.34	37.2 3±3. 01	38.9 1±3 .93	37.46 ±3.38	36.32± 3.47	35.38 ±3.37	34.98± 3.83	35.34 ±3.23	35.43± 3.41	P=0.197 ns [95% CI 0.28 to 2.23]
Creatinine	0.81±0. 48	0.82 ±0.3 4	0.82 ±0. 48	0.86± 0.37	0.82±0. 42	0.88± 0.42	0.91±0 .55	0.83± 0.37	0.73±0. 54	P=0.091 ns [95% CI 0.43 to 1.43]

Table 13: Baseline value of uric acid

	Atenolol			Azilsartan			Chlorthalidone			
Drugs	I/I	I/I		I/I	I/D	D/D	I/I	I/D	D/D	p-value

Uric Acid	5.91±0.96	5.41±0.47		5.83±0.84	5.48±0.43	5.82±0.86	5.48±0.47	5.91±0.96	5.41±0.47	P=0.423 [ns] [95% CI-0.36 to 1.46]

Table 14: Uric acid level after 3 months

	Atenolol			Azilsartan			Chlorthalidone			
Drugs	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Uric Acid	5.34±0.48	5.32±0.43	5.39±3.44	5.48±0.53	5.38±0.41	5.32±0.53	5.47±0.55	5.34±0.48	5.32±0.43	P=0.312 [ns] [95% CI-0.12 to 0.85]

Table 15: Uric acid level after 6 months

	Atenolol			Azilsartan			Chlorthalidone			
Drugs	I/I	I/D	D/D	I/I	I/D	D/D	I/I	I/D	D/D	p-value
Uric Acid	5.39±0.34	5.23±0.11	5.91±3.93	5.52±0.48	5.43±0.23	5.84±0.43	5.32±0.47	5.39±0.34	5.23±0.11	P=0.193 [ns] [95% CI-0.43 to 0.94]

Discussion

Systemic hypertension remains an important risk factor for a variety of cardiovascular, renal and neurological diseases. It is well known that elevated blood pressure (BP) of any degree is a precursor to excessive morbidity and premature mortality. Untreated hypertension predisposes to coronary artery disease (CAD), left ventricular hypertrophy (LVH), congestive heart failure (CHF), chronic kidney diseases (CKD), end - stage renal disease (ESRD), transient ischemic attacks (TIA), and cerebrovascular accidents (CVA). [14] Although systemic hypertension is a risk factor for disease burden, the risk is uneven, heterogeneous, and unpredictable. At the present time, there is no way to determine which patient with an elevated blood pressure (BP) level is at risk and which patient is not; therefore, BP level reduction is indicated and recommended for "all" individuals with hypertension irrespective of their "personal risk". Hence guidelines rightly recommend measures to reduce the BP in all persons with hypertension. [15]

Despite successful treatment of hypertension, some patients experience complications whereas other patients remain free of disease even though their hypertension remains untreated or uncontrolled. This clinical scenario, therefore, raises the possibility of "genetics" in the development of hypertension and/or related complications. Various "genetic" hypotheses have been proposed to explain systemic hypertension but none has been clearly identified. Monogenic explanations have not stood the test of evidence. Hence, it is possible (but not proven) that hypertension may be of polygenic origin. To define hypertension beyond numbers, much work has been done in the areas of genetics, inheritance, and environmental factors. [16]

Angiotensin Converting Enzyme (ACE) gene polymorphism is linked to isolated systolic hypertension (ISH). Renin-Angiotensin-Aldosterone-System (RAAS) is one of the regulatory systems governing circulation, systemic vascular resistance, and kidney function. Thus, it is logical

to assume that inappropriate activation of RAAS elevates systemic BP and may be an aetiopathologic factor in the genesis of hypertension. An upward aberration in the activity of RAAS might lead to an upward shift in the BP level. [17] For the same reason pharmacological measures which block RAAS are widely used to treat hypertension. ACE is
a dipeptidyl carboxy-peptidase - I which activates angiotensin – I through cleavage of carboxy-terminal dipeptide into angiotensin II which causes vasoconstriction and subdues the activity of vasodilators such as bradykinin. Imbalance between forces of vasoconstriction over forces of vasodilation elevates the systemic BP levels, and vascular tone. High (or inappropriate) levels of ACE thus may be associated with hypertension. On the basis of this theory, ACE polymorphism can be considered as a genetic model in the development of hypertension and its complications. [18] The study reported in this issue by Borah and co- workers concludes that Del/Del polymorphism of ACE gene correlated only with isolated systolic hypertension (ISH) but not with systolic/diastolic hypertension or diastolic hypertension. [20]

It is clear that there are inherited and geographical variations in the ACE gene polymorphism which reflect the inconsistency of linking ACE gene to hypertension. For example, even in the Indian subcontinent there is no correlation between ACE gene polymorphism and hypertension in different geographic areas. [21] According to John et al. reveals that among Indians the frequency of the D allele ranges from 0.141 to 0.462 if the population is segregated on the basis of geographic regions; while it narrows to 0.300 to 0.454 if the population is segregated on the basis of ethnicity. Taking a mixed population thus may yield a value anywhere between 0.221 to 0.357 and therefore observed associations may not be actually true for different populations/ethnic groups. [22] Even amongst Asians, there is no consistency among the nations about correlating ACE gene polymorphism to hypertension. Added to this variability, is the factor of altitude and oxygen saturation and ACE gene polymorphism in pulmonary edema. [23]

The findings of D/D polymorphism in ISH as reported by Borah and co-workers add another interesting facet to the pathogenesis of hypertension since D/D polymorphism of ACE gene has been shown to increase vascular stiffness, a hallmark of ISH. There could be an age factor, particularly with CAD risk. For example, ACE I allele has been linked to CAD only in the younger subjects, but not in the older age group. In addition to CAD, chronic kidney disease (CKD) is emerging rapidly as a dangerous threat to public health in India due to escalating prevalence of diabetes and diabetic nephropathy. There appears to be a link between ACE gene (D allele) and diabetic nephropathy in the North Indian population. [24]

Conclusion

Circulating ACE levels show much variability and are genetically determined. An insertion / deletion (I/D) dimorphism has been shown to co-segregate with tissue and serum ACE function, and D allele is associated with elevated ACE levels. Therefore, the ACE gene can be considered as a qualitative trait locus (QTL) modulating ACE levels; ACE I/D dimorphism is a reflection of linkage disequilibrium (LD) with variants located in the ACE gene, implicated in cardiovascular diseases.

References

1. Borah PK, Shankarishan P, Hazarika NC, Mahanta J. Hypertension subtypes and angiotensin converting enzyme (ACE) gene polymorphism in Indian population JAPI 2012;60:11-17.
2. Higaki, J Baba, S Kalsaya, T Sato, N Ishikawa, K Mannami, T Ogala, J and Ogihara, T Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men. The Suita study, Circulation 2000;101:2060-2065.
3. Ashavaid TF, Shalia KK, Nair KG, and Dalal JJ and AT1R gene polymorphisms and hypertension in Indian population. J Clin Lab Anal 2000;14:230-237.

4. Das M, Pal S, and Ghosh A, Angiotensin converting enzyme gene polymorphism (insertion/deletion) and hypertension in adult Asian Indians: a population- based study from Calcutta. India Hum Biol 2008;80:303-12.
5. Hilgers, RH Schiffers, PM Aartsen, WM Fazzi, G E Smits, JF and De Mey, JG, Tissue angiotension- converting enzyme in imposed and physiological flow related, Arlerioscler. Thromb Vasc Biol 2004;24:892- 897.
6. Gupta S, Agrawal BK, Goel RK, and Sehajpal PK. Angiotensin converting enzyme gene polymorphism in hypertensive rural population of Haryana, India. J. Emerg. Trauma Shock 2009;2:150-154.
7. Berg K.E. and Berg K No effect of insertion/deletion polymorphism at the ACE locus on normal blood pressure level or variability. Clin Genet 1991;45:169-174.
8. Fornage, M Amar, CL. Karrdia, S, Sing C F Turner, ST and Boer-winkle, E. Variation in the region of the angiotensin-con-verting enzyme gene influences interindividual differences in blood pressure levels in young white males. Circulation 1998;97:1773-1779.
9. Badaruddoza AJS, Bhawer R, Sawhney NK, and Randhawa K, et al. A Study of Angiotensin Converting Enzyme (ACE) Gene polymorphism in Essential hypertension among a Business Community in Punjab. Int J Hum Genet 2009;9:231-234.
10. Mahboob Morshed, Haseena Khan and Sharif Akhteruzzaman Association between Angiotensin I Converting Enzyme Gene polymorphism and Hypertension in Selected Individuals of the Bangladeshi Population. Journal of Biochemistry and Molecular Biology 2002;35:251-254.
11. Kidd KK, Rajeevan H, Osier MV, Cheung KH, Deng H, Druskin L, Heinzen R, Kidd JR, Stein S, Pakstis AJ, Tosches NP, Yeh CC, Miller PL. "ALFRED the ALlele FREquency Database update." Am J Phys Anthropol. Annual Meeting Issue: Supplement 2003;S36:128.
12. Stobdan T, Ali Z, Khan AP, Nejatizadeh A, Ram R, Thinlas T, Mohammad G, Norboo T, Himashree G, Qadar Pasha M Polymorphisms of renin angiotensin system genes as a risk factor for high altitude pulmonary oedema. J Renin Angiotensin Aldosterone Syst 2011;12:93-101.
13. Andreas Gardemann, Monika Fink, Jurgen Stricker, et al. Ace I/D gene polymorphism; presence of the ACE D allele increases the risk of coronary artery disease in younger individuals. Artherosclerosis 1998;139:153-159.
14. Mitch WE Is the inherited ACE genotype a trump or a joker? J Clin Invest 1995;96:2100-1.
15. Naresh VVS, Reddy A.L.K., Sivaramakrishna G , Sharma PVGK, Vardhan RV, Siva Kumar V. Angiotensin Converting enzyme gene polymorphism in type II diabetics with nephropathy. Indian Journal of Nephrology 2009;19:145-148.
16. Viawanathan V, Zhu Y, Bala K, Dunn S, Snehalatha C Ramachandran A, el al. Association between ACE gene polymorphism and diabetic nephropathy in south Indian patients. J Pancreas 2001;2:83-7.
17. Kumar A, Mohindru K, Sehaipal PK. Angiotensin I converting enzyme polymorphism and diabetic nephropathy in north India. Int J Hum Genet 2005;5:279-83.
18. Rutledge DR, Kubilis P, Browe CS, Ross EA. Polymorphism of the angiotensin I converting enzyme gene in essential hypertensive patients. Biochem Mol Biol Int 1995;35:661-8.
19. Schmidt S. Schone N, Ritz E. Association of ACE gene polymorphism and diabetic nephropathy? Kidney Int 1995;47:1176-81.
20. Kunz R, Bork JP, Fritsche L, Ringel J, Sharma AM. Association between the angiotensin converting enzyme insertion/deletion polymorphism and diabetic nephropathy. A methodological appraisal and systematic review. J Am Soc Nephrol 1998;9:1653-63.
21. Alhenc-Gelas F et al. Distribution of plasma angiotensin I-converting enzyme levels in healthy men; relationship to environmental and hormonal parameters. J Lab Clin Med 1991;117:33-39.
22. Prasad P, Tiwari AK, Kumar KM, Ammini AC, Gupta A, Gupta R, el. al. Chronic renal insufficiency among Asian Indians with type II diabetes. Role of RAAS gene polymorphism. BMC Medical Genetics 2006;7:1-9.
23. Aitkhozhina NA and Lyudvikova, EK, Polymorphism of the Promoter Region of the Angiotensinogen Gene and the Gene for Angiotensin I-Converting Enzyme in Arterial Hypertension and Cardiovascular Disease of the Kazakh Ethnic Group. Russian J of Genetics 2003;39:229-235.

24. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud 14. M, Coin L, *et al*. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; *41* : 666-76.