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ASSOCIATION OF ANGIOTENSINE CONVERTING ENZYME GENE (I/D)POLYMORPHISM WITH HYPERTENSIVE PATIENTS IN NORTH INDIAN POPULATION

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Abstract
Genetic, environmental and demographic factors contribute to the development of essential hypertension. Genetic polymorphism of Rennin-angiotensin-aldosterone system (RAAS) has been extensively studied to determine the genetic susceptibility to hypertension. The insertion/deletion (I/D) angiotensin converting enzyme (ACE) polymorphism has been established as a cardiovascular risk factor in some population, but its association with essential hypertension is controversial. This study sought to determine the association of I/D polymorphism of the ACE gene in North Indian essential hypertensive subjects. A total of 150 clinically diagnosed essential hypertensive patients without any associated diseases. Distribution and allelic frequency of Insertion (I) and Deletion (D) polymorphism at the 287 base pair Alu repeat sequence in the intron 16 of ACE gene were analyzed. The distribution of II, ID, DD genotypes of ACE gene was 24.67%, 31.33% and 40% respectively in essential hypertensive patients. The genotype and allele frequency of ACE gene polymorphism is significantly differed in patients when compared to controls. In conclusion, the I/D polymorphism of ACE gene is associated with North Indian essential hypertension.
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.
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 is 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

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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]

STUDY DESIGN:

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& DISCUSSION:

ACE Gene Polymorphism:

Isolation of DNA:

The DNA was extracted with the use of Qiagen Kit by following user manual protocol. The obtained DNA was estimated through Agarose gel electrophoresis and by the use of spectrophotometer (nanodrop).Concentration and quality assurance of DNAthe quality of the DNA was estimated with the use of 1% Agarose gel electrophoresis and the quality of DNA was also calculated with the use of standard spectrophotometer (nanodrop) at 260 nm and 280 nm ratio.Molecular characterization of polymorphism Angiotensin Converting Enzyme (ACE) Gene, the main objective was to estimate molecular analysis of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism in toxaemia of pregnancy and normotensive mothers at Kanpur region.

ACE gene polymorphism by polymerase chain reaction: To determine the ACE gene genotype (SNP rs4343) of cases and the control groups, the genomic DNA fragments were amplified by PCR. The conditions for DNA amplification were mentioned below:

Primers for polymorphism:

Forward primer

Forward Primer 5'-CTGGAGACCCCCATCCTTTCT-3' Tm=56.00^{o}C

Reverse primer

Reverse Primer 5'-GATGTCGCCATCACATTCGTCAGAT-3' Tm=57.56^{o}C

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The PCR conditions were 95°C for 3min, 35 cycles of 95°C for 30s, 52°C for 30s, 72°C for 1.20 min and final extension at 72°C for 5min.

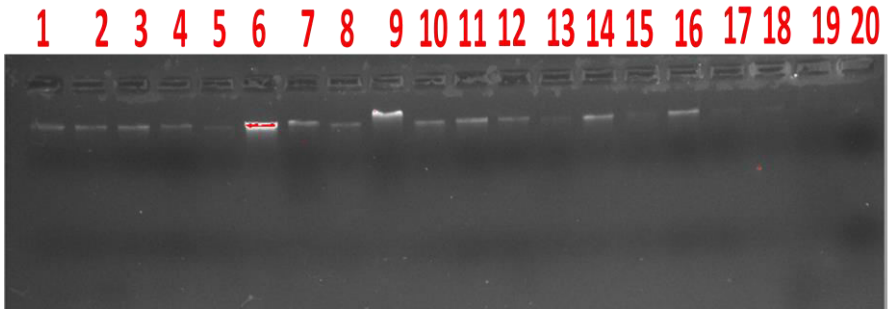

Fig.A) Isolated DNA from whole blood by Qaigen kit method for cases.

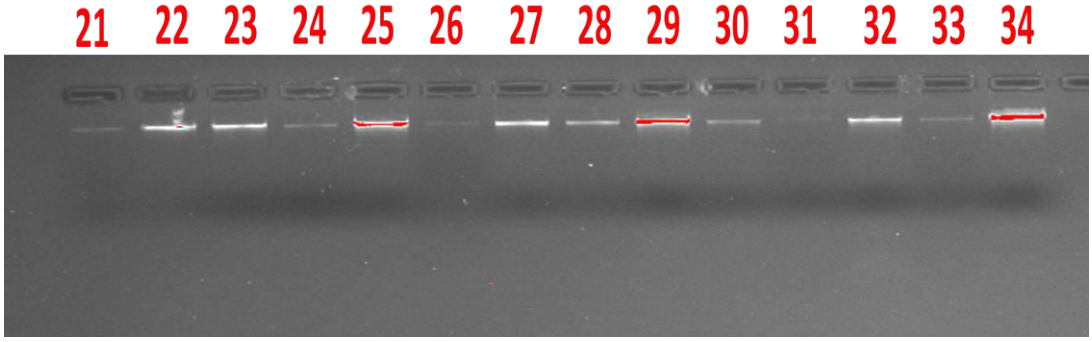

Fig.B) Isolated DNA from whole blood by Qaigen kit method for controls.

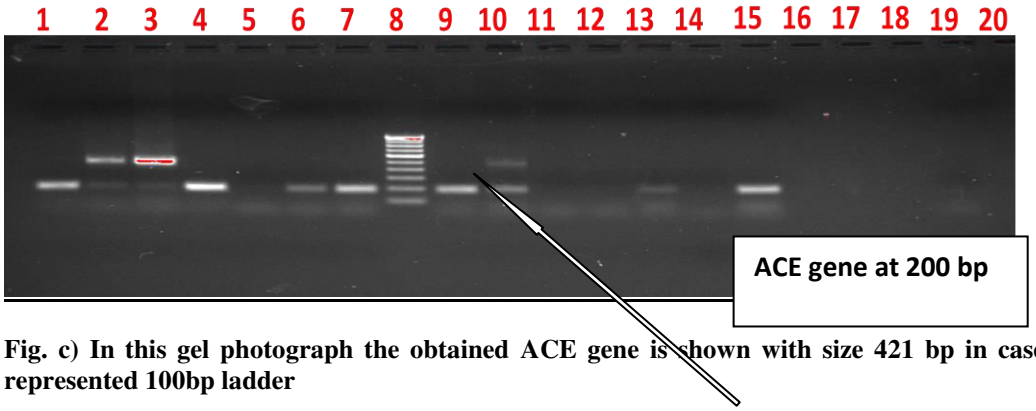

Fig. c) In this gel photograph the obtained ACE gene is shown with size 421 bp in cases, lane no. 8 represented 100bp ladder

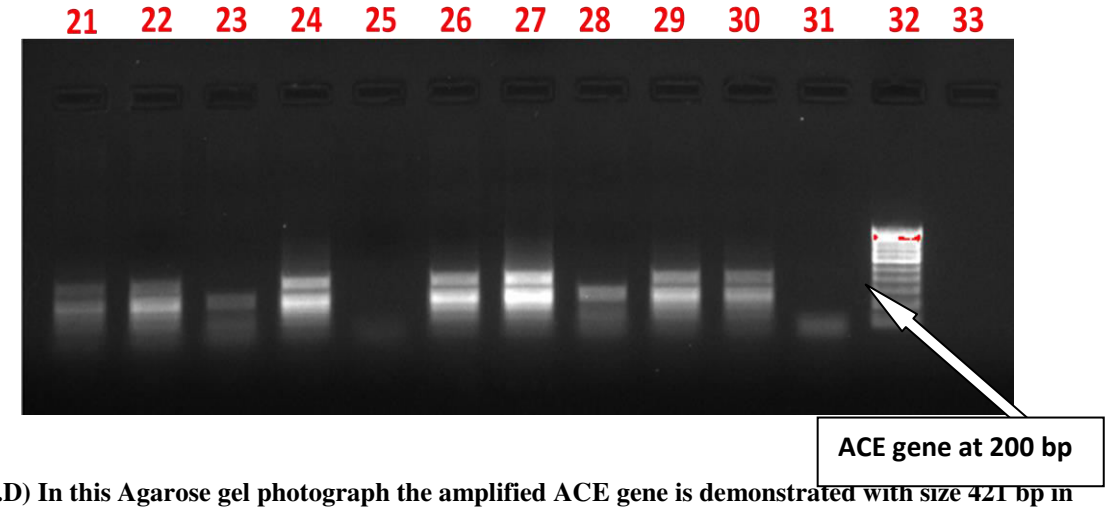

Fig.D) In this Agarose gel photograph the amplified ACE gene is demonstrated with size 421 bp in cases, lane no. 32 represented 100bp ladder

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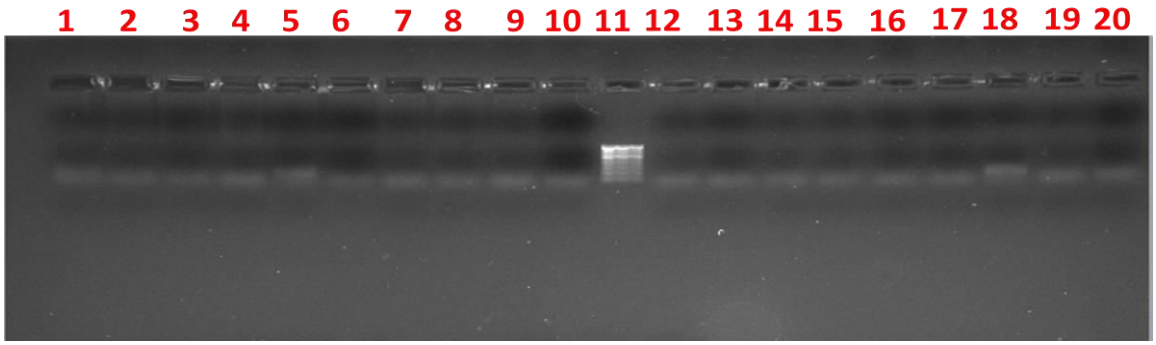

Fig.E) In this Agarose gel photograph the there is no amplification of ACE gene recorded in controls at size 421 bp, lane no. 11 represented 100bp ladder

Restriction digestion (RFLP):

30µl of PCR product was digested with DdeI restriction endonuclease enzyme.Digested product was run in 2.5 % agarose gel containing ethidium bromide. Gel photograph was recorded under UV light in gel documentation system (Bio-Rad).

According to the gel photograph to the sequence we obtained II allele was observed at 421bp and ID allele was observed at 200bp and DD band was observed at 50bp. There were 3 types of genotypes are seen in the ACE gene polymorphism: II homozygous, ID heterozygous and DD homozygous.

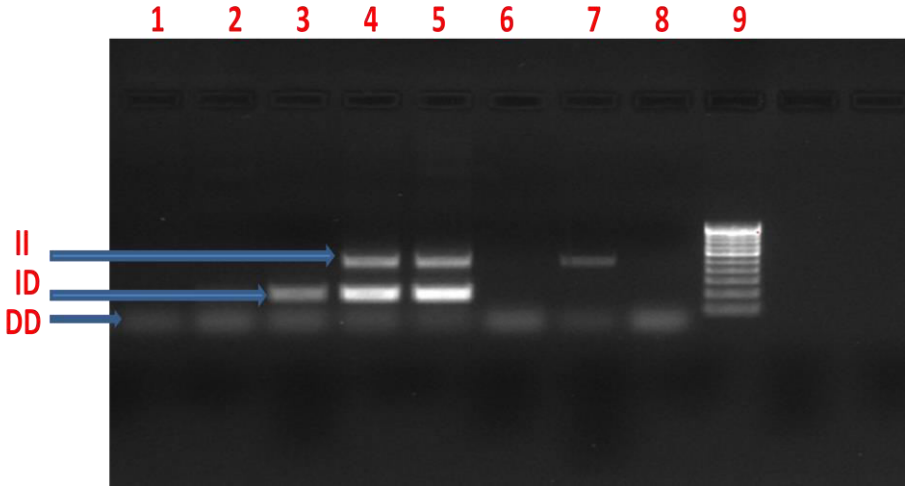

Fig. F) PCR product digested with restriction enzyme (DdeI) for the analysis of restriction fragment length polymorphism (RFLP).

In this study the PCR products were digested with DdeI restriction endonuclease enzyme. The band at 421 bp represented homozygous (II); single band at 200bp denoted as ID heterozygous and last band at 50 bp represented as DD homozygous. Lane no. 9 represented 100bp ladder.

Table- 1) Association of ACE gene variants (SNP 4343) with Hypertension disease

GENOTYPES	OR (95% CI)	P-VALUE
DD vs II	1.51 (0.68-2.97)	0.624
ID vs II	0.45 (0.76- 0.98)	0.002*
II vs II	0.50 (0.38-2.37)	0.357

p<0.05 indicates statistically significant

In table no-1 shown that patients suffering with hypertension have 0.45times more prone to attain ID variant of ACE gene. The p-value has found statistically significant (0.002).

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Table No -2: Genotypes of ACE gene and their frequencies of alleles in the study populations for hypertension cases.

S.N.	Genotype of ACE	Frequency of alleles (hypertension patients)	p-Value	Adjusted OR value	CI (95%) Value
1.	II (37)	24.67%	0.058	Reference	Reference
2.	ID (47)	31.33%	0.050	1.42	1.35-2.67
3.	DD (66)	40.00%	0.043	1.26	1.63-2.59

In this study, ACE gene polymorphisms related with hypertension was recorded the genotype and allele frequencies of the specific SNP (rs4343) in the study group. The genotype frequencies of the ACE gene in cases with hypertension were found in Hardy-Weinberg equilibrium. The rs 4343I/D gene polymorphism of the ACE gene was noticed statistically significant with hypertension, and the ID genotype was noted significantly higher in hypertension cases. Although DD genotype was also found pathogenic in this study because DD genotype p value is<0.05 which is more pathogenic, in this study shows 40% DD frequency of alleles present in out of 150 hypertensive patients.

Conclusion:

In conclusion our results explore the association of DD genotype with essential hypertension. The present study proves the relation between I/D polymorphism of ACE gene and essential hypertension in south Indian population.

Conflicts of interest

There is no conflict of result.

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.

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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.