Original article:

Study of Phenotypic expression on ACE genotype essential hypertensives of South Indian population

Ipsita Choudhury*, Arun Kumar Patra, R.Jothimalar*****

*Department of Biochemistry,Rama Medical College,Kanpur,Uttar Pradesh

**Department of Anesthesia,12 Air Force Hospital,Gorakhpur,Uttar Pradesh

***Department of Biochemistry,Sri RamaChandra Medical College,Porur,Chennai

Corresponding author : Ipsita Choudhury

Abstract

Introduction: Hypertension is a complex disease involving interaction of many risk genes and environmental factors such as obesity, dietary salt intake, alcohol consumption, and stress. Genetic variants of renin angiotensin system (RAS) gene play a significant role in the pathogenesis of essential hypertension and cardiovascular diseases.

Methods: The present study was aimed to study the phenotypic expression on ACE genotype essential hypertensive individuals of South Indian population. Genotyping was performed using a polymerase chain reaction, (PCR) amplification of the intron 16 fragment harboring the 287 bp Alu repeat sequence.

Observation and Result: A significant association was found between the D/D genotypes and essential hypertensives. When the various confounding factors were analyzed, it was found that a significant association was obtained between the non vegetarians and the phenotypic expression of D/D genotypes.

Key words: Angiotensin converting enzyme, Hypertension, Phenotype expression

Introduction

Hypertension is a serious health issue worldwide and essential hypertension, which includes 90–95% of the cases, is influenced by both genetic and environmental factors. It is one of the most important modifiable risk factor for coronary heart disease (the leading cause of death in North America), stroke (the third leading cause), congestive heart failure, end-stage renal disease, and peripheral vascular disease**(1,2).** The Framingham Heart Study has estimated that individual's normotensive at age 55 years have a 70% lifetime risk of developing hypertension **(3)**. Angiotensin converting enzyme (ACE) is a key component of the Renin Angiotensin System .It is responsible for converting the inactive decapeptide, angiotensin I, to the biologic active octapeptide angiotensin II, while inactivating the vasodilator nonapeptide, bradykinin. Angiotensin II contributes to hypertension by its intense vasoconstrictor action, aldosterone stimulation and bradykinin inhibition **(4,5).**

Hypertension is one of the most common complex disorders, with genetic heritability averaging 30%. Among the various genetic causes, the ACE gene polymorphism has been intensively studied.

ACE gene consists of 26 exons and 25 introns and spans 21 kb on chromosome 17q23 .The ACE genotypes include the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence resulting in 3 genotypes (D/D and I/I homozygote, and I/D heterozygote).An I/D (region in intron 16) polymorphism of ACE gene correlates with

circulating ACE plasma activity **(6,7).** Higher plasma ACE activity is observed in subjects with ACE-D/D genotype. A raised plasma ACE activity may elevate blood pressure through increased production of angiotensin II. Studies have demonstrated that ACE Insertion (I) / Deletion (D) polymorphism are associated with common diseases like hypertension, diabetic nephropathy ,coronary heart disease, Tuberculosis , Sarcoidosis(**8**), SLE(**9**), Hypertrophic & Dilated cardiomyopathy (**10**) and Erectile dysfunction(**11**).

Hypertension is a complex disease involving interaction of many risk genes and environmental factors such as obesity, dietary salt intake, alcohol consumption, and stress. Approximately 20 to 60 per cent of the population variability in blood pressure is genetically determined**(12).** In the last decade, a large number of candidate genes have been tested for association with blood pressure and hypertension without convincing results.

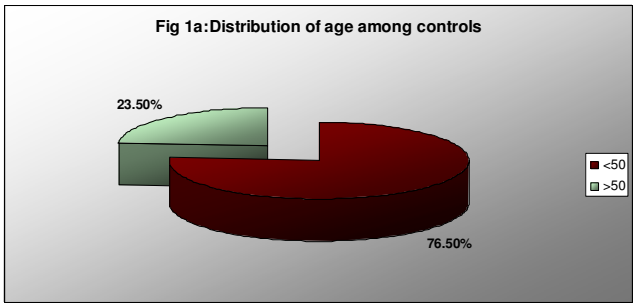
Aim of the study: To analyze the effect of various confounding factors of hypertension on the phenotypic expression of ACE genotypes.

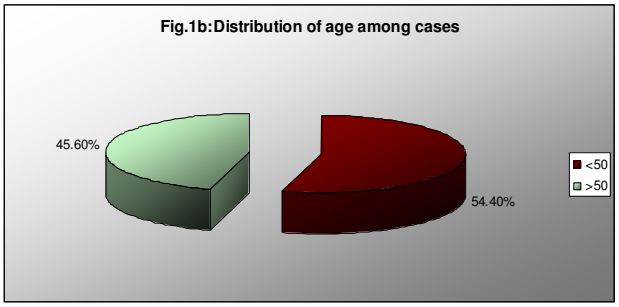
Materials and Methods

The study was conducted on 182 individuals of the out patient department of Medicine at Sri Ramachandra Medical College, Chennai. About 101 clinically diagnosed cases of hypertension were taken as the **cases** against 81 age and sex matched **controls** without any associated illnesses.

A standard questionnaire was prepared and data collected on clinical variables of age, sex, height, weight, family history, smoking and alcohol habits.All the groups had their ACE gene analyzed for the I/D sequence of intron 16 of chromosome 17q23.3 by the standard protocol of DNA isolation, PCR and Agarose gel elctrophoesis. Statistical analysis was performed using the SPSS.15 software programme. Comparison of various parameters like age, sex, diet, smoking, alcoholism and BMI between cases and controls were done using independent 't' test. In bivariate analysis, Pearson Chi square test was applied to check the association between the selected variables. Allele and genotype frequencies were compared using 2 X 2 contingency table using Fisher's exact test. The mean concentration of all numerical values was tested by student's t test or ANOVA test. p value <0.05 was considered statistically significant. Odd's ratio (OR) at 95 % Confidence Intervals (CI) was determined for the disease susceptibility of the patients.

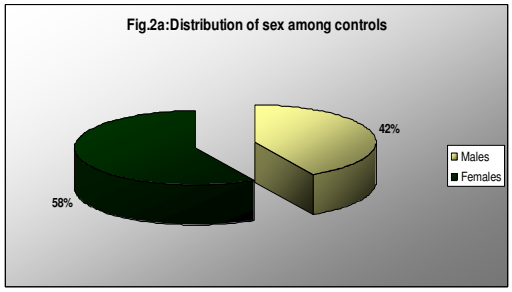
1. Distribution of age

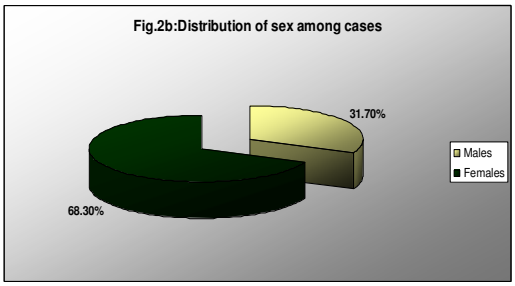




www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

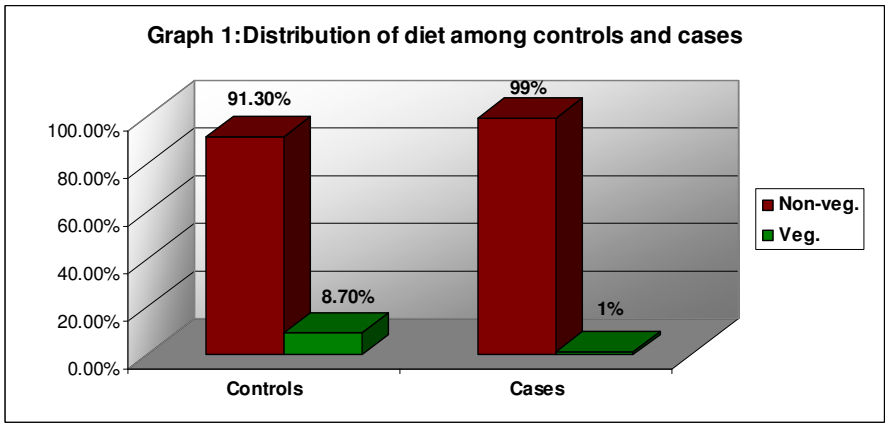
2. Distribution of Sex





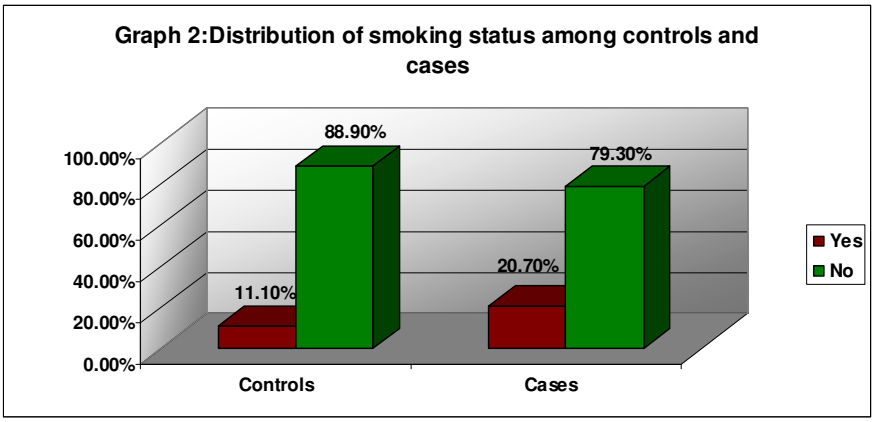
3. Distribution of Diet among Controls and Cases

Among the present study population, the number of non-vegetarians was 174 and vegetarians were 8. Their distribution among cases and controls is;



4. Distribution of Smokers among Controls and Cases

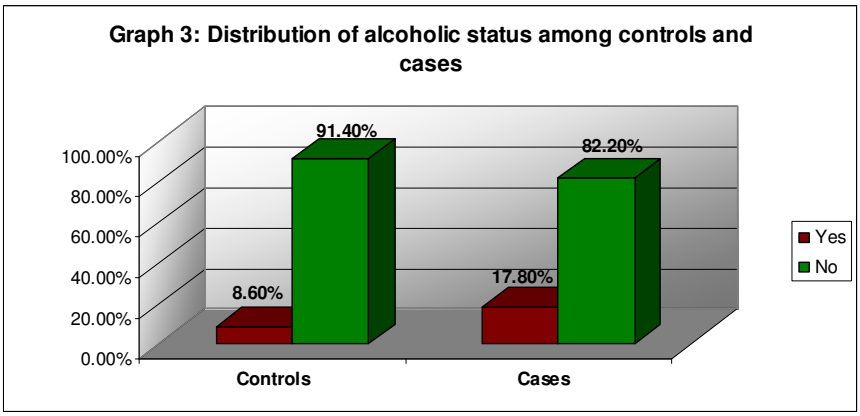
The total number of smokers and non-smokers in this study population were 30 and 152 respectively.



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

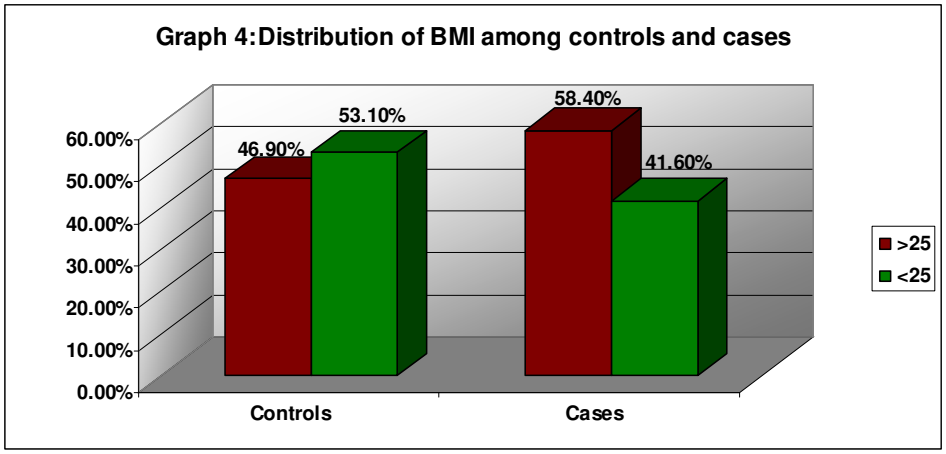
5. Distribution of Alcoholism among Controls and Cases

The number of alcoholics and non-alcoholics in this study were;



6. Distribution of BMI among Controls and Cases

The number of subjects with BMI > 25 were 97 and those with < 25 were 85 respectively. Their distribution between cases and controls were.



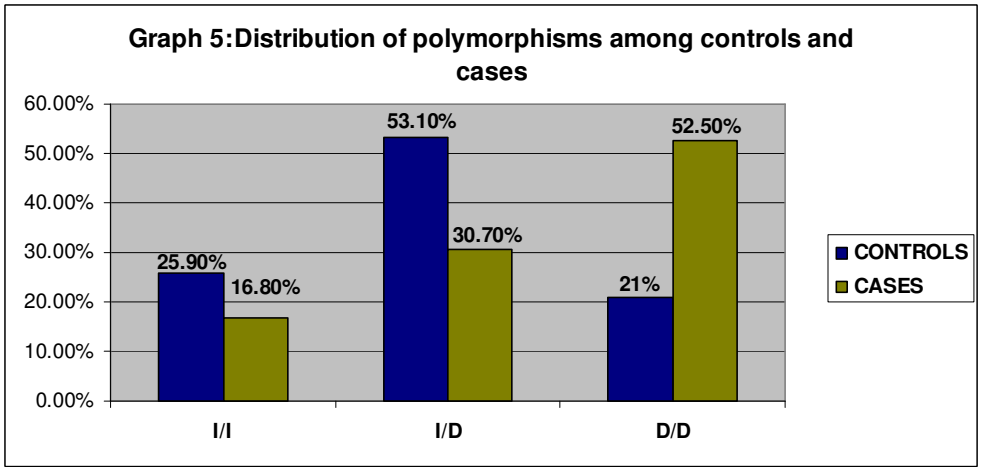
Observations and Results

DNA samples from all the subjects, (101 cases and 81 controls) were amplified for I/D polymorphism in the ACE gene and analyzed. The PCR products were visualized under UV light. D allele was identified at 190 bp and I allele at 490 bp respectively, leading to the formulation of the three major polymorphisms D/D, I/D and I/I. The frequency of all these polymorphisms in association with hypertension and with other variables was statistically analyzed and the results noted.

1. Polymorphism and Hypertension

On genotype analysis the frequency of I/I, I/D and D/D polymorphism in controls were 25.9%, 53.1% and 21.0% and among cases were 16.8%, 30.7% and 52.5% respectively.

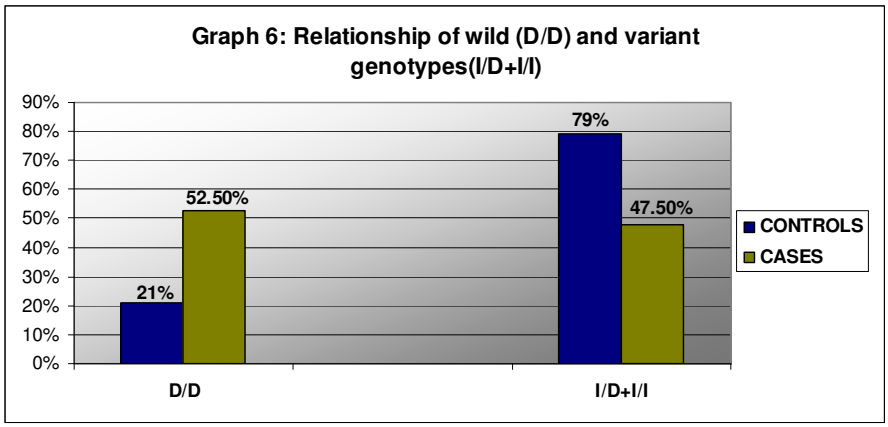
www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858



Pearson's Chi square test was applied to test the association of the various genotypes with hypertension and a significant association was obtained between D/D polymorphism and hypertension with the p value of 0.0005 (< 0.05) .To check the severity and association of D/D genotype with hypertension, a comparison was made between the wild type (D/D) and the variant types (I/D + I/I) .

Odd's ratio for cases/ controls : 4.157

95% Confidence Interval: 2.144-8.060

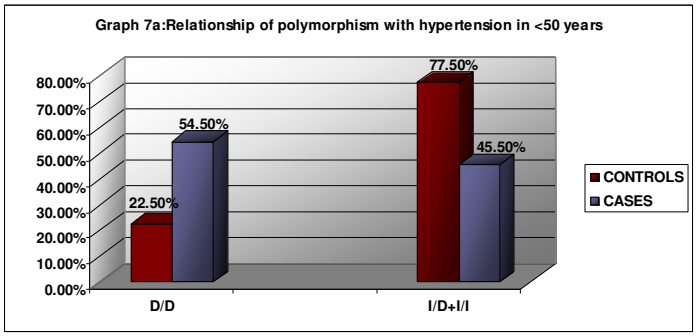


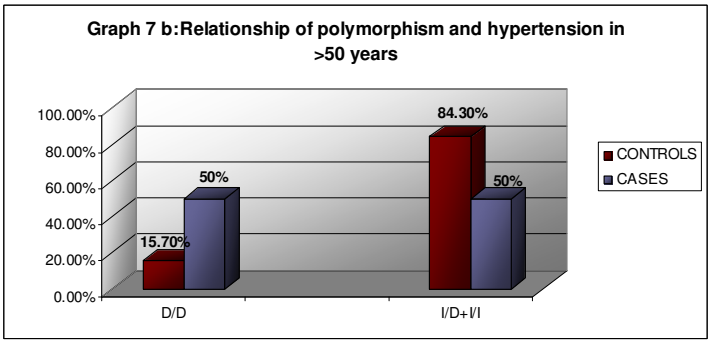
2. Relationship of polymorphism with age: The distribution of all the 3 genotypes between controls and cases were analyzed according to age.

Table 2a: Relationship of polymorphism with age

Age	**Types of ACE Polymorphisms**	**Controls n= 62(%)**	**Cases n=55(%)**
<50	D/D	14(22.5)	30(54.5)
	I/D+I/I	48(77.5)	25(45.5)
Age	**Types of ACE Polymorphisms**	**Controls n= 19(%)**	**Cases n=46(%)**
>50	D/D	3(15.7)	23(50)
	I/D+I/I	16(84.3)	23(50)

p value for <50 years and > 50 years was found to be 0.24(>0.05) and 0.18(>0.05) respectively.





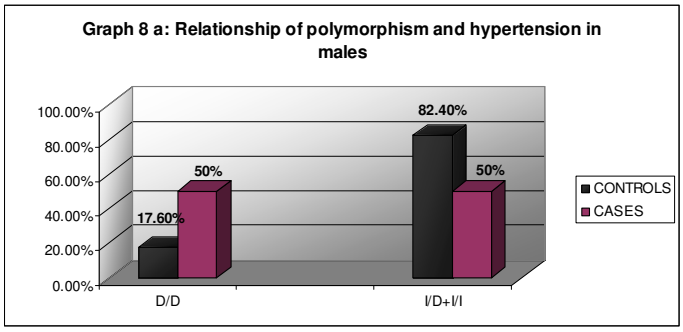
3. Relationship of Polymorphism with Sex

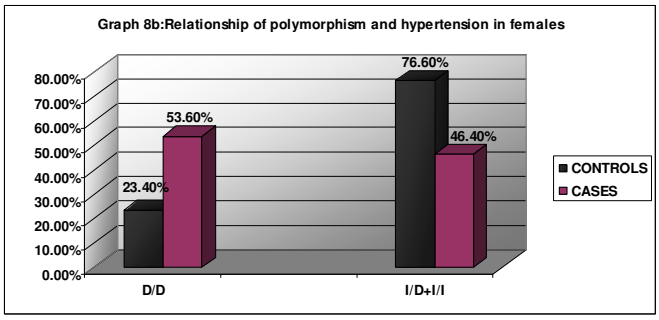
The distribution of all the three genotypes were analyzed in both the sex groups

Table 3a: Relationship of polymorphism with sex

Sex	**Types of ACE Polymorphisms**	**Controls n= 34(%)**	**Cases n=32(%)**
Males	D/D	6(17.6)	16(50)
	I/D+I/I	28(82.4)	16(50)
Sex	**Types of ACE Polymorphisms**	**Controls n= 47(%)**	**Cases n=69(%)**
Females	D/D	11(23.4)	37(53.6)
	I/D+I/I	36(76.6)	32(46.4)

From the above table the p value for males with relation to hypertension was 0.005. For females p value was found to be 0.001





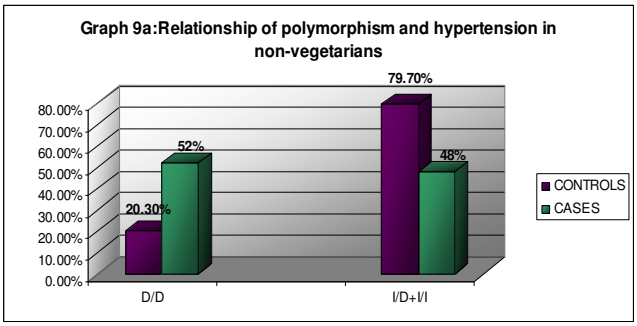
4. Relationship of Polymorphism with Diet

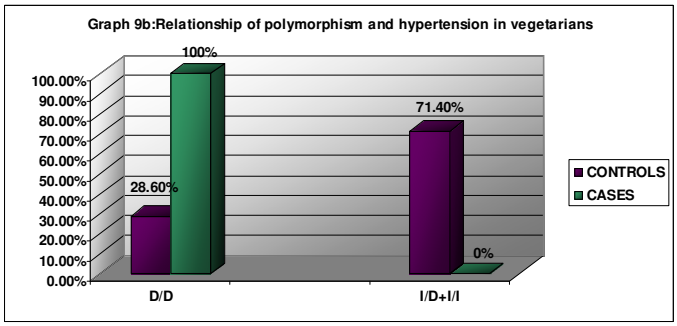
The relationship between the dietary habits and the polymorphism was analyzed by the following table.

Table 4a: Distribution of Diet between controls and cases

Diet	**Types of ACE Polymorphisms**	**Controls n= 74(%)**	**Cases n=100(%)**
Non-vegetarians	D/D	15(20.3)	52(52)
	I/D+I/I	59(79.7)	48(48)
Diet	**Types of ACE Polymorphisms**	**Controls n= 7(%)**	**Cases n=1(%)**
Vegetarians	D/D	2(28.6)	1(100)
	I/D+I/I	5(71.4)	0(0)

p value for Non-vegetarians was found to be 0.0005 (< 0.05) and an Odd's ratio of 4.261. For vegetarians, the p value was obtained to be 0.168 (> 0.05).





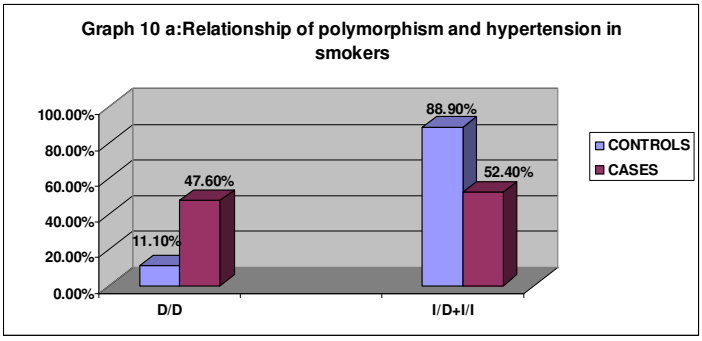
5. Relationship of Smoking with Polymorphism

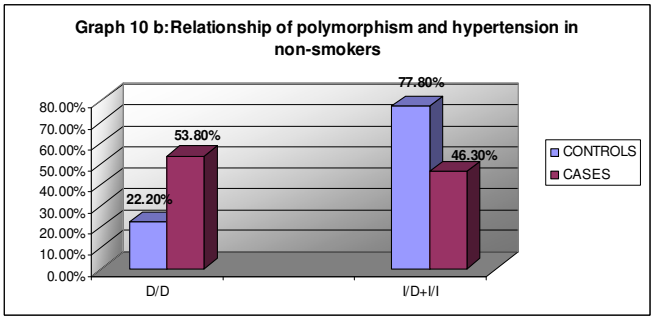
The relationship between smoking and the various polymorphisms were analysed as follows

Table 5a: Distribution of polymorphism among smokers and non-smokers

Smoking status	**Types of ACE Polymorphisms**	**Controls n= 9(%)**	**Cases n=21(%)**
Yes	D/D	1(11.1)	10(47.6)
	I/D+I/I	8(88.9)	11(52.4)
Smoking status	**Types of ACE Polymorphisms**	**Controls n= 72(%)**	**Cases n=80(%)**
No	D/D	16(22.2)	43(53.8)
	I/D+I/I	56(77.8)	37(46.3)

p value for smokers was found to be 0.057 (>0.05)





6. Relationship of Alcoholism with Polymorphism

The distribution of various genotypes with relation to alcoholic status is;

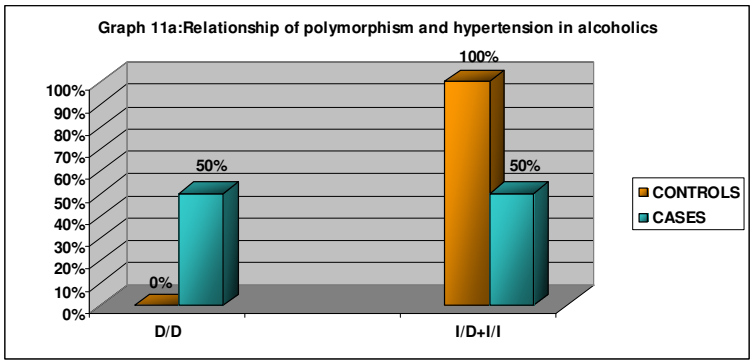
Table 6a: Relationship of Polymorphism and alcohol

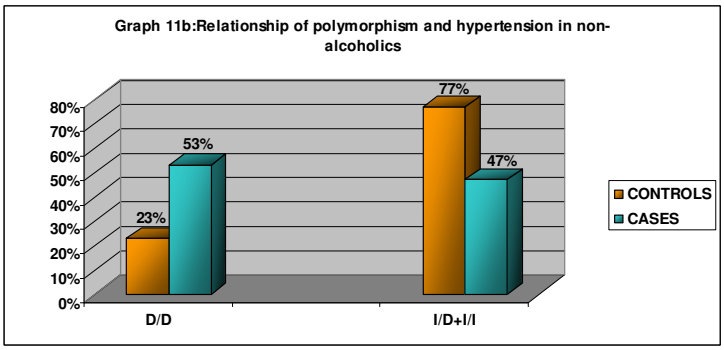
Alcohol status	**Types of ACE Polymorphisms**	**Controls n= 7(%)**	**Cases n=18(%)**
Yes	D/D	0(0)	9(50)
	I/D+I/I	7(100)	9(50)
Alcohol status	**Types of ACE Polymorphisms**	**Controls n= 74(%)**	**Cases n=83(%)**
No	D/D	17(23)	44(53)
	I/D+I/I	57(77)	39(47)

From the above data, the p value was 0.019 (< 0.05) in alcoholics and 0.005(<0.05) in non- alcoholics.

7. Relationship of BMI with Polymorphism

BMI of the cases and controls were



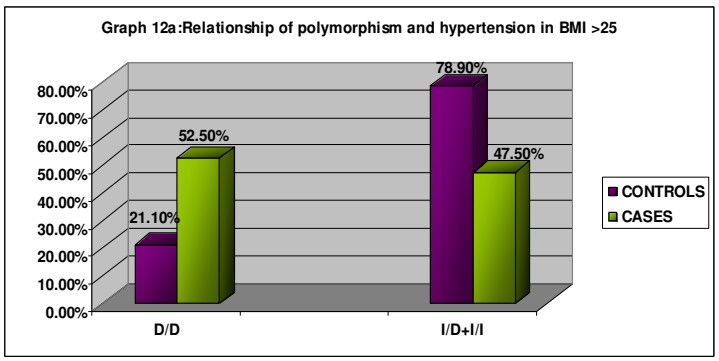


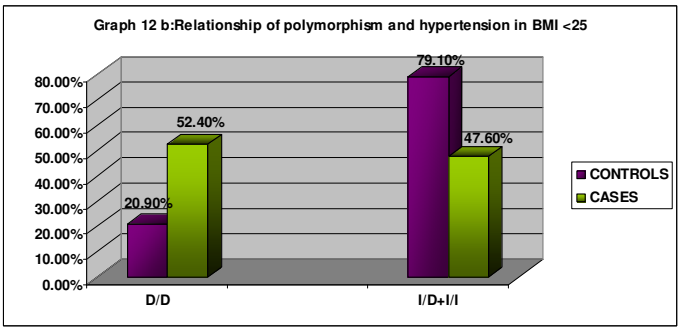
calculated and their polymorphism analyzed. The comparison table is as follows;

Table 7a: Relationship of polymorphism and BMI

BMI	**Types of ACE Polymorphisms**	**Controls n= 38(%)**	**Cases n=59(%)**
>25	D/D	8(21.1)	31(52.5)
	I/D+I/I	30(78.9)	28(47.5)
BMI	**Types of ACE Polymorphisms**	**Controls n= 43(%)**	**Cases n=42(%)**
<25	D/D	9(20.9)	22(52.4)
	I/D+I/I	34(79.1)	20(47.6)

At a BMI > 25, the p value was found to be 0.002 (< 0.05) while at BMI < 25 the p value was found to be 0.003.





Discussion

Essential hypertension (EH) is a polygenic disorder resulting from the interaction of several genetic and environmental factors. Cardiovascular disorders resulted in 2.3 million deaths in the year 1990 and are expected to be doubled by the year 2020. Hypertension accounts for 1.2 million deaths due to coronary heart disease and 0.5 million deaths due to stroke in India (Gupta et al. 2004). The Renin-Angiotensin System (RAS) has a central role in regulating blood pressure and sodium homeostasis. Angiotensin Converting Enzyme is a key enzyme in this system which catalyzes conversion of Angiotensin I to Angiotensin II, a potent vasopressor. Genes encoding components of RAS, including Angiotensinogen (AGT), Angiotensin-converting enzyme (ACE), Angiotensinogen II type-1 receptor (AGTR1), and Renin(-5434 and -5312) have been extensively investigated as genetic determinants of essential hypertension (**13,14**).

The Insertion / Deletion polymorphism in the ACE gene is correlated with the circulating ACE levels. Individuals with the D/D genotype have the highest circulating ACE levels as compared to the I/I genotype (Samani *et al.,* 1994). Various studies have shown association between this ACE polymorphism and several cardiovascular diseases like myocardial infarction (Ludwig *et al*., 1995), left ventricular hypertrophy (Schunkert *et al*., 1994), cardiomyopathy (Raynolds *et al*., 1993) and hypertension(Duru *et al*., 1994; Barley *et al*., 1996; Jeng *et al*., 1997).It has been postulated that the association between the ACE I/D polymorphism and hypertension might be related to gender and ethnicity (Barley *et al*., 1996; Sagnella *et al*., 1999).

In this present study 101 clinically diagnosed hypertensive patients and 81 age and sex matched controls were taken. A number of confounding factors for hypertension like dietary habits, smoking, alcohol and BMI were studied and analyzed. The distribution of all these variables between the hypertensives and controls were studied and their relationship with the three genotypes, I/I, I/D and D/D analysed.

On genotype analysis the frequency of I/I, I/D and D/D polymorphism among non-hypertensives (Controls) was 25.9%, 53.1% and 21.0%. The frequency among hypertensives (Cases) was 16.8%, 30.7% and 52.5% respectively (Graph 5). On statistical comparison of D/D genotypes between the controls and cases, the cases showed a significant increase of frequency. (p value =0.0005). As per (Graph 5), an Odd's ratio of 4.157 was obtained. This showed that in this South Indian population, subjects with D/D genotypes are four times more prone for the development of hypertension. The 95% Confidence Interval (CI) was found to be 2.144-8.060. Since it did not include 1, the result was confirmed to be statistically significant.On comparison of the individual frequencies of the three genotypes (Graph 6), it was found that D/D genotypes were more prone to develop hypertension with an Odd's ratio of 3.85(D/D vs I/I) and 4.32 (D/D vs I/D). The association between ACE D/D allele with hypertension has given contrasting results world-wide. A significant association of ACE D/D allele with hypertension with African Americans, Chinese and Japanese population has already been established (Duru *et al* , 1994 ; Chiang *et al* 1996 ; Morise *et al* 1994 ;Nakno *et al* 1998 ; Morshed *et al* 2001 **(15-19)** .However other studies have failed to show a positive association (Alaitin *et al* ; Maguchi *et al* ; Pamies *et al* 1999; Dazida *et al* 2001; Mondry *et al* 2005) **(20-23).** It has been suggested that these inconsistencies

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

may be due to the difference in background of the population characteristics. Some studies showed that I allele is associated with hypertension in the Australian and Pakistani population (Zee *et al* 1992; Ismail *et al* 2004). The heterogeneity in association of ACE I/D polymorphism with hypertension may be due to varied ethnicity (Barley *et al*, 1994) or various other genetic and environmental factors implicated in the regulation of blood pressure (Guyton *et al*, 1981). A variation in a single etiological factor could lead to difference in blood pressure and hypertension.

Hypertension varies with age. In industrialized societies, blood pressure increases steadily during the first two decades. In children and adolescents, blood pressure is associated with growth and maturation. Blood pressure "tracks" over time in children and between adolescence and young adulthood. The systolic and diastolic blood pressure increases progressively with age until 55 years after which the diastolic blood pressure tends to decrease. The consequence is a widening of pulse pressure (the difference between systolic and diastolic blood pressure) beyond age 60 **(24).** The frequency of D/D genotypes in hypertensive of <50 years and >50 years were 54.5% and 50% respectively (Table 2a).No significant association was found between <50 years group and the phenotype (p value-0.24). Similarly, no significant association was obtained between >50 years group and phenotypic expression (pvalue-0.18).This showed that in this study population, age did not influence the expression of D/D genotype. This result corroborates with the study performed on Punjabi population in North India (Randhwa *et al*) (**25**).

In India, the average systolic blood pressure and diastolic blood pressure is higher for men than for women during early adulthood, although among older individuals the age-related rate of rise is steeper for women. Consequently, among individuals age 60 and older, systolic blood pressures of women are higher than those of men **(26).** This present study was aimed to study the effect of sex on the phenotypic expression. The frequency of D/D genotype in hypertensive males and females were 50% and 53.6% respectively (Table 3a). A significant association was found between the males and phenotype (P-0.0005).Similarly, a significant association was obtained between females and the phenotype (p value-0.001). This concludes that sex does not influence the phenotypic expression of the D/D genotype. Contradictory results have been seen worldwide. While in one Turkish population, the study showed that female sex may affect the phenotypic expression (Sipahi *et al*), another study showed that male sex influenced the phenotypic expression (Morshed *et al*) (**27,28**).

Diet plays a very important role in the causation of hypertension. People consuming diets rich in sodium and deficient in potassium with high saturated fat intake are more prone to develop hypertension. Among the 182 subjects taken for our study the number of non-vegetarians were prominently high with 174 people compared to only 8 vegetarians (Table4a).A significant association was found between the non vegetarians and the phenotype (p- 0.0005) .No significant association was found between the vegetarians and the phenotype (p-0.168) .This showed that, in this study population non vegetarians are at more risk of developing hypertension if they possess the D/D genotype. Similar studies have been done in Czech population where equivalent results were seen (**29)** . Tobacco use is the most common cause of avoidable cardiovascular mortality worldwide **(30).** There are

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

now 1.3 billion cigarette smokers, 82 percent in developing countries, and if current practices continue, there will be an estimated one billion tobacco-related deaths during the 21st century. The immediate noxious effects of smoking are related to sympathetic nervous over activity, which increases myocardial oxygen consumption through a rise in blood pressure, heart rate, and myocardial contractility **(31).** The nicotine present in the smoke causes decreased oxygen supply to the heart, increased blood pressure and heart rate ,increase in blood clotting and damage to the cells that line the coronary arteries and other blood vessels. The frequency of D/D genotypes in smokers were 47.6% and non-smokers were 53.8% (Table 5a).No significant association was obtained between the smokers and phenotypic expression (p-0.057).This showed that smoking is an independent risk factor and did not alter the occurrence of hypertension in subjects with D/D genotype. The higher percentage of D/D genotypes in non-smokers can be explained by the fact that majority of our subjects were females who were non-smokers. This study corroborated to two other studies performed earlier **(32,33).**

Alcohol and hypertension are directly related to each other. Research studies indicate that people who tend to have alcohol on a daily basis (even if it is one or two drinks) have a risk of developing hypertension than those who don't consume alcohol. Studies conducted in America further indicated that moderation in drinking can lower a chance of developing cardiovascular diseases. Alcohol has a direct vasoconstrictor effect, though in most vascular beds this is seen at near lethal concentration. Alcohol withdrawl hypertension is more clearly linked with pressor substances and clinically the syndrome resembles a heightened sympathetic activity. Plasma noradrenaline concentration, renin activity, and concentration of aldosterone and cortisol are raised during withdrawl though only cortisol concentration correlates significantly with blood pressure. By predisposing to various cardiovascular diseases alcohol related hypertension is an important cause of morbidity and mortality. In this present study, among the total number of 182 subjects, 157 were nonalcoholic and the rest alcoholics (Table 6a). Among them the frequency of alcoholics and non alcoholics with D/D genotype were 50% and 53%. A significant association was obtained between the alcoholics and the phenotypic expression (p -0.019) Similarly , a significant association was also found among the non alcoholics (p-0.001) This showed that alcohol did not influence the expression of D/D genotypes.

Obesity and weight gain are strong, independent risk factors for hypertension. It has been estimated that 60% of hypertensive are >20% overweight. Among populations, hypertension prevalence is related to dietary salt intake, and the age-related increase of blood pressure may be augmented by a high salt intake. Obesity is the forerunner for the development of atherosclerosis of major vessels in our body. Hypertensive patients have stiffer arteries, and atherosclerotic patients may have particularly high systolic blood pressures and wide pulse pressures as a consequence of decreased vascular compliance due to structural changes in the vascular wall. In this present study, the weight and height of each individual was taken and the BMI calculated. The number of subjects with BMI > 25 were 97 and those with < 25 were 85 respectively (Table 7a). The frequency of BMI > 25 and <25 among cases were 52.5% and 52.4% .A significant association was obtained between BMI >25 and the

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

phenotypic expression (p- 0.002).A significant association was also obtained between BMI <25 and the phenotype (p-0.001) .This showed that BMI also do not influence the phenotypic expression of D/D genotypes. This study contradicts to a study performed by Maja *et al* which showed that BMI may influence the phenotypic expression of hypertension **(34).** All these variations may be explained in this population to be due to differences in ethnicity.

Conclusion:

i) A statistically significant association was obtained between ACE D/D genotype and hypertension in this study population

ii) A significant association was obtained between the non vegetarians and the phenotypic expression of D/D genotypes.

iii) No significant association was observed between the frequency of age, sex, smoking ,alcohol or BMI on the phenotypic expression of ACE D/D genotypes

References

1. Kannel WB, Wilson PWF. Cardiovascular risk factors and hypertension.In:Izzo Jl,Black HReds. :Hypertension Primer(Second edition):The essentials of high blood pressure 1999;335:765-774
2. Mc Mohan S,Peto R,Cutler J, et al. Blood pressure, stroke, coronary heart disease:Part1.Prolonged differences in blood pressure: Prospective observational studies corrected for regression dilution analysis.Lancet1990;335:765-774
3. Vasan R S,Larson MG,Leip E P, et al. Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham heart study. Lancet 2001;358:1682-1686
4. Navar LG,Lewis L,Hymel A, et al. Tubular fluid concentrations and kidney contents of angiotensins I and II in anesthesized rats .J /am /soc /Nephrol 1994;5:1153-1158
5. Atiyeh BA,Arant BS Jr,Henrich WL,et al.In vitro production of Angiotensin II by isolated glomeruli. Am J Physiol Renal1995;268:F266-F272
6. HubertC,Houot AM,CorvolP,et al.Association between ACE gene I/D polymorphism and primary hypertension in Turkish patients of Trakya region.Biol Chem 1991;266:15377-15383
7. Mattei MG, Hubert C, Alhenc Gelas F et al.ACE gene is on chromosome 17.Cytogenet cell genet 1989;51:1041-1045
8. Yuji Takemoto, Mitsunori Sakatini, Seijyu Takani et al. Association between AII receptor gene polymorphism and serum ACE activity in patients with sarcoidosis. Thorax 1998;53:549-562
9. Saeed M,Mekan SF,Rabbani MA.ACE gene polymorphism and lupus disease severity:A promising link .Annals of Rheumatic disease 2005;64:164-165
10. Taranjit Singh, Subramanium PP.Ace I/D polymorphism in Indian patients with hypertrophic and dilated cardiomyopathy. Mol and Cellular Boch.2008;311:1-2
11. Viroj wiwanitkit. Correlation between ACE I/I polymorphism and erectile dysfunction : an appraisal. Sexuality and Disability 2005;23:2-4
12. Ward R. Familial aggregation and genetic epidemiology of blood pressure. New York: Raven Press; 1995.
13. Manunta,Paolo,Bianchi.Are the new single nucleotide polymorphisms relevant for hypertensive populations?Journal of Hypertension 2002;20(12):2335-2336
14. Thiol B,Weber AB.Genes for essential hypertension.Hype,help or hope? J.Clin Hypertens (Greenwich) 2000;2: 187-193

15. Duru K,Farrow S,Wang J ,et al.Frequency of deletion polymorphism in the gene for ACE is increased in African Americans with hypertension.Am J Hypertension 1994;7:759-762
16. Chiang FT.Chern TH,Lai ZP,et al.Age and gender dependent association of ACE gene with essential hypertension in a Chinese population 1996;10:823-826
17. Morise T,Takeguchi Y,Takeda R 1994.ACE polymorphism and essential hypertension.Lancet;343:125
18. Nakno Y,Oshima T,Hiraga H,et al.Genotype of ACE is a risk factor for early onset of essential hypertension Japanese population.J Lab Clin Med 1998;13:502-506
19. Mahboob Morshed.Association between ACE polymorphism and hypertension in selected individuals of Bangladeshi populations.Journal of Biochem and Mol. Biology 2002;35(3):251-254
20. Alaatin Y.No association between deletion type ACE gene polymorphism and left ventricular hypertrophy in hemodialysis patients.Nephron 2000;84:130-135
21. Maguchi.ACE polymorphism in essential hypertensive patients in Japanese population.Angiology 1996; 47(7):643-648
22. Pamies AE,Palmero PC,Garcia LR,et al.Effect of angiotensinogen M235T and the ACE I/D polymorphism on arterial hypertension and cardiovascular risk factors .Med Clin1999;113(51):164-168
23. Dazida G,Sobatyl J,Puzniak A,et al.Polymorphism of ACE and Ang II receptor type I gene in essential hypertension in Polish population .Clin research 2001;7 :1236-1241
24. Gumar H Anderson.Effect of age on hypertension:Analysis of over 4800 referred hypertensive patients.1990;10:286-297
25. Randhwa NK,Kumar A,Malhotra K,et al. Association study of ACE I/D polymorphism with hypertension in Punjabi population.Int.H.Genet.2006;6(4):317-321
26. Reddy S S.Prevalence and risk factors of hypertension in adults in an urban slum.Indian Journal of community medicine;30(3):2005-2009
27. Sipahi T,Budaki M,SEn S.Association between ACE gene Insertion / deletion polymorphism and primary hypertension in Turkish patients of trakya region.Biotechnol & biotechnol 2006;20(2):104-108
28. Morshed M,Khan H.Association between ACE gene polymorphism and hypertension in selected individuals of Bangladeshi population
29. Julie Bienertova-Vasku, [1] Petr Bienert,[1] Lenka Sablikova et al. Effect of ID ACE gene polymorphism on dietary composition and obesity-related anthropometric parameters in the Czech adult population. Genes Nutr. 2009 Sep; 4(3): 207–213.
30. Teo KK,Ounpe S,Hawken S,et al.Tobacco use and risk of MI in 52 countries in the interheart study.A case control study.Lancet 2006;368:647
31. Najem B,Houssier A,Pathak A,et al.Acute cardiovascular and sympathetic effects of nicotine replacement therapy. Hypertension 206;47:1162
32. Schut,Anna FC,Sayed et al.Smoking dependent effects of ACE gene I/D polymorphism on Blood pressure.Journal of Hypertension 2004;22(2):313-319
33. Ramaswamy,Rajendranath.Lack of association of ACE polymorphism and premature MI in Mauritian Indians.Clinical genetics 1996;50(6):551-554
34. Maja Bansali,Tatzana sharer. Gene polymorphism of RAS and early development of hypertension. Am J Hypertens 2006;19: 837-842