INTRODUCTION

Hypertension (HTN) is a result of interaction between complex environmental and genetic factors. The cardiovascular diseases and chronic kidney disease could be the presenting manifestation of hypertension. Various factors such as usage of tobacco, alcohol, excess intake of salt, physical inactivity and genetic predisposition ultimately leads to hypertension. In 1980, there were about 600 million HTN patients, which rose to 1 billion in 2008. Approximately, 40% of individuals aged 25 and above were diagnosed with HTN worldwide. Every year HTN account for 9.4 million deaths worldwide.

The epoxygenases of cytochrome CYP2J2 and CYP2C subfamilies catalyze oxidative metabolism of arachidonic acid into metabolites, which may have an influential role in hypertension. Endothelial CYP enzymes are shown...
genes on chromosome 10 respectively. So far 17 variant alleles for HTN, identified unclear. 17,18 Literature search also shows that there are no studies involving these polymorphisms on the role of CYP epoxygenases are demonstrated by many studies. Inhibition of CYP epoxygenases decreased renal sodium excretion and increased blood pressure in rats fed with high salt diet. It is shown that EETs have potent vasodilatory actions in various vascular beds such as coronary and renal. The activation of calcium dependent potassium channels and hyperpolarization of smooth muscle cells are attributed for vasodilatory actions of EETs.7,8 The EETs also play a role in regulation of natriuresis by inhibiting the reabsorption of sodium in the proximal tubule and sodium and water in collecting ducts.9 Further, inhibitors of soluble epoxide hydrolases, which hydrolyse EETs to corresponding dihydroxyeicosatrienoic acid are shown to reduce blood pressure and progression of renal injury in rodent models of hypertension.10,11

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The allelic frequency of CYP2C8, CYP2C9, and CYP2J2 genes varies between ethnic groups.12,13 The CYP2J2 gene is located on chromosome 1, CYP2C8 and CYP2C9 genes on chromosome 10 respectively. So far 17 variant alleles for CYP2J2,14,57 variant alleles for CYP2C9 and 13 variant alleles for CYP2C8 gene has been identified out of which CYP2J2*7, CYP2C8*2, CYP2C8*3, CYP2C9*2, and CYP2C9*3 are most common polymorphisms. All these polymorphisms lead to decreased enzyme activity.15,16 Therefore, due to the decreased enzyme activity, there may be decreased formation of EDHF, which may lead to disease susceptibility. Although there are many studies, which showed the influence of genes in regulation of HTN, identification of the candidate genes still remain unclear.17,18 Literature search also shows that there are no studies involving these polymorphisms on the susceptibility of HTN in South Indian population. Thus, the present study aimed to explore the influence of these functional polymorphisms on development of HTN in our population.

METHODS

Subjects

The case-control study consisted of 279 hypertensive patients and 321 healthy controls. All the study subjects were unrelated ethnic Tamilians from families who were residing in South India for at least three generations and spoke any of the four South Indian languages. Subjects were aged between 30 and 60 years of either gender recruited from inpatients and outpatients ward of Jawaharlal Institute of Postgraduate Medical Education and Research Hospital, Pondicherry, India. Standardized datasheet was used to know about the status of their smoking, alcohol, drug intake, and lifestyle. The investigation was approved by Institute Human Ethics Committee. The subjects were explained with study procedure in detail and written informed consent was obtained.

Genotyping

A volume of 5 ml of blood was collected from all subjects in polypropylene tubes with anticoagulant (100 μl of 10% ethylenediaminetraacetic acid). Lipid profile was done from plasma obtained after centrifugation. The deoxyribonucleic acid was extracted by standard phenol–chloroform method and was stored at −20°C. The genotyping of CYP2C8*2 and CYP2C9*2 alleles was done by polymerase chain reaction (PCR)–restriction fragment length polymorphism method. Amplification of CYP2C8*2 was done by forward F5′AAAGTAAAAGAAACAACCAGC3′ and reverse primers R5′AAATCCCTTGAATTTACA3′. Similarly, forward and reverse primers for CYP2C9*2 were F5′TACAAATACATG3′ and R5′CTAACAACAGACTCATAATG3′. The PCR products of CYP2C8*2 and CYP2C9*2 were digested using MboI and AvaII restriction enzymes. Agarose gel (1%) was used to check for amplified PCR product and enzyme-digested products of CYP2C8*2 and CYP2C9*2 were electrophoresed on 8% and 12% polyacrylamide gels, respectively. Genotype was identified using the band pattern (Table 1). Real-time-PCR allelic discrimination method was performed for genotyping of CYP2C8*3, CYP2C9*3 and CYP2J2*7. The kits for amplification, allele discrimination and the real-time-thermocycler were purchased from Applied Biosystems, Foster City, CA, USA.

Statistical analysis

The analysis of genotype data was carried out with the SPSS software; version 16. Student’s t-test was used to compare the demographic details with continuous variables.
and Chi-square test was used to compare the dichotomous variables of the study groups (cases and healthy controls). The differences in genotype and allele frequencies were compared using Fisher’s exact test. The confounding factors were adjusted and the risk of HTN was estimated by performing logistic regression analysis with low risk genotype as the reference groups. $P < 0.05$ was considered as statistically significant.

**RESULTS**

The demographic details of the study subjects are shown in Table 2. The study participant’s age, body mass index, systolic blood pressure, diastolic blood pressure, smoking behavior, total cholesterol, triglycerides, high density lipoproteins, and low density lipoproteins cholesterol were significantly different between the cases and healthy controls ($P < 0.05$). Smokers were higher in cases when compared with the healthy controls (15.8% vs. 7.2%).

The multi-logistic regression odds ratio (OR) of CYP2J2*7, CYP2C8*2, CYP2C8*3, CYP2C9*2, CYP2C9*3 polymorphisms, and HTN compared between cases and healthy controls are shown in Table 3. The table also shows the genotype and allele frequency of the polymorphisms in the South Indian population. The CYP2C9*2 CT + TT genotype between the cases and the controls was significantly different in their distribution ($P = 0.04$) and the crude OR showed 2.4-fold risk for HTN. The risk further increased to 3.2-fold after adjusting for the confounding factors, but there was no statistical significance. The CYP2C9*2 T allele also showed a significant difference when compared between the cases and the healthy controls ($P = 0.04$). Similarly, CYP2C9*3 and CYP2C8*2 showed increased risk after adjusting for confounding factors, but not significantly associated with HTN.

**DISCUSSION**

This is the first study to explore the association of CYP polymorphisms and hypertension in South Indian population. CYP allelic variants were not significantly associated with hypertension. However, the previous studies have shown varied results of association of CYP gene polymorphisms with hypertension. Dreisbach et al. demonstrated that lack of association between CYP2C8, CYP2C9, and CYP2J2 polymorphisms with risk of HTN in the African American population. Whereas, King et al. showed that CYP2J2*7 was significantly associated with hypertension in Caucasian males and Caucasians without a family history of hypertension. However, they did not include CYP2C9 gene polymorphisms in their analysis. Further this study also showed no association between CYP2C8 and hypertension. But, in the present study, although CYP2C9*2, CYP2C9*3 and CYP2C8*2 showed 3.2-, 1.2- and 2.4-fold risk for HTN it did not attain statistical significance.

There are studies in Han Chinese, Russian, Caucasian, and Saudi Arabian populations, which reported that CYP2J2*7 was associated with risk of hypertension. This is contradicting with the present study and the study done in African American population. This probably may be due to differences in ethnicity, which is an important factor attributed to clinical outcomes. Pharmacogenetic variations in genes encoding CYP enzymes are increasingly important as it elucidates inter- and intra-ethnic differences owing to the therapeutic response, adverse events, prognosis, and disease susceptibility. Apart from ethnic differences the factors such as dietary habits, family history, and socioeconomic status may also play a major role in HTN. Our finding is of prime importance in hypertensives given that CYP genes are also involved in metabolism of antihypertensive drugs. Thus CYP genetic polymorphisms involved in varied response to antihypertensives may not be associated with hypertension. This is in accordance with previous studies. However, similar studies in larger and different populations must be carried out to conclude the role of CYP genes in association with hypertension.
CONCLUSION

Our study results show that CYP2C8, CYP2C9, and CYP2J2 gene polymorphisms were not associated with the risk of hypertension in South Indian population.

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