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Original Research Paper

Pediatrics

GENETIC POLYMORPHISM OF CYP450 1A1 IN ISCHEMIC STROKE - A CASE CONTROL STUDY IN A TERTIARY CARE HOSPITAL

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KEYWORDS

INTRODUCTION:

Stroke is often referred to as "brain attack" to denote the fact that it is caused by a lack of blood supply to the brain [1] . According to the Centre for Disease Control, stroke is the third leading cause of death[2] . Athero thrombotic etiology is the most common.

Cytochrome P 450s are a super family of hemoproteins which release metabolites that have vasodilating properties and mediate the anti atherogenic effect. [3]

This study is aimed to determine whether CYP1A1 gene polymorphism influences the risk of developing ischemic stroke.

Certain cytochrome P450s exist in polymorphic forms (genetic isoforms), One of the family member is CYP 1A1 which is involved in the metabolism of polycyclic aromatic hydrocarbons and other molecules [4].

The cytochrome P 450 1A1 (CYP1A1) release metabolites that may cause upregulation of endothelial nitric oxide synthase expression and activity, antiapoptotic effects in endothelial cells, and anti-inflammatory and antiangiogenic effects, all of which suggest a potential anti atherosclerotic effect [5].

Our study aims to evaluate CYP1A1 polymorphisms in both ischemic stroke patients and healthy volunteers in order to understand whether these mutations are associated with stroke and to determine susceptibility to ischemic stroke in south Indian population . The aim of this study is comparison of genotype and allele frequencies between ischemic stroke and control group and to determine if there is any association between specific genotypes and ischemic stroke and to evaluate if there is association between various conventional risk factors and particular genotype

MATERIALS AND METHODS

This study was carried out during the period of March 2012 to October 2012. It was carried out in two groups, apparently healthy controls and patients with ischemic stroke. The study sample comprised of 129 cases of ischemic stroke patients. Patients were chosen from the presenting with symptoms suggestive of cerebrovascular accident were identified and investigated to confirm the diagnosis. Computed tomography scans and magnetic resonance imaging were used to evaluate the cause of stroke. Stroke of hemorrhagic cause were excluded. This study was approved by the institutional ethical committee and written informed consent was obtained from all the participants in the study.

Conventional risk factors such as hypertension and diabetes were noted. Hypertension was defined according to Joint National Committee VII, as a systolic blood pressure >140mmHg and/or a diastolic blood pressure >90mmHg based on the average of the two blood pressure measurements. Diabetes was diagnosed in both according to American Diabetes Association. [6]. 126 age and sex matched controls were recruited from Master health check up

5ml of peripheral venous blood was withdrawn and 2ml was transferred to EDTA tube and mixed thoroughly. Buffy coat was separated and DNA extraction of the samples were performed by using a kit. Extracted DNA was identified by 1% agarose gel electrophoresis. 523 bp fragment of CYP1A1 gene was amplified using, Forward primer – CCA TTT TGG GAG GTT CTT GA Reverse primer _ CCT GAA CCC CAT TCT GTG TT. Master Mix consists of Reaction buffer Tris Hcl , MgCl2, dNTP's and Taq polymerase. Amplification was carried out in an Applied Biosystems thermal cycler with the following cycling conditions. Initial denaturation - 940 C -5min , 30 cycles of Denaturation - 940 C – 1 min, Annealing - 610 C – 1min, Extension - 710 C – 1min and Final extension at 720 C - 10 min. Amplified product – amplicons of 523 bp was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder.

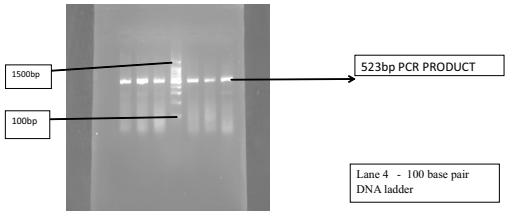
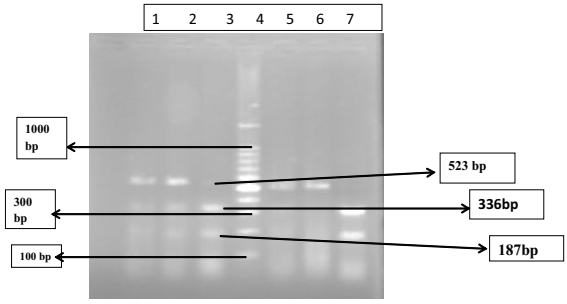


FIG 1 PCR PRODUCT

CYP 1A1 gene polymorphism was detected by digestion of the PCR amplified product with the MspI restriction enzyme followed by run in 2.0% agarose gel electrophoresis. T allele does not have the restriction site hence will yield a 523 bp fragment. C allele has the restriction site, hence gets cleaved to give187 bp and 336 bp fragments. Heterozygous individuals (TC) have 523 bp, 187bp, 336 bp fragments. Analysis was done using a 100bp DNA ladder . Lipid profile was done by the following methods : Total cholesterol – CHO-PAP Method, Triglycerides – GPO-PAP METHOD, HDL - Direct Method . LDL was calculated by Friedwald formula.



Lane 4- 100bp DNA Ladder

Lane 3 – TC, Lane 6- TT, Lane 7- CC STATISTICS

Association between genotypes and stroke was examined by using odds ratio (OR) with 95% confidence interval (CI) and chi-square ($\chi 2$) analysis . All the statistical tests were two-sided and were considered significant at p value <0.05. In addition, conventional risk factors including hypertension, diabetes, obesity and cigarette smoking were recorded for both groups.

Table 1 shows the comparison of various risk factors, age and gender distribution among the patients and the controls.

Table 1

S.No	Parameter	Cases n = 129	Controls n =126	p value
1	Age(years)	56.88±19.82	56.54±19.78	0.72
2	Males n(%)	81(62.8%)	67(53.2%)	0.12
3	Hypertension n(%)	84(65.1%)	54(42.9%)	0.001
4	Diabetes n(%)	78(58.9%)	42(33.3%)	0.0001
5	Smokers n(%)	62(48.1%)	35(27.8%)	0.001
6	Obesity n(%)	67(51.9%)	50(39.7%)	0.05
7	Total Cholesterol(mg/dl)	241.22±86.3	227.82±83.46	0.01
8	TGL (mg/dl)	167.96±114.76	215.98±109.7	0.0001
9	HDL Cholesterol(mg/dl)	36.24±12.72	50.76±19.1	0.0001
10	LDL Cholesterol(mg/dl)	171.2±88.74	133.86±92.24	0.0001

Among the stroke patients, 62.8% (n=81) were males, which was found to be higher when compared to controls 53.2% (n=67) and the percentage of females was found to be 37.2% (n=48) in patients and 46.8% (n=59) in the healthy persons.

The frequency of diabetic individuals in stroke patients (58.9%) was significantly higher than the frequency of the diabetics in controls (33.3%), p<0.001. The percentage of smokers in patient group was 48.1% and 27.8% in healthy controls (p=0.001). Likewise, obesity was observed in 51.9% of patients which was higher than that of controls (39.7%), p=0.05.

According to the clinical laboratory tests given in Table 1, the level of LDL cholesterol was significantly higher in ischemic stroke patients (171.2 ± 88.7mg/dl) when compared to controls (133.8 ± 92.2 mg/dl, p=0.0001); while the level of HDL-cholesterol of patients was significantly lower (36.2 ±12.7mg/dl) than that of controls (50.7 ±19.1mg/dl, p=0.0001). There was also significant difference in the total cholesterol levels of patients (241.2 ±86.3mg/dl) and controls (227.8 ±83.4mg/dl, p= 0.01).The triglycerides level in patients (167.9±114.7mg/dl) was found to be slightly lower in patients than in controls (215.9 ±109.7mg/dl, p=0.0001).

The percentage of hypertensives in stroke patients was found to be 65.1% in patients with ischemic stroke and 42.9% in controls. Smokers were found to be more in number in cases compared to healthy persons.

129 stroke patients and 126 control subjects were genotyped for CYP1A1 gene polymorphism. The genotype distribution among the patients and controls are shown in Table 2. The distribution of genotypes was in Hardy–Weinberg equilibrium.

Table 2

Genotype distribution of CYP1A1 polymorphism among patients and controls

Genotype	Stroke n (%)	Control n (%)	OR	95 %CI	P-Value
TT	74 (57.30)	79 (62.70)	1.2493	0.7561 - 2.0641	0.38
TC	43 (33.30)	44 (34.90)	1.0732	0.6394 - 1.8012	0.79
CC	12 (9.30)	3 (2.38)	3.4597	1.6931 - 7.0695	0.02*

*- Significant

57.4% of stroke patients and 62.7% of controls had homozygous TT genotype. The percentage of heterozygous subjects were 33.3% and 34.9 % in the patients with ischemic stroke and controls respectively. The percentage of the CC genotypes of CYP1A1 polymorphism in patients with ischemic stroke was found to be 9.3% and 2.4% in controls. Among the three genotypes, CC homozygote showed a increased risk of stroke by three times, X2=5.54, (OR=3.4597,95%CI=1.6931 -7.0695,p=0.02), while TT (OR=1.2493,95% CI=0.7561-2.0641,p=0.38) and TC (OR=1.0732,95% CI=0.6394-1.8012, p=0.79) genotypes were not found to be significant .

CC genotype subjects were found to be more among the smokers in patient category. Among the TT and TC genotypes, the percentage of smokers was found to be high in controls than in patients . However the associations are not statistically significant (p=0.52). Among the non smokers, there was no significant difference between the various genotypes.

The proportion of TC genotype was found to be significantly higher in controls (50.0%) than in patients(26.2%). Among the normotensives, the proportion of TT genotype was significantly higher in controls(75%) than in patients(48.9%), (p = 0.004) when compared to the other genotypes.

Among 117 obese individuals, the proportion of TT genotypes were slightly higher in the patients (61.2%) than the controls (56%) and also the CC genotype was slightly higher among the patients (11.7%) than the controls(4%).

DISCUSSION

Stroke occurs when a blood clot blocks an artery that carries blood from the heart to the body, thereby interrupting blood supply to an area of the brain. It is the third leading cause of death worldwide (World Health Organization Fact Sheet 2009;)[7] Stroke causes a great economical burden for the community as a whole because of the disability associated with it and expenditure of lot of resources.

Cytochrome P450s are oxygen-reacting hemeproteins and the most important components of xenobiotic metabolizing system .They have the ability to oxidize, peroxidize and reduce various substances and drugs in an oxygen and NADPH-dependent manner[8,9].

The cytochrome P450 1A1 gene (CYP1A1 gene) is part of the cytochrome P450 supergene family. This enzyme is responsible for the metabolism of a large number of xenobiotics . Several polymorphisms in the CYP1A1 gene have been described and it has been proposed that these polymorphisms affect the function of the enzyme by acting at genetic level. There is increasing evidence of the presence of an association between some of these mutations and various kinds of cancer and other diseases like cerebrovascular diseases[10,11]

In this study, we aimed to investigate one important CYP1A1 polymorphism,m1 as risk factor for ischemic stroke in south Indian population. Conventional risk factors and lipid parameters were also

evaluated in stroke patients and healthy control groups.
Hypertension, heart disease, cigarette smoking, diabetes, obesity, waist-to-hip ratio, atrial fibrillation, high lipid profile, physical inactivity and over consumption of alcohol can be modified [12-15] . In this study, the prevalence of conventional risk factors like hypertension, diabetes, smoking and obesity in the patient group were found to be significantly higher than the control group .

In our study, total cholesterol levels were higher in stroke patients (241.2±86.3) when compared to control group (227.8 ± 83.4 mg/dl) and the difference was significant (P=0.01). So, we found that total cholesterol constitute a significant risk for stroke. Most of the studies have found that high levels of HDL-cholesterol decrease the risk of ischemic stroke [16,17]. According to our findings, the level of HDL-cholesterol of patients was significantly lower (36.2 ±12.7 mg/dl) than that of controls (50.8 ± 19.1 mg/dl, p=0.0001); while the LDL-cholesterol was significantly higher in cases (171.2 ± 88.7 mg/dl) when compared to controls (133.8 ±92.2 mg/dl, P=0.0001).

The CYP1A1 m1allele has a T C mutation in the 3 non-coding region, which affects enzyme activity. It is important to note that in this study, the mutant C allele seems to increase the risk of stroke since it has higher allele frequency in the patient group. In some studies it has been expressed that the noncoding region is important for RNA stability and expression of gene products and CYP1A1 gene expression is modulated by post-transcriptional and post-translational mechanisms [18].

In the present study, there was a significant difference between patients and controls in terms of genotype . Therefore, CYP1A1 gene polymorphism is a significant risk factor for stroke. The following facts from animal studies may be the possible reason behind this mutation causing ischemic stroke

ROLE OF CYP IAI IN ATHEROSCLEROSIS

CYP1A1 catalyze oxygenation of hydrocarbon procarcinogens present in foods and smoke to reactive moieties that adduct cellular macromolecules in vascular smooth muscle cells (VSMCs) and induce oxidative stress. CYP1A1-encoded enzymes are expressed in vascular endothelium and smooth muscle, with considerably higher levels of activity present in endothelium. In VSMCs, expression of CYP1A1 and CYP1B1 may influence oxidative metabolism of exogenous and endogenous substrates and promote formation of reactive oxygen species and electrophilic intermediates. These effects may shift redox status and culminate in oxidative vascular injury.

It is known that CYP1A1and CYP1B1are differentially regulated in VSMCs, with constitutive and inducible expression at the mRNA and protein level strongly influenced by Ahr(aryl hydrocarbon receptor) phenotype and mitogenic status. CYP-derived peroxidation byproducts, such as oxygenated linoleic acid derivatives and oxysterols, are present in human atherosclerotic lesions. Human lesions also contain high levels of isoprostanes and peroxidation products of arachidonic acid. The formation of oxidative byproducts in VSMCs(vascular smooth muscle cells) and the leakage of superoxide during repeated catalytic cycles may be influenced by constitutive and inducible expression of CYP1B1.Thus, in VSMCs, cellular mechanisms involving actions of the Ahr govern the expression of CYPs involved in atherogenesis.[19]

Among the diabetics and nondiabetics there was no statistical significance in the genotype distribution.Among the hypertensive group, the proportion of TC genotype was 26% in stroke patients, which was significantly lower than that of the controls (50.0%),P=0.004 .In non hypertensives ,the percentage of TT genotype is higher in controls(75%) than in patients(49%) which suggests that the genotype may be protective factor against stroke , p=0.004 and there was no other statistically significant association.There was no statistically significant difference in genotype distribution in smokers and nonsmokers.

Among obese individuals, the proportion of TC genotypes (38.0%) were slightly higher in the controls than the stroke patients (28.3%), P=0.27; the proportion of TT and CC genotypes were slightly higher in cases when compared to controls. These observations were also not significant. Therefore it was found that there was no statistically significant association between the genotypes and the risk factors.

CONCLUSION

In the present study ,the frequency of the C allele was significantly found in patients with stroke than controls which demonstrate that mutant C allele may confer the risk for stroke.

CYP1A1 gene polymorphism was also analyzed with respect to groups of hypertension, diabetes, smoking and obesity. We found no significant association of individual genotypes with the various risk factors except for TT genotype which may be protective in patients without hypertension. We conclude that CC genotype is a significant risk factor for ischemic stroke and there was no association of specific genotypes with the risk factors.

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