Full Length Research Paper

Role of GSTM1 Gene polymorphism and its association with Coronary Artery Disease

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Coronary artery disease (CAD) is one of the leading causes of mortality and morbidity worldwide. Complex interplay of environmental and genetic factors has been known to contribute to CAD pathophysiology. Variations in several genes have been found to be associated with risk for developing CAD in different populations. Glutathione S-transferases (GSTs) play a primer role in cellular defense against electrophilic chemical species and radical oxygen species. This study was designed to assess the GSTM1 gene variant in 120 patients with coronary artery disease (CAD) as compared to equal number of controls. DNA was isolated from the blood samples collected and subjected to PCR with specific GSTM1 specific primers. The frequency of GSTM 1 gene was in 73.3% of cases or patients with CAD and 61.6% in control subjects and the frequency of null genotype was 26.6% in CAD cases and 38.3% in control group. There was no significant association of GSTM1 gene (null) polymorphism (χ^2 = 3.72, P = 0.053, OR = 1.17, 95% CI = 0.95 – 3.07) with CAD in our study. However, diabetes and smoking were significantly associated with CAD (p < 0.001).

Key words: GSTM1, CAD, genetic polymorphism.

INTRODUCTION

CAD (Coronary artery disease) epidemic in India has entered into an epidemiological transition phase. There has been an alarming nine-fold increase in urban and over two-fold increase in AD among rural population over the last four decades; surprisingly, majority of CAD population in India present with malignant form and significantly affecting the young ones, that is, less than 40 years of age. At present 25% death among Indians are attribu-table to CAD (Maity, 2003). Over the past 150 years, there have been numerous efforts to explain the complex events associated with the development of CAD. In this endeavor, three distinct hypotheses have emerged that are currently under active investigation. One of the hypotheses stresses the importance of oxidative modification in the plague formation (Stocker Roland and John F. Keaney, Jr., 2004). Oxidative stress plays an important role in the development of atherosclerotic disease, while antioxidants may delay or prevent

various steps in atherosclerosis (Liebson and Amsterdam, 1999).

Polymorphisms that affect key pro- and antioxidant enzymes might alter the susceptibility to oxidative stress–mediated injury and now the use of genetic epidemiology for the study of oxidative stress–related genes has received attention towards CAD. One of the principal pathways to develop vascular complications is the production of ROS (Johansen et al., 2005). ROS can stimulate oxidation of LDL, forming ox-LDL, which is not recognized by the LDL receptor leading to foam cell formation (Da Ros et al., 2005). Recent studies suggest that the common variant in the glutathione S-transferase (GST) M1 (GSTM1) and T1 (GSTT1) gene is associated with the risk of smoking-related Coronary Artery Disease (CAD). Intra-ethnic as well as inter-ethnic differences are known to impact the frequencies of GST gene polymorphisms, thus influen-cing its interactive effect with tobacco smoking on CAD risk (Wang et al., 2008). The glutathione S-transferases (GST), a superfamily of phase II metabolic enzymes play an important role in the cellular mechanism of detoxification by conjugating reactive electrophilic compounds with soluble glutathione (Strange

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et al., 2000). In addition, these enzymes are also believed to play a crucial role in the protection of DNA from oxidative damage. The genes encoding the mu class of enzymes are organized in a gene cluster on chromosome 1p13.3 and are known to be highly polymorphic. These genetic variations can change an individual's susceptibility to carcinogens and toxins as well as affect the toxicity and efficacy of certain drugs. GSTM1 products catalyze the conjugation of glutathione to oxide derivatives of polycyclic aromatic hydrocarbons, the main carcinogens found in tobacco smoke. These products are important in the detoxification of naturally occurring monohalomethanes, dichloromethanes and ethylene oxides (Hanna et al., 2001). Polymorphisms in the GSTT1 and GSTM1 genes are caused by a deletion, which consequently results in virtual absence of enzyme activity, especially in individuals with deletion in both genes (null genotype). Many studies have demonstrated great concordance (> 95%) between the genotype and phenotype (Zhong et al., 1991; Bruhn et al., 1998). In view of the importance of this polymorphic gene, we undertook this study to see if the genotypes of this gene have any bearing on the disease progression or disease susceptibility.

MATERIALS AND METHODS

Selection criteria

This study included 120 patients with angiographically diagnosed CAD patients (88 males and 32 females) consecutively admitted to the hospital with proven coronary artery disease (more than 50% stenosis affecting at least one vessel) during 2007 - 2008. The controls were those who came to the hospital with pain in the chest but did not have a history of angina pectoris or Myocardial Infarction (MI) and they showed a normal electrocardiogram (79 males and 41 females), in whom angiographic examination excluded the presence of coronary artery disease. This study therefore represents positive cases as CAD subjects and the negative cases as control subjects.

Patient's characteristics

All the patients and controls were interviewed and epidemiological data/demographic data were recorded in a structured ques-tionnaire. Each participating in-patient with CAD was interviewed to determine variables, such as smoking, alcohol use, family history (≥ 1 first-degree relatives with MI or coronary artery disease), and to obtain consent for blood sample for genetic analysis. In addition, detailed chart abstractions were performed to collect relevant laboratory and clinical data. A total of 120 CAD patients included in the current study agreed to participate and to provide blood sample for genetic analysis. Informed consent was obtained from all patients and controls, as required by the institutional ethical committee.

Determination of risk factors

For CAD risk factors, the following definitions were used: subjects were defined as hypertensive if their blood pressure was > 140/90 mm Hg or if they were receiving any antihypertensive treatment; those with a history of diabetes or who were receiving any anti diabetic drugs and fasting glucose levels > 120 mgs/dl were considered to be diabetic. Smoking history was recorded as either none or current smokers. A positive family history was the presence of a first degree relative with coronary artery disease at the age of < 55 years for men and < 60 years for women.

Protocol approval

The study and research protocol was approved by Mahavir hospital ethical committee.

Angiographic study

All patients underwent coronary angiography. Coronary stenosis was considered significant in the presence of a luminal diameter narrowing of > 50% of at least one pericardial coronary artery. The severity of coronary artery disease was expressed by the number of affected vessels (one, two, or three vessel disease) and also by means of the Duke scoring system —a prognostic index that includes the number of diseased major vessels, the presence of left main coronary artery disease, the percentage narrowing of the major vessels and involvement of the left anterior descending coronary artery particularly when the proximal segment shows severe stenosis (> 95%). The Duke score ranges from 0 – 100 (0 = no disease, 100 = the most severe disease).

Statistical analyses

We determined whether the distribution of the GSTM 1 genotypes was in Hardy-Weinberg equilibrium using chi-squared analysis, as described (Emery et al., 1976). Comparisons of genotype and allele frequencies between cases and control subjects were performed by an X^2 test. Statistical significance was accepted at $P < 0.05$. All analyses were done using EPI 6 software (Epi info6 CDC).

Genotyping of GSTM1 gene

Genomic DNA was extracted from whole fresh blood using standard phenol/chloroform methodology with ethanol precipitation. PCR was carried out using purified DNA. The primer sequences used for amplification were:

Forward primer   5'- TT CCT TAC TGG TCC TCA CAT CTC - 3'
Reverse primer   5'- TCA CCG GAT CAT GGC CAG CA - 3'

The final concentration of the PCR mixture contained 1.5 mM MgCl2, 50 mM KCl (Bio-Serve Hyderabad), 10 mM Tris-HCl (pH 8.8), 0.1% gelatin, 0.3 mM each of dNTPs (SIGMA), and 2U Taq DNA (Bioserve Hyderabad) polymerase in each 50 μl reaction mix. DNA amplification was carried out in a thermo cycler (TAKARA) using 100 ng of genomic DNA and the condition of PCR were 45 s at 94°C (denaturation), 30 s at 56°C (annealing), and 30 s at 72°C (elongation) for 35 cycles. The amplified DNA fragments were run in 2% agarose gel, stained with ethidium bromide and visualized in a gel documentation system. The presence or absence of GSTM1 gene was detected by the presence or absence of a band of 206 bp (corresponding to GSTM1).

RESULTS

The base line demographics of the patients (n = 120) and

Table 1. Demographic characteristics of patients and controls.

	Cases (n = 120)	Controls (n = 120)	p-value
Age; Males (21 - 59)	52.21 ± 13.13	50.3 ± 18.5	0.81
Age; Females (20 - 60)	48.9 ± 12.12	50.62 ± 9.92	0.67
Alcoholics %	12%	4%	< 0.001
Diabetics %	25%	10%	< 0.001
Hypertension %	41%	12%	< 0.001
Smokers %	32%	9%	< 0.001

Values are represented as mean ± SD: p < 0.05 in comparison to control group.

Table 2. Genotypic frequencies of GST gene null polymorphism in patients and matched control groups.

Category	GST genotype		Total
	Null (%)	Present (%)	
Cases	32 (26.6)	88 (73.3)	120
Controls	46 (38.3)	74 (61.6)	120

	Value	Df	P-value
Pearson chi-square	3.72	1	0.053

Significant P < 0.05; non-significant P > 0.05.

controls (n = 120) are presented in Table 1. The mean age of patients at sampling was around 50 years. Consumption of alcohol and tobacco, hypertension and diabetes were significantly associated with CAD (p < 0.001). From the GSTM1 genotyping it was observed that the frequency of null allele was 26.6% in CAD cases as compared to 38.3% in controls whereas GSTM 1 gene was present in 73.3% of CAD cases and 61.6% in control subjects. Statistical analysis showed no significant association of GSTM1 null polymorphism (χ^2 = 3.72, P = 0.053, OR = 1.71, 95% CI = 0.95 – 3.07) with CAD (as in Table 2.).

DISCUSSION

The present study was conducted to look into the relationship between the genetic polymorphism of glutathione S-transferase mu 1 gene and CAD, which is involved in the metabolism of ROS and detoxifying xenobiotics. The contribution of the GST supergene family to oxidative stress resistance is well established (Hayes and Strange, 1995) and therefore the absence of one or more of the GST enzymes would result in increased ROS-mediated damage.

Although there are very few studies that associate GST polymorphism with CAD, interestingly one report from North India has been published (Girisha et al., 2004) regarding the association of CAD and GST. It is now known that the frequency of GSTM 1 allele varies in different ethnic populations. Wilson et al. (2000) have published two similar studies, one in migrant South Asians and the other in Caucasians where GSTM1 null phenotype were associated with lesser incidence of CAD (P = 0.029 OR = 0.63), but no significant association was observed between the GSTT1 wild genotype and CAD (P > 0.05) in South Asian population.

In Turkish population GSTT1 null genotype was reported to be associated with smoking status and heart disease. It is possible that the decreased activity of GST affects the various mechanisms of DNA damage, including those mediated by tobacco and oxidative stress. As tobacco smoking is known to induce DNA damage, the relationship of polymorphism of enzymes involved in the metabolism of genotoxins with development of CAD has been looked into specifically by Tamer et al. (2004). Tobacco smoking has been shown to cause DNA damage and induce formation of DNA adducts.

In our study population, the frequency of smokers are more in cases when compared to controls (32% V 9%) significantly associated with CAD (< 0.001). GST polymorphism may act differently in different ethnic groups, ethnic differences in the prevalence of the GSTM1 null genotypes have been reported to vary between 22 – 35% in Africans, 38 – 67% in Caucasians and 33 – 63% in East Asian populations (Rebbeck, 1997). The Pacific islanders (Oceania) have the highest reported frequency, GSTM1*2 ranges from 64% to as high as 100% in Kiribati natives. The GSTT1*2 genotype varies from 10 – 18% in Caucasians (Nelson et al., 1995)

to 58% in the Chinese (Le et al., 1995). Since there is so much of diversity in relation to the GSTM1 polymorphism between ethnic groups, our results showing no association of CAD with GSTM1 follows this trend.

The complex etiology around cardiovascular disorders and the multiple environmental conditionings are most of the times not evaluated together. In most cases, CAD has a multifactorial genetic basis, involving a number of genes and environmental factors interacting to determine whether or not the disease will develop. It is difficult to determine the role of polymorphism of different proteins involved in the inflammation in CAD. The studies of this kind have limitations in establishing the causal role in the disease. However, these studies contribute to the scientific evidence in elucidating the etiology of complex multifactorial diseases like CAD and hopefully provide a step in the search for candidate genes.

Therefore, the inherited genes generally predispose to a greater or lesser extent of CAD, but it is the environmental factors (e.g. cigarette smoking, obesity, hypertension, sedentary habits) interacting with the individual's genotype that determine whether or not CAD will develop (Jamil et al., 2009). Since it's a mulfactorial disease it requires genome wide SNP analysis of multiple genes associated with it as well as search of novel gene polymorphism involved in angiogenesis and metabolic pathways like mevalonate pathway, Lipid metabolism, inflammatory and thrombotic pathways along with oxidative stress can help in understanding the etiology of CAD in a better way.

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