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Myeloperoxidase Variants (at SNP -463 G/A) and the Risk of Coronary Artery Disease (CAD): a Case Control Study

KEYWORDS

Dr.G. Rajashekar Reddy	Dr.K. Laxman Rao	Dr. Vinod K unni
Associate Professor, Dept. of Cardiology	Prof & HOD, Dept of Cardiology, Deccan College of Medical Sciences	Assistant Professor, Dept of Cardiology

Dr. Parvaiz Kadloor	Nausheen Fatima
PG(DM), Cardiology, Deccan College of Medical Sciences	Deccan College of Medical Sciences

Dr. Parveen	* Dr. M.Ishaq
Salar-E-Millat Research centre, Princess Esra Hospital, (DCMS) Hyderabad	Salar-E-Millat Research centre, Princess Esra Hospital, (DCMS) Hyderabad *Corresponding author

ABSTRACT *The aim of the study was to evaluate the role of single nucleotide polymorphism (SNP) at -463 G/A position by PCR in predisposition to coronary artery disease. A total of 69 CAD patients to an equal number of healthy subjects were included in the study. The frequency of AA genotype in patients was 1.44% compared to 5.79% in healthy controls. Odd ratio analysis revealed a OR=0.24 indicating that persons with AA genotypes have about 76% reduced risk of CAD.*

Introduction:
Cardiovascular disease (CAD) has been reported to be the leading cause of deaths among patients suffering from non-communicable diseases. The major risk factors that contribute to CAD include hypertension, type 2 diabetes mellitus and hyperlipidemia. (1, 2) Apart from these, another important predisposing factor in genetic susceptibility. The role of genetic factors was established by observations like familial incidence of CAD as well as high sibling recurrence risk (3,4). Several genes have been indentified that are likely to have a role in susceptibility to CAD; such genes are considered as possible candidate genes for disease susceptibility (5). Studies pertaining to such candidate genes have main relied on single nucleotide polymorphisms (which affected gene expression quantitatively) particularly those genes that code for anti-oxidant enzymes as well as those that code for pro-oxidant enzymes like NADPH oxidase, endothelial nitric oxide synthase and myeloperoxidase (MPO) (6).

In the present study we analyzed a genetic variant of MPO as a biomarker of risk prediction for CAD. MPO is a heme enzyme which is found to play an important role in intracellular bactericidal activity (7). During this process MPO generates several reactive oxygen species leading to oxidative stress (8). MPO converts H_2O_2 into hypochlorus acid (HOCL) which has the following damaging effects) it oxidises LDL and chemically interacts with HDL thereby rendering it pro-atherogenic. Apart from this HOCl also reacts with nitric oxide and consequently reduces bioavailability of this muscle relaxant (9). In view of its above adverse affects MPO is considered as a marker of risk prediction for CAD (10). Zhang et al was first who demonstrated that increased MPO activity in plasma was observed in patients with CAD (10). Later it was shown that the plasma activity of MPO was related to genotypes. Thus maximum plasma MPO enzyme activity was observed in subjects with GG genotype, intermediate in individuals with AG genotype and least in those with AA genotype. Hence the present study was carried out to evaluate the role of polymorphic variants of MPO at SNP -463 G/A in predisposition to CAD due to paucity of information on this aspect in CAD patients from India.

Materials and Methods: The study was approved by Institutional Review Board. A total of 69 CAD patients were selected consecutively from the Intensive care Unit of the Department of Cardiology, Princess Esra Hospital, Hyderabad. A total of 69 CAD patients were selected for the study. All the cases were angiographically verified for CAD. The study subjects were 41 with CAD with Diabetes mellitus and 28 were CAD patients without Diabetes. Sixty nine healthy subjects served as controls. Demographic details of the patients and controls were recorded in a proforma.

Genotyping of Myeloperoxidase variants at SNP -463 G/A
Five ml of venous blood was collected in vaccutainers with EDTA from patients and controls and stored at -20^{0}C until use. Genomic DNA was isolated from stored blood samples according to the method of Lahiri et al (11). SNP at -463 G/A was determined employing sequence specific primers: Forward primer [5' CGG TAT AGG CAC ACA ATG GTG AG 3'] and reverse primer [5' GCA ATG GTT CAA GCG ATT CTT C 3']. PCR was carried out according to the method described by Nikpoor et al (12). The 350 bp amplified product obtained was digested overnight by restriction enzyme ACil and RFLP (restriction fragment length polymorphism) pattern was determined by electrophoresis on a 2.5% Agarose gel.

Statistical Analysis
Statistical analysis was performed using SPSS 18.0 (SPSS In., Chicago, USA) software. Frequency statistical and chi square tests were performed on the data collected.

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Results

Genotypic and allelic frequencies of MPO polymorphism at -463 G/A in the total CAD patients and controls are provided in Table 1. It was observed that the frequencies of GG and AG genotypes were comparable in the patients and healthy controls groups; it was 76.81% and 21.73% for GG and AG respectively in CAD patients compared to 72.46% GG and 21.73% AG in controls. Considerable difference was noted in the percentage frequency of AA homozygotes, it was 1.44% in CAD patients and 5.79% in the controls, indicating that the frequency of AA homozygotes was nearly four times less frequent in CAD patients than in controls.

The CAD patients were sub-grouped into those with T_2DM and without T_2DM in order to investigate what pattern of association emerges. It was observed that the GG genotype was more frequent in CAD with T_2DM (table 1). Hence individuals with GG genotype have an increased risk of 70% of developing CAD with T_2DM (O.R=1.70) (Table 2). The frequency of AG genotype was 32.14% in CAD without T_2DM compared to 21.73% in controls indicating increased risk of CAD without T_2DM for individuals with AG genotype. However, these results need to be confirmed by further studies.

Discussion

The significance of MPO genetic variants at -463 G/A can be realized from the fact that these variations are related to plasma MPO activity (13).MPO levels have been shown to be not only independent predictor of endothelial dysfunction but also risk of CAD and its further progression to events like myocardial infarction (14).

An important conclusion drawn in the present study is that in persons carrying AA genotype the risk of CAD is reduced by 76% in relation to individuals who have GG and AG genotypes (O.R.=0.24) (Table 2). The observed protection in AA individuals may be attributed to reduced levels of MPO enzyme activity in the plasma and hence minimum damage to vascular system due to reduced production of hypochlorus acid. Similar results were also reported by Nikpoor et al 2014 who demonstrated that in an autosomal recessive model AA homozygotes showed decreased risk of CAD (12). In another study on patients with premature CAD, Zhong C et al (2009) concluded that AA genotype is a protective factor against premature CAD (15).

Conclusion:

It is concluded that single nucleotide polymorphism at position -463 G/A in the promoter region of MPO gene may be associated with risk of coronary artery disease. An important conclusion drawn in the present study is that in persons carrying AA genotype the risk of CAD is reduced by 76% in relation to individuals who have GG and AG genotypes.

Table1. Genotypic and allelic frequencies of Myeloperoxidase SNP (-463 G/A) in CAD patients and Healthy Controls.

Category	Total No.	Genotype			Allelic frequencies	
		GG	AG	AA	G	A
*Total CAD Patients	69	53 (76.81%)	15 (21.73%)	1 (1.44%)	0.85	0.15
CAD with T_2 DM	41	34 (82.92%)	6 (14.63%)	1 (2.43%)	0.90	0.10
CAD without T_2 DM	28	19 (67.85%)	9 (32.14%)	0 (0%)	0.84	0.16
*Controls	69	50 (72.46%)	15 (21.73%)	4 (5.79%)	0.83	0.17

*The genotypic frequencies of total CAD patients and Controls were within Hardy-Weinberg Equilibrium.

Table2. Analysis of Odds Ratio for assessment of risk of CAD

Category	Genotypes compared	Odds Ratio
Total CAD *Vs Controls	**AA** *Vs* AG+GG	0.24
CAD with T_2DM *Vs* Controls	**GG** *Vs* AG+AA	1.7
CAD without T_2DM *Vs* Controls	**AG** *Vs* GG	1.57
CAD+T_2DM *Vs* Controls	**AG** *Vs* GG	0.58

**Vs*= Versus

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