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Research Brief

The potential influence of *GSTT1* null genetic polymorphism on coronary artery disease: A pilot study in a South Indian cohort

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ABSTRACT

Background: Indians are known to have the highest rates of coronary artery disease (CAD), with the conventional risk factors failing to explain the increased risk. Possible candidate genes to study both the environmental and genetic risk associated with CAD is the glutathione S-transferase (GST) family, as it is involved in detoxification.
Methods: This case–control assessed the association between *GSTM1* and *GSTT1* polymorphisms in Indian patients with CAD. Fifty patients with CAD and 50 healthy volunteers were genotyped for the two polymorphisms by polymerase chain reaction. The genotype frequencies between the groups were compared, where a p-value of less than 0.05 was considered as statistically significant.
Results: There was a significant inverse association between *GSTT1* null polymorphism and CAD susceptibility.

1. Introduction

Coronary artery disease (CAD) is one of the most prevalent types of heart disease responsible for approximately 17.8 million deaths worldwide annually. Considered as a complex genetic disease, it is caused by both environmental triggers and genetic predisposition.[1] While the genes conferring susceptibility to CAD are largely unknown, previous studies have determined that glutathione S-transferases (GSTs) may contribute to the risk of developing CAD.[2]

GSTs are phase II enzymes which detoxifies substrates that may increase the risk of CAD.[2] It also protects the cell against damage from reactive oxygen species (ROS).[2] The functional activity of these enzymes is partly influenced by gene polymorphisms, particularly those of glutathione S-transferase mu 1 (*GSTM1*) gene and glutathione S-transferase theta 1 (*GSTT1*). The deletion polymorphism of *GSTM1* and *GSTT1* genes, commonly termed as null variant, results in the absence of functional enzyme.[3] This causes reduced detoxification of xenobiotic substances and lower antioxidant activity, thereby facilitating cellular damage by free radicals.[3] These variants have been proposed to arise by homologous recombination of the left- and right-repeated sequences, resulting in a 54 kb and 16 kb deletion that includes the entire two genes.[3] Considering the limited studies with a consensus on the role of these polymorphisms in CAD, this study was conducted to investigate whether the polymorphisms of these two genes or their combinations have any effect on the disease susceptibility.

2. Methods

2.1. Subjects and study design

For this case–control hospital based study, 50 patients with clinically confirmed CAD were recruited from the Department of Cardiology for the case group. Those with a history of severe hepatic and renal diseases, acute or chronic inflammatory diseases, immunological diseases, neoplastic diseases, cancer, and a history of recent major surgical procedures were not included. The control group of 50 age- and sex-matched subjects who did not have any history of cardiovascular disease (CVD), hypertension, diabetes, or any other chronic diseases were recruited from the Department of General Medicine. This study was approved by the Institutional Ethics Committee (CSP/19/JAN/75/27). After informed consent from the participants, 2–3 mL of peripheral blood from each patient was drawn into EDTA anticoagulation tubes.

2.2. Analysis of selected gene polymorphisms

Genomic DNA was isolated from peripheral blood leukocytes using

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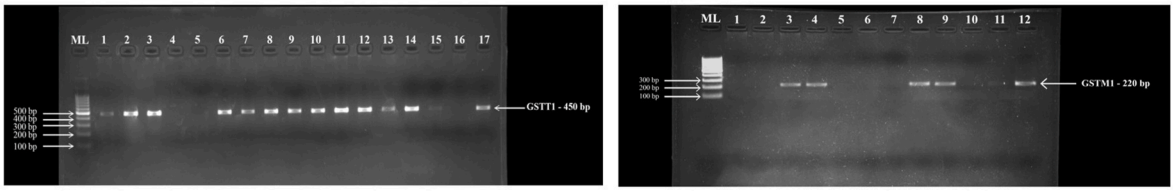

A B

Fig. 1. A: Representative image of PCR analysis of *GSTT1* gene polymorphism - ML:100bp DNA ladder; Controls: Lane 1–8; Cases: Lane 9–17; Genotype *GSTT1*+: Lanes 1,2,3,6–14,17; Genotype *GSTT1*-: Lanes 4,5,15,16. B: Representative image of PCR analysis of *GSTM1* gene polymorphism - ML:100bp DNA ladder; Controls: Lane 1–6; Cases: Lane 7–12; Genotype *GSTM1*+: Lanes 3,4,8,9,12; Genotype GSTM1-: Lanes 1,2,5–7,10,11.

the blood DNA isolation kit (Qiagen, Hilden, Germany). A standard PCR reaction was performed using the primer sequences 5′ACTCCCT GAAAAGCTAAAGC 3' (forward) and 5′GTTGGGCTCAAATATACGGTGG 3' (reverse) for *GSTM1* and 5' –TTCCTTACTGGTCCTCACATCTC 3' (forward) and 5′TCACCGGATCATGGCCAGCA 3' (reverse) for *GSTT1*.[4] The PCR products were visualized by 2% agarose electrophoresis and a 450bp or 220bp band indicated the presence of *GSTT1* or *GSTM1* genotype respectively (Fig. 1A and B).

2.3. Statistical analysis

The independent and combined genotype frequencies in the case and control groups were compared for significance using chi-square test and odds ratio (OR; at 95% confidence interval (CI)). A p-value less than 0.05 were considered as statistically significant.

3. Results

3.1. Characteristics of the study population

The demographics and the clinical characteristics of the study population analysed showed significant differences between the two groups for high-density lipoprotein (HDL), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), plasma glucose, creatinine, and blood urea nitrogen (Table 1).

3.2. Genotype distribution and allele frequencies

The strength of association between CAD risk and the null polymorphisms of *GSTT1* and *GSTM1* were evaluated (Table 2). While there was a significant association between *GSTT1* null polymorphism and CAD susceptibility, no significant association was observed between *GSTM1* null polymorphism and CAD susceptibility. A combined genotype analysis was performed by comparing the frequency of either none of the polymorphism present or at least one of the genotypes present with the frequency of both genotypes present. No statistically significant association was observed with each genotype combination and the risk of CAD.

4. Discussion

Indians are known to have the highest rates of CAD, with the conventional risk factors failing to explain this increased risk.[5] Though numerous studies have been conducted to find the interplay between genetics and environment in CAD, a conclusive result is still elusive. Possible candidate genes to study both these risks are the GSTs, as these are the main genes involved in detoxification mechanisms of the body. While some population studies have already investigated the associations between GST variants and CAD[6–13] the results are contradictory.

This study demonstrated that the null polymorphism of *GSTT1* had an inverse association with CAD. This was also reported in a study in North India.[9] On the other hand, no association was observed with the *GSTM1* null genotypes. A comparable study targeting *GSTM1* null polymorphism revealed that it had no significant association with CAD in a South Indian population, which supported the finding of this study.[10] However, two similar studies conducted on a North Indian population had an opposing result.[11,12] The authors demonstrated that *GSTM1* null polymorphism revealed a two-fold increased risk of developing CAD. Data from Chinese, Saudi Arabian, and Western Iranian

Table 1
Demographics and clinical parameters of the study cohort.

Parameter	Control (n = 50)	Case (n = 50)	p
Mean age (y)	55.7 ± 7.6	58.8 ± 10.2	0.08
Sex	Male: 35 (70%) Female: 15 (30%)	Male: 31 (62%) Female: 19 (38%)	–
Mean LDL (mg/dL)	118.5 ± 25	117.5 ± 40.8	0.8
Mean HDL (mg/dL)	50.6 ± 12.5	33.18 ± 7.7	<0.0001[a]
Mean BMI (kg/m^2)	20.5 ± 3	27.8 ± 5.6	<0.0001[a]
SBP (mm Hg)	120.1 ± 16.5	128.9 ± 19.3	0.01[a]
DBP (mm Hg)	75.6 ± 10.9	79.4 ± 10.8	0.01[a]
Mean plasma glucose (mg/dL)	85.7 ± 7.8	165 ± 61.7	<0.0001[a]
Mean creatinine (mg/dL)	0.8 ± 0.17	1.15 ± 0.41	<0.0001[a]
Mean blood urea nitrogen (mg/dL)	13.7 ± 6.3	17.9 ± 8.0	0.005[a]
Mean total count of WBCs (cells/μL)	129.3 ± 44.5	139.8 ± 44.7	0.2420

Data are reported as mean ± standard deviation or percentage.
[a] Statistically significant ($p < 0.05$), p-values were obtained by the unpaired Student's t test and chi-square analysis, wherever applicable.
n: number of individuals; LDL: low-density lipoprotein; HDL: high-density lipoprotein; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2
Risk analysis of *GSTM1* and *GSTT1* genotypes in the study cohort for CAD.

Genotypes	Controls (n = 50 (%))	Cases (n = 50 (%))	p	OR (95% CI)
GSTM1 genotypes				
GSTM1+ (Positive -wildtype)	33 (66)	33 (66)	reference	
GSTM1- (Null -variant)	17 (34)	17 (34)	1	1.0 (0.44–2.29)
***GSTT1* genotypes**				
GSTT1+ (Positive -wildtype)	21 (42)	32 (64)	reference	
GSTT1- (Null -variant)	29 (58)	18 (36)	0.03[a]	0.40 (0.18–0.91)
***GSTM1* and *GSTT1* – combined genotypes**				
Positive for both genes	23 (32)	32 (39)	reference	
Positive for at least one gene	33 (45)	33 (40)	0.37	0.72 (0.35–1.48)
Null for both genes	17 (23)	17 (21)	0.45	0.72 (0.30–1.69)

GSTM1: glutathione S-transferase mu-1; *GSTT1*: glutathione S-transferase theta-1; n: number of individuals; CAD: coronary artery disease; OR: odds ratio; CI: confidence interval; [a]Statistically significant ($p < 0.05$).

populations also suggested that *GSTM1* and *GSTT1* null polymorphisms were risk factors for CAD.[6–8] Analysis of the combined effect of GSTs genotypes on CAD showed that different combinations of genotypes did not affect CAD risk. The frequency of the double nulls in our control individuals was 23%, which was similar to the previous reports in an Asian control population (24.6%)[14] but contradictory between Iranian (11.8%),[7] Caucasian (10.4%)1[3] and Western Iranian (10.2%)[8] populations. Differences in ethnicity, nutrition, lifestyle and socioeconomics might explain these inconsistent results.

Undoubtedly, there are some limitations in this pilot study. Since all the participants were recruited from the same hospital and the sample size was relatively small, the possibility of selection bias cannot be ruled out. However, as ethnicity is a factor that affects the association of these polymorphisms to the pathophysiology of a disease, it can be concluded that the results of this study are relevant to the South Indian population.

5. Conclusion

The findings from this pilot study have demonstrated that the presence of the *GSTT1* null genotype has an inverse association with CAD. If corroborated by further studies, this polymorphism may find potential use as genetic marker for risk assessment of CAD.

What is already known?

Previous studies have determined that GSTs may contribute to the risk of developing CAD.

What this study adds?

As ethnicity is a factor that affects disease association, the findings from this pilot study have demonstrated that the presence of the GSTT1 null genotype has an inverse association with CAD among Indians.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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