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GSTP1 rs1695 polymorphism, oxidative stress markers, and antioxidants in coronary artery disease

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Abstract---Oxidative damage is among the essential factors in the progression of cardiovascular disease. Objectives: This study aims to evaluate the molecular role of GST genotypic polymorphism involved in the development of CAD. This study also aimed to compare the levels of oxidative stress and antioxidant markers in subjects with CAD with age and sex-matched controls. Result: There was no significant difference in allele frequency ($p= 0.85$) or genotype frequency ($p= 0.85$) between the examined case and control groups. Compared to healthy controls, F2-Isoprostanes and MDA levels were considerably elevated in individuals with coronary artery disease. CAD patients' GST, SOD, Vitamine E, and Vitamin C levels were considerably lower than in normal control subjects. Conclusion: This study observed that oxidative stress markers were significantly higher, whereas, in CAD patients, enzymatic and nonenzymatic-enzymatic antioxidants were significantly lower. This study could not find a good connection between GSTP1 gene polymorphism rs1695 and coronary artery disease.

Keywords---oxidative stress markers, antioxidants, coronary artery disease.

Introduction

The most prevalent cardiovascular condition is coronary artery disease (CAD). It has a complex etiology, including several lifestyle and environmental variables. Some cardiovascular disease risk factors, such as hypertension, hyperlipidemia, smoking, diabetes, obesity, lack of physical activity, poor nutrition, and stress, are controllable. In contrast, age, sex, and family history cannot be altered [Hajar 2017, Pencina et al. 2019]. Heart disease may also occur because of an imbalance between reactive oxygen species (ROS) production and the body's innate antioxidant defense mechanism, leading to oxidative stress and, ultimately, cardiovascular disease. Isoprostanes and malondialdehyde are examples of ROS products that may be measured to detect oxidative stress (MDA). Natural antioxidants like vitamin A and alpha-tocopherol (vitamin E) and antioxidant enzymes like SOD, catalase, and glutathione peroxidase assist in maintaining this equilibrium [Lobo et al. 2010].

More than one gene variant is implicated in the increased risk of developing coronary artery disease. These enzymes, called glutathione S-transferases (GST), play an essential function in reducing oxidative stress and protecting the cell. Reduced GST activity raises the risk of oxidative stress and inflammatory diseases, including coronary artery disease (CAD), as a result. Single nucleotide polymorphism (SNP) rs1695 is located at position 313 in exon 5 of the GSTP1 gene, a functional SNP [Reszka 2011]. The GST genes are susceptible to CAD because of genetic variation, which results in a loss of enzyme activity.

There has been much attention to uncovering novel genetic variations that may be utilized as oxidative stress indicators and assist predict the risk of oxidative stress-related illnesses. This study investigates the variations of oxidative stress and antioxidant markers in subjects with CAD compared to their age and sex-matched controls. This study also aimed to evaluate the molecular role of GST genotypic polymorphism involved in the development of CAD.

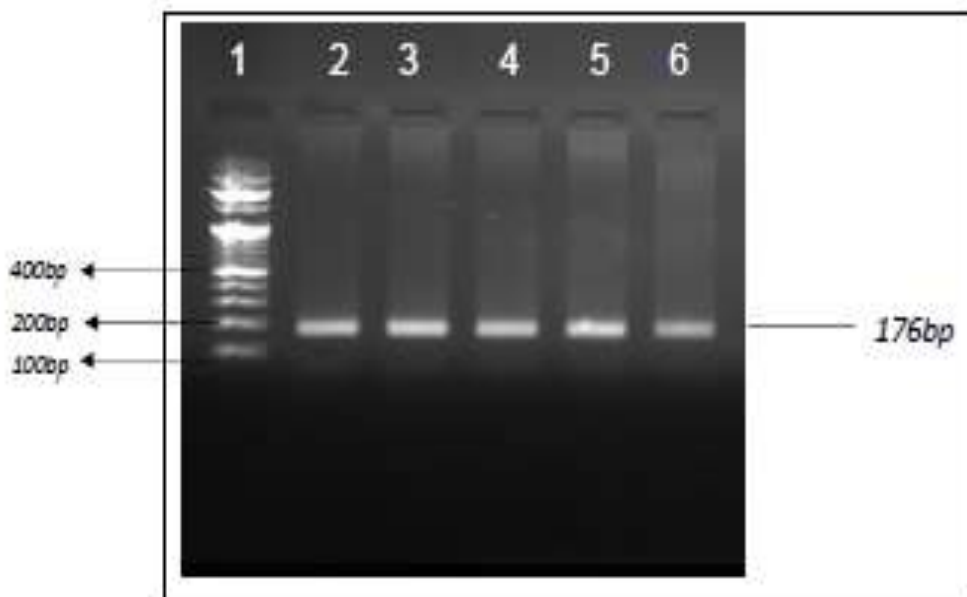
Materials and Methods

This study was conducted in Saveetha Medical College, Tandalam, Chennai. The Research and Ethics Committee of the Institute accepted the research procedure. A total of 62 patients with coronary artery disease were included in the research, all of whom were chosen from the Institute's patient database. The 62 healthy people in the control group were of similar age and gender. Proformas were used to capture all pertinent clinical and other data. Each participant had five milliliters of venous blood taken, and commercially available kits were used to investigate oxidative stress indicators and antioxidants. A genetic study of GSTP1 SNPs was carried out using the following protocols.

Genomic DNA Isolation: 2ml of peripheral blood samples were used to extract genomic DNA using the QIAamp DNA Blood Midi Kit (QIAGEN). Fluorometer was used to measure all materials, including the extracted DNA. The samples ranged in concentration from 23ng/ul to 66ng/ul, with the average being around the 23ng/ul mark. USING A POLYMERASE CHAIN REACTION, the SNP region was successfully amplified using the custom-designed primers in a thermal cycler

(Eppendorf). A 176bp product was amplified using the primers 5'ACCCAGGGCTCTATGGGAA3' and 3'TGAGGGCACAAGAAGCCCCT5". All 124 samples were amplified to a satisfactory level. There is a gel picture shown in Fig.1.

Figure 1. GSTP1 rs1695 polymorphism PCR amplification of 176bp fragment



Sequencing -After the PCR, the product was purified, Sequencing PCR was carried out using this purified product, and finally, a sequencing reaction was performed in a genetic analyzer. Mann-Whitney test was performed to compare the oxidative stress and antioxidants of the study subjects and the control subjects.

Results

This study selected 62 clinically proven CAD patients as test subjects, including 75.0% males and 25.0 % females. Sixty-two normal control subjects were also selected, consisting of 66.1% males and 43.9% females. All subjects were taken within the age group of 30 to 75 years. The age group of the study subjects ranged from 30 to 71, with a mean age of 51.9. The observed mean age of the control subjects was 47.43 (ages ranging from 35-75).

Oxidative Markers- MDA, F2- Isoprostanes, were estimated and compared between test and control groups and given in table 3. F2- Isoprostanes and MDA levels were significantly high in CAD patients compared to normal controls. Enzymatic antioxidants - GST and superoxide dismutase (SOD) and nonenzymatic-enzymatic antioxidants - Vitamin-C and Vitamin-E were estimated and compared between test and control subjects and given in table 1 and figure 2,3,4,5,6,7. GST, SOD, Vitamine E, and Vitamin C levels were significantly low in CAD patients compared to normal control subjects.

Table 1. Comparison of Oxidative Stress Markers and antioxidant markers of subjects

Test	Test	Control	W-Value	P
F2- Isoprostanes	173.4±86.8	28.9±19.4	2007.00	0.000
MDA	25.2±12.2	5.23±6.03	2134.50	0.000
GST	21.0±17.4	75.9±32.8	5491.50	0.000
SOD	57.1±38.2	485±620	5339.50	0.000
VIT E	7.85±5.11	64.4±21.3	5784.00	0.000
VIT C	11.0±7.32	95.9±40.9	5778.00	0.000

Values are mean ±SD

Figure 2. Comparison of F2iso of subjects

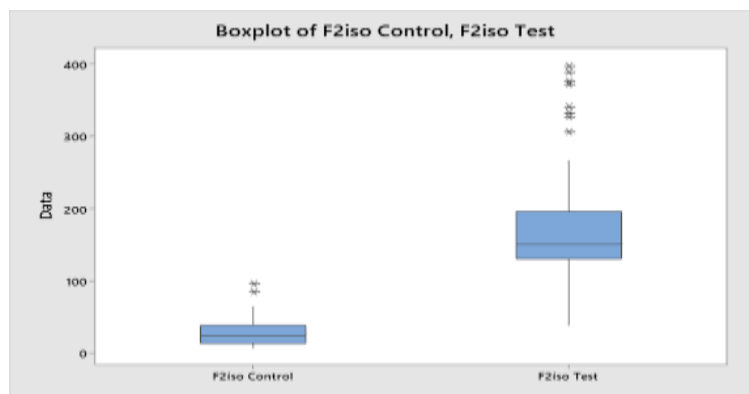


Figure 3. Comparison of MDA of subjects

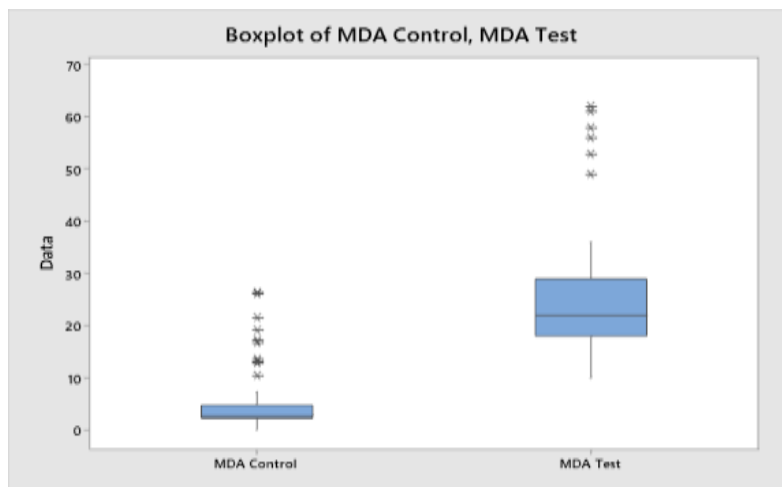


Figure 4. Comparison of SOD of subjects

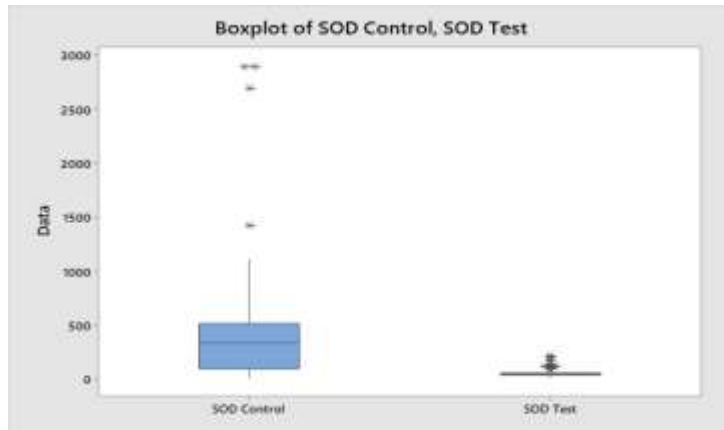


Figure 5. Comparison of GST of subjects

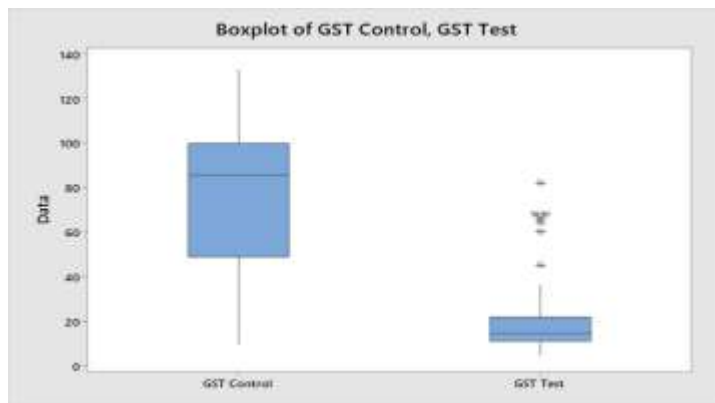


Figure 6. Comparison of VITC of subjects

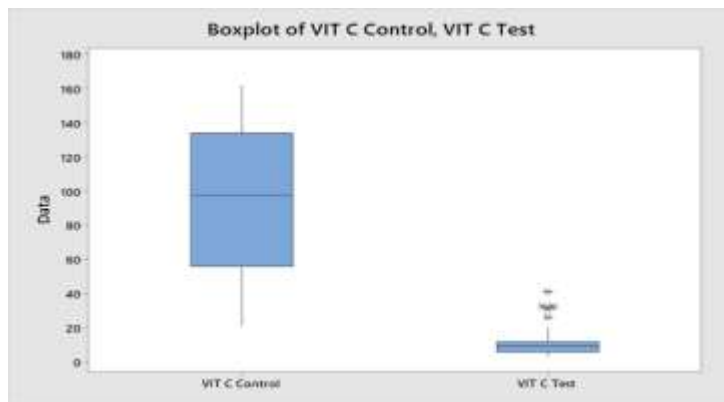
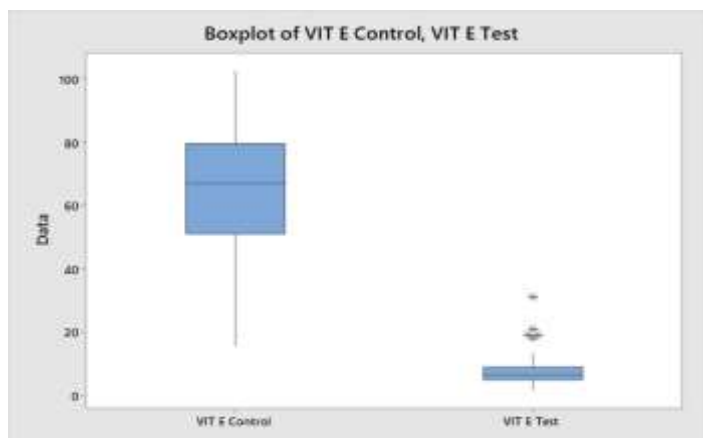


Figure 7. Comparison of VIT E of subjects



Genotype study of rs1695 polymorphism.

The rs1695 polymorphism was found in 62 patient samples, and 34 were found to be homozygous for the 'AA' genotype, 26 to be heterozygous for the 'AG,' and just two to be 'GG.' A total of 38 of the 62 control samples had the AA genotype, 22 had the AG genotype, and two had the GG genotype. Table 8 lists the genotype and allele frequencies for the individuals studied thus far. However, there was no significant difference between the patients and controls in allele frequency ($p=0.85$) or genotype frequency ($p=0.85$).

Table 2. Genotype and allele frequencies (in brackets) of rs1695 SNP polymorphism in case and control groups

rs1695	N	Genotypes			Alleles	
		AA	AG	GG	A	G
Control	62	38 (0.61)	22 (0.36)	2 (0.03)	98 (0.79)	26 (0.21)
Cases	62	34 (0.54)	26 (0.41)	2 (0.03)	94 (0.76)	30 (0.24)
Total	124	72 (0.58)	48 (0.38)	4 (0.03)	192 (0.77)	56 (0.22)

Discussion

The present study compared oxidative stress markers- MDA and F₂-isoprostanes, enzymatic antioxidants GST and SOD, nonenzymatic-enzymatic antioxidants Vitamin C and Vitamin E, and GSTP1 rs1695 polymorphism in CAD patients with normal healthy controls. Lipid peroxidation (LP) is indicated by Malondialdehyde (MDA). MDA measures may be used to track the course of atherosclerosis, which is linked to oxidative stress [Ayala et al. 2014]. According to previous research, CAD patients had higher levels of MDA than healthy individuals [Bhat et al. 2012]. It has emerged in recent years as one of the most sensitive and accurate indicators of lipid peroxidation in vivo, the measurement of F₂-isoprostanes (IsoPs). IsoPs measurements have been used in a wide range of clinical

investigations. F2-isoprostanes have been shown to have a clear correlation with cardiovascular disease (CVD). High levels of F2-isoprostanes in the urine or blood, according to Zhang [2013], maybe a non-specific sign of cardiovascular disease. Patients with coronary artery disease (CAD) were shown to have a considerably more significant amount of F2-isoprostanes than those who did not have the disease. In comparison to the usual control group, CAD patients had significantly higher levels of F2-isoprostanes in our research.

Bastani et al. [2018] reported decreased activities of antioxidant enzymes and a decreased concentration of antioxidant factors in patients with CAD. These present data are also concordant with Bastani et al. [2018]. We observed decreased activity of SOD and GST and decreased levels of Vitamin C and Vitamin E in CAD patients compared with normal controls. Mn-SOD gene expression was lower in CAD patients than in controls in research by Nini et al. [2019]. It was found to be controlled by blood nonenzymatic-enzymatic antioxidant status. Vitamin C deficiency may be a risk factor for people with coronary artery disease, according to Torkzaba et al. [2020]. Several studies have linked decreased enzyme activity to atherosclerosis [Banerjee and Vats, 2014]. According to Pourkeramati et al. [2020], there is a correlation between CAD risk variables and GST changes. [2] There was no significant difference in the frequency of alleles and genotypes between the two groups tested in this research ($p=0.85$).

Conclusion

The present study observed that oxidative stress markers were significantly higher, whereas enzymatic and nonenzymatic-enzymatic antioxidants were significantly lower in CAD patients. MDA and F2-isoprostanes were considerably higher in CAD patients than in healthy controls; however, SOD levels were significantly lower, GST levels were lower, and the vitamins E and C levels were significantly lower. This research found no significant differences in allele frequencies or genotype frequencies between the study's patients and controls. The GSTP1 gene polymorphism rs1695 was not associated with coronary artery disease.

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