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Molecular analysis of the *GSTT1* gene polymorphism in patients with clinical manifestation of atherosclerosis

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ABSTRACT. Atherosclerosis is a chronic inflammatory disease formed by the accumulation of lipids in the innermost layer and large-caliber artery (tunica intima). This accumulation, along with platelet factors, stimulates the proliferation of muscle cells in this region. Over than 400 genes may be related to the pathology since they regulate endothelial function, coagulation, inflammation, metabolism of amino acids, lipids, and carbohydrates. Glutathione S-transferases (GST) are enzymes that catalyze the polymorphic detoxification of metabolites produced by oxidative stress within the cells, which is induced by reactive oxygen species. GSTs are one of the defense mechanisms against oxidative stress damage. Due to genetic, cultural, and environmental factors, the rate of atherosclerosis is higher; however, an early diagnosis is crucial

for the prevention and treatment of several complications related to the disease. The present study aimed to analyze the frequency of *GSTT1* genotypes regarding the presence or absence of the polymorphism in patients with clinical manifestation of atherosclerosis. We collected 200 samples of peripheral blood of patients with the previous diagnosis of atherosclerosis based on clinical examination and imaging, and 100 samples of peripheral blood to compose the control group of patients without clinical manifestation of atherosclerosis. The polymorphism was assessed by PCR and analyzed on the agarose gel stained with 2.0% ethidium bromide. The frequency of the *GSTT1* gene polymorphism was compared using the chi-square test ($P < 0.05$) and the G-test. In the case group, we detected 85.5% of patients with the *GSTT1* genotype present and 14.5% of patients with the null genotype. A significant difference was observed between groups (case *vs* control) for the presence of the *GSTT1* polymorphism. According to the analysis of the variable alcohol consumption, we found that in the case group the presence of the *GSTT1* gene was higher in individuals who reported not drinking alcohol. In this study, the presence of the *GSTT1* gene polymorphism in male patients with atherosclerosis was 1.5 times higher when compared to female patients. Regarding the variable time of smoking, we found that this genotype was more frequent in smokers for both case and control groups.

Key words: *GSTT1*; Polymorphism; Atherosclerosis; PCR

INTRODUCTION

Atherosclerosis is a leading cause of death worldwide, its presence even in severe forms was found in Egyptian mummies over than 3500 years ago, and thus should not be seen as only of a disease of modern times (Ruffter, 2005). Several factors, such as the eradication of infectious diseases and lifestyle changes, led to an increase in life expectancy and a better understanding of this sort of diseases by health professionals. Gottlieb et al. (2001) claimed that coronary artery disease (CAD) and cerebrovascular accident (CVA) are the main sequelae of atherosclerosis, although they were only recognized as health problems at the beginning of the century.

The word atherosclerosis comes from the Greek *atero*, which means gruel, and sclerosis, which means hardening (Gottlieb et al., 2005). Atherosclerosis is a slow and progressive disease as a consequence of a series of highly specific molecular and cellular responses (Hackam and Anand, 2003).

Usually, acute manifestations such as unstable angina (UA) and acute myocardial infarction (AMI) are triggered by a destabilization of atherosclerotic plaque, with a significant reduction of the vessel diameter due to the accumulation of thrombus and consequent development of the disease (Gottlieb et al., 2005).

According to the World Health Organization (WHO), there were 16 million deaths in 2002; 7.2 million of them were related to cardiovascular disease. It is estimated an increase between 35 and 40 million deaths in 2020 (Guimarães et al., 2006). Cardiovascular diseases are

responsible for about 15 million deaths each year worldwide, despite the advanced preventive and therapeutic strategies, representing considerable higher costs in health care (Libby, 2010).

An acute coronary event is the first clinical manifestation of atherosclerotic disease in at least half of the individuals. The identification of asymptomatic patients is essential to achieve effective prevention and individual therapeutic goals.

In Brazil, cardiovascular diseases accounted for 65.0% of deaths in the adult working-age population, from 30 to 69 years old, and they were also responsible for 40.0% of early retirements between 2000 and 2009 (Nogueira et al., 2010).

A set of classic and emerging risk factors have been correlated to the pathogenesis of vascular diseases (Gottlieb et al., 2005). The more of these factors a patientshows the higher the probability of the individual to develop any cardiovascular event. On the other hand, a more strict control of the patient lifestyle to reduce the number of risk factors associated with CAD, less chance the patient have of developing cardiovascular diseases (Lanas et al., 2007).

The Brazilian Society of Cardiology points out that several genes have been linked to cardiovascular diseases in recent years. The use of genotyping for risk evaluation is not recommended; however, studies suggest that in the near future it will possibly be used to identify high-risk individuals. Genetic diseases can be investigated by the use of molecular markers once the procedure is performed non-invasively (Lękawa-Ilczuk et al., 2011).

Over 400 genes may be related to atherosclerosis, and they also take part in the regulation of endothelial function, lipids, carbohydrates, inflammation, metabolism of amino acids, and coagulation. Some of the genes involved are CYP1A, GST (glutathione S-transferase), Apo-E, and ENSO (Marinković et al., 2013).

The GST comprises a multifactorial family of enzymes that catalyze the nucleophilic attack of the reduced form of glutathione (GSH) to compounds that have an electrophilic atom of hydrogen, carbon, or sulfur. Mammals show three different families of GSTs: mitochondrial, microsomal, and cytosolic (Bolt and Thier, 2006).

There are seven specific classes of GSTs; they are distinguished by their amino acid sequences: Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta, the latter is a membrane bound protein. Among the cytosolic proteins, *GSTM1*, *GSTT1*, and *GSTP1* show the highest correlation with susceptibility to cancer (Tsai et al., 2005a,b).

The Theta class of the GST subfamily consists of two genes, *GSTT1* and *GSTT2*, is located on chromosome 22q11.2 and separated by approximately 50 kb. Both genes have five intron-exon boundaries, but there is only 55.0% identity to the amino acid sequence.

The GSTT1 often shows a homozygous deletion polymorphism, which is called the null genotype (Young et al., 2010). GSTs are involved in xenobiotic metabolism and detoxification including environmental carcinogens, reactive oxygen species, and therapeutic agents. The *GSTT1* enzyme metabolizes small reactive hydrocarbons such as ethylene oxide (Nørskov et al., 2011). These hydrocarbons are the cause of mutations in DNA, resulting in cellular transformation and proliferative clones; such compounds are present in cigarette smoke and they are involved in carcinogenesis and atherogenesis (Marinković et al., 2013).

Oxidative stress plays an important role in atherosclerosis since it promotes the excessive release of free radicals leading to a state of endothelial dysfunction. LDL oxidation increases the phosphorylation of the tyrosine kinase; it also affects the production of nuclear factor kappa B, transcription activating factors and protein-1. All these processes and molecules are involved in the atherogenic processes (Zivković et al., 2014).

The interaction of genetic, environmental components, and the inflammatory response

based on scientific evidence has shown that mechanisms involved in the onset of atherosclerotic disease are extremely complex (Hackam and Anand, 2003).

Due to genetic, cultural, and environmental factors, the rate of atherosclerosis is higher; however, an early diagnosis is crucial for the prevention and treatment of several complications related to the disease. The feasibility of carrying out therapies for prevention of cardiovascular diseases and their adverse outcomes highlights the importance of identifying individuals at low, medium, and high risk of developing the disease within a given population and also the implementation of effective medical intervention before the clinical manifestation of the disease since many individuals develop cardiovascular problems even in the absence of risk factors (Gottlieb et al., 2005). Therefore, it is necessary to understand the basic biology of atherosclerosis to provide better clinical support that could improve the practice of preventive medicine and provide benefits to public health. The present study aimed to analyze the frequency of genotypes of the *GSTT1* gene from patients showing clinical manifestations of atherosclerosis in the city of Goiânia.

MATERIAL AND METHODS

The present study was a case-control research. Blood samples were collected from October 2014 to February 2015 and patients were referred to the Cardiology and Vascular Surgery Center at the AngioGyn Clinic in Goiânia. We collected 200 peripheral blood samples (15 mL) of patients with a previous diagnosis of atherosclerosis based on clinical and imaging examination: eco-color Doppler, angiography and/or digital angiography, computed tomography angiography (CTA), and/or cineangiography; to compose the control group we collected 100 blood samples (also 15 mL) from patients without clinical manifestations of atherosclerosis and/or negative results for non-invasive imaging tests - carotid eco-color Doppler without evidence of atheromatous plaque or myointimal thickening. The blood sample was processed and stored at -20°C for later use.

The research was approved by the National Commission on Ethics in Research and the National System of Information on Ethics in Research Involving Human Beings (protocol No. 35321614.3.0000.0037). All patients answered a questionnaire regarding name, smoking and drinking habit, ethnicity, use of medicine, previous surgery, and they all signed the Informed Consent Form.

Regarding the smoking habit, both case and control groups were arranged in three different sub-groups: current smokers, patients who never smoked, and ex-smokers. Based on scientific consensus, smokers are defined as every person who makes regular use of at least one type of tobacco products. Former smokers or ex-smokers are defined as the individual who in the past made use of at least one of the tobacco products and currently does not smoke. Patients within the ex-smoker group had stopped smoking for at least 15 years, a definition according to the Brazilian Medical Association (AMB, 2013).

The peripheral blood samples collected were subjected to molecular analysis to verify the presence of the *GSTT1* gene polymorphism. Molecular genetic analysis was performed at the Replicon-PUC-Goiás. DNA extraction from samples of peripheral blood was conducted according to the instructions of Kaswi® (Genomic DNA Purification Kit). Then, the samples were subjected to quantification using NanoVue™ Plus Spectrophotometer according to the manufacturer's instructions; relevant samples had DNA concentration greater than 5 ng/µL. DNA purity was measured by absorbance at 260 nm with a ratio of 1.8. DNA samples were

kept at a temperature of -20°C until the amplification by polymerase chain reaction (PCR) method was performed. PCR was performed to detect polymorphisms of the *GSTT1* gene in a laminar flow cabinet to avoid contamination. The final volume was 25 μL, according to the protocol proposed by Frare (2011). As a positive control for PCR, we used blood from an individual with confirmed presence of the *GSTT1* polymorphism.

To analyze the polymorphism of *GSTT1*, we consider the absence of amplification as the null genotype and the presence of amplification confirms that the individual has at least one of the alleles. The analysis was always performed in duplicate, and we considered null genotype after three repetitions of the analysis with the same result.

The PCR product was subjected to agarose gel electrophoresis in 1.5% Tris-borate-EDTA solution (TBE) at a concentration of 1X through an electric field of 10 V/cm. The gel was stained with ethidium bromide (5 mg/mL) and then displayed on the video gel using the Gel Doc™ XR documentation system. Table 1 shows the sequences of primers used for the amplification of target region of the gene.

Table 1. Molecular sequence of the *GSTT1* primer.

Primer	Sequence	Size
GSTT1	F: 5'-TTCCTTACTGGTCCTCACATCTC-3'	480 bp
	R: 5'-TCACCGGATGGCCAGCA-3'	

Abdel-Rahman et al. (1996).

Statistical analysis

The data were obtained from the analysis of the *GSTT1* gene polymorphism regarding the study groups were tabulated in spreadsheets of the Excel® software (2010). The chi-square test and the G-test were applied to investigate possible associations between molecular analysis of polymorphism and atherosclerosis. For the statistical performance of the tests, we used the edition 5.3 of BioEstat® (Civil Society Mamirauá/MCT - CNPq).

RESULTS

The distribution of the *GSTT1* gene polymorphism was analyzed in the case group (patients with clinical manifestations of atherosclerosis) and in the control group (patients without clinical manifestations of the disease) as described in Table 2. The average age of patients in the case group (N = 200) was ±61 years, and the average age of patients in the control group (N = 100) was ±50 years.

Table 2. Frequency distribution of *GSTT1* null in case and control groups.

Groups	Present		Null		Total		P*
	N	%	N	%	N	%	
Case	171	85.5	29	14.5	200	100	<0.0001
Control	65	65.0	35	35.0	100	100	

*Chi-square test.

In the case group (N = 200), 85.5% (171/200) of patients with atherosclerosis showed the presence of the *GSTT1* gene and 14.5% (29/200) of patients presented the null genotype. In the control group (N = 100), 65.0% (65/100) of patients showed the presence of the *GSTT1*

gene and 35.0% (35/100) presented the null genotype. The frequency the *GSTT1* gene in patients with atherosclerosis was 1.3 times higher compared to the control group, and this result is statistically significant (P < 0.0001; Table 2).

The distribution of the polymorphism of the *GSTT1* gene in male and female subjects is described in Table 3. The frequency of the *GSTT1*-presentpolymorphism in male subjects was 89.0% (81/91) and the frequency of the null genotype was 11.0% (10/91) for the case group. In the control group, the *GSTT1* gene was present in 56.6% (30/53) of patients, and 43.4% (23/53) of them showed the null genotype. This result is statistically significant (P = 0.005; Table 3). The frequency of the *GSTT1*-present genotype in male patients with atherosclerosis was 1.5 times higher compared to female patients.

Table 3. Distribution of the *GSTT1* polymorphism regarding gender in case and control groups.

Gender	Present		Null		Total		P*
	N	%	N	%	N	%	
Male Case group	81	89.0	10	11.0	91	100.0	0.005
Control group	30	56.6	23	43.4	53	100.0	
Female Case group	90	82.6	19	17.4	109	100.0	
Control group	35	74.5	12	25.5	47	100.0	0.224

*Chi-square test.

In females, the presence of the *GSTT1* gene polymorphism was found in 82.6% (90/109) of the patients, and the null genotype was found in 17.4% (19/109) of them. In the control group, 74.5% (35/47) of patients showed the *GSTT1* genotype present and 25.5% (N = 12/47) showed the null genotype. This result is not statistically significant (P = 0.224) (Table 3).

Table 4 compares the habit of drinking alcohol in patients of the case group and the control group and its relationship with the *GSTT1* gene polymorphism. The analysis of 19 (9.5%) patients in the case group who consume alcohol beverage showed 100.0% (19/19) of the *GSTT1*-present genotype, and none of the patients in this group (0/0) showed the *GSTT1*-null genotype. From 181 (95.5%) patients who did not consume any alcohol beverage, 84.0% (152/181) showed the *GSTT1*-present genotype, and in 29 (29/181) patients (16.0% of the total), we found the *GSTT1*-null genotype, which showed a statistically significant difference (P = 0.0122).

Table 4. Comparison of the *GSTT1* polymorphism frequency regarding alcohol consumption in the case and control groups.

Variables	Alcohol consumption					
	Yes		No		P*	OR (min-max)
	N	%	N	%		
Case group						
Present	19	100.0	152	84.0	0.0122*	
Null	0	0.0	29	16.0		
Total	19	100.0	181	100.0		
Control group						1.25 (0.6707-2.3536)
Present	14	70.0	52	65.0	0.5904	
Null	6	30.0	28	35.0		
Total	20	100.0	80	100.0		

*G-test.

In the analysis of 20 patients (20.0%) from the control group who reported drinking alcohol, we detected 70.0% (14/20) of patients showing the *GSTT1*-present genotype, and 30.0% (6/20) of patients had the *GSTT1*-null genotype. From 80.0% (80/100) of control patients who claimed not to consume alcohol, 65.0% (52/80) had the *GSTT1*-present genotype and in 28 (28/80) patients, 35.0% had the *GSTT1*-null genotype. This result was not statistically significant (P = 0.5904; OR = 1.2564).

In this study, we found that the *GSTT1*-present genotype was higher in patients who declared to consume alcohol beverage regardless the group showing the clinical manifestation of the disease or the control group (Table 4).

Table 5 shows the comparison of the *GSTT1* gene polymorphism among smokers and ex-smokers (>15 years) in atherosclerosis and control groups regarding the period of tobacco consumption. The 53 patients in the atherosclerosis group (if) the GSTT1 gene is present in individuals who use tobacco.

Table 5. Comparison of the *GSTT1* polymorphism frequency regarding the period of tobacco consumption in case and control groups.

Variables	Tobacco consumption							
	GSTT1-present				*GSTT1*-null			
	Smokers		Ex-smokers		Smokers		Ex-smokers	
	N	%	N	%	N	%	N	%
Case group								
<10 years	2	4.0	6	17.0	0	0.0	0	0.0
10 to 20 years	3	6.0	13	37.0	3	20.0	3	43.0
>20 years	48	90.0	16	46.0	12	80.0	4	57.0
Total	53	100.0	35	100.0	15	100.0	7	100.0
P*	<0.001*				0.5468*			
Control group								
<10 years	2	15.0	5	50.0	1	11.0	0	0.0
10 to 20 years	2	15.0	5	50.0	2	22.0	1	100.0
>20 years	9	70.0	0	0.0	6	67.0	0	0.0
Total	13	100.0	10	100.0	9	100.0	01	100.0
P*	0.006*				0.2615*			

*G-test.

In the case group, the presence of the *GSTT1* gene was identified in 53 patients who use tobacco products and in 35 patients considered ex-smokers (≥15 years). From these, only 4.0% (2/53) reported smoking in a period less than 10 years, 6.0% (6/53) between 10 and 20 years, and 90.0% (48/53) for more than 20 years; 17.0% (6/35) smoked for a period less than 10 years, 37.0% (13/35) between 10 and 20 years, and 46.0% (16/35) for more than 20 years, which showed a statistically significant difference (P = 0.001). In subjects who consume tobacco and ex-smokers in the study group, there was no statistically significant difference (P = 0.5468) regarding the null genotype.

In the control group, the presence of the *GSTT1* gene was identified in 13 patients who reported using tobacco products and in 10 patients considered ex-smokers (≥15 years). From these, 15.0% (2/13) reported smoking in a period less than 10 years, 15.0% (2/13) between 10 and 20 years, and 70.0% (9/13) for more than 20 years; 50.0% (5/10) reported smoking in a period less than 10 years, 50.0% (5/10) between 10 and 20 years, and no patient claimed the use of tobacco (0/0) for more than 20 years; there was a statistically significant difference (P = 0.006). In subjects who consume tobacco and ex-smokers in the case group, there was no statistically significant difference (P = 0.2615) regarding the null genotype.

DISCUSSION

The knowledge of genetic polymorphisms that predispose or exacerbate CAD is an important tool for primary prevention, diagnostic, treatment, and genetic counseling in cardiology (Sabino, 2004). Molecular biology is of great importance in understanding complex and multifactorial diseases such as CAD. This approach launches a new form of assessment of CAD and stimulates the development of new techniques, diagnostic methods, and therapeutic approaches, interfering in a late clinical outcome of the patient (Hayek et al., 2004).

Studies suggest that these techniques are likely to be used for the identification of individuals at high risk of developing atherosclerosis (Xavier et al., 2013). Marinković et al. (2013) highlight the importance of genetic polymorphisms involved in the biotransformation and atherosclerosis to better study the expression of the gene and then elucidate their interaction to the environment. Several studies have been related to the polymorphism of *GSTT1* in patients with atherosclerosis (Hayek et al., 2006; Manfredi et al., 2007; Maciel et al., 2009; Bazo et al., 2011; Taspinar et al., 2012).

In the present study, the *GSTT1* polymorphism was significant in both control and case groups, and the polymorphism frequency in patients with atherosclerosis was 1.3 times higher than in controls. A study conducted in São Paulo by Bazo et al., (2011) also detected a greater frequency of the *GSTT1* genotype in patients undergoing angiography diagnosis of CAD. In the Indian population, a greater presence of the *GSTT1* genotype in patients with CAD confirmed by angiography was also detected (Girisha et al., 2004). Zivković et al. (2014) also reported the presence of the *GSTT1* polymorphism in 346 patients diagnosed with advanced atherosclerosis in Serbia.

Taspinar et al. (2012) reported the prevalence of *GSTT1*-null genotype in patients with CAD 8.9 times higher when compared to controls. Another study conducted in Brazil showed that the prevalence of *GSTT1*-null genotype was higher in patients from the city of Vitória subjected to coronary angiography (Maciel et al., 2009). Manfredi et al. (2007) also identified the prevalence of the null *GSTT1* genotype associated with a three times greater risk for the development of coronary artery disease in patients with type II diabetes and smokers from Italy showing a large vessel obstruction.

In our study, we observed that the presence of the *GSTT1* gene polymorphism was statistically significant for male patients in both case and control groups. We found that the frequency of presence of the *GSTT1* gene in male patients with atherosclerosis was 1.5 times higher when compared to female patients. A study with a group of 871 Brazilian patients (Maciel et al., 2009) showed that the presence of the *GSTT1* gene is greater in the male population, which corroborates our results. Likewise, Schreiber et al. (2013) also found that 76.0% of patients had a higher frequency of the *GSTT1*-present genotype in males within the Brazilian population they studied. A study conducted with Chinese patients (Wang et al., 2012) found no significant differences between male and female in both control and case groups regarding the risk of stroke. The difference in results can be explained by the population used in each study, and it is noteworthy to say that the Brazilian population is marked by a high degree of miscegenation (Suarez-Kurtz et al., 2014).

Analysis of the variable alcohol consumption showed that the atherosclerosis group with the *GSTT1*-present genotype was higher in individuals who do not drink alcohol beverage (P = 0.0122). A study conducted with the population of Hakka in southern China by Pan et al. (2011) showed an association of family history of various chronic diseases,

among them hypertension, with the presence of the *GSTT1* gene, which was also higher in patients who reported not consuming alcoholic beverages. However, Girisha et al. (2004) found no significant differences (P = 0.0826) associated with the consumption of alcohol and the polymorphism of the *GSTT1* gene in patients from India with CAD. Pinheiro et al. (2012), studying patients from Brazil, found no significant differences regarding possible biochemical and clinical changes and alcohol consumption among individuals with type 2 diabetes mellitus associated with the presence of the gene or the *GSTT1*-null genotype.

Some studies have linked tobacco use and DNA damage due to the relationship of polymorphisms of enzymes involved in metabolism genotoxins with the development of CAD (Tamer el al., 2004). Manfredi et al. (2007) point out that individual variability in the onset and development of CAD in smoker patients may be the result of genetic polymorphism of enzymes involved in the metabolism of xenobiotics. Furthermore, several genetic variations may interact with cellular damage caused by smoking increasing peroxidation of lipids leading to an even higher atherogenic effect (Hayek et al., 2006).

Olshan et al. (2003) evaluated the effects of smoking and the *GSTT1* gene polymorphism on the development of atherosclerosis risk according to the Atherosclerosis Risk in Communities Study (ARCS). Patients were randomly selected from four different US communities between the years of 1987 and 1989 based on the thickness of the intima layer of the artery. The authors concluded that the presence of the *GSTT1* genotype might be related to a possible interaction with the development of disease in individuals who smoked in a period equal to or greater than 20 years based on the increase of the intima thickness. Grignoli et al. (2009) studied the population of Araras in Brazil and found that 61.3% of patients who had smoked for more than 10 years showed a higher frequency of the *GSTT1*-present genotype. More than 60 compounds found in tobacco are classified as carcinogenic according to the International Agency for Research on Cancer (IARC). According to the Brazilian Medical Association (AMB, 2013), the risk of cancer increases with the duration of smoking and the number of cigarettes or other tobacco products used. Besides, tobacco being a risk factor for cancer is also an important factor that hinders the treatment and control of general neoplasias (AMB, 2013).

Some studies have linked several genetic polymorphisms involved in the mechanisms of biotransformation and carcinogenesis to polycyclic hydrocarbons; however, the data are still scarce (Marinković et al., 2013). Two groups of enzymes are involved in the metabolization process of chemical compounds present in cigarette and alcohol beverages: the enzymes of oxidative metabolism (Phase I) and the conjugation reaction enzymes (Phase II). Oxidative enzymes of Phase I, particularly the enzymes of the superfamily of cytochrome P450 (CYP), convert several compounds to highly reactive metabolites. The Phase II enzymes act to inactivate the products of Phase I, turning them into hydrophilic compounds that are capable of being excreted due to their combination with endogenous substrate (glutathione, sulfate, glucose, acetate) by the action of GSTs, UDP-glucoronyltransferases, and N-acetyltransferases (Taioli, 2008).

Marinković et al. (2013) pointed out that data on the association of atherosclerosis and genetic polymorphisms are still controversial due to several aspects such as the study design and the number of individuals investigated. Also, Hayes et al. (2005) point out that the *GSTT1* polymorphism can increase or decrease the sensitivity of an organism causing an inflammatory or even carcinogenic process involved in the development of atherosclerosis.

In the present study, we found a significant difference regarding the *GSTT1*

polymorphism. The frequency of the *GSTT1* gene in patients with atherosclerosis was 1.3 times higher compared to the control group. The presence of the *GSTT1* polymorphism was 1.5 times higher in male patients with atherosclerosis when compared to female patients.

According to the variable alcohol consumption, the presence of the *GSTT1* genotype was higher in individuals who reported not drinking alcohol within the case group. Regarding the time of smoking, the presence of the *GSTT1* genotype was statistically significant in smokers from both control and case groups.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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