Polymorphisms of GSTM1 and GSTT1 genes in symptomatic atherosclerotic patients with hypertension and/or type 2 diabetes mellitus

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ABSTRACT

Atherosclerosis is a chronic inflammatory condition and originates due to the accumulation of lipids in the innermost layer of the arteries. It is often related due to other underlying diseases, such as systemic arterial hypertension and type 2 diabetes mellitus, which may be the cause or may contribute to a worse prognosis of atherosclerosis. Such diseases also have a genetic imprint on their genesis, such as the GSTM1 and GSTT1 gene polymorphisms. The present study aimed to perform the molecular analysis of GSTM1 and GSTT1 gene polymorphisms in atherosclerotic hypertensive patients, atherosclerotic patients with diabetes, atherosclerotic patients with hypertension and diabetes, and the control group. We analyzed 100 samples of hypertensive atherosclerotic individuals, 54 atherosclerotic individuals with diabetes and 23 atherosclerotic individuals with hypertension and diabetes. The samples were subjected to DNA extraction, then PCR and analyzed on 1.5% agarose gel stained with ethidium bromide. The results were compared through the Chi-Square Test. We found a significant frequency of the GSTM1 present genotype in the hypertensive atherosclerotic group

(p=0.025), the present form of the GSTT1 gene was significant in all of the three groups studied (p=0.006 in the hypertensive atherosclerotic group, p = 0.003 in the diabetic atherosclerotic group and p=0.039 in the hypertensive and diabetic atherosclerotic group). The results show a relationship between the present genotype of GSTM1 and hypertension, and that the present form of GSTT1 is related to the development of atherosclerosis, regardless of the related cofactor.

KEY WORDS: Atherosclerosis; Hypertension; Diabetes; Polymorphism; GSTM1; GSTT1

INTRODUCTION

Atherosclerosis is a chronic inflammatory condition and originates due to the accumulation of lipids in the innermost layer (tunica intima) of the arteries, whether they are small, medium or large. The accumulation of lipids together with platelet factors contribute to the proliferation of the muscle cells of this region. A plaque made up of muscle cells, leukocytes and lipids originated at this site, can lead to the arterial lumen narrowing and subsequently progress to a fibrosis and calcification of the plaque. The growth of this plaque may cause an obstruction of the artery and thereby lead to local ischemia (Zattar et al., 2013; Polonsky et al., 2017).

The atherosclerotic disease begins in childhood with progression in adolescence and adulthood. The atherosclerotic lesions are influenced by several risk factors that are divided into modifiable and non-modifiable. Modifiable or reversible factors are dyslipidemia, hypertension, eating habits, smoking, diabetes mellitus, obesity and sedentary lifestyle. The non-modifiable or irreversible are age, sex, and heredity (Tolfrey, 2002; McMaham et al., 2006).

Atherosclerosis is related to other basic diseases, such as systemic arterial hypertension (SAH) and diabetes mellitus type 2 (DM2). These can be the cause or may contribute to a worse prognosis of atherosclerosis. Such pathologies also have a genetic imprint on their genesis, and like atherosclerosis, they can be caused by mutations in several genes. Genetic alterations associated with environmental factors may result in genetic polymorphisms, these polymorphisms may contribute to the development of atherosclerosis, hypertension and diabetes, concomitantly (Xavier et al., 2013).

SAH is a chronic multifactorial disease in which the maintenance of blood pressure levels is greater than 140 mmHg for systolic and 90 mmHg for diastolic pressure (Cesarino et al., 2008). The disease is related to intrinsic factors such heredity, sex, age and race and the most relevant extrinsic factors are smoking, sedentary lifestyle, obesity, stress, dyslipidemia and diet. It causes a series of alterations in several organs like heart, brain, kidneys and blood vessels, and also causes modifications in the metabolism, being one of the main causes of morbimortality in the present days (Boing et al, 2007; Gontijo et al., 2012).

DM2 is characterized by poor insulin production or resistance of cells to this hormone, resulting in high levels of glucose in the bloodstream, which can compromise the whole organism. DM2 is responsible for a large number of deaths worldwide. As with atherosclerotic disease, factors such as age, sex, ethnicity, lifestyle and heredity as well as environmental interactions play an important role in the pathogenesis of DM2 (Johnson et al., 2017). According to the International Diabetes Foundation (IDF), DM2 affects more than 400 million people worldwide nowadays and it is estimated that in the year of 2040 the number of people living with DM2 will be around 640 million (Marín-Peñalver et al., 2016).

A factor that is directly associated with the development of atherosclerotic disease, especially in patients with modifiable risk factors, such as SAH and DM2, is the accumulation of reactive oxygen species. Failures in antioxidant systems lead to oxidative stress, so the accumulation of toxic substances in the artery wall tends to increase, contributing to the onset of atherosclerosis. The accumulation of ROS is avoided through the action of enzymes, such as those from the Glutathione-S-Transferases family (GSTs), which act in the processes of elimination of xenobiotics and cellular detoxification. GSTs confer the ROS greater hydrophilic capacity, turning xenobiotics more easily to metabolized and eliminated from the organism (Zhang et al., 2012; Costa et al., 2016).

Genetic alterations, such as variations in the base sequences encoding GSTs, are responsible for the lack of protein coding or the encoding of deficient enzymes. This genetic factor seems to be strongly related to a greater susceptibility to atherosclerosis, SAH and DM2 (Etemad et al., 2016). Mammal GSTs are grouped into three families: cytosolic, mitochondrial and microsomal. These intracellular enzymes act in phase II of xenobiotic metabolism, catalysing the nucleophilic attack of the reduced form of glutathione to substances that

have in their composition a carbon, a hydrogen or an electrophilic sulfur atom. Thus, they inhibit the action of endogenous and exogenous toxins on the cells, preventing these toxins from damaging cellular DNA (Song et al., 2012).

Factors such as genetic polymorphisms may be related to the development of atherosclerosis, hypertension and diabetes. Genetic polymorphism is the simultaneous occurrence of different genotypes, which are resulted from the combination of alleles of the same locus, and may or may not result in different phenotypes. However, to consider a gene locus as polymorphic, it is necessary that the most common allele does not present a population frequency greater than 99%, and the other polymorphous allele has a frequency equal to or greater than 1% (Utiyama et al., 2004).

The literature points out that more than 400 genes may be related to atherosclerosis, since they are involved in the regulation of endothelial function, coagulation, inflammation, amino acid metabolism, lipids and carbohydrates. Among the candidate genes we can highlight the GSTM1 and GSTT1 gene polymorphisms belonging to the GST Family (Marinkovic et al., 2013).

The GSTM1 gene is located on chromosome 1q13.3 and encodes a protein of the same name, belonging to the GST family. This gene, responsible for the coding of the GSTM1 isoenzyme is polymorphic and may present a null allele, and thus encode a protein with reduced metabolic capacity or does not encode the protein (Sharma et al., 2000)

From 20 to 50% of individuals express the mutated form of the GSTM1 gene, such mutation is known as homozygous gene deletion. This deletion occurs when one or more nucleotides are deleted from the DNA sequence. Epidemiological studies indicate that individuals with the homozygous GSTM1 deletion are at high risk of developing various types of diseases, such as breast cancer, skin cancer, arterial hypertension, among others (Morais et al., 2008).

O gene GSTT1 foi mapeado no cromossomo 22q11.13, possui dois alelos: um alelo presente (GSTT1*1) e a sua forma nula (GSTT1*0). Several studies indicate that the GSTT1*0 allele is responsible for a total or partial suppression of GSTT1. This enzyme activity is closely linked to the cellular detoxification process (Sharma et al., 2000). It is estimated that about 20 to 60% of individuals present GSTT1 deletion (Morais et al., 2008).

The presente study aimed to perform the molecular analysis of GSTM1 and GSTT1 gene polymorphisms in atherosclerotic hypertensive patients, atherosclerotic patients with diabetes, atherosclerotic patients with hypertension and diabetes, and the control group.

METHODOLOGY

Type of study and sample collection

The present study is a case-control study in which peripheral blood samples were collected from 100 hypertensive atherosclerotic patients (Group 01: G1), 54 atherosclerotic patients with type 2 Diabetes Mellitus (Group 02: G2) and 23 atherosclerotic individuals with hypertension and type 2 diabetes mellitus (Group 03: G3). Patients were at least 38 years old. Blood samples were collected at the Angiogyn Clinic in the city of Goiânia, which serves patients from both private health systems and the Unified Health System (SUS). For the control group, peripheral blood samples were collected from 100 patients over 38 years of age who were non-hypertensive, free from DM2 and who did not present a diagnosis of atherosclerotic disease based on clinical criteria and/or imaging tests. The study was approved by the Research Ethics Committee of the Pontifícia Universidade Católica de Goiás/PUC Goiás (Number 35321614.30000.0037) and all study participants signed the Free and Informed Consent Term.

DNA extraction

From the collected samples, the extractions of the DNA were carried out following the instructions of the purification Kasvi® Kit (Genomic DNA Purification Kit, Lot: 11600223). After extraction, samples were subjected to quantification on the NanoVue ™ Plus (GE, Cambridge, UK) spectrophotometer according to the manufacturer's instructions, with relevance only to samples whose quantification results in relation to DNA concentration were higher than 5ng/µl.

Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction was performed for amplification of the GSTM1 and GSTT1 genes. For GSTM1 amplification a pair of primers were used, the foward sequence F: 5 'ACCCCAGGGCTCTATGGGAA 3' and the reverse sequence R: 5 'TGAGGGCACAAGAAGCCCTT 3', with a molecular size of 216 base pairs (bp). For the GSTT1 analysis we used the forward primer F: 5

'TTCCTTACTGGTCCTCACATCTC 3' and the reverse primer R: 5 'TCACCGGATCATGGCCAGCA 3' with 481bp (Rohr et al., 2004). After PCR, the polymorphism analysis was performed through 1.5% agarose gel electrophoresis. The fractions were stained with ethidium bromide (5mg / mL) and visualized in the BIORAD Photodocumentator (Bio-Rad, Hercules, California, USA).

Statistical analysis

Statistical analysis was performed using the chi-square statistical test using the BioEstat software version 5.3, were considered significant results whose "p" was less than 0.05.

RESULTS

In the analysis of GSTM1 gene polymorphism in G1 (hypertensive atherosclerotic), 73% (73/100) presented the GSTM1 genotype, in the control group the present genotype was observed in 58% (58/100) of the samples. The result was statistically significant, p=0.025. For the GSTT1 gene polymorphism in G1, 82% (82/100) of the samples presented the GSTT1 genotype and in the control group the present form was found in 65% (65/100), the p value was p = 0.006, and the result was statistically significant (Table 1).

Table 1. Distribution of the frequency of *GSTM1* and *GSTT1* gene polymorphisms in atherosclerotic hypertensive group and control.

Genotype | Groups	G1 (N=100)		Control (N=100)		*pValue
	N	%	N	%	
GSTM1+	73	73	58	58	
GSTM1-	27	27	42	42	
Total	100	100	100	100	*p*=0.025
GSTT1+	82	82	65	65	
GSTT1-	18	18	35	35	
Total	100	100	100	100	*p*=0.006

*Chi-square test

In group 2 (atherosclerosis with diabetes mellitus type 2), in the GSTM1 polymorphism analysis, 63% (34/54) of the samples presented the GSTM1 genotype, the frequency of the present form in the control group was 58% (58/100), p=0.549. The result was not statistically significant.

In the analysis of the GSTT1 gene polymorphism in G2, the present form was observed in 87% (47/54) of the cases, whereas in the control group the present GSTT1 genotype was observed in 65% (65/100) of the individuals. The difference between these two groups was significant (p=0.003) (Table 02).

Table 2. Distribution of the frequency of GSTM1 and GSTT1 gene polymorphisms in atherosclerotic patients with type 2 diabetes mellitus and in the control group.

Genotype | Groups	G2 (N=54)		Control (N=100)		*pValue
	N	%	N	%	
GSTM1+	34	63	58	58	
GSTM1-	20	37	42	42	
Total	54	100	100	100	*p=0.549*
GSTT1+	47	87	65	65	
GSTT1-	7	13	35	35	
Total	54	100	100	100	p=0.003

*Chi-square test

Regarding the atherosclerotic subjects with hypertension and type 2 diabetes mellitus (G3), 23 samples were analyzed. The GSTM1 genotype was present in 56.5% (13/23) of the analyzed samples; for the control group the present form was detected in 58% (58/100) of the individuals, with no significant difference (p = 0.897).

As for the GSTT1, the frequency of the present genotype was 87% (20/23) in the analyzed DNA samples; in the control group the frequency for the present form was 65% (65/100), with p = 0.039. The result was statistically significant (table 3).

Table 3. Distribution of GSTM1 and GSTT1 gene polymorphism frequency in atherosclerotic patients with hypertension and type 2 diabetes mellitus and the control group.

Genotype | Groups	G3(N=23)		Control (N=100)		*pValue
	N	%	N	%	
GSTM1+	13	56.5	58	58	
GSTM1-	10	43.5	42	42	
Total	23	100	100	100	*p=0.897*
GSTT1+	20	87	65	65	
GSTT1-	3	13	35	35	
Total	23	100	100	100	*p=0.039*

*Chi-square test

DISCUSSION

Regarding the GSTM1 gene polymorphism in the hypertensive atherosclerotic group, the presence of the GSTM1 allele was 1.2 more frequent than the control group. This result indicates a possible relationship between the presence of the GSTM1 gene and hypertension in patients with atherosclerosis. For G2 and G3, the relation of GSTM1 and the variables analyzed was not significant.

Abbas et al. (2014), analyzed the relationship between GST polymorphisms and their connection with hypertension in North India. They observed a frequency of 65.94% of the GSTM1 genotype in the case group, indicating a possible relationship between GSTM1 and hypertension, a result consistent with the present study. Grignoli et al. (2010) evaluated smoking patients in the state of São Paulo and observed a higher frequency of the GSTM1 genotype in 68% of the analyzed DNA samples, being one of the main risk factors for hypertension.

However, other studies found a higher frequency of the null GSTM1 genotype when evaluating the GSTM1 polymorphisms in hypertensive patients. According to Hussain et al. (2012), samples from hypertensive patients in the United Arab Emirates showed the frequency of the null GSTM1 genotype was 60%. Miranda-Vilela et al. (2010), analyzed Brazilian hypertensive patients and found that the GSTM1 null genotype was more frequent, in 68.7% of the samples.

In the analysis of the GSTT1 gene polymorphism, for the three groups studied, atherosclerotic with hypertension, with diabetes or with both comorbidities, the GSTT1 present genotype was more frequent, thus suggesting that the GSTT1 gene is related to atherosclerosis, regardless of the comorbidities analyzed.

Marinho et al. (2007) found that the GSTT1 genotype was present in 86% of the analyzed samples, and concluded that null GSTT1 genotype has a protective effect against hypertension and that carriers of the GSTT1 genotype are more likely to develop hypertension and other diseases such as atherosclerosis.

Taspinar et al. (2012) analyzed the GSTT1 polymorphism and the relationship with risk factors for coronary artery disease in a population of Turks, also found a higher frequency of the present GSTT1 genotype, reported in 77.0% of the analyses. A higher frequency of the GSTT1 genotype was also found in the work of Girisha et al. (2004), with atherosclerotic patients from North India, with a frequency 92.34%.

However, other studies have discordant results and show a higher frequency of null genotypes, Wang et al. (2012), analyzing samples from the Chinese population, found that the null genotype of GSTM1 and GSTT1 were more frequent, 52.7% and 51.1%, respectively, regarding the risk for ischemic stroke.

In the study of atherosclerotic patients with the type 2 diabetes mellitus, the present genotypes of GSTM1 and GSTT1 were more frequent. However, in G2 and G3 these results were not significant for GSTM1 in relation

to the control group. GSTT1 gentype was statistically significant in G2 and G3, being associated with atherosclerosis. According to our results the GSTM1 and GSTT1 present genotypes are not associated with the DM2. According to Stoian et al. (2015), GSTM1 and GSTT1 are associated with the risk of DM2. The present genotype of the genes appeared in 52.4% and 82.1% of the cases, respectively. However, in relation to the control group, the result was not significant and corroborates the findings of the present study. Afrand et al. (2015) evaluated the GSTM1 and GSTT1 polymorphisms in Iranian patients with metabolic syndrome. They observed that the present GSTM1 genotype was found in 49.0% of the samples and the GSTT1 present genotype was 72.5%. Their results were not statistically significant when compared to the control group. l

The work of Raza et al. (2014), evauated the GSTM1 and GSTT1 gene polymorphisms in diabetics in Northern India. The researchers found the GSTM1 genotype present in 46.39%, a statistically significant result, which is in disagreement with our findings. The present GSTT1 was observed in 95.87%, but in relation to the control group the result was not significant. According to their results, there was a possible association between diabetes and the present gnotype of GSTT1, which is in agreement with the present study.

In a study conducted in South India by Rao et al. (2014), the researchers analyzed DNA samples from men and women with DM2 and also found the present genotypes of GSTM1 and GSTT1 more frequently. For the GSTM1 polymorphism, the present genotype was reported in 61.66% of men and 60.48% of women and in the analysis of the GSTT1 gene the frequency of the present genotype was 73.33% for men and 72.58% for women. In the comparison with the control group the GSTM1 genotype was not significant, being in agreement with the present study and the present form of GSTT1 was significant, disagreeing with our results.

According to Bomfim et al. (2015), when conducting a meta-analysis to identify the main factors associated with atherosclerosis, it was observed that hypertensive and diabetic individuals presented a greater predisposition to the disease, confirming the importance of the present study.

The literature points out that on certain occasions the metabolism of some xenobiotics by GSTs results in products that cause metabolic changes and may be linked to the development of atherosclerosis, hypertension and DM2, and therefore, the non-coding of these proteins, that is, the genotype would be a protective factor against these diseases (Marinho et al., 2007).

Thus, it is concluded from the results of this study and in the literature findings that the present genotype of the GSTM1 gene may influence the pathogenesis of arterial hypertension in atherosclerotic patients, that the GSTT1 genotype present are related to the development of atherosclerosis independent of the associated cofactors and that the present forms of GSTM1 and GSTT1 even though they are more prevalent in the case groups, have no relation to type 2 diabetes mellitus.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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