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Association between the glutathione S-transferase P1 (*GSTP1*) *Ile105Val* gene polymorphism in obese and overweight patients over 60 years

Associação entre o polimorfismo Ile105Val *do gene da glutationa S-transferase P1 (*GSTP1*) em pacientes obesos e com sobrepeso acima de 60 anos*

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ABSTRACT

Introduction: Obesity is related to the possibility of a number of metabolic damage associated with oxidative stress. The enzymes of the glutathione S-transferase (GST) family have the function of promoting detoxification; however, polymorphisms in the glutathione S-transferase P1 (*GSTP1*) gene generate less efficient alleles as well as a decrease in their amount and activity. **Objective:** This study aimed to analyze the frequency of the alleles (A and G) and the genotypes of the *GSTP1 Ile105Val* gene polymorphism, and its association with obesity in the elderly. **Materials and methods:** This was a cross-sectional study involving 232 subjects aged between 60-98 years, of both sexes, originating from southern Brazil. The volunteers were categorized according to the body mass index (BMI) in three groups: normal weight ($n = 52$), overweight ($n = 133$), and obese ($n = 47$). Anthropometry was evaluated and the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genetic analysis, from peripheral blood samples. **Results:** The allelic frequency in the elderly obese group was 37.2% for A and 62.8% for G allele, and the genotypic frequency observed was AA 8.5%, AG 57.4% and GG 34.1%. Both the G allele as the GG and AG genotypes were significantly higher in the obese group compared to the other groups ($p < 0.001$). **Conclusion:** A higher prevalence of the G allele was observed in elderly obese group, responsible for encoding an abnormal enzyme and consequent reduction of antioxidant defenses, which contribute to inflammation process and obesity in the elderly.

Key words: obesity; oxidative stress; glutathione transferase; genetic polymorphism.

INTRODUCTION

Obesity is characterized by an excessive accumulation of body fat (adipose tissue), regional or generalized distributed, presenting multifactorial etiology. It is one of the most important problems that the Brazilian and global public health currently face. The latest data show that 17.9% of the Brazilian population is obese, with growing estimates every year among all age groups and both sexes[1, 2].

Several chronic diseases such as type 2 diabetes *mellitus* (DM2), biliary disease, coronary artery disease (CAD), hypertension, osteoarthrosis, and dyslipidemia, are associated with obesity[3]. This occurs because the adipose tissue is an active metabolic endocrine organ that secretes several immunomodulatory and proinflammatory substances that interfere directly in the insulin-dependent processes, such as glucose homeostasis and lipid metabolism[4].

These conditions are even more important when they occur in the elderly, since increase in age is a risk factor which alone favours the prevalence of chronic diseases[5]. In the last two decades, the rate of obesity has increased dramatically among older adults, regardless of gender, race and education level[6]. Data from the Surveillance of Risk and Protective Factors for Chronic Diseases by Telephone Survey (Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico [Vigitel]), released in 2013, show that the aging of the population is one of the factors for the increase of diabetes

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in the country. The sample of 53 thousand Brazilians showed that among young Brazilians aged between 18-24 years, less than 1% are living with diabetes; however in the elderly aged over 65 years, 22% have the disease[7].

The health status of the geriatric population presents several different patterns, which are the result of throughout life experiences[8], considering that obesity is influenced by lifestyle, nutritional status, physiological, metabolic, psychological and biochemical changes[5]. Furthermore, there are also genetic implications on body weight control. The genetic programming and control are able to produce the physiological maintenance of a certain amount of fat for each individual[9].

Evidence suggests that polymorphisms in the glutathione S-transferase (*GST*) genes, a enzyme which belongs to a group of multigene producer of detoxification enzymes that defend the cells against a wide variety of toxic insults, such as chemicals and oxidative stress, may contribute to the development of DM2 associated with obesity or overweight[10].

The glutathione S-transferase P1 (*GSTP1*) gene is located on the long arm of chromosome 11 and is characterized by a polymorphism in exon 5, at codon 105. Such genetic change results in the substitution of adenine for guanine (A/G) in the deoxyribonucleic acid (DNA) coding sequence, triggering a substitution of isoleucine residue for valine (*Ile/Val*) at the end product of protein, which is linked to the reduction of enzyme activity[11].

Accordingly, individuals who carry less efficient alleles of detoxification enzymes (*GST*), as the G allele of the A/G polymorphism in exon 5 of *GSTP1*, are subject to lower production or inefficient activity of these detoxification enzymes, which favors the development of obesity, since this disease is strongly associated to oxidative imbalance. Moreover, it can contribute to the development of obesity-related comorbidities, such as DM2, cancer and Parkinson's[12-14].

Upon the facts presented, this study aimed at determining the allelic and genotypic frequencies and the relationship between *GSTP1 Ile105Val* gene polymorphism with the development of obesity in obese or overweight patients aged over 60 years.

MATERIALS AND METHODS

Study design

This was a cross-sectional study. Samples of 232 elderly subjects were analyzed, patients aged between 60-98 years of both sexes from southern Brazil were classified according to the body mass index (BMI) (kg/m^2): < 25 (normal weight), between 25 and 29.9 (overweight) and ≥ 30 (obese)[15]. They were recruited from the Department of Biological Sciences and Health at the Universidade do Oeste de Santa Catarina (UNOESC), campus of São Miguel do Oeste (SC), in the period from April to August 2015. The intervention protocol was submitted for revision and approval of the Human Research Ethics Committee of the UNOESC, nº 219.091.

Anthropometric measurements

The techniques used to obtain the anthropometric measurements were performed according to the Anthropometric Standardization Reference Manual[16]. Three measurements were performed, the average between them was considered valid.

The stature was measured in centimeters (cm) using wall stadiometer, Professional ES2020 Sanny®. Weight was measured in kilograms (kg) using a platform scale, brand G-techC, model Glass 180. The BMI was expressed in units of kg/m^2, calculated as Weight (kg)/Height (m)^2, according to the Sociedade Brasileira de Obesidade.

Processing of samples and genotyping of *GSTP1 Ile105Val* gene polymorphism

DNA was obtained from peripheral blood samples. The samples were processed at the Molecular Biology Laboratory of the UNOESC.

The test for the *GSTP1 Ile105Val* gene polymorphism was performed as described by Harries *et al.* (1997)[17], using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The sequences of the primers used in the PCR reaction were the following:

P105 Forward – 5'-ACC CCA GGG CTCTAT GGG AA-3'

P105 Reverse – 5'-TGA CAC GGG AAGAAG CCC TC-3'.

The Alw26I (BsmAI) restriction enzyme was used. The samples were applied in agarose gel ultrapure 4% stained with ethidium bromide (140 v for 25 minutes) and subsequently visualized on the transilluminator. In the *GSTP1 Ile105Val* gene polymorphism, the A allele appeared blunt ended restriction enzyme, with a band at 176 base pairs (bp); the G allele showed two bands at 91 bp and 85 bp.

Statistical analysis

The allelic and genotypic frequencies of the population were calculated and expressed as a percentage. Data were assessed

to determine whether the frequencies are in Hardy-Weinberg equilibrium. We performed the χ^2 (chi-square) statistical test with the SPSS software version 19.0, and p-values < 0.05 were considered significant.

RESULTS

The genotype frequencies of the *GSTP1* polymorphism are shown in **Figures 1**, **2** and **3**. As shown in Figure 1, the genotypic frequency according to the gender was AA 9.1%, AG 56.8%, and GG 34.1% for women, while in men was AA 11%, AG 57%, and GG 32%, with no significant difference ($p = 0.8674$). A significant difference between the BMI and the genotypic frequency ($p = 0.0001$) was observed, and the distribution found was AA 40.4%, AG 59.6%, and GG 0% for BMI < 25; AA 7.4%, AG 58.6%, and GG 34% for BMI between 25-29.9; and AA 8.5%, AG 57.4%, and GG 34.1% for BMI ≥ 30, data shown in Figure 2. There was no significant difference when compared to genotypic frequency and age ($p = 0.2939$), the distribution found was AA 3.2%, AG 71%, and GG 25.8% for 60-69 years; AA 11.8%, AG 52.1%, and GG 36.1% for 70-79 years; and AA 8.8%, AG 61.4%, and GG 29.8% for ≥ 80 years, values shown in Figure 3.

The allele frequencies of the *GSTP1* polymorphism are shown in the **Table**. The allelic frequency according to gender and age showed no significant difference ($p = 0.7001$; $p = 0.9536$, respectively). Regarding the BMI, the frequency was 70.2% for the A allele and 29.8% for the G allele. Allele frequency according to the BMI was: BMI < 25, A 36.8% and G 63.2%; BMI between 25-29.9, A 37.2% and G 62.8%; BMI ≥ 30, A 38.6% and G 61.3%, a statistically significant difference ($p = 0.001$) was observed. The total sample is not in the Hardy-Weinberg equilibrium, $p < 0.05$.

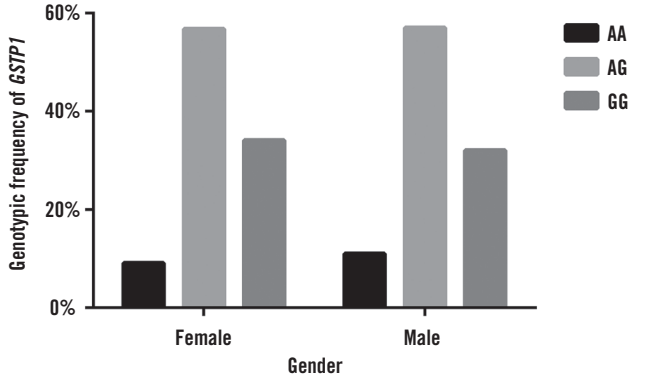

FIGURE 1 – *Genotype distribution of* GSTP1 *polymorphism in relation to gender. Chi-square test* p = *0.8674*

GSTP1: *glutathione S-transferase P1.*

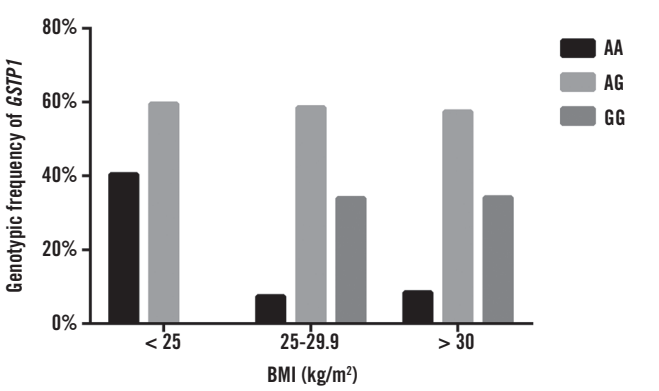

FIGURE 2 – *Genotype distribution of* GSTP1 *polymorphism in relation to BMI. Chi-square test* p = *0.0001*

GSTP1: *glutathione S-transferase P1; BMI: body mass index.*

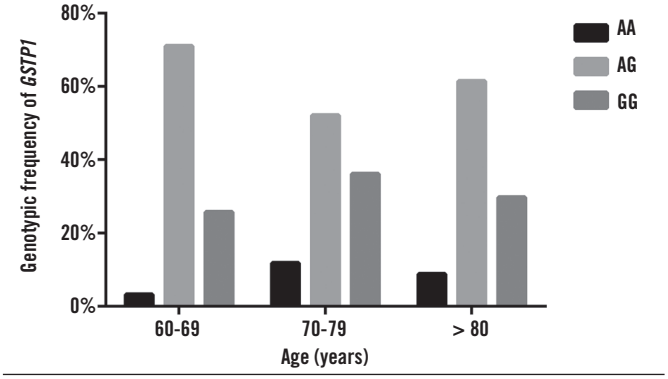

FIGURE 3 – *Genotype distribution of* GSTP1 *polymorphism in relation to age. Chi-square test* p = *0.2939*

GSTP1: *glutathione S-transferase P1.*

TABLE – Allele frequencies of *GSTP1* according to gender, age, BMI and total sample

Variables	Alleles		*p*
	A	G	
Female	37.5%	62.5%	0.7001
Male	39.5%	60.5%	
BMI (kg/m²)			
< 25	70.2%	29.8%	0.001
25-29.9	36.8%	63.2%	
≥ 30	37.2%	62.8%	
Age (years)			
60-69	38.7%	61.3%	0.9536
70-79	37.8%	62.2%	
≥ 80	39.5%	60.5%	
Total	38.6%	61.3%	

GSTP1: *glutathione S-transferase P1; BMI: body mass index.*

DISCUSSION

Obesity, characterized by abnormal or excessive deposition of adipose tissue, causes oxidative stress and interferes with the homeostasis of adipocytes. Since it is strongly linked to a high-fat diet in combination with low intake of antioxidants, the premise that oxidative stress should also be a factor that induces the obese state is well established and has been presented in previously published studies[13, 18].

GST include a family of isoenzymes known for their ability to catalyze the glutathione (GSH) conjugation on a wide variety of endogenous and exogenous electrophilic compounds, playing a crucial role in the detoxification process, promoting the maintenance of cell integrity and protection against damage in the DNA[19].

Epidemiological studies show that allelic variants of *GSTP1 Ile105Val* gene polymorphism, which encode the P1 isoenzyme, create differences in individual susceptibility to several inflammatory diseases. Codon 105 comprises part of the active site of *GSTP1* enzyme for binding reactive electrophiles, and this enzyme, when presented in its wild form (A allele), performs functions such as metabolism of halogenated compounds, low molecular weight molecules and reactive epoxides. However, this region is characterized by a mutation in which there are genetic exchanges of A/G. When this exchange occurs, the final protein is defined by the substitution of isoleucine amino acid for valine, and its substrate-specific catalytic activity and thermal stability will be affected, becoming substantially less effective in its activity[20, 21].

The present study has associated *GSTP1 Ile105Val* gene polymorphism to obesity, evaluating the BMI, age and gender of persons aged over 60 years. The choice of an elderly sample was due to the fact that there are few studies relating this polymorphism with the development of obesity in the elderly, considering that Brazil is experiencing a demographic transition characterized by the exponential increase in the proportion of elderly.

The results presented in this study showed a positive association between the *GSTP1 Ile105Val* gene polymorphism and the BMI, and observed a strong interaction in these variables. It should be noted that the GG genotype showed important and significant increase in the elderly overweight and obese population. Regardless of gender, elderly patients who have at least one G allele are 2.4 times more likely to be obese compared to the AA genotype.

Assuming that the G allele, allelic variant resulted from this polymorphism generates a less effective enzyme in the detoxification role; individuals with this allele (AG or GG genotype) would have a biological oxidative imbalance, genetically determined. This condition can affect the metabolism of adipocytes, favoring the obesity status[22], and the development of chronic diseases such as cardiovascular disease, dyslipidemia, systemic arterial hypertension (SAH), insulin resistance (IR), DM2 and some types malignant neoplasm[23, 24]. As the G allele is associated with low efficiency of *GSTP1* enzyme, the results described emphasize that the oxidative stress caused by chronic reduction of *GST* can induce a pattern of obesity and its comorbidities.

This study also found that increase in age, i.e., the evaluation of this polymorphism in the long-lived elderly (≥ 80 years), did not presented association with the frequency of the G allele in the *GSTP1* gene, demonstrating that this polymorphism does not correlate with longevity, and rather with a predisposition to increased body weight in elderly patients. This factor is of concern because the accumulation of body fat, especially in the abdominal area can cause a number of relevant clinical complications in the elderly.

Kim and Hong (2012)[25] analyzed the *GSTP1 Ile105Val* polymorphism in 560 individuals aged ≥ 60 years, exposed to air pollutants such as sulfur dioxide (SO_2) and ozone (O_3), and assessed their association with IR markers. Individuals with AG or GG genotypes showed strong relationship with increased susceptibility of potential harmful effects of these pollutants, as well as among the IR markers. These findings suggest that the ability to capture oxygen free radicals induced by the exposure to air pollution has genetic predisposition, highlighting the important role of this polymorphism in the body's defenses.

Some limitations of our study should be recognized. First of all, the cross-sectional study presents weaknesses for the association between obesity predisposition, anthropometric markers and polymorphism; a longitudinal follow-up study would be suitable for this kind of investigation. A second limitation that deserves consideration is the fact that the study was carried out in a specific population of a southern state in Brazil, so that a strong regional focus may be appropriate.

However, it is noteworthy that currently approximately 23.5 million individuals in the Brazilian general population are comprised of elderly (people over 60 years)[26]. The ageing population has led to a reorganization of the health system, as this population requires a particular care, which is a challenge due to the chronic diseases they present[27]. For this reason, the need to study the factors that contribute to the development of chronic non-communicable diseases associated with age is widening[28], in particular the genetic, reinforcing the importance of this study in this specific population.

CONCLUSION

The results of this study suggest an association between the G allele (AG and GG genotypes) and obesity in the elderly subjects, highlighting that the individuals that carry at least one G allele have a higher susceptibility to developing obesity. The possible biological explanation for this association could be a chronic state of *GST* imbalance present in patients with AG and GG, which could affect the metabolic differential and hormone modulation pathways due to high levels of free radicals, triggering a positive feedback between oxidative stress – obesity – oxidative stress.

RESUMO

Introdução: *A obesidade está relacionada com a possibilidade de numerosos danos metabólicos associados ao estresse oxidativo. As enzimas da família glutationa S-transferase (GST) têm como função promover a detoxificação, entretanto, polimorfismos no gene da glutationa S-transferase P1 (*GSTP1*) geram alelos menos eficientes, bem como diminuição da sua quantidade e atividade.* ***Objetivo:*** *Este estudo teve como objetivo analisar a frequência dos alelos (A e G) e dos genótipos do polimorfismo* Ile105Val *do gene* GSTP1*, além de sua associação à obesidade em idosos.* ***Materiais e métodos:*** *Tratou-se de um estudo transversal, o qual envolveu 232 indivíduos com idades entre 60 e 98 anos, de ambos os sexos, oriundos da região Sul do Brasil. Os voluntários foram caracterizados de acordo com o índice de massa corporal (IMC) em três grupos: peso normal (*n = *52), sobrepreso (*n = *133) e obesos (*n = *47). A antropometria foi avaliada, e a técnica de reação em cadeia da polimerase-polimorfismo no comprimento de fragmentos de restrição (PCR-RFLP) foi usada para análise genética a partir de amostras de sangue periférico.* ***Resultados:*** *A frequência alélica no grupo de idosos obesos foi de 37,2% para o alelo A e 62,8% para o G, e a frequência genotípica observada, de AA 8,5%, AG 57,4% e GG 34,1%. Tanto o alelo G quanto os genótipos GG e AG foram significativamente maiores no grupo obeso quando comparados com os dos demais grupos (*p < *0,001).* ***Conclusão:*** *Observou-se maior prevalência do alelo G no grupo de idosos obesos, responsável pela codificação de uma enzima anormal e consequente diminuição das defesas antioxidantes, que contribuem para o processo inflamatório e a obesidade em idosos.*

Unitermos: *obesidade; estresse oxidativo; glutationa transferase; polimorfismo genético.*

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