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*Original article*

# Glutathione-S-transferase P1 polymorphism and oxidative stress markers in women infected by human papilomavirus

*Polimorfismo da glutationiona-S-transferase P1 e marcadores de estresse oxidativo em mulheres infectadas por papilomavírus humano*

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**Abstract**

**Objective**: to analyze the genetic polymorphism of GSTP1 located on chromosome 11q13.2 exon 5 (Ile105Val) and its association with oxidative stress markers and HPV infection. **Methods**: DNA were extracted from cervical scrapes for HPV identification by PCR, genotyping using HPV-Screening PapilloCheck®, and from whole blood for analysis of polymorphisms by RFLP-PCR. Oxidative stress was analyzed by measuring the serum levels of thiobarbituric acid reactive species, carbonyl groups, thiol groups, and catalase activity. **Results**: of all HPV-positive women, 75% had simple infections. Viral types 16 and 45 were the most frequent. A total of 56.25% of women had variant Ile105Val GSTP1 genotype (Ile/Val or Val/Val) with a heterozygous prevalence (43.75%). Among the HPV-positive women, the presence of the allelic variant led to a higher risk of developing cervical lesions (OR = 29.44). Women infected with HPV (especially the high-risk type) showed lower catalase activity and an increase in carbonyl groups. Considering the presence of variant genotypes Ile/Val and Val/Val, the infected women presented an increase in some oxidative stress markers, and HPV-negative women showed a decreased antioxidant capacity. **Conclusion**: these results indicate the potential influence of variant GSTP1 genotype on increased oxidative stress and susceptibility to cervical lesions in women infected with HPV.

**Keywords**: gstp1; reactive oxygen species; HPV; cervical injury.

**Resumo**

**Objetivo**: analisar o polimorfismo genético de GSTP1 localizado no cromossomo 11.q13.2, exon 5 (Ile105Val) e associar a marcadores de estresse oxidativo e infecção por HPV. **Métodos**: o DNA foi extraído de raspados cervicais para identificação do HPV por PCR e genotipagem utilizando HPV-Screening PapilloCheck® e de sangue total para análise do polimorfismo por RFLP-PCR. O estresse oxidativo foi analisado através dos níveis séricos das espécies reativas ao ácido tiobarbitúrico, grupos carbonil, grupos tiol e atividade da catalase. **Resultados**: 75% das mulheres HPV positivas apresentaram infecções simples e os tipos virais 16 e 45 foram mais frequentes. 56,25% das mulheres apresentaram o genótipo variante Ile105Val GSTP1 (Ile/Val ou Val/Val) com prevalência de heterozigotos (43,75%). Entre as mulheres HPV positivas, a presença do variante alélico levou a um maior risco de desenvolver lesões cervicais (OR = 29,44). Mulheres infectadas com HPV (especialmente de alto risco) apresentaram menor atividade da catalase e aumento dos grupos carbonila. Considerando a presença dos genótipos variantes Ile/Val e Val/Val, as mulheres infectadas apresentaram aumento dos marcadores de estresse oxidativo e as mulheres HPV negativas apresentaram diminuição da capacidade antioxidante. **Conclusão**: estes achados sugerem uma possível influência do genótipo variante GSTP1 no aumento do estresse oxidativo e suscetibilidade a lesões cervicais em mulheres infectadas com HPV.

**Palavras-chave**: gstp1; espécies reativas de oxigênio; hpv; lesão cervical.

## Introduction

Human papillomavirus (HPV) is responsible for one of the most common sexually transmitted infections and activates oncogenes such as E6 and E7, capable of inducing the malignant transformation of infected cells.[1, 2] In clinical practice, HPVs are classified into high and low oncogenic risk. The low-risk HPV subtypes 6, 11, 40, 42, 43, 44, and 55 cause genital warts and dysplasia, while high-risk HPVs include subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 69, which cause cervical intraepithelial neoplasia.[3]

Cervical cancer is the third most common type of cancer among Brazilian women and the fourth leading cause of death from cancer among women worldwide. There is scientific evidence that the formation of tumors, commonly found in HPV-positive women, is the result of the overproduction of reactive oxygen species (ROS) in the host cell and/or tissue microenvironment.[5, 6] The accumulation of ROS can result in deleterious effects, including lipid peroxidation, protein oxidation, and DNA damage. Lipid peroxidation disrupts the normal structure and function of lipid bilayers, changing the permeability and fluidity of membranes, while protein changes caused by ROS include fragmentation, incorrect folding, protein-protein cross-links, and carbonyl production. On the other hand, oxidative stress can lead to different lesions in DNA, including the direct modification of nucleotide bases, apurinic/apyrimidinic training sites, single-strand breaks, and, much less frequently, double-strand breaks.[7, 8] However, antioxidants may contribute to the prevention of oxidative damage caused by HPV[9].

Glutathione-S-transferases belong to a family of enzymes that prevent redox imbalance and hence DNA damage.[10] These enzymes are involved in the detoxification metabolism of carcinogens, and seven classes have been described: alpha, mu, pi, sigma, theta, omega, and zeta.[11] Some homozygous variant genotypes, seen mainly in the "mu" (e.g. GSTM1), "theta" (e.g. GSTT1), and "pi" (e.g. GSTP1) classes, are associated with reduced enzymatic activity and stability. This can lead to the exposure of cells to carcinogens, which can increase the risk of developing cancer.[12, 13]

Low levels of antioxidant enzymes, including glutathione-S-transferase, were found in patients with cervical cancer[14]. The deficiency of certain antioxidants has been reported to influence the course of HPV infection. ROS leads to the activation of AP-1, an underlying factor in the expression of viral oncoproteins E6 and E7, favoring the proliferation of HPV[15]. The aim of this study was to analyze the genetic polymorphism of GSTP1 located on chromosome 11q13.2 exon 5 (Ile105Val substitution reduces enzyme activity[13]) and its association with oxidative stress markers and HPV infection.

## Materials and Methods

### *Patient samples*

A cross-sectional study with 84 women aged between 18 and 60 years (simple random sampling) was performed in public health centers in Olinda-Pernambuco, Brazil. The exclusion criteria were as follows: sterectomized patients; infection with HIV; pregnant women; history of transplant; and patients treated with immunomodulators. The inclusion criterion was women aged ≥18 years. The research was approved by the Ethics Committee on Human Research of the Health Science Center of the Federal University of Pernambuco - CEP / CCS / UFPE (registration no. 275/08).

### *DNA extraction of samples*

DNA extraction was performed using cervical scrape samples and 300 µL of whole blood in EDTA using the Wizard® Genomic DNA Purification Kit from Promega according to the manufacturer's instructions.

### *Identification of HPV DNA*

DNA extracted from cervical samples was subjected to polymerase chain reaction (PCR) using the primers MY09/11 (5'-CGTCCMARRGGAACTGATC-3', 5'-GCMCAGGGCATAAYAATGG-3') and GP5+/6+ (5'-TTTGTTACTGTGGTAGATACTAC-3', 5'-GAAAAATAAACTGTAAATCATATTC-3').[16,17] The final volume of the reaction was 12.5 µL (1 µL of DNA extracted, 1 µL of each primer (10 pmol), 3.25 µL of ultrapure water, and 6.25 µL of GoTaq Green Master Mix (Promega®)). The amplification conditions were as follows: 94°C for 3 min, 34 cycles of denaturation at 95°C for 1 min, annealing for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The MY09/11 primers

were annealed at 55°C, whereas the GP5+/6+ primers were annealed at 45°C. The HPV16 plasmid pBR322 was used as the positive control, while ultrapure water was used as the negative control. The bands were visualized on agarose gel containing ethidium bromide under ultraviolet light.

β-globin was used as a reporter gene to ensure the quality of DNA extraction by the amplification of a 268-pb region with specific primers (5'-GAAGAGCCAAGGACAGGTAC-3' and 3'-CAACTTCATCCACGTTCACC-5'). The reaction was performed using a final volume of 12.5 µL (1 µL of extracted DNA, 1 µL of each primer (10 pmol), 3.25 µL of ultrapure water, and 6.25 µL of GoTaq Green Master Mix (Promega®)). The PCR conditions were as follows: initial denaturation for 10 min at 94°C followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min. The final extension step was performed at 72°C for 7 min. Ultrapure water was used as a negative control, while the DNA extracted from human blood was used as a positive control.

***Genotyping HPV***

HPV-Screening PapilloCheck® (Greiner Bio One, Germany) was used to genotype HPV present in cervical samples by amplification of a region of 350 pb of the HPV E1 gene using 24 type-specific probes to identify HPV genotypes, 6 genotypes of low-risk oncogenic (06, 11, 40, 42, 43, 44/55), and 18 genotypes of high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82).

***GSTP1 polymorphism***

PCR-restriction fragment length polymorphism (PCR-RFLP) was performed from DNA extracted from the whole blood samples in EDTA using the primers 5'-ACCCCCGGGCTCTATGGGAA-3’ and 5’-CACCAAAGATGAGGGCCCCT-3'. The final reaction volume was 12.5 µL (1 µL of extracted DNA, 1 µL of each primer (10 pmol), 3.25 µL of ultrapure water, and 6.25 µL of GoTaq Green Master Mix (Promega®)). The PCR parameters used were as follows: initial denaturation at 94ºC for 5 min followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 59ºC for 90 s, extension at 72ºC for 90 s, and a final extension at 72ºC for 7 min. The PCR products were treated with a fast digest restriction enzyme (Alw26I; Thermo Fisher Scientific) for 5 min at 37°C. The bands were visualized on a 1% agarose gel containing ethidium bromide under ultraviolet light[18]. 329 and 113-bp bands corresponding to the wild genotype (Ile/Ile), a 216-pb band, and another band corresponding to thick bands (107 and 113 bp) for the homozygous variant genotype (Val/Val). All bands were visualized in the heterozygous variant genotype (Ile/Val). The 107- and 113-bp bands was confirmed by 10% polyacrylamide gel using eight samples (one wild-type genotype, three heterozygous variants, and four homozygote variants).

***Cytopathologic analysis***

Immediately after collection, cervical samples were transported and stored in absolute ethanol. All samples were processed using the conventional methodology for cytopathological analysis.[19] The nomenclature of the Bethesda system was used for the classification of samples: negative for squamous intraepithelial lesions and malignancies (NILM), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL).[20] Any samples not adequate for analysis (e.g. broken blade, presence of blood and inflammatory exudates, thick areas, poor fixation, contamination, and all parameters that could hamper the interpretation of the sample) were excluded. The final cytologic diagnoses were confirmed by consensus of two independent pathologists.

***Oxidative stress markers***

Protein carbonyl groups were determined from the serum by spectrophotometry at 370 nm (reaction with 2,4-dinitrophenylhydrazine).[21] The thiobarbituric acid reactive species (TBARS) were measured as a marker of lipid peroxidation by spectrophotometry at 532 nm, as described by Draper and Hadley.[22] The total thiol content was measured at 412 nm, as described by Bulaj et al.[23] Catalase activity was determined by the rate of decrease of absorbance of hydrogen peroxide at 240 nm, as described by Aebi.[24]

**Statistical analysis**

Data are expressed as the mean ± standard deviation (SD). Normality analysis was performed using the Kolmogorov–Smirnov test. The Chi-square test, Mann-Whitney test, and Kruskal-Wallis test with Dunn's post-test were used for comparison between groups. All analyses were performed using GraphPad PRISM® version 4.03. $P < 0.05$ was considered statistically significant.

**Results**

***Patient profiles***

The average age of the 84 women included in the study was 35.5 years. The distribution of the frequency of variants (Ile105Val) of the heterozygous genotype of GSTP1 (Ile/Val) was 46.43% (39/84), the wild genotype (Ile/Ile) was 42.86% (36/84), and the homozygous genotype (Val/Val) was 10.71% (9/84). HPV infection was found in 38% (32/84) of the samples. The distribution of the frequency of the variant genotype (heterozygous and homozygous) among HPV-positive women was 56.25% and among HPV-negative women was 57.69%. The presence of cytological lesions in HPV positive women was 18.75% (6/32) and among them, 31.25% (10/32) were smokers. In the cytopathological analysis, we found a higher frequency of normal results (92.85%, 78/84). However, between injuries, LSIL was the most common (Table 1). In total, 75% (24/32) of women presented simple infection and 25% (8/32) multiple infections. High-risk HPV was detected in 75% (24/32) of the infected samples (Table 2).

**Table 1**. Characteristics of the 84 women enrolled in the study

| | **HPV +** | | **HPV -** | |
|---|---|---|---|---|
| **Genotype** | ***n*** | **(%)** | ***n*** | **(%)** |
| Heterozygous allelic variant (Ile/Val) | 14 | (43.75) | 25 | (48.07) |
| Homozygous allelic variant (Val/Val) | 4 | (12.5) | 5 | (9.61) |
| Allelic variant total (Ile/Val + Val/Val) | 18 | (56.25) | 30 | (57.69) |
| Wild (Ile/Ile) | 14 | (43.75) | 22 | (42.31) |
| Total | 32 | (100) | 52 | (100) |
| **Cytological analysis** | | | | |
| HSIL | 2 | (6.25) | - | - |
| LSIL | 4 | (12.5) | - | - |
| Normal | 26 | (81.25) | 52 | (100) |
| Total | 32 | (100) | 52 | (100) |
| **Age** | | | | |
| 40-49 years | 5 | (15.62) | 17 | (32.69) |
| <39 or >50 years | 27 | (84.37) | 35 | (67.31) |
| Total | 32 | (100) | 52 | (100) |
| **Smoking** | | | | |
| Smoking | 10 | (31.25) | 9 | (17.03) |
| Non-smokers | 22 | (68.75) | 43 | (82.69) |
| Total | 32 | (100) | 52 | (100) |

**Table 2**. Distribution of the frequency of the HPV genotypes and the presence of multiple and single infections

| **HPV genotypes** | ***n* (%)** |
|---|---|
| **Single infections** | ***n* for HPV type** |
| 42/70/82/56/35/43 | 1/1/1/1/1/1 (18.75%) |
| 53/73 | 2/2 (12.5%) |
| 11/6 | 3/3 (18.75%) |
| 16/45 | 4/4 (25%) |
| Total | 24 (75%) |
| **Multiple infections** | ***n* for HPV types** |

| | |
|---|---|
| 16, 18/39, 56, 16/45, 56, 66/52, 59, 44, 55/16, 59, 58/6, 82 | 1/1/1/1/1/1 (18.75%) |
| 56, 66 | 2 (6.25%) |
| Total | 8 (25%) |

***GSTP1 polymorphism and HPV infection***

No association was observed between the GSTP1 polymorphism and susceptibility to HPV infection. When the variant genotype was associated with smoking, the risk of HPV infection was 2.5 times greater, although this finding was not statistically significant (Table 3). However, HPV-positive women who had the variant genotype (Ile/Val or Val/Val) showed a risk 29 times greater ($P < 0.05$) to present cytological lesions (Table 3). In this analysis, the frequency of the Ile/Val genotype and Val/Val genotype were combined since the frequency of the Val/Val genotype was low in both infected women and the controls.

**Table 3**. Association between the GSTP1 genotype with HPV infection and cervical lesions

| GSTP1 Genotypes | Clinical conditions | | $X^2$ | OR (95% CI) | *P* |
|---|---|---|---|---|---|
| **Genotype** | **HPV presence** | **HPV absence** | 0.26 | 0.8 (0.31–1.99) | 0.608 |
| Variant (Ile/Val) | 14 | 25 | | | |
| Variant (Val/Val) | 4 | 5 | 0.02 | 0.8 (0.06–11.00) | 0.880 |
| Variant (Ile/Val, Val/Val) | 18 | 30 | 0.02 | 0.94 (0.39–2.29) | 0.896 |
| Wild (Ile/Ile) | 14 | 22 | | Reference | |
| **Genotype and smoking** | | | | | |
| Variant (Ile/Val, Val/Val) and smoker | 5 | 4 | 1.54 | 2.5 (0.57–10.92) | 0.214 |
| Variant (Ile/Val, Val/Val) and non-smoker | 13 | 26 | | Reference | |
| **Genotype of HPV-infected women** | **Abnormal cytology** | **Normal cytology** | | | |
| Variant (Ile/Val, Val/Val) | 2 | 0 | 4431 | 29.44 (1.20–720.5) | 0.035 |
| Wild (Ile/Ile) | 4 | 26 | | Reference | |
| **Genotype** | **High-risk HPV infection** | **Low-risk HPV infection** | | | |
| Variant (Ile/Val) | 10 | 4 | 0.01 | 0.6 (0.12–3.82) | 1 |
| Variant (Val/Val) | 3 | 1 | 0.28 | 0.8 (0.06–11.0) | 0.59 |
| Variant (Ile/Val, Val/Val) | 13 | 5 | 0.01 | 0.7 (0.13–3.66) | 1 |
| Variant (Ile/Ile) | 11 | 3 | | Reference | |
| | **HPV presence** | **HPV absence** | 0.26 | 0.8 (0.31–1.99) | 0.608 |
| | 14 | 25 | | | |

**Abbreviations**: HPV: human papillomavirus; OR: odds ratio; CI: confidence interval

***Oxidative stress markers***

Statistically significant results were found for the increase in carbonyl groups and decrease in catalase activity in the presence of HPV (Figure 1b and d), especially in high-risk HPV infection (Figure 2b and d), increase in lipid peroxidation and carbonyl groups among HPV-infected women with variant GSTP1 genotype (Figure 3a and b, respectively), and decrease of thiol groups and catalase activity among uninfected women with variant GSTP1 genotype

(Figure 3c and d, respectively).

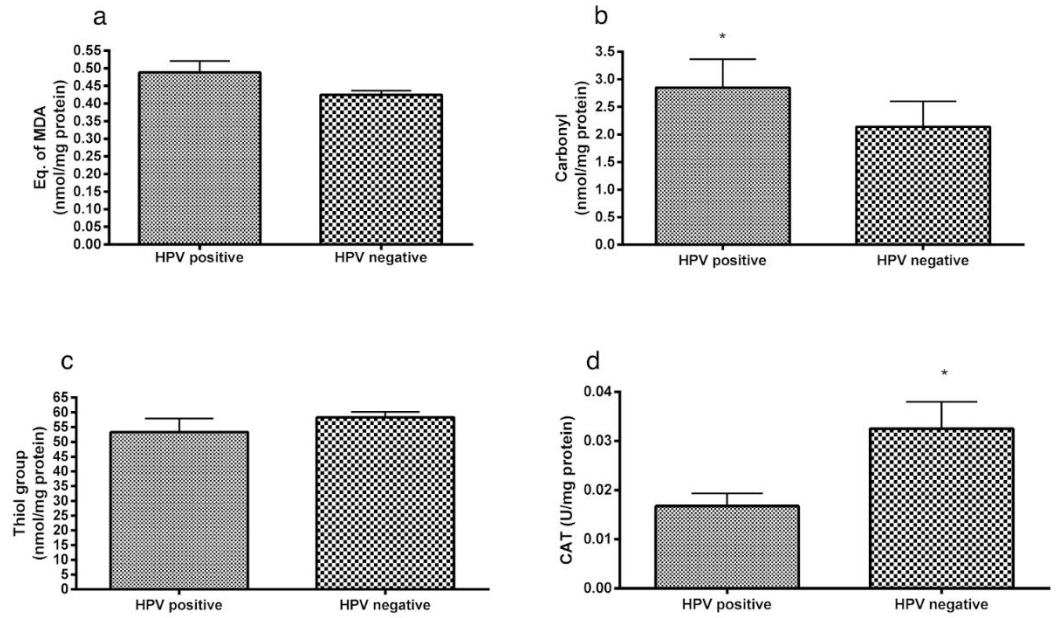


**Figure 1**. Lipid peroxidation (MDA, malondialdehyde) (a), carbonyl group (b), thiol group (c), and enzymatic activity of catalase (CAT) (d) of women infected with HPV or not. *P < 0.05.

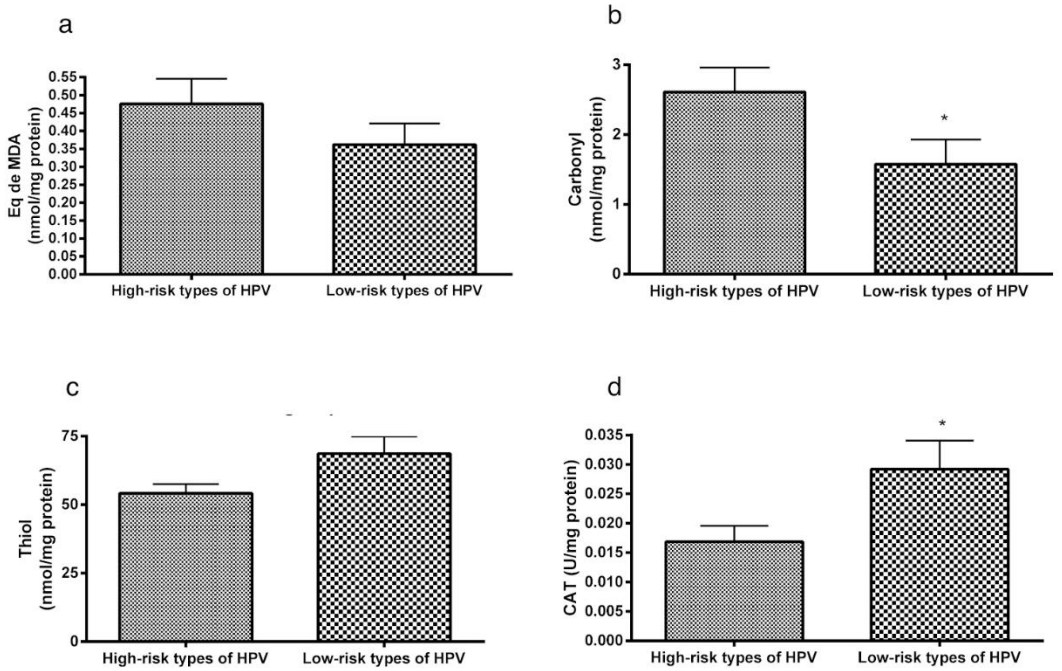


**Figure 2**. Lipid peroxidation (MDA, malondialdehyde) (a), carbonyl group (b), thiol group (c), and enzymatic activity of catalase (CAT) (d) of women infected with high- or low-risk oncogenic HPV. *P < 0.05.

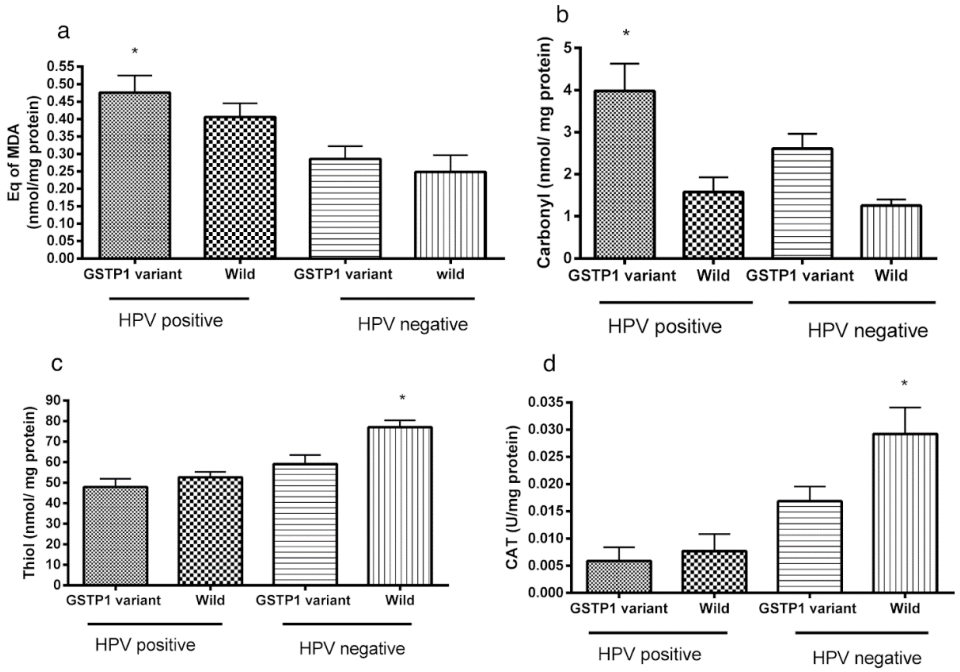


**Figure 3**. Lipid peroxidation (MDA, malondialdehyde) (a), carbonyl group (b), thiol group (c), and enzymatic activity of catalase (CAT) (d) of women infected with high- or low-risk oncogenic HPV and different GSTP1 genotypes. *P < 0.05.

## Discussion

De Aguiar *et al.*[25] analyzed the distribution of genotypes and variant allele frequencies of *GSTM1*, *GSTT1*, and *GSTP1* and correlated these results with the risk of cancer in Porto Alegre, southern Brazil. For the *GSTP1* polymorphism, the genotype frequencies were 44% for the Ile/Ile genotype, 44% for the Ile/Val genotype, and 12% for the Val/Val genotype, similar to those found in our study. In their study, they found a significant association between the combination of the variant (*GSTP1*) or null genotypes (*GSTM1* and *GSTT1*), cervical lesions, and mammographic density in postmenopausal women. The frequency distribution of the *GSTP1* variant allele that we found (46.43% Ile/Val + 10.71% Val/Val) was also higher than that of the wild genotype (42.86%). Similar results were found in studies by Barcelos *et al.*[26] and Sohail *et al.*,[27] held in Brazil and India, where the population of heterozygous variant genotype was higher than that of other genotypes (49% (156/321) and 51% (22/43), respectively).

Studies relating to infections and *GSTP1* polymorphisms are very scarce. In analyses related to malaria, Sohail *et al.*[27] found a higher percentage of the heterozygous genotypes associated with infection by *P. vivax* (46.15%), while in patients infected with *P. falciparum*, a higher percentage of the wild genotype (65.38%) was found. However, in both infections, the lowest percentage was found for the variant homozygous genotype (23.07% for infection by *P. vivax* and 11.53% for *P. falciparum*).

We did not find an association between the *GSTP1* genotypes and HPV infection. Millikan *et al.*[28] also found that the variant *GSTP1* genotype was not associated with an increased susceptibility to breast cancer in North Carolina (USA). Curran *et al.*[29] studied an Australian Caucasian population of 129 patients with breast cancer and found no major presence of the variant *GSTP1* genotype in patients with the disease. Previously, Helzlsouer *et al.*[30] had associated mutations and/or invalidity in the subtypes of the GST superfamily with an increased susceptibility to breast cancer in Maryland (USA), especially in women after menopause (OR = 2.50). Mitrunen *et al.*[31] also reported an increased risk of breast cancer in the presence of the *GSTP1* variant (Ile105Val) genotype (OR = 3.96) in Finland. Finally, this allelic variant was associated with an increased risk of glioma, principally in smokers.[32]

Polymorphisms or hypermethylation in *GSTP1* are frequent in cancer, and lead to decreased expression. Therefore, *GSTP1* is considered a tumor suppressor gene. However, in many types of cancer,

for example, colon and lung cancer, *GSTP1* is overexpressed and not methylated. These different findings lead to the need for specific interpretations of the degree of expression of this gene, as well as additional analyses of other molecules involved in metabolic pathways of GSTs. These enzymes have cytoprotective and regulatory functions, acting on the proliferation and death of cancer cells.[33, 34]

GSTs are enzyme systems that protect cells against the by-products of oxidative stress, and belong to one of three families of widely distributed proteins: mitochondrial, microsomal, and cytosolic. The cytosolic and mitochondrial GST families are comprised of soluble enzymes, while microsomal GSTs consist of proteins associated with membranes and have no structural similarity to the other two families.[35] The activities of cytosolic GSTs are 5 to 40 times larger than those of mitochondria. They represent the largest family of transferases, exert thiol transferase activity, and are capable of reducing trinitrate, acid dehydroascorbic, and acid monometilarsonic.[35, 36]

Three of the GST genes, *GSTP1, GSTM1,* and *GSTT1* (cytosolic GSTs), showed functional polymorphisms that are often present in the general population, and which may decrease or abolish enzyme activity. However, the combination of these polymorphisms with environmental factors may contribute to the increased risk of diseases.[37, 38] Accordingly, our results suggest that the presence of a variant genotype (Ile/Val or Val/Val) in women with HPV infection can increase the risk of developing cervical lesions. However, further analysis will be needed due to the low number of cervical lesions found; this is one of the limitations of this study.

The results obtained by Schellekens *et al*.[39] corroborate our own findings in terms of the distribution of infection by one HPV type and by two or more types. In the present study, 25% of the samples were found to have multiple infections. However, our findings differed in terms of the frequency of HPV genotypes, since we found a greater number of infections related to HPV 16 and 45 for simple infections and an increased number of infections related to HPV 56 and 66 for multiple infections (Table 2). In this respect, Schellekens *et al*.[39] found a higher percentage of HPV 16 and 18 related to single and multiple infections. However, it must be taken into account that they tested cervical cancer specimens, in which high-risk oncogenic HPV is frequent. In our study, patient samples were taken during routine examinations, and only 6 showed cytological abnormalities (2 HSIL and 4 LSIL).

The introduction of carbonyl groups into the amino acid residues of proteins is a hallmark of oxidative modification.[21] We found an increase in carbonyl groups and a decrease in the antioxidant response (catalase activity) in women infected with HPV, mainly by high-risk oncogenic HPV. Catalase decomposes hydrogen peroxide into less-reactive gaseous oxygen and water molecules, and plays critical roles in defending against oxidative stress, which reflects an imbalance in the redox status.[24] Nirmala and Narendhirakannan[40] also found increased levels of carbonyl groups and a decrease in the catalase enzyme activity in the plasma of women with cervical cancer compared to those without cancer. Naidu *et al*.[41] observed an increase in the serum lipid peroxidation in patients with cervical cancer compared to healthy individuals. Borges *et al*.[42] conducted a study on Brazilian women living near the Amazon River and found a significant association between increased oxidative stress indicator (lipid peroxidation) and HPV infection.

The oncogenes E6 and E7 of HPV induce chronic inflammation through various mechanisms. This inflammation leads to a redox imbalance due to pro-inflammatory cytokines that activate protein kinase-mediated signaling pathways, leading to the formation of ROS. ROS has a detrimental effect on proteins, lipids, and nucleic acids, causing damage to cells.[15, 43] HPV 16 neoplastic progression has been associated with an increasingly oxidant environment.[44] HPV 18 E2, for example, can enhance mitochondrial biogenesis by upregulating the mitochondrial transcription factor A and interacting with the respiratory chain, thereby increasing reactive oxygen species (ROS). In a similar way, Song *et al*.[45] found that the overexpression of E6 in cervical carcinoma cells increased ROS levels and mitochondrial membrane polarization, leading to apoptosis, indicating that viral replication is stimulated and can promote carcinogenesis. Deshpande *et al*.[46] observed the effect of flax oil (FO) in a mouse ectopic model of cervical cancer and found that the expression of

oncoproteins (E6 and E7) was decreased**,** plasma antioxidant capacity was increased, and tumor growth was reduced.

In our study, we observed that, among HPV-infected women, those with the variant *GSTP1* genotype showed an increase in both of the oxidative stress indicators analyzed (lipid peroxidation and carbonyl groups). Among the uninfected women, those with the *GSTP1* variant genotype showed a decrease in the thiol groups and catalase activity. The amount of thiol groups, particularly in proteins, contributes to the plasma antioxidant capacity.[23] This finding suggests that the variant *GSTP1* genotype may contribute, together with HPV, to increased oxidative stress, which favors viral proliferation and increases the risk of cell damage. This polymorphism may also increase the probability of successful HPV infection in women with decreased antioxidant defenses. However, HPV infection or smoking alone can cause oxidative damage and may be confounding factors when polymorphisms in *GSTP1* are analyzed and associated with oxidative stress.

The amino acid change (Ile105Val) in both homozygous and heterozygous variants of *GSTP1* may result in decreased protein activity, a loss in affinity to electrophilic compounds, and an increased risk of neoplasms[47]. However, our findings indicate that women infected by HPV with the variant *GSTP1* (Ile105Val) genotype are at greater risk of redox imbalance, and consequently of cytological lesions. Taken together, our results support other findings in the literature that highlight anti-inflammatory and antioxidant agents as promising therapeutic compounds against HPV infection.[40, 43, 48]

## Conclusion

The findings presented in this study demonstrated that protein oxidation increased and catalase activity decreased in women infected with HPV, particularly those with high-risk oncogenic HPV. We found a significant association between the presence of the GSTP1 allelic variant (Ile105Val) and cervical injury among infected women. The presence of a variant genotype led to an increased expression of oxidative stress markers (lipid peroxidation and protein oxidation) among women with HPV infection and decreased antioxidant capacity (thiol groups and catalase enzyme activity) in women not infected by HPV.

**Conflicts of interest:** Nothing to declare.

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