Original Research Article

Association between *GSTP1* (rs1695) and Human Papillomavirus infection in women from southern Brazil

ABSTRACT

Background: Polymorphic variations in enzyme genes involved in the detoxification of oxidative stress products such as glutatations have been associated with cervical carcinogenesis. However, little is known about how to affect human papillomavirus (HPV) infection, a key factor in neoplasia. **Objective:** The objective was to investigate the possible association between the GSTP1 (rs1695) polymorphism and susceptibility to viral infection in women treated by the public health system in Southwest Paraná, Brazil. **Methodology:** We carried out a case-control study involving 140 women, of which 39 were infected with HPV and 101 were not. The GSTP1 (rs1695) polymorphism was detected by the Tetra Amplification Refractory Mutation System (T-ARMS) method using occurrence in the polymerase chain in 138 of them. To our knowledge, this study was the first carried out in Southern Brazil that sought the association between rs1695 and susceptibility to HPV infection. **Results:** The results showed a significant difference between the variants. The A allele (Ile) was protective against therapy both alone (OR: 0.175; 95% CI 0.071-0.434; $P < 0.001$) and in homozygosity (OR: 0.237; 95% CI 0.091-0.616; $P < 0.003$). On the other hand, the G (Val) allele was defined as a risk factor for HPV infection (OR: 4.22; 95% CI 1.623-10.989; $P < 0.003$), increasing the risk by approximately four times; in heterozygosity (AG), the risk of viral infection was even higher (OR: 5.714; 95% CI 2.303-14.180; $P < 0.001$). **Conclusion:** Our findings showed that the G allele and the AG genotype are specific for the risk of HPV infection in the study population.

eywords: Human papillomavirus; Genetic polymorphism; Glutathione S-transferase. Sexually Transmitted Infections.

1. INTRODUCTION

Sexually transmitted infections (STI) caused by human papillomavirus (HPV) are the most prevalent worldwide [1]. HPV is a virus with strong epithelial tropism, which leads to condylomas and lesions. While many of the HPV manifestations tend to disappear within six months to a year, high-risk oncogenic strains of HPV (HR-HPV) can persist and cause cervical cancer [2, 5]. The virus accounts for 5% of all human cancers, particularly anogenital (e.g., cervical, vaginal, vulvar, penile, and anal) and oropharyngeal cancers [1].

The major HR-HPV strains found in cervical tumors are 16, 18, 31, 51, and 52 [6, 7], with viral genetic material present in almost all cases [6, 8-10]. The current consensus is that the

presence of HPV is an important, but not the only, factor in cancer progression [11]. Host-related genetic factors (e.g., single nucleotide polymorphisms [SNPs], which are variations in DNA sequence detected at relevant sites in the genome) have been studied in relation to cancer susceptibility and risk for STIs [12-14].

Glutathione S-transferases (GSTs) are a group of mitochondrial, microsomal, and cytosolic enzymes encoded by genes that detoxify metabolites and toxic compounds and protect tissues from oxidative damage, especially from xenobiotics [15-18]. Eight classes of GSTs have been identified: alpha (α), mu (μ), pi (π), theta (θ), sigma (σ), kappa (κ), omega (ω), and zeta (ζ) [17].

The *GSTP* (pi) gene is located on chromosome 11q13.2 and encodes the P1 isoenzyme, which plays a role in cellular protection during apoptosis by metabolizing low molecular weight halogenated compounds [19]. The gene has nine exons. One of the most studied SNPs is the A (adenine) to G (guanine) transition at nucleotide 313, codon 105 of exon 5 (rs1695), which results in substituting the amino acid isoleucine by valine (Ile → Val) [20]. This nonsynonymous substitution alters the catalytic activity of *GSTP1*, where the allelic variation leads to reduced enzymatic function in many tissues as well as the inability to inactivate mutagenic agents, allowing sensitization of cells to free radicals [20-22].

Polymorphic variations in genes encoding enzymes involved in detoxifying exogenous and endogenous oxidative stress products belonging to the GST family have been studied to understand their association with cervical carcinogenesis [23, 24]. However, few studies have associated GST SNPs with HPV infection [14, 19, 25, 26], although these SNPs are targets for understanding the detoxification of harmful compounds in other viral infections [27, 28].

Researchers, including us, conducted a systematic review of polymorphisms in other glutathione genes (i.e., *GSTM1* and *GSTT1*) and STIs and showed that the null *GSTT1* allele is more common than the null *GSTM1* allele in women infected with HR-HPV [14]. Following the same line of investigation, our group showed that in women from Southwest Paraná, Brazil, the deleted *GSTT1* allele reduced their odds of viral infection (OR_{adj} 0.219; 95% CI 0.078-0.618; *P*=0.004) compared to those with the ancestral allele [14]. This study was important for advancing the understanding of GST polymorphisms in HPV-caused STIs; other SNPs in this family may have important virus-related implications that have not yet been explored. Therefore, the aim of the study was to investigate whether the *GSTP1* polymorphism (rs1695) is associated with HPV infection in women served by the public health system in Southwest Paraná, Brazil.

2. METHODOLOGY

2.1 Characteristics of the study participants and sampling

A case-control study was designed with 138 women from a population of 423 who use the Brazilian Unified Health System (*Sistema Único de Saúde* [SUS]) for routine gynecological consultations in the city of Francisco Beltrão, state of Paraná, Brazil. Data were collected between September and October 2017. Collection sites included four Basic Health Units of the Family Health Strategy (*Antonio de Paiva Cantelmo*, *Cristo Rei*, *Industrial*, and *Pinheirinho*), a reference unit for outpatient gynecological and obstetric care, the Women's Institute, and the oncology center of the city. All participants had the inclusion criteria of having had their first sexual intercourse, excluding pregnant women. The case and control groups were age-matched (± 3 years) and included 39 women with positive HPV detection

and 101 women without viral infection. Individual interviews were conducted using a lit erature-based structured questionnaire to characterize the profile of the participants [29, 30].

During the gynecological visit, women underwent collection of material for Papanicolaou cytology, and the endocervical brush used for the collection was placed in a microtube with 2 mL of saline solution and kept at a temperature of -20ºC for subseq uent analyses [14, 28, 31]. Biological material storage, extraction, virus detection, and *GSTP1* polymorphism (rs1695) analysis were performed at the Laboratory of Molecular Biology and Human Cytogenetics of the State University of Western Paraná, in the city of Francisco Beltrão, state Paraná.

The study was approved by both the Human Research Ethics Committee and the National Research Ethics Commission (Opinion No. 2,254,450 and Certificate of Ethical Appraisal No. 72983817.5.0000.0107). After receiving detailed information about the research, women who agreed to enroll in the study signed the informed consent form to confirm their participation.

2.2 DNA Isolation and HPV Detection

From the stored initial sample, a 200 µl aliquot was taken for isolation of total genomic DNA according to the manufacturer's extraction protocol "Silica Columns DNA Extraction and Purification Kit" (*Nova Biotecnologia*, São Paulo, Brazil). The quality of the extraction was determined by amplification of a 268 bp segment of the human β-globin gene by polymerase chain reaction (PCR), synthesized with the *primers* GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') e PC04 (5'- CAACTTCATCCACGTTCACC-3') [32, 33].

For molecular detection of HPV, specific *primers* were used for in vitro synthesis of the coding region of the virus L1 gene, using *primers* MY09 (5'-CGTCCMAARGGAWACTGATC-3') e MY11 (5'-GCMCAGGGWCATAAYAATGG-3'), which amplify a 450 bp fragment; both procedures followed the same PCR conditions proposed previously [14, 33]. A HeLa cell DNA sample containing the HPV 18 genome was used as a positive control for virus detection. All amplicons were analyzed by 2% agarose gel electrophoresis under a potential difference of 150 volts for half an hour, visualized under ultraviolet (UV) light, and photodocumented.

2.3 GSTP1 Single Nucleotide Polymorphism (rs1695)

The Tetra Amplification Refractory Mutation System (T-ARMS) method via PCR for genotyping the *GSTP1* SNP (rs1695) was based on another study [34], with some adaptations. The primer pairs used were:

Forward outer 5'-CAGGTGTCAGGTGAGCTCTGAGCACC-3' and reverse outer 5'-ATAAGGGTGCAGGTTGTGTCTTGTCCCA-3' for the A allele (Ile) and;

Forward inner A/Ile 5'-CGTGGACCTCCTCCGCTGCAAATCCA-3' and reverse Inner 5'-GCTCACATAGTTGGTGTAGATGAGGGATAC-3' for the G allele (Val).

The final volume of each PCR reaction was 25 µl, with 10 mM Tris-HCl, 2 mM MgCl2, 0.1 mM dNTPs, 0.5 µM of each primer, 1.25 U Taq DNA Polymerase (*Ludwig Biotecnologia Taq DNA Polymerase*, Brazil), and total DNA added at the end (50 ng/µl). Cycling steps included: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 35 seconds, 62°C for 35 seconds, 72°C for 50 seconds, and a final extension at 72°C for 10 minutes. The amplicons generated were 233 bp for the A allele, 290 bp for the G allele, and 467 bp for the

external *primers* or control band (Figure 1). Of the total number of participants (140), two controls did not amplify fragments of the gene studied, being excluded from the description for the polymorphism, possibly due to loss of quality of the sample collected (but which were maintained in the characterization of the participants)

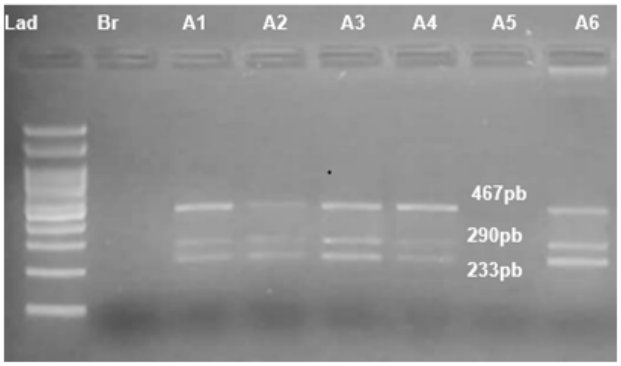

Figure 1 – Polymorphic profile of fragments generated for *GSTP1*. The A Allele (233pb), the G Allele (290pb), and control band (467pb).

2.4 Statistical analysis

Data were tabulated and analyzed using the Statistical Package for the Social Sciences (SPSS), version 24.0. Absolute and relative frequencies of variables were determined to characterize cases and controls. Allelic and genotypic frequencies were determined, and Hardy-Weinberg equilibrium (HWE) was tested using the chi-squared test (χ^2) for the rs1695 polymorphism, having two controls not ampliified in a previous step, thus the results of 39 cases and 99 controls. Yates' correction for continuity and Fisher's exact test were used to test the association between SNP and HPV infection ($p < 0.20$). Logistic regression analysis was performed, and the odds ratio (OR) was determined considering α less than 5% ($p < 0.05$) and 95% confidence interval (CI). In the logistic regression, marital status, smoking habits, contraceptive use, and current cytology results were included as confounders.

3. RESULTS

The sociodemographic, behavioral, and gynecologic characteristics of the study participants are shown in Table 1. Most of **the** cases (59%, median = 33) and controls (61.4%, median = 42) were younger than 40 years, which represents a population of young women. White race was predominant in both groups, which is typical for the Southwest Paraná. Marital status showed that the majority were in a stable relationship or married and had more than eight years of education. Lifestyle habits showed low alcohol consumption and low frequency of female smokers in both groups.

Age at first sexual intercourse showed that 69.2% of cases and 75.2% of controls started their sexual life at age 18 or younger. Two or more lifetime sexual partners were reported for 69.2% of cases, but most women did not have a new partner in the last year. Contraceptive use was most commonly reported, but condom use was less commonly reported by the participants, a condition that exposed them to various STIs. Although most participants did not have a history of STIs, HPV infection was the most common (10%) among those who did. This means that 7.7% of the case group were already infected with the virus, as were 10.9% of the women in the control group. With regard to cervical abnormalities, these were found mainly in the cases but without statistical significance. Low-grade squamous intraepithelial lesion (LSIL) had the highest prevalence (7.7%), followed by high-grade

squamous intraepithelial lesion (HSIL) and atypical squamous cells of undetermined significance (ASC-US), both present in 5.1% of cases. In terms of cervical microbiota, *Gardnerella vaginalis* was the most common in the population studied.

Table 1. Sociodemographic, behavioral, and gynecological characteristics of women treated in the city of Francisco Beltrão, state of Paraná.

Variables	Sociodemographic characteristics			
	Cases (n = 39)	**Controls (n = 101)**	**Total**	***P****
Age (mean ± SD)	39.64 (± 15.19). range: 19 to 68 years	40.34 (± 13.81). range: 19 to 74 years		
≤ 40 years	23 (59%)	62 (61.4%)	85 (60.7%)	0.945
> 40 years	16 (41%)	39 (38.6%)	55 (39.3%)	
Race				
White	30 (76.9%)	79 (78.2%)	109 (77.9%)	1
Others	9 (23.1%)	22 (21.8%)	31 (22.1%)	
Marital status				**0.073**
Married or in a stable relationship	24 (61.5%)	79 (78.2%)	103 (73.6%)	
Single, divorced, or widowed	15 (38.5%)	22 (21.8%)	37 (26.4%)	
Schooling				0.841
≤ 8 years	14 (35.9%)	33 (32.7%)	47 (33.6%)	
> 8 years	25 (64.1%)	68 (67.3%)	93 (66.4%)	
Compartmental patterns				
Alcohol intake				0.799
Yes (up to twice in a week)	19 (48.7%)	45 (44.6%)	64 (45.7%)	
No	20 (51.3%)	56 (55.4%)	76 (54.3%)	
Smoking habit				**0.141**
Never smoked	26 (66.7%)	81 (80.2%)	107 (76.4%)	
Yes/ex-smoker	13 (33.3%)	20 (19.8%)	33 (23.6%)	
Sexual and gynecological patterns				
Age at first intercourse				0.61
≤ 18 years	27 (69.2%)	76 (75.2%)	103 (73.6%)	
> 18 years	12 (30.8%)	25 (24.8%)	37 (26.4%)	
Number of sexual partners in lifetime				0.504
Only 1	12 (30.8%)	39 (38.6%)	51 (36.4%)	
Two or more	27 (69.2%)	62 (61.4%)	89 (63.3%)	
Number of sexual partners in the last year				0.477
None	28 (71.8%)	80 (79.2%)	108 (77.1%)	
One or more	11 (28.2%)	21 (20.8%)	32 (22.9%)	
Parity				0.948
No children	9 (23.1%)	21 (20.8%)	30 (21.4%)	
At least one child	30 (76.9%)	80 (79.2%)	110 (78.6%)	
Contraceptives				**0.191**
No/never used	7 (17.9%)	31 (30.7%)	38 (27.1%)	
Yes, and have used	32 (82.1%)	70 (69.3%)	102 (72.9%)	
Time of use				0.405
Does not use	9 (23.1%)	33 (32.7%)	42 (30%)	
≤ 1 year	3 (7.7%)	3 (3%)	6 (4.3%)	

≤ 5 years	11 (28.2%)	21 (43.6%)	32 (22.9%)	
> 5 years	16 (41.0%)	44 (43.6)	60 (42.9%)	
Use of preservative				0.288
Yes/sometimes	18 (46.2%)	35 (34.7%)	53 (37.9%)	
No	21 (53.8%)	66 (65.3%)	87 (62.1%)	
Oral sex practice				0.909
Yes	15 (38.5%)	36 (35.6%)	51 (36.4%)	
No	24 (61.5%)	65 (64.4%)	89 (63.6%)	
History of sexually transmitted infections				0.663
Yes	4 (10.3%)	15 (14.9%)	19 (13.6%)	
No	35 (89.7%)	86 (85.1%)	121 (86.4%)	
Recent history of vaginitis				0.5
Yes	17 (43.6%)	36 (35.6%)	53 (37.9%)	
No	22 (56.4%)	65 (64.4%)	87 (62.1%)	
Cytopathology report				**0.003**
Negative for neoplasm	31 (79.5%)	99 (98.0%)	130 (92.9%)	
LSIL	3 (7.7%)	1 (1.0%)	4 (2.9%)	
HSIL	2 (5.1%)	—	2 (1.4%)	
ASC/AGC	1 (2.6%)	—	1 (0.7%)	
ASC-US	2 (5.1%)	—	2 (1.4%)	
Microbiological analysis				0.256
Normal	30 (77%)	76 (75.2%)	106 (75.7%)	
Gardnerella vaginalis	6 (15.4%)	15 (14.9%)	21 (15%)	
Candida albicans	—	5 (5.0%)	5 (3.6%)	
Unsatisfactory	3 (7.7%)	5 (5.0%)	8 (5.7%)	

LSIL – Low-Grade Squamous Intraepithelial Lesion; HSIL – High-Grade Squamous Intraepithelial Lesion; ASC/AGC – Atypical Squamous or Glandular Cells; ASC-US – Atypical Squamous Cells of Undetermined Significance. *P** = Significance of the χ^2 test, considered to be less than 0.20 (P < 0.20).

Regarding the *GSTP1* polymorphism (rs1695), the distribution of allelic and genotypic frequencies in HPV-infected participants and controls is described in Table 2. Genotypic frequencies are presented in HWE. In the study population, the A allele was more common than the G allele, with frequencies of 63.04% and 36.96%, respectively. Among the homozygous genotypes, AA had a frequency of 35.5% and GG 9.4%, and the heterozygous combination AG was the most frequent with 55.1%. In two controls, there was no amplification of *GSTP1*, so the final number for the group was 138 women.

Table 2. Observed allele and genotype frequencies and Hardy-Weinberg Equilibrium in the population.

SNPs	**Genotypes**				**Alleles**	
***GSTP1* (rs1695)**	**AA**	**GG**	**AG**	**0.156**	**A (Ile/ancestral)**	**G (Val/variant)**
Observed number	49	13	76		174	102
Observed frequency	35.50%	9.40%	55.10%		63%	37%
Expected frequency (HWE)	39.70%	13.70%	46.60%		NA	NA

GSTP1 - Glutathione S-Transferase P1. χ^2 test with 95% significance for HWE. NA (not applicable).

The results showed a significant difference in *GSTP1* (rs1695) polymorphic variants between women with and without STI. The A allele (OR: 0.175; 95% CI 0.071-0.434; *P* < 0.001), either in isolation or in homozygosity (OR: 0.237; 95% CI 0.091-0.616; *P* < 0.003), showed protective effects against viral infection. In contrast, the G allele was shown to be a risk factor for STI (OR: 4.22; 95% CI 1.623-10.989; *P* < 0.003), increasing the odds of infection more than fourfold compared to those with the A allele. For the allelic combinations, a similar

result was obtained for the heterozygous genotype (AG), which increased the susceptibility of women to infection by almost six times (OR: 5.714; 95% CI 2.303-14.180; *P* < 0.001) compared to homozygous genotypes (Table 3).

Table 3. Distribution of allele and genotype frequencies of the *GSTP1* SNP (rs1695) in the study population.

Polymorphism	Women in the study (n = 138)	Cases (n = 39)	Control (n = 99)	*P* - value*	OR (95% CI)	*P* - value**
Alleles rs1695						
A (ancestral)	174 (63.04%)	44 (56.41%)	130 (65.66%)	< 0.001	0.175 (0.071-0.434)	< 0.001
G	102 (36.96%)	34 (43.59%)	68 (34.34%)	0.004	4.22 (1.623-10.989)	0.003
Genotypes (rs1695)						
AA	49 (35.5%)	6 (12.2%)	43 (87.8%)	< 0.001	1 (*Reference*)	
GG	13 (9.4%)	1 (7.7%)	12 (93.3%)		0.191 (0.024-1.520)	0.118
AG	76 (55.1%)	32 (42.1%)	44 (57.9%)		5.714 (2.303-14.180)	< 0.001

P *: Significance of the chi-squared test (*P* < 0.20). Consider the line for the sum of cases and controls in the X2 distribution; *P* **: Significance of logistic regression (*P* < 0.05).

4. DISCUSSION

To date, this is the first Brazilian study conducted in the Southern Brazil to investigate the association between the SNP rs1695 of the *GSTP1* gene and susceptibility to HPV infection. The persistent state of viral infection and the factors that contribute to the entry of the virus into the body strengthen the assumption that evasion of host defense and detoxification mechanisms is an important step in HPV-related tumor progression [5], supporting the aim of the study.

The results of the study suggest that susceptibility to HPV infection may be associated with the G allele and its heterozygous state for the rs1695 polymorphism of the *GSTP1* gene. Conversely, the A allele, when isolated and in its homozygous form, may be considered a protective factor against viral infection in the participants.

Previous studies by the research group showed that the theta class (*GSTT*) of GSTs showed a protective association against HPV infection in women with nullity for the *GSTT1* gene (OR_{adj} 0.219; 95% CI; 0.078-0.618; *P* = 0.004) [14]. In the same study, no association was found for *GSTM1* variations. The results of the *GSTP1* rs1695 SNP, analyzed in the same geographical area, indicate a higher risk of infection in women with the G allele alone (OR_{adj} 4.22; 95% CI 1.623-10.989; *P* = 0.003) or in heterozygosity (AG) (OR_{adj} 5.714 95% CI 2.303-14.180; *P* < 0.001). The A allele (ancestral) was characterized as a protective factor against STIs, as was the AA genotype (OR_{adj} 0.237 95% CI 0.091-0.616; *P* < 0.001). Interestingly, patients with the homozygous genotype (GG) showed no significant association, suggesting that only one copy of the G allele influences viral infection.

A study conducted in the state of Pernambuco, Brazil [19] found no independent association between rs1695 and infection risk; however, it highlighted a synergy between oral contraceptive use and viral infection with the SNP, the combination of which may lead to the emergence of cervical lesions because of the persistence of viral infection. Similarly, other studies found no association between the rs1695 polymorphism and cervical cancer or susceptibility to HPV infection [16, 26, 35].

An important finding **has highlighted** that Brazilian women with the AG and GG genotypes for the rs1695 SNP and infected with HPV have a 29-fold increased risk of developing cervical lesions when associated with smoking [26]. A meta-analysis found that the AA genotypic variant of *GSTP1* was not associated with the risk of developing cervical lesions [27], similar to our findings, where the allelic and homozygous presence of A was not associated with susceptibility to STIs. The *GSTP1* gene is involved in the metabolism of various carcinogenic substances, such as those found in cigarettes (polycyclic aromatic hydrocarbons), and the impairment of *GSTP1* enzymatic activity in the respective genotypes affects the detoxification of harmful substances in the body [36].

The present study focused on verifying the association between the SNP and viral infection, and when a possible interaction with smoking was included, no association was demonstrated. On the other hand, a possible loss or impairment of enzymatic function in the presence of the G allele may favor HPV infection, according to the results presented. The altered activity of glutathione because of genetic variations increases the risk of cancer, possibly by reducing cellular protection against damage and DNA mutations generated by the restriction of enzymatic activity. Decreased activity of antioxidant enzymes, including GSTs, has been described in patients with cervical cancer. The deficiency of these enzymes favors the progression of HPV infection by activating the adaptor complex AP-1, a fundamental factor for the expression of the viral oncoproteins E6 and E7, which are involved in the proliferation of HPV [26].

The vast majority of studies **sought** to understand the interference of genetic polymorphisms with the development or evolution of various types of cancer, including cervical cancer [20-23]. In addition to neoplasms, SNPs have also been studied in a number of clinical conditions, such as diabetes [37, 38], metabolic syndrome [39], and pre-eclampsia [40, 41]. However, the results are contradictory. In Thai women, the presence of the G allele in homozygosity or heterozygosity for rs1695 contributed to a reduction in the risk of developing cervical cancer [25]. A meta-analysis conducted by Indian researchers presented a different conclusion, confirming that the presence of the G allele contributes to a higher susceptibility to various types of cancer [42]. Our focus was to analyze *GSTP1* (rs1695) in relation to susceptibility to HPV infection, which makes the results presented here important since the presence of the virus is the main factor for lesions and cervical carcinogenesis [4, 5].

The high prevalence of HPV infection in women classified as young adults has been demonstrated in several studies [6, 7, 26]. In Brazil, the prevalence rate of HPV infection was 53.6% in 2020 [7]. Another study reported HR-HPV subtypes in Brazilian women at 28.4% [43], while the estimated prevalence in North American women aged 20 to 40 years was 42.5% [44]. On the other hand, a Brazilian study found a low prevalence (6.8%) [45] in women from the same geographic region as the present study. The findings that the G allele was less frequent than the A allele and that it increases susceptibility to STIs allows us to conclude that the reduced prevalence of viral infection corresponds to the risk generated by the allelic form. Although there is still no molecular basis for this relationship, knowing the genetic profile of the population can characterize groups with greater susceptibility to viral infection and contribute to the prognosis and evolution of the infection.

Although an association between the risk of HPV infection and the characteristics of our participants could not be demonstrated, other studies have shown that sexual behaviors (e.g., age at first intercourse, number of sexual partners, and lifetime parity) tend to increase susceptibility to HPV infection [46, 47]. Furthermore, it was not possible to establish associations between the presence or absence of viral infection and cervical lesions because only a small fraction of cases and controls had them.

Although the study did not consist of a large sample of participants, care was taken to match cases and controls to minimize or eliminate research limitations.

5. CONCLUSION

Our results showed that the G allele and the heterozygous form of the rs1695 SNP of the *GSTP1* gene are risk factors for HPV infection in women. Therefore, women from Southwestern Paraná with only the G allele and with the AG genotype, respectively, are four to six times more susceptible to HPV infection compared to the control group. Further studies regarding the association of the rs1695 polymorphism of the *GSTP* gene in larger samples would be important to understand results such as those found in this study.

CONSENT

All authors informed consent.

ETHICAL APPROVAL

All authors declare that all experiments were examined and approved by the ethics committee of the State University of Western Paraná under opinion No. 2.254.450 and, therefore, were carried out in accordance with the ethical standards established in the Declaration of Helsinki of 1964.

Declaration of role played by each co-author – should be included
Acknowledgement – to be included thanking any one or group that helped the authors in the research or write up of the article
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REFERENCES
REFERENCES MUST BE CONSISTENT AND WRITTEN IN FULL

1. Haręża DA, Wilczyński JR, Paradowska E. Human Papillomaviruses as Infectious Agents in Gynecological Cancers Oncogenic Properties of Viral Proteins. IJMS. 2022; 23(3):1818.
2. Leto MGP., Santos Júnior GF Porro, A. M., & Tomimori, J. Human papillomavirus infection: Etiopathogenesis, molecular biology and clinical manifestations. An Bras Dermatol. 2011; 86(2):306–17.
3. Balasubramaniam SD, Balakrishnan V, Oon CE, Kaur G. Key Molecular Events in Cervical Cancer Development. Medicina. 2019; 55(7): 384.
4. Muñoz N, Castellsagué X, de González AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine. 2006; 24:S1–10.
5. Lebeau A, Bruyere D, Roncarati P, Peixoto P, Hervouet E, Cobraiville G, et al. HPV infection alters vaginal microbiome through down-regulating host mucosal innate peptides used by Lactobacilli as amino acid sources. Nat Commun. 2022; 13(1):1076.

6. Sargent A, Bailey A, Almonte M, Turner A, Thomson C, et al. Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. Br J Cancer. 2008; 98(10):1704–9.
7. Wendland EM, Villa LL, Unger ER, Domingues CM, Benzaken AS. POP-Brazil Study Group, et al. Prevalence of HPV infection among sexually active adolescents and young adults in Brazil: The POP-Brazil Study. Sci Rep. 2020; 10(1):4920.
8. Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol. 2015; 25(S1):2–23.
9. Graham SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clin Sci; 2017; 131(17):2201–21.
10. Kombe Kombe AJ, Li B, Zahid A, Mengist HM, Bounda GA, Zhou Y, et al. Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation. Front Public Health. 2021; 8:552028.
11. McBride AA. Human papillomaviruses: diversity, infection and host interactions. Nat Rev Microbiol. 2022; 20(2):95–108.
12. Deng N, Zhou H, Fan H, Yuan Y. Single nucleotide polymorphisms and cancer susceptibility. 2017; 8(66): 110635-649.
13. Shao W, Xia H, Lan Q, Gu J, Huang H, Zheng F, et al. Polymorphism rs2682818 participates in the progression of colorectal carcinoma via miR-618-TIMP1 regulatory axis. Sci Rep. 2021; 11(1):23186.
14. Bortolli APR, Vieira VK, Treco IC, Pascotto CR, Wendt GW, Lucio LC. GSTT1 and GSTM1 polymorphisms with human papillomavirus infection in women from southern Brazil: a case–control study. Mol Biol Rep. 2022; 49(7):6467–74.
15. Oakley A. Glutathione transferases: a structural perspective. Drug Metab Rev. 2011; 43(2):138–51.
16. Kiran B, Karkucak M, Ozan H, Yakut T, Ozerkan K, Sag S, et al. *GST* (*GSTM1* , *GSTT1* , and *GSTP1*) polymorphisms in the genetic susceptibility of Turkish patients to cervical cancer. J Gynecol Oncol. 2010; 21(3):169.
17. Hollman A, Tchounwou P, Huang HC. The Association between Gene-Environment Interactions and Diseases Involving the Human GST Superfamily with SNP Variants. IJERPH. 2016; 13(4):379.
18. Ferreira DC, Jr VM, Cunha KSG, Janini MER, Curvedo JAR. Cytochrome P450 enzymes and their correlation with the risk for developing mouth cancer – a condition of art. R. Ci Méd Biol. 2007; 6(2):223-232.
19. Chagas BS, Gurgel APAD, Júnior SSLP, Lima RCP, Cordeiro MN, Moura RR, et al. Research Article Synergic effect of oral contraceptives, GSTP1 polymorphisms, and high-risk HPV infection in development of cervical lesions. Genet Mol Res [Internet]. 2017; 16(3): 16039742.
20. Elhoseiny S, El-Wakil M, Fawzy M, Rahman AA. GSTP1 (Ile105Val) Gene Polymorphism: Risk and Treatment Response in Chronic Myeloid Leukemia. JCT. 2014; 05(01):1–10.
21. Parl FF. Glutathione S-transferase genotypes and cancer risk (2005) Cancer Letters 221(2):123–9.
22. Ansolin PL, Damin DC, Alexandre CP. Polymorphism of Glutathione S-transferase M1, T1 and P1 and Association with Clinicopathological Aspects in Colorectal Carcinoma. Rev bras Coloproct. 2010; 30(3): 281-88.
23. Zhen S, Hu CM, Bian LH. Glutathione S-Transferase Polymorphism Interactions with Smoking Status and HPV Infection in Cervical Cancer Risk: An Evidence-Based Meta-Analysis. Coleman WB, organizador. PLoS ONE. 2013; 8(12):e83497.
24. Sharma V, Sonkar SC, Singhal P, Kumar A, Singh RK, Ramachandran VG, et al. Functional impact of allelic variations/haplotypes of TNF-α on reproductive tract infections in Indian women. Sci Rep. 2021; 11(1):627.

25. Phuthong S, Settheetham-Ishida W, Natphopsuk S, Ishida T. Genetic Polymorphism of the Glutathione S-transferase Pi 1 (GSTP1) and Susceptibility to Cervical Cancer in Human Papilloma Virus Infected Northeastern Thai Women. Asian Pac J Cancer Prev. 2018; 19 (2), 381-85.
26. Galvão AM et al. Glutathione-S-transferase P1 polymorphism and oxidative stress markers in women infected by human papilomavírus. Brazilian Journal of Research in Health Sciences. 2021; 8(15):3-13.
27. Tian S, Yang X, Zhang L, Zhao J, Pei M, Yu Y, et al. Polymorphic variants conferring genetic risk to cervical lesions support GSTs as important associated loci. Medicine. 2019; 98(41):e17487.
28. Aguiar ES de, Giacomazzi J, Schmidt AV, Bock H, Saraiva-Pereira ML, Schuler-Faccini L, et al. GSTM1, GSTT1, and GSTP1 polymorphisms, breast cancer risk factors and mammographic density in women submitted to breast cancer screening. Rev bras epidemiol. 2012; 15(2):246–55.
29. Nonnenmacher B, Breitenbach V, Villa LL, Prolla JC, Bozzetti MC. Identification of human papillomavirus by molecular biology in asymptomatic women. Rev Public Health. 2002; 36(1):95–100.
30. Oliveira GR, Vieira VC, Barral MFM, Döwich V, Soares MA, Conçalves CV, et al. Risk factors and prevalence of HPV infection in patients at Basic Health Units and a University Hospital in Southern Brazil. Rev Bras Ginecol Obstet. 2013; 35(5):226–32.
31. Treco IC, Vieira VK, Silva JC da, Treco FR, Ferreto LED, Lucio LC. Prevalence and factors associated to cervical changes in units from the Single Health System. Rev Gaúcha Enferm.. 2021; 42:e20200233.
32. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic Amplification of β-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia. Science. 1985; 230(4732):1350–4.
33. Trugilo KP, Cebinelli GCM, Berti FCB, Okuyama NCM, Cezar SF, Sena MM, et al. Polymorphisms in the TGFB1 signal peptide influence human papillomavirus infection and development of cervical lesions. Med Microbiol Immunol. 2019; 208(1):49–58.
34. Hashemi M, Eskandari-Nasab E, Fazaeli A, Bahari A, Hashemzehi NA, Shafieipour S, et al. Association of Genetic Polymorphisms of Glutathione-S-Transferase Genes (*GSTT1* , *GSTM1* , and *GSTP1*) and Susceptibility to Nonalcoholic Fatty Liver Disease in Zahedan, Southeast Iran. DNA and Cell Biology. 2012; 31(5):672–7.
35. Palma S, Novelli F, Padua L, Venuti A, Prignano G, Mariani L, et al. Interaction between glutathione-S-transferase polymorphisms, smoking habit, and HPV infection in cervical cancer risk. J Cancer Res Clin Oncol. 2010; 136(7):1101–9.
36. Zhao E, Hu K, Zhao Y. Associations of the glutathione S-transferase P1 Ile105Val genetic polymorphism with gynecological cancer susceptibility: a meta-analysis. Oncotarget. 2017; (25):41734–9.
37. Azarova I, Bushueva O, Konoplya A, Polonikov A. Glutathione S-transferase genes and the risk of type 2 diabetes mellitus: Role of sexual dimorphism, gene-gene and gene-smoking interactions in disease susceptibility. J of Diabetes. 2018; 10(5):398–407.
38. Montazeri-Najafabady N, Dabbaghmanesh MH, Namavar Jahromi B, Chatrabnous N, Chatrsimin, F. The impact of GSTM1 and GSTT1 polymorphisms on susceptibility to gestational diabetes in Iranian population. J. Matern Fetal Neonatal Med. 2022; 35(8): 1451–1456.
39. Oliveira JS, Boery R NOS. Association of polymorphic variants with metabolic syndrome: An integrative review. J Vasc Bras. 2018; 17(2):141–147.
40. Sljivancanin Jakovljevic T, Kontic-Vucinic O, Nikolic N, Carkic J, Soldatovic I, Milasin, J. Glutathione-S-transferase M1 polymorphism and pro-inflammatory cytokines tumour necrosis factor-α and interleukin-1β are associated with preeclampsia in Serbian women. Am J Reprod Immunol. 2019; 81(5):e13105.

41. Feyda Nursal A, Mervan Aytac H, Sentürk Ciftci H, Sila Yazar M, Oyaci Y, Pehlivan, M, Pehlivan S. TNF-α-308 G/A variant may be associated with bipolar disorder in a Turkish population. Arch Clin. 2020; 47(6).
42. Mandal RK, Mittal RD. Glutathione S-Transferase P1 313 (A > G) Ile105Val Polymorphism Contributes to Cancer Susceptibility in Indian Population: A Meta-analysis of 39 Case–Control Studies. Ind J Clin Biochem. 2020; 35(1):8–19.
43. Miranda, A. E., Silveira, M. F., Travassos, A. G., Tenório, T., Val, I. C. C., Lannoy, L., Mattos Junior, H. S., & Carvalho, N. S. High-risk papillomavirus infection among women living with human Immunodeficiency virus: Brazilian multicentric study. J Med Virol. 2017; 89(12), 2217–23.
44. Hariri S, Unger ER, Sternberg M, Dunne EF, Swan D, Patel S, et al. Prevalence of Genital Human Papillomavirus Among Females in the United States, the National Health and Nutrition Examination Survey, 2003–2006. J Infect Dis. 2011; 204(4):566–73.
45. Bortolli APR, Treco IC, Vieira VK, Pascotto CR, Ferreto LED, Wendt GW, et al. Prevalence of HPV and associated factors in a population of women living in southern Brazil. Braz J Microbiol. 2022; 53:1979–86.
46. Tay SK, Oon LLE. Prevalence of cervical human papillomavirus infection in healthy women is related to sexual behaviours and educational level: a cross-sectional study. Int J STD AIDS. 2014; 25(14):1013–21.
47. Mangieri LFL, Cezar-dos-Santos F, Trugilo KP, Watanabe MAE, de Jaime Curti RR, Castilha EP, et al. Cross-Sectional Analysis of Human Papillomavirus Infection and Cytological Abnormalities in Brazilian Women. Pathogens. 2023; 12(1):148.