GSTT1 and GSTM1 Polymorphisms with Human Papillomavirus Infection in Women from Southern Brazil: A Case-control Study

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Research Article

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Abstract

Background Some important factors for the most common sexually transmitted infection (STI) in the world caused by human papillomavirus (HPV) are well defined as early sexual activity, use of contraceptives, tobacco smoking, immunological and genetic factors. This study aimed to investigate the relationship between polymorphisms, GSTM1 and GSTT1, and HPV infection and associated risk factors in a group of women assisted in the public health system in southwestern Paraná, Brazil.

Methods and results A case-control study was designed, in which the case group consisted of 21 women with HPV, matched by age, with the control group, 84 women without the virus. Viral detection was conducted via Polymerase Chain Reaction (PCR) and GSTM1 and GSTT1 genotyping by Multiplex PCR. The results showed that the GSTT1 null allele was a protective factor against infection (OR_{adj} : 0.219; 95% CI 0.078-0.618; $p=0.004$). No relationship was observed for the GSTM1 gene. The smoking habit was defined as a risk factor (OR_{adj} : 3.678; 95% CI 1.111-12.171; $p=0.033$), increasing the chance for the presence of HPV by up to 3.6 times.

Conclusion In short, this study showed in an unprecedented way, the relationship between GSTM1 and GSTT1 genetic polymorphisms and HPV. The relationship found portrays protection from viral infection and not from susceptibility in the female population of southern Brazil.

Introduction

The Human Papillomavirus (HPV) is a virus capable of infecting both the skin and mucous membranes, comprising about 220 distinct subtypes [1]. From these, 40 have the potential to infect the anogenital tract, characterizing it as responsible for the most common sexually transmitted infection (STI) in the world [2]. Among the subtypes, 12 are defined as high risk for the development of cancer, including cervical (CC), with emphasis on 16 and 18 [3]. This type of cancer is the fourth most frequently diagnosed among women and the fourth leading cause of cancer death in the female population, with a global estimate of 604,000 new cases and 342,000 deaths in 2020 [4]. Although, HPV infection is the main risk factor for cervical cancer, alone, it is most often an insufficient event [5].

Several factors that play an important role in viral biology and disease progression are well defined in the literature, such as ethnicity [6], early onset of sexual activity [7], use of contraceptives [8], exposure to tobacco [6, 9], co-infection with more than one viral subtype [10], oxidative stress [11], immunological and genetic factors [12].

The cytosolic glutathione-S-transferase (GST) belong to a family of enzymes involved in phase II of xenobiotic metabolism and are divided into eight gene classes: alfa (α), mu (μ), pi (π), theta (θ), sigma (σ), kappa (κ), omega (ω) and zeta (ζ) [13]. The GSTM1 and GSTT1 genes located on chromosome 1p13.3 and 22q11.23, respectively, exhibit insertion and deletion polymorphism, and the null allele characterizes the absence of enzymatic function [14]. This condition has been related to oxidative stress, which therefore may be associated with pathologies such as diabetes mellitus, prostate cancer [15], breast [16]

and cervical cancer [17] and also, favor the replication of some pathogens such as the Acquired Immunodeficiency Virus (HIV) in different ethnic groups [18].

Although there are gaps, it is known that genes involved in the metabolism of xenobiotics can influence the natural history of HPV subtypes, especially those with high oncogenic risk [17]. Although several studies point to a relationship between the GSTM1 and GSTT1 null genotypes and cervical cancer [19] there are controversies among population groups, restricting the generalization of such association [20]. An Indian study found a higher frequency of GSTM1 and GSTT1 null alleles in women with cervical cancer infected with the virus [21]. However, most studies prioritize the relationship between deleted alleles with events triggered by viral infection and not with susceptibility to HPV infection [17, 22]. Sudenga et al. [17] found an association between HPV16 infection and GSTM1 and GSTT1 nullity, and between GSTM1 deleted with a lower persistence (or higher clearance) of the high-risk subtype.

In the recent systematic review, conducted by the authors of the present research, were gathered works that indirectly addressed the relationship between SNPs, GSTM1 and GSTT1 and high-risk HPV infection in women with or without cervical alterations [23]. Although still inconclusive, the study pointed to an expressive frequency of the GSTT1 null allele in women infected with high-risk HPV subtypes compared to the deleted GSTM1. Due to inconsistencies on the subject and the absence, so far, of studies on the direct relationship between SNPs and viral infection, the present study aimed to investigate the relationship between polymorphisms, GSTM1 and GSTT1, with the infection caused by HPV and associated risk factors in a group of women assisted by the public health system in southwestern Paraná, Brazil.

Materials And Methods

Study population and sampling

A case-control study was conducted with 105 women who attended Health Units (HU) for routine gynecological consultation in the municipality of Francisco Beltrão, State of Paraná, Brazil, between September and October 2017. The HU included in the study were Family Health Strategy (FHS) Antônio de Paiva Cantelmo, Cristo Rei, Industrial, Pinheirinho and a reference in gynecology and obstetrics at an outpatient level, the Women's Institute (*Instituto da Mulher* - IM). All participants had the inclusion criteria of having had their first sexual intercourse, excluding women in the condition of pregnant women. The case and control groups were matched by age (± 2 years) comprising, respectively, 21 women with positive detection for HPV and 84 with no viral infection.

In addition to the GSTM1 and GSTT1 polymorphism, potential factors associated with HPV infection, such as tobacco smoking, alcohol consumption, use of contraceptives and vaginal infection, all with potential xenobiotic characteristics, were evaluated. In addition to race, a relevant variable when considering the investigated polymorphisms. All information on these variables were obtained through interviews conducted individually from a validated questionnaire [24].

During the gynecological consultation, the women underwent collection of material for the Pap smear, and the endocervical brush used in the collection was placed in a microtube with 2mL of saline solution, kept at a temperature of -20°C until virus detection [24]. The storage of biological material, extraction of genetic material, viral detection and GST polymorphisms were conducted at the Molecular Biology Laboratory of the *Universidade Estadual do Oeste do Paraná*, in Francisco Beltrão.

The project was approved by the Research Ethics Committee involving Human Beings (REC) and the National Research Ethics Committee (CONEP) through opinion No. 2.254.450 and CAAE: 72983817.5.0000.0107. After receiving information about the research, the women who agreed to participate signed the Free and Informed Consent Form (FICF) (Appendix I) to be included in the study.

Total DNA extraction and HPV detection

A 200µl aliquot of the original sample was used to isolate the total genetic material, following the extraction and purification protocol “Biological Fluid/Blood Genomic DNA extraction kit” – “Purelink® Genomic DNA Mini Kit” (Invitrogen by Thermo Fisher Scientific, Carlsbad, California) according to the manufacturer’s instructions and stored in a freezer at -20°C. For molecular detection of HPV, specific primers were used for in vitro synthesis of the coding region of the virus L1 gene, MY09 (5'-CGTCCMAARGGAWACTGATC-3') and MY11 (5'-GCMCAGGGWCATAAYAATGG-3'), producing a fragment of 450 bp. The final volume of each PCR (Polymerase Chain Reaction) reaction was 25 µl, containing, 10 mM Tris-HCl, 2 mM MgCl₂, 0.1 mM dNTPs, 0.5 µM each primer, 1,25U of Taq DNA polymerase (Ludwig Biotechnology Taq DNA polymerase, Brazil), adding 3.5µl of total DNA at the end (50ng/µl). To control the extraction and synthesis process, PCR of a 268bp segment of the human β-globin gene was performed in all samples, using primers GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') and PCO4 (5'-CAACTTCATCCACGTTACAC-3') [25]. The amplifications of both genomes were processed in thermocycler Applied Biosystems Veriti Thermal Cycler (ThermoFisher Scientific, Germany), following the steps: 10min at 94°C, followed by 37 cycles of 1 min at 94°C; 1min at 55°C and 1 min at 72°C; finishing with extension for 10 min at 72°C [25]. As a positive control for viral detection, a DNA sample from HeLLa cells (HPV 16) was included). All amplicons were fractionated via 2% agarose gel electrophoresis, stained with ethidium bromide, visualized under ultraviolet light (UV) and photodocumented.

GSTM1 and GSTT1 genotyping

Genotyping was performed by Multiplex PCR. The primer pairs used were 5'-GAACTCCCTGAAAAGCTAAAGC-3' (forward) e 5'-GTTGGGCTCAAATA TACGGTGG-3' (reverse) for GSTM1 and 5'-TTCCTTACTGGTCCTCACATCTC-3' (Forward) and 5'-TCACCGGATCATGGCCAGCA-3' (reverse) for GSTT1. The first produces an amplicon of 219bp and the second of 459bp [26]. PCR conditions included initial denaturation at 94°C for 5min, followed by 35 cycles of 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute, ending at 72°C for 10 minutes. The genotypes were fractionated via 2% agarose gel electrophoresis, stained with ethidium bromide, visualized and photodocumented. The presence of amplicons characterizes the functional alleles and the absence of null alleles.

Statistical analysis

The genotypic frequencies, GSTM1 and GSTT1, and the variables tobacco smoking, alcohol consumption, use of contraceptives, vaginal infection and race were determined and compared between cases and controls using the Chi-square test (X^2), with continuity correction for Yates and Fisher. Variables with a significance value less than 0.20 were used for multivariate logistic regression, with a 95% confidence interval and $p < 0.05$, to determine the risk factors for STIs. All statistical analyzes were performed using the software Statistical Package for Social Sciences (SPSS) version 24.0.

Results

The genotypic pattern of the GSTM1 and GSTT1 genes can be seen in Figure 1 and the allele frequency distribution are shown in Table 1. The GSTM1 null allele (GSTM1-) was more frequent both in cases (57.1%) and in controls (75.0%). In contrast, the GSTT1 null (GSTT1-) was less frequent (38.1%) in the case group when compared to the GSTT1+ allele (61.9%) and was more frequent in the control group (73.8%). Furthermore, the most common allelic combination in women with HPV was GSTM1-/GSTT1+ and in the control group it was the double deletion.

Table 1
Distribution of GST genotypes among the cases and controls of a group of women from the municipality of Francisco Beltrão, Paraná.

Polymorphism	Cases (n=21)	Controls (n= 84)	Total (n= 105)
GSTM1			
Null/GSTM1-	12 (57.1%)	63 (75.0%)	75 (71.43%)
Present/ GSTM1+	9 (42.9%)	21 (25.0%)	30 (28.57%)
GSTT1			
Null/GSTT1-	8 (38.1%)	62 (73.8%)	70 (66.7%)
Present/GSTT1+	13 (61.9%)	22 (26.2%)	35 (33.3%)
Combinations			
GSTM1+/GSTT1+	6 (28.6%)	15 (17.9%)	21 (20%)
GSTM1+/GSTT1-	3 (14.3%)	7 (8.3%)	10 (9.53%)
GSTM1-/GSTT1+	7 (33.3%)	7 (8.3%)	14 (13.33%)
GSTM1-/GSTT1-	5 (23.8%)	55 (65%)	60 (57.14%)

Table 2 concentrates the results of the bivariate analysis (Chi-square test, X^2) suggesting associations between GST polymorphisms, life habits, vaginal infection, and race between groups. The choice of

variables was based on the possible relationship with xenobiotic metabolism, which is directly linked to GST genes. All categories established for the GST polymorphism suggested associations in the study (GSTM1, $p=0.177$; GSTT1, $p=0.004$; Combinations, $p=0.002$). As well as the smoking habit ($p=0.040$), the presence of recent vaginal infection ($p=0.132$) and race ($p=0.043$).

Although both the use of contraceptives and the consumption of alcohol did not show a significant association, 81% and 61.9% of women with HPV, respectively, use or have used the medication and consume some type of alcoholic beverage (Table 2).

Table 2

– Genetic polymorphism, life habits, vaginal infection and race associated with HPV in women from the municipality of Francisco Beltrão, Paraná.

Municipality of Francisco Beltrão, Paraná.					
Variables	Cases (n= 21)		Controls (n= 84)	Total	p value
GSTM1					
Null/GSTM1-	12 (57.1%)		63 (75.0%)	75 (71.4%)	0.177 ^b
Present/ GSTM1+	9 (42.9%)		21 (25.0%)	30 (28.6%)	
GSTT1					
Null/GSTT1-	8 (38.1%)		62 (73.8%)	70 (66.7%)	0.004 ^b
Present/GSTT1+	13 (61.9%)		22 (26.2%)	35 (33.3%)	
Combinations					
GSTM1+/GSTT1+	6 (28.6%)		15 (17.9%)	21 (20.0%)	0.002 ^c
GSTM1+ /GSTT1-	3 (14.3%)		7 (8.3%)	10 (9.5%)	
GSTM1-/GSTT1+	7 (33.3%)		7 (8.3%)	14 (13.3%)	
GSTM1 - /GSTT1-	5 (23.8%)		55 (65.5%)	60 (57.1%)	
Contraceptive					
No/Never used	4 (19.0%)		17 (20.2%)	21 (20.0%)	1.000 ^c
yes and already used	17 (81.0%)		67 (79.8%)	84 (80.0%)	
Smoking habit					
Never smoked	14 (66.7%)		74 (88.1%)	88 (83.8%)	0.040 ^b
Yes/ex-smoker	7 (33.3%)		10 (11.9%)	17 (16.2%)	
Alcohol consumption					
No	8 (38.1%)		42 (50.0%)	50 (47.6%)	0.464 ^b
Yes	13 (61.9%)		42 (50.0%)	55 (52.4%)	
Vaginal Infection					
No	10 (47.6%)	55 (65.5%)	65 (61.9%)	0.132 ^a	
Yes	11 (52.4%)	29 (34.5%)	40 (38.1%)		

Notes: ^aPearson's chi-square test; ^bChi-square with Yates's correction for continuity; ^cFisher's exact test. Associations that followed for multivariate analysis with p<0.20.

Variables	Cases (n= 21)	Controls (n= 84)	Total	<i>p</i> value
Race				
White	14 (66.7%)	72 (85.7%)	86 (81.9%)	0.043 ^b
Others	7 (33.3%)	12 (14.3%)	19 (18.1%)	
Notes: ^a Pearson's chi-square test; ^b Chi-square with Yates's correction for continuity; ^c Fisher's exact test. Associations that followed for multivariate analysis with $p < 0.20$.				

Table 3 shows the variables that remained in the multivariate analysis and defined an explanatory model for viral infection. The GSTT1 null allele was defined as a protective factor (OR_{adj} : 0.219; 95% CI 0.078-0.618; $p < 0.004$). In other words, women who have GSTT1 deletion are less susceptible to HPV infection than the control group. On the other hand, smoking habit was recognized as a risk factor, increasing the chance of viral infection up to 3.6 times (OR_{adj} : 3.678; 95% CI 1.111-12.171; $p < 0.033$). No interaction was observed between variables.

Table 3

– Predictive factors for HPV infection in a group of women living in the municipality of Francisco Beltrão, Paraná, based on multivariate logistic regression analysis.

Variables	OR _{adj}	95% CI	p value
GSTM1			
Null	0.960	(0.289 - 3.189)	0.947
Present	1 (Reference)		
GSTT1			
Null	0.219	(0.078 - 0.618)	0.004
Present	1 (Reference)		
Smoking habit			
Yes/ex-smoker	3.678	(1.111- 12.171)	0.033
Never smoked	1 (Reference)		
Recent Vaginal Infection			
Yes	2.140	(0.745 - 6.145)	0.158
No	1 (Reference)		
Race			
Others	1.825	(0.537- 6.208)	0.335
White	1 (Reference)		
Notes: OR _{adj} = value obtained from the Multivariate Logistic Regression with p<0.05 and R ² _{Nagelkerke} = 0.20 or 20%.			

Discussion

As far as we know, the results of the present study have shown, in an unprecedented way, that the GSTT1 null allele was characterized as a protective factor against viral infection, that is, women with GSTT1 deletion are less susceptible to HPV infection than the control group. While smoking habit was defined as a risk factor, increasing the chance of viral infection up to 3.6 times.

In general, individuals with GSTM1 and GSTT1 null genotypes are mainly predisposed to damage caused by oxidative stress [27]. Although there are gaps, it is known that genes involved in the metabolism of xenobiotics can influence the natural history of HPV subtypes, especially those with high oncogenic risk [17]. Individuals with GSTM1 and GSTT1 null alleles do not conjugate metabolics during phase II of

xenobiotic metabolism, increasing damage caused by oxidative stress, facilitating infection and viral replication [13].

The GSTM1 and GSTT1 SNPs have been reported in various pathologies such as rheumatoid arthritis, age macular degeneration, prostate cancer, lung cancer, cervical cancer, schizophrenia [28, 29, 30]. In addition, a recent study suggests that patients with severe acute respiratory syndrome caused by Sars-cov-2 (COVID-19) with GSTT1 null genotype had higher mortality compared to those with the presence of the active allele [31]. Although several studies point to a relationship between the deletions, GSTM1 and GSTT1, and risk for cervical cancer [19, 32], there is still controversy among population groups, restricting the generalization of such association [20]. In this context, it is reiterated that the vast majority of studies prioritize the relationship between the deleted alleles with the course and not with the susceptibility of the infection [22, 17].

Several studies suggest that individuals with homozygous deletion of both genes have limitation or incapacity in the production of their enzymes, decreasing the ability of the detoxification process and increasing the malignant transformation of cells promoting carcinogenesis [33]. Among the cancers related to the presence of the virus are head and neck [34] and cervical cancer [19].

The findings of the present study showed no relationship between the GSTM1 polymorphism and viral infection. However, it showed for the first time that the deleted GSTT1 decreases the chances of HPV infection, offering protection to women with the null genotype (GSTT1-). Research conducted in patients with HIV suggests that deletions in the GSTM1 and GSTT1 genes lead to slower disease progression [18]. Iorio et al. [35] reinforce that GSTT1- is a protective genetic marker for allergic rhinitis. In other work, both the GSTT1 deleted allele and the combined form with the GSTM1 null are protective factors and reduced the susceptibility to schizophrenia in the Chinese population [29].

Chen e Nirunsuksir [36] observed that HPV infection behaves differently according to GST genotypes. The product of GSTT1 is believed to protect the host's DNA against mutations [37]. Foppoli et al. [38] reported that high-risk subtypes are able to modulate and counteract the effects of increased levels of ROS (reactive oxygen species) in infected cells. That is, viral oncoproteins allow infected cells to survive in a hostile oxidizing environment, preventing the oxidation of anti-apoptotic and detoxifying enzymes. E6 and E7 modulate cellular microRNAs that regulate genes associated with the antioxidant response, suppression of OS-induced apoptosis and regulation of antioxidant enzymes and compounds [39].

The activity of several antioxidant proteins, such as those from the catalases family, peroxiredoxins, quinone oxidoreductase-1 and superoxide dismutase (SOD), can be disrupted by viral infections [39]. Furthermore, the expression of oncoproteins is associated with high levels of detoxifying enzymes, such as GSTs and GSH, providing the host cell with an escape system from oxidative damage and resisting programmed cell death [39].

One of the only works with results similar to those presented was that by Lee et al. [22], who found a protective relationship of the GSTT1 null allele in women infected with high-risk HPV, even with the

development of cervical cancer. However, they did not evaluate the relationship of susceptibility to infection. A recent systematic review suggested a risk association between null GSTT1 and infection by high-risk HPV subtypes [23]. Numerous works reiterate the probable influence of GSTM1 and GSTT1 null alleles for the progression of several types of cancer, including cervical cancer [19, 28]. However, this finding is still not unanimous. The approach of these polymorphisms with the infection by HPV and other viral agents are scarce, however, they are fundamental for the understanding of results like the ones presented.

Unlike the GST polymorphism, smoking habit was identified as a risk factor for viral infection. In the present study, the risk of HPV infection in smokers and ex-smokers is 3.6 higher compared to those who never smoked. A Finnish study revealed a 1.76-fold higher susceptibility to infection, especially of subtype 16, in female smokers [40]. Another study, conducted in Greece, showed that women who smoke are 1.7 times more likely to be infected [41]. Several studies bring tobacco smoking as a risk factor that increases the risk of the presence of HPV in different populations [42]. Tobacco smoking affects the immune system, increases the risk of new virus infections, as well as the persistence of HPV [43]. Simen-Kapeu et al. [43] reported that young female smokers have limitations in the production of antibodies to fight high-risk oncogenic subtypes. A similar finding was revealed in a study by Bergqvist et al. [40]. However, the influence of smoking in the initial phase of HPV infections is not yet fully understood as to cervical carcinogenesis [44]. Tobacco metabolites present in uterine cervical mucus decrease the quantity and function of epithelial Langerhans cells, producing a local immunosuppressive effect, leading to the host's inability to develop an effective immune response, maximizing the risk of viral and persistent infections, such as those of HPV [45, 46]. Tobacco consumption can also exacerbate the carcinogenic action of the virus by inhibiting INF- γ , TNF- α and mutations in the p53 gene, preventing apoptosis of the infected cell, favoring the progression of infection to cervical lesions and cancer [47]. Furthermore, some components of cigarettes such as benzopyrene, nicotine and derivatives damage the cervical epithelium, favoring viral infection [47].

In this work, it can be inferred that the smoking habit characterized the exposure to xenobiotics in the female population and proved to be a predictive factor for the risk of viral infection. Tian et al. [33] suggested a relationship between GSTT1 deletion and the chance of cervical changes in smokers. However, in the findings of this and other studies [19], no interaction was observed between GST polymorphisms and smoking in the investigated population. Still, the study has some limitations. The small number of women with HPV, interfering with the statistical power of the analyses. Condition that can be solved by expanding the sample and including other populations to validate the results presented. However, the case-control study also showed strengths points and allowed us to conclude that tobacco smoking is an important risk factor for viral infection. Furthermore, it was the first work to effectively assess the relationship between GSTM1 and GSTT1 and STI caused by HPV, demonstrating that the GSTT1 null allele can protect women from infection.

Conclusion

The study conducted in the municipality of Francisco Beltrão, in the State of Paraná, revealed unprecedented results and characterized the GSTT1 null allele as a protective factor against viral infection, that is, women who have GSTT1 deletion are less susceptible to HPV infection than the control group. While smoking habit was defined as a risk factor, raising up to 3.6 times the chance for viral infection.

In this sense, it is worth other studies that can expand the sample, allowing dividing the groups between GSTT1-/GSTT1+ genotypes and smokers and non-smokers, and see if there is a relationship between them.

Declarations

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Compliance with ethical standards

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Conflict of interest

All authors declare that they have no conflict to interest.

Author Contribution

Conceptualization: Ana Paula Reolon Bortolli and Léia Carolina Lucio; Methodology: Ana Paula Reolon Bortolli, Valquíria Kulig Vieira, Indianara Carlotto Treco, Claudicéia Risso Pascotto, Guilherme Welter Wendt, and Léia Carolina Lucio; Formal analysis and investigation: Ana Paula Reolon Bortolli, Guilherme Welter Wendt, and Léia Carolina Lucio; Writing - original draft preparation: Ana Paula Reolon Bortolli and Léia Carolina Lucio; Writing - review and editing: Ana Paula Reolon Bortolli, Valquíria Kulig Vieira, and

Consent to Participate

All authors informed consent.

Consent to Publish

All authors approved the version to be published and agree to be accountable for all aspects of the work in ensuring related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figures

Figure 1

Electrophoresis of PCR products from the co-amplification of GSTM1 and GSTT1. Ethidium bromide stained 2% agarose gel. Lanes (L): L1-L5, L7, L9 (Fig.1A) were positive for GSTM1; L6, L8, L13, L14 (Fig. 1A) and L18, L19 AND L22 (Fig.1B) were GSTM1 an GSTT1 deletion genotype. Lanes 11, L12, (Fig. 1A) and L21, L23 (Fig.1B) were positive for GSTM1 and GSTT1; L20 were positive for GSTM1 genotype (Fig.1B). Lanes 15 and 17 were negative control (Fig. A and B) and L10 and L16 were 100bp molecular weight marker (Fig. A and B).