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Impact of *glutathione S-transferase* genes polymorphisms on human papillomavirus infection and precancerous lesions in West African women

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Genetic polymorphisms of certain classes of glutathione S-transferase (GST), enzyme responsible for the biotransformation of drugs and xenobiotics, have been associated with risk of several cancers such as cervical cancer. The aim of this study is to investigate the impact of *glutathione S-transferase M1* and *T1* deletion on high-risk human papillomavirus (HR-HPV) infections and on dysplasia. A case-control study was carried out on 1069 endocervical samples from West African women including 482 HR-HPV positive and 139 patients had cervical lesions according to visual inspection with acetic acid and Lugol (VIA/VILI) screening. Deletion of the *GSTM1* and *GSTT1* genes was determined using conventional PCR and genotypes of HR-HPV by real-time PCR. An association with a reduced risk for HR-HPV infection was observed in Ivorian population with *GSTT1*-null (OR = 0.61, 95% CI = 0.40 - 0.92, p= 0.02) and *GSTM1*-active/*GSTT1*-null genotypes (OR = 0.56, 95% CI = 0.35 - 0.90, p= 0.02). In West African, women with *GSTT1*-null genotype had 1.72-fold higher risk for infection with HPV66 (p= 0.044) and reduced risk (OR = 0.39) for HPV35. Whereas women with *GSTM1*-null/*GSTT1*-active genotype had 2.32-fold higher risk for HPV18 infection (p= 0.042). *GSTT1*-null genotype was associated to cervical lesions in West African with a reduced risk (OR = 0.63, p= 0.017). The results of the present study demonstrate that *GSTT1*-null could be associated with cervical lesions and HPV35 infection with reduced risk. *GSTM1*-null associated with *GSTT1*-active could play a role in increasing the risk for HPV18 infection.

Key words: Cervical cancer, *GSTM1*, *GSTT1*, HR-HPV, West Africa.

INTRODUCTION

Cervical cancer is a major challenge for developing countries. The human papillomavirus (HPV) is considered as the main etiological agent responsible for cervical cancer (Walboomers et al., 1999). In 2018, the global incidence of cervical cancer was estimated at 570,000 cases with 311,000 deaths (Bray et al., 2018). About 85% of cases related to cervical cancer occur in low-income countries (Chuang et al., 2016; Randall and Ghebre, 2016). The disease was the fourth most common diagnosed cancer in women worldwide and the second in terms of incidence and mortality in developing countries (Bray et al., 2018). The highest incidence and mortality rates are recorded in Southern Africa followed by East and West Africa (Guinea, Burkina Faso and Mali) (Bray et al., 2018). According to WHO, cervical cancer related mortality might increase by 42% to reach 442,926 deaths in 2030 (WHO, 2015). The largest increase will occur in low- and middle-income countries and could be due to specific factors influencing HPV infection and cervical cancer in sub-Saharan Africa. Persistent HPV infections remain the main cause of precancerous lesions and cervical cancer (Walboomers et al., 1999; Schneider et al., 1992). Despite the fact that HPV infected women are at high risk for cervical cancer, not all infected women develop the disease as the infection is most often detected in asymptomatic people (Wheeler et al., 1993). Only a minority would develop cancer; the neoplastic lesions can regress spontaneously. Malignant transformation occurs during a period of 15 to 20 years in the infected epithelium of the cervix (Zur Hausen, 2002) and also involve the host genetic factors in the progression of the disease.

Among these genetic factors there is mounting evidence about glutathione S-transferase (*GST*) gene polymorphisms. Glutathione S-transferase (*GST*) is a polymorphic enzyme involved in the conjugation of reduced glutathione to harmful electrophilic compounds and in chemo-resistance to anticancer agents. Isoenzymes of *GST* are involved in detoxification of carcinogen and play a very important role in the cellular defense system. *GSTs* belong to the family of cytosolic enzymes (GST, EC 2.5.1.18) divided into 8 classes: *mu* (*GSTM*), *alpha* (*GSTA*), *pi* (*GSTP*), *theta* (*GSTT*), *zeta* (*GSTZ*), *sigma* (*GSTS*)), *kappa* (*GSTK*) and *omega* (*GSTO*) (Hayes and Pulford, 1995, Hayes and Mclellan, 1999). GST enzymes are soluble with a molecular mass of about 25 kDa. The most studied polymorphisms of *GST* genes consist of *mu*, *theta* and *zeta* classes and the subclasses are mainly *GSTM1*, *GSTT1*, *GSTP1*. The present study will focus on the polymorphisms of the *GSTM1* and *GSTT1* genes in West African women. So far, a single nucleotide polymorphism (G2619C at 534 position 7 exon) or a complete deletion of the gene was reported for *GSTM1* subclass with three known alleles: *GSTM1*A, GSTM1*B* and *GSTM1*0*. The first two alleles differ by a single nucleotide in exon 7 of the gene with no influence on the enzyme activity. The null allele *GSTM1*0* also called *GSTM1*-null results in the absence of the *GSTM1* enzyme. People with complete deletion of the *GSTM1* gene seem unable to metabolize epoxides or quinones (Hayes and Pulford, 1995; Hayes and Strange, 2000). The frequency of the null allele *GSTM1*0* is estimated at 50% in Caucasians and 27% in Asian population. *GSTT1* gene polymorphisms (A310C) result in the substitution of threonine residue into proline at position 104 of the amino acid sequence (Eaton and Bammler, 1999) with three alleles named *GSTT1*A*, *GSTT1*B* and *GSTT1*0*. The latter also called *GSTT1*-null differs from the first two alleles by an absence of enzymatic activity with a frequency estimated at 20 and 61% in Caucasian and Asian population respectively. Insufficient detoxification caused by gene polymorphism of the metabolizing enzymes or dysregulation in the elimination system of toxins (oxidative stress) from the body can lead to increased exposure to reactive carcinogenic derivatives and contribute to the malignant cellular reaction in women infected with HPV.

According to studies, oxidative stress was associated with an increase in viral replication in cells infected (Scholz et al., 1996), and the increase in oxidants has been associated with neoplastic progression of HPV16 (De Marco et al., 2012). Several studies in our laboratory have identified a number of high-risk HPVs associated with cervical lesions in West Africa other than 16 and 18 (Zohoncon et al., 2020). The importance of studying *GSTM1*, *GSTT1* genes deletion and high-risk HPV infection and precancerous lesions is very necessary. Several studies have investigated the association of *GSTM1* and *GSTT1* deletion in the acquisition of precancerous lesions and study cervical cancer in different countries with the exception of Africa. In this first pioneering study of a population of women from several West African countries, we have examined and verified the hypothesis that the deletions of *GSTM1* and *GSTT1* are associated with HR-HPV infection and the precancerous lesions.

MATERIALS AND METHODS

Ethical aspects

The women recruited gave their free and informed consent to participate in the study according to the Helsinki Declarations. The research protocol was approved by the Ethics Committee for Health

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Research (CERS) of Burkina Faso on January 10, 2018 with reference number 2018-01-012. The information obtained from the patient are kept strictly confidential. The results were used for a better therapy of women.

Type, site and study population

The study population consisted of 1,069 samples randomly selected from 2,133 endocervical samples collected from women in the general population of five West African countries, namely Benin, Burkina Faso, Côte d'Ivoire, Niger and Togo. HPV sampling and genotyping were carried out in 2017 as part of an earlier study funded by the *"Agence Universitaire de la francophonie"* (AUF). Ten cities were selected for sample collection in the five countries according to their importance in terms of population density and geographic location.

This is a cross-sectional, case-control study. We considered the high-risk HPV positive samples as the cases and the high-risk HPV negative samples as the controls. The distribution of the number of samples by city was: 99 cases and 111 controls chosen from 234 samples in Ouagadougou (Burkina Faso), 63 cases and 76 controls from 239 samples in Kara (Togo), 183 cases and 214 controls from 484 samples in Abidjan-Bouaké-Yamoussoukro (Côte d'Ivoire), 108 cases and 134 controls among 484 samples in Parakou-Cotonou-Borgou/Alibori-Abomey Calavi (Benin) and 29 cases and 52 controls chosen from 250 samples in Niamey (Niger). The sample size in each country was calculated according to the prevalence of HPV in the country. In total, there were 482 cases and 587 controls. Among the 1069 samples selected, 139 patients were carriers of lesions and 897 patients were without lesions according to the VIA/VILI tests.

Cervical specimen collection and screening for precancerous lesions

After sensitizing the study respondents about how to prevent HPV infection and cervical cancer risk, and obtaining free and informed consent from the women, a questionnaire was administered to the women to collect their socio-demographic, behavioural and clinical information. Endocervical swab samples were taken from the uterus of the respondents using a sterile cotton swab and a single use speculum; behind screening was done for visual inspection using acetic acid and Lugol (IVA/VILI) to determine cervical lesions or dysplasia in the women. An examination could specify the lesions types were not known. Patients with a positive VIA or VILI test were considered to have cervical lesions or dysplasia, and those with a negative VIA / VILI were considered to have normal cytology.
The samples obtained were immersed in a transport medium from the DNA-Sorb-A kit (Sacace Biotechnologies, Como, Italy) and stored at -20°C in the laboratory of the various sites. In CERBA/LABIOGENE (Pietro Annigoni Biomolecular Research Center / Molecular Biology and Genetics laboratory) of Ouagadougou (Burkina Faso), DNA was extracted, high-risk HPV genotypes were characterized and *GSTM1* and *GSTT1* deletion was done.

DNA extraction

The DNA of the endocervical samples was extracted using the commercial kit called "DNA-Sorb-A" from sacace biotechnologies® according to the manufacturer's protocol. The extracted DNA was stored at a temperature of -20°C in order to carry out PCR amplifications.

HR-HPV detection

HR-HPV was detected with the HPV Genotypes 14 Real-TMQuant kit (SACACE Biotechnologies®, Italy) using real-time multiplex PCR test for detection of 14 high-risk genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). For PCR amplification, each DNA sample, to be amplified was distributed in 4 tubes at the rate of 10 µl/tube. Each tube previously contained 15 µl of the reaction mixture and target specific genotypes. Positive and Negative controls given by the supplier have been performed following the same procedure.

The reaction mixture of 15 µl in the 4 tubes was composed of a mixture of Hot Start DNA, PCR-buffer-FRT and respectively the primers L1, E6 and E7 of the target regions of 3 to 4 HR-HPV and internal control (PCR-mix-1 16, 18, 31, IC; PCR-mix-1 39, 45, 59, IC; PCR-mix-1 33, 35, 56, 68; PCR-mix-1 51, 52, 58, and 66). The amplification program was 1 cycle of 95°C for 15 min, followed by 5 cycles of 95°C for 5 s, 60°C for 20 s and 72°C for 15 s and finally 40 cycles of 95°C for 5 s, 60°C for 30 s and 72°C for 15 s. The results were interpreted using Microsoft Excel program software called HPV Genotypes 14 Real-TM.xls (SACACE Biotechnologies®, Italy) according to the manufacturer's protocol.

GSTM1 and GSTT1 polymorphisms characterization

The method used for the genotyping of *GSTM1* and *GSTT1* is a conventional multiplex PCR described by Chen et al. (Chen et al., 1997). The primers used were _F': 5'GAACTCCCTGAAAAGCTAAAGC-3' and R' : 5'GTTGGGCTCAAATATACGGTGG-3' for GSTM1; _F' : 5'-TTCCTTACTGGTCCTCACATCTC-3' and R' : 5'-TCACCGGATCATGGCCAGCA-3' for GSTT1 ; _F ': 5'CAACTTCATCCACGTTCACC-3' and R' : 5'GAAGAGCCAAGGACAGGTAC-3' for internal control (β-globine). Each well contained 10µl of Taq Gold 360 Master Mix Ampli, 1µl of each primer, 7µl of sterile water, 2µl of DNA. The PCR amplification program began with a denaturation step at 94°C for 5 min followed by 35 cycles at 94°C for one min, 57°C for 1 min and 72°C for one minute, ending with an extension at 72°C for 7 min.

PCR amplification products were revealed by 3% agarose gel electrophoresis containing ethidium bromide. The bands of 215 bp, 480 bp and 268 bp were allocated to *GSTM1*, *GSTT1*, and β-globin, respectively (Figure 1). The absence of PCR products corresponding to *GSTM1*, *GSTT1* was considered to be zero genotype and invalid PCR in the absence of PCR products corresponding to β-globin.

Statistical analysis

Our data were analyzed by Excel 2016 software, SPSS Statistics 25.0.0.0, Epi Info 7.2.2.6. The confidence interval was set at 95% and the fisher test was used for the comparison. The difference was statistically significant for $p < 0.05$.

RESULTS

Sociodemographic characteristic of the study population

The present study concerned 1069 samples. Table 1 shows the sociodemographic characteristic of the study population. Their ages range from 15 to 72 years with a mean of 35.48 ± 9.6 years. The median age was 35 years. 25-34 and 35-44 age groups were the most

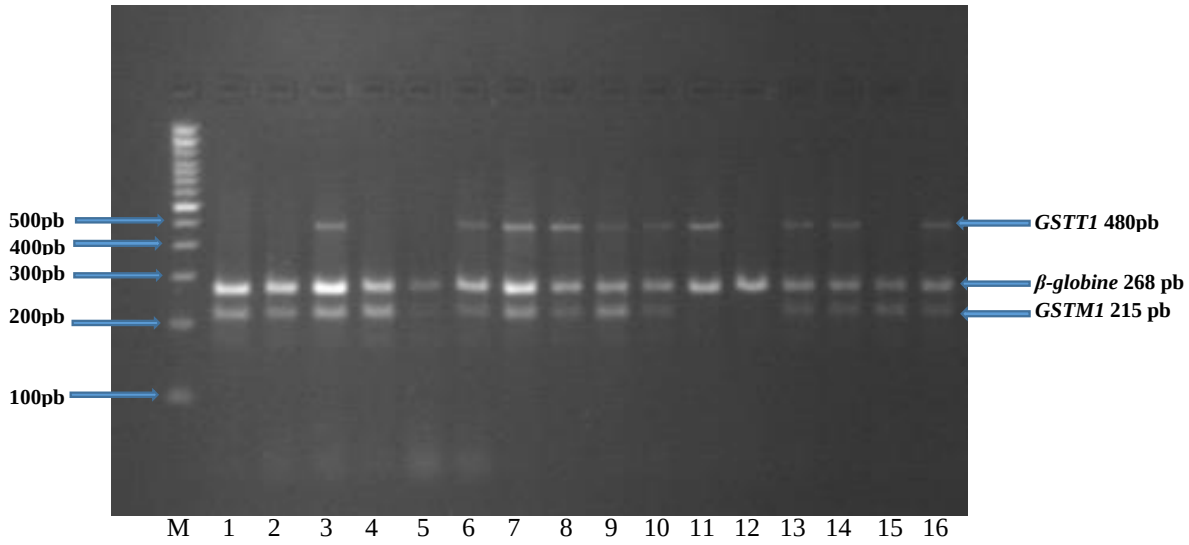

Figure 1. PCR-Multiplex Electrophoresis Gel. M: Molecular weight marker (100 bp). Samples 5 and 12: Double genotypes null of *GSTM1* and *GSTT1*. Samples 1; 2; 4 and 15: Genotypes of *GSTM1* present and *GSTT1* null. Samples 3; 6; 7; 8; 9 and 10: Double present genotypes of *GSTM1* and *GSTT1*. Sample 11: *GSTM1* null and GSTT1 present.

represented with frequencies of 36.30 and 35.45%, respectively. The study population was made of 64.36% of women who were less than 30 years old. The married wives in our study represented 71.66% of the study population. Women who had their first sexual intercourse between 15 and 24 years old represented 78.77% (842/1069). The number of sexual partners ranged from zero to more than two. About 29% of women said they were on contraception, 13.94% had contracted a sexually transmitted infection during their life, 0.84% said they were diagnosed with HIV. VIA/VILI tests were negative in 86.16% of the study population. HR-HPV infections accounted for 45.09% (28.16 single infections and 16.93% multiple infections). The most frequent HR-HPV in cases and controls were HPV 52 (Table 2)

GSTM1, GSTT1 polymorphism and HPV infection in western Africa

In our study 29 (2.71%) samples were considered invalid after PCR because no band was detected during electrophoresis migration. One thousand and forty (1040) samples produced valid results during PCR testing. Table 3 shows *GSTM1* and *GSTT1* and HR-HPV infection in West Africa. The presence of the *GSTM1* and *GSTT1* was observed in 74.52 and 55.87% of the population, respectively. The absence of *GSTM1* also called *GSTM1*-null was observed in 25.48% (265/1040) of which 25.79% (147/570) were in the controls and 25.11% (118/470) were in positive cases for HR-HPV (OR = 0.96; 95% CI = 0.72 - 1.27; p= 0.83). That of the *GSTT1* also called *GSTT1*-null is 44.13% with 45.79% in controls and 42.13% in positive cases for HR-HPV (OR = 0.86; 95% CI = 0.67 - 1.10; p= 0.2).

In the *GSTM1* and *GSTT1* associations, we found 40.87% (425/1040) of *GSTM1*-active/*GSTT1*-active, 10.67% of *GSTM1*-null/*GSTT1*-null (OR = 0.96; 95% CI = 0.63 - 1.46; p= 0.91); 14.71% and 33.75% of *GSTM1*-null/*GSTT1*-active (OR = 0.75; 95% CI = 0.52 - 1.10; p= 0.15) and *GSTM1*-active/*GSTT1*-null (OR = 0.75; 95% CI = 0.56 - 0.99; p= 0.05) respectively in the population. The analysis had shown in the general population non-significant associations of glutathione-S-transferase deletion and HR-HPV infection (Table 3).

On the other hand, statistically significant associations in our population was observed in carrying out of the *GSTM1*-null/*GSTT1*-active genotype with a 2.32-fold higher risk in HPV 18 infection compared to *GSTM1*-active/ *GSTT1*-active in the study population (95% CI = 1.06 - 5.08; p= 0.042). Carriers of the *GSTT1*-null genotype had a 1.72-fold higher risk of HPV 66 infection compared to other HPV genotypes (CI = 1.02 - 2.90; p= 0.044). However, there was a decrease in HPV 35 infection for women with the *GSTT1*-null genotype in the study population (OR = 0.39; 95% CI = 0.19 - 0.78; p= 0.008) (Table 5).

GSTM1 and GSTT1 polymorphism and HR-HPV infection in different countries of study

Table 4 reveals the distribution of the deletion polymorphisms of *GSTM1* and *GSTT1* in the different countries of our study. *GSTM1*-active and *GSTT1*-active were 77.75 and 62.15% respectively in Côte d'Ivoire,

Table 1. Sociodemographic data of cases and controls.

Variable	HR-HPV, n(%)	Controls, n(%)	Total, n (%)	p-value
Total	482 (100,00)	587 (100,00)	1069 (100.00)	
Mean age	35.25 ± 10.03	35.66 ± 9.2	35.48 ± 9.6	
Age groups (years)				0.077
≤ 24	60 (12.45)	64 (10.90)	124 (11.60)	
25-34	185 (38.38)	203 (34.58)	388 (36.30)	
35-44	162 (33.61)	217 (36.97)	379 (35.45)	
45-54	52 (10.79)	85 (14.48)	137 (12.82)	
≥ 55	23 (4.77)	16 (2.73)	39 (3.65)	
Unknown	(0.00)	2 (0.34)	2 (0.19)	
Marital status				0.000
Single	152 (31.54)	116 (19.76)	268 (25.07)	
Divorced	1 (0.21)	2 (0.34)	3 (0.28)	
married	315 (65.35)	451 (76.83)	766 (71.66)	
Widow	13 (2.70)	15 (2.56)	28 (2.62)	
Unknown	1 (0.21)	3 (0.51)	4 (0.37)	
Age of 1st intercourse (years)				0.000
< 15	22 (4.56)	29 (4.94)	51 (4.77)	
≥ 25	15 (3.11)	22 (3.75)	37 (3.46)	
15 ≤ X ≤ 24	407 (84.44)	435 (74.11)	842 (78.77)	
Unknown	38 (7.88)	101 (17.21)	139 (13.00)	
Number of partner				0.880
0	6 (1.24)	9 (1.53)	15 (1.40)	
1	419 (86.93)	514 (87.56)	933 (87.28)	
≥ 2	53 (11.00)	61 (10.39)	114 (10.66)	
Unknown	4 (0.83)	3 (0.51)	7 (0.65)	
Use of contraception				0.351
No	238 (49.38)	278 (47.36)	516 (48.27)	
Yes	136 (28.22)	174 (29.64)	310 (29.00)	
Unknown	108 (22.41)	135 (23.00)	243 (22.73)	
Sexual infection				0.894
No	61 (12.66)	79 (13.46)	140 (13.10)	
Yes	66 (13.69)	83 (14.14)	149 (13.94)	
Unknown	355 (73.65)	425(72.40)	780 (72.97)	
HIV infection				
Negative	116 (24.07)	125 (21.29)	241 (22.54)	
Positive	5 (1.04)	4 (0.68)	9 (0.84)	0.633
Inconnu	361 (74.90)	459 (78.02)	819 (76.61)	
VIA/VILI				
Negativef/Negative	378 (78.42)	543 (92.50)	921 (86.16)	
Negative/Positive	5 (1.04)	5 (0.85)	10 (0.94)	
Positive/Negative	7 (1.45)	6 (1.02)	13 (1.22)	0.000
Positive/Positive	91 (18.88)	29 (4.94)	120 (11.23)	
Unrealize	1 (0.21)	4 (0.68)	5 (0.47)	

Table 2. Epidemiology data of cases.

HPV type	Number (% in women infected)	Number (% in all people)
HPV 52	92 (19.09)	92 (8.61)
HPV 68	84 (17.43)	84 (7.86)
HPV 66	70 (14.52)	70 (6.55)
HPV 45	68 (14.11)	68 (6.36)
HPV 31	65 (13.49)	65 (6.08)
HPV 56	61 (12.66)	61 (5.71)
HPV 58	59 (12.24)	59 (5.52)
HPV 51	55 (11.41)	55 (5.14)
HPV 39	51 (10.58)	51 (4.77)
HPV 35	47 (9.75)	47 (4.40)
HPV 59	43 (8.92)	43 (4.02)
HPV 18	42 (8.71)	42 (3.93)
HPV 16	13 (2.70)	13 (1.22)
HPV 33	11 (2.28)	11 (1.03)
HPV- multiple infection		
No	301 (62.45)	301 (28.16)
Yes	181 (37.55)	181 (16.93)

Table 3. *GSTM1* and *GSTT1* and HR-HPV infection in West Africa.

Genotype	Controls, n (%)	HPV+, n (%)	Total, n (%)	OR (95%IC) *p*-value
GSTM1(+)	423 (74.21)	352 (74.89)	775 (74.52)	Ref
GSTM1(-)	147 (25.79)	118 (25.11)	265 (25.48)	0.96 (0.72-1.27) 0.83
GSTT1(+)	309 (54.21)	272 (57.87)	581 (55.87)	Ref
GSTT1(-)	261 (45.79)	198 (42.13)	459 (44.13)	0.86 (0.67-1.10) 0.25
GSTM1(+)/GSTT1(+)	218 (38.25)	207 (44.04)	425 (40.87)	Ref
GSTM1(-)/GSTT1(-)	58 (10.18)	53 (11.28)	111 (10,67)	0.96 (0.63-1.46) 0.91
GSTM1(-)/GSTT1(+)	89 (15.61)	64 (13.62)	153 (14.71)	0.75 (0.52-1.10) 0.15
GSTM1(+)/GSTT1(-)	205 (35.96)	146 (31.06)	351 (33.75)	0.75 (0.56-0.99) 0.05
Total	570	470	1040	

(-) Null; (+) Active; Ref: reference.

73.68 and 52.19% in Benin, 73.43 and 51.21% in Burkina Faso, 72.79 and 50.74% in Togo, and 66.67 and 56.41% in Niger. *GSTM1*-null, *GSTT1*-null, *GSTM1*-active/*GSTT1*-null, *GSTM1*-null/*GSTT1*-active, and *GSTM1*-null/*GSTT1*-null in this study did not show any significant difference between positive cases of HR-HPV and controls in the population of Burkina Faso, Togo, Benin and Niger. On the other hand, in the Ivorian population, we observed a significant difference by comparing the cases with the controls for *GSTT1*-null and the association between *GSTM1*-active/*GSTT1*-null. Their frequency in the population was respectively 37.85% (OR = 0.61; 95% CI = 0.40-0.92; p= 0.02) and 29.41% (OR = 0.56; 95% CI = 0.35-0.90; p = 0.02) with reduced risk of infection (OR <1). *GSTM1*-null in Niger, the associations *GSTM1*-null/*GSTT1*-null in Niger and in Togo had marginal risks of 2.25; 2.28 and 2.60 respectively.

GSTM1, GSTT1 polymorphisms and HPV infection in cervical lesions or dysplasia

Table 6 shows the distribution of HR-HPV infection and the deletion polymorphisms studied as a function of the results of the VIA/VILI tests. In women, HR-HPV infection was significantly associated with cervical lesions with the risk of 3.98-fold higher compared to women with negative HPV (95% CI = 2.67-5.93; p<0.0001). *GSTT1*-null had statistically significant frequencies with a reduced risk of cervical lesions compared to *GSTM1*-active (OR = 0.63; 95% CI = 0.43 - 0.91; p = 0.01). Unlike the *GSTT1*-null genotype,

Table 4. GSTM1 and GSTT1 polymorphism by country of study.

Countries	Variables	Controls [n(%)]	HR-HPV+ [n(%)]	Total [n(%)]	OR	95%IC	P-value
	GSTM1(+)	164(77.73)	140(77.78)	304(77.75)	ref		
	GSTM1(-)	47(22.27)	40(22.22)	87(22.25)	0.99	0.61-1.60	1.00
	GSTT1(+)	120(56.87)	123(68.33)	243(62.15)	ref		
	GSTT1(-)	91(43.13)	57(31.67)	148(37.85)	0.61	0.40-0.92	0.02
Cote d'Ivoire	GSTM1(+)/GSTT1(+)	92(43.60)	97(53.89)	189(48.34)	ref		
	GSTM1(-)/GSTT1(+)	28(13.27)	26(14.44)	54(13.81)	0.88	0.48-1.61	0.75
	GSTM1(+)/GSTT1(-)	72(34.12)	43(23.89)	115(29.41)	**0.56**	**0.35-0.90**	**0.02**
	GSTM1(-)/GSTT1(-)	19(9.00)	14(7.78)	33(8.44)	0.69	0.33-1.47	0.45
	Total	211(100)	180 (100)	391(100)			
	GSTM1(+)	94(72.87)	74(74.75)	168(73.68)	ref		
	GSTM1(-)	35(27.13)	25(25.25)	60(26.32)	0.90	0.49-1.64	0.76
	GSTT1(+)	67(51.94)	52(52.53)	119(52.19)	ref		
	GSTT1(-)	62(48.06)	47(47.47)	109(47.81)	0.97	0.57-1.65	1.00
Benin	GSTM1(+)/GSTT1(+)	49(37.98)	41(41.41)	90(39.47)	ref		
	GSTM1(-)/GSTT1(+)	18(13.95)	11(11.11)	29(12.72)	0.73	0.30-1.72	0.52
	GSTM1(+)/GSTT1(-)	45(34.88)	33(33.33)	78(34.21)	0.87	0.47-1.61	0.75
	GSTM1(-)/GSTT1(-)	17(13.18)	14(14.14)	31(13.60)	0.98	0.43-2.23	1.00
	Total	129 (100)	99(100)	228(100)			
	GSTM1(+)	73(67.59)	79(79.80)	152(73.43)	ref		
	GSTM1(-)	35(32.41)	20(20.20)	55(26.57)	0.52	0.27-0.99	0.058
	GSTT1(+)	57(52.78)	49(49.49)	106(51.21)	ref		
	GSTT1(-)	51(47.22)	50(50.51)	101(48.79)	1.14	0.66-1.96	0.67
Burkina Faso	GSTM1(+)/GSTT1(+)	32(29.63)	37(37.37)	69(33.33)	ref		
	GSTM1(-)/GSTT1(+)	23(21.30)	12(12.12)	35(16.91)	0.45	0.19-1.04	0.09
	GSTM1(+)/GSTT1(-)	41(37.96)	42(42.42)	83(40.10)	0.88	0.46-1.67	0.74
	GSTM1(-)/GSTT1(-)	12(11.11)	8(8.08)	20(9.66)	0.57	0.20-1.58	0.31
	Total	108(100)	99(100)	207(100)			
	GSTM1(+)	56(76.71)	43(68.25)	99(72.79)	ref		
	GSTM1(-)	17(23.29)	20(31.75)	37(27.21)	1.53	0.71-3.27	0.33
	GSTT1(+)	38(52.05)	31 (49.21)	69(50,74)	ref		
	GSTT1(-)	35(47.95)	32(50,79)	67(49.26)	1.12	0.57-2.19	0.86
Togo	GSTM1(+)/GSTT1(+)	26(35.62)	22(34.92)	48(35.29)	ref		
	GSTM1(-)/GSTT1(+)	12(16.44)	8(12.70)	20(14.71)	0.78	0.27-2.27	0.79
	GSTM1(+)/GSTT1(-)	30(41.10)	22(34.92)	52(38.24)	0.86	0.39-1.91	0.84
	GSTM1(-)/GSTT1(-)	5(6.85)	11(17.46)	16(11.76)	2.60	0.78-8.63	0.15
	Total	73(100)	63(100)	136(100)			
	GSTM1(+)	36(73.47)	16(55.17)	52(66.67)	ref		
	GSTM1(-)	13(26.53)	13(44.83)	26(33.33)	2.25	0.85-5.92	0.13
	GSTT1(+)	27(55.10)	17(58.62)	44(56.41)	ref		
	GSTT1(-)	22(44.90)	12(41.38)	34(43.59)	0.86	0.34-2.19	0.81
Niger	GSTM1(+)/GSTT1(+)	19(38.78)	10(34.48)	29(37.18)	ref		
	GSTM1(-)/GSTT1(+)	8(16.33)	7(24.14)	15(19.23)	1.66	0.46-5.92	0.52
	GSTM1(+)/GSTT1(-)	17(34.69)	6(20.69)	23(29.49)	0.67	0.20-2.23	0.56
	GSTM1(-)/GSTT1(-)	5(10.20)	6(20.69)	11(14.10)	2.28	0.55-9.36	0.29
	Total	49(100)	29(100)	78(100)			

(-) Null; (+) Active.

Table 5. Association between GSTT1, GSTM1 polymorphisms and HPV type.

Polymorphisms	Women others	HPV35 infected women	OR (95%IC) p-value	Total
GSTT1 (+)	547	34	ref	581
GSTT1 (-)	448	11	0.39 (0.19-0.78) **0.008**	459
Total	995	45		1040
	Women others	**HPV18 infected women**	**OR (95%IC) p-value**	**Total**
GSTM1(+)/GSTT1(+)	410	15	ref	425
GSTM1(+)/GSTT1(-)	337	14	1.13 (0.54-2.38) 0.849	351
GSTM1(-)/GSTT1(+)	141	12	2.32 (1.06-5.08) **0.042**	153
GSTM1(-)/GSTT1(-)	110	1	0.2 (0.03-1.90) 0.213	111
Total	998	42		1040
	HPV others	**HPV66 infected women**	**OR (95%IC) p-value**	**Total**
GSTT1(+)	241	31	ref	272
GSTT1(-)	162	36	1.72 (1.02-2.90) **0.044**	198
Total	403	67		470

Table 6. Association between HPV infection, GSTM1, GSTT1 polymorphisms and VIA/VILI results.

Variable		*VIA/VILI*				Total
		Unrealized	Without dysplasia	With dysplasia	OR (95%IC) p-value	
HPV infection	Negative	3	530	37		570
	Positive	1	367	102	3.98 (2.67-5.93) **<0.0001**	470
GSTM1	Active	3	672	100		775
	Null	1	225	39	1.16 (0.78-1.73) 0.46	265
GSTT1	Active	1	489	91		581
	Null	3	408	48	0.63 (0.43-0.91) **0.01**	459
Total		4	897	139		1040

Table 7. IVA/IVL, *GSTM1*, *GSTT1* and HPV infection.

		GSTM1			GSTT1			
	IVA/IVL	**Null**	**Active**	**OR (95%IC) p-value**	**Null**	**Active**	**OR (95%IC) *p*-value**	**Total**
HPV-	Unrealized	1	2		2	1		3
	Without dysplasia	134	396		243	287		530
	With dysplasia	12	25	1.41 (0.69-2.90) 0.33	16	21	0.89 (0.45-1.76) 0.86	37
	Total	147	423		261	309		570
HPV+	Unrealized	0	1		1	0		1
	Without dysplasia	91	276		165	202		367
	With dysplasia	27	75	1.09 (0.66-1.79) 0.79	32	70	0.55 (0.35-0.89) **0.01**	102
	Total	118	352		198	272		470

GSTM1-null and the double null genotype did not have statistically significant results. However, a risk of 0.55 was found in the double genotype *GSTM1*-active/*GSTT1*-null compared to *GSTM1*-active/*GSTT1*-active genotype in cervical lesions (95% CI = 0.35-0.86; p = 0.009). Table 7 presents the HPV, GST statuses as a function of the test results; women carrying *GSTT1*-null genotype, and HPV positive had a statistically significant result. Cervical lesions are linked to reduction in risk compared to *GSTT1*-active (OR = 0.55; 95% CI = 0.35-0.89; p = 0.01).

GSTM1, GSTT1 deletion and socio-demographic characteristic

The study did not show significant results of the respondents' socio-demographic characteristic such as age, marital status, age of 1st intercourse, contraception using, sexual partner, sexual infection, HIV infection with deletion of *GSTM1* and *GSTT1*.

DISCUSSION

The present study aims to assess the risk of *Glutathione S-Transferase M1* and *T1* polymorphisms in HR-HPV infection and cervical lesions or dysplasia in women. In literature, very few studies have been carried out on HR-HPV infection in relation to the deletions of *GSTM1* and *GSTT1*. Our study is the first in sub-Saharan Africa and West Africa particularly, where HPV infection is very common.

In this study, 29 samples (02.71%) were invalid after PCR testing. Different studies on polymorphisms have largely used blood samples (Ueda et al., 2010; Agodi et al., 2010). In our study, we used endocervical cells samples. There were very few samples, with a low DNA concentration when checked with the nanodrop. Endocervical cells could explain this low DNA concentration after extraction. This would therefore lead to illegible bands after electrophoresis.

Oxidative stress, according to studies, had been associated with an increase in viral replication (Scholz et al., 1996; Koike, 2009). According to De Marco et al. (2012), increased oxidants are associated with the neoplastic progression of HPV16 (De Marco et al., 2012). Several factors are responsible for oxidative stress. The genetic factors are the most crucial and influential. The main enzyme P450 cytochromes, catalyze the different oxidation reactions in phase I, producing oxidative stress by the metabolic activation of chemical carcinogens and xenobiotics (Lang and Pelkonen, 1999). The products resulting from this phase are reactive electrophilic intermediates and can cause lipids, proteins and DNA damage (Shackelford et al., 2000). Glutathione S-transferases, one of the groups of phase II enzyme, neutralize these reactive electrophiles by conjugating them with glutathione, making them more soluble in water (Ketterer et al., 1993; Wilce and Parker, 1994; Armstrong, 1997), and thus eliminating oxidative stress. The deletions of genes by these enzymes could lead to the persistence of oxidative stress, causing viral multiplication, DNA damage by reactive electrophiles attacks or chromosomal instability, and consequently the carcinogenic process.

According to Ueda et al. (2010) certain high-risk HPV-infections were associated with polymorphisms of glutathione S-transferase. Our study did not show an association between the *GSTM1*-null, *GSTT1*-null genotypes and HR-HPV infection in West Africa population. These results are similar to those found by Agodi et al. (2010) in Italy. Their results and ours suggested that the deletion of *GSTM1*, *GSTT1* genotypes is not associated in general with HPV-infection. However, particularities could exist according to the HR-HPV types.

The study also showed that the double *GSTM1*-active/*GSTT1*-null genotype was associated with HR-HPV infection, particularly in Côte d'Ivoire, with a relative risk always <1 (OR = 0.56). These results, although significant, showed no increased risk of HR-HPV infection, but rather a decrease in risk. The double *GSTM1*-active/*GSTT1*-null genotype could protect women from HPV-infection. *GST*s could modulate signal transduction pathways involved in cell survival and apoptosis, by controlling the activity of protein members of the mitogen-activated kinase family (MAPK) (Laborde, 2010; Singh, 2015). In normal conditions, ASK1 would be sequestered by *GSTM1* enzyme and forming the *GSTM1*/ASK1 complex. In oxidative stress conditions or heat shock, there is dissociation of the complex and activation of ASK1 and subsequently the induction of the apoptosis process. It could contribute to kill cell-infection although GSTT1 is an exposure factor.

In addition, in the general population of study, by comparing each type of HR-HPV in the study population and each type of HR-HPV with the other remaining HR-HPV, there is an association with increased risk between infection with HPV18 and double genotype *GSTM1*-null/*GSTT1*-active (OR = 2.32; p = 0.042). Agodi et al. (2010), as in this study, did not find a significant association between the suppression of the *GSTM1*, *GSTT1* genes and infection with HPV16 despite the fact that this is considered the most common in premalignant and malignant cervical lesions (Muñoz et al., 2003). The study also showed an association between HPV66 infection and the *GSTT1*-null genotype with an increased risk (OR = 1.72; p= 0.04) and another association between HPV35 infection and the *GSTT1*-null genotype (OR = 0.39; p= 0.008) with risk reduced. These results differ from those found by Ueda et al.(2010) in Japan who reported a significant association between HPV16/18 infection and the *GSTT1*-null genotype (p= 0.029) compared to the other HR-HPV. However, we have not found in the literature an association between HPV66 and HPV35 infection with *GSTT1*-null genotype. This could be explained by the very few number of studies on HR-HPV infection and polymorphism of glutathione S-transferase. This peculiarity of HPV66, HPV35 association with *GSTT1*-null in West Africa could also be due to the diversity of emergence of other high-risk HR-HPV (than HPV16/18), which are more frequent in these countries (Zohoncon et al., 2020).

In literature according to several studies and reviews, *GSTT1*-null and *GSTM1*-null could be associated with the development of cervical cancer (Lee et al., 2004; Ueda et al., 2005; Ueda et al., 2010; Wang et al., 2011; Liu and

Xu, 2012; Sun and Song, 2016; Liu et al., 2017). However, our data were collected by questionnaires and the visual inspection tests were done with acetic acid and lugol (VIA/VILI) for the detection of cervical lesions or dysplasia in the endocervix. Lack of confirmations of the lesions types and grade by biopsy after the VIA/VILI tests constitutes a limitation of the study. Although the IVA/IVL screening tests recommended for low-resource countries are necessary, the results are not very specific like those of histology or cytology.

Regarding the deletions of *GSTM1* and *GSTT1* genes and the cervical lesions, there is an association between *GSTT1*-null genotype and cervical lesions by VIA/VILI detection (p= 0.01) with a decrease in risk (OR = 0.63). Satinder et al. (2017) in India, also found a reduced risk linked to cervical cancer (OR = 0.5; p= 0.04) in carriers of the *GSTT1*-null genotype. The GSTs, according to certain studies do not lead to detoxification complete (van Bladeren, 2000). The conjugate could be reversible or not stable and could lead to high toxic metabolite. It was demonstrated that the GST-dependant conjugation involving GSTT1-null enzyme for certain substrate such as hydrocarbon mono- or di-halogens would lead to very reactive electrophile responsible for carcinogenesis (Hayes and Pulford, 1995, van Bladeren, 2000). In view of these results, the *GSTT1*-null genotype could contribute to a reduction in the risk of acquiring lesions and cancer of the cervix.

Our results showed an absence of association between *GSTM1*-null and cervical lesions (p= 0.46). Several studies have shown this absence in association between the *GSTM1*-null genotype and the intraepithelial lesions in Italy (Agodi et al., 2010; Palma et al., 2010), India (p= 0.67) (Sharma et al., 2015), and Serbia (p= 0.07) (Stosic et al., 2014). An absence of *GSTM1*-null association was also noted in India in Squamous cell carcinomas (SCC) and adenocarcinoma (AC) (Abbas et al., 2013; Satinder et al., 2017) and in cervical cancer in Turkey (p= 0.73) (Kiran et al., 2010). In addition, in women with SCC and AC in Brazil, Tacca et al. (2018) determined survival of 80.0% in women with *GSTM1*-active and 73.3% in women with *GSTM1*-null after a 60-month follow-up and also found statistically insignificant results (p= 0.368). These results suggested that the genotype *GSTM1*-null could not be associated with lesions and cervical cancer.

In terms of gene-gene interactions, the study showed a reduction in the risk of cervical lesions (OR = 0.55; p = 0.009) for carriers of the *GSTM1*-active / *GSTT1*-null double genotype. Furthermore, Satinder et al. (2017) found an OR <1 also statistically significant in India for the double genotype *GSTM1*-active / *GSTT1*-null for cervical cancer (OR = 0.4; p= 0.02) and SCC (OR = 0.4; p= 0.04). This is contrary to our study which focused on cervical lesions by IVA/IVL detection. The double *GSTM1*-active/*GSTT1*-null genotype would contribute, just like the *GSTT1*-null genotype to reduce the risk of cervical lesions according to our study. *GSTT1* and *GSTM1* could play an important role in oxidative stress or in the metabolization of xenobiotic or regulation cell, which could partially compensate for the absence of one of two.

The enzymes responsible for the metabolism of carcinogens would be important risk modifiers in carcinogenesis (Sheweita, 2000). Thus, in the case of persistent infection with HR-HPV, oxidative damage to DNA caused by enzyme deletion could indeed serve as a mechanism to facilitate the integration of HPV, and subsequently, carcinogenesis (Williams et al., 2011). In the study, in women infected with HR-HPV, there were no statistically significant results (p= 0.79) in cervical lesions by VIA/VILI detection in carriers of *GSTM1*-null genotype. Nunobiki et al. (2015) also showed statistically insignificant results (p= 0.35) of the *GSTM1*-null genotype in intraepithelial lesions de haut grade (HSIL) in Japan among women infected with HPV. The mean results of the *GSTM1*-null genotype could not contribute to cervical lesions in women with HPV-infection.

GSTT1-null genotype had statistically significant results (OR = 0.55; p= 0.01) in women with HR-HPV+ and its risk could be reduced for cervical lesions. However in Japan (Nunobiki et al., 2015), Hungary (Cseh et al., 2011) and India (Joseph et al., 2006), they found an increased risk. In the studies by Nunobiki et al. (2015) *GSTT1*-null was significantly associated with low grade to high grade lesions in HR-HPV positive patients in Japan (OR = 3.45). According to the studies of Cseh et al. in Hungary after 7 years following women with HPV positive, a risk of 1.89-fold higher was attributed to the development of HSIL compared to controls. Joseph et al. determined in India a 19.25-fold higher risk of acquiring invasive cancer and HSIL in women with *GSTT1*-null genotype, and HPV16 positive compared to controls with normal cytology and low-grade lesions.

As for the double null genotype, we did not find significant results in West Africa for HPV infection and cervical lesions by VIA/VILI detection. In India, Romania and Serbia, non-significant results for the interactions of both null alleles were demonstrated (Stosic et al., 2014; Sharma et al., 2015; Daniel et al., 2016). However GSTs role was demonstrated in cells (Laborde, 2010; Gao et al., 2011; De Marco et al., 2012; Singh, 2015; Wang et al., 2016). Cseh et al. (2011) in Hungary after 7 years of follow up determined a relative risk of 2.35 of HSIL development for women infected with HR-HPV carrying a double null genotype compared to those who have at least one genotype present. Environmental factors could more or less contribute as a cofactor to cervical lesions. It would be more interesting to study the environmental factors as a source of xenobiotic contribution in detoxification genes deletions relation, which could play an important role.

The different case/control studies on the deletions of *GSTM1* and *GSTT1* by other authors, in addition to performing biopsies unlike ours, included the action of

passive and active smoking and exposure to wood smoke (Palma et al., 2010; Abbas et al., 2013; Stosic et al., 2014; Sharma et al., 2015; Satinder et al., 2017; Tacca et al., 2018). Thus, these authors determined statistically significant results between cancer and smoking (Abbas et al., 2013; Tacca et al., 2018); exposure to wood smoke (Satinder et al., 2017), and between cancer, smoking, and *GSTM1* and *GSTT1* polymorphisms (Sobti et al., 2006; Sharma et al., 2015). In this study, socio-demographic characteristics of the respondents such as smoking and exposure to wood smoke were not included. Two other authors carried out a study on a patient followed-up in Hungary and Brazil (Cseh et al., 2011; Tacca et al., 2018). The size of our samples was disproportionate between the number of women positive for at least one of the positive VIA/VILI tests and those with negative VIA/VILI (139/897), which also does not allow analysis by country.

Conclusion

Our results suggest a reduction in the progression of high-risk HPV infection in the Ivory Coast Republic, in carriers of *GSTT1*-null and of the double *GSTM1*-active/*GSTT1*-null in women with HR-HPV infected. Among women with HR-HPV infection, *GSTT1*-null genotype could reduce the risk of progression of HPV35 infection and increase HPV66 infection in West Africa. The risk of progression of HPV18 infection would be favored by the *GSTM1*-null/*GSTT1*-active genotype in HR-HPV positive women from West Africa. The risk reduction in the acquisition or progression of cervical lesions by VIA/VILI detection could be justified by carrying out the *GSTT1*-null genotype in the study population and women with HPV infection. *GSTM1*-active/*GSTT1*-null may be associated with the reduction of precancerous lesions in West Africa. A study including other genetic cofactors, environmental and confirmed lesions and cancer, would be necessary to shed more light on the various factors influencing carcinogens of cervical cancer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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