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Distinct distribution of HPV types among cancer-free Afro-Caribbean women from Tobago

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Abstract

Human papillomavirus (HPV), a sexually transmitted virus causes cervical carcinomas, and is associated with ~36% of oropharyngeal tumours where HPV16 is the predominant genotype. The cervical cancer incidence rate in Trinidad and Tobago is about two times higher than the worldwide rate. We have for the first time determined the prevalence and type distribution of cervical HPV infections among cancer-free Afro-Caribbean women from Tobago, and compared it with the HPV subtypes observed in their oral cavity. Thirty-five per cent of the women were cervical HPV positive. The most common high-risk type detected in the cervix was HPV45 rather than HPV16 and 18. The prevalence of HPV infection in the oral mucosa was 6.6%. The distribution of HPV genotypes in healthy Tobagonian women is different from that reported in studies conducted in European and North American populations. This may have important implications for vaccine introduction in this and other Afro-Caribbean countries.

Keywords: *Human papillomavirus, HPV16, HPV45, oral infection, cervix, concurrent infection, women*

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Introduction

Human papillomavirus (HPV) is aetiologically linked to >95% of cervical and other anogenital cancers (zur Hausen 1996). The prevalence of high-risk HPV infection in the cervix is highest in developing countries (Clifford et al. 2005), and corresponds to high incidence and mortality rates of cervical cancer in these countries (Parkin et al. 1999, Pisani et al. 1999). Cervical cancer incidence in the Caribbean and more specifically in the island Republic of Trinidad and Tobago (TT) is approximately two times higher than worldwide (age-standardized rate per 100 000: Caribbean 32.6, TT 27.1 vs. worldwide 16.2) (Ferlay et al. 2004).

There are 15 high-risk (carcinogenic) and three probably high-risk HPV genotypes that are associated with cervical cancer development (Munoz et al. 2003),

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Table I. Human papillomavirus (HPV) genotypes and risk classification[a].

Risk classification	HPV types
High risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82
Probable high risk	26, 53, 66
Low risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108

[a]Based on the risk classification by Munoz et al. (2003).

summarized in Table I. The type-specific prevalence of HPV infections in Afro-Caribbean populations is not well described. A recent International Agency for Research on Cancer (IARC) pooled analysis of HPV infections among women with normal cytology showed that there is a significantly larger proportion of HPV16 observed in European women than in sub-Saharan Africa (Clifford et al. 2005). However, no data were reported in Afro-Caribbean populations. Among the 26 Caribbean islands, to date only one study in Jamaica, reported HPV prevalence in healthy cancer-free women (Figueroa et al. 1995). The prevalence of HPV was 58/202 (28.7%) and among the high-risk HPV types detected, HPV16 and 18 were the most frequent. However, for 62% of these women, the HPV genotypes could not be characterized. Therefore, the true type-specific prevalence of high-risk HPV infections may have been underestimated due to the methodology used for HPV genotyping. In Cuba, the prevalence of HPV among 60 HIV-negative women was 21.7%. However, the HPV testing was only performed for women with a clinical lesion visualized after acetic acid testing and the most frequently detected high-risk HPV types were HPV33 and 51 (Rodriguez et al. 2005).

A HPV vaccine for prevention of cervical cancer has been recently approved by the US Federal Drug Administration (FDA) and targets the most common high-risk HPV types, 16 and 18. It is expected that implementation of this vaccine would prevent approximately 70% of all cervical cancers worldwide (Clifford et al. 2006). However, it is hard to predict the success of this vaccine in countries where the HPV subtypes are unknown.

In our study, we have for the first time determined the HPV prevalence and described the type distribution of cervical infections among cancer-free Afro-Caribbean women from Tobago. We have compared these results with previous reports in other populations in order to assess the potential impact of the HPV vaccine in the prevention of cervical cancer. Since HPV16 infections have also been found to be associated with a subset of head and neck tumours, particularly in the oropharynx (Gillison et al. 2000, Ragin et al. 2006). We will also for the first time, determine the prevalence of oral HPV infection and of HPV subtypes, and compare it with cervical HPV prevalence and subtypes in this Afro-Caribbean population.

Materials and methods

Study population

This study is part of a collaborative project between the Tobago Cancer Society and the University of Pittsburgh Graduate School of Public Health and Cancer Institute. Healthy women ($n=216$), aged 18–65 years, were recruited from the general population on the island of Tobago between July and September 2004 by means of posters, flyers, public service announcements on television and radio, word of mouth,

and a series of cancer information sessions conducted throughout the island during the month of July. Of the 216 women who were recruited, three were excluded because they had total hysterectomies (i.e. did not have an intact cervix) and one refused to participate after reading the informed consent document. The total number of women who were included in this study was 212. All subjects were seen at the Tobago Cancer Society office under a protocol approved by the University of Pittsburgh Biomedical Institutional Review Board (IRB) and the IRB of the Division of Health and Social Services, Tobago House of Assembly. All study participants provided written informed consent to participate in this study.

Data and specimen collection

A gynaecological examination and routine medical screening for cervical cancer (including a conventional Pap test) sponsored by the Tobago Cancer Society (TCS) was carried out for all study participants. Cytological diagnosis of the Pap test was performed by an off-site clinical laboratory used routinely by the TCS. De-identified results were forwarded to the University of Pittsburgh for data entry. Abnormal cervical cytology includes diagnoses of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and cancer. After informed consent, medical, demographic, social, cancer-related and sexual lifestyle data were collected by a self-administered standardized questionnaire. To ensure privacy, the participants were allowed to complete their questionnaires in a private room and asked to enclose the completed forms in a sealed envelope which was returned to the University of Pittsburgh for data entry. All questionnaires and envelopes were de-identified and labelled with a subject ID to ensure confidentiality.

Blood, cervical and oral epithelial cells were collected from each participant at the time of the gynaecological visit, processed and stored at -20°C, then later shipped on dry ice to the University of Pittsburgh where all samples were stored at -80°C until testing. Cervical epithelial cells were collected using a Rovers® Cervex-brush® (Fisher Scientific, Pittsburgh, PA, USA), and placed immediately into 1 ml of cell lysis buffer (Gentra Systems, Minneapolis, MN, USA), then frozen at -20°C. Oral epithelial cells were obtained from an oral mouthwash sample. The participants were asked to gargle toward the back of the throat for 30 sec. with 10 ml of Scope mouthwash (Procter & Gamble, Cincinnati, OH, USA). The 5-ml aliquots of the oral cells were centrifuged and the cell pellet was stored at -20°C. Blood (30 ml) was collected, and the plasma and cells were aliquoted and stored at -20°C.

Tobacco and alcohol exposure

A participant was categorized as a non-smoker if they were self-defined as a lifetime non-smoker, or had not smoked in the past 30 days and/or had not smoked more than 100 cigarettes in their lifetime (if the self-defined smoking status was missing). Short-term former smokers were self-defined former smokers who had smoked within the last 30 days. Long-term former smokers were self-defined former smokers who had not smoked within the last 30 days. Active smokers were self-defined as smokers who currently smoked. Smoking exposure was also determined for all participants by measuring plasma cotinine levels using a standard enzyme-linked immunosorbent assay (ELISA) (Bio-quant, San Diego, CA, USA). The assay was performed

according to the manufacturer's instructions. A standard concentration curve was generated using six standards (0, 5, 10, 25, 50 and 100 ng ml^{-1}). Cotinine concentration values for the unknown samples were obtained by interpolation from the curve. Subjects with cotinine levels of 0–5 ng ml^{-1} were classified as having no environmental tobacco smoke (ETS) exposure; while, subjects with 5–10 ng ml^{-1} were classified as positive for ETS exposure and those with cotinine levels >10 ng ml^{-1} were classified as smokers.

Alcohol exposure was determined from the questionnaire data. A participant was categorized as having alcohol exposure if they ever consumed beverages with alcohol. Because of the alcohol content of most mouthwashes (e.g. Scope ($\sim$14 wt% alcohol) and Listerine ($\sim$22% alcohol)) we also evaluated the prevalence of mouthwash use. A participant was classified as having used mouthwash if the practice occurred on a regular basis at least once a week for 6 or more months at a time.

HPV testing and genotyping

DNA was extracted from the 1-ml cervical cell lysates and oral cell pellets from 5 ml of mouthwash using the Puregene DNA purification kit (Gentra Systems, Minneapolis, MN, USA). Polymerase chain reaction (PCR) amplification of a β-globin gene fragment was performed using the PCO4 and GH20 primers (Saiki et al. 1986) to confirm that the extracted DNA was amplifiable. All samples were positive for the β-globin amplicon ($n = 212$). HPV status was determined by a nested PCR assay using consensus HPV primers PGMY09/11/HMB01 (Gravitt et al. 2000) and GP5+/6+ primers (de Roda Husman et al. 1995). DNA amplification was performed using the LightCycler® FastStart DNA Master^{PLUS} SYBR Green I kit on a LightCycler® 2.0 system (Roche Diagnostics, Indianapolis, IN, USA). All DNA amplification reactions were set up according to the manufacturer's protocol and were performed in a separate room from that used for the DNA amplification steps. For all HPV-positive samples, confirmation of the appropriately sized amplicon was carried out by running an aliquot of each sample on a 10% PAGEr® polyacrylamide gel (Cambrex Bio Science, Rockland, ME, USA), at 250 V for 55 min. The gel was stained with the highly sensitive GelStar® nucleic acid stain (Cambrex Bio Science) and the 150 bp GP5+/6+ amplicon was visualized using a Clare Chemical Dark Reader transilluminator (Clare Chemical Research, Dolores, CO, USA).

All samples positive with an HPV gel band were genotyped using the Linear Array HPV Genotyping kit (Roche Diagnostics). The assay involved amplification of samples by PCR using a master mix which contained biotin-labelled primers for the detection of 37 HPV genotypes as well as the human β-globin gene. The PCR products were chemically denatured and hybridized for 30 min at 53°C to linear array strips which contained specific and one cross-reactive oligonucleotide probes for the HPV genotypes as well as a high and low concentration of a β-globin probe. The HPV genotypes were identified when visualized using streptavidin–horseradish peroxidase conjugate and a substrate solution containing hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine which yielded a blue precipitate at the probe positions where hybridization occurred (Figure 1A).

Nine samples were positive by nested PCR but did not hybridize to any of the HPV-specific oligonucleotide probes on the Linear Array strip. HPV genotyping of these samples was accomplished by pyrosequencing (Biotage, Foxboro, MA, USA)

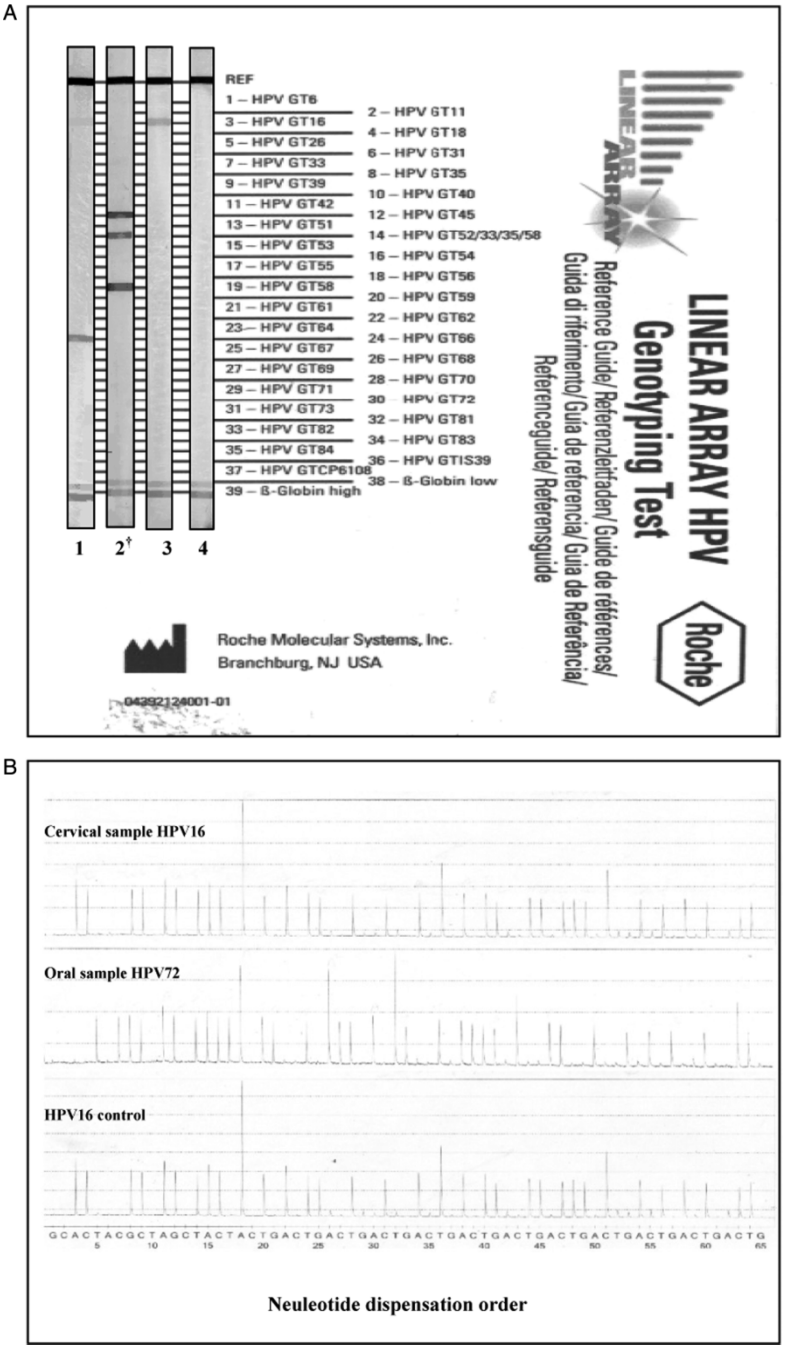

Figure 1. Human papillomavirus (HPV) genotyping was performed using (A) a Roche Linear Array Genotyping kit or (B) pyrosequencing. (A,1) HPV-positive oral sample infected with HPV16 and 66, (2) HPV-positive cervical sample infected with HPV58 and 45; †unable to rule out HPV52 due to cross-reactivity of the HPV58 probe, (3) HPV-positive control cell line infected with HPV16, (4) HPV-negative control cell line.

(Figure 1B). Briefly, the nested PCR was performed as described above but with the GP6+primer biotinylated at the 3′ end (Gharizadeh et al. 2001). The PCR product was denatured to obtain single-stranded DNA, followed by pyrosequencing using the GP5+as the sequencing primer. To identify the HPV genotype, sequencing results were aligned with the expected sequences for the 37 most common anogenital HPV genotypes or blasted against HPV sequences in the National Center for Biotechnology Information (NCBI) database. HPV-risk classification, summarized in Table I, was based on the epidemiological classification of HPV types that are associated with cervical cancer (Munoz et al. 2003). The HPV types that do not fall into any of these three categories were classified as undetermined risk.

Statistical analysis

All of the statistical analyses were performed using Intercooled STATA (version 8.2) software (StataCorp LP, College Station, TX, USA). Cross-tabulations were analyzed by the χ^2 test or Fisher's exact test, where appropriate and used to identify variables associated with oral and cervical HPV status. Odds ratios were calculated to assess the association between demographic variables and oral or cervical HPV status. The relative odds of demographic variables with cervical and oral HPV infection were adjusted for potential confounders (age, age at first coitus and marital status) using a logistic regression model. The age was stratified according to the median age of the population (42 years).

Results

Population demographics

Demographics, personal behaviour and HPV status in the cervix and oral mucosa are summarized in Table II. The population consisted of 212 women aged 18–65 years (median 42) and the majority (83%) was of Afro-Caribbean descent. Fifty-one per cent (109) were married, 35% (74) were single and 13% (27) were widowed or divorced. Sixty-six per cent of the women had ever consumed alcohol. A large proportion of women were self-reported non-smokers (88%), and, based on plasma cotinine levels, 78% had no measurable tobacco exposure. Forty-one per cent of the population reported that their age at first coitus was 18 years or less and the lifetime number of sexual partners ranged from 1 to 15 (<3 partners, 30.2%; 3–15 partners, 60.4%). About 59% of the women reported that they practiced oral sex and 71% (151/212) reported that their partners performed oral–vaginal sex.

HPV infections in the cervix and oral mucosa

After adjusting for age, marital status and age at first coitus, as expected, younger ($\leq$42 years) unmarried women were more likely to be HPV positive in the cervix than older, married women (single: odds ratio (OR) 3.1, 95% confidence interval (CI) 1.5–6.2; age $\leq$42 years: OR 1.8, 95% CI 1.0–3.4). We found no association between demographic variables and a positive oral HPV status (data not shown).

Cervical HPV infection was detected in 75 (35.4%) of the women. Table III summarizes the HPV-type distribution for the cervix and oral mucosa. Among the 75 women with cervical HPV infection, 43 (57.3%) carried at least one high-risk or

Table II. Population demographics, personal behaviour and human papillomavirus (HPV) status in healthy Tobagonian women ($n = 212$).

	No.	Overall %		No.	Overall %		No.	Overall %
Age (years)			History of cancer			Oral sex[b]		
<42	101	47.6	No	199	93.9	Never	60	28.3
42–65	111	52.4	Yes	7	3.3	Ever	126	59.4
			Not reported	6	2.8	Not reported	26	12.3
Race			Family history of cancer[a]					
Afro-Caribbean	176	83.0	No	141	66.5	Oral receptive sex[c]		
East Indian	4	1.9	Yes	27	12.8	Never	35	16.5
Mixed	15	7.1	Unknown/unsure	20	9.4	Ever	151	71.2
Not reported	17	8.0	Not reported	24	11.3	Not reported	26	12.3
Marital status			Age at 1st coitus (years)			Cervical cytology		
Single	74	34.9				ASCUS	3	1.4
Married	109	51.4	<18	86	40.6	LSIL	3	1.4
Widowed	7	3.3	18–30	105	49.5	Normal	204	96.2
Other	20	9.4	Not reported	21	9.9	Missing	2	1.0
Not reported	2	0.9						
Smoking status			Lifetime no. partners			Presence of HPV: Oral		
Non-smoker	186	87.7						
Short-term former smoker	5	2.4	<3	64	30.2			
Long-term former smoker	7	3.3	3–15	128	60.4	No	198	93.4
Active smoker	2	0.9	Not reported	20	9.4	Yes	14	6.6
Not reported	12	5.7						
Smoking exposure (cotinine levels)			Partners in last 12 months			Presence of HPV: Cervix		
Negative ETS	166	78.3	0	17	8.0	No	137	64.6
Positive ETS	36	17.0	1–4	168	79.3	Yes	75	35.4
Smoker	8	3.8	Not reported	27	12.7			
Not done	2	0.9						
Mouthwash use			Condom			HPV status (cervical/oral)		
No	89	42.0	Never	82	38.7	−/−	130	61.3
Yes	84	39.6	Sometimes	96	45.3	+/−	68	32.1
Not reported	39	18.4	Always	7	3.3	−/+	7	3.3
			Not reported	27	12.7	+/+	7	3.3

Table II (*Continued*)

	No.	Overall %		No.	Overall %		No.	Overall %
Alcohol			Oral contraceptives					
Never	40	18.9	Never	81	38.2			
Ever	139	65.6	Ever	120	56.6			
Not reported	33	15.6	Not reported	11	5.2			
Total	212	100.0	Total	212	100.0	Total	212	100.0

[a]Family cancer history refers to a first-degree relative with a history of cancer in the cervix, mouth, tongue, throat, voicebox, windpipe, oesophagus or lung; [b]female oral–penile contact; [c]male oral–vaginal contact. ETS, environmental tobacco smoke; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions.

Table III. Human papillomavirus (HPV)-type distribution in the cervix and oral mucosa of healthy Tobagonian women.

	Cervical HPV status (%)	Total HPV+(%)	Oral HPV status (%)	Total HPV+(%)
HPV negative	137 (64.6)		198 (93.4)	
HPV positive	75 (35.4)	100	14 (6.6)	100
At least one HR and/or probable HR	43 (20.3)	57.3	3 (1.4)	21.4
LR and/or UD only	32 (15.1)	42.7	11 (5.2)	78.6
Type-specific prevalence among HPV positive[a]				
High risk				
16	5 (6.7)		2 (14.3)	
18	2 (2.7)		0 (0.0)	
31	4 (5.3)		1 (7.1)	
33	2 (2.7)		0 (0.0)	
35	5 (6.7)		0 (0.0)	
39	4 (5.3)		0 (0.0)	
45	8 (10.7)		0 (0.0)	
51	2 (2.7)		0 (0.0)	
52[b]	5 (6.7)		0 (0.0)	
56	2 (2.7)		0 (0.0)	
59	2 (2.7)		0 (0.0)	
68	3 (4.0)		0 (0.0)	
73	4 (5.3)		0 (0.0)	
Probable high risk				
53	3 (4.0)		0 (0.0)	
66	6 (8.0)		1 (7.1)	
Low risk				
70	6 (8.0)		8 (57.1)	
54	9 (12.0)		0 (0.0)	
42	3 (4.0)		0 (0.0)	
44	2 (2.7)		0 (0.0)	
72	2 (2.7)		0 (0.0)	
81	2 (2.7)		0 (0.0)	
CP6108	1 (1.3)		0 (0.0)	
Undetermined risk				
62	5 (6.7)		2 (14.3)	
32	1 (1.3)		1 (7.1)	
61	6 (8.0)		0 (0.0)	
84	5 (6.7)		0 (0.0)	
83	4 (5.3)		0 (0.0)	
55	3 (4.0)		0 (0.0)	
69	3 (4.0)		0 (0.0)	
58	2 (2.7)		0 (0.0)	
71	2 (2.7)		0 (0.0)	
GA6053	1 (1.3)		0 (0.0)	

HR, high-risk HPV infection; LR, low-risk HPV infection; UD, infection with HPV of undetermined risk; [a]some women were counted more than once because of multiple infections; [b]unable to rule out HPV52 infections in five subjects.

probable high-risk HPV type either as a single or multiple infection. Overall, 25 of the 75 women with cervical HPV infection (33%) were infected with multiple HPV types (data not shown). The most prevalent high-risk HPV type was HPV45, which was present in 8/75 (10.7%) women; HPV16 was detected in the cervix of only 5 of the 75 women (6.7%).

For the oral mucosa, only 14/212 (6.6%) women were HPV infected (Table III). Of these, only three carried high-risk HPV infections (HPV16 (1), HPV66 (1) and HPV31 (1)), while the majority, 11/14 (78.6%), carried HPV types of low risk or undetermined risk (low-risk type, HPV72 (8) and undetermined risk types, HPV62 (2) and HPV32 (1)). Although HPV16 was detected in only two of the 14 women with oral HPV infections, this was the most prevalent high-risk infection observed.

Among the women with oral HPV infections, 7/14 (50%) had concurrent infections in both the oral mucosa and cervix. A comparison of the HPV-type distribution in both the oral mucosa and cervix for women who were HPV positive at both sites is summarized in Table IV. The majority of subjects did not carry the same HPV types, except for one subject who was infected with HPV16.

Age-specific distribution of high-risk cervical HPV infections

The highest prevalence of high-risk HPV infections was observed for women aged 25–34 years (30%) while the lowest prevalence was seen among women 55 years and older (7%). The number of lifetime Pap smears ranged between 0 and 15 with a median of 2 (data not shown). The majority of women 169/199 (85%) reported having only 1–5 Pap smears in their lifetime (data not shown).

Discussion

Our results show a high prevalence of cervical HPV infection in Tobagonian women (35.4%), consistent with what has been reported in other healthy Afro-Caribbean women. Figueroa et al. (1995) reported that the prevalence of cervical HPV infection in 202 Jamaican women who were attending a sexually transmitted disease (STD) clinic was 28.7%. Our result confirms that developing countries have a higher burden of cervical HPV infection compared with that of developed countries (Castellsague et al. 2001). A pooled analysis of HPV infection in 11 countries (15, 613 subjects) showed that overall, HPV16 and 18 are the most common viral genotypes detected in the cervix of healthy women, although not in sub-Saharan African populations (Clifford et al. 2005). Furthermore, the proportion of subjects of African descent included in that study was only 5%. In our study, HPV45 was the most common high-risk type detected rather than HPV16 and 18. Therefore, at least in Tobago, the distribution of HPV genotypes among healthy women is different from what is expected based on studies conducted in European and North American populations.

Table IV. Type of human papillomavirus (HPV) infection in the oral mucosa and cervix in women with concurrent infections ($n = 7$).

HPV type (oral mucosa)	HPV type (cervix)
16[a]	16[a]
16, 66[a]	70
31[a]	66[a], 71
32	73[a]
62	31, 52[a]
72	61, 62, 81
72	83

[a]Infection with high-risk HPV type(s).

Based upon the observed prevalence of cervical HPV16 and HPV18 infections in the women from our study, the potential benefit from the HPV vaccine is evident. However, for Tobago, where other high-risk HPV types are more prevalent, the level of impact which this vaccine may provide in protecting women from cervical cancer is still unclear and should be investigated. In the Caribbean population, a small study conducted in Jamaica in 1996 reported that the type-specific prevalence of HPV45 DNA among 174 colposcopy patients was 12% (Rattray et al. 1996). There was no HPV45 detected in patients with benign/reactive lesions. However, HPV45 was increasingly prevalent in patients with cervical neoplasia (ASCUS (10%), CIN1 (10%), CIN11 (11%)) and highest in high-grade lesions and invasive cancer (CIN111/CA (15%)), while HPV16 was the most common high-risk infection in patients with high-grade lesions and invasive cancer (36%). The true type-specific prevalence of high-risk HPV infections may not be completely correct for this particular study, due to the methodology used for HPV genotyping. In fact, there was a high prevalence of HPV types that could not be characterized (18%). Nevertheless, an important suggestion was that there may also be geographic variations in the distribution of HPV genotypes among cancer-free women and among women with cervical dysplasia.

To our knowledge, this is the first study to report oral HPV prevalence in healthy Afro-Caribbean women in comparison with cervical HPV prevalence. Three other studies have compared the prevalence of oral and cervical HPV infection in women, but they were conducted in other geographic regions – USA (Smith et al. 2004b, Fakhry et al. 2006) and Italy (Badaracco et al. 1998). The prevalence of HPV infection in the oral mucosa in our population is lower than that of the cervix (6.6% vs. 35.4%). Smith et al. (2004b) collected oral rinse specimens from 577 healthy US women and reported an oral HPV prevalence of 2.4% vs. 29% for the cervix. Similar to our findings, a distinct difference in HPV prevalence was observed between the oral mucosa and the cervix. Fakhry et al. (2006) also reported comparisons of HPV prevalence in 221 paired cervical and oral rinse specimens from women with and without HIV infection. The oral HPV prevalence in that study was slightly higher than we observed in our study; nonetheless, there was a distinct difference between oral and cervical HPV prevalence: in HIV-negative subjects, 9.9% had HPV-positive oral samples while 44.9% had HPV-positive cervical samples. In contrast, in an Italian study population, Badaracco et al. (1998) combined samplings of visible lesions and normal mucosa from both sites and reported an oral HPV prevalence of 38% vs. 33% in the cervix. The small proportion of women with concurrent oral–cervical infection in our study makes it difficult to interpret the true significance of the risk associations. Nonetheless, these preliminary data have for the first time provided the prevalence of specific HPV types detected in the cervical and oral mucosa of healthy Tobagonian women. Larger studies are needed to investigate further the dynamics of HPV16 infection in the oral mucosa of cancer-free individuals, keeping in mind that HPV16 is the most common viral type detected in HPV-positive oral cancers.

One limitation of the present study is that the facility in Tobago was not equipped for cell counting, therefore introducing the potential for a false-negative HPV result due to inadequate cell numbers. To address this limitation, DNA adequacy was evaluated by testing the samples for the β-globin gene, which was positive in all samples. The use of oral exfoliated cells for HPV testing has been established by others to yield a higher number of, and a more representative sample of,

DNA-containing nucleated cells compared to other collection methods (Lawton et al. 1992, Kreimer et al. 2004, Smith et al. 2004a).

The main strength of our study is that it shows that the prevalence of cervical HPV infection in cancer-free Afro-Caribbean women is different from that found in studies of US and European populations. There are very few studies of HPV infection conducted in developing countries compared with developed countries. Therefore we have added supporting data related to the high burden of HPV infection in women of African descent. Since the HPV vaccine specifically prevents cancers caused by HPV16 and 18, studies are needed to assess the impact of the current vaccine in this population where the prevalence of genotypes other than HPV16 and 18 are predominant in the general population.

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