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Concurrent Infections With Human Papillomavirus and Cervical Intraepithelial Lesions: What Is the Relationship?

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Abstract

Background: Human papillomavirus (HPV) infection is necessary for cervical dysplasia and cervical cancer to develop, but infection with HPV is not predictive of which women will develop cervical squamous intraepithelial lesions (SILs) or cancer. This study examines the relationship between the number of concurrent HPV infections and risk of SIL as well as the variations in HPV types in a diverse population.

Methods: IRB approval was obtained. Women presenting for gynecologic exam were recruited to participate. ThinPrep samples were sent for cytological evaluation, and cervical cells were obtained for HPV screening and typing (INNO-LIPA genotyping kit); medical information was recorded into a Microsoft Access database. Data analysis was performed using JMP statistical software.

Results: Seven hundred nineteen women were recruited to participate; race/ethnic distribution was 79.6% for African-American/Black and 14.2% for Caucasian/White with an average age of 31.4 years. Of the patients, 27.5% were HPV-positive, and the average number of HPV types present at the time of the Pap test was 2.55. There was no difference in the number of concurrent HPV infections when stratified via race/ethnicity and cervical cytology/pathology. Regardless of race/ethnicity and cytology and pathology, the three most common high-risk types of HPV were 52, 16, and 39.

Conclusions: Abnormal cytology/pathology did not vary with number of concurrent HPV infections.

Keywords: HPV; Cervical squamous intraepithelial lesions; Cervical cancer

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Introduction

Despite the fact that there are well-established screening programs for cervical precancerous lesions or squamous intraepithelial lesions (SIL) and cervical cancer, and the etiologic agent, human papillomavirus (HPV), has been identified, cervical cancer rates have plateaued over the past 10 years. In the United States, the Centers for Disease Control and Prevention (CDC) reported 11,955 new cases and 4,217 deaths attributable to cervical cancer in 2013 [1].

Additionally, significant health disparities continue to exist for cervical cancer incidence and mortality. Cervical cancer incidence and mortality are 40-60% higher in African-Americans/Blacks and Hispanic/Latinas as compared to Caucasian/White women [2-5]. The disparities persist among races even after controlling for differences in prior hysterectomy, screening and access to health care [2-8]. Differences also exist in survival rates between African American and Caucasian women even when controlled for stage of cancer at diagnosis, histological grade and type [1-8].

The majority of women who develop high grade cervical SIL after acquisition of HPV do not have any of the known risk factors (smoking, multiple sexual partners, etc.). This study examines if concurrent HPV infections are associated with an increased risk in high grade SIL lesions.

Materials and Methods

Study population

IRB approval was obtained through the Palmetto Health Institutional Review Board. Patients presenting for routine annual gynecologic exam or colposcopy at Palmetto Health Women's Center were recruited to participate in the study; all patients were managed and referred to colposcopy according to published and established guidelines by the American Society for Colposcopy and Cervical Pathology (ASCCP [9, 10]). ThinPrep Pap samples were sent for routine cytological evaluation. Cervical cell samples were obtained for HPV screening and typing (real-time PCR and INNO-LIPA genotyping kit). DNA was extracted from the cervical cells of study participants to ascertain the HPV positivity rate as well as for specific HPV typing. All

Table 1. Demographics

	% (no.)
Race/ethnicity	
African-American/Black	79.6 (572)
Caucasian/White	14.2 (102)
Hispanic/Latina	4.7 (34)
Asian/other/unknown	1.5 (11)
Age (years)	
Range	21 - 64
Mean	31.4
Age at first pregnancy	
Range	12.0 - 36.0
Mean	20.6
Body mass index (BMI)	
Range	16.2 - 68.9
Mean	31.7

samples were initially screened for the presence of any HPV type, utilizing a real-time PCR amplification protocol optimized in our laboratories with L1 HPV consensus primers GP5+/6+ to generate a 150-base pair fragment with real-time PCR using the ICycler IQ detection system [5, 6]. All the HPV-positive samples underwent specific HPV typing through a PCR-based hybridization protocol from Innogentics (INNO-LiPA HPV genotyping) that allows for the detection of 27 HPV types [5, 6].

Statistical analyses

Medical information was abstracted from the participant's medical record and recorded into a Microsoft Access database (Microsoft Office, Redmond, WA). Fisher exact test and Student's *t*-test were utilized for analysis of categorical and continuous data, respectively using a P value < 0.05 for statistical significance. Data analysis was performed with JMP statistical software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA).

Results

A total of 734 women were recruited to participate in this study with 15 women excluded from the final analysis because cervical cells were unable to be obtained, leaving a total of 719 participants in our final analysis (Table 1). Ages of participants ranged from 21 to 64 years (mean: 31.4). Racial/ethnic distribution was 79.6 % for African-American/Black, 14.2% for Caucasian/White, 4.7% for Hispanic/Latina, and 1.5% for Asian/other/unknown. Body mass index (BMI) ranged from 16.2 to 68.9, with a mean of 31.7 (Table 1). Age, race/ethnic distribution, and BMI did not differ from the general population receiving care at Palmetto Health Women's Center.

Table 2. Cytology and Pathology From Cervical Biopsies

	%
Pap test cytology	
Negative	74.5
ASCUS	8.0
LSIL	12.0
HSIL/ASC-H	3.0
AGS	0.6
Pathology from cervical biopsy	
CIN1	61.1
CIN2/3	38.2
Cancer	0.7

The Pap test cytology distribution was 74.5% negative, 8% atypical cells of undetermined significance (ASCUS), 0.6% atypical glandular cells (AGS), 12% low-grade squamous intraepithelial lesions (LSIL), and 3% high-grade squamous intraepithelial lesions (HSIL) (Table 2). All women with abnormal cytology were referred for colposcopy according to ASCCP and ACOG guidelines. The distribution of pathology from colposcopy-directed biopsies was 61.1% LSIL, 38.2% moderate/high-grade SIL, and 0.7% cancer (Table 2). There was only one case of squamous cell carcinoma of the cervix in our study participants. There was no difference in Pap test or pathology distribution when comparing African-Americans, Caucasians, and Hispanics (data not shown).

All cervical samples (regardless of cytology) were initially screened for the presence of any HPV type through a real-time PCR amplification protocol with L1 HPV consensus primers GP5+/6+ [5, 6]. Overall 27.8% of study participants were found to be HPV-positive. When broken down by race/ethnicity, 75.5% of those that were HPV-positive were African American, 20.7% Caucasian and 3.5% Hispanic/Latina. There were more Caucasian women who were HPV-positive com-

Table 3. Race/Ethnicity and HPV

Race/ethnicity	Total population, % (no.)	HPV-positive, % (no.)
African-American/Black	79.6 (572)	75.5 (151)
Caucasian/White	14.2 (102)	20.6 (41)
Hispanic/Latina	4.7 (34)	3.5 (7)
Asian/other/unknown	1.5 (11)	0.5 (1)
Total	100 (719)	100 (200)

pared to what was expected from the general study population ($P < 0.0472$, Table 3).

All the HPV-positive samples underwent specific HPV typing through a PCR-based hybridization protocol (INNO-LiPA HPV genotyping) that allows for the detection of 27 HPV types. High-risk HPV types accounted for 82.4% of all the HPV infections, and low-risk HPV types were found in 14.6% of the infections; the other 3% were undetermined HPV types by our assay. The majority of women had multiple HPV infections at the time of the Pap test. The average number of HPV types present at the time of the Pap test was 2.55 (SD 1.96; range 1 - 13). There was no difference in the number of HPV types when stratified by race/ethnicity or age (Table 4). Furthermore, the number of HPV infections did not vary according to cytology (Table 4) or cervical pathology (Table 4); there was a trend towards less HPV infections in women with CIN3 versus CIN2, 2.07 and 2.96, respectively, that did not reach statistical significance ($P = 0.07$).

Regardless of race/ethnicity and cytology, the three most common high risk types of HPV were 52, 16, and 39, regardless of race/ethnicity (Table 5). We found that White/Caucasian women with HSIL had HPV16 more frequently than African-American women with the same lesion with an odds ratio of 10.3 (95% CI: 2.15 - 49.23; $P = 0.03$). Additionally, Caucasian/White women with CIN2 were more likely to have HPV33 than Black/African-American women ($P < 0.0065$). Although there were several other trends in HPV type frequency, no other differences in HPV type were statistically significant.

Table 4. Concurrent HPV Infections

	HPV infections (no.)
Age	
21 - 24	2.57
25 - 29	2.48
> 30	2.47
Race	
African	2.67
American/Black	2.32
Caucasian/White	1.57
Hispanic/Latina	
Cytology	
Negative	2.56
ASCUS	2.65
LSIL	2.61
HSIL/ASC-H	2.26
Cervical pathology	
CIN1	2.57
CIN2	2.96
CIN3	2.07

Discussion and Conclusions

Significant health disparities exist for cervical cancer; Black/African-American and Hispanic/Latina women have twice the mortality rate as White/Caucasian women. Access to health care does account for some of these disparities but cannot account for the entire discrepancy. We have shown that in the college-age demographic, Black/African-American women have more persistent HPV infections than White/Caucasian

Table 5. High-Risk HPV Type Distribution

	HPV52	HPV16	HPV39	HPV18	HPV45	HPV51	HPV33	HPV35
Any cytology								
African-American	16.3%	11.1%	9.4%	7.2%	1.6%	7.5%	6.2%	6.5%
Caucasian	16.9%	16.9%	10.8%	4.6%	7.7%	7.7%	4.6%	1.5%
Negative cytology								
African-American	14.9%	10.6%	9.2%	9.2%	2.8%	7.1%	5.7%	6.4%
Caucasian	15.4%	15.4%	15.4%	7.7%	15.4%	7.7%	0.0%	7.7%
CIN1								
African-American	14.7%	11.8%	7.8%	4.9%	1.0%	6.9%	7.8%	4.9%
Caucasian	20.8%	4.2%	8.3%	0.0%	12.5%	12.5%	0.0%	0.0%
CIN2								
African-American	22.4%	10.2%	14.3%	8.2%	0.0%	10.2%	4.1%	6.1%
Caucasian	20.0%	26.5%	6.7%	7.7%	0.0%	0.0%	20.0%	0.0%
CIN3								
African-American	20.0%	13.3%	6.7%	0.0%	0.0%	6.7%	6.7%	20.0%
Caucasian	7.7%	30.8%	15.4%	7.7%	0.0%	7.7%	0.0%	0.0%
CIN2/3								
African-American	21.9%	10.9%	12.5%	6.3%	0.0%	9.4%	6.2%	9.4%

women [5, 6]. Persistence of HPV is the main precursor to developing severe SIL and cervical cancer. It is unclear why White/Caucasian women are able to eliminate HPV from their cervix more readily than African-American women.

This study examined if concurrent HPV infections were a risk factor for developing SIL. The study population was recruited from patients who receive their care at Palmetto Health Women's Center; this group consisted of a high percentage of African-American patients with an average age of 31 years and a primarily low income.

We found that abnormal cytology and pathology in our study participants did not vary by race/ethnicity; this finding is in contrast to what we found in a younger population (college-age students from Carolina Women's Care Study [6, 7]) where African-American young adults were more likely to have abnormal cytology than their Eastern European/Caucasian counterparts (OR: 1.58).

About 28% of our study participants were HPV-positive, and this did not vary by race/ethnicity. Multiple concurrent HPV infections were common, but there was no correlation of number of HPV types with race/ethnicity or abnormal Pap test cytology or cervical pathology in patients undergoing a colposcopy. Most interestingly, regardless of race or cytology/pathology, the three most common HPV types were 52, 16, and 39. White/Caucasian women with CIN3 were more likely to have HPV16 infection as compared to Black/African-American women which, in turn, were more likely to have HPV52 and HPV35. The recently FDA approved 9-valent vaccine provides immunity to HPV31, 33, 45, 52, and 58 in addition to 6, 11, 16, and 18; this does not cover HPV39, or HPV35 which are high-risk types, but these types are thought to rarely lead to cervical cancer [11]. Of note, there has been some important evidence that indicates HPV39 may be associated with anal cancers [12] as well as cervical cancers in certain ethnic groups [13, 14]. This was one of the most common types of HPV found in our diverse study population, and anal cancers are increasing in prevalence. Currently, no screening guidelines for HPV type exist for anal cancer in low-risk populations.

Our data show that the number of concurrent HPV infections is not likely to play a role in developing cervical SIL and cervical cancers. These findings support the concept that molecular biomarkers are of greater importance when predicting HPV persistence and high-grade cervical SIL than clinical characteristics, and are more likely to account for health disparities found in cervical cancer [15].

Conflicts of Interest

Authors report no direct conflicts of interest for this manuscript or experiments presented in this study. LBS has been a consultant for Inovio Pharmaceuticals in the past year and poses no conflicts of interest in these studies and manuscript.

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