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Cervical cytology and multiple type HPV infection: A study of 8,182 women ages 31–65

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Abstract

Objective—Determine the rates of single and multiple type Human Papillomavirus (HPV) infection in women in the United States ages 31–65 with known cervical cytology results.

Methods—Type-specific HPV analyses were conducted using the first samples of women who had HPV typing performed by Access Genetics as part of cervical cancer screening between July 2007 and May 2011. Women 31–65 years at testing with associated abnormal cytology results were included. The odds of abnormal cytology (compared to normal results) for multiple vs. single HPV infections were calculated for each cytology sub-type and odds ratios (OR) and 95% confidence intervals (CI) are reported.

Results—The analysis included 8,182 women. The majority (67.7%) had ASCUS cervical cytology. A total of 329 (4.0%) were positive for 2 or more HPV types. For all cervical cytology subtypes considered (ASCUS, ASCUS-H, LSIL or HSIL), women with multiple type infections were more likely to have abnormal cytology (compared to normal cytology) with the highest OR associated with HSIL (OR 1.81 (1.26–2.60)). When analyzing HPV type 16 alone, women with

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AUTHOR CONTRIBUTIONS
Dickson wrote the research paper, with edits from Geller, Vogel, and Downs. Vogel performed the statistical analysis for the research project. Downs is the guarantor of the research project, and has overseen all portions of the project's fruition.

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Elizabeth L. Dickson, MD: No potential conflicts
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multiple type infections were more likely to have abnormal cytology, with the highest OR associated with HSIL cytology (OR 2.98 (1.57–5.64)). Few women had HPV type 18 infections and no results reached statistical significance. Results based on phylogenic family organization focusing on the alpha 9 phylogenic family showed similar results as HPV type 16.

Conclusions—Women ages 31–65 with multiple type HPV infections were more likely to have abnormal cytology than those with single HPV type infections.

Keywords

HPV; Epidemiology; Cervical Cytology; Multiple Infections

Introduction

Cervical cancer continues to be a global concern. The American Cancer Society estimates 12,340 new cases of invasive cervical cancer will be diagnosed, and 4,030 women will die of cervical cancer in 2013 in the United States [1]. The link between Human Papillomavirus (HPV) and cervical cancer has been well established; of the more than 100 types of HPV identified, 13–15 oncogenic subtypes are associated with the majority of cases of cervical cancer [2].

While most HPV infections are characterized by spontaneous viral clearance, there are some infections which are persistent in nature. Previous work found that 39% of women who had consecutive HPV testing as a component of cervical cancer screening had persistent HPV infection, of which 34% were high risk HPV types [3]. Persistent high risk HPV infection is a strong predictor for the development of CIN 2/3 and invasive cervical cancer [4].

Multiple-type HPV infections have been described more consistently since analysis of type-specific HPV results with PCR assays, which have been found to have higher analytical sensitivity [5]. In large studies of populations from Costa Rica and Italy, multiple-type HPV infection prevalence range from 24.8% to 52.6% among all HPV positive tests [6, 7]. There continues to be discussion as to whether these infections occur randomly, or as a result of interactions between HPV types [5]. This in part is due to the debate over the accuracy of type-specific testing modalities to distinguish between HPV types and whether the testing modalities can accurately determine if two distinct HPV types are present. The prevalence of persistent infection, which is difficult to establish, could also be involved in multiple infections, and therefore whether both infections occurred at the same time is unknown. Few studies have evaluated the interactions of HPV multiple-type infections on cervical disease risk [7].

To address this question, we sought to determine the rates of single and multiple type HPV infection in women with known cervical cytology results. We examined the frequency of multiple-type infection with high risk HPV types in a large population of women ages 31–65 referred for HPV testing as a component of cervical cancer screening in the United States.

Materials and Methods

Study Population

Prior to the initiation of this investigation, approval was granted by the University of Minnesota's Institutional Review Board. We analyzed data from women ages 31–65 who had HPV typing performed by Access Genetics (Eden Prairie, MN) between July 2007 and May 2011. Access Genetics offers medical diagnostic services, including HPV testing. In addition to reporting the presence or absence of high-risk HPV types, they perform PCR-based HPV typing. Data from 47 labs across the country which use Access Genetics for HPV typing were analyzed. Most frequently, HPV typing was performed after an abnormal pap test or an abnormal colposcopy, as per ASCCP guidelines [3]. Patient age at testing, laboratory location, and test media type were the only demographic information available.

Specimen Analysis

Specimen analysis was performed at Access Genetics as previously described [8]. Samples were processed within 48 hours of receipt. DNA was extracted by salt precipitation in standard methods previously described, and the genomic DNA amplification was performed per the methods described by Resnick et al [9]. The products of PCR amplification were analyzed by polyacrylamide gel electrophoresis and the HPV positive samples were subjected to genotyping by restriction endonuclease fragment analysis.

Interpretation of the HPV genotypes was based on the pattern of resulting bands for each enzyme, which was compared to the genomic maps of each viral type. The pattern of restricted DNA bands for each of the known HPV viral types has been described previously [9]. The 22 types considered high-risk were HPV-16, −18, −26, −31, −33, −35, −39, −45, −51, −52, −53, −56, −58, −59, −66, −67, −68, −69, −70(LVX160), −73(MM9), −82(MM4, IS39), and −85. Low-risk HPV types in this study were HPV-6, −11, −32, −40, −42, −44, −54, −55, −61, −62, −64, −71(CP8061), −72(CP4173, LVX100), −74, −81(CP8304), −83, −84(MM8), −87, −89(CP6108), and −91 [10]. The subtypes listed in parentheses were combined with their primary type for analysis. All of the other detected HPV types were categorized as unclassified (unknown risk).

Statistical Methods

The first sample for HPV type evaluation with a conclusive negative or positive result from each woman collected between July 2007 and May 2011 were considered, and therefore ensured that women were only included once in analysis. Data were limited to those women who were 31–65 years old at testing and had corresponding cytology results. The demographic information available was age at testing, laboratory location (Western, Southern, North Central, and Eastern United States) and test media type (SurePath, ThinPrep, or other). All available patient demographic and clinical characteristics were summarized using descriptive statistics; means ± standard deviations and percentages are presented. All HPV types were analyzed separately. The odds of abnormal cytology (compared to normal results) for multiple versus single HPV infections were calculated for each cytology sub-type and odds ratios (OR) and 95% confidence intervals (CI) are reported.

HPV types were also analyzed according to their phylogenic viral species [11]. Based on characteristics of the HPV genome, specifically the L1 nucleotide sequences, HPV types are grouped into α species, 1–15. Comparison of phylogenic species was limited to the α-9 specie (including types 16, 31, 33, 35, 52, 58, 67) and α-7 specie (including types 18, 39, 45, 59, 68, 70). Rates of infection and multiple infections were calculated by virus specie. The odds of abnormal cytology, compared to normal results, for multiple versus single HPV infections were calculated for all possible pairs of phylogenic viral species using the statistical methods described above.

Statistical analyses were performed using SAS version 9.3 (Cary, NC). Due to the exploratory nature of this analysis, confidence intervals were not adjusted for multiple comparison testing.

Results

Available demographic data are shown in Table 1. Samples were included from all regions of the United States, with the greatest number of samples received from the North Central US (46.2%). The majority of women (n=5,402; 66.0%) had ASCUS cervical cytology.

Women with multiple type HPV infections were more likely to have abnormal cytology showing ASCUS, ASCUS-H, LSIL or HSIL (Table 2). When analyzing specimens for HPV 16, women with multiple type infections including HPV 16 were more likely to have abnormal cytology, with the highest OR in the HSIL group those having (OR 2.98 (1.57–5.64). Few women had infections including HPV type 18 and no results reached statistical significance.

Comparison of phylogenic species was limited to the α-9 specie (including types 16, 31, 33, 35, 52, 58, 67) and α-7 specie (including types 18, 39, 45, 59, 68, 70). In comparison to the normal group, the α-9 phylogenic family results were similar to findings with HPV type 16, however, the results become more attenuated in all but the ASCUS group. The α-7 specie data were sparse and insufficient for statistical tests.

Discussion

In this study we found that HPV was present in 1,936 of 8,182 (23.7%) women ages 31–65 referred for HPV testing who had associated cytology results. This prevalence is similar to those found in other large studies of women tested for HPV at the time of cytology testing. In the ARTISTIC trial of 24,510 women, 27.3% of women under the age of 30 had high risk HPV types, whereas only 6.1% of women over the age of 30 had high risk HPV types at the time of cytology testing [12]. The Costa Rica Vaccine trial of 5,871 sexually active women found HPV was present in 2,478 women (50.0%), with 1,983 women (33.8%) having oncogenic HPV types [7]. In contrast to our study, sexual activity status was known in both of these trials.

Our results demonstrate increased odds of having abnormal cervical cytology if multiple-type HPV infections are present, with the highest OR found in the HSIL cytology groups when compared to normal cytology. This is consistent with a longitudinal study by Trottier

et al., where 2,113 women were followed for four years with serial cytology and HPV testing: 12.3% were infected with multiple HPV types at initial screening, and 22.3% were infected during the first four years of follow-up [13]. In that same study, patients infected with two or more high risk HPV types had an increased risk of any grade SIL, even after excluding women with persistent infections. To date, the relationship between multiple HPV type infections and the progression to cervical cancer is unknown; however, competitive or cooperative interactions between HPV types could increase the risk of progression to cervical cancer [8]. In the Costa Rica Vaccine trial, women with multiple infections were at significantly increased risk of both CIN2+ and HSIL+ when compared with single infection, with the highest risk being in those having multiple oncogenic types and those from the α-9 specie [7]. Our findings are consistent with these data.

It is unknown whether the HPV types in multiple infections occur in a random fashion or if a competitive or cooperative relationship exists. Our recent paper suggests that multiple type infections occur more frequently than would be expected by chance alone [8]. In an analysis of the Guanacaste Study of HPV Natural History, infections with multiple HPV types occurred more often than expected by chance; however, after controlling for lifetime number of sexual partners, age, and specific HPV type prevalence, the cases with multiple type infections were small [14]. This study, however, does not discuss cervical cytology or the risk of multiple-type infections based on the cytology results. There has been discussion of clustering of HPV types based on the L1 region genetic similarity; however, many believe this to be an artifact of HPV detection methods [15]. A Danish study of 5014 women tested for 35 HPV genotypes, and for the 15 HPV types of primary interest (which included HPV types 16 and 18), almost all pairs occurred more frequently than expected by random chance alone [16]. This relationship held after correcting for common risk factors and mode of transmission. Regardless of whether multiple-type HPV infection is random, understanding the impact of these types of infections is critical in the era of HPV vaccination.

The similarities between members of HPV phylogenic families suggests the potential partial cross-protection for other members of the α-9 (including HPV 16, 31, 33, 35, 52, 58, and 67) and the α-7 families [17]. In the current analysis, multiple-type HPV infection with members of the α-9 family was associated with ASCUS, LSIL and HSIL cytology. This concurs with the Chaturvedi et al study, who found an increased risk of HSIL with multiple-type infections from the α-9 family [7]. The increased risk of abnormal cytology in these studies could be a result of interactions between these family members, yet identified. Trottier et al. hypothesizes that HPV types in the α-9 specie could exert oncogenic effects and impact the cervical epithelia infected with other HPV types [13]. Continued examination of the epidemiology of phylogenic HPV families will help to understand the changing prevalence of specific HPV types with continued increase of vaccination.

Retrospective analyses have inherent limitations. The main strength of this retrospective study is the large number of samples with known cytology data; unfortunately we do not have data on other characteristics of the women that may suggest increased risk of multiple-type infections, such as sexual activity. A study by Querec et al noted that certain epidemiologic factors could affect multiple type infections, such as age, smoking, lifetime number of sexual partners, and frequency of sexual intercourse [18]. However, we do not

have these data for our study. Despite this, our results are similar to those studies where the demographic data are included [12, 14]. As different techniques evolve for detection of type-specific HPV, each previous detection method is scrutinized. It is possible that PCR test results may be affected by differences in sensitivity of the testing method for different HPV types, though our findings are similar to others. We analyzed a wide range of high risk HPV types, and therefore, could have detected more multiple type infections than if the number of high risk HPV types were limited. A recent study on laser capture micro-dissection reveals new insight onto the HPV and cytology relationship [19]. This was not performed on these samples, and therefore we do not know the causality of the HPV multiple types and the cytology results reported. We analyzed the first reported result for each patient, which may represent persistent infection. Determination if infections were concurrently versus sequentially acquired was not possible. Finally, while this is a large sample, the number of women with multiple infections by cytology group was small in some cases.

In conclusion, in this sample of 8,182 women with known cytology results, we show higher rates of abnormal cervical cytology in women with multiple-type infections, especially those which included HPV-16 and the α-9 family. Further prospective investigations are needed to verify and determine the mechanism of these multiple-type infections and the progression to abnormal cervical cytology and cervical cancer.

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Research Highlights

- Women over the age of 30 with multiple type HPV infections are more likely to have abnormal cytology
- Women with multiple type HPV infections including HPV 16 had the highest OR associated with HSIL cytology
- Continued study necessary to identify the impact of multiple type HPV infections on abnormal cytology

Table 1

Available Demographic and Clinical Characteristics for all Patients.

	N	%
TOTAL	**8182**	**57.9**
Laboratory Location		
Southern US	780	9.5
North Central US	3778	46.2
Eastern US	361	4.4
Western US	3263	39.9
Specimen		
SurePath	5271	64.4
ThinPrep	2760	33.7
Other	151	1.9
Cytology		
Normal	1923	23.5
ASCUS	5402	66.0
ASCUS-H	74	0.9
LSIL	618	7.6
HSIL	57	0.7
Other	105	1.3
Carcinoma	3	0.04
Positive for HPV Infection		
None	6246	76.3
1 infection	1607	19.6
2 infections	283	3.5
3 infections	36	0.4
4 infections	10	0.1
	N	**Mean (SD)**
Age (years)	8182	43.4 (8.7)

Table 2

HPV Infections and Cytology Result – all Odds Ratios in comparison to Normal group.

	Normal	ASCUS		ASCUS-H		LSIL		HSIL	
Variable	**N (%)**	**N (%)**	**OR (95% CI)****	**N (%)**	**OR (95% CI)****	**N (%)**	**OR (95% CI)****	**N (%)**	**OR (95% CI)****
Any HPV Type									
Single Infection	157 (8.2)	1029 (19.1)	1.00	27 (36.5)	1.00	332 (53.7)	1.00	32 (56.1)	1.00
Multiple Infections	21 (1.1)	196 (3.6)	**1.31 (1.07–1.62)**	3 (4.1)	**1.73 (1.08–2.77)**	96 (15.5)	**1.77 (1.57–2.01)**	9 (15.8)	**1.81 (1.26–2.60)**
HPV Type 16									
Single Infection	13 (0.7)	124 (2.3)	1.00	7 (9.5)	1.00	44 (7.1)	1.00	11 (19.3)	1.00
Multiple Infections	8 (0.4)	48 (0.9)	**1.74 (1.05–2.89)**	1 (1.4)	**2.50 (1.08–5.80)**	18 (2.9)	**1.60 (1.19–2.14)**	2 (3.5)	**2.98 (1.57–5.64)**
HPV Type 18									
Single Infection	5 (0.3)	33 (0.6)	1.00	1 (1.4)	1.00	10 (1.6)	1.00	4 (7.0)	1.00
Multiple	1 (0.1)	10 (0.2)	1.09	0 (0.0)	NE	9 (1.5)	0.97	2 (3.5)	2.11
Infections			(0.39–3.06)				(0.56–1.69)		(0.89–5.02)
Oncogenic* Types									
Single Infection	72 (3.7)	568 (10.5)	1.00	19 (25.7)	1.00	180 (29.1)	1.00	31 (54.4)	1.00
Multiple Infections	4 (0.2)	47 (0.9)	1.27 (0.85–1.89)	2 (2.7)	1.44 (0.81–2.55)	28 (4.5)	**1.33 (1.10–1.62)**	2 (3.5)	**2.59 (1.50–4.47)**
α9 Species									
Single Infection	49 (2.6)	412 (7.6)	1.00	16 (21.6)	1.00	122 (19.7)	1.00	26 (45.6)	1.00
Multiple Infections	4 (0.2)	16 (0.3)	**2.20 (1.34–3.61)**	1 (1.4)	1.42 (0.66–3.02)	6 (1.0)	**1.46 (1.04–2.04)**	2 (3.5)	**1.88 (1.06–3.33)**
α7 Species									
Single Infection	16 (0.8)	133 (2.5)	1.00	4 (5.4)	1.00	52 (8.4)	1.00	7 (12.3)	1.00
Multiple Infections	0 (0.0)	4 (0.1)	NE	0 (0.0)	NE	2 (0.3)	1.48 (0.79–2.75)	0 (0.0)	NE

* HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68–73

** Compared to Normal

NE = not estimable; Bold value indicate that odds ratio does not include 1.0 ($p<0.05$).