Risk factors and the association of multiple HPV infections on pre-invasive cervical lesions and invasive cervical cancers among women with HPV infection in Senegal, West Africa

Simeon Junior Adiunegiya

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Stephen E Hawes
Monisha Sharma

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Abstract

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Simeon Junior Adiunegiya

Chair of Supervisory Committee:
Stephen E Hawes
Department of Epidemiology

Infection with multiple HPV types may increase or reduce the risk of pre-invasive cervical lesions and invasive cervical cancers. We conducted a secondary analysis of data to assess the risk factors of infection with multiple HPV types, and the association of infection with multiple HPV types and pre-invasive cervical lesions and invasive cancers among 1202 women who tested positive for HPV serology in 3 clinics in Senegal. We used Poisson regression analysis with robust standard error to calculate risk estimates and 95% CI. We did not find any significant association between different socio-demographic characteristics and behavioral factors associated with infection with multiple HPV types. We also did not find any significant association of infection with multiple HPV types and pre-invasive cervical lesions and invasive cervical cancers. We did, however, find a positive trend towards multiple HPV infection in pre-invasive cervical lesions and a negative trend in invasive cervical cancers.

Introduction

Cancer of the uterine cervix is the fourth leading cause of morbidity and mortality among women globally, with particularly high rates in low-middle-income countries where routine screening and Human Papillomavirus (HPV) vaccination are not widely available[1]. It is well established that certain types of high-risk HPV are responsible for the vast majority of pre-invasive cervical lesions and invasive cervical cancers[2],[3].

Co-infection with multiple HPV types is common, particularly among sexually active women below the age of 25 years. About 20% of women with a normal cervix have been found to have multiple HPV types[4]. Despite this, very few studies have investigated the association between infection with multiple HPV types and cervical cancer oncogenesis, and those conducted have shown contrasting evidence. Quint et al found that the risk of cervical oncogenesis among women with multiple HPV infection was dependent on the HPV type. In their study that looked at histological micro-dissection of cervical intraepithelial neoplasia (CIN) lesions, they found each HPV type with an independent biological lesion[5]. This was also observed in a similar study in South Africa where higher viral load for specific high-risk HPV types correlated with cervical oncogenesis among women with invasive cervical cancers[6]. Several observational studies postulate that infection with multiple types of HPV, including high-risk HPV types, may increase risk of cervical cancer due to synergistic effect[7],[8]. Other studies have suggested that women who are infected with multiple HPV types may have lower risk of cervical cancer due to inter-genotypic interaction or antibody cross-reaction[9],[10].

In addition, the risk factors of infection with multiple HPV types have not been well understood as only few studies have investigated this association. As a sexually transmitted infection, the risk factors of infection with multiple HPV types have been assumed to be related to sexual activity as in single HPV infection such as early sexual debut, multiple sexual partners, marital status, and HIV infection[11],[12]. Given the high burden of multiple HPV infection and the lack of consistency in the literature, it is necessary to investigate the risk factors and potential impact of infection with multiple HPV types on cervical cancer oncogenesis. In this cross-sectional study, we investigate the risk factors of infection with multiple HPV types and the association of multiple HPV types with the presence of pre-invasive cervical lesions and invasive cervical cancers among women with HPV infection in Senegal, West Africa.

Material and Methods

Study population

We conducted a secondary analysis of data involving 1202 women 18 years and older enrolled in 3 observational studies conducted between 2003 and 2010, in 3 outpatient clinics in Dakar, Senegal. The first is the Hypermethylation Study, a prospective cohort study among women who attended cervical screening at Fann University Hospital infectious disease clinic, and Pikine outpatient clinic. The second is the Chlamydia Study, where enrolled women were referred to the oncology clinic at Dantec Hospital with presumed cervical cancer. In the third study, participants were screened for cervical disease at Dantec Hospital and Fann Hospital.

All participants were previously unscreened for cervical cancer and were unvaccinated against HPV. In our analysis, we included participants who had a positive HPV DNA test and had results for cervical cytology and/or cervical histology. We excluded participants who tested positive for HIV serology.

Collection of specimens and study procedures

Women attending the clinics were invited to participate in the study, consented, and interviewed by local researchers to complete questionnaire forms that captured their demographics, socioeconomic characteristics, and medical history. Information collected among others included age, age at first sex, age at first pregnancy, number of pregnancies, marital status, number of cowives, number of lifetime sexual partners, conceptive use, education level and smoking status.

Cytological screening, HPV DNA Detection and Typing

Baseline cervical swabs specimens were obtained for HPV DNA testing and cervical cytology. Screening for HPV infection was done using a generic probe and typing for up to 38 HPV types was conducted using Luminex, which is described by Feng et al in a previous study[13]. In a subset of study participants (all participants at Dantec with presumed cancer and those enrolled into longitudinal studies at Pikine and Fann), cervical biopsies were obtained for histological assessment of cervical disease. All three studies were approved by institutional boards of the University of Washington, Seattle, and the University of Dakar, Senegal.

Statistical methods

Single HPV infection was defined as having one HPV DNA type in a cervical swab specimen. Multiple HPV infection was defined as having two or more co-infection of HPV DNA types at the time of cervical swab. We excluded participants who tested positive for HPV DNA but without a detectable known type(s) of HPV, as assessment of having single vs. multiple HPV types was not possible in those individuals.

We classified cervical results as normal, pre-invasive lesions, and invasive cancers based on cervical cytology and/or histology. Normal was defined as having normal, atypical, or atypical squamous cells of undefined significance (ASCUS). Pre-invasive lesions were defined as low-grade squamous intra-epithelial lesion (LSIL), high grade squamous intra-epithelial lesion (HSIL), cervical intraepithelial neoplasia (CIN1, CIN2, CIN3) or carcinoma in-situ (CIS). Invasive cancers were invasive cancer, adenocarcinomas, or other histological cancer types of cervical cancers.

We performed Poisson regression with robust standard error to assess 1) factors associated with infection with having multiple HPV types and 2) the association between infection with multiple HPV types and pre-invasive cervical lesions and invasive cancers. We used a confirmatory approach for the former and exploratory approach for the latter.

To determine the risk factors of multiple HPV infection, we conducted univariate and multivariate Poisson regression with robust standard errors among participants with normal cervical finding and compared to single HPV infection. As this was a confirmatory analysis, our

analysis of demographic and behavioral factors was based on a priori hypothesis informed by current literature.

To determine the association between infection with multiple HPV types and pre-invasive cervical lesions and invasive cervical cancers, we conducted univariate and multivariate regression models comparing each stratum to normal cervical finding. We first performed univariate and bivariate models to identify potential confounders, effect modifiers, and then fitted two multivariate models. All statistical analysis was conducted in R version 4.2.3.

Results

Characteristics of study population

Of the 1202 participants, 630 (52.0%) were enrolled at Dantec Hospital, 448 (37.0%) at Pikine outpatient clinic, and only 123 (10.0%) were enrolled at Fann University Hospital (Table 1). Women recruited at Dantec hospital were the oldest and had high number of pregnancies, while those at Fann Hospital were the youngest, who were more likely to delay their first sex after 18 years and had fewer children. Across the three study sites, most women were married and in polygamous relationship, no formal education, and did not use any form of contraceptives.

Table 1: Demographic characteristics of participants by study site

	Dantec	Fann	Pikine	Overall
N (%)	630 (52)	123 (10)	448 (37)	1202
Age (mean (SD))	52 (12.02)	39 (10.77)	46 (10.49)	49 (12.15)
Age at first sex (mean (SD))	17 (2.75)	19 (4.51)	18 (4.15)	18 (3.70)
Number of pregnancies (mean (SD))	7 (3.11)	4 (3.43)	6 (3.59)	7 (3.45)
Lifetime partners (mean (SD))	1 (0.88)	2 (1.59)	2 (2.15)	2 (1.57)
Marital status (%)				
Married monogamy	171 (27.1)	38 (30.9)	147 (32.8)	356 (29.6)
Married polygamy	243 (38.6)	37 (30.1)	223 (49.8)	503 (41.8)
Separated/ other	198 (31.4)	26 (21.1)	73 (16.3)	296 (24.6)
Single	5 (0.8)	22 (17.9)	5 (1.1)	32 (2.7)
NA	13 (2.1)	0 (0.0)	1 (0.2)	15 (1.2)
Education level (%)				
None	529 (84.0)	45 (36.6)	240 (55.6)	814 (67.7)
Primary	71 (11.3)	32 (26.0)	148 (33.0)	251 (20.9)
Secondary	16 (2.5)	30 (24.4)	56 (12.5)	102 (8.5)
University	1 (0.2)	14 (11.4)	2 (0.4)	17 (1.4)
NA	13 (2.1)	2 (1.6)	2 (0.4)	18 (1.5)
Contraceptive use (%)				

	No	604 (95.9)	92 (74.8)	390 (87.1)	1087 (90.4)
	Yes	16 (2.5)	30 (24.4)	58 (12.9)	104 (8.7)
	NA	10 (1.6)	1 (0.8)	0 (0.0)	11 (0.9)

HPV infection and cervical cytology/ histology

Of the 1202 women in our sample, 455 (37.9%) had infection with multiple HPV types (Table 2). Overall, 524 (43.6%) women classified as normal, 86 (7.2%) had pre-invasive lesions, and 592 (49.7%) had invasive cervical cancers.

Table 2: HPV infection and cervical cytology/histology

	Single HPV Infection (%)	Multiple HPV Infection (%)	Overall (%)
Normal	302 (57.6)	222 (42.3)	524 (43.6)
Pre-invasive cervical lesion	46 (53.5)	40 (46.5)	86 (7.2)
Invasive cervical cancer	399 (67.4)	193 (32.6)	592 (49.7)
Overall	**747 (62.1)**	**455 (37.9)**	**1202**

Table 3: Unadjusted and adjusted risk ratio of multiple HPV infections and various factors among women with a normal cervix (N=524)

		Unadjusted		Adjusted	
		Risk ratio	**95% CI**	**Risk ratio**	**95% CI**
Age (years)	Age <45	Reference			
	Age >=45	1.20	0.92, 1.56	1.04	0.75, 1.44
Age at first sex (years)	Below 18	Reference			
	18 and above	0.94	0.72, 1.22	1.05	0.78, 1.40
Number of lifetime partners	One	Reference			
	Two or more	1.2	0.92, 1.56	1.14	0.86, 1.52
Number of pregnancies	0-3	Reference			
	4 - 7	1.06	0.73, 1.53	0.97	0.64, 1.47
	>7	1.24	0.91, 1.71	1.11	0.75, 1.65
Contraceptive use	No	Reference			
	Yes	0.81	0.55, 1.21	0.93	0.60, 1.45
Education level	None	Reference			

	Primary	0.78	0.57, 1.06	0.83	0.60, 1.14
	Secondary	0.91	0.62, 1.34	0.97	0.64, 1.49
	University	0.30	0.07, 1.23	0.36	0.08, 1.62
Marital status	Single	1.07	0.53, 2.15	1.54	0.68, 3.49
	Married monogamy	Reference			
	Married polygamy	1.32	0.96, 1.80	1.25	0.89, 1.74
	Separated/ other	1.33	0.90, 1.97	1.29	0.85, 1.74

**Adjusted risk ratio after adjusting for age, age at first sex, number of lifetime sexual partners, number of pregnancies, education level, marital status, contraceptive use, and study site.*

Risk factors of multiple HPV infection

We did not find a significant association between demographic and sexual behavior characteristics and infection with multiple HPV types in univariate and multivariate analysis (Table 3). We did however, observed a trend towards infection with multiple HPV types among women who had two or more lifetime sexual partners, higher parities, and were either single, married in polygamous relationship or were separated. We also observed a negative trend for multiple HPV infection among women who used contraceptives or attained at least a primary education.

Table 4: Unadjusted and adjusted risk ratio of multiple HPV infections and risk of pre-invasive cervical lesions and invasive cancer

	Unadjusted risk ratio			***Adjusted risk ratio**		
Cervical histology/cytology	Risk Ratio	95% CI	**P-value	*Risk Ratio	95% CI	**P-value
Normal	Reference			Reference		
Pre-invasive cervical lesion	1.15	0.76 - 1.77	0.47	1.15	0.73 – 1.82	0.49
Invasive cervical cancer	0.82	0.69 - 0.97	0.01	0.96	0.78 – 1.18	0.19

**Risk ratio after adjusting for age, age at first sex, education level, marital status, and study site. We adjusted for number of lifetime sexual partners and number of pregnancies in our initial model. **P-value with robust standard error.*

Risk of pre-invasive lesions and invasive cancers

We did not find any significant association between infection with multiple HPV types and pre-invasive cervical lesions and invasive cancers in multivariate analysis (table 4). In univariate analysis, we found significantly lower likelihood of invasive cancers among women infected with

multiple HPV types (p-value = 0.01, RR 0.82, 95% CI 0.69, 0.97). However, this was no longer statistically significant when we adjusted for other factors in our multivariate analysis. We observed a positive trend for pre-invasive lesions (RR 1.15).

Discussion

Risk factors of infection with multiple HPV types

In multivariate regression analysis, we found no independent factors which distinguished infections with single HPV type and infections with multiple HPV type, even after controlling for factors related to age, socio-demographic and sexual behavior. This observation is consistent with current body of evidence that sexual activity appears to be the primary driver of HPV infection and does not differential from infection with multiple HPV types[14],[8],[15],[16].

Our finding that age does not influence infection with multiple HPV types further supports this notion that level of sexual activity perhaps is the primary predictor of HPV infection. However, one study found that age significantly influence acquisition of multiple HPV types, but this involved young women between 16 -25 years, where prevalence and incidence is highest[15]. Given that multiple HPV infection has a bimodal age distribution with first peak below 25 years, and second peak after 45 years[4], our study captures both high-risk sub-populations and perhaps reflects the risk in general population.

An important observation was that women who had at least a primary education somewhat had a lower risk of having multiple HPV infection compared to women who had no education. There is evidence to support that sexual education in schools may lead to reduction in various sexually transmitted infections (STI), although findings have been inconsistent, and limited number of research have been done specifically on HPV infection[17]. Similarly, consistent with other studies, contraceptive use including condoms appear to not provide any protection against HPV infection as transmission by non-penetrative sexual contact is highly possible[18],[19].

In contrast, we found that having more than one lifetime sexual partner, being single, married in polygamous relationship, or separated, had a positive (but non-significant) trend toward infection with multiple HPV types, compared to being married in monogamous relationship. Previous studies observed significant association between multiple sexual partners, and being either single, in casual relationships, or divorced and multiple HPV infection, although these studies involved younger age women 15 – 25 years old[16],[15].

Risk of pre-invasive lesions and invasive cancers

We did not find a significant association between infection with multiple HPV types and pre-invasive cervical lesions and invasive cervical cancers, after adjusting for demographic and sexual behavioral factors. However, we did observe a positive trend for pre-invasive lesions among women with multiple HPV infections. In contrast, women with multiple HPV infections had a lower risk of invasive cancers which was significant but was attenuated when adjusted for other factors.

The heterogenous observation in risk estimate between pre-invasive lesions and invasive cancers has been documented in other studies[20],[9]. Indeed, one can imagine that having multiple infections on the cervix might result in more lesions, but both are most commonly transient. Only persistent lesions progress to cancer, and it's unlikely that multiple separate lesions would progress in the same women. This is justified by the high prevalence of multiple HPV infection among younger women, with common transmission through sexual activity, but decreases in older women[4],[14]. As almost 90% of women infected with HPV is cleared within 1-2 years, only the most prevalent, oncogenic types persist in invasive cancers[21],[22].

With regards to invasive cancers, we found that women with multiple HPV infection had lower risk of invasive cancer compared to single HPV infection and was statistically significant but was no longer statistically significant after we adjusted for socio-demographic and sexual behavioral factors. We observed neither a positive nor negative trend for invasive cancers among women infected with multiple HPV types compared to infection with single HPV infection. This is consistent with women who had pre-invasive lesions elsewhere[20].

This perhaps points towards more type-specific intergenotypic interaction as observed in other studies, with most oncogenic and prevalent types driving cervical cancer carcinogenesis. Indeed, this was supported in studies that found each HPV infection to be associated with a biologically independent lesion. This observation was further supported in a study that found corresponding high viral load of oncogenic type in invasive lesions[5],[6].

Although our study did not explore the impact of HPV type-specific combination, other studies have postulated that inter-genotypic interaction may confer increased risk of cervical carcinogenesis from mechanisms involving synergistic interactions, while others observed a protective mechanism[7]. With regards to protective mechanisms, it is possible that competition interaction or more effective immune response may be triggered by multiple infection leading to reduction in high-risk cervical lesions in various patterns[9]. Indeed, one study found that HPV type 72 and type 81 displayed the strongest positive relationship, while HPV type 33 and 66 displayed the strongest negative interaction[23].

Strengths

To our knowledge, this was the largest study in the region, involving previously unscreened and unvaccinated women with pre-invasive lesions and invasive cervical cancers. Additionally, the primary exposure and outcome of interest were objective measurements based on cytology and or/ histology diagnosis and our findings can be assumed to reflect the true estimate in this population.

Limitations

Although our study points towards no association between infection with single and multiple HPV infection, it needs to be evaluated with caution in the following context. Firstly, as this was a cross-sectional study one cannot ascertain the sequence of exposure between multiple HPV types, and subsequent causation for the observed cervical disease outcome. Secondly, we had

small number of women with pre-invasive lesions, and thus underpowered to detect any significant difference in risk between single and multiple HPV infection associated with that outcome. Furthermore, there is a potential possibility of non-differential misclassification for precancerous lesions as cytology has low sensitivity[25],[26]. Finally, several risk factors were not included in this secondary analysis as they were not captured in the studies - number of sexual partners of participants or were excluded - oral contraceptive use and tobacco smoking, because of low prevalence.

Conclusion

In summary, we found that socio-demographic and sexual behavioral factors did not differentiate women who were infected with multiple HPV types from those infected with a single HPV infection. This supports the prevailing understanding of common transmission of HPV through sexual activity. We also did not observe any significant competitive interaction or synergistic effect of multiple HPV infection on cervical cancer carcinogenesis in women with pre-invasive lesions and invasive cancers. Inter-genotypic interaction appears to be the most plausible explanation for the heterogenous observation in infection with multiple HPV types, and worth further investigation. Finally, given the high burden of HPV infection and invasive cancers in this population, screening and vaccination against HPV should be expended to include the most vulnerable younger population.

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