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Short communication

HPV genotypes in women with squamous intraepithelial lesions and normal cervixes participating in a community-based microbicide study in Pretoria, South Africa

H.M. Said [a,b,*], K. Ahmed [a,b], R. Burnett [c], B.R. Allan [c], A.-L. Williamson [d,e], A.A. Hoosen [a,f]

[a] Faculty of Health Science, Department of Medical Microbiology, University of Pretoria, South Africa
[b] Faculty of Medicine, Department of Microbiological Pathology, University of Limpopo, South Africa
[c] School of Public Health, University of Limpopo, South Africa
[d] Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa
[e] National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa
[f] National Health Laboratory Service, Pretoria Academic Hospital, Pretoria, South Africa

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ABSTRACT

Background: Little is known regarding the human papillomaviruses (HPV) genotypes prevalent in women in South Africa, a country with a high incidence of cervical cancer.
Objective: To determine the prevalence and HPV genotypes in women with squamous abnormalities and normal cervixes participating in a community-based microbicide study.
Study design: A total of 159 cervical specimens, including 56 specimens from women with abnormal cytology (cases) and 103 randomly selected specimens from women with normal cytology (controls), were collected. HPV was detected by consensus PCR primers and HPV genotypes were determined by Roche Linear Array® HPV genotyping assay.
Results: HPV genotypes were found in 91% of cases and 40% of controls ($p < 0.005$). High-risk HPV was detected in all high-grade squamous intraepithelial lesions (HSILs), 69% of low-grade squamous intraepithelial lesions (LSILs), 57% of atypical squamous cells of undetermined significance (ASCUS), and 86% of ASCUS in which HSIL could not be excluded (ASCUS-H), and 73% of HPV positive controls. HPV-35 was the predominant genotype in HSILs; HPV-18 in ASCUS; HPV-58 in ASCUS-H and HPV-16 in LSILs and controls.
Conclusion: High-risk HPV prevalence was high in both cases and controls. HPV genotype distribution in HSILs was different from that reported worldwide and from other studies in South Africa.

1. Introduction

Human papilloma virus (HPV) is one of the commonest causes of sexually transmitted infections.[1] Certain high-risk (HR) HPV types had been identified as the primary cause of cervical cancer.[2] South Africa is one of the countries with high incidence of cervical cancer, with an overall incidence rate of 30 per 100,000 per year.[3] HPV-16 is the most prevalent genotype in cervical cancers worldwide, being detected in about 50% of cervical cancers, followed by HPV-18 which is detected in about 10%.[4] However, geographical variation in HPV genotype distribution occurs in the different regions of the world.[5]

Despite the high prevalence of cervical cancer in South Africa, little data is available with regard to HPV prevalence, especially in the Pretoria region. There is no information on the major HPV genotypes associated in women with squamous abnormalities and women with cytologically normal cervixes. Information on HPV prevalence and HPV genotype distribution is essential for developing cervical cancer prevention strategies. The present study was conducted to determine the prevalence of HPV genotypes in women in the Pretoria region with normal or abnormal cervical cytology.

2. Methods

2.1. Subjects and samples

The study protocol was approved by the institutional Research Ethics Committee. The study participants were women who were participating in a phase III microbicide study. As part of the microbicide study, each woman received a gynecological examination, at which time a Pap smear was obtained with cytobrush. For the present study, 159 cytobrush specimens were collected. Fifty-six specimens with a Pap smear diagnosis of squa-

* Corresponding author at: PO Box 30583, Sunnyside, Pretoria 0132, South Africa. Tel.: +27 12 319 2250; fax: +27 12 321 9456.
E-mail address: ahlammdd@yahoo.com (H.M. Said).

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Table 1
Prevalence of HPV genotypes in study population.

Single infection		Multiple infection							
1 type	No.	2 types	No.	3 types	No.	4 types	No.	>4 type	No.
16	4	16,18	1	18,58,69	1	6,18,45,59	1	18,31,39,45,53,66,70	1
18	5	16,39	1	16,33,68	1	18,53,56,58	1	6,16,18,58,66,70	1
26	6	16,45	1	26,51,58	1	53,66,68,70	1	18,35,45,66,73	1
31	1	16,53	1	16,51,59	1	18,39,58,70	1		
35	7	16,59	1	16,52,68	1	16,51,58,66	1		
39	4	16,66	1	26,31,52	1				
45	2	16,68	1	45,70,82	1				
51	4	16,70	1	35,53,59	1				
52	4	18,51	1						
53	1	18,69	1						
56	1	31,39	1						
58	5	31,53	1						
59	1	31,73	1						
66	1	35,45	1						
68	1	35,58	1						
		35,59	2						
		35,68	2						
		39,45	1						
		39,59	1						
		53,58	1						
		53,82	1						
		56,58	1						

mous abnormalities were selected from these as study cases, and the remaining 103 women with normal cytology were used as controls. The cytobrushes were stored dry at −70 °C until processing.

2.2. HPV detection and genotyping

For isolation of DNA, the dry cytobrushes were suspended in 1 ml of phosphate buffered solution (PBS, pH 7.4). The MagnaPure LC Isolation station (Roche Diagnostics, USA) was used for DNA extraction as described by the manufacturer.

After DNA isolation, specimens were analyzed by Roche Linear Array® HPV genotyping assay (Roche Molecular Diagnostics, USA) according to manufacturer's instructions. The method is based on broad-spectrum PCR-based system coupled with a reverse line-blot array. It is capable of detecting and genotyping of 37 different HPV genotypes along with assessing human β-globin.

2.3. Data analysis

HPV genotypes were classified based on the study by Munoz et al., and the following genotypes were regarded as HR-HPV geno-

Table 2
Distribution of HPV genotypes in cases in relation to the cytological diagnosis.

HPV genotypes	ASCUS	ASCUS-H	LSIL	HSIL	Total	
	(*n* = 23) %	(*n* = 7) %	(*n* = 16) %	(*n* = 8) %	(*n* = 54) %	(95% CI)
HR-HPV						
16	4 (17)		4 (25)	1 (13)	9 (16)	(0.0903–0.2874)
18	4 (17)	1 (14)	4 (25)	1 (13)	10 (19)	(0.1038–0.3084)
31	4 (17)				4 (7)	(0.0292–0.1756)
33			1 (6)		1 (2)	(0.0033–0.0977)
35	2 (9)	1 (14)	5 (32)	4 (50)	12 (22)	(0.1319–0.3494)
39	2 (9)		1 (6)		3 (6)	(0.0191–0.1511)
45	2 (9)		1 (6)		3 (6)	(0.0191–0.1511)
51	2 (9)	1 (14)			3 (6)	(0.0191–0.1511)
52	2 (9)		1 (6)		3 (6)	(0.0191–0.1511)
56	2 (9)	1 (15)			3 (6)	(0.0191–0.1511)
58	1 (4)	3 (43)	3 (19)	2 (25)	9 (17)	(0.0903–0.2874)
59	2 (9)		2 (13)	1 (13)	5 (9)	(0.0402–0.1991)
68	1 (4)	2 (29)	3 (19)		6 (11)	(0.0519–0.2219)
69			2 (13)		2 (4)	(0.0102–0.1253)
70	1 (4)		2 (13)		3 (6)	(0.0191–0.1511)
73	1 (4)		1 (6)		2 (4)	(0.0102–0.1253)
82			1 (6)		1 (2)	(0.0033–0.0977)
Probably HR-HPV						
26	1 (4)	1 (14)			2 (4)	(0.0102–0.1253)
66	3 (13)		3 (19)	2 (25)	8 (15)	(0.077–0.2659)
LR-HPV						
6	3 (13)				3 (6)	(0.0191–0.1511)
61	1 (4)		2 (13)		3 (6)	(0.0191–0.1511)
62	1 (4)		1 (6)		2 (4)	(0.0102–0.1253)
71			1 (6)		1 (2)	(0.0033–0.0977)
72			1 (6)		1 (2)	(0.0033–0.0977)
81	1 (4)		1 (6)		2 (4)	(0.0102–0.1253)
83	2 (9)		1 (6)		3 (6)	(0.0191–0.1511)

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Table 3
Distribution of HPV genotypes in controls in relation to the cytological diagnosis.

HPV genotypes	Total (n = 99) % (95% CI)
HR-HPV	
16	8 (0.0415–0.1514)
18	5 (0.0218–0.1128)
31	2 (0.0056–0.0707)
35	2 (0.0056–0.0707)
39	7 (0.0347–0.1388)
45	6 (0.0281–0.126)
51	5 (0.0218–0.1128)
52	3 (0.0104–0.0853)
58	5 (0.0218–0.1128)
59	3 (0.0104–0.0853)
68	1 (0.0018–0.055)
70	3 (0.0104–0.0853)
82	1 (0.0018–0.055)
LR-HPV	
62	5 (0.0218–0.1128)
71	1 (0.0018–0.055)
72	1 (0.0018–0.055)
81	4 (0.0158–0.0993
84	2 (0.0056–0.0707)
CP6108	1 (0.0018–0.055)

types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 70, 73, and 82); 3 as probable HR-HPV genotypes (26, 53, and 66); and 6 were classified as Low-risk (LR) HPV genotypes (6, 61, 62, 71, 72, 81, 83, 84, and CP6108).[6]

Estimates were obtained for the prevalence of HPV in the different risk groups and the 95% confidence intervals were reported. The odds ratios (OR) at 95% confidence intervals (CI) was calculated. Chi-square test was performed to test the significance of the difference in the prevalence of HPV in cases and controls. The significance level was set at 5%.

3. Results

Of the 159 specimens tested, 153 were positive for β-globin internal control indicating adequate specimen integrity, DNA extraction and PCR amplification. The negative controls in the test revealed no evidence of contamination. The 6 specimens with undetectable β-globin DNA were excluded from the analysis. These included two specimens from cases (1 HSIL and 1 ASCUS-H) and four specimens from controls. Overall, HPV was detected in 89/153 (58%) specimens. Of these, 46/89 (52%) had only one HPV genotype and 43/89 (48%) contained multiple (2–8) HPV genotypes (Table 1).

3.1. HPV prevalence and genotype distribution in different cytological categories

As expected the prevalence of HPV was significantly higher in cases 49/54 (91%) compared to controls 40/99 (40%) (OR = 14.46 (95% CI 4.95–45.38), $p < 0.005$). The cases included 23 ASCUS, 8 ASCUS-H, 16 LSIL and 9 HSIL. In this study 28 different HPV genotypes were detected. The distribution of individual HR-HPV and LR-HPV genotypes in different cytological categories in cases and controls is shown in Tables 2 and 3.

4. Discussion

The prevalence of HR-HPV genotypes in both cases and control was relatively high considering that the study population was women volunteers from a community. The prevalence of HR-HPV increased with increasing cytological grade. In the controls, 73% of the HPV positive specimens were HR-HPV types. In a study conducted by Allan et al., the prevalence of HR-HPV types in women with normal cytology was 10.9%, but that study was done using the Digene Hybrid Capture 2, which tests for 13 HPV types and is not as sensitive as the genotyping assay we used.[7]

The HPV genotype distribution in LSIL, ASCUS and in women with normal cytology was similar to that reported in previous studies. However, the most prevalent genotypes in HSILs were HPV-35 followed by 58 and 66, which differ from a previous study conducted in South Africa by Kay et al., in Cape Town, and other studies, where the most prevalent genotypes in HSILs were HPV-16 and 18.[8,4,9] This difference could represent regional variations and differences in the populations studied. The present study population was women participating in a vaginal microbicide study, who were women from the community without any specific risk factors other than the high HIV prevalence in this community, which was reported to be 25%.[10] The high HIV prevalence could explain the observed HPV genotype distribution, since HIV-infected women are reported to acquire a broader spectrum of HPV genotypes other than HPV 16 or 18.[11–13] Most previous studies in South Africa were conducted in low HIV prevalence areas. This finding is epidemiologically important because the existing vaccine may not be as efficacious in this region of South Africa. Therefore, large scale population-based studies on the HPV genotype distribution in high HIV prevalence areas are needed to investigate the role of these genotypes in the etiology of SIL and cervical cancer.

In this study, multiple HPV genotypes were detected in about half (48%) of the HPV positive samples. The presence of multiple HPV genotypes may suggest repetitive exposure to multiple HPV genotypes due to high-risk sexual behavior. It could also be due high prevalence of HIV in the community. Pervious studies reported that multiple HPV genotypes are more common among HIV-infected population. [14,15]

In conclusion, the study has shown a high prevalence of HR-HPV in both cases and controls. Unlike other studies, HPV-35 followed by 58 and 66 were the most frequent genotypes in HSILs confirming geographical variation in HPV genotype distribution. This might diminish the efficacy in this region of the currently available HPV vaccine. Further studies on HPV prevalence and genotype distribution in South Africa are needed to investigate the oncogenic potential of these HPV genotypes.

Conflict of interest

There is no conflict of interest, the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere. All authors have the manuscript have consented to publication and if the article is accepted for publication, manuscript proofs returned by the corresponding author will be approved by all authors. There is no financial or personal relationship with other people or organizations that could inappropriately influence the submission of the manuscript.

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