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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)