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Genital Human Papillomavirus Genotypes in Northwestern Tanzania

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Using MY09-MY11 PCR and human papillomavirus (HPV) typing by reverse blot hybridization, we found a 34% cervical HPV prevalence among 561 pregnant women in Tanzania. One hundred three of 123 women (84%) with typeable samples harbored high-risk oncogenic strains. HPV type 16 (HPV-16) was the most prevalent subtype (18%) among HPV-infected women and among women with cervical neoplasia (3 of 19). A multivalent vaccine for HPV-16, -18, -31, -33, and -35 would be necessary to prevent 50% of the neoplasia in this population.

Cervical cancer is the most common female cancer in sub-Saharan Africa, with age-standardized incidence rates being fourfold higher in eastern and southern Africa than in North America and Europe (9). High-risk oncogenic genital human papillomaviruses (HR-HPV) have been shown to be the causative agents of cervical cancer and precancerous or squamous intraepithelial lesions (SIL) (2, 13). Because cytological or HPV screening would be logistically difficult and expensive in most African countries, immunization against HR-HPV offers the greatest hope for a long-term solution to cervical cancer. On the basis of epidemiological data showing that HPV type 16 (HPV-16) and HPV-18 are the most frequently detected types in cervical carcinomas worldwide (1, 2), vaccines currently in development use HPV-16 and -18 L1 (the major capsid protein) virus-like particles (VLP) as immunogens (6). Furthermore, the inclusion of HPV-6 and -11 VLP as supplementary antigens to help prevent genital warts and cervical intraepithelial neoplasia is being pursued (C. J. N. Lacey, personal communication).

Since there is no evidence of cross-protection between HPV types in natural infection or induced by L1 VLP, the development of multivalent vaccines would be desirable (6). We have conducted a study among a sample of pregnant women attending a large urban antenatal clinic in northern Tanzania to determine the prevalence of cervical HPV infections and their associations with neoplasia and human immunodeficiency virus (HIV) (7), and we report here a detailed HPV genotype distribution and its associations with neoplasia and genital warts.

Detailed characteristics of the study population, the samples taken, and the basic laboratory investigations have been described previously (7). We enrolled a systematic sample of consenting women attending a busy antenatal clinic in Mwanza, Tanzania, from April to December 1994. Women were interviewed and underwent genital and pelvic examinations, during which serum, vaginal, and cervical samples (including a Pap smear) were collected. Free treatment was provided to all participants with positive sexually transmitted disease symptoms or tests.

Informed written consent was sought, and the patients were seen by a female clinician in a private room. Patients were treated on the spot for their sexually transmitted infections according to the Tanzanian national guidelines. Patients were counseled on low-risk sexual behavior, offered condoms, and given a seven-day follow-up appointment for their comprehensive laboratory results, at which time additional treatment was provided for infections that were not covered by syndromic treatment at the initial visit. All treatments and investigations were provided free of charge. Women willing to know their HIV serostatus were counseled at the clinic, and appropriate referrals to the then-existing care services were made for HIV-positive individuals.

This study was approved by the Tanzanian National AIDS Control Programme and the Tanzanian National Institute for Medical Research (NIMR) Coordinating Committee. The study complied with ethical regulations of the World Health Organization and the London School of Hygiene & Tropical Medicine.

HPV PCR samples were stored at −70°C until shipment and testing at Imperial College, London, United Kingdom, according to a previously described methodology (7). The biotinylated primers MY09-MY11, HBB01, and GH20-PC04 were utilized to enable detection of positive PCR products by a 27-genotype reverse blot hybridization assay, as previously described (4), with confirmation for HPV-6, -11, -16, -18, and -31 with type-specific PCR primers (12).

Six hundred sixty women with a mean age of 23.4 years (standard deviation, 5.1; range, 15 to 44) were enrolled. The majority were married (92%) and had low education levels (70% with no or only primary schooling). They had a mean gravidity of 2.7, and few reported more than one sexual partner in the previous year (10%). Prevalences of HIV (15%), active syphilis (8%), and cervical infections with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* (7.5%) were high. External genital warts were observed in 20 women (3%).

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TABLE 1. HPV genotype distribution in all HPV DNA-positive women and in HPV DNA-positive women with cervical neoplasia in Mwanza, Tanzania

HPV genotype	All HPV-positive women (n = 190)		HPV-positive women with cervical neoplasia (n = 19)	
	No. (%) of women	% of isolated types (no. of typeable samples, 191)	No. (%) of women	% of isolated types (no. of typeable samples, 17)
High-risk types				
16	34 (18)	18	3 (16)	18
58	23 (12)	12	1 (5)	6
MM7	19 (10)	10	0	0
33	20 (11)	10	3 (19)	18
18	13 (7)	7	1 (5)	6
31	8 (4)	4	2 (11)	12
51	7 (4)	4	2 (11)	12
68	5 (3)	3	0	0
59	4 (2)	2	1 (5)	6
MM4	4 (2)	2	0	0
35	3 (2)	2	2 (11)	12
55	3 (2)	2	1 (5)	6
52	2 (1)	1	1 (5)	6
9	2 (1)	1	0	0
56	2 (1)	1	0	0
26	2 (1)	1	0	0
39	1 (1)	0.5	0	0
45	1 (1)	0.5	0	0
Low-risk types				
6	14 (7)	7	0	0
53	12 (6)	6	0	0
11	5 (3)	3	0	0
54	4 (2)	2	0	0
66	2 (1)	1	0	0
MM8	1 (1)	0.5	0	0
Unknown types	67 (35)		7 (37)	

The prevalence of HPV infection was 34% (190 of 561 patients) among women who had samples adequate for both HPV PCR analysis and cervical cytology. Of these infected women, 67 (35%) had genotypes that were not characterized by the typing system. The relatively frequent detection of such uncharacterized HPV types by current HPV amplification systems has been noted by other investigators. For example, in a study in New Mexico of a population where HIV infection was infrequent, Peyton et al. (10) found that uncharacterized types constituted 24% of all genotypes. Genotyping was performed in our study for the remaining 123 women, from whom 191 distinct HPV isolates from 24 different genotypes were obtained. One hundred two women (54%) harbored high-risk oncogenic strains, with 46 women (37% of those with known genotypes) having multiple genotypes (Tables 1 and 2). The most common genotypes found were HPV-16 (34 women, 18%), HPV-58 (23 women, 12%), HPV-MM7 (19 women, 10%), HPV-33 (20 women, 11%), and HPV-18 (13 women, 7%) (Table 1).

Nineteen HPV-positive women had cervical neoplasia, nine of whom had high-grade SIL (HSIL). Twelve women had known genotypes, and all harbored HR-HPV types only. Seventeen infections were detected in these 12 HPV-positive women with cervical neoplasia, 4 of whom had multiple HPV types (Table 1). HPV-16 and -33 were the most common types identified in the subset of women with cervical neoplasia (5 of 19 women, including a woman with dual HPV-16 and -33 infection) and among the women with HSIL (2 of 9 women, including the dually infected woman) (Table 3). Of the 67 women with unknown HPV genotypes, 3 had low-grade SIL (LSIL) (all of whom were HIV positive) and 4 had HSIL (none were HIV positive).

TABLE 2. Prevalence of single and multiple HPV infections

Infection type	No./total no. tested (%)			
	HPV positive		HPV positive with cervical neoplasia	
	Women[a]	Typeable samples	Women[b]	Typeable samples
Single infections	77/123 (63)	77/191 (40)	8/12 (67)	8/17 (47)
Multiple infections	46/123 (37)	114/191 (60)	4/12 (33)	9/17 (53)
Multiple infections with:				
2 genotypes	29/46	58/114	3/12	6/9
3 genotypes	12/46	36/114	1/12	3/9
4 genotypes	5/46	20/114	0/12	0/9

[a] Sixty-seven of 190 women had unknown genotypes and were excluded from these calculations.
[b] Seven of 19 women with cervical neoplasia had unknown genotypes and were excluded from these calculations.

A vaccination strategy targeting only HPV-16 or -18 in this population would prevent only 4 of 19 cases of cervical neoplasia and 2 of 9 cases of HSIL. However, a multivalent vaccine against HPV-16, -18, -31, -33, and -35 would protect against 9 of 19 cases of cervical neoplasia and 5 of 9 cases of HSIL (Table 3), and would have prevented infection among 32% of women (61 of 190 patients). Further work is needed to identify unknown genotypes and to give form to a vaccination strategy.

Overall, only 19 women (10%) were infected with HPV types commonly associated with genital warts (HPV-6 and -11). Six of these women had genital warts (32%), compared with just 1 of 171 women without these genotypes ($P < 0.001$).

TABLE 3. Distribution of HPV genotypes in 19 HPV DNA-positive women with cervical neoplasia in Mwanza, Tanzania

Individual	HPV genotype(s)	Cervical neoplasia	HIV status
1	18	HSIL	−
2	33	HSIL	−
3	33	LSIL	−
4	35	HSIL	−
5	35	LSIL	−
6	52	LSIL	−
7	55	LSIL	−
8	59	LSIL	+
9	16, 31	LSIL	−
10	16, 51	LSIL	−
11	31, 51	HSIL	+
12	16, 33, 58	HSIL	−
13	Unknown	LSIL	+
14	Unknown	LSIL	+
15	Unknown	LSIL	+
16	Unknown	HSIL	−
17	Unknown	HSIL	−
18	Unknown	HSIL	−
19	Unknown	HSIL	−

True population-based studies of HPV infection in Africa have been scarce (3, 11), and few have provided detailed reports on HPV genotypes (3, 5). A recent study conducted in Mozambique (3) found that HPV-35 was the most prevalent type among HPV-positive women (16 of 96, 17%) and among women with cervical neoplasia (7 of 23, 30%) or HSIL (4 of 22, 18%), while HPV-16 and -18 were found only in 13 and 9% of women with neoplasia and 10 and 0% of women with HSIL or carcinoma, respectively. It was concluded that the effect of an HPV-16-based vaccine in preventing cervical neoplasia would be low, and in this Mozambican population, four genotypes (HPV-16, -18, -35, and -39) would need to be considered to prevent at least 70% of neoplasia cases. A study of women with HSIL from Zimbabwe showed HPV-16, -58, -18, and -52 to be the most common genotypes, but these women had a high prevalence of HIV infection (78%) and multiple HPV infections (68%) (5). We found in our study that cervical neoplasia was exclusively associated with oncogenic HPVs. However, a vaccination strategy targeting HPV-16 and HPV-18 would prevent only 21% of cervical neoplasia and HSIL cases. Multiple types (HPV-16, -18, -31, -33, and -35) would need to be included to achieve satisfactory protection from cervical cancer in this population. Although infrequent, HPV-6 and -11 subtypes were closely associated with genital warts, and a vaccine containing these genotypes would prove useful.

The conclusions of these African studies have been limited by their small sample sizes. Furthermore, their cross-sectional design does not allow for the capture of the dynamic nature of HPV infections, with their hallmark features of persistence, regression, and clearance (8, 14). Finally, the impact of HIV, both on the persistence of oncogenic HPV types and on the spectrum of HPV genotypes in invasive cervical cancer in Africa, has not been properly assessed. However, these studies draw attention to the diversity of HPV types associated with SIL in Africa and to the fact that currently designed vaccination strategies may ignore the burden of infection and associated disease in one of the most affected regions of the world.

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