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ORIGINAL ARTICLE

Risk of being seropositive for multiple human papillomavirus types among Finnish and Ugandan women

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Abstract

Although infections with multiple human papillomavirus (HPV) types have been reported widely, more information is needed on the occurrence of the different types. We determined the distribution of seroprevalences to multiple HPV types in Finland and Uganda to compare the epidemiology of the different HPV types in the 2 populations. Serum samples were obtained from 2784 Finnish and 1964 Ugandan women (mean ages 22 y and 25 y, respectively) of whom 44% and 57%, respectively, had antibodies to at least 1 of the 7 HPV types (6, 11, 16, 18, 31, 33, 45) tested ($p < 0.001$). Multiple HPV antibody positivity was common. HPV45-seropositive Finns had a higher risk of having antibodies to other high-risk HPV types: HPV18 (odds ratio (OR) = 10.9), HPV31 (OR 6.1), HPV33 (OR 12.2), than their Ugandan counterparts: HPV18 (OR 3.4), HPV31 (OR 2.2), HPV33 (OR 3.3). Increased estimates for being double antibody-positive were also noted among HPV18- and HPV16-seropositive women, but there were no major differences between HPV16-seropositive Finns and Ugandans. In addition to biological and behavioural factors, iatrogenic and societal factors (screening vs no screening) may also result in the different occurrence of infections with the high-risk HPV types in Finland and Uganda.

Introduction

Cervical cancer is the second most common malignancy in women worldwide, accounting for 9.8% of all female cancers [1]. Infection with high-risk human papillomavirus (hrHPV) is the major (necessary) cause of cervical cancer [2]. Geographical differences in the hrHPV types between countries and within countries have been readily reported [3,4], however little is known about the occurrence of multiple HPV types at the individual or at the population level, e.g., across countries.

Although, several studies have reported multiple HPV infections using DNA techniques, they have had limitations in elaborating the epidemic situation. Infected individuals usually clear the virus within 6–18 months and concomitant HPV infections identified represent only a proportion of HPV infections acquired within a y [5]. If generated, HPV antibody levels are usually stable up to 10 y after a genital HPV infection [6], thus HPV seropositivity can be used to estimate lifetime cumulative HPV exposure [7]. We determined distributions of seroprevalences to multiple HPV types among Finnish and Ugandan pregnant women to compare the epidemiology of the different HPV types in the 2 populations.

Materials and methods

Study design and populations

We performed an HPV seroprevalence study among antenatal clinic attendees in Finland and Uganda. In Finland, all pregnant women donate blood during their first trimester to the Finnish Maternity Cohort (FMC) for the screening of congenital infections and may

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consent to further serological use of the samples for health-related research [4]. The FMC was established in 1983 and by 2007, 750,000 (98%) pregnant women had donated serum to this cohort with a total of approximately 1,500,000 serum samples stored at −25°C at the National Institute for Health and Welfare, Oulu, Finland. For this study, we included a random subset of women who were pregnant between the y 1995 and 2003.

In Uganda, pregnant women attending hospitals covering the central region of the country – Entebbe, Nsambya and Naguru health centres – were enrolled following informed consent. Entebbe Hospital is a district referral hospital in a semi-urban setting, about 40 km from Kampala. It serves women within a distance of about 80 km including the islands in Lake Victoria. Nsambya Hospital is a tertiary, private (non-profit) referral hospital in the suburbs of Kampala. It serves women of all income levels in private and public clinics within a distance of about 50 km. Naguru is a public health centre, about 20 km from Kampala, which serves women from nearby suburbs.

Women included in the study from Uganda were enrolled in 2 time frames. The first group comprised women less than 25 y of age who participated in a follow-up study of young primiparous pregnant women at the Naguru Health Centre [8] between May and November 2004. The second group was enrolled between October and December 2008 at the Entebbe and Nsambya hospitals during their first antenatal visit of the current pregnancy. Information about HPV was included in the routine health information given by trained midwives and nurses. All attending women (study participants or not) were encouraged to seek cervical examination at postnatal clinics (such a service could not be offered during enrolment). The participating women consented for the use of the donated sample to serological HPV studies. All the samples from Uganda were shipped frozen to the National Institute for Health and Welfare, Oulu, Finland for serological analyses.

Laboratory analysis

Serum IgG antibodies were analysed for 7 genital HPV types: 6, 11, 16, 18, 31, 33 and 45, by applying specific virus-like-particles (VLPs) in direct enzyme-linked immunosorbent assays (ELISAs) [5,9]. These VLPs were generously donated by Dr Kathrin Jansen (HPV types 6, 11 and 16, Merck Research Laboratories, Philadelphia, PA, USA) and Dr Francis Dessy (HPV types 18, 33, and 45, GlaxoSmithKline Biologicals, Rixensart, Belgium). HPV31 VLPs were produced as described [7,9].

All sera were analysed for human immunodeficiency virus (HIV) antigen and antibody in the Oulu laboratory by the Abbott Combo test (Abbott Ireland, Diagnostic Division, Sligo, Ireland). HIV serology was also done for the Ugandan women using rapid tests in the antenatal clinic laboratories at enrolment [8].

Sera from Finnish women were analysed for cotinine to assess their smoking habit (smokers >20 ng/ml), using a commercial kit (STC Technologies, Bethlehem, PA, USA) as described [9].

Ethical approval

The study was approved by the institutional review boards at the National Institute for Health and Welfare (THL), Finland, and at the Uganda Virus Research Institute, St Raphael of St Francis Hospital Nsambya, and the Uganda National Council of Science and Technology.

Statistical analysis

A descriptive analysis was done for age, smoking and HIV status in both study populations using mean, standard deviation (SD) and proportions. We used Mantel–Haenszel and logistic regression for estimating crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) for multiple HPV infections. All analyses were done using Stata 8 (Stata Corp., College Station, Texas, USA).

Results

A sub-sample of 3251 women with a mean age of 22 y (range 14–28 y, SD 3) and 2053 women with a mean age of 23 y (range 14–48, SD 5) were enrolled from Finland and Uganda, respectively. Out of the enrolled women, 2780 (Finland) and 1964 (Uganda) had adequate serum samples for all the analyses.

Both the overall HPV seroprevalence (for at least 1 HPV type) and multiple HPV seroprevalence (for at least 2 HPV types) were lower among the Finnish (44% and 22%, 1222 and 619 of 2780) than among the Ugandan women (57% and 30%, 1118 and 588 of 1964; $p < 0.001$). In Finland, the overall HPV seroprevalence peaked at 20–24 y of age, but the multiple HPV seroprevalence was still increasing at 25–29 y of age (Figure 1). In Uganda both the overall and multiple HPV seroprevalences peaked in women under 20 y of age (Figure 1).

HIV prevalence was zero among the Finnish women and 7% among the Ugandan women. Overall HPV and multiple HPV seroprevalences were 74% (104/140) and 53% (74/140), respectively, among HIV-infected Ugandan women as compared to 56% (1014/1824) and 28% (514/1824), respectively, among HIV-negative Ugandan women. Twenty-three percent of the

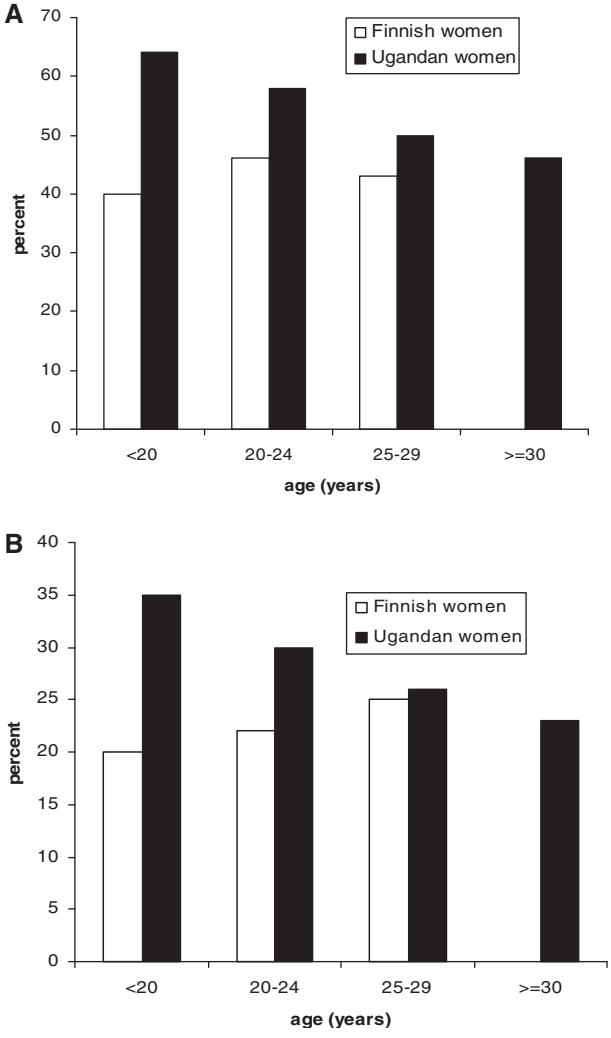

Figure 1. (A) Seroprevalence (occurrence of antibodies) of HPV types (at least 1 of 7 genital HPV types: 6, 11, 16,18, 31, 33, 45) by age among pregnant women in Finland (n = 1222 of 2780; stratified by age: $n_{<20}$ = 258 (648), $n_{20–24}$ = 689 (1496), $n_{25–29}$ = 275 (638), $n_{>30}$ = none), and in Uganda (n = 1118 of 1964; $n_{<20}$ = 385 (598), $n_{20–24}$ = 421 (724), $n_{25–29}$ = 195 (389), $n_{>30}$ = 117 (253)). (B) Seroprevalence (occurrence of antibodies) of multiple HPV types (at least 2 of 7 genital HPV types: 6, 11, 16, 18, 31, 33, 45) by age among HPV pregnant women in Finland (n = 619 of 2780; stratified by age: $n_{<20}$ = 132 (648), $n_{20–24}$ = 325 (1496), $n_{25–29}$ = 162 (636), $n_{>30}$ = 0), and in Uganda (n = 588 of 1964); $n_{<20}$ = 212 (598), $n_{20–24}$ = 215 (724), $n_{25–29}$ = 103 (389), $n_{>30}$ = 58 (253)).

Finnish women and 3% of the Ugandan women smoked. In the following analyses, the risk of being seropositive for multiple HPV types was adjusted for age, HIV positivity (in Uganda) and smoking (Finland).

The prevalences of low-risk (lr) HPV types 6 and 11, and 6/11 combined were lower (12%, 11%, and 17%) among the Finnish women as compared to the Ugandan women (30%, 26%, and 44%). In Finland, HPV16 was the most predominant high-risk (hr) HPV type with a seroprevalence of 21%, followed by HPV18 (14%), HPV31 (12%), HPV33 (11%) and HPV45 (5%). In Uganda the ranked order of hrHPV seroprevalences was: HPV33 (23%), HPV16 (21%), HPV31 (16%), HPV45 (11%) and HPV18 (8%). Half (51%, 619) of the 1222 HPV-seropositive Finnish women had antibodies to more than 1 HPV type as follows: 2 types 26% (321), 3 types 12% (143), 4–7 types 13% (155). Of the 1118 HPV-seropositive Ugandan women, 53% (588) had antibodies to more than 1 HPV type: 2 types 30% (331), 3 types 14% (156), 4–7 types 9% (101).

Finnish women who were HPV45-seropositive (F-HPV$_{45}$), had higher adjusted risk estimates of having antibodies to most of the other HPV types (HPV18, 31 and 33) (Table I, upper right half) than comparable Ugandan women (U-HPV$_{45}$) (Table I, lower left half): F-HPV$_{45}$:HPV18 (OR 10.9, 95% CI 5.3–23) vs U-HPV$_{45}$:HPV18 (OR 3.4, 95% CI 2.3–5.0); F-HPV$_{45}$:HPV31 (OR 6.1, 95% CI 2.8–13.4) vs U-HPV$_{45}$:HPV31 (OR 2.2, 95% CI 1.6–3.0); and F-HPV$_{45}$:HPV33 (OR 12.2, 95% CI 5.8–26) vs U-HPV$_{45}$:HPV33 (OR 3.3, 95% CI 2.4–4.5). This was also true for antibodies to HPV31 and HPV33 among HPV18-seropositive Finnish women (F-HPV$_{18}$) (Table I, upper right half) vs HPV18-seropositive Ugandan women (U-HPV$_{18}$) (Table I, lower, left half), albeit with somewhat overlapping confidence intervals: F-HPV$_{18}$:HPV31 (OR 5.2, 95% CI 3.0–9.0) vs U-HPV$_{18}$:HPV31 (OR 3.1, 95% CI 2.2–4.4); F-HPV$_{18}$:HPV33 (OR 6.9, 95% CI 4.1–11.7) vs U-HPV$_{18}$:HPV33 (OR 3.3, 95% CI 2.3–4.6). In general, among the HPV16-seropositive women, increased risk estimates for being seropositive for a second HPV type were observed, but major differences were not observed between the Finns and the Ugandans (Table I).

Discussion

The overall HPV seroprevalence was significantly higher among Ugandan women than among Finnish women. On the other hand, we observed remarkably increased risk estimates for being double HPV antibody-positive among HPV45- and HPV18-seropositive Finnish women as compared to the Ugandan women.

Seroprevalence indicates cumulative incidence of different HPV types in an individual [4–6]. Our population-based approach and large sample sizes yielded a unique possibility to compare HPV epidemiology in 2 culturally, genetically and socio-economically different female populations. However, our study had limitations: Not all HPV-infected individuals develop antibodies or the antibody response is delayed. Thus, even though highly specific, HPV serology has a sensitivity range of between 40% and 70% [5,10]. Also the number of HPV type-specific antibodies tested was restricted to 7 types (6, 11, 16, 18, 31, 33, and 45) and excluded individuals who might have been positive for some other HPV types commonly reported

Table I. Odds ratios with 95% confidence intervals of being seropositive to another HPV type if seropositive to a defined HPV type among pregnant Finnish (upper right half, $n = 2780$) and Ugandan (lower left half, $n = 1964$) women, with HPV-seronegative women as the reference group.

	6/11	16	18	31	33	45
6/11						
Negative		1	1	1	1	1
Positive	NA	2.4 (1.6–3.7)	3.1 (1.9–4.9)	3.4 (1.9–5.8)	2.2 (1.3–3.7)	1.8 (0.8–4.1)
16						
Negative	1		1	1	1	1
Positive	1.9 (1.5–2.5)	NA	6.7 (4.3–10.5)	4.7 (2.8–7.9)	7.0 (4.3–11.4)	6.5 (3.2–13.3)
18						
Negative	1	1		1	1	1
Positive	2.0 (1.3–3.0)	5.3 (3.8–7.5)	NA	5.2 (3.0–9.0)	6.9 (4.1–11.7)	10.9 (5.3–23)
31						
Negative	1	1	1		1	1
Positive	2.2 (1.6–2.9)	3.8 (2.9–5.0)	3.1 (2.2–4.4)	NA	4.5 (2.5–8.2)	6.1 (2.8–13.4)
33						
Negative	1	1	1	1		1
Positive	3.0 (2.3–3.8)	4.6 (3.6–5.8)	3.3 (2.3–4.6)	3.5 (2.7–4.5)	NA	12.2 (5.8–26)
45						
Negative	1	1	1	1	1	
Positive	1.7 (1.2–2.4)	2.0 (1.4–2.7)	3.4 (2.3–5.0)	2.2 (1.6–3.0)	3.3 (2.4–4.5)	NA

NA, not applicable.
Adjusted for age, smoking (Finland and Uganda) and HIV positivity (Uganda).

among African populations (such as 51, 52 and 58). Moreover, the cross-sectional approach does not allow inferences about the order of the HPV infections.

In line with other studies, the prevalences of overall HPV infections and multiple HPV infections, as indicated by serum antibodies, were high in both study populations [4,7,10,11,12]. The overall HPV seroprevalence was higher among both HIV-positive and HIV-negative Ugandan women than among Finnish women, especially in women under 20 y of age. In Uganda, the mean age at sexual debut is 16.2 y [13]; in Finland it is 16.6 y [14]. There may, however, be a difference between the 2 countries in the number of sex partners of the males [15,16]. Increased risk-taking behaviour (early sex, unprotected and multiple sexual partners) has been reported in young women [17,18], which makes them vulnerable to sexually transmitted diseases, including multiple HPV infections. In addition to behavioural and socio-economic differences, the observed overall difference between the 2 populations could also be partially due to the very high prevalence of smoking in the youngest age group of Finnish women (51%) as compared to Ugandan women (10%), since smoking impairs the HPV antibody response [11].

The most important finding of this study was the identification of remarkably higher risk estimates of having or having had double HPV infections (as indicated by HPV antibodies) in Finnish women seropositive for either HPV45 or HPV18 compared to similar Ugandan women. The causes may not be viral or genetic. European HPV18 and HPV16 variants persist longer in European women, while African HPV18 and HPV16 variants persist longer in African women [19]. On the other hand, HPV18 and HPV45 belong to the same phylogeny, and are primarily associated with lesions higher in the genital tract leading to cervical adenocarcinoma. Its incidence is increasing in Western countries due to the inability of Pap-smear screening to detect the precursor lesions [1]. Forty y of organized cervical screening in Finland may have favoured the occurrence of HPV18/45 in the sexually active female population. There is recent evidence of the epidemic spread of HPV45 in the female Finnish population [20]. Moreover both age-specific seroprevalences of the overall and multiple HPV types revealed that Finnish women are at risk of acquiring new hrHPV infections over a long time [4,21]. Taken together these factors might explain the increased occurrence of especially these 2 HPV types in the double hrHPV infections in Finland.

In conclusion, societal, iatrogenic and behavioural factors may explain the observed differences in the occurrence of multiple HPV infections between the Finnish and Ugandan female populations.

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