HPV infection in women with and without cervical cancer in Conakry, Guinea

BACKGROUND: Cervical cancer incidence in western Africa is among the highest in the world.

METHODS: To investigate human papillomavirus (HPV) infection in Guinea, we obtained cervical specimens from 831 women aged 18–64 years from the general population of the capital Conakry and from 77 locally diagnosed invasive cervical cancers (ICC). Human papillomavirus was detected using a GP5+/6+ PCR-based assay.

RESULTS: Among the general population, the prevalence of cervical abnormalities was 2.6% by visual inspection and 9.5% by liquid-based cytology. Fourteen of 15 high-grade squamous intraepithelial lesions were visual inspection-negative. Human papillomavirus prevalence was 50.8% (32.1% for high-risk types) and relatively constant across all age groups. Being single or reporting ≥3 sexual partners was significantly associated with HPV positivity. HPV16 was the most common type, both among the general population (7.3%) and, notably in ICC (48.6%). HPV45 (18.6%) and HPV18 (14.3%), the next most common types in ICC, were also more common in ICC than in HPV-positive women with normal cytology from the general population.

CONCLUSION: The heavy burden of HPV infection and severe cervical lesions in Guinean women calls for new effective interventions.

The Republic of Guinea in western Africa has a population of approximately 9.4 million, of which 2 million live in the capital of Conakry. The country shows high birth rates (5.8 children per woman) and communicable diseases, notably malaria, which predominate as causes of death. Estimates of cervical cancer incidence and mortality rates in western Africa are among the highest in the world (Ferlay et al., 2004), and in Conakry, cervical cancer is by far the most common malignancy in women (Koulibaly et al., 1997).

Establishing the viral aetiology of cervical cancer has raised the hopes for primary and secondary prevention through human papillomavirus (HPV) vaccination (Kahn and Burk, 2007) and HPV DNA test-based screening (Cuzick et al., 2006), respectively. The rationale and planning of such measures require population-based epidemiological data on overall and type-specific HPV prevalence in women with and without cancer. To this end, the International Agency for Research on Cancer (IARC) has carried out surveys in representative samples of women worldwide (Clifford et al., 2005).

We report on HPV prevalence survey among a representative sample of the general female population in Conakry, Guinea, as well as in a corresponding sample of Guinean women with invasive cervical cancer (ICC).

MATERIALS AND METHODS

Study methods were similar to those used for earlier IARC HPV Prevalence Surveys (Clifford et al., 2005). In the study area of Gbessia Port, a densely populated district of Conakry, we aimed to enrol approximately 100 women from the general population in each 5-year age group between 15 and 19 and 60 and 64 years. All mentally and physically competent women, regardless of their marital and sexual activity status, were eligible. Women were enumerated at their homes by local community workers and invited to the Gbessia Port Health Centre (Centre de Santé de Gbessia Port) between April and December 2006 to participate in the study.

Of the 1725 invited women, 440 (25.5%) did not accept the invitation, mainly because they apparently did not understand the need for gynaecological examination in the absence of symptoms. Acceptance was similar in all the age groups, but owing to the age structure of the population, relatively few women over 44 years of age could be invited (life expectancy of women in Guinea was estimated in 2004 to be 55 years [www.who.int]). In addition, among the 462 women below age 25 years who accepted the
invitation to the study clinic, 213 (of whom 148 were self-declared virgins) were unwilling to undergo a pelvic examination, and opted to provide only a blood sample. Hence, no cervical cell specimen was collected for these 213 women; an additional 73 women aged 25 years or older did not undergo a pelvic examination mainly because of heavy menstrual bleeding.

A structured questionnaire was administered by one of the five nurses in the local dialect covering socio-demographic characteristics, reproductive and menstrual factors, sexual habits, including those of the participants and their husbands’ and lifetime use of contraceptive methods.

A total of 999 women underwent a pelvic examination by one of the three midwives. Three women presented with an advanced cervical cancer; one of which did not allow for collection of an adequate cervical cell sample. Among the remaining 996, a sample of exfoliated cervical cells for liquid-based cytology and HPV testing was collected. A cytobrush, after insertion into the endocervical canal, was rotated gently at 180° to collect cells, was then placed with its cellular material in a vial containing PreservCyt media (Cytyc-Hologic, Marlborough, MA, USA). The cervix was then inspected with acetic acid (VIA) and visual inspection with Lugol’s iodine (VILI), with results reported according to the IARC criteria (Sankaranarayanan and Wesley, 2003). Slides for liquid-based cytology were prepared using a Thin Prep 3000 processor (Cytyc-Hologic), stained according to manufacturer’s instructions and read at the Department of Pathology at the Vrije University Medical Center, Amsterdam, the Netherlands. Cytological diagnosis was formulated according to CISOE-A standards and was translated into the Bethesda 2001 terminology system (BulkJ et al, 2004). Confirmatory biopsies, when available, were read (MK) in the Centre National d’Anatomie Pathologique, Centre Hospitalier Universitaire (CHU) de Donka.

Women presenting with ICC at the gynaecological clinic of the CHU de Donka, Conakry, were identified between January 2006 and March 2007. The collection of a tumour biopsy for study purposes was possible for all 99 women diagnosed with ICC; most were classified as stage II (51 women) or III (44 women). Biopsies were fixed with buffered formalin, embedded in paraffin (MH), then read (MK) in the Centre National d’Anatomie Pathologique, CHU de Donka. HPV testing was available for 41 women with ICC, of which eight (19.5%) were HPV-positive; among 19 women with ICC below age 45 years, six were HPV-positive.

All participants, whether from the HPV prevalence survey or those with ICC, signed informed consent forms according to the recommendations of the IARC and the CHU de Donka ethical review committees, both of which approved the study.

HPV testing was performed on exfoliated cervical cells and ICC biopsies in the Department of Pathology at the Vrije University Medical Center. DNA was extracted from the PreservCyt sample using magnetic beads (Macheri-Nagel, Duren, Germany) on a robotic system (Hamilton, Germany), according to the manufacturer’s instructions. Invasive cervical cancer biopsies were sectioned using a ‘sandwich’ approach, whereby inner tumour sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue. One or more five lM sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue. One or more five lM sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue. One or more five lM sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue. One or more five lM sections were destined for HPV testing and outer sections for histological confirmation of tumour tissue.

Beta-globin PCR analysis was performed first to confirm the presence of human DNA in all specimens (Rod Hasman et al, 1995). The overall presence of HPV DNA was determined by performing a general primer GRS +6 – mediated PCR, which permits the detection of a broad spectrum of genital HPV types at the subplicope level (Jacobs et al, 2000). Human papillomavirus positivity was assessed by hybridisation of PCR products in an enzyme immunoassay using two HPV oligoprobe cocktails that, together, detect the following 44 HPV types: HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (JS39 and MM4 subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), cand85, 86, cand89 (equivalent to CP6108) and JC9710. Subsequent HPV typing was performed by reverse line blot hybridisation of PCR products, as described earlier (van den Brule et al, 2002). In this typing format, all aforementioned HPV types were individually genotyped, except for the uncommon HPV types 32, 83, 84, 85, 86 and JC9710. Oligoprobes representing the latter types were loaded as a pool on the reverse line blots and consequently were genotyped as a pool. Human papillomavirus types considered high risk for this analysis that comprised HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 (Munoz et al, 2003); all other HPV types were considered low risk.

Statistical analysis

Odds ratios (ORs) for HPV positivity and corresponding 95% confidence intervals (CIs) were calculated by means of unconditional logistic regression equations, adjusted for age group (15 – 24, 25 – 34, 35 – 44, 45 – 54, 55 – 64 years), marital status (never- or ever-married) and lifetime number of sexual partners (1, 2 and ≥ 3), as appropriate. The statistical significance of trends for ORs was assessed by considering the categorical variable as a continuous variable in the logistic model. Prevalence ratios and corresponding 95% CIs were used to compare the relative frequency of the most common HPV types in HPV-positive women with ICC with that among cytologically normal HPV-positive women from the HPV prevalence survey.

RESULTS

Of 996 women who provided cervical cell samples, 84 had inadequate HPV DNA results (77 β-globin-negative, seven missing), and an additional 81 had inadequate cytology, leaving 831 women with valid results for both HPV and cytology. Among them, 79 (9.5%) had abnormal cytological findings, including 48 (5.8%) atypical squamous cells of undetermined significance (ASCUS), 16 (1.9%) low-grade squamous intraepithelial lesions (LSIL) and 15 (1.8%) HSIL.

Overall HPV prevalence was 50.8% (78.5% and 47.9% among women with and without cervical abnormalities, respectively, Table 1). The corresponding age-standardised prevalence to the world population was 51.5% (95% CI: 48.0 – 55.0). In total, 275 (33.1%) women had single-type and 147 (17.7%) had multiple-type infections. Prevalence of high-risk and low-risk types (32.1, 30.5%, respectively) was similar. The commonest high-risk types with normal cytology were HPV16 (6.7%), 45 (4.7%), 52 (4.0%), and 18, 35 and 58 (3.2% each). HPV66, 42 and 81 were the most commonly detected low-risk types. HPV61 was also the commonest type (13.9%) among women with cervical abnormalities, of whom 59.5% had high-risk types. The prevalence of high-risk HPV types in women with HSIL or worse was 73.3% (data not shown).

Twenty-one women had abnormalities suspected at VIA/VILI. The correlation between the results of VIA/VILI and those of liquid-based cytology and HPV testing was poor (Table 2). VIA/VILI identified one HSIL (that was shown to be ICC by histology), but missed 14 of 15 HSIL, 15 of 16 LSIL and all 48 ASCUS. The proportion of suspected abnormalities at VIA/VILI did not differ significantly between high-risk HPV-positive (7/264; 2.7%) and low-risk HPV-negative (14/558; 2.5%) women (χ^2 = 0.0146, P = 0.904).

Of the 21 women with suspected abnormalities at VIA/VILI, 17 accepted referral to colposcopy and 10 had colposcopy-directed biopsies, none of which showed lesions except the aforementioned ICC. Repeated attempts were also made after cytological findings became available (i.e., approximately one year and a half after the
initial visit) to recall the 14 women who had no abnormality detected at VIA/VILI but HSIL found at liquid-based cytology. Three women, however, had moved far from Conakry, one was not found at her address, one had died of a disease other than cervical cancer and one was in the third trimester of pregnancy. Of the eight biopsies taken, one was inadequate, three showed cervical intraepithelial neoplasia 1 and the other four showed no cervical lesion.

Figure 1A and B show, respectively, the prevalence of HPV (any HPV type, HPV16 and/or 18 and any high-risk types and low-risk types, separately) and of VIA/VILI and cytological abnormalities by age group. Human papillomavirus prevalence was 56.1% among women younger than 25 years, and dropped to 45.3% in women aged 35–44 years, before increasing again up to 55% in women aged 45 years or older. Age-specific patterns were similar for the prevalence of HPV16 and/or 18 and any high-risk type. When 157 women who had never been married (75.8% of whom were $<25$ years) were excluded, HPV prevalence below age 25 years decreased to 46.3% (Figure 1A). VIA/VILI and cytological abnormalities increased up to age 35–44 years and then declined.

Table 3 shows the relationship between HPV positivity and various characteristics of participants according to two different models. In the age-adjusted model, significant differences in HPV positivity were observed by age group (OR for 35–44 vs $<25$ years $=0.65; 95\%\ CI: 0.44–0.95$), marital status (OR in single vs married women $=1.60; 95\%\ CI: 1.04–2.45$) and number of lifetime sexual partners (OR for $\geq3$ vs 1 partner $=1.71; 95\%\ CI: 1.03–2.84$). These three associations were slightly attenuated in the adjusted model.
model additionally adjusted, as appropriate, for marital status and sexual partners.

Education, number of births, age at first sexual intercourse, husbands’ polygamy or extra-marital sexual relationships and lifetime use of hormonal contraceptives were unrelated to HPV positivity in either adjustment model (Table 3) as were smoking habits, condom use (reported by only 16 women) and history of spontaneous or induced abortions (data not shown). Only six (0.7%) participants reported having had previous cervical cancer screening.

Beta-globin-negative ICC biopsies (22) were excluded; among the remaining 77 ICC biopsies with valid HPV results, 8 were adenocarcinoma, one adenosquamous, and 79 squamous-cell carcinoma. The median age of women with ICC was 45 years (range: 23–80 years). Seven ICC biopsies, including one adenocarcinoma, were HPV-negative. Type-specific normal HPV prevalence in 70 HPV-positive ICC and 360 cytologically normal HPV-positive women from the HPV prevalence survey are compared in Table 4. HPV16 was found in 34 (48.6; 95% CI: 36.4–60.8%) HPV-positive ICC biopsies. The next commonest types were HPV45 (18.6; 95% CI: 10.3–29.7%) and 18 (14.3; 95% CI: 7.1–24.7%). Prevalence ratios in women with ICC vs cytologically normal women were 3.5 (95% CI: 2.2–5.5) for HPV16, 2.1 (95% CI: 0.9–4.6) for HPV18, 1.9 (95% CI: 0.9–3.7) for HPV45 and 0.8 (95% CI: 0.5–1.2) for high-risk types other than 16 or 18. Multiple-type infections were much less common in HPV-positive women with ICC than in HPV-positive women with normal cytology (Table 4). Among the five ICC biopsies deriving from HIV-infected women with a valid HPV result, HPV16, 45, 18 were found in two, two and one woman, respectively.

**DISCUSSION**

Our large study, the first on HPV infection in Guinea, showed a very high (50.8%) prevalence of HPV, and nearly no history of cervical cancer screening. Approximately one-third of women in our survey were infected with high-risk HPV types and four prevalent ICC were discovered. The age-standardised HPV prevalence we found in Conakry (51.5%) was considerably higher than that observed in earlier studies performed using the same HPV testing protocol in areas at high risk for cervical cancer such as South America (12–18%) and India (17%) or even another western African population in Nigeria (27%) (Franceschi et al., 2006). It is also of the highest HPV prevalence reported in any study in western Africa (Xi et al., 2003; Wall et al., 2005; Lack et al., 2005) or other parts of sub-Saharan Africa (de Sanjosé et al., 2007; Castellsague et al., 2008), with the exception of those that included only, or a majority of, HIV-positive women (Sahasrabuddhe et al., 2007). Differences in age and sensitivity of PCR testing methods, however, limit the direct comparability with studies other than the IARC HPV Prevalence Surveys.

Nearly half of study women reported having two sexual partners or more in their lifetime, and over two-thirds reported husband’s...
polygamy or extra-marital relationships. These indicators of high-risk sexual behaviour can help to explain the elevated HPV prevalence in this study. Conversely, on account of the very high frequency of HPV infection that emerged in our study, it is not surprising that HPV-negative and -positive women did not differ by various characteristics, including education level, except for a moderately increased infection risk in single women and women who reported three sexual partners or more in their lifetime.

An influence of undetected HIV infection on high HPV prevalence in our study women cannot be ruled out, as the protocol of the IARC HPV Prevalence Surveys does not include HIV testing. In 2004, a survey of pregnant women estimated the prevalence of HIV to be 4.2% nationally, and 6% in Gbessia Port, Conakry; this seemed to be fairly uniform across age groups, implying a well-established epidemic, but was somewhat higher among unmarried women (9.2%) (Ministère de la Santé Publique, 2005). HIV infection among tested women with ICC (20%) was high considering the limited life expectancy of people with HIV/AIDS during the study period (Franceschi and Jaffe, 2007).

One of the main aims of the IARC HPV Prevalence Surveys concerns the variations of age-specific and type-specific HPV prevalence by geographical region. The age-specific curve of HPV prevalence in Guinea resembled the age curves reported earlier in Nigeria (Thomas et al., 2004), India (Franceschi et al., 2005) and China (Dai et al., 2006; Li et al., 2006; Wu et al., 2007), but differed from the steep decrease in prevalence seen by age in high-resource countries (Franceschi et al., 2006; De Vuyst et al., 2009). A modest peak in women below age 25 years in Guinea was accounted for by

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### Table 3: ORs for HPV positivity and corresponding 95% CIs according to selected characteristics among 831 women (Conakry, Guinea, 2006–2008)

| Characteristics | N women | N (%) | ORb | 95% CI | ORc | 95% CI |
|-----------------|---------|-------|------|--------|------|--------|
| **Age group (years)** |         |       |      |        |      |        |
| <25             | 214     | 120   | 56.1 |        |      |        |
| 25–34           | 212     | 100   | (47.2)| 0.70   | 0.48–1.02 | 0.78 | 0.51–1.21 |
| 35–44           | 212     | 96    | (45.3)| 0.65   | 0.44–0.95 | 0.70 | 0.44–1.12 |
| 45–54           | 123     | 70    | (56.9)| 1.03   | 0.66–1.62 | 1.19 | 0.71–2.00 |
| 55–64           | 70      | 36    | (51.4)| 0.83   | 0.48–1.42 | 1.00 | 0.54–1.84 |
| χ² for trend    |         |       |      | 0.13   | 0.716 | 0.51   | 0.473 |
| **Education**   |         |       |      |        |      |        |
| None            | 411     | 209   | (50.9)|        |      |        |
| Primary         | 176     | 86    | (48.9)| 0.93   | 0.65–1.34 | 0.91 | 0.63–1.32 |
| Secondary or higher | 244   | 127   | (52.1)| 1.03   | 0.74–1.43 | 0.94 | 0.66–1.32 |
| χ² for trend    |         |       |      | 0.13   | 0.716 | 0.51   | 0.473 |
| **Marital status** |       |       |      |        |      |        |
| Married         | 586     | 285   | (48.6)|        |      |        |
| Single          | 157     | 95    | (60.5)| 1.60   | 1.04–2.45 | 1.55 | 1.00–2.41 |
| Widowed/divorced | 88     | 42    | (47.7)| 0.89   | 0.55–1.43 | 0.88 | 0.54–1.42 |
| **Number of births** |     |       |      |        |      |        |
| 0               | 170     | 85    | (50.0)|        |      |        |
| 1–2             | 226     | 122   | (54.0)| 1.32   | 0.87–2.00 | 1.45 | 0.93–2.27 |
| 3–5             | 225     | 103   | (45.8)| 1.06   | 0.66–1.69 | 1.32 | 0.79–2.19 |
| ≥6              | 208     | 111   | (53.4)| 1.31   | 0.77–2.26 | 1.64 | 0.93–2.90 |
| χ² for trend    |         |       |      | 0.12   | 0.790 | 1.29   | 0.256 |
| **Age at 1st intercourse (years)** |     |       |      |        |      |        |
| ≥18             | 216     | 112   | (51.9)|        |      |        |
| 16–17           | 455     | 231   | (50.8)| 0.92   | 0.66–1.27 | 0.90 | 0.64–1.25 |
| ≤15             | 133     | 64    | (48.1)| 0.78   | 0.50–1.21 | 0.78 | 0.50–1.22 |
| χ² for trend    |         |       |      | 1.12   | 0.790 | 1.29   | 0.256 |
| **Lifetime sexual partners** | |       |      |        |      |        |
| 1               | 454     | 227   | (50.0)|        |      |        |
| 2               | 273     | 136   | (49.8)| 1.08   | 0.78–1.48 | 1.06 | 0.77–1.46 |
| >3              | 76      | 46    | (60.5)| 1.71   | 1.03–2.84 | 1.64 | 0.98–2.72 |
| χ² for trend    |         |       |      | 3.23   | 0.072 | 3.07   | 0.080 |
| **Polygamous husband** | |       |      |        |      |        |
| No              | 196     | 93    | (47.5)|        |      |        |
| Yes             | 621     | 323   | (52.0)| 1.31   | 0.93–1.84 | 1.30 | 0.91–1.86 |
| **Husband’s extra-marital sexual relationships** | |       |      |        |      |        |
| No              | 103     | 52    | (50.5)|        |      |        |
| Yes             | 701     | 357   | (50.9)| 1.11   | 0.72–1.72 | 1.10 | 0.70–1.72 |
| **Use of hormonal contraceptive** | |       |      |        |      |        |
| Never           | 740     | 371   | (50.1)|        |      |        |
| Ever            | 90      | 50    | (55.6)| 1.30   | 0.84–2.04 | 1.15 | 0.72–1.83 |

*aSome figures do not add up to the total because of a few missing values. *bAdjusted for age group, as appropriate. *cAdjusted for age group, ever/never married and lifetime number of sexual partners (1,2, >2), as appropriate. *dIncluding nine divorced women. CI = confidence intervals; HPV = human papillomavirus; N = number; OR = odds ratios.
Table 4  Prevalence of selected HPV types in 70 HPV-positive women with ICC\(^a\) and 360 HPV-positive women with normal cytology (Conakry, Guinea, 2006–2008)

| HPV type | ICC (N = 70) | Normal cytology (N = 360) | ICC.normal cytology |
|----------|--------------|--------------------------|---------------------|
|          | Total (%)    | Total (%)                | Prevalence ratio (95%CI) |
| 16       | 34 (48.6)    | 50 (13.9)                | 3.5 (2.2–5.5)        |
| 18       | 10 (14.3)    | 24 (6.7)                 | 2.1 (0.9–4.6)        |
| 16/18    | 44 (62.9)    | 73 (20.3)                | 3.1 (2.1–4.6)        |
| Other high-risk types |                       |                          |                     |
| 33       | 2 (2.9)      | 22 (6.1)                 | 0.5 (0.05–1.9)       |
| 35       | 3 (4.3)      | 24 (6.7)                 | 0.6 (0.1–2.1)        |
| 39       | 2 (2.9)      | 7 (1.9)                  | 1.5 (0.1–7.7)        |
| 45       | 13 (18.6)    | 35 (9.7)                 | 1.9 (0.9–3.7)        |
| Any high-risk type other 25 (35.7) | 169 (46.9)         | 0.8 (0.5–1.2)         |
| Low-risk type |                       |                          |                     |
| X        | 2 (2.9)      | 30 (8.3)                 | 0.3 (0.04–1.4)       |
| Multiple infections | 3 (4.3)       | 119 (33.1)               | 0.1 (0.03–0.4)       |

\(^a\)Seven HPV-negative ICC are not included. \(^b\)Includes one single HPV30 infection.

With respect to the relative importance of different HPV types, HPV16 was the commonest type overall, that is, 13.9%, 17.7% and 48.6%, respectively, in cytologically normal and abnormal women and in women with ICC (Clifford et al., 2005; Smith et al., 2007). Noteworthy, however, the high proportion of HPV45 (18.6%; 95% CI: 10.3–29.7%) is consistent with a meta-analysis of HPV type distribution in ICC from western Africa (Smith et al., 2007).

The very heavy burden of HPV infection and severe cervical lesions in Guinea calls for new effective interventions. The quality of VIA/VILI should be greatly improved, or other types of screening used (e.g., rapid HPV test, Qiao et al., 2008), whereas screen-and-treat approaches should be encouraged for recalling women. With respect to the potential benefit of vaccination, the fraction of ICC preventable in Guinea (62.9%; 95% CI: 50.5–74.1) by a vaccine including HPV16/18, is compatible with the worldwide estimate (Smith et al., 2007), but would be substantially improved if some cross-protection between HPV16/18 and HPV45 was confirmed to exist (Barr and Sings, 2008; Jenkins, 2008).

ACKNOWLEDGEMENTS

The authors thank Dr Rengaswamy Sankaranarayanan for valuable comments. This work was supported by the Bill and Melinda Gates Foundation, Seattle, WA, USA (grant number 35537).

Conflict of interest

The authors declare no conflict of interest.

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Epidemiology
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