Short Communication

Human papillomavirus infection in women with and without cervical cancer in Karachi, Pakistan

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BACKGROUND: No data exist on the population prevalence of, or risk factors for, human papillomavirus (HPV) infection in predominantly Muslim countries in Asia.

METHODS: Cervical specimens were obtained from 899 married women aged 15–59 years from the general population of Karachi, Pakistan and from 91 locally diagnosed invasive cervical cancers (ICCs). HPV was detected using a GP5 + /6 + PCR-based assay.

RESULTS: The prevalence of HPV in the general population was 2.8%, with no evidence of higher HPV prevalence in young women. The positivity of HPV was associated with women's lifetime number of sexual partners, but particularly with the age difference between spouses and other husbands' characteristics, such as extramarital sexual relationships and regular absence from home. The HPV16/18 accounted for 24 and 88% of HPV-positive women in the general population and ICC, respectively.

CONCLUSION: Cervical cancer prevention policies should take into account the low HPV prevalence and low acceptability of gynaecological examination in this population.

To date, there are no data on the population prevalence of, or risk factors for, human papillomavirus (HPV) infection in predominantly Muslim countries in Asia, where sexual mores differ from many other world populations (Wellings *et al*, 2006). These data are essential to assess the potential relevance of HPV vaccination and HPV test-based screening to invasive cervical cancer (ICC) prevention in the region, as well as to identify any changes in risk occurring in young generations. Thus, a study of women with and without cervical cancer was carried out in Karachi, Pakistan, according to the standardised protocol of the International Agency for Research on Cancer (IARC) HPV Prevalence Surveys (Clifford *et al*, 2005), which was approved by both the IARC and local ethical review committees.

METHODS

A total of 3882 married women aged 15–59 years living in Orangi, a densely populated suburb of Karachi, were visited at their homes and invited to join the study, with the aim to enrol ~100 women in

each 5-year age group. Participation rates were 24.1, 25.7, 25.1 and 23.6% among women aged 15–24, 25–34, 35–44 and 45–59 years, respectively. All participants signed an informed consent form and were administered a questionnaire. In all, 915 participants came to the study clinic located in the Sindh Government Qatar Hospital, where a sample of exfoliated cervical cells was collected and placed into PreservCyt media (Hologic, Marlborough, MA, USA) for HPV testing and liquid-based cytology.

In parallel, formalin-fixed tumour biopsies were retrieved from women presenting with histologically confirmed ICC between 2004 and 2008 to the Ziauddin and Aga Khan University Hospitals, Karachi. After exclusion of 40 biopsies that were β-globin negative and/or without histological evidence of tumour, 91 ICCs remained (79 squamous cell, 3 adeno, 4 small cell and 5 other or unspecified carcinomas).

Liquid-based cytology and HPV testing were carried out at the Vrije University, Amsterdam, the Netherlands. A general primer GP5 + /6 + -mediated PCR was used for the detection of 44 genital HPV types (Jacobs *et al*, 2000). Subsequent HPV typing was carried out by reverse-line blot hybridisation of PCR products (van den Brule *et al*, 2002). Odds ratios (ORs) for HPV positivity were calculated by unconditional logistic regression adjusted for age, with OR trends assessed by considering categories as continuous variables.

RESULTS

Among 899 women from the general population with valid cytology and HPV results, HPV prevalence was 2.8% (Table 1). Cervical abnormalities were diagnosed in 2.4% of women, of whom 27.3% were HPV-positive. They included 19 atypical squamous cells of undetermined significance (4 HPV-positive), 1 low-grade squamous intraepithelial lesion (HPV16/66-positive) and 2 high-grade squamous intraepithelial lesions (1 HPV-negative; 1 HPV16-positive, later revealed to be ICC). HPV16 was confirmed as the most common type among women with both normal (0.5%) (Clifford et al, 2005) and abnormal (9.1%) cytology. HPV16 was also the predominant HPV type (75.8%) in ICC, followed by HPV18 (6.6%) and 45 (4.4%) (Table 1).

Having $\geqslant 2$ lifetime sexual partners was reported by 5.6% of women, and was significantly associated with HPV positivity (OR = 3.36; 95% confidence interval (CI): 1.08–10.41), as was being a working woman (OR = 3.01; 95% CI: 1.26–7.21) (Table 2). In addition, HPV infection was strongly associated with husbands' characteristics, such as extramarital sexual relationships (OR = 4.40; 95% CI: 1.83–10.56), absence from home > 7 nights per month (OR = 4.84; 95% CI: 1.30–17.95) and a $\geqslant 10$ year age difference between spouses (OR = 6.88; 95% CI: 1.48–31.94) (Table 2). Husbands' characteristics were correlated with each other, but associations were unaffected by adjustment for women's lifetime number of sexual partners.

The positivity of HPV did not vary significantly by age (Table 2). Neither were there any significant associations with education level, marital status, number of full-term pregnancies, age at sexual debut (Table 2), language/ethnic group (87.5% Urdu speakers), birth outside Karachi (44.9%), use of condom (19.7%), hormonal contraceptives (16.7%) and intrauterine device (7.2%), tubal ligation (5.2%), history of spontaneous (35.2%) or induced (11.5%) abortions, smoking (1.7%) or previous Pap smear (1.1%) (data not shown).

DISCUSSION

This study disclosed a very low burden of HPV infection in the general female population of Karachi, considerably lower than that found using similar protocols in nearby India (17%) (Franceschi et al, 2005), China (15–18%) (Dai et al, 2006; Li et al, 2006; Wu et al, 2007) and Nepal (9%) (Sherpa et al, 2010), and > 10-fold lower than in sub-Saharan Africa (e.g., 51% in Guinea, also a predominantly Muslim country) (Keita et al, 2009). There was no evidence of higher prevalence in younger women, confirming the flat age-specific curve seen in other low-resource countries in Asia and Africa (Franceschi et al, 2006), although at much lower HPV prevalence. An HPV prevalence of 27.3% in cervical abnormalities is also low compared with high-resource settings (IARC, 2005), highlighting problems in specificity of even gold-standard cytology in settings of very low HPV prevalence.

The HPV16 accounted for three-quarters of ICC in Pakistan, confirming the high prevalence of HPV16 observed in ICC from the Indian subcontinent compared with other world regions (Franceschi et al, 2003; Basu et al, 2009; Gheit et al, 2009). Indeed, our data would suggest that the predominance of HPV16 over other high-risk types might be even greater in settings of low HPV exposure.

Major study strengths include a large population-based sample, use of a standardised and well-validated HPV test allowing comparisons with similar studies around the world (Clifford et al, 2005) and with a concurrent series of ICC from the same area. The main limitation, which was nevertheless an important finding in itself, was the low participation rate, for which the principal reasons were lack of husband's permission and/or lack of appreciation of screening in the absence of symptoms. As HPV infection is asymptomatic, it is unlikely that participation

Table 1 Prevalence of HPV types in 899 women from the general population and 91 women with ICC in Karachi, Pakistan

HPV type	General population — Normal cytology: Sing.	Mult.	Total (%)	Abnormal cytology: Sing.	Mult.	Total (%)	All (%)	ICC All (%)
N			877			22	899	91
HPV+	16	3	19 (2.2)	3[a]	3[b]	6 (27.3)	25 (2.8)	83[c] (92.2)
High-risk								
16	3	1	4 (0.5)	1[a]	1[b]	2 (9.1)	6 (0.7)	69 (75.8)
18	—	—	—	—	—	—	—	6 (6.6)
33	—	—	—	—	—	—	—	1 (1.1)
35	2	—	2 (0.2)	—	—	—	2 (0.2)	—
45	—	—	—	—	1	1 (4.5)	1 (0.1)	4 (4.4)
51	1	1	2 (0.2)	—	—	—	2 (0.2)	—
56	2	—	2 (0.2)	—	—	—	2 (0.2)	2 (2.2)
59	1	—	1 (0.1)	—	—	—	1 (0.1)	1 (1.1)
68	—	—	—	—	1	1 (4.5)	1 (0.1)	—
Any	9	2	11 (1.3)	1	3	4 (18.2)	15 (1.7)	81 (89.0)
Low-risk								
6	2	—	2 (0.2)	1	—	1 (4.5)	3 (0.3)	—
40	—	1	1 (0.1)	—	—	—	1 (0.1)	—
42	2	2	4 (0.5)	—	—	—	4 (0.4)	1 (1.1)[d]
43	—	—	—	—	1	1 (4.5)	1 (0.1)	—
54	1	—	1 (0.1)	—	—	—	1 (0.1)	—
66	—	—	—	—	1[b]	1 (4.5)	1 (0.1)	1 (1.1)
67	—	1	1 (0.1)	—	—	—	1 (0.1)	—
69	—	—	—	—	—	—	—	1 (1.1)
70	—	2	2 (0.2)	—	—	—	2 (0.2)	—
84	1	—	1 (0.1)	—	—	—	1 (0.1)	—
CP6108	1	—	1 (0.1)	—	1	1 (4.5)	2 (0.2)	—
Any	7	3	10 (1.1)	1	3	4 (18.2)	14 (1.6)	3 (3.3)
X	—	—	—	1	—	1 (4.5)	1 (0.1)	1 (1.1)

Abbreviations: HPV = human papillomavirus; ICC = invasive cervical cancer; mult. = multiple; sing. = single. [a]Including one woman with high-grade squamous intraepithelial lesion or ICC. [b]Including one woman with low-grade squamous intraepithelial lesion. [c]Including three multiple infections (16/18, 16/18/66, 16/69), which are each counted separately. [d]One ICC was positive for a low-risk type only.

was highly correlated to HPV positivity. However, hesitancy to undergo gynaecological examination would be a strong obstacle to any future cervical cancer screening efforts in Pakistan (Imam et al, 2008). Furthermore, vaginal examination was acceptable only to married women, as in similar surveys in Asia (Franceschi et al, 2005; Dai et al, 2006; Sherpa et al, 2010). Nevertheless, 98.2% of women reported an age at sexual debut concurrent with, or just after, age at marriage, suggesting that marriage may be a good proxy of sexual debut in this population.

In conclusion, the low HPV prevalence in this study is consistent with the low ICC risk estimate from Karachi (7.5/100 000 cases annually; rarer than the breast, mouth, ovary and oesophagus) (Curado et al, 2007) and other predominantly Muslim countries in Asia (Curado et al, 2007) and, reassuringly, there is no evidence that HPV prevalence is higher in young generations of urban women. However, 80% of the ICC that do develop in this population (of which one was newly diagnosed by our study) are attributable to HPV16/18. These findings should be taken into account when considering the cost-effectiveness of ICC prevention modalities in a population in which the burden of HPV and other sexually transmitted infections (Zaheer et al, 2009) seems low, but the burden of many other chronic infectious diseases (e.g., tuberculosis (Hasan et al, 2009) and hepatitis C virus (Ahmad, 2004)) is high.

Table 2 ORs for HPV positivity and corresponding 95% CIs according to selected characteristics among 899 women in Karachi, Pakistan

| Characteristic | N women[a] | HPV-positive | | OR[b] | 95% CI |
		N	%		
Age (years)					
<25	199	4	2.01	1	—
25–34	235	7	2.98	1.50	0.43–5.19
35–44	246	6	2.44	1.22	0.34–4.38
≥45	219	8	3.65	1.85	0.55–6.24
χ^2_1 for trend				0.71	P=0.4
Education level					
Secondary or higher	209	3	1.44	1	—
Primary or middle	319	6	1.88	1.35	0.33–5.49
Illiterate	371	16	4.31	3.22	0.88–11.84
χ^2_1 for trend				4.31	P=0.04
Occupation					
Housewife	774	17	2.20	1	—
Employed	125	8	6.40	3.01	1.26–7.21
Marital status					
Married, husband has one wife	784	20	2.55	1	—
Married, husband has more than one wife	44	2	4.55	1.69	0.38–7.51
Separated or divorced	26	2	7.69	3.36	0.74–15.36
Widow	45	1	2.22	0.70	0.09–5.61
Number of full-term pregnancies					
0	110	3	2.73	1.24	0.31–4.98
1–3	302	7	2.32	1	—
≥4	480	15	3.13	1.21	0.39–3.78
χ^2_1 for trend				0	P=1
Lifetime number of sexual partners					
1	845	20	2.37	1	—
≥2	50	4	8.00	3.36	1.08–10.41
Age at sexual debut (years)					
≤15	176	7	3.98	1	—
16–18	333	6	1.80	0.46	0.15–1.41
≥19	389	12	3.08	0.83	0.30–2.29
χ^2_1 for trend				0.01	P=0.9
Age difference with husband[c]					
0–4 years	269	2	0.74	1	—
5–9 years	366	10	2.73	3.66	0.79–16.87
≥10 years	193	10	5.18	6.88	1.48–31.94
χ^2_1 for trend				7.25	P=0.007
Husband's extramarital sexual relationships[c]					
No	693	12	1.73	1	—
Yes	134	10	7.46	4.40	1.83–10.56
Husband away from home >7 nights per month[c]					
No	801	19	2.37	1	—
Yes	26	3	11.54	4.84	1.30–17.95

Abbreviations: CI = confidence interval; HPV = human papillomavirus; OR = odds ratio. [a]Some figures do not add up to the total because of few missing values. [b]Adjusted for age. [c]Restricted to currently married women only (N = 828).

Conflict of interest

The authors declare no conflict of interest.

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