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CANCER

Risk factors of invasive cervical cancer in Mali

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Background Cervical cancer is the most common cancer in women in Mali and the second commonest cause of cancer mortality.

Methods As part of an international effort to evaluate the role of human papillomavirus (HPV) in the aetiology of cervical cancer, we conducted a hospital-based case-control study in three medical centres in Bamako during 1994–1995. A total of 82 cases (invasive cervical cancer patients) and 97 controls matched to the cases for age were included. Information on risk factors was collected through personal interview. Serum antibodies to HPV 16, 18 and 31 virus like particles (VLP) were detected using ELISA assays. Polymerase chain reaction was used to detect HPV DNA in frozen biopsies of cases.

Results Human papillomavirus 6, 18, 31 VLP were detected in 60.4% of cases and 45.4% of controls ($P = 0.03$). Overall, HPV DNA was identified in 96.9% of the cervical cancer cases. Risk factors for cervical cancer were parity >10 versus <5 children ([odds ratio] OR = 4.8, 95% CI : 1.5–14.7), never having practised vaginal douching (OR = 17.6, 95% CI : 4.2–74.7), re-using home-made feminine napkins (OR = 45.9, 95% CI : 8.8–238.7) and having a husband with more than two wives (OR = 5.3, 95% CI : 1.3–21.3).

Conclusions These data provide further evidence on the role of HPV in cervical cancer and show that high parity and poor genital hygiene conditions were the main co-factors for cervical cancer in this population with prevalent HPV infection.

Keywords HPV, HPV VLP, Africa, cervical cancer

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Cervical cancer is the most common cancer in women in Mali with an age-standardized incidence rate of 24.4 per 100 000 women and is the second commonest cause of cancer mortality.[1]

The contribution of human papillomavirus (HPV) infection in the development of cervical cancer is established and detection of HPV DNA has been shown to be the strongest risk factor for cervical cancer world-wide.[2,3] Human papillomavirus is an endemic infection in sub-Saharan Africa.[4–6] However, the number of surveys reported is scarce and so far no natural history studies have been reported. Studies in other settings have shown that although most of the infections are likely to regress, in a proportion of women the infection persists and increases the likelihood of developing cervical cancer. The co-factors that may intervene in the development of cervical cancer among HPV infected women are still the subject of research. It has been proposed that early age at sexual initiation, high parity, smoking and use of oral contraceptives may be relevant factors that modulate the oncogenic effect of HPV infection. The nature of the HPV infection (type, viral load, genetic variants, DNA integration…) as well as the immune status of the subject may also influence the natural history of the infection. Other factors have also been reported in specific countries as markers of women at high risk of cervical cancer, which may indicate specific opportunities for preventive actions. For example, poor hygiene has been related to cervical cancer in China and in Africa[7,8] but not in more affluent countries.[9]

This study in Bamako, Mali, was undertaken as part of an international effort, co-ordinated by the International Agency for Research on Cancer (IARC), to describe the contribution of HPV and other factors in cervical carcinogenesis. Bamako has a population of over 900 000. Hospitals in Bamako offer cancer care to the whole country. In Bamako there is one of the few

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population-based cancer registries in Africa. It is located within the Pathological Department of the Institut National de Recherche en Santé Publique.

Material and Methods

The study took place in two hospitals in Bamako: Gabriel Touré and Point G, and in the Family Planning Clinic, Protection Maternelle et Infantile (PMI) located near to Bamako. Cases of invasive cervical cancer were identified between 1994 and 1995 at first visit to the study centres. Patients were informed of the study objectives and invited to participate. All cases had to be verified by histology and were recruited before treatment. Controls were identified and selected from the same hospital as case subjects and were matched to the cases by 5-year age group. Eligible participants were in the age range 18–80 years and had to be in good physical and mental condition in order to provide reliable answers. Patients were not eligible as controls if the main diagnosis at entry was any of the following diseases: ano-genital cancer, cancers of the breast, oral cavity, oesophagus, lung, bladder or liver; cardiovascular diseases, chronic bronchitis, emphysema or a sexually transmitted disease. Hysterectomy and previous conization were also exclusion criteria.

Study subjects were interviewed by any of the three trained interviewers during their visit to the hospital. A detailed structured questionnaire was used to describe sexual behavioural patterns, reproduction and contraceptive practices, smoking habits and the socioeconomic and cultural background of the participants. Efforts were made to keep the interviewers blind to the clinical diagnosis of the participants.

Cases of cervical cancer provided a biopsy and two cervical scrapes. Controls provided two cervical scrapes using the standard Ayre spatula and cytobrush. Biopsies were kept in liquid nitrogen without any additives. The cells were first used to prepare a Pap smear. The remaining cells on the spatula were eluted in phosphate-buffered saline (PBS), pelleted and kept at –20°C. At the end of the fieldwork, samples were shipped to IARC and afterwards to the Academisch Ziekenhuis Vrije University in Amsterdam for HPV testing.

A blood sample was obtained for both cases and controls. Sera aliquots were produced and kept at –20°C until they were sent to the Faculté de Pharmacie in Tours for HPV serological detection.

Husbands of cases and controls were also invited to participate. However, only 18 men agreed to participate and the data is not considered further.

Protocols were accepted by IARC's Ethical Committee.

HPV DNA detection

Cervical scrapes

Cell suspensions of cervical exfoliated cells were centrifuged for 10 minutes at 3000 g. The cell suspensions were resuspended in 1 ml 0.01 M Tris–HCl buffer and stored at –80°C until use. The HPV DNA detection was performed on crude extracts as earlier described.[10]

Biopsies

For HPV detection the sandwich method was used.[11] Briefly, a series of sections were cut from the frozen biopsies of which the outer sections were haematoxylin stained and analysed histologically to confirm the presence of cancer tissue. To analyse the quality of the target DNA, samples were pre-screened by polymerase chain reaction (PCR) using β-globin specific primers spanning 209 base pairs.[10] Only β-globin PCR positive samples were analysed further.

Human papillomavirus testing was performed by PCR enzyme immunoassay (EIA) using Generic Primer (GP)5+/bio 6+ primer mediated PCR, as earlier described.[12,13] Fifteen high-risk HPV types (16,18,31,33,35,39,45,51,52,56,58,59,66, 68 and 73) and six low-risk HPV types (6,11,40,42,43 and 44) could be demonstrated in one test at the subpicogram level. Moreover, amplification products were analysed for the individual HPV types. GP5+/bio6+ PCR products were also analysed by conventional Southern blot hybridization with a radioactive-labelled general probe.[12] The PCR EIA positive samples were also positive after Southern blot analysis. Southern blot positive samples that were negative in the PCR EIA were labelled as HPV-X.

HPV serology

The HPV virus like particles (VLP) were produced in SF21 insect cells using a recombinant baculovirus system and purified by isopycnic banding on a CsCl gradient (Combita *et al*. in preparation).[14] The VLP observed at densities 1.27–1.30 were diluted in PBS (pH 7.4) and then used in the following ELISA test. Microtitre plates (Maxisorp, Nunc, Life Technologies) were coated either with 200–800 ng/well of purified HPV VLP (test well) or newborn bovine serum (control well). The plates were incubated at 4°C overnight. After four washes with PBS-0.1% Tween 20, non-specific binding sites were blocked by incubation for 30 minutes at 37°C with PBS-1% newborn bovine serum. The blocking solution was replaced by 100 µl of human sera diluted 1/10 in 5 × PBS containing 10% NBS and 2% Tween 20. Following incubation at 45°C for 50 minutes and 4 washes, bound antibodies were detected with mouse anti human IgG antibodies covalently linked to horse radish peroxidase and 100 µl of substrate solution containing 0-phenylene diamine and H_2O_2 were added after incubation at 45°C for 50 minutes and 4 washes. The reaction was stopped after 30 minutes by addition of 100 µl of 4N H_2SO_4 and optical densities (OD) (492 nm) were read with an automated plate reader. The cut-off values for positivity were set up at 0.2 and 0.4 (OD of the test well minus OD of the control well).

Statistical analyses

Standard methods of statistical analysis were used to estimate the association between risk factors, including markers of HPV infection, and cervical cancer. Odds ratios (OR) and 95% CI were calculated using unconditional logistic regression. Logistic regression models were adjusted for age group, area of birth, genital douche, age at first sexual intercourse and HPV serostatus. Some models were adjusted also for availability of toilet inside the house and parity.

Results

Participation rates

Of the 100 cases and 100 controls identified, 2 cases and 2 controls refused to participate and 16 cases and one control could not be interviewed. Thus, 82 cases and 97 controls were included in the final analysis.

Medical information

Seventy-nine cases (96.3%) were diagnosed as squamous carcinomas. In the three remaining cases, the diagnosis was done on a clinical basis only. The distribution of tumour stages (TNM) was: 32.9% T1, 49.4% T2, 7.6% T3, 10.1% T4/M1. The distribution of the medical conditions of the controls was: 25.8% diseases of the digestive system, 12.4% endocrine nutritional and metabolic diseases, 15.5% diseases of circulatory system, 5.2% injury and poisoning. The remaining subjects were distributed in different categories, all of them accounting for less than 5% of the control group.

Socio-demographic characteristics

The mean age of both cases and controls was 47 years, almost all women were Muslims (97%) and about a third had been born in Bamako. Table 1 shows the distribution of cases and controls in relation to selected socio-demographic characteristics. Among the variables explored, cases were statistically more likely to report a semi-urban place of birth compared to a rural or urban birthplace ($P = 0.049$). The majority of women were married and about a quarter were widowed. Few women reported school attendance with a lower percentage among cases (17% for cases and 21% for controls). Lack of facilities in the home, such as running water, toilet, TV, refrigerator, telephone, video, stereo, motorbike or car were more commonly reported by controls (37.1% for controls, 9.8% for cases $P = 0.004$).

For the following analysis, age, area of birth, home facilities and HPV VLP were considered as potential confounders and therefore kept in the logistic models.

Table 1 Distribution of invasive cervical cancer cases and controls by selected socio-demographic characteristics in Mali

	Cases		Controls	
	N	%	N	%
Total	82		97	
Mean age	46.9	(12.3)	46.9	(12.1)
Age (years)				
25–37	22	26.8	26	26.8
38–45	21	25.6	23	23.7
46–56	18	22.0	26	26.8
>56	21	25.6	22	22.7
P-value				0.95
Area of birth				
Rural	43	52.4	55	61.1
Urban	25	30.5	31	34.4
Semi-urban	14	17.1	4	4.4
Unknown	0		7	
P-value				0.049
Marital status				
Married	58	70.7	68	70.1
Cohabiting	1	1.2	1	1.0
Separated, divorced	3	3.7	7	7.2
Widowed	20	24.4	21	21.6
P-value				0.94
School attendance				
Yes	14	17.1	20	20.6
No	68	82.9	77	79.4
P-value				0.54
No. of facilities in the house[a]				
>5	14	17.1	18	18.6
3–4	29	35.4	15	15.5
1–2	31	37.8	28	28.9
None	8	9.8	36	37.1
P-value				0.004

[a] Ownership within the family of any of the following: running water, toilet, TV, refrigerator, telephone, video, stereo, motorbike and car.

HPV

The results on HPV DNA for cases and controls in exfoliated cells and for cases in biopsies are shown in Table 2. Among cases, 65 out of 82 tested β-globin positive and of these, 96.9% were HPV positive. Among 91 controls, 79 tested negative for β-globin (86.8%) and 4 of the 12 β-globin positive were HPV positive. Table 2 summarizes the HPV types detected. HPV 16 was the commonest type detected followed by HPV 18. Low-risk HPV types were not detected in any of the biopsies studied. Seven case-women were positive for multiple high-risk types. Among the four controls HPV positive, one was positive for HPV 18 and the other three were positive for an unknown HPV type.

Human papillomavirus VLP at the cut-off point of 0.2 for any of the three types tested were present in almost all women (95%). The prevalence and OR of HPV VLP using 0.4 as a cut-off point are described in Table 2. Cases were slightly more likely to be HPV VLP positive than controls (OR = 1.8). The HPV VLP 16 was also more common among cases than controls (OR = 1.7) but no statistical difference was observed between case

Table 2 Human papillomavirus (HPV) DNA and HPV 16, 18 and 31 virus like particles (VLP) among invasive cervical cancer cases and controls in Mali

	Cases		Controls		
HPV infection	No.	(%)	No.	(%)	**OR (95% CI)**
HPV DNA					
Negative	2	(3.1)	8	(66.7)	1
Positive	63	(96.9)	4	(33.3)	63 (9.9–400.6)
HPV 16	31	(50.0)		(0)	–
HPV 18	8	(12.7)	1	(25.0)	32 (2.4–427.7)
HPV 33	1	(1.6)	0	(0)	–
HPV 35	1	(1.6)	0	(0)	–
HPV 45	7	(11.1)	0	(0)	–
HPV 51	4	(6.3)	0	(0)	–
HPV 58	2	(3.2)	0	(0)	–
HPV 73	2	(3.2)	0	(0)	–
HPV X	0	(0)	3	(75.0)	–
HPV multiple types	7	(11.1)	0	(0)	–
β-globine negative	2		79		
No result	15		6		
Positive for VLP antibodies to:[a]					
HPV 16	47	(49.0)	35	(36.1)	1.7 (1.0–3.0)
HPV 18	7	(7.3)	7	(7.2)	1.0 (0.3–3.0)
HPV 31	24	(25.0)	20	(20.6)	1.3 (0.6–2.5)
Any HPV	58	(60.4)	44	(45.4)	1.8 (1.0–3.3)

[a] Cut-off point of optical density of 0.4 nm.

and control seropositivity to VLP 18 or VLP 31. Seroresponse to HPV 31 was frequently observed but no HPV 31 DNA had been detected in the cervical biopsies.

Reproductive and sexual characteristics

Mean age at first marriage (15 years for cases; 16 years for controls) and age at first sexual intercourse (15 years for both cases and controls) were almost the same for both cases and controls. The mean age of the first partner was 30 years for both cases and controls. This represents an age difference between couples of 16 years for the cases and 15 for the controls.

Monogamy was more common among controls (48.5%) than cases (37.8%) and a quarter of both cases and controls reported having had a casual partner (Table 3). Cases and controls reported the practice of anal intercourse to a similar extent (16%).

All women had at least one pregnancy except for one control, and a substantial proportion of women had more than five pregnancies (85.4% of the cases and 62.5% of the controls). Risk of cervical cancer was strongly related to parity. The risk increased with increasing number of pregnancies (*P* for linear trend 0.004). Women with more than 10 children had a nearly five-fold risk (OR = 4.77, 95% CI : 1.54–14.7) of cervical cancer compared to women with one to five children. Intercourse after pregnancy was generally delayed for at least one month. Women resuming sexual relations 6 months or more after giving birth were at an increased risk for cervical cancer (OR = 11.24, 95% CI : 1.7–74.9), although estimates are based on few observations. Only three women declared ever having been engaged in prostitution (two cases and one control).

About 41% of the control women reported a monogamous husband versus 26% of the cases. Polygamy increased by two-fold the risk of cervical cancer but the estimate was at the limit of statistical significance. Risk increased with increasing number of wives within the family. Other characteristics related to

Table 3 Risk of invasive cervical cancer by reproductive and sexual patterns in Mali

	Cases		Controls				
	N	%	N	%	OR[a]	OR[b]	95% CI
Age at first marriage (years)							
>18	12	14.6	17	17.5	1.00	1.00	
16–17	13	15.9	23	23.7	0.87	0.58	(0.17–2.07)
15	37	45.1	24	24.7	2.58	2.94	(0.80–10.75)
<15	20	24.4	33	34.0	0.97	0.76	(0.16–3.65)
Test for trend							0.30
Age at first sexual intercourse (years)							
>15	19	23.2	25	25.8	1.00	1.00	
15	34	41.5	31	32.0	1.53	1.50	(0.59–3.81)
14	15	18.3	24	24.7	0.87	0.73	(0.25–2.07)
<14	14	17.1	17	17.5	1.13	0.83	(0.30–2.34)
Test for trend							0.39
No. of regular sexual partners							
1	34	41.5	56	57.7	1.00	1.00	
>1	48	58.5	41	42.3	1.98	1.50	(0.76–2.96)
No. of casual partners							
None	60	73.2	73	76.0	1.00	1.00	
1	11	13.4	12	12.5	1.12	0.71	(0.26–1.89)
2–3	7	8.5	10	10.4	0.85	1.20	(0.38–3.80)
>3	4	4.9	1	1.0	5.02	3.57	(0.34–36.91)
Unknown	0		1				
Test for trend							0.51
Lifetime sexual partners							
1	31	37.8	47	48.5	1.00	1.00	
2–3	39	47.6	44	45.4	1.37	1.16	(0.57–2.37)
>3	12	14.6	6	6.2	3.02	2.39	(0.73–7.80)
Test for trend							0.19
Anal intercourse							
Never	68	84.0	79	84	1.00	1.00	
Ever	13	16.0	15	16.0	1.01	1.52	(0.55–4.22)
Unknown	1		3				
Intercourse during menstruation							
Ever	15	18.3	18	18.9	1.00	1.00	
Never	67	81.7	77	81.1	1.07	1.01	(0.41–2.52)
Unknown	0		2				

Table 3 Continued

	Cases		Controls				
	N	%	N	%	OR[a]	OR[b]	95% CI
No. of pregnancies							
0–5	12	14.6	37	38.1	1.00	1.00	
6–8	26	31.7	24	24.7	3.37	2.80	(1.07–7.33)
9–10	27	32.9	22	22.7	3.83	3.30	(1.23–8.88)
>10	17	20.7	14	14.4	3.90	4.77	(1.54–14.74)
Test for trend							0.004
Intercourse during pregnancy							
Never/rare	19	23.5	24	25.8	1.00	1.00	
Regularly	62	76.5	69	74.2	1.12	1.26	(0.56–2.82)
Unknown	1		4				
Intercourse after pregnancy							
Immediately	3	3.7	13	14.1	1.00	1.00	
1–3 months	59	73.7	56	60.9	4.85	4.08	(0.97–17.13)
4–6 months	6	7.5	19	20.7	1.52	1.31	(0.24–7.15)
>6 months	12	15.0	4	4.3	13.95	11.24	(1.69–74.86)
Unknown	2		5				
Test for trend							0.19
Polygamous husband							
No	21	25.9	40	41.2	1	1.00	
Yes	60	74.1	57	58.8	2.27	2.17	(1.0–5.0)
Unknown	1		0				
No. of wives in the women's house							
1	25	41.7	35	61.4	1	1.00	
2	18	30.0	15	26.3	1.76	2.2	(0.8–6.5)
>2	17	28.3	7	12.3	3.41	5.3	(1.3–21.3)
Unknown	22		40				
Test for trend				0.02			
Female circumcision							
No	4	4.9	7	7.2	1	1.00	
Yes	78	95.1	90	92.8	1.60	1.29	(0.3–5.8)
Type of circumcision							
Clitoris ablation	29	37.2	86	95.6	1	1.00	
Cut prepuce clitoris	11	14.1	3	3.3	14.83	60.9	(4.14–896.0)
Unknown	38	48.7	1	1.1	119.22	NA	
No circumcision	4		7				

[a] Adjusted for age.

[b] Adjusted for age, area of birth, genital douche, age at first sexual intercourse, HPV serostatus.

polygamy were generally not answered, such as knowledge of a specific type of sexually transmitted infection in the other wives.

Female circumcision was a common practice for both cases and controls (95.1% of the cases and 92.8% of the controls). When type of circumcision was explored, about half of the cases but only 1% of the controls could not specify the type of circumcision. Women who were submitted to cutting of the prepuce of the clitoris were at a 15-fold (OR = 14.8, 95% CI : 4.14–896) increased risk of cervical cancer as compared to women that reported ablation of the entire clitoris. The age range of circumcision was 1–16 years and the median age was 6 years old.

Hygiene patterns

Several genital hygiene patterns showed an association with cervical cancer (Table 4). The estimates of the OR were adjusted in this Table for parity and availability of inside toilet at home. Women were asked whether they forced water or liquid soap into the vagina when they washed their genital area. Most cases reported never carrying out such procedures (96% for cases versus 70% of the controls) resulting in a 17-fold increased risk for cervical cancer in those who never practised vaginal douching. Women were also asked whether they took special care in cleaning their genitals when washing themselves. Lack of particular care in cleaning the genitals increased 5.6 times the risk of cervical cancer.

Table 4 Risk of invasive cervical cancer by hygienic patterns in Mali

	Cases		Controls				
	N	%	N	%	OR[a]	OR[b]	95% CI
Vaginal douche							
Ever	3	3.7	29	29.9	1.00	1.00	
Never	78	96.3	68	70.1	11.96	17.6	(4.2–74.7)
Unknown	1	0					
Special care in washing genitals							
Always	37	46.2	80	82.5	1.00	1.00	
Not always	43	53.7	17	17.5	5.63	5.64	(2.5–12.8)
Unknown	2		0				
Use of home-made sanitary napkins							
Never	0		13				
Unknown	12		4				
Ever: Years of use	70		80				
<20 years	12	17.1	23	29.1	1.00	1.00	
20–25 years	17	24.3	21	26.6	1.79	1.57	(0.78–7.66)
26–34 years	17	24.3	18	22.8	3.04	5.19	(1.31–20.38)
>34	24	34.3	17	21.5	8.70	8.05	(1.68–38.64)
Unknown	0		14				
Test for trend							0.002
Sanitary napkins' condition							
Always clean	36	48.0	91	97.8	1.00	1.00	
Re-used	39	52.0	2	2.2	50.68	45.93	(8.84–238.68)
Unknown	7		4				

[a] Adjusted for age.

[b] Adjusted for age, availability of toilet inside the house, parity and HPV serostatus.

Commercial sanitary napkins or tampons were almost non-existent in this population. A strong association was found for women who had used sanitary napkins for more than 25 years versus those who had used them for less than 20 years. Reporting re-using sanitary napkins was almost only restricted to cases and the resulting OR was one of the strongest found in the analysis (OR = 46). No association was found between HPV VLP and hygiene practices (data not shown).

Discussion

This is the first case-control study on HPV and cervical cancer carried out in Mali and one of the few in sub-Saharan Africa. Although Mali is resource-poor, the country has one the few cancer registries in Africa which has been of invaluable help in cancer control activities.[15] Nearly all cases (96.9%) harboured HPV DNA sequences and about 60% of them had a serological response to VLP of HPV types 16, 18 and 31. The prevalence of HPV serum antibodies confirms that Malian women are widely exposed to HPV infections throughout their lives. Differences in the presence of antibodies or antibody titres between cases and controls was only observed when the cut-off point of the optical density was set at 0.4 nm. The serological response to capside proteins is generally present while HPV infection is persistent in the cervical cells for a certain period or during the development of high-grade lesions.[16] The high HPV VLP prevalence observed in Malian control women contrasts with the comparable seroprevalence levels observed in other non-African populations. For example, De Gruijl *et al.*[17] in Holland showed that HPV VLP were present at the cut-off point of 0.2 in 17% of women with a normal Pap smear, while in Mali at the same cut-off point, HPV VLP were detected in more than 90% of the control women.

Human papillomavirus DNA could not be detected in the exfoliated cells of the control population because the majority of samples did not pass the DNA quality control test of amplification of the β globine gene. The most likely interpretation was that disruption in the cold chain might have occurred. Studies undertaken in such resource-poor settings may benefit from using transport mediums that are less vulnerable to changes in temperature.[5,17] Serological response to HPV was used in this study as the best surrogate to control for the presence of HPV infection when estimating the potential effect of factors other than HPV in the development of cervical cancer. Assessment of HPV exposure by serological assays is, however, less accurate than HPV DNA detection by PCR assays and consequently our investigation of co-factors is hampered by lack of a proper control of the strong effect of HPV.

Parity

In this population, high parity was a strong risk factor for cervical cancer. Our data confirm previous work[18,19] that high-parity women are at high risk for cervical cancer. The effect of parity in cervical cancer development seems to be mainly detectable in countries with high fertility rates and is rarely found in low-parity countries. Taking into account that almost all women in Mali have been exposed to HPV infections, the

strong association with parity observed in this study may reflect an additional effect to the one produced by the HPV infection. Parity is probably a good marker of the oestrogen-hormonal environment throughout the fertile years of a women as well as a marker of repeated cervical trauma among highly parous women. It is not known whether hormones intervene in cervical carcinogenesis but oestradiol has been reported to induce immortalization of HPV infected cells.[20]

Poor genital hygiene

The results of this study indicate that poor hygiene is linked to an increased risk of cervical cancer. It is unclear whether genital hygiene patterns contribute by facilitating HPV infection and its persistence or rather that they are an indicator of other sexual characteristics that induce HPV acquisition or progression. In the study presented, poor genital hygiene conditions refer to conditions rarely seen in affluent societies. Malian women commonly report repeated use of menstrual pads and describe the used pads as 'not always clean'. A majority of the women do not have access to chlorinated water, or tap water and their personal hygiene is carried out under restricted conditions. These results are in agreement with those in China and Morocco. Poor hygiene may be one of the explanations of the observation that low socioeconomic status is consistently found as a risk factor for cervical cancer.[21]

Husband's polygamy

The sexual behaviour of women's sexual partners has been shown to influence the risk of cervical cancer. Increased number of sexual partners or common use of prostitutes by the husband affects the probability of HPV transmission to the female partner and thus increases the risk of subsequent cervical cancer. This effect has been particularly described in populations in which female monogamy was common.[22] In Mali, as in many other African countries, polygamy is common particularly among Muslims. Our data show that polygamy was more common among women with cervical cancer and that cases were more likely to share a household with more than two wives. We had almost no data directly provided by the husbands (only 18 answered the questionnaire) and thus we could not control for the sexual behaviour of the husband. The adverse effect of polygamy in this population may well reflect higher promiscuity of the husbands. In this community age at first marriage is close to age at first sexual intercourse for women. The approximately 15-year gap between age at first marriage between women and men may increase the likelihood of HPV infection at the time of first intercourse due to the husband's previous sexual experiences. This cultural trait has also been observed in other African countries[8] and may be a key determinant of the HPV infection among young women.

Female circumcision

Genital mutilation is a practice widely used in many African countries. Circumcision in Mali was reported by 93% of the women, which represents one of the highest prevalence rates in the world. In some other African countries female circumcision is now outlawed and the practice of circumcision is becoming less prevalent.[23] Circumcision is generally performed early in life, under conditions that commonly lead to local infection. There was a considerable proportion of case-women that did not specify the type of circumcision. Complete female mutilation was protective as compared to partial circumcision. Women who had partial circumcision were more likely to have more sexual partners and to start earlier sexual intercourse.

In conclusion, 96.7% of cervical cancer cases harboured oncogenic HPV types. Detection of anti-HPV VLP shows that the background prevalence of HPV infections in Mali is high. Within a population widely infected with HPV, poor social conditions, high parity and poor hygienic conditions were the main cofactors for cervical cancer. These results suggest that preventable actions for cervical cancer in Mali need to focus on health education: preventing childbearing lesions, delaying sexual relations after a traumatic childbirth and promoting hygienic measures including use of clean menstrual pads.

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KEY MESSAGES

- Human papillomavirus (HPV) DNA is present in almost all invasive cervical cancer cases in Mali.
- A large proportion of Malian women have been in contact with HPV infections resulting in a detectable serological response against HPV capside proteins.
- In Mali, risk factors for invasive cervical cancer were high parity (>10 children), having a husband with more than two wives and poor genital hygiene conditions.

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