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Human Papillomavirus Infection and Risk Determinants for Squamous Intraepithelial Lesion and Cervical Cancer in Japan

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A case control design was used to investigate human papillomavirus (HPV) prevalence and risk factors associated with development of cervical squamous intraepithelial lesion (SIL) and cervical cancer (CC) in Japan. One hundred and twenty-three women with histologically confirmed SIL or CC were compared to a control group of 778 cytologically normal women. With the use of a polymerase chain reaction (PCR)-based method for detection of low-risk (types 6 and 11) and high-risk (types 16, 18, 31, 33, 35, 52 and 58) HPVs, a high prevalence of HPV infection was observed in smokers among the controls. Logistic regression analysis demonstrated that high-risk HPV infection was the most significant risk determinant for LSIL (OR=9.4, 95% CI=4.5–19), HSIL (OR=77, 95% CI=28–217) and CC (OR=97, 95% CI=35–269). It also showed that unmarried women, women married for 5 to 19 years and smokers represented high risk groups for SIL, while smokers and women with a history of many pregnancies/parities had increased risk for CC. Smoking was the only HPV infection-independent factor for CC, suggesting that smoking may have a carcinogenic effect on the cervix. Since neither history of other cancer nor family cancer history was associated with SIL or CC, genetic factors appear to play little role in cervical carcinogenesis. The risk for cervical neoplasia due to HPV infection increased after marriage in Japan, suggesting a role for husbands as carriers of HPV transmission. Protection from high-risk HPV infection may be of greatest importance for prevention of cervical cancer.

Key words: HPV — Risk factors — SIL — Cervical cancer — Japanese women

Human papillomaviruses (HPVs) form a group of small DNA viruses which cause ano-genital cancers. More than 35 distinct types are thought to infect the genital mucosal epithelium and 20 or more types of HPV are cancer-associated.[1)] Sensitive and reliable polymerase chain reaction (PCR) methods for detection of HPV DNA in cervical swab samples have made feasible large-scale epidemiological studies of the role of HPV in cervical neoplasia. Many such studies have demonstrated a strong association of HPV infection with cervical neoplasia.[2)] Infection with certain HPVs such as HPV 16 and HPV 18 has been shown to put patients at high risk for cervical cancer and its precursor lesions.[1–3)]

Cervical neoplasia has been considered a sexually transmitted disease. This conclusion has been supported by epidemiologic observations that a women's life-time number of sexual partners, her age at commencement of sexual activity and the numbers of sexual partners of her male partners are directly related to her risk of cervical cancer. Recent studies using PCR-based methods more clearly revealed the characteristics of sexually transmitted HPV infection.[4–8)] However, not all PCR-based studies in various populations have uniformly reproduced such results. While some studies show a strong association between HPV infection and sexual activity,[9–11)] others have found only a weak association between them[12)] or no association at all.[13)] Another study found a strong association between HPV infection and sexual activity only for infection with high-risk and not with low-risk types of HPV.[14)]

HPV is classified into low-, intermediate- and high-risk types, and only HPV types belonging to the latter two groups are strongly associated with development of high-grade cervical neoplastic lesions.[1,3)] Many recent studies have also shown that not only HPVb16 or 18, but also other high- and intermediate-risk types as well may be associated with cervical cancer[15)] or its precursor lesions.[16,17)] Thus, the use of a method capable of detecting a variety of HPV types is important in assessing the causative role of HPV in cervical neoplasia.

Although HPV is the most important etiological factor in the development of cervical cancer, infection with HPV is not sufficient for the final step in malignant progression. It has been found that not all human cell lines immortalized with HPV are tumorigenic and that latent periods of many years precede the establishment of cervical cancer. Other exposure or host factors may be involved in the final step of malignant transformation.

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Some studies have suggested that pregnancy[18–20] and oral contraceptive use[5] increase the risk of HPV infection, while others have suggested that smoking increases the risk of squamous intraepithelial lesion (SIL)[9, 21] or cervical cancer.[22, 23]

A large number of epidemiological studies in Europe and America have elucidated the role of HPV infection and other risk factors in the pathogenesis of cervical cancer. However, there has been no large-scale epidemiological study of HPV infection in Japan. Since Japan is geographically distant from western countries and has different culture and customs, the risk determinants for cervical cancer in Japanese women might differ from those in western countries. This is the first report of a large-scale epidemiological study of HPV infection and cervical neoplasia performed in Japan.

MATERIALS AND METHODS

Study population In order to conduct a population-based cohort study, a random sample of 2560 eligible women (16–82 years of age) was drawn from the general female population of the Ishikawa and Toyama municipal regions. All these women were enrolled between April, 1995 and August, 1996 in the Department of Obstetrics and Gynecology, School of Medicine, Kanazawa University or Toyama Prefectural Central Hospital. They were interviewed individually by trained interviewers. Patient evaluations included a pelvic examination with a Pap smear and colposcopic examination with colposcopically-directed cervical biopsy in women showing an abnormal Pap smear.

Cases All the cases, which were histologically confirmed, were selected from cytologically abnormal women in the cohort. This case group of 123 women included 40 women with LSIL (low-grade squamous intraepithelial lesion), 52 with HSIL (high-grade squamous intraepithelial lesion), and 31 with CC (cervical carcinoma, including 26 cases of squamous cell carcinoma and five cases of adenocarcinoma).

Controls From the remaining eligible women in the cohort who were cytologically normal at enrollment, we randomly chose a sample of 778 women from the same population that generated the cases, matched by age (within 5 years with the cases). A control subject was defined as a woman with no past or current evidence of cervical diseases on Pap smear. Women with vulvar or vaginal condyloma or other sexually transmitted diseases were excluded from the control group.

Sample collection The cervical scraped cells were collected with a cytobrush at the transformation zone of the uterine cervix of all subjects. The samples were divided in two, one part for Pap test and the other for detection of HPV DNA. The latter samples were suspended in phosphate-buffered saline and stored at −30°C until DNA purification. All procedures were carried out by experienced gynecologists. The final clinical diagnoses of the women with abnormal cytology were performed by histopathological evaluation of biopsy samples obtained under colposcopic guidance. HPV detection and pathological diagnosis were performed independently of each other.

Cytological and histological evaluations Smears were screened by two cytotechnologists. All possibly abnormal smears and all histologic slides were reviewed independently by two surgical pathologists. Final diagnoses were arrived at by agreement of both pathologists.

The diagnoses were then divided into negative, LSILs, HSILs and invasive cervical carcinoma using the WHO classification[24] and the Bethesda system.[25]

HPV detection and typing using PCR The cervical cells were suspended in 50 m*M* Tris-HCl (pH 8.0) with 10 m*M* EDTA containing 200 mg/ml of proteinase K and incubated at 37°C overnight for cell lysis. DNA was extracted from this lysis solution by the phenol-chloroform method. All utensils used were disposable and were discarded immediately after use (single use for each specimen). Standard methods to avoid and monitor for contamination were used throughout the laboratory analysis.

Primers for a fragment of β-globin gene served as an internal control to assess the sufficiency of template DNA in each specimen in the PCR. Three E6 and E7 consensus primers, pU1M, pU31BM and pU2R, were used for PCR detection of HPV DNA.[26] pU1M and pU2R were originally designed for detecting HPV types 16, 18, 31, 33, 35, 52 and 58, and pU31BM and pU2R for detecting HPV 6 and 11. Approximately 50 ng of sample DNA was added to 50 μl of PCR solution. The PCR procedure was modified slightly from the original one in our laboratory, and was performed as follows: one cycle at 94°C for 5 min, the next 5 cycles at 94°C for 1.0 min, at 45°C for 1.0 min, and 72°C for 1 min, the next 5 cycles at 94°C for 1 min, 48°C for 1 min, 72°C for 1 min, then 20 additional cycles at 94°C for 1 min, at 55°C for 1 min and at 72°C for 1 min, and a final cycle at 72°C for 5 min. Amplified DNA samples were run on 3% 3 Nusieve 1 agarose gel or 12% acrylamide gel in Tris-borate/EDTA buffer, and DNA bands stained with ethidium bromide were viewed by UV monitor camera (Epi-Light UV FA1100, AIC, Japan). HPV typing was performed by restriction fragment length polymorphism analysis of PCR products with *Rsa I, Ava II, Sau 3AI* and *Acc I*, for typing HPV 16, 18, 31, 33, 35, 52, and 58, and with *Rsa I* for typing HPV 6 and 11. The sensitivity of this test was about 0.1 copy per cell of HPV genome. Several samples in which the type of HPV was not determined by the PCR were further analyzed by the Southern blot method with HPV 58 probe under low-stringent conditions.[17] On the basis of the results of recent studies,[12–14] HPV16, 18, 31, 33,

35, 45, 52, 58 and 59 were combined into a high-risk group in the present study. HPV types which could not be identified with the PCR-based and Southern blot methods were included in the undetermined group.

Statistical analyses Using SAS program software, we computed odds ratios as estimates of relative risk with 95% confidence intervals (CI) by unconditional logistic regression.[27,28] Age in years (less than 24, 25–34, 35–44, 45–54, more than 55) was always included in the model. We calculated odds ratios for each factor with adjustment for age, or age together with HPV status (HPV DNA (+), HPV (−)). This procedure provided evidence of whether an association was mediated primarily by HPV infection or by other epidemiological factors.

RESULTS

Prevalent HPV types in Japanese women Before evaluation of risk for cervical neoplasia, we determined the prevalence of HPV infection. The results were summarized in Table I. All subjects were tested for HPV DNA by PCR as described in "Materials and Methods." More than 0.1 copy of viral DNA of low-risk (HPV 6 and 11) or high-risk HPV (HPV 16, 18, 31, 33, 35, 52, and 58) was detectable in the present assay system. Subsequent Southern blot analysis revealed that the types undetermined by PCR alone included HPV types 45 and 59. However, ten cases and six controls were still undetermined after analyses with both PCR and Southern blot. HPV 18, 35, 45, 59 were not detected in the controls, whereas all high-risk types were identified in HSIL and all but HPV 35 in cervical cancer. HPV 16 was the most common type in HSIL and cervical cancer, while HPV 31 and 58 were the most common in the controls and LSIL, respectively.

Of low-risk HPV types, HPV 6 was positive in 3 controls (3/778, 0.4%) and HPV 11 was not detected in any woman tested, while no low-risk types of HPV were identified in LSIL, HSIL or cervical cancer. High-risk HPV DNAs were positive in 24 of 40 (60%) in the LSILs, 48 of 52 (92%) in the HSILs and 26 of 31 (84%) in cervical cancers, while 35 of 778 (4.5%) controls were positive for HPV DNA.

High HPV prevalence groups in Japanese women We determined which groups of women had the highest risk for HPV infection. No difference in HPV prevalence was observed between different age groups of cytologically normal or of abnormal women (Table II). Smoking women in the control group (OR=2.7, 95% CI: 1.1–6.9) had significantly high relative risk for HPV infection. However, no other factors were significantly associated with the risk of HPV infection. Among the normal controls, women married at younger than 19 years of age (OR=2.2, 95% CI: 0.86–5.5) and women with more than three pregnancies (OR=2.5, 95% CI: 0.72–8.6) or with more than three births (OR=2.6, 95% CI: 0.88–7.9) showed high relative risk for HPV infection, but the effects did not reach statistical significance. Also in the group of SILs or CC, women aged 25–34 (OR=3.3, 95% CI: 0.54–19), unmarried women (OR=4.7, 95% CI: 0.5–43) and women married at younger than 19 years of age (OR=7.3, 95% CI: 0.86–67) showed high relative risk, but not significantly so. Different from previous studies, women who were younger at first sexual intercourse did not have increased prevalence of HPV infection.

HPV infection as a risk determinant for cervical neoplastic diseases For age-adjusted analysis, a logistic regression model was used to estimate the importance of HPV infection as a risk factor for low- and high-grade SILs and cervical cancer (Table III). A total of 778 eligible cytologically normal controls, 40 LSIL, 52 HSIL and 31 cervical cancers were included in this analysis.

The relative risks of each HPV group for LSIL, HSIL and cervical cancer were determined. Low-risk HPV types could not be analyzed due to their very low rate of detection. The high-risk HPVs increased the relative risk of LSIL, HSIL and cervical cancer 9.4-, 77- and 97-fold over the control level, respectively, while the undetermined HPV types were associated with 17-, 121- and 46-fold higher relative risk for LSIL, HSIL and cervical

Table I. Prevalence of HPV Infection in Japanese Women

			HPV negative	HPV types											
				6	11	16	18	31	33	35	45	52	58	59	ND
Controls	$n^{a)}=778$	(4.5%)[b)]	743	3	0	8	0	11	1	0	0	2	4	0	6
Cases	$n=123$	(80%)	25	0	0	25	6	14	2	4	2	14	19	2	10
Low-grade SIL[c)]	$n=40$	(60%)	16	0	0	3	1	5	0	1	0	4	8	0	5
High-grade SIL	$n=52$	(92%)	4	0	0	13	2	6	1	3	1	6	9	1	8
Cervical cancer	$n=31$	(84%)	5	0	0	9	3	3	1	0	1	4	2	1	2

a) Number of samples.
b) Prevalence rates.
c) Squamous intraepithelial lesion.

Table II. HPV Prevalence in Japanese Women According to Several Characteristics

	Normal				SIL and CC			
	HPV (+)	HPV (−)	Odd[a]	95% CI	HPV (+)	HPV (−)	Odd	95% CI
Age								
≤24	5	97	1	(reference)	8	3	1	(reference)
25–34	10	250	0.78	(0.26–2.3)	26	3	3.3	(0.54–19)
35–44	6	135	0.86	(0.26–2.9)	21	5	1.5	(0.3–8.2)
45–54	8	143	1.1	(0.34–3.4)	18	8	0.84	(0.18–4.0)
55≤	6	118	0.99	(0.29–3.3)	25	6	1.6	(0.32–7.7)
Age at first intercourse[b]								
≥24 or never	2	62	1	(reference)	8	4	1	(reference)
≤19	3	28	3.3	(0.53–21)	12	2	3	(0.44–20)
20–23	3	73	1.3	(0.21–7.9)	13	6	1.1	(0.23–5.1)
Age at marriage								
≥26	8	173	1	(reference)	18	6	1	(reference)
unmarried	2	69	0.63	(0.13–3.0)	14	1	4.7	(0.5–43)
≤19	12	120	2.2	(0.86–5.5)	22	1	7.3	(0.81–67)
20–25	13	381	0.74	(0.3–1.8)	44	17	0.86	(0.29–2.5)
Married years								
≤4	10	210	1	(reference)	11	3	1	(reference)
5–9 yr	4	94	0.88	(0.27–2.9)	14	3	1.2	(0.2–7.6)
10–19 yr	1	101	0.2	(0.03–1.6)	24	5	1.3	(0.26–6.4)
≥20	17	269	1.3	(0.57–2.8)	42	13	0.88	(0.21–3.6)
Smoking								
non-smoker	29	691	1	(reference)	82	22	1	(reference)
smoker	6	52	2.7	(1.1–6.9)	16	3	1.4	(0.38–5.4)
Pregnancy								
never	3	124	1	(reference)	8	3	1	(reference)
1–2 times	14	321	1.8	(0.51–6.4)	32	11	1.1	(0.25–4.9)
≥3 times	18	298	2.5	(0.72–8.6)	58	11	2	(0.45–8.6)
Parity								
never	5	220	1	(reference)	18	4	1	(reference)
1–2 times	20	356	2.5	(0.91–6.7)	46	17	0.6	(0.182–2.0)
≥3 times	10	167	2.6	(0.88–7.9)	34	4	1.9	(0.42–8.5)
History of other cancer								
never	35	692	1		94	25	1	
ex or current	0	51	NA[c]		4	0	NA	
Family cancer history								
no	25	594	1	(reference)	79	20	1	(reference)
yes	10	149	1.6	(0.74–3.4)	19	5	0.96	(0.32–2.9)

a) Crude odds ratio.
b) Some samples were missed because of loss of information.
c) Not applicable.

Table III. Relative Risk of HPV Infection for Squamous Intraepithelial Lesion (SIL) and Cervical Cancer

HPV type	Control		LSIL				HSIL				Cervical cancer			
	n[a]	(%)[b]	*n*	(%)	Odd[c]	95% CI	*n*	(%)	Odd	95% CI	*n*	(%)	Odd	95% CI
Negative	743	(96)	16	(40)	1	(reference)	4	(8)	1	(reference)	5	(16)	1	(reference)
Low risk	3	(0.4)	0	(0)	NA[d]		0	(0)	NA		0	(0)	NA	
High risk	26	(3.3)	22	(55)	9.4	(4.5–19)	42	(81)	77	(28–217)	24	(77)	97	(35–269)
Undetermined	6	(1)	2	(5)	17	(4.9–61)	6	(12)	121	(30–501)	2	(6)	46	(7.1–294)

a) Number of cases.
b) Incidence.
c) Odds ratio adjusted for age.
d) Not applicable.

Table IV. Relative Risk for Squamous Intraepithelial Lesions (SILs) According to Potential Risk Factors

	Cases	Controls	Odd 1[a)]	(95% CI)	Odd 2[b)]	(95% CI)
Age at first intercourse[c)]						
≥28 or never	10	56	1	(reference)	1	(reference)
≤19	18	62	1.3	(0.29–6.3)	0.48	(0.076–3.0)
20–23	34	162	0.52	(0.14–1.9)	0.34	(0.073–1.6)
24–27	14	98	0.52	(0.11–2.4)	0.43	(0.06–2.99)
Age at marriage						
≥26	19	182	1	(reference)	1	(reference)
unmarried	12	69	3.5	(1.2–10)	2.5	(0.55–11)
≤19	14	132	0.94	(0.41–2.2)	0.43	(0.13–1.3)
20–25	47	395	0.91	(0.51–1.6)	1.1	(0.51–2.3)
Married years						
≤5	9	232	1	(reference)	1	(reference)
5–9 yr	16	113	3.2	(1.2–8.6)	2.8	(0.77–10)
10–19 yr	25	115	5.4	(1.6–18)	2.9	(0.45–19)
≥20	36	318	0.4	(0.03–5.0)	0.19	(0.005–6.5)
Smoking						
non-smoker	85	724	1	(reference)	1	(reference)
smoker	7	54	2.5	(1.1–5.7)	1.6	(0.63–4.0)
Pregnancy						
never	14	128	1	(reference)	1	(reference)
1–2 times	37	340	0.65	(0.33–1.3)	0.65	(0.26–1.6)
≥3 times	41	310	0.56	(0.27–1.2)	0.44	(0.16–1.3)
Parity						
never	24	230	1	(reference)	1	(reference)
1–2 times	50	377	0.72	(0.4–1.39)	0.6	(0.25–1.2)
≥3 times	18	171	0.78	(0.36–1.7)	0.91	(0.28–2.9)
History of other cancer						
never	90	729	1	(reference)	1	(reference)
ex or current	2	49	1.4	(0.4–5.1)	2.1	(0.58–7.7)
Family cancer history						
no	74	622	1	(reference)	1	(reference)
yes	18	156	1	(0.58–1.8)	0.88	(0.41–1.86)

a) Odds ratios adjusted for age.
b) Odds ratios adjusted for age and HPV status.
c) Some samples were missed because of loss of information.

cancer. These findings suggest that infection with both high-risk (HPV 16, 18, 31, 33, 35, 45, 52, 58 and 59) and undetermined types significantly increased the risk for cervical cancer and its precursor lesions (SILs).

Other risk determinants for cervical neoplasia We studied risk factors for cervical neoplasia (SIL and cervical cancer) other than HPV using multivariate logistic regression analysis.

In an age-adjusted analysis for SIL (Odd 1, Table IV), unmarried women (OR=3.5, 95% CI: 1.2–10), women married for 5 to 9 years (OR=3.2, 95%CI: 1.2–8.6), women married for 10 to 19 years (OR=5.4, 95% CI: 1.6–18) and smokers (OR=2.5, 95% CI: 1.1–5.7) had significantly high relative risk (95% CI excluded 1.0 in these groups). However, none of these determinants was significant after adjustment for age together with HPV infection status. This indicates that these determinants were dependent on HPV infection. The age at first sexual intercourse, number of pregnancies and births, history of other cancer and family cancer history did not significantly affect the risk for SILs.

We next examined risk factors for cervical cancer (Table V). Unmarried women (OR=6.1, 95% CI: 1.2–31), women married for 10–19 years (OR=6.6, 95% CI: 1.1–44), smokers (OR=8.6, 95% CI: 2.8–27), women with more than three pregnancies (OR=4.6, 95% CI: 1.1–18), and multiparous women (more than three births) (OR=3.7, 95% CI: 1.1–13) had significantly high relative risks for cervical cancer. All these determinants except smoking were dependent on HPV infection, since they were not significant after adjustment for both age and status of HPV infection. Since the significance of

Table V. Relative Risk for Cervical Cancer According to Potential Risk Factors

	Cases	Controls	Odd 1[a]	(95% CI)	Odd 2[b]	(95% CI)
Age at first intercourse[c]						
≥28 or never	2	56	1	(reference)	1	(reference)
≤20	10	62	1.5	(0.2–10)	4.6	(0.16–136)
20–23	4	162	0.56	(0.04–7.4)	0.69	(0.02–27)
24–27	0	98	NA[d]		NA	
Age at marriage						
≥26	4	182	1	(reference)	1	(reference)
unmarried	2	69	6.1	(1.2–31)	5.6	(0.4–75)
≤19	9	132	2.1	(0.55–8.0)	1.1	(0.25–4.7)
20–25	16	395	1.6	(0.51–4.9)	1.4	(0.47–4.4)
Married years						
≤4	2	232	1	(reference)	1	(reference)
5–9	1	113	3.7	(0.41–33)	1.9	(0.11–30)
10–19	5	115	6.6	(1.1–44)	2.9	(0.38–22)
≥20	22	318	1	(0.05–24)		
Smoking						
non-smoker	23	724	1	(reference)	1	(reference)
smoker	8	54	8.6	(2.8–27)	5.8	(1.8–19)
Pregnancy						
never	2	128	1	(reference)	1	(reference)
1–2 times	4	340	0.41	(0.07–2.5)	0.1	(0.012–1.4)
≥3 times	25	310	4.6	(1.1–18)	2.2	(0.41–12)
Parity						
never	4	230	1	(reference)	1	(reference)
1–2 times	10	377	0.82	(0.22–3.1)	0.5	(0.13–1.9)
≥3 times	17	171	3.7	(1.1–13)	2.5	(0.49–13)
History of other cancer						
never	30	729	1	(reference)	1	(reference)
ex or current	1	49	1.6	(0.33–6.7)	3.5	(0.56–22)
Family cancer history						
no	26	622	1	(reference)	1	(reference)
yes	5	156	0.8	(0.32–2.0)	0.8	(0.29–2.4)

a) Odds ratios adjusted for age.
b) Odds ratios adjusted for age and HPV status.
c) Some samples were missed because of loss of information.
d) Not applicable.

smoking was not affected by this adjustment, it was the only independent risk factor for cervical cancer.

In conclusion, unmarried status, marriage for 10–19 years, and smoking were risk determinants for both SILs and cervical cancer, and multigravidity or multiparity increased the risk for cervical cancer. All these determinants except smoking appeared to be HPV-dependent. Although HPV infection is the most important risk factor for cervical neoplasia among the factors analyzed in the present study, smoking is another independent risk factor.

DISCUSSION

In the present study, HPV DNA was detected in 60% of LSILs, 92% of HSILs and 84% of cervical cancers, but only 4.5% of cytologically normal women were positive for HPV DNA. In a worldwide study using a PCR-based method for detection of the L1 consensus sequence of 20 HPV types, 75–100% of cervical cancers were positive for HPV and 5–20% of specimens from the general population were positive.[15] Using our PCR-based method with E6-E7 consensus primers, HPV 16, 18, 31, 33, 35, 52 and 58 were confidently typed, but other HPV types could not be identified. Some of the undetermined types were found to be HPV 45 and 59, or unknown types on subsequent Southern blot analysis. Our method is able to detect most common HPV types belonging to the intermediate- or high-risk categories.[1,3] The positive rates of HPV DNA in HSILs (92%) and cervical cancers (84%) in the present study also showed that our method

had the same sensitivity as others using the L1 consensus primers.[5, 9]

Geographical differences in prevalence of HPV types have been reported to exist between countries and even within the United States.[10, 11, 29] In the present study, which was carried out in the central-northern area of Japan, we found that HPV 16 was the most common type and that HPV types 31, 52 and 58 were also prevalent in SILs and cervical cancer. On the other hand, HPV 18 was relatively rare in this study, compared with a previous study.[15] Similar patterns of distribution of HPV prevalence were also found in other areas of Japan,[17, 30] suggesting that geographical variation in HPV types is very small within Japan.

The incidence of HPV infection has been reported to be relatively high in sexually active younger women and to decline with increasing age.[5, 31, 32] Such age-related differences in HPV prevalence were not apparent in the present study (Table II). The prevalence of HPV infection in cytologically normal women aged 16 to 24 years was 5.3% (5/95) in the present study, whereas more than 44% of the same age group was positive for HPV DNA in Europe.[4, 5] Many young Japanese probably use barrier contraceptives rather than oral contraceptives, since the latter type is not commercially available in Japan. This may account in part for the relatively low prevalence of HPV infection in the younger generation in Japan.

In our study, smoking increased the prevalence of HPV infection in cytologically normal women, whereas no other factors were associated with the risk of HPV infection. Many previous reports have indicated that lifetime number of sexual partners and number of recent sexual partners, which appear to represent degree of sexual activity, are strongly correlated with HPV infection.[9, 10] Unfortunately, we lacked such information for our subjects, due to difficulty in obtaining correct information concerning such personal matters in Japan. In the present study, younger age at first sexual intercourse, which is also a risk determinant for HPV infection in western countries,[5, 14] appeared not to be a risk factor in Japanese women. Women who smoke were at higher risk for HPV infection in Japan. This may suggest that women who smoke are likely to be more sexually active in Japan. However, we can not rule out the possibility that smoking promotes HPV infection, since smoking may impair the immunoresponse of T lymphocytes.[33]

When we estimated the risk factors for SIL and cervical cancer with logistic regression analysis, unmarried status, marriage for 10–19 years, and smoking increased the risk for both SIL and cervical cancer, whereas multiple pregnancies or parities increased the risk for cervical cancer alone.

In general, unmarried women were thought to be at lower risk of cervical cancer compared with endometrial cancer. However, in the present study unmarried women were at high risk for cervical cancer or its precursor lesions. The reason for this result is unknown, but the women with SIL or CC in this study were not virgins and they appeared to have higher relative risk for HPV prevalence (OR=4.7) than women married at 21–25 years of age (OR=0.86) or at older than 26 (reference), although the difference was not statistically significant (95% CI=0.5–43) (Table II). These findings suggest that unmarried women may be sexually more active than women married at older than 21 years of age. This possibility is also compatible with the result that high relative risk for SIL or CC in unmarried women was dependent on HPV infection (Tables IV and V). Women married for 5–19 years also had high risk for SIL and those married for 10–19 years had high risk for cervical cancer. Since married women are unlikely to have many sexual partners other than their husband in Japan, they may have acquired HPV infection from their husband.[6–8] A recent study also revealed a causal link between male sexual behavior and risk of cervical cancer of wives.[34] This study showed that the number of extramarital sexual partners or prostitutes a man had was associated with risk for cervical cancer in his wife.

The present study also suggested a causative role of smoking in cervical cancer, as suggested in previous studies.[9, 21–23] Smoking was associated with cervical cancer, independently of HPV infection, in our study. The role cigarette smoking plays in the pathogenesis of cervical cancer has not yet been established. The nitroso compounds contained in cigarette smoke have been shown to have a mutagenic effect on uterine mucus.[35] HPV-immortalized cells are transformed to malignant cells when treated with nitrosomethylurea.[36] We have also shown that administration of chemical agents is required for malignant progression in the cervix in an animal model system.[37] The chemical reagents included in cigarette smoke thus appear to be involved in cervical cancer development.

In other studies, number of pregnancies was an independent risk factor for cervical cancer.[38] In the present study too, multigravid/multiparous status was associated with cervical cancer, although these factors dependent on HPV infection. This increased risk for cervical cancer associated with HPV infection suggests that pregnancy and HPV infection may exert synergistic effects in the development of cervical cancer. In contrast, history of other cancer and family cancer history were not associated with increased risk for either SIL or cervical cancer. This suggests that genetic background has little effect on cervical carcinogenesis.

In the present study we found that certain HPV types play a significant etiological role in the development of cervical cancer and its precursor lesion (SIL). Infection

with HPV 16, 31, 33, 35, 45, 52, 58 and 59 is the most important risk factor for cervical cancer in Japan. Unmarried women and those married for 10 to 19 years appear to be at high risk for SIL, dependent on HPV infection. Smoking was the only independent risk determinant for cervical cancer.

HPV testing using PCR-based methods may be expected to be useful as a new diagnostic tool supplementing the Pap test in prediction of risk for cervical cancer. The present finding that 35 of 778 (4.5%) cytologically normal women were positive for high-risk HPV DNA is consistent with this expectation, although the significance of such silent infection with these HPV types remains to be determined. The PCR-based method we used in this study is simple, quick, and relatively inexpensive, and appears to be suitable for routine examinations as well as large-scale epidemiological studies.

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