Original
article

Seropositivity against HPV 16 capsids: a better marker of past sexual behaviour than presence of HPV DNA

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Objectives: To assess if seropositivity to human papillomavirus type 16 capsids is a better marker of sexual history than the presence of HPV DNA.

Study design: A population based age stratified random sample of 234 Norwegian women (mean age 32.8 years, range 20-44) was examined for HPV 16 serum antibodies, cervical HPV DNA, cytology and age in relation to sexual behaviour.

Results: Neither age nor age at first sexual intercourse was associated with HPV 16 antibodies. Adjusted ORs for 4-5, 6-10 and > 10 versus 0-1 lifetime sexual partners, were 13.1 (95% CI 1.5-110.8), 8.2 (1.0-69.6) and 10.5 (1.2-94.0) for HPV 16 seropositivity, respectively; and 2.6 (0.2-27.8), 3.4 (0.4-31.7) and 4.1 (0.4-42.8) for HPV 16 DNA positivity, respectively.

Conclusion: Seropositivity to HPV 16 capsids is positively associated with the number of sexual partners, suggesting that HPV 16 is predominantly sexually transmitted. The fact that serology had a stronger association with number of sexual partners than viral DNA suggests that seroreactivity is a better measure of lifetime history of HPV infection.

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Keywords: human papilloma virus; DNA; capsids; sexual behaviour

Introduction

Human papilloma virus (HPV) is considered to be the major causal factor for dysplasia and cancer of the uterine cervix.¹⁻⁸ HPV is sexually transmissible. However, the quantitative importance of non-sexual transmission is unclear. DNA of the genital types of HPV has been reported to be present in the oral mucosa of children and corresponding serum antibodies to be common in children.^{9,10} Several early studies also failed to find a clear association between sexual activity and detection of genital HPV infection.¹¹⁻¹⁴ These studies did not use reliable PCR techniques for the detection of HPV DNA, and the lack of association may have been due to misclassification of HPV status. More recent studies, using validated PCR, have shown a strong association with the number of sexual partners.¹⁵⁻¹⁸

The need to evaluate the importance of sexual activity for the transmission of oncogenic HPV infections is obvious. To obtain results which are generally applicable, population based studies in women with age corresponding to the ages where high grade dysplasia appears is important. The choice of marker of HPV 16 infection is crucial in relation to whether present infection or lifetime exposure is the question under study. Despite very sensitive techniques for the detection of HPV DNA, a single test for detecting HPV DNA in the cervix may be a false negative owing to sampling errors. Another diagnostic problem is that HPV infections seem to be transient in the majority of cases.^{19,20} Thus no information on past infections is obtained if HPV DNA is no longer present in the tissue. Obtaining reli-

able data on sexual behaviour is also difficult. A potentially useful way of estimating lifetime exposure to HPV is to study serum antibody responses.

The aim of our study was to examine the association between sexual behaviour and presence of antibodies against HPV 16 capsids and to compare that with the estimated association between sexual behaviour and detection of HPV DNA in cytological specimens from the uterine cervix, in a representative sample of the female population in Oslo, Norway.

Materials and methods

STUDY POPULATION

In 1991-2 a population based case-control study was conducted in Oslo, Norway to estimate the association between cervical intra-epithelial neoplasia (CIN) II-III and HPV as detected by PCR.⁶ Women of Norwegian descent, aged 20 to 44 years, and presently living in Oslo were invited. Exclusion criteria were pregnancy, menopause, previous treatment for dysplasia or a history of other gynaecological malignancies. The control group was randomly selected from the Central Population Register and group matched for age to a case series.⁶ Of the traceable women who fulfilled the inclusion criteria, 71.8% were willing to participate. All participants had a cervical smear and a serum sample taken at study entry. Only women with both cytological and serological diagnoses were included in this study.

The 234 women, aged 20-44 years (mean 32.8 years, median 32), included women with

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normal cytology ($n = 208$), women with HPV signs in cytology ($n = 20$) and women in whom CIN II-III/cancer was diagnosed at study entry ($n = 6$).

SPECIMEN COLLECTION, CYTOLOGICAL EXAMINATION AND DETECTION OF HPV DNA

Serum samples and cervical specimens were obtained according to a standardised protocol at the same visit as the interview took place. Endocervical brushes were used to prepare a smear and were then placed in phosphate buffered saline (PBS) until preparation in the laboratory. Smears were stained by the Papanicolaou method and examined by a trained cytopathologist not informed about laboratory findings or interview data (TS). β Globin positive cytological specimens were analysed with polymerase chain reaction (PCR) using general nested primer pairs detecting HPV types 6, 11, 16, 18, 31 and 33 as well as a number of unknown HPV types.²¹ The preparation of cytological specimens and the typing of the nested positive samples have been described elsewhere.²² The pellets for PCR and serum were stored at -70°C .

HPV SEROLOGY/ELISA

Enzyme linked immunosorbent assay (ELISA) methodology, as described elsewhere,²³ was used to detect antibodies against HPV 16 capsids in serum samples at a 1:30 dilution. The capsids were obtained from Dr John T Schiller, National Cancer Institute, Bethesda, MD, USA and their preparation and characterisation is described in detail elsewhere.²⁴ Antibody levels exceeding a pre-assigned cut off level of 0.100 dOD (difference in optical density) were considered as a positive result.

INTERVIEW DATA/INDEPENDENT VARIABLES

Data on sexual and reproductive history were obtained through a structured interview carried out by a female physician (AOO). The interview took place at the hospital in connection with the physical examination and the sample collection. An exposure by time matrix was constructed for the sexual history. Data on the *number of sexual partners* were obtained in two ways, first by asking the simple question about total number of sexual partners, then by asking for number of partners within 5 year intervals visualised in the exposure by time matrix containing data about major steady relationships and reproductive events. If the index person reported fewer than 10 sexual partners, it was reported with an exact number. If more, it was reported as "about 10 to 15", "about 15 to 20" and "more than 20".

STATISTICAL ANALYSES

The "outlying" very high antibody levels were considered as biologically valid and were not excluded. Because of skewed, non-normal distribution of data, a non-parametric test (Kruskal-Wallis) was applied when the serological data were treated as continuous variables. To describe the distribution of observations, percentiles (50, 75, 90) were used.

For bivariate and multivariate analyses, the dependent variable (serum antibody response) was made dichotomous by using a pre-assigned cut off at 0.100 dOD and an alternative cut off at 0.300 dOD. For bivariate analyses, χ^2 tests were applied. Stratified analyses were performed employing EPI INFO, Version 6.²⁵ The multivariate logistic regression analyses were performed employing SPSS for Windows, release 6.0,²⁶ and the strength of associations were expressed as odds ratios (OR).

The multivariate analyses were restricted to subjects with complete data on both serology and HPV DNA ($n = 222$).

ETHICS

The study was approved by the regional committee for medical research ethics. Informed, written consent was obtained from all participants.

Results

Treated as a continuous variable, seroreactivity against HPV 16 capsids was marginally higher in the youngest women (20–24 years) and lower in ages 25–29 years, compared with women 30 years or older (Kruskal-Wallis, $p = 0.05$). The seroreactivity was not associated with age at first sexual intercourse (table 1). Women with normal cervical cytology tended to have lower levels of antibodies than women with HPV morphology or cervical dysplasia (Kruskal-Wallis, $p = 0.06$). Subjects with fewer than four partners had lower levels of antibodies (Kruskal-Wallis, $p < 0.01$) (table 1). Treated as a dichotomous variable, 39 women (16.7%) had serum immune response above the pre-assigned cut off level at 0.100 dOD.

The proportion of women with antibody response above 0.100 was significantly lower in the category with normal cytology than in the categories of cytological features of HPV or CIN (χ^2 6.8, $p = 0.03$). Age at first sexual intercourse did not influence the seropositivity in the pooled sample or in the age groups; 20–29, 30–34 and 35–44, separately (data not shown). Comparing women who reported four or more lifetime sexual partners with women with 0–3 partners, the ORs for seropositivity in the three age strata were 5.9 (0.7–129), 5.1 (0.6–113) and 3.6 (0.7–26), respectively. The crude OR for all strata was 4.7, whereas the Mantel-Haenszel weighted OR was 4.6 (1.5–15.9). Hence, interaction or confounding from age on the association between the number of sexual partners and seropositivity against HPV 16 was not observed. In the pooled sample, a dose-response relation between the number of sexual partners and antibody response above 0.100 was found ($\chi^2 = 6.8$, 1DF, p for trend = 0.009).

Age at first cohabitation, a measure of the start of a more regular sexual life, was not related to the lifetime number of sexual partners and was not further analysed in the models.

Three of 17 women (17.6%) with HPV type

Table 1 Median, 75 and 90 percentiles for HPV 16 capsid antibodies and distribution of positives (cut off level of 100 dOD × 1000) according to cytological diagnosis, age, age at first sexual intercourse, number of sexual partners and presence of HPV DNA in uterine cervix in a population based sample of women

	N	HPV 16 capsid antibodies (dOD × 1000)			Kruskal-Wallis p value*	HPV 16 capsid seropositivity		
		Median	75	90		Pos	%	χ ² p value
ALL	234	0.0	5.3	184.0		39	16.7	
Cytological diagnosis					0.06			0.03
Normal	208	0.0	0.0	153.7		30	14.4	
HPV	20	0.0	155.8	396.1		7	35.0	
CIN/cancer	6	1.0	509.5			2	33.3	
Age (years)					0.05			0.13
20–24	15	0.0	124.0	412.0		5	33.3	
25–29	69	0.0	0.0	79.0		6	8.7	
30–34	71	0.0	25.0	271.2		15	21.1	
35–39	50	0.0	10.5	181.8		8	16.0	
40–44	29	0.0	17.0	264.0		5	17.2	
Age first intercourse					0.99			0.96
<17	78	0.0	14.0	163.4		13	16.7	
17–18	76	0.0	1.5	176.3		12	15.8	
19+	80	0.0	3.0	249.2		14	17.5	
No of sexual partners					<0.01			<0.01†
0–1‡	32	0.0	0.0	0.0		1	3.1	
2–3	40	0.0	0.0	71.2		3	7.5	
4–5	45	0.0	110.5	268.4		12	26.7	
6–10	69	0.0	10.5	173.0		11	15.9	
>10	48	0.0	111.0	408.0		12	25.0	
HPV DNA§					0.99			0.96
Negative	184	0.0	7.8	178.5		30	16.3	
16	17	0.0	24.5	467.0		3	17.6	
Others	21	0.0	38.5	160.8		3	14.3	

*Kruskal-Wallis test (corrected for ties) for the continuous variable.
†Mantel-Haenszel test for linear association.
‡Two women with zero number of sexual partners.
§N = 222. "Other" includes HPV type 6/11, 18, 31, 33, and unknown types.

16 DNA had a serum antibody level above 0.100 dOD. Of the 205 subjects who were either HPV DNA negative or positive for other HPV types, 33 (16.1%) were seropositive.

In multivariate logistic regression analysis, age, age at first sexual intercourse and number of sexual partners were included into the same model (table 2). With the age group 30–34 as reference, no significant differences in serum immune response between the age groups were found (table 2, model 1). Women with early sexual debut showed no increased risk for being seropositive. The adjusted OR for seropositivity against HPV 16 (> 0.100 dOD) was 13.1 (95% CI 1.5–110.8) for subjects reporting 4–5 lifetime sexual partners, 8.2 (95% CI 1.0–69.6) for those reporting 6–10 partners and 10.5 (95% CI 1.2–94.0) for subjects reporting more than 10 partners as compared with those with 0–1 partners (table 2, model 1). When HPV 16 DNA was studied in

one group and the subjects who were positive for other types were categorised together with the HPV negative subjects, neither an association with age nor with sexual history was found (table 2, model 2). For HPV DNA, all types combined (table 2, model 3), a marginally significant association with younger age (20–24 and 25–29) was found. An association with HPV DNA (any types) was seen when the reported number of sexual partners exceeded five but was only significant for more than 10 partners; OR 7.8 (95% CI 1.5–41.7). A major contribution came from the few women positive for HPV type 6/11 and 18, of whom three of four and two of three reported 10 or more partners, respectively.

Discussion

We have found that antibodies against HPV 16 capsids is a strong marker for past sexual

Table 2 Adjusted odds ratio for the association between age, age at first sexual intercourse, number of sexual partners and HPV as measured by antibodies to HPV 16 capsids or by HPV DNA in uterine cervix as detected by PCR, in 222 women

	Model 1				Model 2				Model 3			
	HPV 16 capsid seropositivity*				Presence of HPV 16 DNA				Presence of HPV DNA (any types)			
	Pos	Neg	adjOR†	(95% CI)	Pos	Neg‡	adjOR†	(95% CI)	Pos	Neg	adjOR†	(95% CI)
Age (years)												
20–24	5	8	3.2	(0.8–12.6)	2	11	3.2	(0.5–20.3)	4	9	4.5	(1.0–19.3)
25–29	5	59	0.4	(0.1–1.1)	7	57	1.8	(0.5–6.3)	15	49	2.2	(0.9–5.6)
30–34	15	53	1.0		5	63	1.0		10	58	1.0	
35–39	6	42	0.5	(0.2–1.4)	3	45	0.7	(0.2–3.4)	5	43	0.6	(0.2–2.0)
40–44	5	24	0.8	(0.3–2.8)	0	29	0.0		4	25	0.9	(0.2–3.3)
Age at first intercourse												
<17	11	63	0.6	(0.2–1.5)	5	69	0.4	(0.1–1.5)	14	60	0.4	(0.2–1.1)
17–18	11	59	0.7	(0.3–1.9)	5	65	0.5	(0.1–1.8)	8	62	0.3	(0.1–0.9)
19+	14	64	1.0		7	71	1.0		16	62	1.0	
No of sexual partners												
0–1	1	29	1.0		1	29	1.0		2	28	1.0	
2–3	3	37	2.9	(0.3–30.8)	3	37	2.3	(0.2–23.6)	6	34	2.7	(0.5–15.2)
4–5	12	29	13.1	(1.5–110.8)	3	38	2.6	(0.2–27.8)	6	35	3.2	(0.6–18.1)
6–10	11	55	8.2	(1.0–69.6)	6	60	3.4	(0.4–31.7)	12	54	4.5	(0.9–22.8)
>10	9	36	10.5	(1.2–94.0)	4	41	4.1	(0.4–42.8)	12	33	7.8	(1.5–41.7)

*Seropositivity defined as dOD > 0.100.
†Adjusted for age, age at first sexual intercourse and number of sexual partners.
‡Includes HPV DNA negatives and positives for any other types than 16.

activity (number of sexual partners), indicating a primarily sexual route of HPV transmission. This is in line with the results from a Swedish study of female adolescent students. Andersson-Ellstrom *et al* did not find HPV DNA in cervix²⁷ or serum antibodies against HPV 16 capsids²⁸ among virgins. In another study of 130 vaginal samples from Swedish virginal women, two were HPV 6 DNA positive whereas no HPV 16 DNA was found.²⁹ Also an Australian study found no HPV DNA positives in analyses of tampon specimens from virgins.³⁰ However, there are also contradictory findings reported; no significant difference was found in the prevalence of vulvar HPV DNA in virginal and sexually active Chinese women.³¹

In the large clinic based study of randomly selected cytologically normal women, a dose-response relation between number of sexual partners and HPV prevalence was reported. The adjusted prevalence was 16.9% higher in women reporting 10 or more partners compared with women reporting one life-time sexual partner.¹⁵ We found no uniformly increasing trend, but with more than three partners the seroprevalence reached a plateau. An association of seroprevalence with number of partners, that levelled at a high number of partners was also found in another study, although a younger population was studied and the plateau was reached at a higher number of partners (6–10).³² The plateau phenomenon was also observed in a study based on HPV DNA detection.³³ In this a threshold effect between monogamous and non-monogamous women was found, probably because the likelihood of exposure to HPV with each sexual partner was increased in this high prevalence population.³³ The phenomenon may also reflect a “saturation”, that is, a point where most women have been exposed to HPV is reached. We found a trend towards an increasing prevalence of HPV DNA with increasing number of lifetime sexual partners. A significant association was observed only for women reporting more than 10 partners. Possibly, past HPV infections may have been eliminated by the time of sample collection and women with many partners may carry HPV DNA because of more recent (re)infections.

In the studies reporting a strong association between HPV DNA and number of partners,^{16–18} the mean age has been markedly lower, pointing to a more recent sexual history with HPV DNA still present. Only one of these cross sectional studies has been carried out in a group that is representative for the general population of young females.¹⁸

Selection bias is a minor problem in our study because sampling was based on personal identification numbers in the comprehensive and complete Norwegian population register. Furthermore, there were relatively few (98 of 348) who did not respond or who refused to participate, and these women did not differ from the attendees with respect to age or marital status (data not shown). None of the attendees refused to answer questions or to give biological specimens. To obtain reliable data

on sexual history may be difficult and may to some extent explain discrepant findings in the literature. We emphasised a confident setting, the consultation including a personal interview by a female practitioner lasting about 45 to 60 minutes. However, the possible bias from interviewer effect cannot be assessed since all interviews were made by one interviewer. Efforts were made to avoid recall bias by obtaining sexual and reproductive history chronologically and in parallel. Since an exposure by time matrix was subsequently filled in, the respondent also had the opportunity to revise answers. All analyses for the detection of HPV DNA and HPV 16 antibodies were performed with the analysing laboratory blinded to the identity of the samples.

A variety of HPV serological assays that are associated with clinical disease and cancer exist.^{34 35} However, serology based on HPV capsids is the assay that shows best agreement with detection of HPV DNA in the uterine cervix of women with asymptomatic HPV 16 infections or cervical intraepithelial neoplasia (CIN).³⁴ Among 54 HPV 16 DNA positive women, 59% had IgG to HPV 16 capsids, whereas only two of 31 HPV DNA negative women showed seropositivity.²⁴ A high specificity is also indicated by the fact that no virgins were seropositive and by seroconversions following the detection of HPV DNA.²⁸ Cross reactivity to other HPV types appears to be limited.^{36 37}

The poor agreement between serology and PCR in this study³⁸ has also been reported by Wideroff *et al*, who found 17% of HPV 16 DNA negative subjects to be seropositive.³⁷ Among women who were found to be HPV 16 DNA positive on only one occasion, either at enrolment or at follow up, 22% were seropositive which corresponds to the 17.6% reported in the present study. It is probable that serology and PCR have different sensitivities for measuring past versus present exposure and transient versus persistent infection to HPV. This assumption is strengthened by the finding of increased seropositivity, to 83%, for subjects who tested HPV 16 DNA positive at two separate occasions.³⁷ Our cross sectional study design with one single cervical sample cannot assess the nature and course of HPV infection. In longitudinal studies it has been shown that seroconversions may occur relatively concomitantly with detection of new HPV DNA.^{28 36} On the other hand, it seems that not all HPV infected individuals will develop antibodies. No seroconversion was observed in one of six girls who acquired cervical HPV 16 DNA during follow up.²⁸ In another study, 22 of 54 HPV 16 DNA positives were HPV 16 L1 ELISA negative.²⁴

In two case-control studies, both reporting no significant relation between the number of sexual partners and serum antibodies to HPV, the serological assays used measured antibody response associated with neoplasia, but not with infection.^{39 40} However, seropositivity against HPV 16 and/or HPV 33 capsids is indeed associated with infection and is also associated with the number of sexual partners

and with sexual intercourse before the age of 17.²⁸

The strong association of seropositivity with the number of sexual partners observed in this study suggests that seropositivity reflecting the past history of exposure to HPV is common. Transient HPV antibody response, that is, loss of seropositivity after clearance of the viral DNA has, however, been described.^{28 36}

In conclusion, the association between serum antibody response to HPV 16 and lifetime number of sexual partners is evidence for a principally sexually transmitted route of HPV 16. Serum immune responses to HPV capsids appears to be a better marker for lifetime HPV exposure than HPV DNA measurements.

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