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Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma: nested case-control study

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Abstract

***Objective*—To study human papillomavirus type 16 in the aetiology of cervical carcinoma.**

***Design*—Within a cohort of 18 814 Finnish women followed for up to 23 years a nested case-control study was conducted based on serological diagnosis of past infection with human papillomavirus type 16.**

***Subjects*—72 women (27 with invasive carcinoma and 45 with in situ carcinoma) and 143 matched controls were identified during the follow up.**

***Main outcome measure*— Relative risk of cervical carcinoma in presence of IgG antibodies to human papillomavirus type 16.**

***Results*—After adjustment for smoking and for antibodies to various other agents of sexually transmitted disease, such as herpes simplex virus type 2 and *Chlamydia trachomatis*, the only significant association was with infection with human papillomavirus type 16 (odds ratio 12·5; 95% confidence interval 2·7 to 57, 2P < 0·001).**

***Conclusion*—This prospective study provides epidemiological evidence that infection with human papillomavirus type 16 confers an excess risk for subsequent development of cervical carcinoma.**

Introduction

Infection with human papillomavirus type 16 (HPV16) is the major factor that has been linked to cervical neoplasia.[1,2] But no prospective studies of infection with human papillomavirus and cervical carcinoma have yet been reported. We have previously reported the risks of cervical carcinoma associated with various other sexually transmitted diseases in a cohort of 18 814 Finnish women followed for 12 years.[3] Only *Chlamydia trachomatis* infection was associated with an increased risk. A recently developed serological assay provides a type restricted measure of infection with human papillomavirus type 16.[4] This and extension of the maximum follow up time to 23 years enabled us (*a*) to determine whether infection is a particularly strong risk factor for subsequent development of cervical carcinoma and (*b*) to determine whether any risk associated with other sexually transmitted infections is independent of the risk associated with exposure to this papillomavirus.

Subjects and methods

Cases and controls were identified as follows. The Finnish Social Insurance Institution carried out a mobile health examination survey among 30 different population groups in various parts of Finland during 1966-72. More than 30 000 women (aged 15 years or more) were invited to a health examination, which included asking about medical history and smoking habits and taking a blood sample. The serum samples of 18 814 women were stored at −20°C.

The population based Finnish cancer registry receives reports of cancer cases from hospitals, pathology laboratories, and physicians throughout Finland. Fewer than 200 cases each of carcinoma in situ (excluding cervical intraepithelial neoplasia) and invasive cervical carcinoma are reported annually. All women who had given blood in the mobile health examination survey during 1968-72 and were free of cancer at the baseline were followed. Those who had cervical carcinoma diagnosed after the baseline examination were identified by linking the data files of the mobile health examination survey and the Finnish cancer registry. Until 1991, 72 cases of cervical carcinoma (27 invasive cervical carcinoma and 45 carcinoma in situ) were diagnosed. Altogether 143 women individually matched for sex, age, and municipality were identified to act as controls. Age was matched by using nearest available matching: in 61 sets the age was exactly matched, in nine sets at least one of the controls differed by one to two years and in two sets by three to four years. Mean (range) age at the baseline was 39·1 (15-83) years and at the diagnosis 49·2 (22-95) years. Mean (range) time (follow up time) between withdrawal of serum and diagnosis of cervical carcinoma was 10·1 (0·7-22·8) years.

IgG antibody analyses were performed by standard enzyme linked immunosorbent assay (ELISA). For human papillomavirus type 16 analysis baculovirus expressed capsids purified by ultracentrifugation and comprising both the L1 and L2 proteins were used with bovine papillomavirus capsids as controls.[4-6] We used *C trachomatis* elementary body to detect chlamydia infection, antigen to lysate from cells infected with herpes simplex virus type 1 for herpes simplex virus, and glycoprotein gG-2 from herpes simplex virus type 2 for herpes simplex virus type 2.[3,7] The same standardised reference serum samples, antihuman IgG enzyme conjugates, and cut off levels were used as in previous studies.[3-7] The specificity of the chlamydia antibodies for *C trachomatis* was confirmed by identifying ELISA positive cases who were microimmunofluorescence positive (> 1:32) for the *C trachomatis* serovars B,E,D/C,J,H,I/G,F,K/ (Washington Research Foundation, Seattle)[8] and for the solely genital *C trachomatis* serovar G,F,K,[9] respectively.

Table 1—*Odds ratios (95% confidence interval) of cervical carcinoma according to presence of IgG antibodies to different sexually transmitted diseases*

Micro-organism	No (%) positive		Odds ratio (95% confidence interval)		
	Cases (n=72)	Controls (n=143)	Unadjusted	Adjusted for smoking	Adjusted for smoking and sexually transmitted disease
Human papillomavirus type 16	17 (24)	3 (2)	13·2 (3·0 to 59)	13·0 (2·9 to 58)	12·5 (2·7 to 57)
Herpes simplex virus*	67 (93)	130 (91)	1·5 (0·5 to 5·2)	1·5 (0·4 to 5·8)	Not done
Herpes simplex virus type 2	11 (15)	37 (26)	0·5 (0·2 to 1·0)	0·5 (0·2 to 1·2)	0·6 (0·2 to 1·4)
Chlamydia†	31 (43)	44 (31)	1·8 (1·0 to 3·5)	1·8 (0·9 to 3·4)	Not done
C trachomatis‡	25 (35)	30 (21)	2·1 (1·1 to 4·0)	1·8 (0·9 to 3·6)	Not done
C trachomatis§	7 (10)	6 (4)	3·4 (1·0 to 11·1)	3·3 (0·9 to 12·1)	3·0 (0·7 to 13·4)

*Antibodies common to herpes simplex virus types 1 and 2.
†Antibodies common to genus *Chlamydia*.
‡Antibodies common to all *C trachomatis* serovars.
§Antibodies specific for genital *C trachomatis* serovars G, F, K.

Relative risks of cervical carcinoma (odds ratios, 95% confidence intervals, and two tailed P values) were estimated by conditional logistic regression for matched sets of case-control triplets (one case, two controls)[10] or by the exact inference methods for contingency tables[11] with EGRET and EPIXACT software (Statistics and Epidemiology Research Corporation, Seattle).

Results

In univariate analyses we found that type restricted antibodies to the papillomavirus and *C trachomatis* were both associated with an increased risk of cervical carcinoma (odds ratio 13·2, P<0·001; 2·1, P<0·05, respectively), but only for papillomavirus was the association highly significant. The risk associated with the presence of antibodies specific for the *Chlamydia* genus was modest and only just conventionally significant (1·8, 2P=0·05), and although the risk associated with the presence of antibodies to the genital *C trachomatis* serovars G,F,K was higher, it too was only just conventionally significant (3·4, P=0·05). Adjustment for smoking had only minor effects on the estimates, but it made all but the papillomavirus association cease to be conventionally significant (each P>0·05; table 1). The papillomavirus association was seen both for patients with a short time from sampling to diagnosis (n=21) and for those with a long time (n=51) (odds ratio for <5 years 8·6, 95% confidence interval 1·0 to 75; for >5 years 18, 2·3 to 142) as well as for patients with invasive cervical carcinoma (n=27) and for those with carcinoma in situ (n=45) (∞, 2·0 to ∞; 6·0, 1·2 to 29·7). Finally, we used a multivariate model to evaluate the relative risk associated with papillomavirus and herpes simplex virus type 2 infections and infections with genital *C trachomatis* serovars G,F,K (table 1). Again only the papillomavirus association remained significant (12·5; P<0·001).

Key messages

- Sexual risk taking behaviour is a risk factor for cervical cancer
- Human papillomavirus type 16 is the main micro-organism linked to the development of cervical cancer
- Prospective studies of infection with this virus and cervical cancer have not been reported because of ethical and clinical difficulties and because diagnosis of past infections with the virus has not been possible
- In this nested case-control study in over 18 000 Finnish women who donated blood to a serum bank 25 years ago we were able to measure past infection with human papillomavirus type 16 with new serological tools
- The results show that infection with the virus confers an increased risk of developing cervical cancer

Discussion

Between 65% and 95% of cervical carcinomas contain human papillomavirus DNA, and about half carry type 16.[12] Also about half of patients with cervical carcinoma are positive for antibodies to papillomavirus type 16.[5,13] In our study nearly a quarter of the women who subsequently developed cervical carcinoma had detectable antibodies to papillomavirus type 16 at the baseline. Possibly additional women may have become infected[14] during the average lag of 10 years (and up to 23 years) between withdrawal of serum and diagnosis of cervical carcinoma.

In line with most cross sectional seroepidemiological studies on cervical carcinoma[12] the multivariate analyses found papillomavirus type 16 to be a far stronger risk factor than any other. Although the analysis for the genital *C trachomatis* serovars G,F,K may have suffered from small numbers, our results suggest that the increased risk associated with other sexually transmitted disease agents may be secondary to papillomavirus type 16, reflecting high risk sexual behaviour.

Presence of antibodies to papillomavirus type 16 increased the risk for subsequent development of both carcinoma in situ and invasive cervical carcinoma. Furthermore, the highest risk was found for cervical carcinoma diagnosed more than five years after serum withdrawal, which is the expected finding if persistent infection with papillomavirus type 16 is causally involved in the aetiology of this disease.

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Continuing transmission of sexually transmitted diseases among patients infected with HIV-1 attending genitourinary medicine clinics in England and Wales

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Abstract

***Objective*—To determine whether those who are aware of being infected with HIV continue to adopt behaviours that place others at risk of HIV infection.**

***Design*—Ongoing survey of current diagnosis of sexually transmitted disease and awareness of HIV infection among patients attending genitourinary medicine clinics.**

***Setting*—Six genitourinary medicine clinics in England and Wales (two in London and four outside) participating in unlinked anonymous HIV serosurveillance during 1990-3.**

***Subjects*—All attenders having blood drawn for syphilis serology for the first time during the calendar quarter of attendance.**

***Main outcome measures*—The proportion of syphilis serology specimens with antibody to HIV-1 detected by unlinked anonymous testing of the residue. The proportion of attenders infected with HIV-1 who remained clinically undetected, and the proportion who had another recently acquired sexually transmitted disease.**

***Results*—Of 85 441 specimens tested, 2328 (2·7%) were positive for antibodies to HIV-1. About 30% of these specimens were from attenders whose HIV-1 infection remained clinically undetected. HIV-1 infection was found to coexist with another recently acquired sexually transmitted disease in 651 attenders, of whom 522 were homosexual or bisexual men. Of these, 245 (47%) already knew themselves to be infected with HIV-1. This proportion increased between 1990 and 1993.**

***Conclusions*—A considerable proportion of patients infected with HIV-1 are not identified by voluntary confidential HIV testing in genitourinary medicine clinics. Substantial numbers of homosexual or bisexual men attending genitourinary medicine clinics continue to practise unsafe sex despite being aware of their infection with HIV-1.**

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Introduction

As the HIV epidemic evolves, important questions concerning current levels of infection, optimal strategies for detecting people infected with HIV in contact with health services, and the effectiveness of prevention programmes remain unanswered.[1] The occurrence of new episodes of other sexually transmitted diseases among people infected with HIV must be a strong indicator for continuing risk of HIV transmission in the sexually active population. If transmission of these other sexually transmitted diseases is also continuing among those who are aware of their HIV infection then this raises questions about the effectiveness of counselling after testing and the potential benefit of policies designed to encourage HIV testing.

The unlinked anonymous survey of HIV seroprevalence among attenders of genitourinary medicine clinics in England and Wales provided an opportunity to look at these questions because it collects data on current diagnoses of sexually transmitted diseases and patients' awareness of their HIV status, as well as the result of unlinked anonymous HIV tests.

Patients and methods

Six genitourinary medicine clinics and associated laboratories were selected by using the criteria of large case load and willingness to participate for a minimum of five years. Approval for the survey was obtained from the Public Health Laboratory Service and local ethics committees. The principles of the method of unlinked anonymous testing,[2] which minimises the participation bias inherent in voluntary confidential HIV case finding,[3-5] were followed. The detailed methods applied in the clinics and that for laboratory tests are described elsewhere.[6]

All attenders, including those known to be positive for antibodies to HIV-1, having blood drawn for syphilis serology for the first time during the calendar quarter of attendance were eligible for inclusion in the survey. If patients declared that they had been diagnosed or were known to have been diagnosed as positive for HIV-1 before the current episode of attendance this was recorded on the survey form.

A limited dataset was collected for each eligible attender and was matched at the coordinating centre with the result of the test for HIV-1 on the unlinked anonymous residue of the syphilis serology sample for that attender. The analyses presented in this paper are based on a dataset that contained the following items: diagnosis of sexually transmitted disease for each attender (coded as on the statistical return (KC60) made by genitourinary medicine clinics to the Department of Health), calendar quarter of clinic attendance, age group, exposure category, previous awareness of being infected with HIV, and unlinked anonymous test result. Data indicating the clinic attended were not included within the database to