Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study

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Abstract

Objectives To investigate the role of human papillomavirus (HPV) in the development of cervical neoplasia in women with no previous cervical cytological abnormalities; whether the presence of virus DNA predicts development of squamous intraepithelial lesion; and whether the risk of incident squamous intraepithelial lesions differs with repeated detection of the same HPV type versus repeated detection of different types.

Design Population based prospective cohort study.

Setting General population in Copenhagen, Denmark.

Participants 10 758 women aged 20-29 years followed up for development of cervical cytological abnormalities; 370 incident cases were detected (40 with atypical squamous cells of undetermined significance, 165 with low grade squamous intraepithelial lesions, 165 with high grade squamous intraepithelial lesions).

Main outcome measures Results of cervical smear tests and cervical swabs at enrolment and at the second examination about two years later.

Results Compared with women who were negative for human papillomavirus at enrolment, those with positive results had a significantly increased risk at follow up of having atypical cells (odds ratio 3.2, 95% confidence interval 1.3 to 7.9), low grade lesions (7.5, 4.8 to 11.7), or high grade lesions (25.8, 15.3 to 43.6). Similarly, women who were positive for HPV at the second examination had a strongly increased risk of low (34.3, 17.6 to 67.0) and high grade lesions (60.7, 25.5 to 144.0). For high grade lesions the risk was strongly increased if the same virus type was present at both examinations (813.0, 168.2 to 3229.2).

Conclusions Infection with human papillomavirus precedes the development of low and high grade squamous intraepithelial lesions. For high grade lesions the risk is greatest in women positive for the same type of HPV on repeated testing.

Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses. Although most infections are transient, the potential health implications are obvious because HPV types 16 and 18 are considered carcinogenic and other types (for example, 31, 33, 35, 39, 45, 52, 56, and 58) have an important role in cervical carcinogenesis.[1]

Most epidemiological evidence originates from cross sectional studies with samples from women taken after the cancer or the squamous intraepithelial lesion was diagnosed. Only few prospective cohort studies with reliable and sensitive methods for HPV testing have assessed the risk of new development of cervical neoplasia by using repeated measurements of genital HPV in their study design.[2–6] Only in such studies can the temporal association between exposure and outcome be evaluated and established. However, the currently available studies included only a few women with high grade squamous intraepithelial lesions.[2–5]

We carried out a prospective follow up study to investigate the role of HPV (detected on two occasions) in the development of cervical neoplasia in women who had no previous diagnosis of cytological abnormalities. We examined whether the presence of viral DNA can predict development of lesions. We also investigated the role of repeated detection of high compared with low risk types and repeated detection of the same virus compared with different types.

Methods

We collected a random sample of 17 949 women aged 20-29 years from the general population in Copenhagen using the central personal registry. Every citizen in Denmark has a unique 10 digit identification number (CPR number), which is universally used in the public administration. These identification numbers, which comprise information on sex and date of birth, are registered in the computerised central personal registry. The register is updated daily and contains information on vital status and migration, including the current address. We invited all eligible women to a

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bmj.com 2002;325:572

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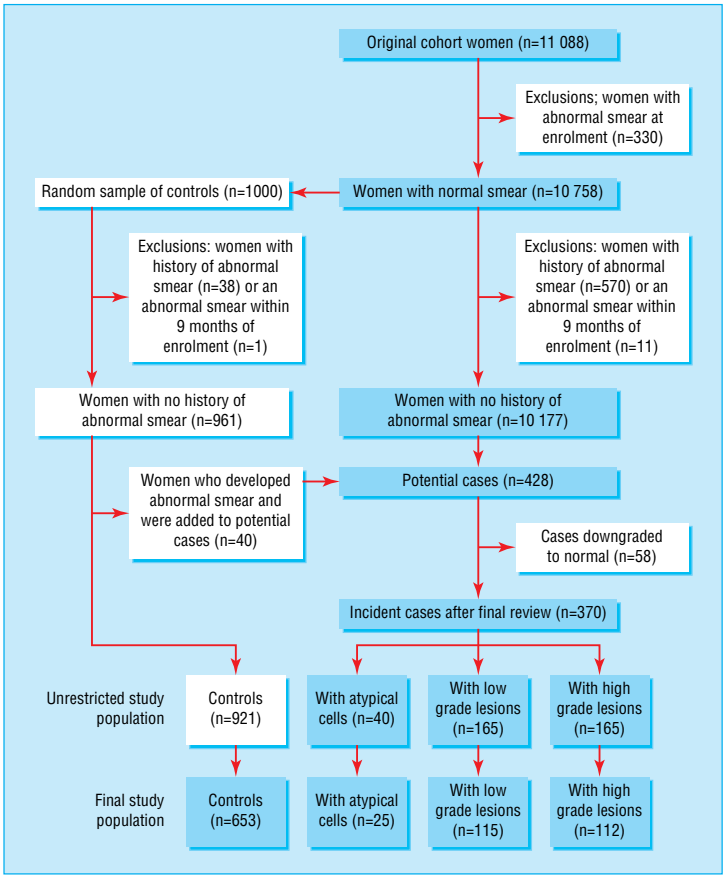
study clinic established at one of the university hospitals in Copenhagen. Recruitment was from May 1991 to January 1993. We included 11 088 women in the study, all of whom gave informed consent. A detailed description of the enrolment procedure is provided elsewhere.[7] The study was approved by the local ethics committee.

Examination at enrolment

At enrolment all 11 088 women were interviewed personally by specially trained female nurses. The nurses collected data on demographic variables, smoking, reproductive background, contraception, sexual habits, previous sexually transmitted diseases, and history of cervical smear tests. The participants also had a gynaecological examination, in which we carried out a smear test and obtained endo-ectocervical cells for detection of HPV DNA. All swabs were placed in a tube with TE-buffer (10 mM Tris-HCl and 1 mM EDTA, pH=8.0). In addition, all participants gave two blood samples. All biological material was kept at −80°C until tested.

Examination at follow up

In October 1993 we invited the entire cohort for a second examination. Initially, the cohort was linked to the central personal register using the CPR number as key identifier. We traced all the women in the cohort using this register and retrieved information on vital status and current address. We invited the women to participate in the second phase in the same order as they were originally enrolled in the study. During the following 18 months (that is, until January 1995) 8656 women (78%) underwent this second examination. Women were interviewed about suspected risk factors for cervical cancer, focusing on the time between enrolment (first examination) and follow up (second examination). We also did a smear test and took cervical swabs for HPV testing (placed in phosphate buffered saline with 0.05% methiolate) and two blood samples from each woman using the same procedure as at the initial examination, all biological material being stored at −80°C.



Schematic overview of overall study design. Final study population comprised 653 women in the control group had at least one normal smear test result at or after the second examination and 25, 115, and 112 women who were diagnosed within three months after the second examination

Passive follow up

We also had the cohort under passive surveillance for occurrence of abnormal cytology. In a high proportion of Danish counties, all cytological and histological diagnoses are registered in a computerised pathology register (the smears taken in the present study were also registered in the pathology register). In November 1995 we linked the original cohort of 11 088 women to the pathology register files, and all women were traced in the register. Although the Danish Board of Health recommends cervical smear testing every three years, many women tend to get screened more often.[8] By means of the pathology register we were able to get information about all such examinations on every woman in our study since their first smear test and up to the date of the register linkage.

Study population

We excluded women with a history or current evidence of cervical neoplasia. The figure shows the different exclusions for the entire cohort. We excluded 11 women in whom cervical neoplasia had been diagnosed in the first nine months (the time was chosen to ensure comparability with another study[4]) to avoid inclusion of potentially prevalent cases in the study (four had atypical squamous cells of undetermined significance, four had low grade lesions, three had high grade lesions). After all exclusions 10 177 women remained in the follow up study.

Identification of potential cases

A total of 428 potential cases developed in the cohort. Of these, we identified 329 at the second examination. The linkage with the pathology register resulted in 99 more women with an incident diagnosis of lesions on the uterine cervix that qualified them as potential cases in the time period between nine months after the first examination (that is, enrolment in the cohort) and November 1995. The cytological diagnoses covered a spectrum from "non-specific viral changes, not further specified," "koilocytosis," and "atypia" to dysplasia (mild, moderate, severe) and carcinoma in situ.

Review and confirmation of case diagnoses

From the files of the pathology register we identified the microscopy number on all abnormal smear results (and biopsies if taken) as well as on the enrolment smear and smears taken and diagnosed as negative during follow up from every potential case. The smear samples and biopsy slides were subsequently located and retrieved from the respective pathology departments. This material was reviewed in a masked fashion by one pathologist (GP) using the Bethesda nomenclature system.[9]

In cases of discrepancy between the original diagnosis and the review diagnosis, another pathologist (MES), who was unaware of any of the two previous diagnoses, reviewed the material. In most cases there was agreement at the first review, and in the remaining cases there was agreement between two of the three pathologists (GP, MES, PAP).

In cases where both cervical smear result and biopsy contributed to the diagnosis, the more severe diagnosis formed the basis of the final diagnosis. Among 428 potential cases, 58 were downgraded to normal in the review procedure, and 370 (86%) had a confirmed diagnosis of incident atypical squamous cells of undetermined significance or cervical neoplasia. This covered 40 with atypical squamous cells, 165 with low grade squamous intraepithelial lesions, and 165 with high grade squamous intraepithelial lesions (figure). Histological examination results to confirm the diagnosis were available in 136 (83%) high grade cases and 60 (35%) low grade cases. None of the smear results that were originally negative at enrolment were upgraded at the review procedure.

Subcohort selection

We randomly selected a sample of 1000 from the 10 758 women in the entire cohort who had cytologically normal results at enrolment. We retrieved the smear samples taken at enrolment and during follow up from the files of the pathology departments, and they were reviewed by one pathologist (PAP). In cases of discrepancy between the original diagnosis and the reviewed diagnosis, another pathologist blindly reviewed the smear (MES).

We excluded 39 women from the subcohort because of previous cervical neoplasia (n=38) or abnormal cytology detected within nine months of enrolment (n=1). During follow up, 40 women had an abnormal smear test result, and we included them in the group of potential cases. This left 921 women without any history of cervical neoplasia (that is, no history of cervical neoplasia before enrolment and no abnormal cervical cytology during follow up). At the review procedure none of the enrolment or follow up smears was upgraded.

Final study population

We excluded cases diagnosed later than three months after the follow up examination. This time limit was chosen so the HPV status at the follow up visit would still reflect the status at diagnosis. In the analyses including HPV status at follow up, we excluded cases that were diagnosed before the follow up examination and in which cervical biopsies or surgical treatment (cone) had been carried out (two with atypical squamous cells, 13 with low grade lesions, 19 with high grade lesions). For us to define women in the subcohort as "cytologically normal" we considered that they had to have a normal cervical smear result at or after the follow up examination. On the basis of these restriction criteria, we excluded 15 women with atypical squamous cells, 46 with low grade lesions, 51 with high grade lesions, and 265 controls from the analyses. We excluded four other women with low grade lesions, two with high grade lesions, and three controls because their cervical swabs were inadequate for HPV analysis. Thus, the final study population comprised 252 incident cases (25 with atypical squamous cells, 115 with low grade lesions, and 112 with high grade lesions) and 653 cytologically normal women (see figure). Among the cases, 191 (76%) women were identified at the second examination and 61 women with an incident diagnosis of cervical neoplasia were identified from the pathology register linkage.

HPV DNA detection

The cervical samples were analysed by the general primer GP5+/6+ mediated polymerase chain reaction-enzyme immunoassay method.[10] Briefly, we added 10 µl of the crude cervical cell suspension to the polymerase chain reaction mixture (10 mM TRIS HCl, pH 8.3; 50 mM KCl; 3.5 mM MgCl2; 1 unit of thermostable DNA polymerase (Amplitaq, Perkin Elmer Cetus, Norwalk, CT); 200 µmol of each dNTP; and 25 pmol of each primer (GP5+ and biotinylated GP6+)). We incubated the mixture for five minutes at 94°C for DNA denaturation, followed by 40 cycles of amplification with a polymerase chain reaction processor (Biomed, Theres, Germany). Each cycle included a denaturation step to 94°C for one minute, an annealing step to 40°C for two minutes, and a chain elongation step to 72°C for 90 seconds. To ensure a complete extension of the amplified DNA we prolonged the final elongation step by four minutes.

We analysed the biotinylated GP5+/6+ polymerase chain reaction products by enzyme immunoassay using HPV high risk (HR) and HPV low risk (LR) oligococktail probes to identify 14 high risk HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and six low risk types (HPV 6, 11, 40, 42, 43, 44). We also typed the high risk and low risk positive swabs individually using specific enzyme immunoassays. In addition, we analysed GP5+/6+ polymerase chain reaction products for the presence of other HPV types not identified by the high risk and low risk enzyme immunoassays; this was done with gel electrophoresis, followed by Southern blot analysis under low stringent conditions with a cocktail probe of different HPV types.[11] We classified samples that were positive by this Southern blot analysis but negative by both high risk and low risk enzyme immunoassay as HPV X positive.

Statistical analysis

We investigated the associations between squamous intraepithelial lesions and HPV DNA detected at the two examinations by multiple logistic regression analyses performed separately for each type of lesion compared with the controls (subcohort). This corresponds to being either cytologically normal or having a specific case type in the full generalised logistic regression model considering all four outcome categories (normal, atypical squamous cells, low grade lesions, and high grade lesions) simultaneously, and makes the estimates directly comparable with case-control studies of any of the single adverse outcomes. We corrected all analyses for age at enrolment as a categorical variable, grouped in yearly intervals. The 95% confidence intervals were based on Wald's test performed on the log transformed odds ratios and back transformed.

We classified HPV types in relation to their association with cervical cancer. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were grouped together in a high risk ("oncogenic") group, and HPV types 6, 11, 40, 42, 43 and 44 were placed in the low risk

("non-oncogenic") group. The uncharacterised HPV types (HPV X) were grouped together with the low risk types. Women with multiple types were grouped according to the highest risk group.

We performed the statistical analyses both on the unrestricted study population (that is, 40 with atypical cells, 165 with low grade lesions, 165 with high grade lesions, and 921 without any history of cervical neoplasia), on the final study population (25, 115, 112, and 653 respectively) (figure), and with intermediate restriction criteria for cases and the subcohort (data not shown). The overall pattern of the results was the same irrespective of the restriction criteria; however, the strength of association in the analyses including HPV status at follow up increased with the severity of the inclusion criteria for the subcohort and decreased with the length of time between the second examination and the case diagnosis (data not shown). We have presented only those results concerning the final (restricted) study population.

Finally, we investigated whether the restrictions leading to the final study population were associated with different distributions of some potential confounders registered at enrolment (age, number of sexual partners, age at first intercourse, and use of oral contraceptives). Thus, we compared the women in the final study population with those who were excluded from the unrestricted study population within the control group and within the case groups, and the respective distributions were nearly the same (data not shown).

Results

Age at enrolment did not differ significantly between women in the cytologically normal group (mean 25.2 years) and women in the total case group (24.8 years), women with atypical cells (24.9 years), and women with high grade lesions (24.9 years). The women with low grade lesions tended to be a little younger (24.3 years).

Table 1 Distribution and prevalence of types of human papillomavirus (HPV) among cases and cytologically normal women who were positive for HPV at enrolment. Figures are numbers (percentages) of women

HPV type	Cytologically normal (n=89)	Atypical cells (n=8)	Low grade lesions (n=63)	High grade lesions (n=89)
6	5 (6)	0	2 (3)	1 (1)
11	1 (1)	1 (13)	0	1 (1)
16	27 (30)	3 (25)	19 (30)	43 (48)
18	11 (12)	2 (17)	7 (11)	10 (11)
31	7 (8)	1 (13)	10 (16)	15 (17)
33	11 (12)	2 (17)	9 (14)	7 (8)
35	1 (1)	1 (13)	1 (2)	1 (1)
39	2 (2)	0	1 (2)	2 (2)
40	0	0	0	0
42	1 (1)	1 (13)	0	0
43	0	0	0	0
44	1 (1)	0	1 (2)	0
45	1 (1)	0	4 (6)	5 (6)
51	1 (1)	0	9 (14)	6 (7)
52	2 (2)	0	4 (6)	5 (6)
56	1 (1)	1 (13)	7 (11)	2 (2)
58	5 (6)	0	5 (8)	5 (6)
59	0	0	1 (2)	2 (2)
66	1 (1)	1 (13)	7 (11)	4 (5)
68	0	0	0	0
X	22 (25)	0	5 (8)	6 (7)

Prevalence of HPV

In both cytologically normal women and all three case groups the most common type of HPV at enrolment was HPV 16 (table 1). Among women positive for HPV, the prevalence was similar among cytologically normal women (30%), women with atypical cells (25%), and women with low grade lesions (30%), but significantly higher in those with high grade lesions (48%). Uncharacterised HPV types were found in about 8% of women with low grade lesions and 7% of women with high grade lesions, whereas nearly a quarter of the cytologically normal women harboured these unknown types. At enrolment, among women positive for HPV, 12% of cytologically normal women, 36% of women with low grade lesions, and 25% of women with high grade lesions had multiple types of HPV.

The prevalence of HPV detected at enrolment in women who were cytologically normal at enrolment and during follow up was 14% (89/653). Among women in whom high grade lesions were subsequently detected, 80% (89/112) were HPV positive at enrolment. The corresponding figures for low grade lesions and atypical cells were 63 (55%) and 8 (32%). At the follow up examination, 11 (50%) women with atypical cells, 92 (89%) women with low grade lesions, and 84 (93%) women with high grade lesions were positive for HPV.

Clearance of HPV

Among cytologically normal women who were positive for HPV at the first examination, 62/87 (71%) cleared that specific HPV infection: 45 became HPV negative and 17 acquired another HPV type. In contrast with this, 21/53 (40%) women who were positive for HPV and had low grade lesions lost the HPV (two became HPV negative and 19 got a new type), and only 17/72 (26%) women who were positive for HPV and had high grade lesions cleared the specific infection (four (6%) became HPV negative and 13 (20%) acquired a new HPV type), such that in 72% of HPV positive women with high grade lesions, the same type(s) was found at both examinations (2% still had uncharacterised HPV types at both examinations).

HPV and cervical lesions

Table 2 shows the odds ratios for incident atypical cells and squamous intraepithelial lesions according to HPV status at enrolment. Compared with women who were HPV negative at enrolment, HPV positive women had a significantly increased risk of developing atypical cells (odds ratio 3.2, 95% confidence interval 1.3 to 7.9), low grade lesions (7.5, 4.8 to 11.7), and high grade lesions (25.8, 15.3 to 43.6). When we divided the women who were HPV positive into a group of low risk or unknown HPV types and a group of high risk or oncogenic types, it was evident that the oncogenic HPV types were associated with the highest risk, especially for high grade lesions, when compared with HPV negative (7.8, 3.1 to 9.4 for low risk or unknown types *v* HPV negative) and (34.5, 19.7 to 60.2 for high risk or unknown types *v* HPV negative).

A similar picture emerged regarding the risk of cervical neoplasia in relation to HPV status at follow up (table 2), with HPV positive women having a strongly increased risk, especially of low grade lesions (34.3, 17.6 to 67.0) and high grade lesions (60.7, 25.5 to

Table 2 Risk of incident cervical neoplasia according to human papillomavirus status at enrolment and at follow up

HPV status	Cytologically normal	Atypical cells		Low grade lesions		High grade lesions	
		No	Odds ratio* (95% CI)	No	Odds ratio* (95% CI)	No	Odds ratio* (95% CI)
Enrolment:							
Negative	564	17	1.0	52	1.0	23	1.0
Positive	89	8	3.2 (1.3 to 7.9)	63	7.5 (4.8 to 11.7)	89	25.8 (15.3 to 43.6)
Low risk and unknown types†	26	0	—	7	3.1 (1.3 to 7.5)	8	7.8 (3.1 to 19.4)
High risk types‡	63	8	4.9 (1.9 to 12.4)	56	9.3 (5.8 to 14.9)	81	34.5 (19.7 to 60.2)
Follow up:							
Negative	498	11	1.0	11	1.0	6	1.0
Positive	122	11	4.1 (1.7 to 10.2)	92	34.3 (17.6 to 67.0)	84	60.7 (25.5 to 144.0)
Low risk and unknown types†	32	4	6.3 (1.8 to 22.2)	13	17.3 (7.1 to 42.5)	8	21.7 (7.0 to 67.6)
High risk types‡	90	7	3.5 (1.3 to 9.5)	79	40.9 (20.6 to 81.3)	76	74.5 (31.1 to 178.7)
Enrolment/follow up:							
Negative/negative	451	9	1.0	7	1.0	2	1.0
Positive/negative	45	2	2.3 (0.5 to 11.2)	2	2.7 (0.5 to 13.3)	4	20.3 (3.6 to 115.3)
Negative/positive	79	6	3.5 (1.2 to 10.5)	39	30.7 (13.0 to 72.5)	14	39.1 (8.6 to 178.1)
Positive/positive	42	5	7.4 (2.3 to 24.3)	51	83.8 (35.1 to 200.2)	68	413.9 (96.3 to 1779.5)

*Adjusted for age.
†HPV 6, 11, 40,42,43,44, X.
‡HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.

Table 3 Risk of incident cervical neoplasia according to different characteristics of human papillomavirus status* at enrolment and follow up

HPV status	Cytologically normal	Atypical cells		Low grade lesions		High grade lesions	
		No	Odds ratio† (95% CI)	No	Odds ratio† (95% CI)	No	Odds ratio† (95% CI)
Enrolment/follow up:							
Negative/negative	451	9	1.0	7	1.0	2	1.0
Positive (high‡)/positive (low§)	5	2	37.1 (4.3 to 317.2)	3	34.0 (6.4 to 180.8)	1	61.9 (4.1 to 941.8)
Positive (low§)/positive (high‡)	5	0	—	3	53.4 (9.5 to 299.2)	2	99.8 (10.8 to 923.7)
Positive (high‡)/positive (high§)	26	2	4.9 (0.9 to 26.7)	42	117.7 (45.2 to 306.8)	58	691.6 (145.3 to 3292.7)
Enrolment/follow up:							
Negative/negative	451	9	1.0	7	1.0	2	1.0
Positive/positive (not identical HPV types)	17	4	16.8 (3.8 to 75.1)	19	73.2 (25.7 to 208.9)	13	192.7 (37.5 to 988.7)
Positive/positive (identical HPV types)¶	19	0	—	29	117.9 (42.5 to 327.4)	48	813.0 (168.2 to 3229.2)

*Women positive only to HPV X at one or both visits excluded from analysis.
†Adjusted for age.
‡HPV types belonging to high risk HPV group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).
§HPV types belonging to low risk HPV group (6, 11, 40, 42, 43, 44).
¶At least one specific HPV type present both at enrolment and at follow up.

144.0). Also here the group of high risk HPV types was responsible for the highest increase in risk.

We also examined the risk of incident cervical neoplasia taking into account the HPV status both at enrolment and at follow up (table 2). We found a similar overall pattern of risk for all three disease categories. However, the most substantial effect was seen for high grade lesions, where the odds ratio was 413.9 (96.3 to 1779.5) when we compared women who were HPV positive at both examinations with women who were negative at both examinations.

When we compared women who were positive at both examinations with those who were positive only at enrolment, the odds ratio for low and high grade lesions increased (low grade 31.6, 7.1 to 140.5; high grade 20.4, 6.6 to 62.9) (data not shown). When we compared the same women with women who had HPV detected only at follow up we observed significantly increased odds ratios for both low grade (2.7, 1.5 to 5.0) and high grade lesions (10.6, 5.1 to 21.8) (data not shown).

Table 3 shows the results for women who stayed HPV negative compared with women who stayed HPV positive. For both low and high grade lesions the highest risk was associated with having a high risk HPV type detected at both visits, though the odds ratio for high grade lesions was the highest (low grade 117.7, 45.2 to 306.8; high grade 691.6, 145.3 to 3292.7).

In addition, we found that for high grade lesions the risk was strongly increased if at least one identical HPV type was present at both examinations (813.0, 168.2 to 3229.2 for being positive for identical types at both examinations *v* being negative for HPV at both examinations, table 3). Even when we carried out an internal comparison of women with identical types at the two visits versus different types at the two visits, we observed a significantly increased risk of high grade lesions (4.2, 1.5 to 12.3) (data not shown). In contrast, we found no significant difference between having identical HPV types or different HPV types at the two examinations in relation to the risk of low grade lesions

(1.6, 0.6 to 4.2). No women with atypical cells had identical types at the two visits.

Finally, we estimated the odds ratio for the association between being repeatedly postitive for HPV and high grade lesions in relation to age. The risk of high grade lesions in women positive for identical types at the two visits compared with women negative at both examinations tended to be stronger in women aged 25-29 years (810, 97 to 6754) than in women aged 20-24 years (567, 63 to 5688) (data not shown).

Discussion

In this prospective follow up study of more than 10 000 cytologically normal 20-29 year old women we found that HPV status at enrolment predicted future development of high grade squamous intraepithelial lesions. In a random sample of the women who stayed cytologically normal during follow up, only 14% were HPV positive at their first visit whereas this applied to 80% of the women who were subsequently diagnosed with high grade lesions. Most women were diagnosed at the second examination, and the HPV status at this examination was also strongly associated with the presence of cytological abnormalities, though the outstanding risk for incident high grade lesions in this study was being repeatedly positive for HPV. We found that women who were positive for HPV DNA both at enrolment and at follow up had an odds ratio of more than 410 for developing high grade lesions compared with women who were HPV negative at both visits. Even when we compared women who were repeatedly positive for HPV with women who were HPV positive at only one of the visits (either the first or the second), they had a significantly increased risk of high grade lesions. Our findings agree with those recently reported from another big cohort study.[4]

Our results provide evidence that HPV infection precedes the development of high grade squamous intraepithelial lesions and support the suggested central role of persistent HPV infection in the development of cervical neoplasia.[12 13] At present there is no general consensus on a definition of persistent HPV infection, and we have no knowledge about the duration of infection required for the development of high grade lesions. In this study it was evident that type specific persistence of HPV was highly associated with high grade lesions, with persistence defined as positivity to the same HPV type at two visits with an interval of two years. It is interesting that for low grade lesions, there was no significant difference in the risk associated with being HPV positive on both occasions with different types and having the same HPV type at both visits, and among the women with atypical cells, none presented with the same HPV type twice. In contrast with this, the risk of high grade lesions was significantly higher in women positive for identical HPV types on both occasions than in such women with different types.

Women with different HPV types detected at enrolment and at follow up still had a substantially increased risk of high grade lesions. Because of the rather long time (about two years) between the two visits in this study, however, the group of women with apparently different HPV types detected at the two examinations may actually contain a group of women with truly type specific persistent HPV infection if, for example, the HPV type detected at the first examination was cleared soon after the visit and the woman subsequently became infected with a new type that persisted and thus was detected at the second examination. Furthermore, the women in our study were young and sexually active, and as such had a high background prevalence and acquisition rate of HPV. The group of women who had the same HPV type detected at both examinations may actually cover different kinds of infection—for instance, in cytologically normal women it may mostly reflect reinfection with the same HPV type, whereas in women with high grade lesions it is likely to reflect type specific persistence. However, we were unable to determine whether repeated type specific HPV positivity was reflecting true persistence or a recurrent HPV infection with the same HPV type as we did not do variant analyses. Thus, we may have underestimated the association between high grade lesions and type specific persistence.

Because the prevalence of HPV among women without cervical lesions decreases with age we expected that the association between HPV and cervical neoplasia would be even stronger among older women. Although the age range in our cohort was quite narrow and the age stratified analyses were based on small numbers we were able to show such an age pattern, though it did not reach significance.

Conclusion

In conclusion, we can confirm previous reports that stated that HPV infection is common in young women and that most infections are transient with high rates of acquisition and clearance. More importantly, we have shown that HPV infection precedes the development of low and high grade squamous intraepithelial lesions and that high risk HPV infection is a good predictor of subsequent high grade lesions in young women. Our data also indicate that HPV is an even better predictor in older women with a lower background HPV prevalence. The outstanding predictor of high grade lesions, however, was being repeatedly positive for HPV with

What is already known on this topic

Persistence of infection with human papillomavirus (HPV) is thought to have a role in the development of cervical neoplasia

Previous studies have included only a few cases of high grade squamous intraepithelial lesions, and few have randomly sampled women from the general population

What this study adds

In women aged 20-29, HPV infection preceded the development of high grade lesions

Persistent HPV infection with a specific HPV type was an indicator of incident high grade lesions among young women in the general population

The association between persistence and high grade cervical lesions was more pronounced among women aged over 25

the same HPV type, in line with the previously suggested hypothesis that persistence of high risk HPV types is strongly associated with the development of high grade lesions.

Contributors: SKK designed the follow up study, organised the data collection at enrolment and at follow up, interpreted the results, and wrote the original and successive drafts of the paper. AJCvdB supervised the HPV analyses and commented on drafts of the paper. GP reviewed all cytological and histological slides and commented on drafts of the paper. EIS participated in the data collection at follow up and commented on all drafts of the paper. MES reviewed the slides and commented on drafts of the paper. BLT planned the statistical analyses, interpreted the results, and commented on every draft of the paper. MS conducted the statistical analyses. JEB advised on the organisation of the data collection and commented on drafts of the paper. PAP supervised the daily diagnostic procedures regarding the cervical cytological examinations and reviewed the normal cervical smears taken at enrolment. CJLMM commented on all drafts of the paper. SKK is guarantor.

Funding: National Cancer Institute (RO1 CA47812) and Danish Cancer Society.

Competing interests: SKK is a consultant for Merck. CM is a consultant for Digene. Mark Sherman was formally a faculty member at Johns Hopkins (participant in the centres of excellence programme developed by Digene).

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(Accepted 23 April 2002)