MAIN RESEARCH ARTICLE

Human papillomavirus and predictors of cervical intraepithelial neoplasia among young mothers in a prospective follow-up study

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Abstract

Objective. To study the incidence times and rates for cervical intraepithelial neoplasia (CIN) and its predictors. *Material and methods.* This is a prospective follow-up study at Turku University Hospital, Finland. The Finnish Family human papillomavirus (HPV) study comprised 329 pregnant women followed up for 3 years. In an extension of the follow-up period, 171 women participated in an additional 3 years follow-up. Cervical scrapings for HPV testing and cervical smears were collected at each follow-up visit (2, 12, 24 and 36 months and 6 years). Following two abnormal cervical smears, colposcopy with biopsies was done. The main outcome measures were actuarial and crude incidence times, incidence rates and predictors of incident CIN. *Results.* During the follow-up period, 10 women (3.2%) developed biopsy-confirmed CIN, and four presented with incident atypical squamous cells suggesting high-grade squamous intraepithelial lesion cytology. The CIN/squamous intraepithelial lesion developed in 74.5 and 66.3 months, with crude incidence rates of 13.4/1,000 and 15.1/1,000 women months at risk, respectively. In multivariate Poisson regression, independent predictors of incident CIN were as follows: high-risk HPV positive at baseline (incidence rate ratio = 5.54; 95% confidence interval 1.02–30.14, $p = 0.048$); type-specific high-risk HPV persistence during follow-up (incidence rate ratio = 5.84; 95% confidence interval 2.28–17.93, $p = 0.0001$); cervical smear cytologically diagnosed for atypical squamous cells of undetermined significance or worse at any follow-up visit (incidence rate ratio = 4.56; 95% confidence interval 2.37–8.78, $p = 0.0001$); and new sexual partner during follow-up (incidence rate ratio = 9.45; 95% confidence interval 1.90–46.97, $p = 0.006$). *Conclusion.* The results indicate that combined use of cervical smear and HPV testing, with prompt referral to colposcopy, enables accurate detection of incident CIN well before progression to invasive cancer. In addition to baseline and persistent high-risk HPV, abnormal cervical smear and new sexual partner are key predictors of incident CIN.

Abbreviations ASCUS, atypical squamous cells of undermined significance; ASCUS+, atypical squamous cells of undetermined significance or worse; CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IR, incidence rate; IRR, incidence rate ratio; LSIL, low-grade squamous intraepithelial lesion; SIL, squamous intraepithelial lesion

Introduction

Of the mucosal human papillomaviruses (HPVs), 15 genotypes are high-risk types based on their clinical behavior (1). Persistent infection with any high-risk type increases the risk of progression to cervical intraepithelial neoplasia (CIN) and cervical cancer (2). Several HPV covariates seem to be involved and of these, current and past smoking consistently increase the risk for CIN (3,4). Other potential cofactors include oral contraceptives, parity and number of sexual partners (5,6). Sex hormones also play a role in the development of cervical cancer (7,8) in addition to genetic and immunological factors (9).

The majority of all HPV infections will clear spontaneously within 2 years (10), but sometimes cytological changes suggest rapid progression within 3–4 months from infection (11–13). It has been estimated that only 10–20% of persistent HPV infections are associated with incident CIN (14). The time from HPV exposure to CIN or worse (CIN2+) varies. However, in most cases this will occur within 3 years of viral persistence (11).

In the ongoing prospective Finnish Family HPV study, a cohort of pregnant women were enrolled in the third trimester, and subsequently followed for 6 years at 6 month intervals. During that time, 10 of these mothers developed incident CIN and in an additional four, cytological abnormality suggesting high-grade squamous intraepithelial lesion (HSIL) was detected. We report the incidence times and rates for these two end points (CIN and CIN/SIL) and analyze the risk factors for incident CIN.

Material and methods

The Finnish Family HPV Study is a prospective cohort study conducted at the Department of Obstetrics and Gynecology, Turku University Hospital and Institute of Dentistry, University of Turku. The study was designed to evaluate the dynamics of HPV infections in mothers, fathers and their infants. A total of 329 women, 131 men and 331 infants to be born were recruited to this study between 1998 and 2002. In total, 171 women participated in an extension of the follow-up for an additional 3 years, 2006, 2007 and 2008.

The women (mean age 25.5 years) in this cohort were mothers-to-be who were recruited after 36 weeks of their index pregnancy (15) and followed up for 3 years. In the extension of the follow-up period, 171 women participated with an additional 3 year follow-up. The Joint Commission on Ethics of Turku University and Turku University Hospital approved the study protocol and its amendments (#2/1998 and #2/2006). Altogether, 329 mothers were enrolled. The flowchart of the study setting has been described earlier (16). Of these women, 308 had at least two visits completed, and thus contribute women month at risk for incident CIN. Some of the women were lost to follow-up during the 6 years, mostly due to family reasons. A total of 171 women completed the whole follow-up of 6 years. A structured questionnaire recording demographic data and risk factors was introduced at baseline and repeated at the 36 month and 6 year follow-up visits.

Cervical scrapings for HPV testing were taken at baseline and at 2, 12, 24 and 36 month and 6 year visits as previously described (16).

A routine cervical smear was obtained immediately after taking the sample for HPV testing from all women at baseline, and at 12, 24 and 36 month and 6 year visits, using the three-sample technique with a wooden spatula and Cytobrush® (Medscand, Malmö, Sweden), as described recently (15). Cervical smears were graded using the 2001 Bethesda System. The management of the women with abnormal cervical smears followed the algorithm used at Turku University Hospital and the national guidelines. Therefore, some of the women had cervical smears taken with both clinical and research indications, and detection of cervical smear abnormalities did not necessarily coincide with the follow-up visits.

Following two cervical smears taken at 6–12 month intervals containing abnormal atypical squamous cells of undetermined significance or worse (ASCUS+), women were referred for colposcopy and biopsies to confirm the cytological findings. All biopsies were fixed in formalin, embedded in paraffin and processed for hematoxylin and eosin-stained sections following routine procedures. The slides were re-evaluated to confirm the diagnosis of CIN ($n = 10$) by an independent reviewer (KS). All women were treated by cone biopsy with large loop excision of the transformation zone and followed according to the protocol of Turku University Hospital.

Human papilloma viral DNA was extracted from cervical scrapings with the high-salt method (17). For detection of high-risk HPV types collectively, DNA was amplified with GP05+/GP06+ primers (18). The polymerase chain reaction products were hybridized with digoxigenin-labelled high-risk HPV oligoprobe cocktail (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56 and 58) (19).

Human papilloma virus genotyping was performed using a Multimetrix kit (Progen Biotechnik GmbH, Heidelberg, Germany), which detects the following 24 low-risk and high-risk HPV genotypes: low-risk HPV 6, 11, 42, 43, 44 and 70; and high-risk HPV 16 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. The test was performed as described in the protocol, except that we re-amplified the earlier polymerase chain reaction product (described above) for biotinylation with GP05+/bioGP06+ primers. The hybrids were analyzed using a Luminex LX-100 analyzer (Bio-Plex 200 System; Bio-Rad Laboratories, Hercules, CA, USA). The median fluorescence intensity of

at least 100 beads was computed for each bead set in the sample. The cut-off value for each run and HPV type was calculated as 1.5 times the background median fluorescence intensity (negative control) plus 5 times median fluorescence intensity. All HPV16-positive samples were retested using the original sample, nested polymerase chain reaction and bead-based HPV genotyping (20) to exclude potential contamination.

Statistical analyses

All statistical analyses were run using SPSS® (SPSS, Inc., Chicago, IL, USA) and STATA (Stata Corp., College Station, TX, USA) software (PASW Statistics for Windows, version 18.0.1 and STATA/SE 11.0). Frequency tables were analyzed using the χ^2-test, with the likelihood ratio or Fisher's exact test for categorical variables. Differences in the means of continuous variables were analyzed using non-parametric (Mann–Whitney *U* or Kruskal–Wallis) tests for two and multiple independent samples, respectively.

The end point of progression was biopsy-confirmed CIN detected in 10 women. Actuarial (all women included) and crude (women with progression) times (months) to the first progression event were calculated from the baseline visit to the point when progression was confirmed, either with histology ($n = 10$) or with a cervical smear of cytological abnormality suggesting HSIL ($n = 4$). Actuarial and crude incident rates (IRs) for CIN were expressed as events per 1,000 women months at risk. For actuarial IRs, the number of progression events, 10 for CIN (and four cases of CIN/SIL) was divided by the total women months at risk, i.e. 17,049 women months accumulated by all 308 women at risk. In crude IRs, only the women with progression events were included, the number of progression events being divided by women months at risk accumulated by those 10 (and four) women only.

To estimate the predictors of progression, we used only the cone biopsy-confirmed histological CIN end point ($n = 10$). Poisson regression analysis (in population-averaged mode) was used for panel data, clustered by mother identification number (mother-ID) and using follow-up visit as the time variable in the panel settings (21). In the univariate Poisson analysis, we first tested all covariates recorded in the baseline questionnaire as well as some pertinent variables from the follow-up questionnaire (such as partner change), previously implicated as potential risk factors of HPV in this cohort (15). In the final multivariate model, only variables that were significant in univariate analysis were entered, and adjusted for age at study entry. All statistical tests were two sided and declared significant at p-value < 0.05 level.

Results

All women were negative at baseline for cytological abnormalities (ASCUS cut-off) or CIN. The mean follow-up time was 55.4 months (SD = 27.5 months; median = 62.7 months; range 1.6–99.0 months), and by the study end point, 10 women (3.2%) had developed a biopsy-confirmed CIN lesion; CIN1 ($n = 2$), CIN2 ($n = 3$) or CIN3 ($n = 5$). In addition, four women presented with atypical squamous cells suggesting HSIL cytology and were included in the combined CIN/SIL group ($n = 14$), representing 4.5% of the women in the cohort.

Progression events are shown in Table 1. The actuarial times for CIN and CIN/SIL were the same, 55.3 months [95% confidence interval (CI) 52.3–58.4]. Crude time, however, was longer for CIN (74.5 months; 95% CI 58.7–90.3) than for CIN/SIL (66.3 months; 95% CI 52.3–80.2), because of a shorter interval required to develop incident SIL.

Also the crude time from the detection of incident high-risk HPV infection until incident CIN could be calculated for four women and it was 70.3 months (95% CI 50.9–89.7). For seven women in the CIN/SIL group, the time from incident high-risk HPV to the combined end point was shorter, at only 47.8 months (95% CI 17.7–77.9).

Univariate (Kaplan–Meier) survival analysis was performed to illustrate the cumulative incidence of CIN and CIN/SIL events, stratified by baseline cervical high-risk HPV DNA status (Figure 1, Figure 2). The cumulative incidence of both CIN and CIN/SIL outcomes was significantly higher among baseline high-risk HPV-positive than -negative women [logrank (Mantel–Cox) test, $p = 0.0001$ and $p = 0.003$, respectively].

While taking into the account all persons at risk (women months at risk), the actuarial IR reflects the rate (per 1,000 women months at risk) at which these women at risk accumulated incident CIN and CIN/SIL events. The

Table 1. Characteristics of progression to cervical intraepithelial neoplasia (CIN) and CIN/squamous intraepithelial lesion (SIL) among 308 women in the Finnish Family HPV study.

			Mean time to progression (months)		Incidence rate per 1,000 women months at risk	
	n/total	Percentage	Actuarial (95% CI)	Crude (95% CI)	Actuarial (95% CI)	Crude (95% CI)
CIN	10/308	3.2	55.3 (52.3–58.4)	74.5 (58.7–90.3)	0.59 (0.22–0.95)	13.42 (5.1–21.7)
CIN/SIL	14/308	4.5	55.3 (52.3–58.4)	66.3 (52.3–80.2)	0.82 (0.39–1.25)	15.09 (7.2–22.9)

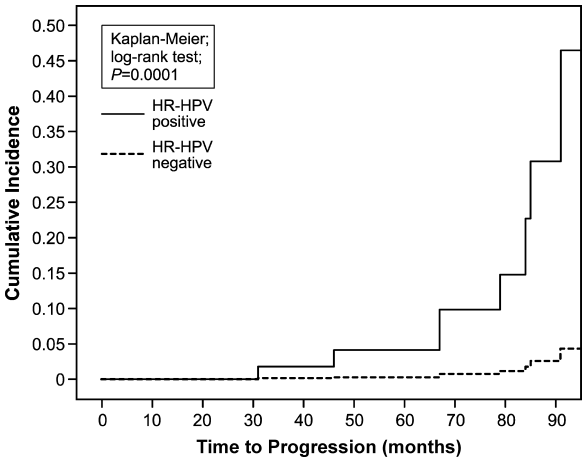

Figure 1. Cumulative incidence of cervical intraepithelial neoplasia in Kaplan–Meier analysis.

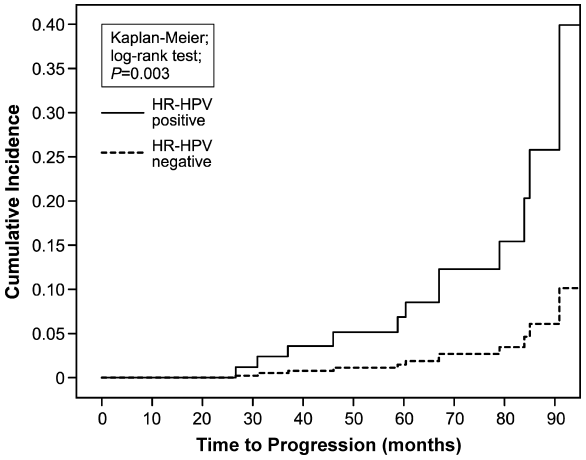

Figure 2. Cumulative incidence of cervical intraepithelial neoplasia/squamous intraepithelial lesion in Kaplan–Meier analysis.

actuarial incidence rate for CIN was 0.59/1,000 women months at risk (95% CI 0.22–0.95) and that for CIN/SIL was 0.82/1,000 women months at risk (95% CI 0.39–1.25). While calculated only for persons with confirmed progression events, the crude incidence rates reflect the rate (per 1,000 women months at risk) at which individual women accumulated such an event: 13.4/1,000 women months at risk for CIN and 15.1/1,000 women months at risk for CIN/SIL.

The progression event documented at the follow-up visit was used as the dependent variable in the Poisson regression analysis for panel data in analysing the predictors. Of all variables tested in univariate Poisson regression analysis (16), only those shown in Table 2 were significant. In univariate Poison analysis, the following four variables were significantly associated with progression events: (1) cervical high-risk HPV DNA positive at baseline; (2) type-specific high-risk HPV persistence; (3) ASCUS+ cervical smear at any follow-up visit; and (4) a new sexual partner during the study period.

When all significant and borderline significant univariate predictors were entered in the multivariate Poison model adjusted for maternal age at study entry, all four variables retained their significance as independent predictors of incident CIN.

Discussion

Persistent high-risk HPV infection is necessary for development of incident CIN lesions. In the present cohort of pregnant women participating in the Finnish Family HPV study, persistent HPV infection was detected in 35% ($n = 115$ of 329) during 6 year follow-up (16). We demonstrate here that persistent high-risk HPV infections, cytologic abnormality in cervical smear and a new partner were independent predictors of incident CIN/SIL lesions, developed by 4.5% of these women.

We found here that the actuarial time for both incident CIN and CIN/SIL was the same, 55.3 months, for both events. The crude time for CIN was nearly 8 months longer than for CIN/SIL (74.5 months vs. 66.3 months). These are 10–15 months longer than reported by Trottier and co-workers (22) and Woodman and co-workers (12), but a longer actuarial time (82.7 months) has also been reported for progression from ASCUS to HSIL (23). These differences are likely to be explained by different study designs. The actuarial time is dependent on the length of the follow-up time, which was longer in the present series, compared with a mean of 29.0 and 45.3 months in previously published studies (12,22). Additionally, the possible impact of pregnancy has to be considered, because all women in our cohort were pregnant at study entry, giving this cohort a unique profile. However, a second pregnancy ($n = 78$) that occurred during the follow-up did not appear to increase the risk for incident CIN or CIN/SIL. It has been shown previously that pregnancy as such may not be associated with a higher risk of incident CIN3 (24); however, conflicting data exist on the role of pregnancy in the natural history of CIN (25,26).

Not unexpectedly, high-risk HPV has a significant impact on the risk of incident CIN, as shown in the Kaplan–Meier survival analysis, where the time difference of 15 and 6 months was observed between high-risk HPV positive and high-risk HPV negative patients for developing incident CIN or CIN/SIL, respectively (Figure 1, Figure 2). Until now, incidence rates of CIN have rarely been reported. The crude incident rate in our cohort was 13.4/1,000 women months at risk for CIN and 15.1/1,000 women months at risk for CIN/SIL. These figures are practically identical with those in a recent study by Trottier and co-workers

Table 2. Predictors of progression of HPV infections in Poisson[1] regression (for panel data) in a univariate mode and adjusted for significant covariates.

Covariates	Incident CIN					
	Crude IRR	95% CI	*p*-Value	@Adjusted IRR	95% CI	*p*-Value
baseline genital high-risk-HPV DNA status (negative ref.)	7.67	2.24–26.30	0.001	5.54	1.02–30.14	0.048
Type-specific persistence (no ref.)	4.19	1.92–9.16	0.0001	5.84	2.28–14.93	0.000
Cervical smear any visit (ASCUS cut-off; no ref.)	4.87	3.53–6.71	0.000	4.56	2.37–8.78	0.000
Same sexual partner during follow-up (yes ref.)	5.24	1.12–24.62	0.036	9.45	1.90–46.97	0.006

[1]Results obtained from panel Poisson regression for count outcomes (log-link), clustered by woman-ID, follow-up visits as time variable, and 95% CI calculated by robust variance estimation; @adjusted for age and all significant univariates in the model; IRR = incidence rate ratio, CI = confidence interval.

for incident CIN in HPV16- and HPV18-positive women; 10.3–13.0/1,000 women months at risk (22).

In this study, Poisson regression was used to analyse what might predict incident CIN (Table 2). There were four significant predictors that remained significant in the multivariate model. Not unexpectedly, women who at baseline were high-risk HPV DNA positive had an increased risk for progression to CIN. Among 20- to 29-year-old women testing positive with Hybrid capture 2, the risk of CIN3 or cervical cancer within 10 years was 13.6% (95% CI 10.9–16.2) (29), and the highest risk was associated with HPV16 (12). In our study, single HPV16 infection was detected in three of 10 women with incident CIN, the remaining seven women having multiple ($n = 4$) or single infections with other high-risk HPV types. However, HPV16 was also included in all four multiple-type combinations, increasing the HPV16 involvement to 70% of CIN and 64.3% of CIN/SIL (data not shown). Indeed, HPV16 has been reported also to play a key role in studies where cytological end points of progression have been evaluated (13).

Another risk factor increasing the probability of progression to CIN was persistent high-risk HPV infection (Table 2). A recent meta-analysis based on 40 studies evaluating the association between HPV persistence and CIN2–3/HSIL (or cervical cancer) disclosed that the relative risk varies from 1.3 to 813.0 (2). It has been implicated that if HPV infection persisted over 6 months or 12 months, it was likely to remain permanent and increase the risk of progressive disease (2). The importance of this finding has increased recently, because over 6 months and over 12 moths viral end points could offer potential new surrogates of progressive disease, to be used instead of histological (CIN2+) end points in future clinical trials with non-HPV16/18 vaccines (2).

The third significant predictor of progression was ASCUS+ cervical smear at any follow-up visit. ASCUS+ detected at an interval of 6 months or longer has been associated with incident CIN1, CIN2 or SIL (27), and in 20% of women with persistent LSIL progressed to HSIL or cervical cancer during 8 years of follow-up (23). With atypical squamous cells suggesting HSIL, this association is even stronger; 40% of women with this finding have been reported to develop CIN2 or worse (28). This substantiates the use of atypical squamous cells suggesting HSIL as a surrogate of progression, as in the present study (CIN/SIL group). This leaves little doubt that persistent high-risk HPV together with cervical smear abnormality (with ASCUS cut-off) is indicative of progressive disease.

The fourth significant predictor was a new sexual partner during the follow-up, shown to significantly (incidence rate ratio > 9) increase the risk for incident CIN. This is likely to be associated with exposure to new HPV infection, as shown in our recent study on this same cohort. A new partner increased the risk of incident high-risk HPV infections (Louvanto K, Rintala MA, Syrjänen KJ, Grénman SE, Syrjänen SM, unpublished observations). Smoking and use of oral contraceptives are other cofactors considered to be associated with CIN. We recently showed that smoking was an independent predictor for incident high-risk HPV but not for CIN2 (29). In the present series with fewer cases, only a borderline association to incident CIN was ascribed to early initiation of oral contraceptive use ($p = 0.082$; data not shown). Smoking clearly had a high incidence rate ratio (>3.4), but the 95% CIs showed wide variation, precluding smoking among the significant predictors in this small series (data not shown).

Taken together, of the 329 young mothers in the Finnish Family HPV study, 10 (3.2%) developed a biopsy-proven CIN within a mean crude time of 74.5 months, and another four women showed cytology-confirmed progression. They had the following predictors of progression similar to those detected in large population-based cohorts: (1) testing high-risk HPV positive at baseline; (2) type-specific high-risk HPV persistence; (3) ASCUS+ cervical smear at any follow-up visit; and (4) a new sexual partner during the follow-up. The data indicate that when any of these factors are identified, the increased risk of CIN/SIL lesions needs to be recognised, even in women who are young, delivered relatively recently, and are possibly pregnant. Combined use of cervical smear and HPV testing with prompt referral for colposcopy enables accurate detection of these lesions well before progression to invasive disease.

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