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Cigarette smoking is an independent risk factor for cervical intraepithelial neoplasia in young women: A longitudinal study ☆

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ABSTRACT

Repeated measurements of smoking, cervical human papillomavirus (HPV) status and sexual behaviour were used to measure the risk of high-grade cervical intraepithelial neoplasia (CIN) in relation to changes in smoking and cervical HPV status, and to explore the impact of smoking on the acquisition and duration of incident cervical HPV infection. Included in this longitudinal analysis are 1485 women aged 15–19 years: 1075 were HPV-negative and cytologically normal at recruitment; 410 were HPV-positive, cytologically abnormal or both, at this time. Women re-attended every 6 months, when samples were taken for cytological and virological examination. Current smoking intensity was associated with an increased risk of high-grade CIN, after controlling for cervical HPV status (compared to non-smokers, hazards ratio (HR) for 10 or more cigarettes per day = 2.21, 95% confidence interval (CI) 1.19–4.12, *p*-trend = 0.008). In women who were HPV-negative and cytologically normal at recruitment, current smoking was not significantly associated with the risk of acquiring a cervical HPV infection, after controlling for life-time number of partners and age of oldest partner (HR = 1.13, 95% CI 0.90–1.41); nor did it prolong the length of time during which HPV could be detected (HR = 1.03, 95% CI 0.78–1.34). Current smoking intensity is an independent risk factor for high-grade CIN in young women, after controlling for cervical HPV infection.

1. Introduction

Cervical human papillomavirus (HPV) infection, a common sexually transmitted disease, is considered a necessary but not sufficient cause of cervical cancer, and attention is now focused on identifying cofactors which modulate its progression to high-grade cervical intraepithelial neoplasia (CIN) and invasive disease.[1] Cigarette smoking has long been suspected of increasing the risk of cervical neoplasia, but an association has been difficult to prove because smoking is strongly correlated with various aspects of sexual behaviour. However, a pooled analysis of 23 case-control and longitudinal studies, which included 13,000 cases and 23,000 controls, has shown that, compared with women who had never smoked, current

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cigarette smokers have a significantly increased risk of carcinoma *in situ* and cervical cancer: this risk increased with the number of cigarettes smoked daily, and persisted when the analyses were restricted to women who tested positive for oncogenic HPV types.[2] This analysis also showed that the risk of cervical neoplasia increased with decreasing age of smoking initiation, but surprisingly not with smoking duration.[2] These observations are intriguing, but beg another question: if early age at smoking initiation is a risk factor for cervical neoplasia, how quickly do smoking-associated changes in the incidence of high-grade CIN manifest themselves? This question can only be answered by a longitudinal study. We have addressed this question using observations made on a cohort of young women who had recently become sexually active and who were free of disease and HPV-negative at study entry. We also explore, in the same study population, the impact of smoking on the acquisition and duration of incident cervical HPV infections.

2. Materials and methods

The study design and characteristics of the study population have been described elsewhere.[3] In brief, 2011 women aged 15–19 years were recruited from a single Birmingham Brook Advisory Centre (a family planning clinic) in Birmingham, United Kingdom, between 1988 and 1992, and asked to return at intervals of 6 months: follow-up ended on 31st August 1997. At recruitment a standardised interview questionnaire was used to construct a detailed social, sexual and behavioural risk factor profile, including smoking and sexual behaviour. At each follow-up visit: sexual and smoking histories were collected or updated; and one cervical sample was taken for cytological examination, followed by another which was stored for subsequent virological examination. All women with an abnormal smear were immediately referred to a colposcopy clinic for histological examination, irrespective of the severity of that abnormality. Colposcopic and cytological surveillance was maintained in these women and treatment postponed until there was histological evidence of high-grade CIN (CIN2 or CIN3), at which point women left the study.

After all clinical follow-up had ended, cervical cytology samples were tested for the presence of HPV DNA using a general primer (GP5+/GP6+) mediated polymerase chain reaction (PCR), and further PCR tests were done with type-specific primers on samples that were HPV-positive after ethidium bromide staining.[3] The study was approved by the appropriate research ethics committee, and informed oral consent was obtained from all women.

2.1. *Statistical analysis*

For this report, the study population is restricted to the subset of all 1485 women who had further follow-up after recruitment. For the analysis of the incidence of HPV infection, its duration, and its association with smoking, the study population was further restricted to an incident cohort, comprising 1075 women who were HPV-negative and cytologically normal at study entry, and who had further follow-up.

Observations on cervical HPV status and high-grade CIN are interval censored; their time of onset is known only to lie in the interval between the date of the visit at which they were first detected and the date of the immediately preceding visit; similarly for the clearance of HPV infection. Analyses were undertaken using a semi-parametric method for modelling interval-censored time-to-event data with time-dependent covariates, as a generalised linear model.[4] Each subject contributed a sequence of binary variables indicating if the event had, or had not, occurred during each interval of observation. The likelihood function from these binary data is equivalent to that from the interval-censored data. The method is based on a proportional hazards model in which covariates are incorporated parametrically, with the logarithm of the baseline hazard function approximated by a smooth function of the observation interval midpoints; it allows for an arbitrary observation scheme, with subjects seen at irregular intervals and on a varying number of occasions, as occurred in this study. Time-to-event was measured from study entry to first detection in incidence analyses, and from the first detection of either any HPV or the relevant HPV type, to clearance, in analyses of duration; a woman was considered to have 'cleared' her infection when she was first tested negative for HPV, or for the relevant HPV type in type-specific analyses. End of follow-up was the earliest of: date of diagnosis of high-grade CIN, date of treatment, or date of last visit.

Time-varying covariates were assigned their current values at each study visit. Cervical HPV status was controlled by constructing three separate time-dependent binary variables which measured whether a woman had ever been exposed to HPV16, HPV18 or other HPV types; HPV16 and HPV18, the two most common high-risk types, were detected in sufficient numbers to make this analysis feasible. Current cigarette smoking status was categorised as never-smoker, ex-smoker and smoker; a 'never-smoker' was a woman who was a non-smoker prior to study entry, and who did not smoke at any time during follow-up; a woman was categorised as an ex-smoker immediately after stopping smoking. Smoking intensity was recorded as a categorical variable, with categories 0 cigarettes smoked per day, 1–9, 10–19, 20–29, 30–39, and 40 or more. Cumulative smoking exposure was measured using pack-years, estimated as the midpoint of each smoking quantity category (with the final category set to 45), multiplied by the length of the interval during which this quantity applied, and accumulated over the lifetime of the woman. For current smokers, the current smoking episode was considered to have begun at the midpoint between the most recent date at which a woman reported having started to smoke and the date of the preceding visit. When testing the statistical significance of linear trends, an indicator variable for ever having smoked, or current smoking status, as appropriate, was also included: this is equivalent to restricting the analysis to ever, or current, smokers, respectively.[5] Sexual behaviour variables available for the analysis were age at first sexual intercourse, age of oldest sexual partner, and life-time number of sexual partners, all treated as continuous variables; only those variables which were significant in multivariate analyses including smoking were retained in the final model. Tests of statistical significance were conducted at the 5% two-sided significance level using

likelihood ratio tests, and 95% confidence intervals (CIs) were constructed as appropriate.

3. Results

For the 1075 women included in the incident cohort, and the 410 in the prevalent cohort who had further follow-up after recruitment, the median age at first sexual intercourse was 16 (range 0–19, i.e. some women were virgins at study entry) and the median number of sexual partners at study entry was two (range 0–21); 94% of women described themselves as Caucasian. During follow-up, 717 women changed partner at least once (range 0–17); 433 (29.2%) women had a partner age 25 years or older before the end of follow-up. Six hundred and ninety-nine women were never-smokers and 58 quit prior to study entry; 540 women were smokers at study entry and 188 first started or recommenced smoking after this time; 599 were still smokers at the end of follow-up.

3.1. *Smoking and the risk of high-grade CIN*

The risk of high-grade CIN was first investigated in the incident cohort of 1075 women who were HPV-negative and cytologically normal at study entry, and who had further follow-up. Twenty-eight women in this incident cohort progressed to high-grade CIN. We have previously reported that the hazards ratio of high-grade CIN after exposure to HPV16 was 8.5 (3.7–19.2) and that after exposure to HPV18 was 3.3 (95% CI 1.4–8.1).[3] Twenty-three women with high-grade CIN tested positive for HPV DNA during follow-up: 12 were current smokers at diagnosis, as were four of five who repeatedly tested negative for HPV. In these analyses, because no events occurred in current ex-smokers, women were categorised as smokers or non-smokers, with non-smokers having a smoking intensity of zero. In a univariate analysis, the risk of high-grade CIN in current smokers was twice that of current non-smokers; and increased significantly with smoking intensity (Table 1). In a multivariate analysis, controlling for past or current exposure to HPV16 or HPV18 or other HPV types, these hazards ratios decreased slightly, but remained significant. No association was found with the other smoking variables. The study population for this analysis was then expanded to include a further 410 women who were cytologically abnormal, HPV-positive, or both, at study entry, and who had further follow-up (the prevalent cohort): 28 progressed to high-grade CIN. When these women were included in the analysis, estimates of hazards ratios associated with smoking were not substantially changed (Table 1). When this analysis was repeated in the incident cohort with a diagnosis of CIN2 as outcome, censoring at the first detection of CIN3,

Table 1 – The association between smoking and the incidence of high-grade cervical intraepithelial neoplasia, controlling for cervical HPV status (n = 1075, 28 events).

	Incident cohort (n = 1075, 28 events)		All women (n = 1485, 56 events)	
	Crude HR (95% CI)	Adjusted HR (95% CI)[a]	Crude HR (95% CI)	Adjusted HR (95% CI)[a]
Have you ever been a cigarette smoker?				
No	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	1.27 (0.60–2.69)	1.06 (0.50–2.28)	1.50 (0.87–2.59)	1.33 (0.77–2.30)
	p = 0.54	p = 0.61	p = 0.13	p = 0.29
Pack-years[b]				
Never-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
(0, 1)	0.71 (0.20–2.52)	0.49 (0.14–1.77)	1.09 (0.48–2.47)	0.94 (0.41–2.14)
[1, 2)	1.55 (0.54–4.44)	1.66 (0.57–4.80)	1.83 (0.90–3.74)	1.56 (0.76–3.19)
[2, 3)	1.37 (0.37–5.02)	1.18 (0.31–4.42)	2.03 (0.89–4.64)	1.79 (0.78–4.07)
[3, +)	1.79 (0.54–5.97)	1.76 (0.52–5.91)	1.43 (0.56–3.64)	1.40 (0.55–3.55)
	p-trend = 0.54	p-trend = 0.28	p-trend = 0.31	p-trend = 0.12
Current smoking status				
Non-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Smoker	2.21 (1.04–4.68)	2.13 (1.00–4.52)	2.19 (1.29–3.74)	2.06 (1.20–3.52)
	p = 0.04	p = 0.05	p = 0.003	p = 0.007
Duration of current smoking episode[b]				
Non-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
(0, 24)	2.07 (0.66–6.45)	1.96 (0.63–6.12)	2.29 (1.02–5.14)	2.20 (0.98–4.94)
[24, 48)	2.91 (1.02–8.35)	2.72 (0.95–7.80)	2.89 (1.36–6.12)	2.62 (1.23–5.57)
[48, +)	1.94 (0.75–5.00)	1.91 (0.74–4.96)	1.85 (0.96–3.58)	1.75 (0.91–3.38)
	p-trend = 0.30	p-trend = 0.37	p-trend = 0.48	p-trend = 0.54
Current smoking intensity				
Non-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
1–9 per day	1.59 (0.60–4.26)	1.68 (0.62–4.51)	2.04 (1.06–3.92)	1.90 (0.99–3.66)
10+	2.87 (1.24–6.66)	2.54 (1.09–5.92)	2.35 (1.27–4.37)	2.21 (1.19–4.12)
	p-trend = 0.002	p-trend = 0.005	p-trend = 0.01	p-trend = 0.008

HPV = human papillomavirus; CIN = cervical intraepithelial neoplasia; HR = hazards ratio; CI = confidence interval.

a Adjusted for exposure to HPV16, HPV18 and other HPV types.

b [a, b) denotes the interval $\geqslant a$ to $<b$.

the hazard ratios associated with current smoking intensity were only modestly attenuated.

3.2. Smoking and the acquisition of cervical HPV infection

We next investigated whether smoking increased the risk of acquiring a cervical HPV infection. This analysis was restricted to 1075 women who were HPV-negative and cytologically normal at study entry, and who had further follow-up. The cumulative incidence at 3 years of a cervical infection with HPV of any type, type 16 and type 18, in these women, was 43.8% (95% CI 40.1–47.5), 10.5% (8.3–12.7) and 6.6% (4.8–8.4), respectively, as reported previously.[3] Four hundred and seven women first acquired a HPV infection during follow-up: 192 before starting to smoke; 198 afterwards; and 17 first reported starting to smoke at the same visit that HPV was first detected. One hundred and ten women were infected with HPV16, and 64 with HPV18. In univariate analyses, there was an increased risk of acquiring HPV associated with current smoking status and with a history of ever having smoked. In multivariate analyses, however, these associations became non-significant after controlling for life-time number of partners and age of oldest partner (Table 2). Similar hazards ratios were found when the analysis was restricted to HPV16 and HPV18 infections. There is no evidence to suggest that the risk of acquiring a HPV infection of any type, or a HPV16 or HPV18 infection, increases with either pack-years of exposure to smoking, or duration of current smoking episode; however, the range of values of these variables is not extensive in this population of young women.

3.3. Smoking and the duration of HPV infection

Finally, we investigated whether smoking prolonged the duration of a HPV infection. This analysis was restricted to the 1075 women who were HPV-negative and cytologically normal at study entry, and who had further follow-up (the incident cohort). The median duration of any HPV infection, HPV16 and HPV18 in these women, was 13.7 (inter-quartile range 8–25.4), 10.3 (6.8–17.3) and 7.8 (6–12.6) months, respectively.[3] Of 328 women with further follow-up after their first HPV infection, 130 were smokers when HPV was first de-

Table 2 – The association between smoking and the incidence of cervical infection with HPV DNA of any type (n = 1075, 407 events), HPV16 infection (n = 1075, 110 events) and of HPV18 infection (n = 1075, 64 events).

	Incidence of any HPV		Incidence of HPV16		Incidence of HPV18
	Crude HR (95% CI)	Adjusted HR (95% CI)[a]	Crude HR (95% CI)	Adjusted HR (95% CI)[b]	Crude HR (95% CI)
Have you ever been a cigarette smoker?					
No	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Yes	1.39 (1.14–1.69)	1.15 (0.93–1.41)	1.40 (0.96–2.05)	1.14 (0.76–1.69)	1.22 (0.74–1.99)
	p = 0.001	p = 0.19	p = 0.08	p = 0.53	p = 0.43
Pack-years[c]					
Never-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
(0, 1)	1.59 (1.25–2.01)	1.36 (1.06–1.74)	1.96 (1.27–3.03)	1.54 (0.99–2.40)	1.38 (0.75–2.54)
[1, 2)	1.16 (0.84–1.58)	0.99 (0.71–1.36)	0.90 (0.46–1.73)	0.72 (0.37–1.41)	0.90 (0.40–2.04)
[2, 3)	1.50 (1.01–2.21)	1.15 (0.77–1.71)	1.82 (0.94–3.54)	1.40 (0.71–2.74)	1.55 (0.64–3.74)
[3, +)	1.03 (0.56–1.90)	0.73 (0.39–1.37)	0.39 (0.10–1.56)	0.27 (0.07–1.10)	0.93 (0.28–3.07)
	p-trend = 0.20	p-trend = 0.04	p-trend = 0.01	p-trend = 0.005	p-trend = 0.94
Current smoking status					
Never-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Ex-smoker	1.41 (1.04–1.93)	1.22 (0.89–1.68)	1.77 (1.03–3.06)	1.48 (0.85–2.58)	1.18 (0.54–2.58)
Smoker	1.38 (1.11–1.70)	1.13 (0.90–1.41)	1.29 (0.86–1.95)	1.04 (0.67–1.59)	1.23 (0.73–2.08)
	p = 0.005	p = 0.37	p = 0.11	p = 0.38	p = 0.73
Duration of current smoking episode[c]					
Never-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Ex-smoker	1.41 (1.03–1.93)	1.22 (0.88–1.67)	1.77 (1.03–3.05)	1.47 (0.84–2.46)	1.18 (0.54–2.58)
(0, 24)	1.50 (1.12–2.01)	1.28 (0.95–1.73)	2.05 (1.23–3.41)	1.68 (1.00–2.83)	1.38 (0.65–2.92)
[24, 48)	1.34 (0.98–1.82)	1.10 (0.80–1.52)	0.69 (0.31–1.52)	0.56 (0.25–1.24)	1.14 (0.50–2.61)
[48, +)	1.30 (0.95–1.78)	1.00 (0.72–1.38)	1.15 (0.64–2.06)	0.88 (0.48–1.62)	1.17 (0.57–2.41)
	p-trend = 0.39	p-trend = 0.15	p-trend = 0.04	p-trend = 0.02	p-trend = 0.38
Current smoking intensity					
Never-smoker	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0	1.41 (1.03–1.93)	1.22 (0.89–1.68)	1.77 (1.03–3.06)	1.48 (0.85–2.58)	1.18 (0.54–2.58)
1–9 per day	1.48 (1.15–1.90)	1.25 (0.97–1.62)	1.28 (0.77–2.10)	1.05 (0.63–1.75)	1.15 (0.60–2.22)
10+	1.26 (0.96–1.67)	0.99 (0.74–1.32)	1.32 (0.79–2.20)	1.03 (0.60–1.75)	1.31 (0.68–2.52)
	p-trend = 0.69	p-trend = 0.31	p-trend = 0.29	p-trend = 0.20	p-trend = 0.98

HPV = human papillomavirus; HR = hazards ratio; CI = confidence interval.

a Adjusted for life-time number of sexual partners and age of oldest partner.

b Adjusted for life-time number of sexual partners.

c $[a, b)$ denotes the interval $\geqslant a$ to $<b$.

tected, and 18 started or recommenced smoking after this time; 12 of these women stopped smoking before the end of follow-up. Two hundred and sixty-nine women cleared their HPV infection during follow-up. Duration of any HPV infection, and of HPV16 infection, was unrelated to smoking; the sample size ($n = 55$) was insufficient for meaningful analysis of the duration of HPV18 (Table 3).

4. Discussion

We have shown that smoking is a risk factor for incident high-grade CIN in young women soon after they become sexually active. Current smokers were twice as likely to be diagnosed with high-grade CIN as non-smokers, after controlling for HPV status; this risk increased with current smoking intensity, and the magnitude of the effect was consistent with that observed in case-control and longitudinal studies performed in older women.[2] However, by making repeated measurements of smoking and HPV status in women who were free of disease and HPV-negative at study entry, we were able to reduce the risk of misclassification of exposure status. We were also able to avoid the residual confounding by time from first HPV exposure which may occur in case-control studies when HPV status is measured at only a single point in time, or which can occur in longitudinal studies when HPV status is only defined at enrolment.[1,2] Under-reporting of smoking behaviour *could* have had an influence on the analysis of this outcome, but would only have resulted in estimates of hazards ratios closer to the null value. We found no excess risk of high-grade CIN in former smokers, consistent with the pooled analysis described already, which concluded that the risk associated with past-smoking remains uncertain.[2]

It has been suggested that smoking could increase the risk of cervical neoplasia by increasing the risk of acquiring a cervical HPV infection. Revealing such an association requires careful consideration of the confounding effect of sexual behaviour, which is strongly correlated with smoking: failure to control for this may result in spurious measures of association.[6] Although we found an increased risk of acquiring a HPV infection associated with smoking, this was attenuated after adjustment for age of oldest partner and life-time number of sexual partners. Under-reporting of sexual behaviour might have explained a *positive* association between smoking and the risk of acquiring a HPV infection, but not the *absence* of an association. Under-reporting of smoking behaviour could have had an influence on the analysis of this outcome,

Table 3 – The association between smoking and the duration of cervical infection with HPV DNA of any type ($n = 328$, 269 events) and of HPV16 infection ($n = 90$, 76 events).

	Duration of HPV of any type Crude HR (95% CI)	Duration of HPV16 Crude HR (95% CI)
Have you ever been a cigarette smoker?		
No	1.00 (Reference)	1.00 (Reference)
Yes	1.05 (0.82–1.35)	1.41 (0.87–2.28)
	$p = 0.68$	$p = 0.15$
Pack-years[a]		
Never-smoker	1.00 (Reference)	1.00 (Reference)
(0, 1)	1.25 (0.91–1.71)	1.49 (0.87–2.58)
[1, 2)	0.92 (0.62–1.36)	1.38 (0.54–3.53)
[2, 3)	0.96 (0.59–1.54)	1.61 (0.70–3.69)
[3, +)	0.88 (0.53–1.47)	0.94 (0.22–4.02)
	p-trend = 0.32	p-trend = 0.89
Current smoking status		
Never-smoker	1.00 (Reference)	1.00 (Reference)
Ex-smoker	1.14 (0.78–1.66)	1.24 (0.65–2.34)
Smoker	1.03 (0.78–1.34)	1.54 (0.90–2.63)
	$p = 0.79$	$p = 0.28$
Duration of current smoking episode[a]		
Never-smoker	1.00 (Reference)	1.00 (Reference)
Ex-smoker	1.14 (0.78–1.66)	1.24 (0.65–2.34)
(0, 24)	1.18 (0.80–1.74)	1.31 (0.61–2.85)
[24, 48)	1.12 (0.73–1.73)	1.11 (0.49–2.49)
[48, +)	0.89 (0.62–1.26)	2.89 (1.25–6.68)
	p-trend = 0.43	p-trend = 0.35
Current smoking intensity		
Never-smoker	1.00 (Reference)	1.00 (Reference)
0	1.14 (0.78–1.66)	1.24 (0.65–2.34)
1–9 per day	1.20 (0.87–1.65)	1.68 (0.89–3.16)
10+	0.86 (0.61–1.22)	1.37 (0.68–2.77)
	p-trend = 0.12	p-trend = 0.98

HPV = human papillomavirus; HR = hazards ratio; CI = confidence interval.
a $[a, b)$ denotes the interval $\geqslant a$ to $<b$.

by biasing estimates of hazards ratios towards the null value. However, our findings are consistent with most other longitudinal studies of incident type-specific HPV infections.[7–10] Although one such study did report an increased risk of incident HPV infection associated with current smoking, after adjusting for the acquisition of a new sexual partner in the preceding 12 months, it did not distinguish between cervical and vulvovaginal infections;[11] another found an increased risk only in HIV-infected women.[12] Recent cross-sectional studies, which of course cannot distinguish between those factors associated with the acquisition of HPV from those associated with its persistence, continue to report inconsistent findings.[13–17] The most compelling of these, which included information on the prevalence of HPV in women recruited from 10 areas in four continents, found that among current smokers, the risk of being HPV-positive increased significantly with smoking intensity.[15] However, a clear dose–response relationship was seen only in women with one life-time sexual partner and the authors suggest that, had they been able to adjust their analysis for other aspects of sexual behaviour, then this association might have been attenuated. We believe that there is, as yet, insufficient evidence to conclude that smoking increases the risk of acquiring cervical HPV infection.

Although smoking is reported to impair the antibody response in women infected with HPV, we found no evidence that smoking prolonged either the duration of infection with any HPV, or specifically that of HPV16.[16,18,19] When measuring duration, we defined the onset of infection as the first detection of HPV DNA or of type-specific HPV DNA in women who tested negative for HPV DNA of any type at study entry; others have defined onset of infection as the first detection of a HPV type not present at baseline. In none of these studies however, was smoking shown to significantly increase the duration of infection.[7,12,20]

In contrast, inconsistent findings have been reported in studies which measure the duration of type-specific infections already present at study entry (prevalent type-specific infections). When the study population is defined in this way, the time of onset of infection, and hence the duration of infection, is unknown, and spurious measures of association may be revealed.[21–23] For example, one study found a significant and substantial reduction in the time to clearance of prevalent HPV16 and related infections in ever smokers compared to never-smokers, but no evidence of an association in those with incident infections.[24]

If smoking neither increases the risk of acquiring HPV infection, nor prolongs the duration of infection, then other mechanisms have to be invoked to explain its association with cervical neoplasia. One stream of enquiry flows from recent observations suggesting that exposure to certain behavioural and environmental factors during critical periods of development can result in persistent epigenetic changes which have phenotypic consequences.[25] Smoking-induced epigenetic changes offer a possible explanation for the associations we have revealed, given that the onset of sexual activity in young women is followed by extensive remodelling of cervical epithelium, a process which is accelerated by cigarette smoking.[26] Such changes are biologically plausible because nicotine and its derivatives are found in the cervical mucus of smokers: *in vitro* studies in untransformed and transformed cell lines show that short-term exposure to nicotine or cigarette smoke extract is followed by changes in the expression of the DNA methyltransferases *DNMT1*, *DNMT3A* and *DNMT3B*.[27–29] Empirical evidence in support of this proposition comes from the observation that aberrant methylation of the tumour suppressor gene, *p16 (CDNK2A)*, is strongly associated with current smoking in women with squamous cell cervical cancers and high-grade CIN.[30] However, for smoking-induced epigenetic changes to be a credible explanation for the increased risk of cervical neoplasia observed in young women, and not simply a consequence of the disease process, it will first be necessary to demonstrate in women who are free of disease, that smoking initiation precedes the appearance of these changes in cervical material. Such studies are underway.

Conflict of interest statement

None declared.

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