Hormonal contraceptive use and smoking as risk factors for high-grade cervical intraepithelial neoplasia in unvaccinated women aged 30-44 years: a case-control study in New South Wales, Australia

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Abstract

Background: Human papillomavirus (HPV) vaccines protect against HPV types 16/18, but do not eliminate the need to detect pre-cancerous lesions. Australian women vaccinated as teenage girls are now entering their mid-thirties. Since other oncogenic HPV types have been shown to be more prevalent in women ≥30 years old, understanding high grade cervical lesions in older women is still important. Hormonal contraceptives (HC) and smoking are recognised cofactors for the development of pre-malignant lesions.

Methods: 886 cases with cervical intraepithelial neoplasia (CIN) 2/3 and 3636 controls with normal cytology were recruited from the Pap Test Register of NSW, Australia. All women were aged 30-44 years. Conditional logistic regression was used to quantify the relationship of HC and smoking to CIN 2/3 adjusted for various factors.

Results: Current-users of HC were at higher risk for CIN 2/3 than never-users [odds ratio (OR)=1.50, 95%CI=1.03-2.17] and risk increased with increasing duration of use [ORs:1.13 (0.73-1.75), 1.51 (1.00-2.72), 1.82 (1.22-2.72) for <10, 10-14, ≥15 years of use; p-trend=0.04]. Ex-users had risks similar to never-users (OR 1.08, 95%CI=0.75-1.57) regardless of duration of use. Current smoking was significantly associated with CIN 2/3 (OR=1.43, 95%CI=1.14-1.80) and risk increased with increasing number of cigarettes/day (p-trend=0.02). Among ex-smokers, the risk of CIN 2/3 decreased with increasing time since quitting (p-trend=0.04).

Conclusions: In this benchmark study, current, long term users of HC and current smokers of ≥5 cigarettes/day were each at increased risk of developing CIN 2/3. Findings support smoking cessation in relation to decreasing the risk of pre-cancerous lesions and reinforce the continuing need for cervical screening for cancer prevention in vaccinated and unvaccinated populations.

Keywords: cervical intraepithelial neoplasia, human papillomavirus; hormonal

contraceptives; smoking; high grade; pre-cancer.

Abbreviations

CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus, IARC: International Agency for Research on Cancer; IUD: intra-uterine device; LSIL: low grade squamous intraepithelial lesion; NSW: New South Wales, PTR: Pap Test Register.

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1. Introduction

Australia was one of the first countries to implement a publicly funded National HPV Vaccination Programme. The programme commenced in 2007 and involved administering 3 doses of the quadrivalent vaccine (Gardasil™ , Merck) to 12-13 year old schoolgirls and until 2009 included a catch-up phase, where women aged up to 26 years were also offered vaccination. In 2013 the vaccination programme was extended to include boys aged 12-13 years and a 2-year catch-up phase for males aged 14-15 years. More than a decade since the implementation of the vaccination programme in Australia, reductions of 65%, 40% and 13% have been observed in histologically confirmed high grade cervical abnormalities in women aged <20 years, 20-24 years and 25-29 years [1], respectively. A 78% fall in population prevalence of vaccine-included HPV types in women 18-24 years [2] and a 73-90% decline in the incidence of anogenital warts in females 12-26 years [3] have also been reported. However, HPV vaccination will not eliminate the need to detect and treat pre-cancerous cervical lesions. In the catch-up phase, adult females were not tested for HPV16/18 infections prior to vaccination and administration of the vaccine after exposure has been found not to impact clearance of existing infections [4]. Also, in its current form, the HPV vaccine does not protect against oncogenic HPV types other than HPV 16 and 18. Although cervical HPV16/18 DNA is more prevalent in women under 30 years of age, studies have shown that other high risk HPV types become prevalent in women above 30 years [5-7]. Furthermore, taking into consideration that the majority of women worldwide have not been vaccinated against HPV, it is still important to understand the occurrence and determinants of high grade cervical lesions in women aged over 30 years.

Infection with oncogenic HPV types is required for the development of high grade cervical intraepithelial neoplasia (CIN) and cervical cancer, however, not all infected women develop pre-cancerous lesions. The International Agency for Research on Cancer (IARC) has classified smoking and combined oral contraceptives as carcinogenic to humans and its evaluation of the evidence has shown a causal association between these agents and cervical cancer [8-10]. Two collaborative analyses of data from international epidemiological studies on the relationship between the pattern of use of these agents and cervical cancer reported elevated risks of cervical cancer and CIN3 associated with smoking and the use of combined

oral contraceptives [11-12]. However, no Australian data were included in the collaboration on smoking and less than 100 cases from Australia were included in the collaboration on oral contraceptives. With temporal changes in the formulation of oral contraceptives in terms of oestrogen dose and progestagen type, investigating the association between pre-cancer and oral contraceptive use in a more recent cohort is warranted. Based on survey data from Australian General Practices, among women aged 35-44 years, 64 out of 1000 consultations were for contraceptive management and over half of these (58%) concerned the use of oral contraceptives[13]. Furthermore, about 14% of women aged 25-44 years reported being current smokers, with higher prevalence found among women living in areas of most disadvantage [14].

The aim of the current study was to measure the effects of hormonal contraceptive use and smoking history on the risk of developing high-grade cervical lesions for Australian women above 30 years of age.

2. Methods

2.1 Setting and Subjects

Data for this analysis were obtained from the Cervical Health Study, described previously [15]. Briefly, women were recruited from the NSW Pap Test Register (PTR) [16]. The PTR was established in 1996 and is a centralised database of NSW cytology results. It contains information on name, address, date of birth and cervical screening history of women who have had a Pap test, and each of their cytology and histology results except those for <1% of women who opt-out. Study recruitment was conducted between December 2006 and July 2011 and women were eligible if they were aged 20-64 years when they entered the study. Preliminary cases were defined as women with high-grade squamous intraepithelial lesions (HSIL), including a cytological prediction of cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) during the study period. The date of the first abnormality was regarded as the date of entry into the study and this test was referred to as the index test. The preliminary cases were frequency-matched by 5-year age band and date of index test to three preliminary controls (women with a normal Pap test result). Preliminary controls were selected at random

from the women meeting these criteria. For preliminary controls, the date of the test which was used to match them to the corresponding preliminary case was referred to as the index test date.

2.2. Definition of cases and controls

Cases and controls were then selected from their corresponding preliminary lists. Women with hysterectomy were excluded since the cervix is generally removed and so the risk of CIN 2/3 is negligible. Incident cases of CIN 2/3 were women with a CIN 2/3 smear cytology index test (i.e. the preliminary cases) that was also confirmed by a histology test within 3 months after the index test. Cases with CIN 2/3 cytology or positive histology within 5 years prior to the index test were excluded since they were considered to be prevalent cases. Controls were women with a normal index smear cytology test and no CIN 2/3 cytology or histology test within 5 years prior to the index test.

For this analysis, cases and controls aged 30-44 years were selected. The age limit of 44 was used as women aged 44 or older are less likely to be using oral contraceptives for prevention of pregnancy. Controls and cases were matched by 5-year age band (30-34, 35-39, 40-44) and date of index test (2-month periods).

2.3 Data collection and measurements

Questionnaires and consent forms were mailed to women who were registered with the NSW PTR and were eligible for the study. A help line was established to respond to participants' queries about the study, consent or assistance with questionnaire completion.
Non-respondents were followed up after two weeks with a repeat mailing.

A self-administered questionnaire sought information on demographic and relevant medical details, hormonal contraceptive use, history of smoking, alcohol consumption, reproductive and sexual history, use of menopausal hormone therapy and cervical screening history. In addition, data from the Pap Test Register were used to ascertain previous frequencies of Pap smears and the corresponding test results. Hormonal contraceptives included the combined pill, progestagen-only pill, injections, IUDs with hormones, implants and vaginal rings.

Current hormonal contraceptive users and/or smokers were defined as those who were using/smoking at the time of having the index Pap smear test or who had stopped less than a year before the date of the index test. Most of the questions used in the questionnaire have been used previously and validated in the UK Million Women Study [17]. Similar questions regarding use of injectable/implanted contraceptives were also included.

Increased attendance for cervical screening has been found to be associated with having children, having ever-used oral contraceptives and not currently smoking [18]. Therefore it is important to adjust for the number of Pap smear tests when assessing the potential risk factors for cervical disease. In Australia, it is recommended that cervical screening is carried out every second year; women with a smear result suggesting a low grade cervical lesion or a possible low grade squamous intraepithelial lesion (LSIL) are recommended to have a repeat cytology test at 12 months after the index smear; those aged over 30 years without a history of negative cytology in the preceding two to three years and with a low grade cervical lesion or a possible LSIL smear result are recommended to have a repeat cytology test within 6 months [19]. Hence, women with prior equivocal smears may have more subsequent smear tests over a relatively short period of time and an increased number of smear tests overall. To account for this, tests conducted up to 1.5 years prior to the index test in this study were not included in the number of prior Pap tests. That is, the number of Pap smear tests was counted for the period 1.5 to 5 years prior to the index text.

2.3 Statistical analyses

All statistical analyses were performed using Stata 11.0 software (StataCorp). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression analysis. Hormonal contraceptive use variables included separately in various regression models were: broad usage (never-user, ex-user, current-user); time since last use (never-user, ≥10yrs, 5-9yrs, 1-4yrs, current-user); and duration of use (never-user, ex-user <10yrs, ex-user 10-14yrs, ex-user ≥15yrs, current-user <10yrs, current-user 10-14yrs, current-user ≥15yrs). Smoking history variables included separately in various regression models were: broad usage (never-smoker, ex-smoker, current-smoker); time since quitting (never-smoker, ≥10yrs, 5-9yrs 1-4yrs, current-smoker); duration of use (never-smoker,

ex-smoker <10yrs, ex-smoker ≥10yrs, current-smoker <10yrs, current-smoker ≥10yrs); and number of cigarettes/day (never-smoker, ex-smoker <5cigarettes/day, ex-smoker ≥5cigarettes/day, current-smoker <5cigarettes/day, current-smoker ≥5cigarettes/day).

Models were adjusted for: parity (0, 1, 2, ≥3 children); age at first sexual intercourse (≥21, 19-20, 17-18, <17 years); number of Pap smears 1.5 to 5 years prior to the index cytology test (≥3, 2, 1, 0); lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10); and number of sexual partners in the last 5 years (0-1, 2, 3-5, ≥6). Models for hormonal contraceptive use were additionally adjusted for smoking (never-smoker, ex-smoker, and current-smoker) and models for smoking history were additionally adjusted for hormonal contraceptive use (never-user, ex-user, and current-user). Body mass index (BMI) and a history of sexually transmitted diseases were not included in the multivariable analyses because adjustment for these factors did not change the estimated odds ratio (and the number of sexual partners and age at first sexual intercourse which were included in the model are strongly associated with sexually transmitted diseases [18,20]). Women with missing data on confounders, hormonal contraceptive broad usage and/or smoking broad usage were excluded from all analyses and tabulations (although 'prefer not to answer' was a response option for some questions and given its own category). Women with missing data on other hormonal contraceptive use or smoking exposure variables (such as duration of use and time since stopping) were excluded from logistic regression analyses which included those variables.

Sexual behaviours such as prior number of sexual partners and age at first sexual intercourse are potentially strong confounders of the effects of both hormonal contraceptive use and smoking on CIN 2/3 because such behaviours are key determinants of exposure to HPV infection and are also often associated with hormonal contraceptive use and smoking [8-12]. Hence, to ensure that our results were not unduly affected by the method used to analyse sexual behaviour, we performed sensitivity analyses accounting for sexual behaviour in three additional ways. First, odds ratios were adjusted for finely-categorised versions of the 3 sexual behaviour covariates: 1) age at first sexual intercourse; 2) lifetime number of sexual partners; and 3) number of sexual partners in last 5 years. Second, odds ratios were adjusted for the 3 sexual behaviour covariates included as continuous variables. Functional forms for

these continuous covariates were modelled as the best-fitting first or second degree fractional polynomials with powers -2, -1, -0.5, 0, 0.5, 1, 2 or 3. Best-fit was determined using the algorithm recommended and described in detail by Royston et al [21]. Third, analyses were restricted to women who reported having only one sexual partner in the last 5 years (514 CIN2/3 cases and 2815 controls).

2.5 Ethics approval

The study was approved by the Cancer Institute NSW Population Ethics Committee; reference number Ref 2004/05/073. All participants completed and signed a patient consent form.

3. Results

Of the 17,968 women who completed and returned questionnaires, 6270 were aged between 30 and 44 years. Of these 6270 women, 2009 had a CIN 2/3 smear cytology index test (preliminary cases) and 4261 had a normal Pap smear result (preliminary controls). Of the 2009 preliminary cases, 1123 were excluded because of prior CIN 2/3 (n=152), hysterectomy (n=10), the absence of a confirmatory positive histology result (n=857) or incomplete data (n=104). Of the 4261 preliminary controls, 625 were excluded because of: prior CIN 2/3 (n=246), hysterectomy (n=13) or incomplete data (n=366). Thus a total of 886 cases and 3636 controls were included in the current study.

Ninety-three percent of controls were either current-users (55%) or ex-users (38%) of hormonal contraceptives (Figure 1). Among controls with data for duration of hormonal contraceptive use, 27% were current–users for 10 or more years. Among cases and controls, 94% and 92% had ever used the pill or the mini pill respectively (Table 1). The proportions of cases and controls who had ever used injections, IUDs with hormones or implants ranged from 6% to 10% per category (Table 1).

Table 1: Types of hormonal contraceptives ever used by cases and controls in NSW 2006-2011.

Hormonal contraceptive:	Controls n=3636 n (%)^	Cases n=886 n (%)^
Any hormonal contraceptive ever used:	**3389 (93)**	**842 (95)**
Pill and/or mini pill	3347 (92)	833 (94)
Injections	321 (9)	85 (10)
IUDs with hormones	306 (8)	57 (6)
Implants	239 (7)	62 (7)
Vaginal ring	21 (1)	6 (1)
No hormonal contraceptive ever used:	**247 (7)**	**44 (5)**

^ Percentages add to more than 100% due to women using more than one hormonal contraceptive type

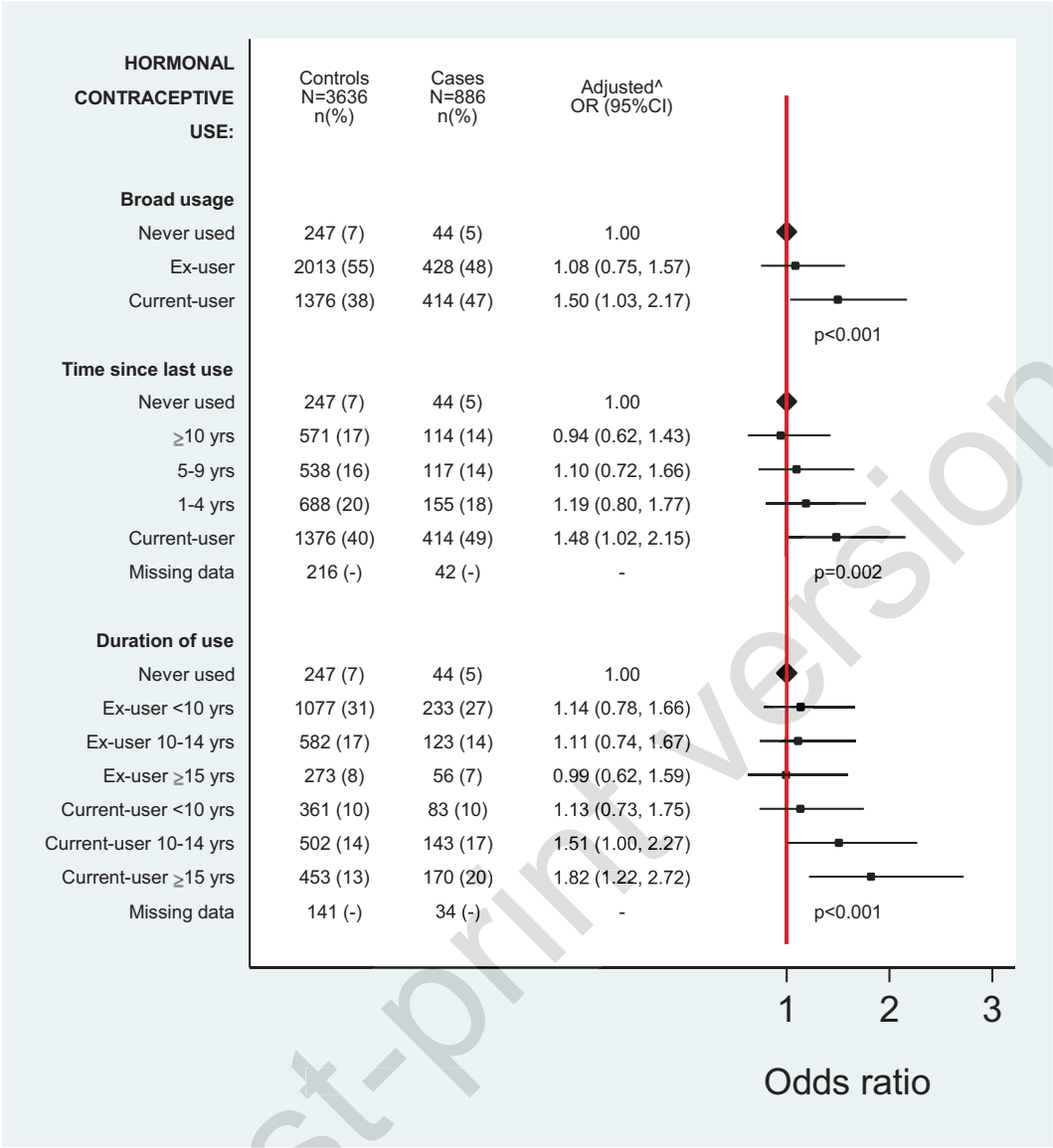
Among controls, never-users of hormonal contraceptives were more likely than ever-users to be nulliparous (26% vs. 23%), to be 21 years or older at first sexual intercourse (41% vs. 17%), to have had 1-2 lifetime sexual partners (51% vs. 26%), to have had 0-1 sexual partners in the last 5 years (82% vs. 78%) and to have had no smear tests 1.5 to 5 years prior to their index test (16% vs. 9%) (Table 2).

Table 2: Demographic characteristics and CIN 2/3 risk factors for control participants, according to hormonal contraceptive use and smoking history.

	Hormonal contraceptives		Smoking	
	Never used n=247	Ever used n=3389	Never smoked n=2105	Ever smoked n=1531
Characteristic	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Age groups (years)				
30-34	105 (43)	1503 (44)	988 (47)	620 (40)
35-39	80 (32)	1146 (34)	694 (33)	532 (35)
40-44	62 (25)	740 (22)	423 (20)	379 (25)
Age: mean, SD	36.6, 4.3	36.2, 4.1	36.0, 4.1	36.6, 4.2
Parity				
0	65 (26)	767 (23)	477 (23)	355 (23)
1	54 (22)	652 (19)	399 (19)	307 (20)
2	76 (31)	1222 (36)	776 (37)	522 (34)
≥ 3	52 (21)	748 (22)	453 (22)	347 (23)
Age at first sexual intercourse (years)				
≥ 21	102 (41)	565 (17)	552 (26)	115 (8)
19-20	48 (19)	479 (14)	371 (18)	156 (10)
17-18	47 (19)	1167 (34)	692 (33)	522 (34)
< 17	27 (11)	1060 (31)	392 (19)	695 (45)
prefer not answer	23 (9)	118 (3)	98 (5)	43 (3)
Lifetime no. sexual partners				
1-2	126 (51)	893 (26)	824 (39)	195 (13)
3-5	38 (15)	728 (21)	465 (22)	301 (20)
6-9	23 (9)	507 (15)	273 (13)	257 (17)
≥ 10	24 (10)	881 (26)	335 (16)	570 (37)
prefer not answer	36 (15)	380 (11)	208 (10)	208 (14)
No. sexual partners in the last 5 years				
0-1	203 (82)	2640 (78)	1713 (81)	1130 (74)
2	8 (3)	266 (8)	145 (7)	129 (8)
3-5	14 (6)	282 (8)	135 (6)	161 (11)
≥ 6	5 (2)	125 (4)	56 (3)	74 (5)
prefer not answer	17 (7)	76 (2)	56 (3)	37 (2)
No. Pap smears 1.5 to 5 years prior				
≥ 3	36 (15)	671 (18)	379 (18)	328 (21)
2	74 (30)	1427 (42)	916 (25)	585 (38)
1	98 (40)	981 (29)	622 (17)	457 (30)
0	39 (16)	310 (9)	188 (5)	161 (11)
No. Pap smears 1.5 to 5 years prior: mean, SD	1.5, 1.1	1.9, 1.2	1.8, 1.1	1.9, 1.3

Broad usage of hormonal contraception, time since last use and duration of use were all found to be associated with CIN 2/3 ($p<0.001$, $p=0.002$ and $p<0.001$ respectively) (Figure 1). Women who were ex-users of hormonal contraceptives had similar risks of CIN 2/3 as never-users [OR=1.08, (95%CI 0.75-1.57)] and the risks for ex-users did not vary significantly according to time since last use ($p=0.29$) or duration of use ($p=0.75$). Risks for current-users were found to be significantly higher than those for never-users [OR=1.50, (95% CI 1.03-2.17)]. Among current-users, risk increased with increasing duration of use, with OR of 1.13 (0.73-1.75), 1.51 (1.00-2.72) and 1.82 (1.22-2.72) for <10, 10-14 and >=15 years of use, respectively (p-trend=0.04). Among women who had used hormonal contraception for more than 15 years or between 10 and 14 years, those who ceased use were significantly less likely to develop CIN 2/3 than current-users in corresponding duration of use categories ($p<0.001$ and $p=0.039$ respectively).

Figure 1: Association between high-grade cervical intraepithelial neoplasia (CIN) 2/3 and hormonal contraceptive use.

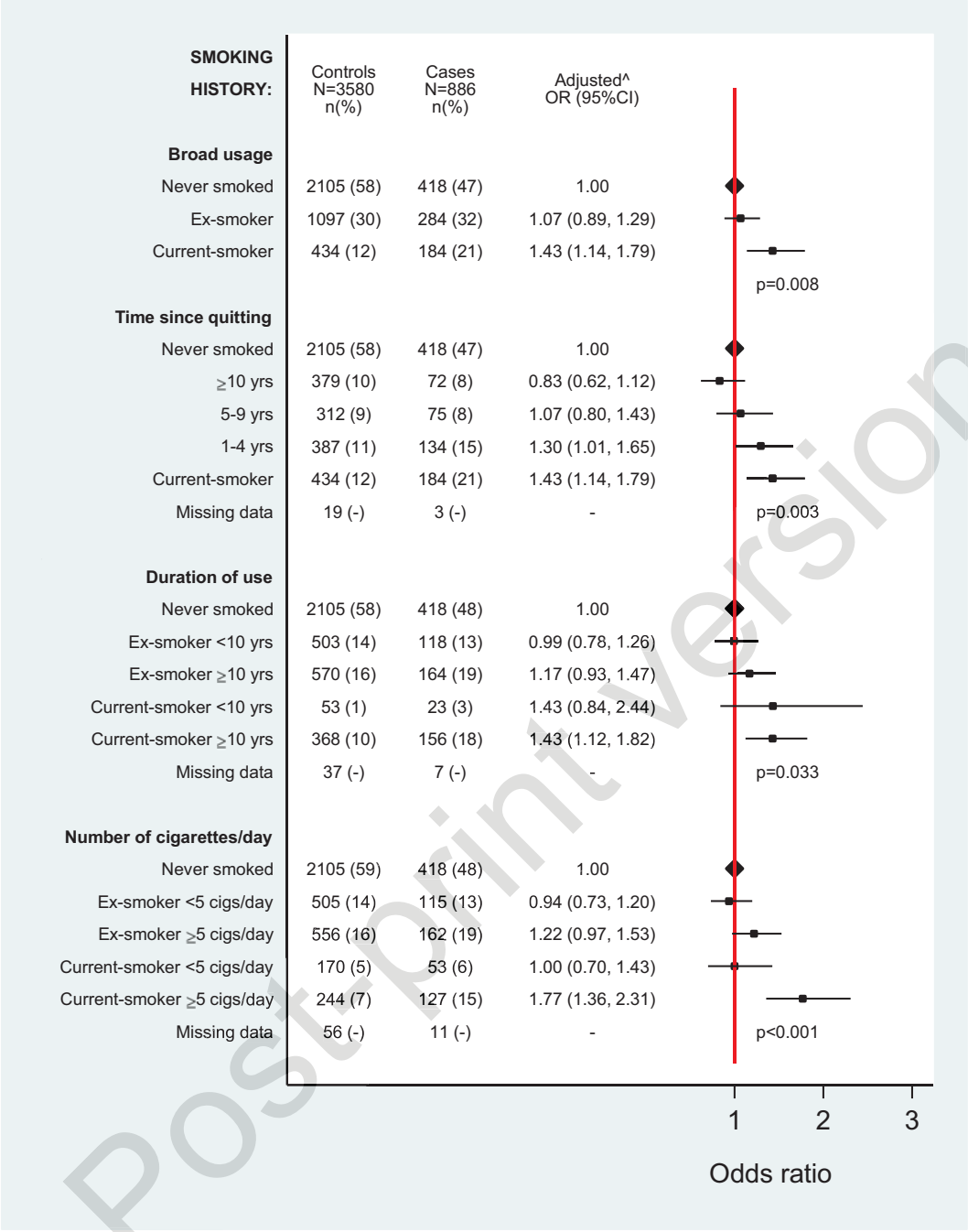


^ Adjusted for smoking broad usage (never-smoker, ex-smoker, current-smoker), parity (0, 1, 2, ≥3), age at first sexual intercourse (≥21, 19-20, 17-18, <17), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

p-values are for tests of global null hypotheses that odds ratios are equal within each variable.

In relation to smoking, 12% and 30% of controls were current-smokers and ex-smokers respectively, in keeping with background rates [14] with 87% of current-smokers with duration information having smoked for 10 years or more (Figure 2). Compared to controls who had ever smoked, never-smokers were more likely to have been 21 years or older at first sexual intercourse (26% vs. 8%), to have had 1-2 lifetime sexual partners (39% vs. 13%), to have had 0-1 sexual partners in the last 5 years (81% vs. 74%), and were less likely to have had no smear tests prior to their index test (5% vs. 11%) (Table 2).

Figure 2: Association between high-grade cervical intraepithelial neoplasia (CIN) 2/3 and smoking history.



^ Adjusted for hormonal contraceptive broad usage (never-user, ex-user, current-user), parity (0, 1, 2, ≥3), age at first sexual intercourse (≥21, 19-20, 17-18, <17), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).p-values are for tests of global null hypotheses that odds ratios are equal within each variable.

For smoking history, broad usage, time since quitting, duration of use and number of cigarettes/day were all found to be associated with CIN 2/3 (p=0.008, p=0.003, p=0.033 and p<0.001 respectively) (Figure 2). Current-smokers had higher risk of CIN 2/3 than never-smokers [OR=1.43, (95%CI 1.14-1.80)] and risks for current smokers varied significantly according to number of cigarettes/day (p=0.02). In particular, current-smokers who smoked ≥5 cigarettes/day were more likely to be diagnosed with CIN 2/3 than never-smokers [OR=1.77, (95%CI 1.36-2.31)]. Current-smokers who smoked ≥5 cigarettes/day were also at higher risk of CIN 2/3 than ex-smokers of ≥5 cigarettes/day (p=0.013). Risks of CIN 2/3 for current smokers did not vary by duration of use (p=0.99). Although ex-smokers had similar risks overall as never-smokers [OR=1.07, (95% CI 0.89-1.29)], there was some evidence that risks for ex-smokers varied according to time since quitting (p=0.04). Specifically, compared with never smokers, some increased risks were found for women who quit smoking 1-4 years ago [OR=1.30, (95%CI 1.02-1.65)] but not for those who quit 5-9 years ago [OR=1.07, (95%CI 0.80-1.43)] or more than 10 years ago [OR=0.83, (95%CI 0.62-1.12)]. Risks of CIN 2/3 for ex-smokers did not vary according to duration of use (p=0.26) or number of cigarettes/day (p=0.07).

There were no significant interactions between smoking and hormonal contraceptive broad usage (p=0.55). Sensitivity analysis indicated that effect estimates did not vary materially when different methods were used to account for sexual behaviour (Supplementary Figures A1 and A2).

4. Discussion

In this Australian study women aged 30-44 years who were current users of hormonal contraceptives were found to have around 50% increased odds of developing high grade CIN compared to women who had never used them, or had used them in the past. The risk of CIN2/3 increased with increasing duration of use among current users, but not among ex-users. Long term use of hormonal contraceptives was relatively common in this population. In addition, the odds of high grade CIN was increased by 43% among current smokers compared to never-smokers and rose with increasing intensity of smoking.

The current study presents the risks of CIN2/3 co-factors for women in their 30s and early 40s. These risks may change in successive cohorts of women offered the HPV vaccine as oncogenic HPV types other than HPV16/18 will become proportionally more prevalent in the population. Similarly, risks may also change in the long-term following administration of the nonavalent vaccine which has recently been approved in Australia for inclusion in the school-based immunisation programme from 2018 onwards [22]. Our findings therefore provide a benchmark against which the effects of smoking and oral contraceptive use can be assessed in future studies. Overall, despite the administration of the HPV vaccine, cervical screening will still be necessary for the early detection and treatment of high risk abnormalities. From December 2017, Australia transitioned from cytology-based screening to primary HPV screening with partial genotyping as part of the renewed National Cervical Screening Program. We have previously reported that transient increases in the detection of CIN2/3 lesions are predicted to occur in the initial three screening rounds due to increased sensitivity of HPV testing compared to cytology, enabling earlier detection of the lesions [23-25]. It should also be noted that within the renewed screening programme women will be differentially managed depending on the HPV types detected. Women positive for HPV16/18 will be referred directly to colposcopy and women positive for other oncogenic types will be triaged according to their liquid base cytology results. These different management pathways will also affect the number of CIN2/3 lesions detected overall. Therefore, in the context of a changing landscape in cervical cancer screening and HPV vaccination, there is a continued need to evaluate the effect of oral contraceptive use and smoking in relation to high grade abnormalities.

In 2007, the International Collaboration of Epidemiological Studies of Cervical Cancer combined individual participants' data from 25 studies involving 16,573 women with invasive cervical cancer, in situ cervical cancer or CIN 3 (cases) and 35,509 women without cervical disease (controls) [11]. The use of individual participant data provided the Collaboration far greater statistical control of sexual, gynaecological and obstetric confounders than previous meta-analyses of published effect estimates [9]. The Collaboration found that the risk of invasive cervical cancer and CIN3/cervical cancer in situ was increased

386 for current users of oral contraceptives [e.g. relative risk of invasive cancer for 5 or more
387 years' use versus never use, 1.90 (95%CI 1.69-2.13)] and declined after use ceased. Our
388 findings are in agreement with results from the Collaboration, and other prospective studies
389 reported subsequently as well as a previously conducted, Australian study of 117 women
390 [26-28]. The Collaboration also found that injectable progestagen-only formulations
391 increased the risk of cervical cancer. Although this was based on a small amount of data on
392 progestagen-only contraceptives this finding was later confirmed by a large South African
393 case-control study [29]. Our data were insufficient for disentangling the potentially different
394 effects of oestrogen-progestagen and progestagen-only formulations, however, we found an
395 increased risk of high grade cervical disease with current use of hormonal contraceptives
396 which is consistent with the findings of the Collaboration. We also found a pattern of
397 increased risk of CIN2/3 with more recent use of hormonal contraceptives for ex-users which
398 is in agreement with findings from the collaboration for CIN3/carcinoma in situ. The
399 confidence intervals for our results, however, were wider by comparison, which could be due
400 to smaller sample sizes.
401
402 The mechanisms by which hormonal contraceptive use increases the risk of cervical
403 neoplasms are not entirely clear. Epidemiological evidence suggests that use of hormonal
404 contraceptives promotes persistence of oncogenic HPV infections [30,31] which could lead to
405 progression to cervical cancer but does not increase the risk of new HPV infections [10-11,
406 30]. In addition, a number of laboratory-based studies have reported hormone-related
407 exposures inducing biological changes consistent with cervical disease progression. For
408 example, studies of the female reproductive tract of HPV16-expressing transgenic mice have
409 shown a possible synergistic mechanism between the oncogenes of HPV16 and chronic
410 oestrogen exposure which in turn modulates squamous cell carcinogenesis [32,33]. More
411 recently, genetic polymorphisms have also been identified that may act synergistically with
412 hormonal contraceptives and HPV infections to promote cervical carcinogenesis [34,35].
413
414 We also found that the risk of high grade cervical disease was higher for current-smokers
415 than never-smokers, increasing with the number of cigarettes smoked per day and with
416 increased duration of smoking. These results are consistent with the largest pooled analysis of

epidemiological studies of cervical cancer ever conducted on the association between tobacco smoking, invasive cervical cancer and CIN3/cervical cancer in situ [12]. A nested case-control study of European women reported similar findings after adjusting for serological markers of exposure to HPV, Chlamydia trachomatis and Human Herpes Virus 2 [36]. Although we found a small increased risk of CIN 2/3 for long-term ex-smokers (>10 years) compared with never-smokers this was not statistically significant, possibly due to small sample size or an attenuation of the risk from combining CIN2 and CIN3 cases together. Nevertheless, our data did show a significant trend of increased risk for ex-smokers with decreasing time since quitting.

The epidemiological evidence for a relationship between tobacco and cervical carcinogenesis is supported by a number of biological studies. Several of these demonstrated malignant transformations of papilloma and cervical tissue from exposure to chemical carcinogens contained in tobacco smoke [11,37]. Other studies have reported that smoking appears to additionally increase the risks of HPV infection and the likelihood of infection persistence through the suppression of cell-mediated immunity [38,39]. In a recent study on progression of HPV infections in adult women, those who smoked were significantly less likely to clear an infection than non-smokers [40]. The plausibility of a causal link between smoking and cervical carcinogenesis is also strengthened by evidence of tobacco-specific carcinogens in the cervical mucus of smokers [41].

This study has several limitations. First, as with all case-control studies using self-reported exposures, our results are potentially affected by recall bias. Second, despite the consistency of the results obtained using four different methods of adjustment for sexual behaviours, the possibility of residual confounding and/or confounding from unmeasured confounders remains. Third, we were unable to investigate associations for CIN3 cases alone as the NSW PTR reports high grade CIN as a combination of grades 2 and 3. Despite the above limitations, the current study has a number of strengths, including a large number of participants recruited from a single source, the NSW PTR, which provided Pap test histories for all participants. The availability of these screening histories in addition to sexual behaviour and other lifestyle characteristics enabled analyses to be adjusted for a range of

448 confounding factors. Furthermore, our findings are consistent with the findings of the two
449 major collaborations.
450
451 **5. Conclusions**
452 This population-based case-control study indicates that among Australian women 30-44 years
453 of age, current users of hormonal contraceptives and current-smokers were at increased risk
454 of developing CIN 2/3, and that longer duration of use and increasing intensity of exposure,
455 respectively, lead to further increase in risk. The evidence from this study also indicates that
456 these increased risks are generally reversible, with risks returning to similar levels as those
457 for never-users and never-smokers within 5 years of stopping/quitting. Our findings support
458 smoking cessation in users to decrease their risk of pre-cancerous lesions. Although we found
459 that contraceptives increase the risk of CIN2/3, combined oral contraceptives have been shown
460 to be protective in the long term against endometrial and ovarian cancers [42,43] in addition to
461 their effective contraceptive properties. Overall, our study reinforces the continuing need and
462 importance of routine cervical screening for cancer prevention in both vaccinated and
463 unvaccinated populations.
464
465
466 **Data Statement**
467 The dataset analysed for the current study is available from the corresponding author on
468 reasonable request.
469

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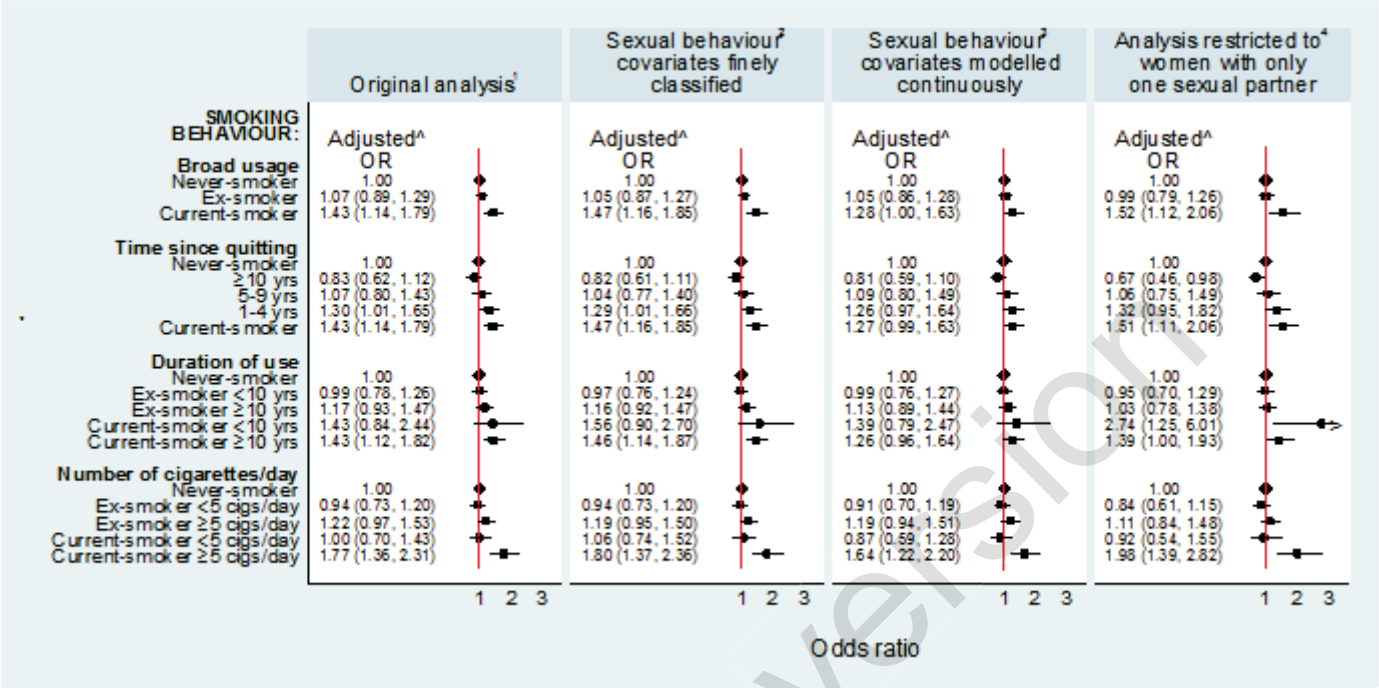
Appendix A

Figure A1: Comparability of odds ratios for hormonal contraceptive use estimated using different methods of accounting for sexual behaviour covariates.



^ All odds ratios adjusted for smoking broad usage (never-smoker, ex-smoker, current-smoker), parity (0, 1, 2, ≥3), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

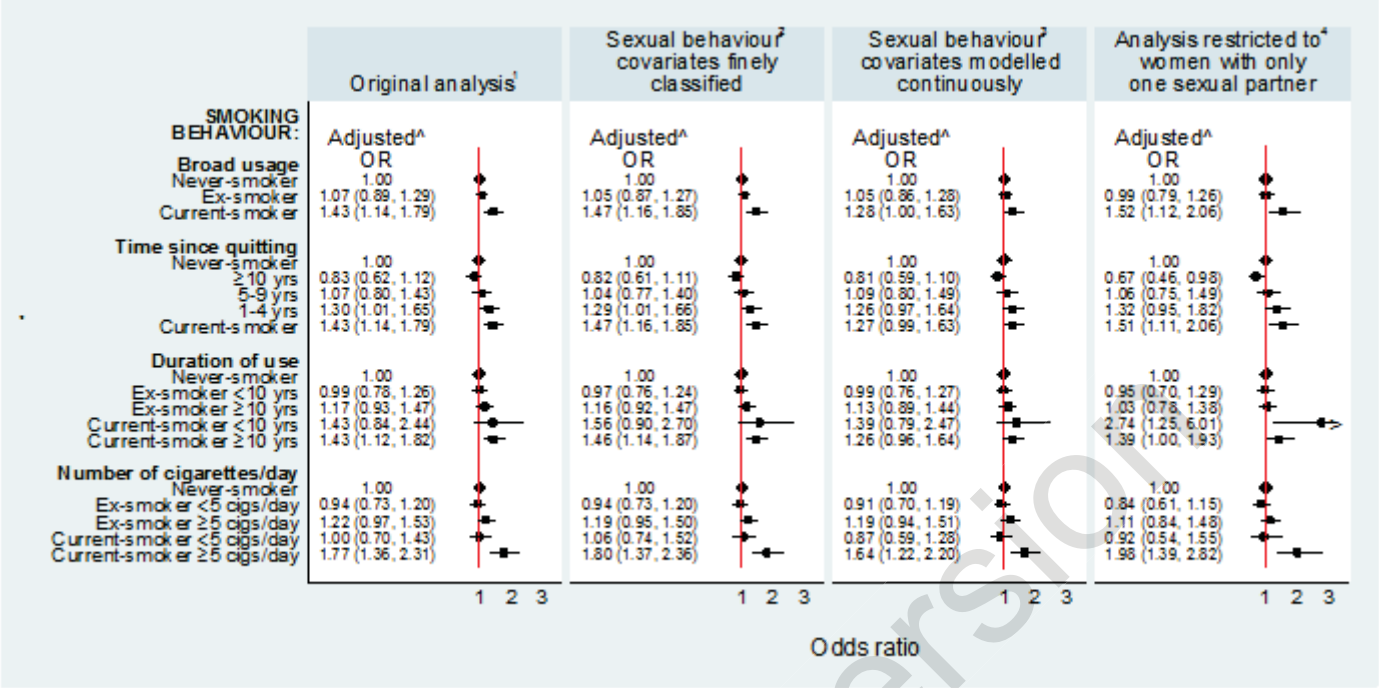
[1] Odds ratios additionally adjusted for age at first sexual intercourse (≥21, 19-20, 17-18, <17, prefer not to answer), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10, prefer not to answer), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6, prefer not to answer). These odds ratios are shown in Figure 1 and redisplayed here for convenience.

[2] Odds ratios additionally adjusted for age at first sexual intercourse (≥29, 28, 27,…..,13, 12, <12, prefer not to answer), lifetime number of sexual partners (1, 2, 3,….,24, ≥25, prefer not to answer), number of sexual partners in last 5 years (0,1, 2, 3,….,10,11, ≥12, prefer not to answer).

[3] Odds ratios additionally adjusted for age at first sexual intercourse (modelled continuously), lifetime number of sexual partners (modelled continuously) and number of sexual partners in last 5 years (modelled continuously). Functional forms for continuous covariates were modelled using fraction polynomials as described in the methods section. Data restricted to the 766 CIN 2/3 cases and 3181 controls who did not select 'prefer not to answer' to any of the 3 sexual behaviour questions.

[4] Odds ratios additionally adjusted for age at first sexual intercourse (≥21, 19-20, 17-18, <17, prefer not to answer), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10, prefer not to answer), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6, prefer not to answer). Data restricted to the 514 CIN II/III cases and 2815 controls who reported having only one sexual partner in the last 5 years.

Figure A2: Comparability of odds ratios for smoking behaviour estimated using different methods of accounting for sexual behaviour covariates.



^ All odds ratios adjusted for smoking broad usage (never-smoker, ex-smoker, current-smoker), parity (0, 1, 2, ≥3), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

[1] Odds ratios additionally adjusted for age at first sexual intercourse (≥21, 19-20, 17-18, <17, prefer not to answer), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10, prefer not to answer), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6, prefer not to answer). These odds ratios are shown in Figure 1 and redisplayed here for convenience.

[2] Odds ratios additionally adjusted for age at first sexual intercourse (≥29, 28, 27,…..,13, 12, <12, prefer not to answer), lifetime number of sexual partners (1, 2, 3,….,24, ≥25, prefer not to answer), number of sexual partners in last 5 years (0,1, 2, 3,….,10,11, ≥12, prefer not to answer).

[3] Odds ratios additionally adjusted for age at first sexual intercourse (modelled continuously), lifetime number of sexual partners (modelled continuously) and number of sexual partners in last 5 years (modelled continuously). Functional forms for continuous covariates were modelled using fraction polynomials as described in the methods section. Data restricted to the 766 CIN II/III cases and 3181 controls who did not select 'prefer not to answer' to any of the 3 sexual behaviour questions.

[4] Odds ratios additionally adjusted for age at first sexual intercourse (≥21, 19-20, 17-18, <17, prefer not to answer), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10, prefer not to answer), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6, prefer not to answer). Data restricted to the 514 CIN II/III cases and 2815 controls who reported having only one sexual partner in the last 5 years.

Table A1: Associations between CIN 2/3 and the potential confounders of the relationships between CIN 2/3 and hormonal contraceptive use and between CIN 2/3 and smoking history.

Characteristic	Controls n=3636	Cases n=886	Adjusted OR^
Parity: n (%) ^			
0	832 (23)	304 (34)	1.00
1	706 (19)	164 (19)	0.97 (0.76, 1.24)
2	1298 (36)	259 (29)	0.96 (0.76, 1.20)
≥ 3	800 (22)	159 (18)	0.97 (0.75, 1.25)
Age at first sexual intercourse (years): n (%) ^			
≥ 21	667 (18)	99 (11)	1.00
19-20	527 (14)	125 (14)	1.24 (0.91, 1.70)
17-18	1214 (33)	329 (37)	1.21 (0.92, 1.61)
< 17	1087 (30)	304 (34)	1.06 (0.79, 1.43)
prefer not answer	141 (4)	29 (3)	0.95 (0.56, 1.63)
No. sexual partners in the last 5 years: n (%) ~			
0-1	2843 (78)	519 (59)	1.00
2	274 (8)	89 (10)	1.50 (1.14, 1.97)
3-5	296 (8)	151 (17)	2.30 (1.81, 2.92)
≥ 6	130 (4)	96 (11)	3.44 (2.53, 4.69)
prefer not answer	93 (3)	31 (3)	1.69 (1.04, 2.73)
Lifetime no. sexual partners: n (%) #			
1-2	1019 (28)	90 (10)	1.00
3-5	766 (21)	183 (21)	2.35 (1.76, 3.13)
6-9	530 (15)	167 (19)	3.10 (2.29, 4.19)
≥ 10	905 (25)	333 (38)	3.26 (2.43, 4.36)
prefer not answer	416 (11)	113 (13)	2.48 (1.75, 3.51)
No. Pap smears 1.5 to 5 years prior: n (%)			
≥ 3	707 (19)	154 (17)	1.00
2	1501 (41)	260 (29)	0.90 (0.72, 1.14)
1	1079 (30)	250 (28)	1.15 (0.91, 1.45)
0	349 (10)	222 (25)	2.99 (2.31, 3.88)

^ Adjusted for hormonal contraceptive broad usage (never-user, ex-user, current-user), parity (0, 1, 2, ≥3), age at first sexual intercourse (≥21, 19-20, 17-18, <17), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

~ Adjusted for hormonal contraceptive broad usage (never-user, ex-user, current-user), parity (0, 1, 2, ≥3), age at first sexual intercourse (≥21, 19-20, 17-18, <17), number of sexual partners in last 5 years (0-1, 2, 3-5, ≥ 6), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

Adjusted for hormonal contraceptive broad usage (never-user, ex-user, current-user), parity (0, 1, 2, ≥3), age at first sexual intercourse (≥21, 19-20, 17-18, <17), lifetime number of sexual partners (1-2, 3-5, 6-9, ≥10), number of Pap smears in 1.5 to 5 years prior to index test (≥3, 2, 1, 0) and by matched design for age (5-year age groups) and date of index test (2-month periods).

~# Odds ratios for the number of sexual partners in last 5 years were not adjusted for lifetime number of sexual partners (and vice versa) to avoid collinearity.