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Oral Contraceptives are not an Independent Risk Factor for Cervical Intraepithelial Neoplasia or High-risk Human Papillomavirus Infections

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Abstract. *Background: Oral contraception (OC) has been proclaimed by the IARC as a risk factor of cervical cancer (CC), on prolonged use by high-risk human papillomavirus (HPV) positive women. However, the available data are far from complete, and more evidence is necessary on the potential confounding effects of sexual behavior and HPV infection. The aim of the present was study to analyse the risk estimates for OC users in order to develop several intermediate end-point markers in cervical carcinogenesis. Patients and Methods: A cohort of 3,187 women, enrolled in a multi-center screening trial in three New Independent States (NIS) of the former Soviet Union (the NIS Cohort Study), was stratified into three groups according to their contraception modes: i) non-users of contraception, ii) non-OC users and iii) OC users. These groups were analysed for predictors of three outcome measures: a) exposure to HR-HPV; b) progression to high-grade cervical intraepithelial neoplasia (CIN2/3 and HSIL); and c) persistence/clearance of HR-HPV and cytological abnormalities during a prospective follow-up. Results: All three groups had an identical prevalence of HR-HPV (HCII and PCR), Pap smear abnormalities and CIN histology, but differed significantly (p=0.0001) with regard to all key variables of sexual behaviour, known as risk factors for CC. Predictors of HR-HPV, CIN2/3 and HSIL were different in the three groups, reflecting these different sexual preferences. Use of OC was not a significant predictor of CIN2/3 or HSIL in HPV-positive or HPV-negative women. Outcomes of cervical disease and HR-HPV infection were unrelated to contraception. In a multivariate regression model, mode of contraception was of no predictive value for either HR-HPV or high-grade CIN. Conclusion: Sexual behaviour is different among OC users, non-OC users and in non-users of contraception; these risk factors predispose women to HR-HPV, high-grade CIN, and determine the outcome of their cervical disease/HR-HPV infection. The use of OC is not an independent risk factor for any of these intermediate end-point markers of cervical carcinogenesis. Failure to record these epidemiological data inevitably leads to erroneous conclusions about the role of OC as an independent risk factor of cervical cancer.*

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Shortly after introduction into general use, oral contraceptives (OC) were implicated as a risk factor with serious health impediments, including a variety of hormone-dependent cancers (1, 2). The first reports on possibly increased risk of cervical cancer (CC) among OC users (1-4) were followed by a large number of epidemiological studies reporting contradictory results regarding OC use as a risk factor for CC (3, 5-21). Furthermore, many reports have failed to establish any increased risk for CC associated with OC use (6, 7, 13, 14, 17-19, 20, 22-28), while others have reported OC use as increasing such a risk (9-11, 12, 15, 21, 29-31).

Following the concept implicating HPV as the most important etiological agent of CC since the late 1980's (32, 33), increasing attention has been focused on interactions between HPV and OC use, raising the question whether OC is an independent risk factor of CC or its involvement is merely confounded by the intimate association of HPV with CC (6, 7, 10, 11, 16, 17, 19, 20, 34-36). In the first published report on risk factors for HPV transmission (in 1984), use/non-use of contraception emerged among the most significant ones (37), but, later on, failed to establish any increased risk for HPV among OC users (38). Since the early 1990's, a sizeable number of studies have been published, reporting either an increased risk of HPV infection among OC users (34-36, 39-44), no risk related to OC at all (8, 16, 38, 45-51), or even a protective effect of OC use on the incidence of HPV infections (52-55).

All these data have been repeatedly reviewed by IARC experts, resulting in two separate monographs (56, 57). In the last (still in press), these experts based their evaluation on the pooled data from 8 IARC multi-center case-control studies comprising 1,561 CC patients and 1,916 controls (57, 58). Compared with never-users, women having used OC for less than 5 years, did not show an increased risk of CC (odds ratio, OR=0.73; 95% CI 0.52-1.03) (58). However, OR for CC was 2.82 (95% CI 1.46-5.42) among OC users for 5-9 years, and 4.03 (95%CI 2.09-8.02) for those used OC for >10 years, leading to the conclusion that long-term use of OC could be a co-factor that increases the risk of CC in women who are positive for HPV DNA. In subsequent reviews, these data have been interpreted with caution (59, 60), however, and even WHO does not recommend any changes in the practices of using oral contraceptives (61).

A cohort study testing 3,187 women for optional screening tools in three New Independent States (NIS) of the former Soviet Union has been recently conducted; almost 900 of these women have been followed-up to assess the natural history of HPV infections (62-64). In this NIS Cohort study, sexual habits and other potential risk factors of CC were also analysed (63). Using women with no contraception and those with non-hormonal contraception as controls, the role of OC use was analysed: i) in predisposing the women to HR-HPV infections, ii) as an independent risk factor for development of high-grade CIN or HSIL (intermediate endpoint markers in cervical carcinogenesis), and iii) as a predictor of HPV persistence during the follow-up.

Patients and Methods

Patients and study design. The subjects and the study design of this European Commission funded cross sectional and cohort study have been published previously (62, 63). The study cohort comprised 3,187 consecutive women attending six different outpatient clinics in three New Independent States (NIS) of the former Soviet Union between 1998-2002. These women were derived from three different groups: (i) cervical cancer screening (=SCR patients); (ii) attendants of gynaecology outpatient clinics with different indications (=GYN patients), and (iii) patients examined at STD clinics (=STD patients). The mean age of the women was 32.6 (±10.7 SD) years (median 30.6, range 15-85 years). All women were informed of the aims of the study (verbally and in writing) and all gave their written consent to participate. Only 90 out of the 3,187 women failed to take a Pap smear, and 100 refused sampling for HCII test.

The study design has been detailed in a series of previous papers (62-64). All eligible women (n=3,097) had Pap smear and were tested for high risk HPV with HCII. In addition, the first 1,500 patients were tested for HPV with PCR and confirmative hybridisation using the same DNA as for HCII, as described previously (65). Patients with Pap test of ASC or higher had biopsy confirmation (62, 63). Based on their HPV and Pap smear status, four sub-cohorts were built up: HPV–/Pap– (n=1,194), HPV–/Pap+ (n=876), HPV+/Pap– (n=315) and HPV+/Pap+ (n=709).

Follow-up. All women who presented with biopsy-confirmed low-grade lesions (HPV-NCIN or HPV-CIN I), were assigned for prospective follow-up, while high-grade lesions were treated, as detailed previously (62, 64). Follow-up at 6-month intervals included examination by colposcopy, Pap smear and punch biopsy (in suspected progression). Cytological samples for HPV testing were collected at each follow-up visit. Altogether, follow-up (FU) data were available on 887 women (Median FU 16.7 months), divided into four sub-cohorts according to their baseline HPV/Pap smear status: HPV–/Pap– (n=120), HPV–/Pap+ (n=128), HPV+/Pap– (n=191) and HPV+/Pap+ (n=444).

These four sub-cohorts were followed-up for the outcome of their cervical lesions (Pap status) and HPV infections. Four possible outcomes were recorded: (a) always Pap (or HPV) negative, (b) incident Pap abnormality (or new HPV), (c) persistent Pap abnormality (or HPV), and (d) cleared disease (or HPV infection). The number of women in these four categories of HPV outcome were: (a) n=134; (b) n=41; (c) n=273; and (d) 270. In addition, 118 women had only one HPV test done. For PAP smear outcome, the figures in the four categories were: (a) n=153; (b) n=141; (c) n=301 and (d) 238, and a group of additional 44 women had only one Pap test performed. The criteria for defining these four outcomes have been described in detail previously (64).

Epidemiological questionnaire. At the first visit, all women who gave their consent to participate in the study were subjected to detailed inquiry concerning the implicated risk factors of HPV, CIN and CC. This structured questionnaire contained 66 separate questions exploring the reproductive history sexual history, current sexual

practices, sexual hygiene, medical history and smoking habits (63). Out of the 3,187 women enrolled in the study, 2,894 (90.8%) agreed to complete this questionnaire, the completeness of the responses rarely falling below 85% (63).

Based on the data recorded on the modes of contraception, the patients were divided into three groups: (i) women using oral contraception (OC) (n=397), (ii) women using other (non-hormonal) contraception modalities (n=1012), and (iii) women who did not use any contraception (n=1374). In all analyses, the risk estimates for OC use to develop the outcome measure were controlled against the two other strata.

Papanicolaou (Pap) smears. Altogether, 3,097 women were subjected to conventional Pap smear, interpreted using the jointly agreed terminology (62). For statistical purposes, this classification was translated to the Bethesda 2001 system (62, 64). Primary screening and interpretation of the smears were done in the NIS laboratories, but all slides were subjected to re-screening by two International Academy of Cytology (IAC)-certified cytotechnologists and interpretation by one cytopathologist (FIAC) in Finland (62).

Directed punch biopsy. Directed punch biopsies (n=497) were taken from all colposcopic abnormalities, according to routine procedures. On histological grading of the lesions, CIN nomenclature was used. The presence of HPV infection was recorded using the accepted morphological criteria (33, 62).

Detection of HPV DNA by hybrid capture II assay. From 3,087 women, the sample for the Hybrid Capture II test was taken from the cervix using the HCII sampling kit (Digene, Silver Springs, MD, USA). The test was performed according to the provider's instructions using the probe panel B which detects 13 high-risk HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The RLU/CO value of 1 pg/ml (approximately 8,000 copies of HPV/test) was used as the cut-off for a positive test (33, 62).

Statistical analyses. Statistical analyses were performed using the SPSS® and STATA software packages (SPSS for Windows, Version 13.0.1., SPSS Inc., Chicago, USA, and STATA/SE 9.2., Stata Corp., Texas, USA). Frequency tables for categorical variables were analysed using the Chi-square test, with likelihood ratio (LR) or Fisher's exact test for significance. Differences in the means of continuous variables were analysed using non-parametric tests (Mann-Whitney, Kruskal-Wallis) or ANOVA, after careful control of the normal distribution (Kolmogorov-Smirnov test). *Post-hoc* tests (LSD, Tukey) were used to assess the differences between the individual strata in ANOVA tests. Logistic regression was used to analyse the power of different co-variates as predictors of the outcome variables (CIN2/3, HR-HPV), calculating crude odd ratios (OR) (and 95% CI). Significant variables in univariate analysis were entered into the multivariate regression models to calculate adjusted ORs (with 95% CI), using the stepwise backward or forward approach and LR (likelihood ratio) statistic for removal testing ($p=0.10$ probability for removal, and $p=0.05$ probability for entry). On particular occasions, confounding was also controlled by calculating the weighted-average of the stratum-specific estimates using Mantel-Haenszel test for common OR (with 95% CI). In all tests, the values $p<0.05$ were regarded as statistically significant.

Results

The key clinical and epidemiological data recorded using the questionnaires are summarised in Table I, stratified according to the three modalities of contraception. Importantly, the three groups were identical with regard to HR-HPV positivity, Pap smear abnormalities and CIN grades. In contrast, the three groups differed significantly ($p=0.0001$) in several important characteristics of their obstetric and gynaecological history and sexual preferences. In most respects, OC and non-OC users were alike, but differed from the group of non-users of contraception, *e.g.* the patient category, number of abortions, age at onset of sexual activity, number of partners during the previous 24 months, STD history, casual sex partners and history of skin and/or genital warts. Although the significant differences represented a minority of the 66 items recorded (not all listed in Table) (63), they included all the key variables of sexual behaviour known as risk factors for CC and its precursors. The results indicate that women with different contraceptive modalities also have a significantly different sexual behaviour.

The three groups were analysed for the predictors of high-grade CIN (CIN2 cut-off) in univariate analysis and the significant predictors are listed in Table II. HSIL Pap smear was the only significant predictor common to all three groups, while the number of deliveries predicted CIN2 in OC users and in women with no contraception. All other predictors were different in the three groups, and the list of significant predictors was more extensive for women without any contraception. When analysed separately for HPV-positive and HPV-negative women, use of OC was not a significant predictor of CIN2/3 in either group; OR=0.98 (95% CI 0.53-1.82) and OR=0.92 (95% CI 0.10-8.85), respectively. This is another indicator that the factors explaining the detection of CIN2/3 in these three groups are different.

When HSIL was used as the end-point marker (Table III), there were considerably fewer significant predictors in the three groups. In addition to young age (protective), the most significant predictor common to all groups is a positive HCII test ($p=0.0001$), with OR varying between 15.7 and 26.2 (not computable in OC users, because all HSIL lesions were HCII-positive). When analysed separately for HPV-positive and HPV-negative women, the use of OC was not a significant predictor of HSIL; OR 1.42; 95% CI 0.64-3.15.

The significant predictors of HR-HPV infections in the three groups are summarised in Table IV. As compared with the data in Table II and Table III, many more predictors are equally strong in all three groups. Years of age below 35, being an STD or GYN patient and HSIL Pap test, are all highly significant predictors of HR-HPV, while previous pregnancy is a significant protective factor against

Table I. *Key epidemiological characteristics related to mode of contraception.*

Characteristic	Users of oral contraceptives (n=397)	Users of other contraception (n=1,012)	Women with no contraception (n=1,374)	Significance *p*-value
Mean age (95%CI)#	31.3 (30.4-32.2)	31.7 (31.1-32.3)	33.8 (33.2-34.5)	**0.0001**
Patient category				
STD	22.9% (91/397)	24.3% (246/1012)	19.4% (266/1374)	
GYN	20.9% (83/397)	18.2% (184/1012)	26.6% (366/1374)	**0.0001**
SCR	56.2% (223/397)	57.5% (582/1012)	54.0% (742/1374)	
HPV positive (HCII test)	33.9% (132/389)	32.4% (323/998)	31.7% (429/1353)	0.709
HPV positive (TaqMan assay)	41.0 (153/373)	39.3% (374/951)	38.8% (510/1315)	0.738
PAP smear				
HSIL or worse	2.3% (9/386)	1.3% (13/1000)	1.8% (24/1344)	0.382
LSIL or worse	9.1% (35/386)	8.2% (82/1000)	8.0% (108/1344)	0.812
ASCUS or worse	21.0% (81/386)	17.2% (172/1000)	15.8% (212/1344)	0.061
Cervical biopsy				
CIN3 or cancer	13.0% (9/69)	13.1% (20/153)	16.5% (35/212)	0.599
CIN2 or worse	26.1% (18/69)	25.5% (39/153)	25.9% (55/212)	0.994
CIN1 or worse	47.8% (33/69)	47.1% (72/153)	44.8% (95/212)	0.869
Ever been pregnant	69.7% (276/396)	74.8% (755/1009)	79.3% (1088/1372)	**0.0001**
No. of deliveries (M±SD)#	0.62 (±0.722)	0.84 (±0.82)	0.94 (±0.92)	**0.0001**
Ever had miscarriages	15.6% (60/385)	13.4% (132/986)	17.2% (231/1341)	**0.040**
Ever had abortions	55.6% (214/385)	54.1% (533/986)	55.6% (743/1337)	0.747
Number of abortions (M±SD)#	1.95 (±1.37)	1.92 (±1.33)	2.24 (±1.74)	**0.0001**
Age at first sexual intercourse#	18.79 (±2.78)	18.92 (±2.71)	19.38 (±3.06)	**0.0001**
Sexual habits regular ever since	46.1% (177/384)	51.7% (502/971)	51.8% (692/1337)	0.123
Currently, only one sex partner	85.5% (337/394)	85.3% (855/1002)	82.6% (1107/1340)	0.140
No. partners during previous 2 yrs#	1.92 (±2.43)	2.04 (±3.20)	1.47 (±1.50)	**0.0001**
Ever had venereal disease	18.0% (70/388)	18.8% (186/991)	12.0% (162/1345)	**0.0001**
Sexual practices: oral sex	70.2% (259/369)	58.3% (543/931)	46.0% (557/1211)	**0.0001**
Sexual practices: anal sex	17.0% (56(329)	13.1% (107/815)	11.1% (121/1093)	**0.019**
Casual sexual partners	20.0% (76/380)	20.2% (196/970)	10.8% (145/1345)	**0.0001**
Casual contacts domestic	53.6% (52/97)	52.6% (131/249)	41.8% (118/282)	**0.022**
Casual contacts abroad	7.9% (18/227)	9.1% (55/607)	3.0% (24/811)	**0.0001**
Bide/douche at intercourse	97.7% (381/390)	95.4% (955/1001)	94.7% (1263/1334)	**0.028**
Douche requested from the partner	90.0% (352/391)	87.6% (877/1001)	87.7% (1170/1334)	0.397
History of skin or genital warts	29.7% (111/374)	29.6% (291/982)	22.4% (296/1323)	**0.0001**
History of previous CIN	6.4% (22/342)	8.0% (68/855)	7.8% (86/1096)	0.630
Ever taken Pap smear	43.8% (147/336)	42.7% (370/866)	37.7% (447/1186)	**0.028**
Time since the last Pap test (months)#	10.80 (±10.23)	11.21 (±10.42)	12.82 (±14.54)	0.291
Previous Pap test normal	62.7% (89/142)	71.3% (258/362)	73.9% (359/486)	**0.039**
Current smoker	24.6% (96/391)	27.6% (275/995)	26.7% (360/1349)	0.501
If yes, for how long (yrs)#	7.30 (±5.4)	7.65 (±5.58)	8.99 (±6.97)	0.075
Ever been smoker	19.0% (55/289)	18.6% (132/710)	21.5% (202/941)	0.315
How long did you smoke (yrs)#	4.36 (±2.89)	4.63 (±4.00)	5.73 (±4.66)	0.047
Time since stopped smoking (months)#	32.92 (±31.70)	54.55 (±63.70)	44.43 (±64.03)	0.133
Sexual partner regular smoker	60.1% (226/376)	55.0% (529/961)	59.1% (755/1278)	$p=0.098$

#Kruskal-Wallis test.

HR-HPV. Several other variables are significant predictors in two out of the three groups, and additional few predict HR-HPV in only one of the groups, implicating marked differences in the sexual habits and other recorded epidemiological variables between the three groups. Interestingly, treatment of cervical erosion is strongly protective in women with no contraception and those using non-OC, but not among OC users.

The outcomes of cervical disease and HPV infection as determined by repeated Pap test and HPV-testing with HCII are shown in Table V. All three groups are practically identical in their baseline HPV/Pap status ($p=0.440$), and no differences could be established between the three groups. This suggests that the mode of contraception (or no use of any) is not a significant determinant of the outcome of cervical disease or HR-HPV infections.

Table II. *Significant predictors of high-grade CIN as related to the use of contraception.*

Co-variate	Users of oral contraceptives		Users of other contraception		Women with no contraception	
	OR (95% CI)	*p*	OR (95%CI)	*p*	OR (95% CI)	*p*
Age <35 yrs					0.25 (0.13-0.48)	0.0001
Patient category (GYN)					10.35 (2.98-35.95)	0.0001
HCII test+	7.74 (0.89-66.62)	0.057*	2.60 (0.87-7.71)	0.072*	2.74 (1.31-5.91)	0.009
HSIL PAP	27.27 (2.97-249.9)	0.001*	33.60 (4.09-275.7)	0.0001	21.19 (5.87-76.48)	0.0001
Ever been pregnant					3.02 (1.27-7.15)	0.012
No. of deliveries	2.53 (1.14-5.61)	0.022			1.45 (1.06-1.99)	0.020
Onset of sexual activity			0.86 (0.76-0.99)	0.034	0.89 (0.80-0.99)	0.036
No. of recent (previous 12 months) sex partners					1.89 (1.10-3.23)	0.019
Ever had an STD					1.27 (1.09-1.42)	0.026
Casual sex contacts					0.11 (0.015-0.88)	0.010
Previous PAP taken					2.63 (1.33-5.19)	0.006
Previous PAP normal			0.22 (0.06-0.83)	0.028		
Partner current smoker					0.49 (0.26-0.93)	0.035
Cervical erosion treated	3.85 (1.09-13.65)	0.032				

#CIN2 cut-off; *Fisher's exact test.

Table III. *Significant predictors of HSIL as related to the use of contraception.*

Co-variate	Users of oral contraceptives		Users of other contraception		Women with no contraception	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age <35 yrs	0.23 (0.05-0.93)	0.033	0.22 (0.07-0.72)	0.008	0.14 (0.04-0.42)	0.0001
Patient category (GYN)			11.39 (1.41-91.94)	0.002		
HCII test+	NC	0.0001*	26.2 (3.39-202.3)	0.0001*	15.7 (4.66-53.06)	0.0001*
No. of deliveries	2.56 (1.19-5.51)	0.016				
Previous PAP taken	NC[1]	0.007				
Partner current smoker					0.33 (0.14-0.79)	0.011
Cervical erosion treated			4.69 (1.01-21.85)	0.035		

*Fisher's exact test; NC, not computable, all cases HCII+; NC[1], not computable, one empty cell.

The significant predictors of HR-HPV in univariate and multivariate analysis are listed in Table VI. Several of the variables were highly significant ($p=0.0001$) in univariate analysis, but importantly, neither the mode of contraception nor hormonal contraception (use/no use) were of any predictive value. All of these highly significant predictors were entered in a multivariate model (including the contraception data), and only 4 of these variables proved to be independent significant predictors: age <35 yrs, patient category, HSIL (all with $p=0.0001$), and being a current smoker ($p=0.001$). As expected, the mode of contraception or OC use were of no predictive value in this multivariate analysis.

Finally, multivariate analysis was performed to disclose the independent predictors of high-grade CIN (Table VII). Only two out of the almost 70 variables tested proved to be significant in the final regression model: (i) patient category (protective when STD used as reference); (ii) HCII result (HR-HPV detection). Importantly, the two variables recording contraception were not included among those 5 independent predictors of high-grade CIN.

Discussion

According to a recent IARC monograph, oral contraceptives were implicated as a risk factor for CC, particularly when used for prolonged periods by women infected with HPV (57, 58). It is important to emphasize, however, that this interpretation was based on only 8 IARC-sponsored case-control studies (58-61), and the interpretation of the data has been subjected to critical reviews (60, 61). However, when the 8 studies were extended to cover 28 eligible case-control studies, the long

Table IV. *Significant predictors of #high-risk HPV as related to the use of contraception.*

Co-variate	Users of oral contraceptives		Users of other contraception		Women with no contraception	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age <35 yrs	3.53 (2.08-6.00)	0.0001	3.05 (2.21-4.20)	0.0001	2.99 (2.32-3.86)	0.0001
Patient category:						
SCR	Reference		Reference		Reference	
STD	2.32 (1.38-3.88)	0.0001	2.21 (1.61-3.03)	0.0001	2.70 (2.01-3.64)	0.0001
GYN	2.55 (1.49-4.35)		1.85 (1.30-2.62)		2.06 (1.57-2.71)	
HSIL PAP	NC	0.0001*	26.2 (3.39-202.3)	0.0001*	15.7 (4.66-53.06)	0.0001*
Ever been pregnant	0.60 (0.39-0.95)	0.036	0.65 (0.48-0.88)	0.006	0.64 (0.49-0.85)	0.002
No. of deliveries			0.72 (0.61-0.86)	0.0001	0.78 (0.69-0.89)	0.0001
Early onset of sexual activity			1.06 (1.01-1.12)	0.015	1.07 (1.02-1.16)	0.001
Ever had an STD					1.43 (1.02-2.02)	0.044
Oral sex			1.35 (1.02-1.82)	0.046		
Casual sex contacts	3.17 (1.27-7.90)	0.017				
Good sexual hygiene (bide)			0.42 (0.23-0.76)	0.005		
Partner's good hygiene					0.69 (0.49-0.98)	0.046
Previous CIN	2.59 (1.04-6.44)	0.041				
Previous PAP normal	0.42 (0.21-0.87)	0.018				
Current smoker			1.39 (1.04-1.87)	0.026	1.37 (1.06-1.76)	0.018
Partner current smoker	1.87 (1.18-2.94)	0.006				
Cervical erosion treated			0.73 (0.55-0.99)	0.043	0.63 (0.49-0.82)	0.001

#Hybrid Capture II assay; *Fisher's exact test; NC, not computable.

Table V. *Clinical outcome of cervical lesions and HR-HPV infections as related to the use of contraception.*

Characteristics	Users of oral contraceptives (n=134)	Users of other contraception (n=298)	Women with no contraception (n=359)	Significance *p*-value
Baseline disease status				
HPV–/Pap–	11.2% (15/134)	15.4% (46/298)	14.9% (53/355)	
HPV–/Pap+	15.7% (21/134)	11.7% (35/298)	15.2% (54/355)	0.440*
HPV+/Pap–	26.1% (35/134)	21.8% (65/298)	18.9% (67/355)	
HPV+/Pap+	47.0% (63/134)	51.0% (152/298)	51.0% (181/355)	
Clinical outcome of lesions				
Always Pap-negative	14.3 (19/133)	20.5% (61/298)	17.0% (61/359)	
Incident abnormal PAP	20.3% (27/133)	14.4% (43/298)	14.8% (53/359)	
Persisting abnormality	33.8% (45/133)	31.2% (93/298)	35.9% (129/359)	
Cleared abnormal Pap	24.8% (33/133)	28.9% (86/298)	26.7% (96/359)	0.548**
One abnormal Pap only	4.5% (6/133)	4.0% (12/298)	5.0% (18/359)	
Fluctuating course	2.3% (3/133)	1.0% (3/298)	0.6% (2/359)	
Outcome of HR-HPV infections				
Always HPV-negative	14.2 (19/134)	13.4% (40/298)	17.1% (61/356)	
Incident HR-HPV	8.2% (11/134)	3.7% (11/298)	4.8% (17/356)	
Persisting HR-HPV	29.9% (40/134)	30.2% (90/298)	32.3% (115/356)	0.133*
Cleared HR-HPV	31.3% (42/134)	34.2% (102/298)	28.1% (100/356)	
Only one HPV+ test	6.7% (9/134)	12.8% (38/298)	12.9% (46/356)	
Fluctuating course	9.7% (13/134)	5.7% (17/298)	4.8% (17/356)	

#Hybrid Capture II assay; *Chi-Square test with LR statistic; **Fisher's exact test.

duration of OC use still remained a risk factor for CC, but ORs were not particularly impressive, and the 95% CI frequently spanned across 1.0, making these figures not significant or only marginally significant (60).

An examination of published literature could equally justify the opposite conclusion, because the studies failing to establish OC use as an independent risk factor of CC (6, 7, 13, 14, 17-20, 22-28) far outnumber those (9-12, 15, 21, 29-31)

Table VI. *Predictors of HR-HPV infections in univariate and multivariate regression analysis.*

Univariate regression analysis		
Co-variates	Crude OR (95% CI)	Significance *p*-value
Age	1.064 (1.055-1.073)	0.0001
Age <35 yrs	3.19 (2.63-3.74)	0.0001
Patient category		
Screening patient	Reference	0.0001
STD-patient	2.49 (2.07-3.01)	
Gynaecological outpatient	1.98 (1.64-2.39)	
HSIL Pap	19.81 (7.84-50.02)	0.0001
High-Grade CIN (CIN2 and above)	2.68 (1.33-5.39)	0.002
Any grade of CIN (Y/N)	3.19 (1.83-5.54)	0.0001
Any contraception (Y/N)	1.05 (0.89-1.23)	0.539
Oral contraception (Y/N)	1.09 (0.87-1.37)	0.448
Ever been pregnant (Y/N)	0.63 (0.53-0.76)	0.0001
No. of deliveries	0.77 (0.70-0.85)	0.0001
Ever have abortions	0.82 (0.69-0.96)	0.013
Ever have miscarriages	0.70 (0.56-0.89)	0.003
Early onset of sexual activity	1.06 (1.03-1.09)	0.0001
Sexual practices: cunni-lingus	1.25 (1.05-1.49)	0.012
Partner's good hygiene at intercourse	0.70 (0.55-0.89)	0.004
Previous Pap normal	0.73 (0.54-0.97)	0.030
Diabetes treated with insulin	6.18 (1.24-30.67)	0.018
Current smoker	1.39 (1.17-1.67)	0.0001
Partner current smoker	1.21 (1.03-1.44)	0.019
Cervical erosion treated	0.69 (0.58-0.83)	0.0001
Time since erosion treated (conf. by age!)		0.0001
Less than 1 year	Reference	
2-5 years	0.84 (0.62-1.13)	0.255
6-10 years	0.64 (0.45-0.90)	0.012
More than 10 years	0.53 (0.39-0.72)	0.0001

Multivariate regression analysis*		
Co-variates	Adjusted OR (95% CI)	Significance *p*-value
Age <35 yrs	2.91 (2.30-3.68)	0.0001
Patient category	1.31 (1.16-1.49)	0.0001
HSIL Pap	107.16 (14.25-805.76)	0.0001
Any contraception	0.95 (0.88-1.04)	0.295
Oral contraception (Y/N)	1.03 (0.79-1.37)	0.800
Ever been pregnant (Y/N)	1.28 (0.97-1.67)	0.071
No. of deliveries	0.93 (0.80-1.07)	0.342
Early onset of sexual activity	0.99 (0.96-1.03)	0.342
Current smoker	1.46 (1.17-1.82)	0.001
Cervical erosion treated	0.88 (0.72-1.08)	0.233
Time since erosion treated	1.01 (0.99-1.03)	0.206

*All highly significant ($p=0.0001$) co-variates in univariate analysis were entered in the model.

Table VII. *Predictors of high-grade CIN* in multivariate regression analysis.*

Multivariate regression analysis				
Co-variates	Adjusted OR	95% Confidence interval		Significance *p*-value
		Lower bound	Upper bound	
Patient category**	0.49	0. 29	0. 83	**0.008**
HCII test result	4.05	1.14	14.44	**0.031**
Ever been pregnant	1.92	0.57	6.43	0.285
No. of deliveries	1.05	0.67	1.74	0.826
Ever had abortions	0.42	0.16	1.06	0.069
Oral contraception (Y/N)	1.18	0.42	3.30	0.750
Onset of sexual activity	1.11	0.98	1.26	0.077
No. of partners (past 12 months)	0.79	0. 49	1.27	0.334
Ever had an STD	1.49	0. 40	5.48	0.544
Casual sexual partners	2.00	0.44	9.05	0.366
Ever taken Pap smear	1.09	0. 47	2.53	0.830
Current smoker	2.40	0. 34	16.74	0.374
Ever been smoker	1.20	0. 44	3.24	0.718
Partner current smoker	1.54	0.61	3.912	0.357
Ever had cervical erosion	0.97	0.44	2.17	0.953

*CIN2 cut-off; **STD category as reference.

reporting such an association. This association of OC use and CC is made far more complex by the strong causal link of HR-HPV types to CC (32, 33). This is because HPV infections are closely related to the sexual behaviour of women (and their partners), and these adopted sexual habits are in turn closely linked with individual women's preferences for contraception modes. Thus, any study claiming a causal association between OC use and CC should be able to control for the confounding effect of both HR-HPV and these sexual habits. As emphasized in the published commentaries (59-61), the IARC analysis managed to control (to some extent) for the confounding by HPV, but controlling for the variables of sexual behaviour of these women (and their partners) is far more complex, not the least because of the incomplete data recorded in these separate studies (57, 58). As highlighted by Skegg (59), another potential caveat in these analysis (58) could be that the logistic regression models can become unstable when adjusted for too many confounders and interaction terms, potentially leading to over-estimation of the relative risks.

The present approach to analysing the risk of OC use in the development of several intermediate end-point markers of cervical carcinogenesis is different from the traditional case-control designs used in the IARC studies (57, 58, 60, 61). Importantly, the IARC study completely omitted the data on other contraceptive measures by simply stating that these are unrelated to either the disease of interest (CC) or to the use of OC (58). As pointed out above, use of OC and other contraception, or opting not to use any, are certainly interlinked, because they represent different options in the

decision-making of individual women, reflecting their complex and dynamic socio-behavioural patterns, which (as core components) include their adopted sexual habits. We strongly believe that it is these behavioural patterns that confer the true risk of developing CC rather than the use of OC itself, which is only one of the several surrogate markers of these patterns. The three following hypothesis were tested and confirmed in the present study: (i) to demonstrate that the sexual behaviour is indeed different, among OC users, non-OC users and non-users of contraception; (ii) those different habits (irrespective of OC use) are the risk factors predisposing these women to HR-HPV, development of high-grade CIN (HSIL), and also influence on the outcome of their cervical disease/HR-HPV infection; and (iii) that the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis.

Importantly, the three groups demonstrate an identical prevalence of HR-HPV, Pap smear abnormalities and different CIN lesions (Table I). This strongly argues against the concept that OC increases the risk of CC and its precursors. These data are consistent with the majority of the published papers which fail to demonstrate such an association (6, 7, 13, 14, 17, 18-20, 22-28), and contradicts others (9-11, 12, 15, 21, 29-31) reporting increased prevalence of CIN/HSIL or CC among OC users. On the other hand, these three groups in our study differed significantly ($p=0.0001$) with regard to several key variables of sexual behaviour known to be risk factors for CC and its precursors (Table I). This is true with regard to their patient category, number of abortions, age at the onset of sexual activity, number of partners during the previous 24 months, ever having an STD, casual sex partners, as well as history of genital warts. These data clearly implicate that women with different contraceptive preferences have also adopted significantly different patterns of sexual behaviour and/or present with divergent gynaecological and obstetric histories. These observations fully confirm our first hypothesis, while demonstrating that the sexual behaviour is different among OC users, non-OC users and non-users of contraception. Importantly, the risk of HR-HPV, CIN2/3 (or HSIL) is not dependent on the mode of contraception. On the other hand, we could not confirm the single report (66), where use of OC was shown to be protective against high-grade CIN.

As expected, having HSIL is the single most significant determinant of CIN2/3 in all three groups in univariate analysis (Table II). Otherwise, the significant predictors of high-grade CIN are different in the three groups, being another indication of the complex behavioural patterns associated with the different contraception modalities. Failure to collect these data and to recognise their potential confounding effect in multivariate modelling, will inevitably lead to erroneous conclusions about the role of OC as a risk factor of CIN (CC), which might explain the discrepant results reported in the literature (5-31). Positive HCII test was the single most significant predictor of HSIL in all three groups, followed by the protective effect of younger age. As reported in the IARC study, OC was a risk factor of CC only in HPV-positive women (57, 58), but we could not confirm this. When the risk of CIN2/3 and HSIL was analysed separately among HPV-positive- and HPV-negative women, OC was not a significant predictor of CIN2/3 or HSIL in either group. This is clearly shown when the Mantel-Haenszel test was used to control for the confounding by HPV on the OC-HSIL or OC-CIN2/3 association, resulting in non-significant (common) OR in both cases (OR=1.27, 95% CI 0.58-2.78 and OR=0.98, 95% CI 0.54-1.77), respectively), thus, failing to demonstrate any confounding effect of HR-HPV. Hence, use of OC is not associated with increased risk of either CIN2/3 or HSIL in HPV-positive or HPV-negative women in the present study.

The predictors of HR-HPV detection in the three groups were further analysed (Table IV). Only four of these risk factors are shared by all three groups: age <35 yrs, patient category, HSIL Pap and ever been pregnant, all being highly significant determinants of HR-HPV infection. Another significant joint (protective) factor proved to be the treatment of erosion, when controlled in the Mantel-Haenszel test, with common OR=0.71 (95% CI 0.59-0.85) ($p=0.0001$). Again, these data implicate that, despite these shared risk factors, there is marked variability in these predictors of HR-HPV infection among the three groups, emphasising the importance of recording these data in studies exploring the role of OC use as a risk factor of CC. These different factors determine the exposure of the women to HR-HPV differently among OC users, non-OC users and non-users of contraception. Some of these might be even protective and could explain why CIN2/3 is not different in HPV-negative and HPV-positive OC users (see above).

Part of our second hypothesis implies that factors, other than the modes of contraception, are likely to determine the outcome of cervical disease and course of HR-HPV infections. The first prospective reports addressing the effects of OC on disease and/or viral outcome in the cervix have been published only recently (67-73). Not unexpectedly, the data are controversial and in part contradictory. While some studies have found OC use to increase persistent HR-HPV infections (67, 70), others have failed to confirm this (73), and in another, HPV clearance was actually faster among those who had ever used OC (71). In one study, OC use was suspected to promote progression of CIN (68), but two others could not ascribe any effect on disease progression (69) or clearance of LSIL for OC use (72). The present setting enabled us to assess (by serial PAP smears and HCII assays) both disease outcome and course

of HPV infection in relation to contraception (Table V). The outcome of clinical disease and HR-HPV infection was surprisingly similar among OC users, non-OC users and non-users of contraception. This clearly implicates that the outcome of cervical disease and HR-HPV infection is not determined by the modalities of contraception, which is in agreement with the reports that failed to link HPV persistence (73), disease progression (69) or LSIL clearance (72), to the use of OC. All the other predictors of disease outcome and course of HPV infections have been analysed in a series of recent reports from this cohort (64, 74-76).

One of the well established intermediate end-point markers of cervical carcinogenesis is persistent HR-HPV infection, being the single most important risk factor of CC (32, 33). One of the plausible mechanisms whereby OC use could contribute to the claimed increased risk of CC (57, 58) could be by increasing the susceptibility of OC users to oncogenic HPV types (59). Whether this is the case, however, remains highly controversial. Approximately an equal number of studies have reported an increased risk of HPV infection among OC users (34-36, 39-44), and fail to establish any such risk (8, 16, 38, 45-51). Still a few others have found OC use as protective against incident HPV infections (52-55). The results of the present study are in agreement with those where OC did not increase the prevalence of HR-HPV infections among the users (8, 16, 38, 45-51). In our series, only the commonly agreed risk factors of HR-HPV appeared as significant predictors in both univariate and multivariate regression models. Importantly, the mode of contraception or the use/non-use of OC did not appear among the factors that either increase or protect against HR-HPV in these women (Table VI). Exactly the same results were obtained in the final multivariate regression model, where all variables were separately tested by two software packages (SPSS & STATA/SE), using high-grade CIN as the dependent variable (Table VII). These data unequivocally show that the mode of contraception in general and the use/non-use of OC in particular did not significantly increase or decrease the risk of high-grade CIN in this cohort of 3,187 women. Thus, our data fail to confirm the studies reporting that OC use increases the risk of CC and/or its precursors (9-12, 15, 21, 29-31). Instead, our observations substantiate those reports where no increased risk for CC could be ascribed to the use of OC (6, 7, 13, 14, 17-20, 22-28).

To conclude, the present observations fully confirm our three hypothesis, while demonstrating that: (i) the sexual behaviour is different among OC users, non-OC users, and non-users of contraception; (ii) these different risk factors predispose the women to HR-HPV, development of high-grade CIN (HSIL), and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status; and (iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. The implications of these observations are straightforward: failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions about the role of OC as an independent risk factor of cervical cancer and its precursors.

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