Articles

Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study

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Summary

Background Use of oral contraceptives could increase risk of cervical cancer; however the effect of human papillomavirus (HPV), the main cause of cervical cancer, is not usually taken into account. We aimed to assess how use of oral contraceptives affected risk of cervical cancer in women who tested positive for HPV DNA.

Methods We pooled data from eight case-control studies of patients with histologically confirmed invasive cervical carcinoma (ICC) and from two studies of patients with carcinoma in situ (ISC). Information about use of oral contraceptives was obtained from personal interviews. Effects were estimated as odds ratios, with logistic-regression models adjusted for possible confounders.

Findings 1465 of 1561 (94%) patients with ICC, 211 of 292 (72%) with ISC, and 255 of 1916 (13%) controls were positive for HPV DNA. Compared with never-users, patients who had used oral contraceptives for fewer than 5 years did not have increased risk of cervical cancer (odds ratio 0·73; 95% CI 0·52–1·03). The odds ratio for use of oral contraceptives was 2·82 (95% CI 1·46–5·42) for 5–9 years, and 4·03 (2·09–8·02) for use for 10 years or longer, and these risks did not vary by time since first or last use.

Interpretation Long-term use of oral contraceptives could be a cofactor that increases risk of cervical carcinoma by up to four-fold in women who are positive for cervical HPV DNA. In the absence of worldwide information about HPV status, extra effort should be made to include long-term users of oral contraceptives in cervical screening programmes.

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Introduction

Since use of exogenous steroid hormones became more widespread in the 1960s, concern has been raised about the safety of such treatment in patients with neoplastic diseases. Suspicions that use of oral contraceptives is associated with cervical cancer have been raised in many epidemiological studies.[1] Human papillomavirus (HPV) has an important role in causation of cervical cancer,[2,3] and is probably a prerequisite for development of the disease, among other factors. Exogenous female hormones such as those used in combined oral contraceptives have been proposed as cofactors.[4]

Most investigations in case-control studies of the relation between cervical cancer risk and use of oral contraceptives have been of either population or hospital-based control women, without accurate information about the women's HPV status. 80–95% of controls did not have HPV DNA and thus, were probably not susceptible to cervical cancer, irrespective of their exposure to oral contraceptives. Absence of information on HPV could thus have biased estimates of the association to unity. Statistical analyses restricted to women who are positive for HPV DNA should help to assess the role of suspected cofactors in the presence of a strong cause such as HPV.[4–8]

Between 1985 and 1997, the International Agency for Research on Cancer (IARC) used similar protocols in case-control studies of invasive cervical cancer in eight countries and of carcinoma in situ in two. Because assessment of HPV DNA in cervical cells was reliable, these studies have provided a unique opportunity to investigate the role of HPV cofactors. Here, we present a pooled analysis of these studies, restricted to patients and controls who were positive for HPV DNA.

Methods

Data collection

We analysed the results of studies done in Thailand,[9] the Philippines,[10] Morocco,[11] Brazil,[12] Peru[13] (unpublished), Paraguay,[14] Colombia,[2,15] and Spain.[2,15] Incidence of cervical cancer varied greatly between countries. In Spain and Colombia, two further studies of cervical carcinoma in situ (ISC) were done simultaneously with those of invasive cervical cancer (ICC).[16,17] Because ISC is accepted as an immediate precursor of ICC, we have included these studies in our analysis. A previous analysis of the present data did not detect differences in risk factors between patients with ISC and those with ICC.[18]

Details of all studies have already been reported.[2,9–19] Eligible cases were incident, had histologically confirmed disease, had ICC or ISC, were resident in predefined study regions or were attending the reference hospitals, had had no previous treatment for the disease, and had agreed to participate in the study. Expert pathologists reviewed cytological and histological slides and confirmed diagnosis. Because we restricted our analyses

to patients with squamous-cell carcinomas, we excluded 135 women with cervical adenocarcinoma or adenosquamous carcinoma.

In the studies of ICC in Spain and Colombia, controls were population-based; all the others assessed patients who were hospital-based. In all studies, the number of controls and patients were matched in each 5-year age group. For hospital-based studies, patients with disorders potentially related to known cervical cancer risk factors (eg, other anogenital tumours; tumours of the breast, oral cavity, oesophagus, lung, bladder, or liver; and tobacco-related diseases) were excluded as controls. All protocols were approved by the IARC and the local ethics and research committees, and all patients gave written informed consent.

Questionnaire

Women were interviewed face-to-face by trained interviewers, who used a structured questionnaire. Proxy interviews were not accepted. We tabulated information about life-long use of contraceptives from a life-time calendar, with periods defined by changes in contraceptive pattern. For each time of contraceptive use, the method used and the age at starting and stopping was recorded. From these tables, we computed ever use, age at start, total duration of use, time since first use (latency), and time since last use (recency). The questionnaire used in Paraguay[14] only included ever use of oral contraceptives and duration of use. Thus, patients and controls from Paraguay were excluded from some analyses. Oral contraceptives could not be accurately distinguished from other hormonal-type contraceptives. However, use of contraceptives based on injectable progestagens was generally less than 5% of total hormone contraceptive use in the countries studied.[4] Thus, although in some studies a few women might have used other hormonal contraceptives, for simplicity, we will always refer to oral contraceptive use.

Detection of HPV DNA

Two scrapes of exfoliated cells were taken with a conventional wooden spatula. After preparation of one smear for Papanicolaou staining and reading, the remaining cells were eluted in saline, centrifuged, and frozen at –20°C or –60°C until shipment to the laboratories for HPV testing. Details of the hybridisation assays are provided in the individual reports.[2,9–17] HPV DNA detection and typing were done in central laboratories which used PCR amplification methods that targeted a small fragment of the *L1* gene. Quality of DNA was assessed with β-globin primers. HPV DNA with a negative amplification of β-globin was not detected in 430 patients and 478 controls, and these women were excluded from our analysis. MY09 and MY11 consensus primers were used in Colombia and Spain,[19] and GP5+/6+ primers in the remaining studies. Presence of HPV in PCR products was assessed with low-stringency Southern blot hybridisation with various probes that are specific to HPV.[20] For viral genotyping, PCR products were successively hybridised with oligonucleotide probes for 33 HPV types.[20,21] Samples that tested positive for HPV DNA but did not hybridise with any of the 33 probes were labelled HPVX.

Where biological material was available, E7 primers for 14 high-risk HPV types[3] were then used to reamplify all samples from patients who were positive for the β-globin gene and classified as HPVX or as HPV DNA negative; all samples from controls classified as HPVX; and a subsample of specimens from controls who were β-globin-positive and HPV DNA negative. This procedure meant that 32 patients were reclassified as positive for HPV DNA. Standard precautions were taken to keep the risk of false-positive results in the PCR reactions to a minimum.[22] All PCR assays were done masked to the case or control status. Because some retests were done at completion of fieldwork in all study regions, numbers of patients and controls who were positive for HPV in our analysis sometimes differ from those originally reported.

Statistical analysis

We fitted logistic regression models to individual data,[23] and investigated associations between disease status and exposure with likelihood ratio tests. We assessed trends for quantitative variables with an increasing score for every level of the categorised variable. Every level was compared with never users. Summary odds-ratios and their corresponding 95% CIs were calculated. When more than two groups were compared, CIs were calculated by treating relative risk as floating absolute risk.[24] This method assigns a variance to the reference category and reduces unwanted correlation between coefficients, thereby diminishing corresponding variances. No change is made, however, to the estimates of risk ratios.

All analyses were adjusted by centre, age (five categories), educational level (three categories), number of Papanicolaou smears during life (three categories: none, one to five, and six or more), number of births (linear trend with five categories), number of sexual partners during life (linear trend with three categories), age at first sexual intercourse (linear trend with five categories), and interaction terms of centre with age, education, and lifetime number of Papanicolaou smears. We investigated other potential confounders, but judged adjustment for these to be unnecessary because they were unrelated either to disease or to use of oral contraceptives, or because they did not change the odds ratio estimates for oral contraceptives. These confounders were race, rural area of residence, marital status, use of contraceptive methods other than oral contraceptives (eg, condom, intrauterine devices, others), smoking habits, and seropositivity to *Chlamydia trachomatis* and herpes simplex-2 virus. We measured heterogeneity among centres with a likelihood ratio test that compared the model of interaction between centres and exposure to the model that measured the main effects only.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

1853 patients with squamous cell carcinoma and 1916 controls were included in the original studies (table), of whom 1676 and 255, respectively were positive for HPV DNA, and were thus included in our analysis. The proportion of controls who were positive for HPV DNA ranged from 5% in Spain to 22% in Morocco. The proportion of patients who were positive for HPV DNA ranged from 70% in Colombia to 97% in Paraguay.

Women participating in these studies had high parity (median of five [IQR 3–7] births in patients and four [2–6] in controls), and were mostly monogamous (973 [58%] of 1676 patients and 182 [71%] of 255 controls); 461 (28%) patients and 48 (19%) controls had never been to school. 740 (44%) and 106 (42%), respectively,

	Controls	HPV tested		HPV positive		HPV prevalence		Age*		Ever use of oral contraceptives†		Duration of use of oral contraceptives‡	
		Patients (n=1853)	Controls (n=1916)	Patients (n=1676)	Controls (n=255)	Patients	Controls	Patients	Controls	Patients (n=1676)	Controls (n=255)	Patients	Controls
Invasive carcinoma													
Thailand	H	339 (18%)	261 (14%)	327 (21%)	41 (16%)	96%	16%	50 (41–59)	48 (36–56)	132 (40%)	20 (49%)	4 (2–8)	2 (1–4)
Philippines	H	331 (18%)	381 (20%)	319 (19%)	35 (14%)	96%	9%	47 (38–55)	47 (40–58)	66 (21%)	12 (34%)	2 (1–5)	2 (1–3)
Morocco	H	175 (9%)	176 (9%)	170 (10%)	38 (15%)	97%	22%	50 (41–60)	42 (32–48)	76 (45%)	16 (42%)	5 (2–8)	2 (1–3)
Brazil	H	169 (9%)	196 (10%)	164 (10%)	34 (13%)	97%	17%	51 (45–61)	49 (41–57)	61 (37%)	10 (29%)	4 (2–10)	2 (1–3)
Peru	H	171 (9%)	175 (9%)	163 (10%)	31 (12%)	95%	18%	47 (40–59)	49 (42–63)	47 (29%)	5 (16%)	5 (2–10)	2 (1–3)
Paraguay	H	106 (6%)	91 (5%)	104 (6%)	18 (7%)	98%	20%	48 (40–57)	47 (36–54)	11 (11%)	7 (39%)	2 (2–4)	3 (2–4)
Colombia	P	111 (6%)	126 (7%)	87 (5%)	22 (9%)	78%	18%	44 (36–56)	48 (39–60)	35 (40%)	8 (36%)	12 (8–16)	15 (10–17)
Spain	P	159 (9%)	136 (7%)	131 (8%)	8 (3%)	82%	6%	54 (45–64)	55 (46–62)	31 (24%)	0 ..	12 (8–14)	.. ..
Total with invasive carcinoma		1561 (84%)	1542 (80%)	1465 (87%)	227 (89%)	94%	15%	49 (41–59)	47 (38–57)	459 (31%)	78 (34%)	5 (2–10)	2 (1–4)
Carcinoma in situ													
Colombia	C	135 (7%)	181 (9%)	96 (6%)	19 (8%)	71%	11%	34 (29–44)	36 (26–44)	60 (63%)	9 (47%)	8 (6–13)	6 (2–14)
Spain	C	157 (9%)	193 (10%)	115 (7%)	9 (4%)	73%	5%	33 (29–38)	35 (31–38)	86 (75%)	5 (56%)	5 (3–11)	9 (8–11)
Total carcinoma in situ		292 (16%)	374 (20%)	211 (13%)	28 (11%)	72%	7%	34 (29–40)	36 (28–40)	146 (69%)	14 (50%)	7 (3–12)	8 (2–13)
Overall total		1853	1916	1676	255	90%	13%	47 (38–57)	46 (36–56)	605 (36%)	92 (36%)	5 (2–10)	3 (1–6)

H=hospital, P=population, C=clinic. *Median (IQR) among women who were positive for HPV. †Of those who were HPV positive. ‡Median (IQR) years among women who are positive for HPV and use oral contraceptives.

Distribution of patients with invasive and in situ cervical carcinoma and control women by various characteristics and centre

had never had a Papanicolaou smear; and 136 (8%) patients had received six or more Papanicolaou smears compared with 50 (20%) controls. In studies of ICC, 520 (31%) patients who tested positive for HPV DNA had ever used oral contraceptives. The number ranged from 11 (11%) of 104 in Paraguay to 76 (45%) of 170 in Morocco; the number of controls was 87 (34%), and ranged from none of eight in Spain, to 20 (49%) of 41 in Thailand. As expected because of the young age of the women, more women in the ISC studies in Spain and Colombia[16] had ever used oral contraceptives than in other countries. In Colombia, 60 (63%) of 96 patients and nine (47%) of 19 controls who were positive for HPV had ever used oral contraceptives, compared with 86 (75%) of 115 and five (56%) of nine, respectively, in Spain.

Women who were positive for HPV and who had ever used oral contraceptives were almost 1·5 times more likely to develop cervical cancer than controls (figure 1). The odds ratio for ICC, was almost half that for ISC (figure 1). Ever use of oral contraceptives was not associated with cervical cancer in Paraguay, the Philippines, and Thailand, whereas an increase in risk was recorded in the other centres (figure 1). The test for heterogeneity among studies was marginally significant (p=0·052).

Average duration of use of oral contraceptives among users was 6·1 years (SD 5·0), with a range from 1 to 21 years. Women from Spain and Colombia had used oral contraceptives for longer than those from other countries (p<0·0001; table). Figure 2 shows no increase in risk of cervical neoplasia for duration of oral contraceptive use for up to 4 years (odds ratio 0·73; 95% CI 0·52–1·03). However, use of oral contraceptives for longer than

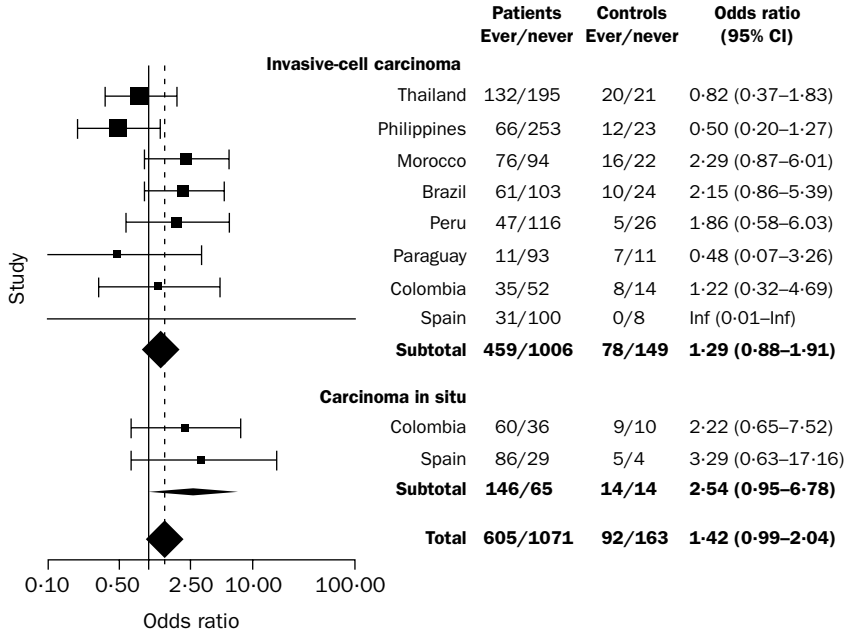

Figure 1: **Risk of cervical neoplasia associated with ever use of oral contraceptives**
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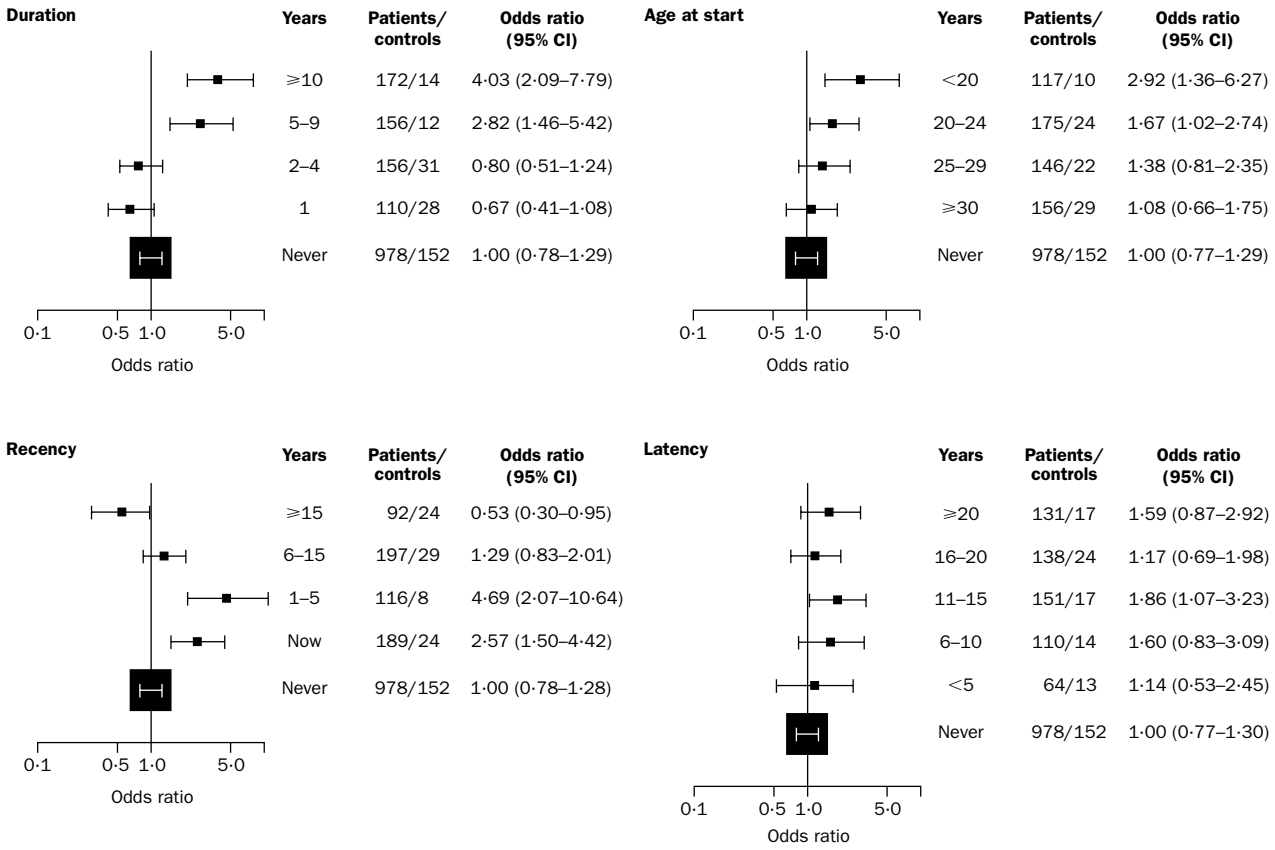

Figure 2: **Risk of cervical neoplasia associated with duration, age at start, recency, and latency of use of oral contraceptives**

5 years was significantly associated with cervical cancer (3·42; 2·13–5·48; p<0·001; figure 2). Figure 3 shows the odds ratio estimates for use of oral contraceptives for 5 years or longer. Odds ratios were more homogeneous among studies for long duration (heterogeneity test p=0·14) than for ever-use of oral contraceptives. Women who had used oral contraceptives for longer than 5 years had a four-fold increase in risk of developing ICC, and were 2·87 times more likely to develop ISC (figure 3). The pooled odds ratios for ICC did not differ from those of ISC (p=0·15).

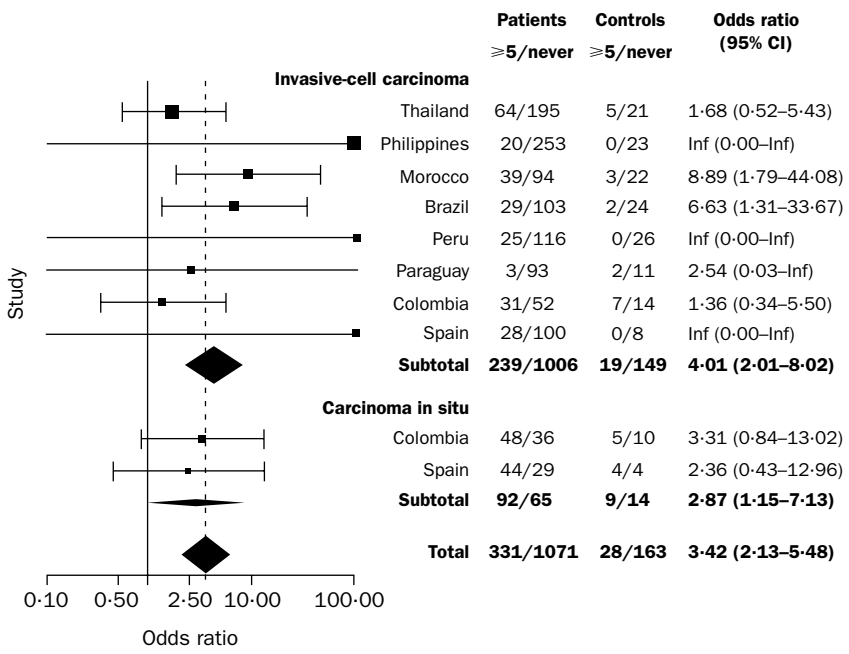

Figure 3: **Risk of cervical neoplasia associated with use of oral contraceptives for 5 years or longer**
Inf=infinite.

Analysis unadjusted for duration of use showed that women starting oral contraceptives before age 20 were almost three times more likely to develop cervical cancer than controls, but risk was not significantly increased in those who first used oral contraceptives at age 25 years or older (figure 2). These effects were homogeneous among centres (p=0·12) and between studies of ICC and of ISC (p=0·39). However, age at first use was related to duration (Pearson r=–0·26 among users of oral contraceptives), and combined analysis of both variables showed that risk was more likely to be determined by duration of oral contraceptive use than by age at first use (figure 4). The age at which women started to use oral contraceptives was not significantly associated with cervical cancer after adjustment for duration of use (p=0·41).

Women who had used oral contraceptives within the past 5 years were almost three times more

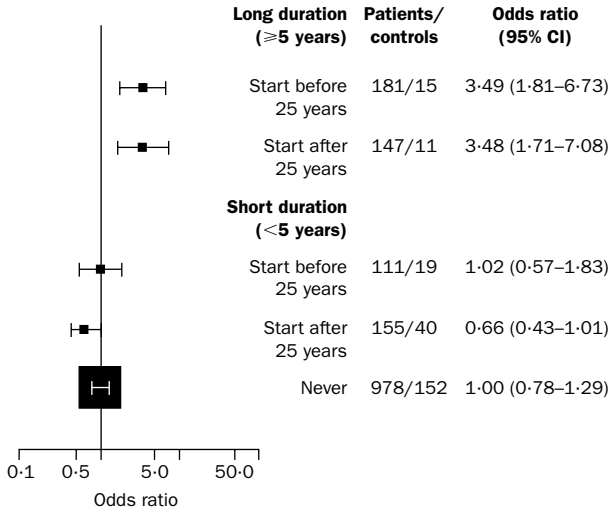

Figure 4: **Risk of cervical neoplasia associated with duration of use of oral contraceptives, stratified by age at start of using oral contraceptives**

likely to develop cervical cancer than controls (odds ratio 2·97; 95% CI 1·82–4·85; figure 2). Recency of use was strongly correlated with duration of use (Pearson r=–0·44). Of women who had used oral contraceptives for less than 5 years, only those who had used them within the past 5 years (recently) had a significantly higher risk of developing cervical cancer than controls, although this association was only of borderline significance (odds ratio 1·98, 95% CI 0·93–4·21; figure 5). The odds ratios for development of cervical cancer fell to levels close to those seen in never users in women who had stopped taking oral contraceptives at least 6 years ago (figure 2). This effect was homogeneous among centres (p=0·16) and between the two types of cancer (p=0·45). However, in women who had used oral contraceptives for 5 years or more, an increased risk persisted for 5–14 years after stopping use (figure 5). After 15 years of stopping, risk of developing cervical cancer was almost three times greater than in never users. However, care should be taken in interpretion of these estimates since our results were based on only 21 patients and two controls, and had a broad CI.

Time since first use of oral contraceptives was not linearly associated with the risk of cervical cancer (figure 2). Only patients who had first used oral contraceptives 11–15 years ago were significantly more likely to develop cervical cancer than controls (figure 2). This small effect of time since first use of oral contraceptives was not seen after allowance for duration of use (p=0·68; figure 6). Women who had used oral contraceptives for 5 years or longer had a greater risk of developing cervical cancer, irrespective of the time since first use (figure 6).

Most (89%) of the HPV types identified among patients and controls were in the high-risk type group, 2% were in the low risk group, and 8% were detected by the generic probe, but could not be typed. When analysis was restricted to patients and controls who were positive for high-risk HPV types, our results did not change: for ever use of oral contraceptives the odds ratio was 1·41 (95% CI 0·89–2·22) and for use of oral contraceptives for 5 years or longer was 3·69 (1·83–7·74; compare with figures 1 and 3, respectively).

We assessed the risk of testing positive to HPV associated with use of oral contraceptives using all available controls (255 positive for the virus, and 1661 negative). Overall, no association was recorded. For ever use of oral contraceptives, the odds ratio of being positive for HPV was 1·03 (0·74–1·42), and the test for trend for oral contraceptive duration of use was not significant (p=0·38). Prevalence of positivity was 13% in patients who had never used oral contraceptives, 8% for 5–9 years, and 10% for 10 years or longer. The odds ratio for HPV infection associated with use of oral contraceptives for 10 years or longer compared with never users was 0·73 (0·39–1·39).

To assess the effect of restriction of analyses to women who were positive for HPV, we repeated the analysis for all patients and controls and adjusted also for the status of the virus. Ever use of oral contraceptives in such an analysis had an odds ratio of 1·13 (0·86–1·49; compare with figure 1). Risk of cervical cancer rose with increase of duration of use of oral contraceptives, although all odds ratios were systematically lower than those recorded for women who tested positive for HPV. The odds ratio for

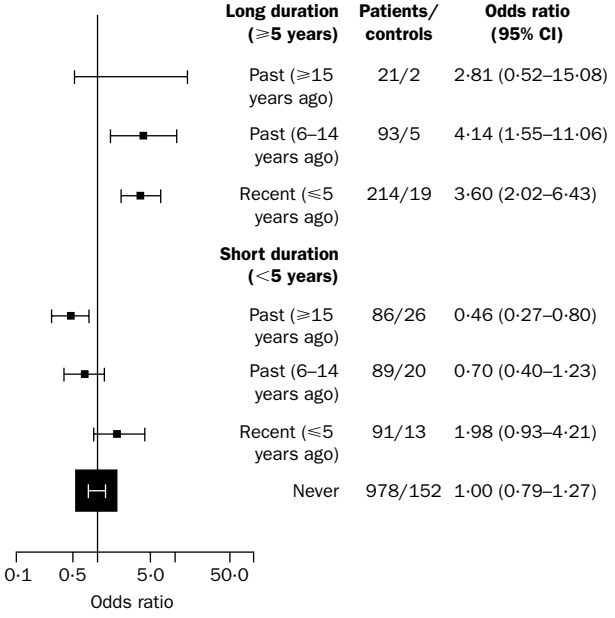

Figure 5: **Risk of cervical neoplasia associated with duration of use of oral contraceptives, stratified by recency**

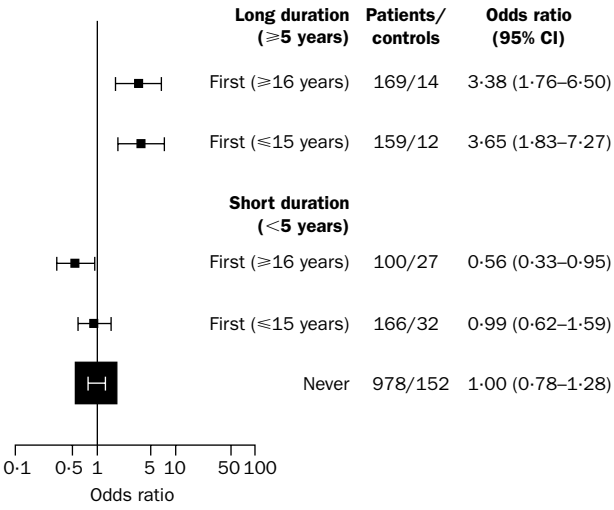

Figure 6: **Risk of cervical neoplasia associated with duration of use of oral contraceptives, stratified by latency**

use of oral contraceptives for less than 5 years was 0·77 (0·59–1·01); for 5–9 years 1·30 (0·89–1·88); and for 10 years or longer 1·87 (1·32–2·66).

Analysis restricted to patients and controls who were negative for HPV showed an odds ratio of 0·83 (0·54–1·27), for ever use of oral contraceptives. For duration of use of oral contraceptives, the odds ratios were 0·77 (0·48–1·23) for less than 5 years, 0·56 (0·30–1·03) for 5–9 years, and 1·09 (0·70–1·71) for 10 years or longer.

As a sensitivity analysis of the modelling process, we investigated the effect of the potential confounding variables used for adjustment in the models. The odds ratios for duration of use of oral contraceptives adjusted only for age and centre were 0·71 (0·54–0·95) for less than 5 years, 2·25 (1·28–3·98) for 5–9 years, and 2·18 (1·27–3·75) for 10 years or longer. Variables associated with sexual behaviour (number of partners and age at first sexual relationship) or parity did not change these estimates by much. Addition of the number of Papanicolaou smears had a negative confounding effect. The odds ratios for duration of use increased to 0·70 (0·52–0·95) for less than 5 years, 2·42 (1·33–4·40) for 5–9 years, and 2·93 (1·64–5·23) for 10 years or longer. The interaction terms of centre with age, education, and number of Papanicolaou smears further increased these estimates to the values reported in figure 2.

Discussion

Our analysis suggests that risk of invasive squamous cervical cancer and ISC for women who tested positive for HPV DNA is increased three-fold if they have used oral contraceptives for 5 years or longer. Because the prevalence of oral contraceptive use and other risk correlates varied between countries, that odds ratios among ever users of oral contraceptives showed some heterogeneity between studies was not surprising. The increase in risk for women who had used oral contraceptives for 5 years or longer, however, was more consistent across studies than that for ever use, and was similar between the types of cervical cancer investigated. Because of the low prevalence of oral contraceptive users in some regions, assessment of the residual effect of the three indices of timing of use other than duration was difficult. Among patients who had used oral contraceptives for 5 years or longer, however, risk of development of cervical cancer was not substantially changed by time since last (or first) use, or by age at first use.

Results of several studies[4] of oral contraceptive use and squamous-cell ICC showed a small increase in the risk ratio associated with long duration of use, although many had broad CIs and were not significant. In a meta-analysis[1] of 51 studies, risk ratios associated with ever use of oral contraceptives were 1·5 (95% CI 1·3–1·8) for ISC and 1·2 (1·1–1·4) for ICC. Results of cohort studies generally have higher risk ratios than case-control studies, and both types of study showed a clear dose-response effect of increasing risk with increased duration of use. In a 25-year follow-up study[25] of 46 000 British women, mortality from cervical cancer was increased 2·5-fold (1·1–6·1) in women who either were presently using, or had recently used, oral contraceptives, after adjustment for parity, social class, and smoking. The conclusions of these studies are similar to ours, but the strong effect of HPV was not taken into account fully. Also, in some of these studies, biases associated with sexual behaviour, screening, and, most notably, HPV infection, could not be ruled out.[26]

Few studies, other than those that have been re-evaluated in our present pooled analysis, have provided specific information on use of oral contraceptives and cervical neoplasia among women who are positive for HPV. Negrini and colleagues[5] reported that use of oral contraceptives was unrelated to risk of abnormal changes or low-grade squamous cell intra-epithelial lesion, but was associated with increased risk of high-grade squamous cell intra-epithelial lesion. This result, however, was based on only four patients and 66 controls who had tested positive for HPV DNA in Southern blots. Kruger-Kjaer and colleagues[7] investigated 71 patients with high-grade squamous cell intra-epithelial lesions and 155 controls who tested positive for HPV DNA in a PCR-based assay.

A risk ratio of 3·8 (1·0–14·0) was reported for 9 years or more of sex life without barrier contraceptive use, but the risk ratio for use of oral contraceptives among women who were positive for HPV was not shown.[7] Lacey and colleagues[6] compared 89 patients with cervical-intraepithelial neoplasia (CIN) grade 3 and 154 with ICC with 48 controls who had tested positive for HPV DNA in a PCR-based assay, and recorded no significant association with oral contraceptive use or duration of use. Deacon and colleagues[8] compared oral contraceptive use in 199 patients with CIN 3 and 181 controls, all of whom were HPV-positive. The odds ratio for 8-year or more use of oral contraceptives was 1·5 (0·8–2·9), and was lower, but not incompatible with our results. Therefore, our present analysis of 1676 patients with ICC or ISC and 255 controls who were all HPV DNA positive includes most of the information presently available about the effect of use of oral contraceptives on cervical carcinoma in the presence of HPV cervical infection. The relation between risk of ICC or ISC and oral contraceptives that we have recorded seems consistent with oral contraceptives promoting some step in the process of HPV-related cervical carcinogenesis. However, in agreement with results of previous studies of low-grade squamous-cell intra-epithelial lesion[4] or HPV carriage,[27] our results do not lend support to the hypothesis that oral contraceptives have a role in facilitation of infection or persistence of HPV. In fact, use of oral contraceptives was not related to HPV-positivity among controls, after allowance for education, indicators of sexual activity, and screening history.

Our results are unlikely to be accounted for by chance, especially in long-term users of oral contraceptives. Recall bias can be introduced if patients and controls differ in their reporting of use of oral contraceptives. However, reporting of short-term use for patients did not differ from that of controls. Low refusal rates among population[15] and clinic controls,[17] and the inclusion of many different diseases that are unrelated to oral contraceptive use among hospital controls,[9–14] should have reduced the effect of potential selection bias. Furthermore, our findings for use of oral contraceptives did not differ systematically by type of control group.

Unfortunately, the questionnaire was not designed to gather enough detail about the type of hormonal contraceptive used. Independent surveys[4] done in the study regions showed that most women took combined oral contraceptives and only a few (less than 5%) took progestagen-only contraceptives. Such a trend was not seen in Paraguay, in which 5% of women took progestagen-only contraceptives, in Peru (8%), and in Thailand (12%). Combined injectable preparations (containing both oestrogen and a progestagen) have been widely used in some Latin American countries and could increase these figures for some countries. A review of

three studies[28] that analysed the association between progestagen-only methods such as depot-medroxyprogesterone and cervical cancer concluded that risk of cancer was not raised, possibly helping to account for the lower risk estimates seen in some countries such as Thailand.

To keep the potential for confounding to a minimum, in addition to adjustment for age and centre, all odds ratios were adjusted for education, indicators of sexual activity, and screening history—ie, variables that were correlated with disease status and, in some studies, with use of oral contraceptives. Although smaller in magnitude, the odds ratios adjusted only for age and centre were similar to those adjusted for these other variables and still significant. The number of Papanicolaou smears women had received and its interaction with centre were the terms that most changed the estimates.

Exposure to possible cofactors for cervical cancer varies substantially between countries. In less-developed countries, women are frequently exposed to sexually transmitted diseases other than HPV. However, preliminary analysis of available data on serum antibodies to herpes simplex-2 virus and *Chlamydia trachomatis* in our study population suggest that the effects of these infections do not account for the association between long-term oral contraceptive use and cervical cancer.

Proper account of HPV effect was the main concern in this analysis, since high-risk HPV types are the main cause of cervical neoplasia,[2] and they can be detected in 99% of patients with ICC.[3,29] We focused, therefore, on patients and controls who were positive for HPV, instead of adjusting for HPV status, as has been done previously. We had chosen our analytical strategy on the basis that oral contraceptives would not increase cervical cancer risk in absence of HPV. This design had the drawback of reducing the number of controls and increasing the potential for selection bias. However, prevalence of women who were positive for HPV was not associated with ever use of oral contraceptives and did not fall significantly with duration of use of oral contraceptives in controls.

Any misclassification of a strong cause of cervical neoplasia such as HPV is a crucial issue. For detection of HPV DNA, we used highly sensitive methods.[3,20] In fact, cervical carcinomas that are HPV negative are so rare,[3] that we were tempted to judge most of them as false-negatives. Had we made this assumption, and included all women who were positive for HPV as having cervical cancer, our results would not have changed—the odds ratio for use of oral contraceptives for 5 years or longer would have been 3·1 (95% CI 1·8–5·2; compare with figure 3). We did not systematically re-test for HPV controls, since a pilot study in Peru[13] showed that few, if any, women without cervical lesions would have tested positive with E7-primer PCR. Classification of a few HPV-positive controls as being HPV negative would have weakened the modification effect of HPV on use of oral contraceptives. Exclusion of the 32 patients who tested positive for HPV after E7 PCR primer retesting did not change our results, since this reclassification was independent of oral contraceptive use.

Irrespective of advances in methods for detection of HPV, interpretation of HPV-positivity among controls remains difficult, and how much one measurement of HPV DNA can show chronic carriage of the virus among women not affected by cervical neoplasia is unclear. Retesting for HPV would probably have meant than some HPV-positive controls, but not patients, had become negative if they had cleared the infection. Other women would probably have shown newly acquired infections.[30] Controls who were positive for HPV and who had recently had new sexual partners are, for instance, more likely to be affected by transient infections than those who had not.[8] Results of cohort studies[27,30] have suggested that most infections with high-risk HPV types in women aged older than 30 years (91% of our controls) are persistent. Furthermore, that some misclassification of HPV-positivity or over-representation of women with a high-risk profile among controls who were HPV-positive can fully account for the duration-dependent association with oral contraceptive use is unlikely. Restriction of our analyses to women who are HPV-positive did affect the odds ratios—the odds ratio for use of oral contraceptives for 10 years or longer was about 4 in women who were HPV-positive, but 1·1 in those who tested negative.

We think that our results lend support to the existence of an association between oral contraceptives and HPV, and suggest that studies not restricted to women who were positive for HPV could have underestimated the effect of oral contraceptives. Our results could help women who have persistent HPV infection to balance benefits (prevention of pregnancy and cancers of the ovary and corpus uteri)[4] and harms of long-term oral contraceptive use, and suggest that long-term users of oral contraceptives should be included in cervical screening programmes.

Contributors

V Moreno and S Franceschi did the analysis and wrote the report. N Muñoz, F X Bosch, and R Herrero, were the main researchers of the original studies, designed the multicentric analysis, and helped to elaborate the report. K V Shah did the virology determinations in the studies of Spain and Colombia, and participated in design of the multicentric analysis. C J L M Meijer and J M M Walboomers did the virology determinations of the rest of the studies.

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Conflict of interest statement

C J L M Meijer has acted as a consultant for Digene Laboratories.

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