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Review

Environmental co-factors in HPV carcinogenesis

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Abstract

Epidemiological studies have shown that only a small fraction of women infected with oncogenic HPV types will eventually progress to high-grade intraepithelial lesions (HSIL) and cervical cancer (CC). Because infection by oncogenic HPVs is a necessary but not a sufficient cause of CC, it has been assumed that other factors, acting in conjunction with HPV, influence the risk of transition from cervical HPV infection to cervical malignancy. This paper reviews the epidemiological evidence for the role of environmental co-factors in HPV carcinogenesis as assessed from selected studies that report associations within a well-defined HPV-DNA positive group. Co-factors assessed include parity, use of oral contraceptives, tobacco smoking, infection with other sexually transmitted diseases, and dietary and nutritional factors. Based on the evidence provided by the largest epidemiological studies that using sensitive detection methods allowed for the effects of HPV, it can be concluded that, among HPV positive women, high parity, long-term OC use, smoking, and co-infection with other sexually transmitted agents are the most consistently identified environmental co-factors likely to influence the risk of progression from cervical HPV infection to HSIL and invasive CC. There is limited evidence for a role of dietary factors in HPV carcinogenesis. On-going epidemiological studies will shed more light into the role of these and other co-factors, but if confirmed, these conclusions may imply that multiparous women, women who are smokers, and women on long-term OC use, might need a closer cytological and HPV surveillance than women in the general population.

Keywords: Cervical cancer; HPV; Co-factors; Smoking; Parity; Oral contraceptives

1. Introduction

Epidemiological studies have shown that only a small fraction of women infected with oncogenic HPV types will eventually progress to high-grade intraepithelial lesions (HSIL) and cervical cancer (CC). Because infection by oncogenic HPVs is a necessary but not a sufficient cause of CC (Walboomers et al., 1999), it has been assumed that other factors, in conjunction with HPV, modulate the risk of transition from cervical HPV infection to cervical malignancy.

Most of the sexual behaviour parameters that were associated with CC in past studies have been re-assessed in studies that considered the strong influence of HPV by including information on markers of HPV infection, including detection of

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HPV-DNA in cells or tissue, or presence of HPV serum antibodies. Soon after the introduction of these markers into research protocols, it became clear that most of the key risk factors linked to sexual behaviour merely reflected the probability of HPV exposure and acquisition. Other variables such as the estimates of age at first exposure (as indicated by age at first sexual intercourse or age at first marriage), are still under evaluation as many studies show an independent effect even after adjusting for the subject's HPV status. In addition, several environmental factors historically related to CC are currently being re-assessed. These include hormonal factors (use of OCs and multiparity), infection with other STDs (HSV-2, *Chlamydia trachomatis*, and HIV), tobacco smoking, and dietary factors. Other non-environmental cofactors being also considered include those related to the host's immune response and those related to the virus itself, such as HPV genotype, co-infection with other HPV types, HPV variants, viral load, and viral integration.

Investigation of the role of such factors, once the central role of HPV has been recognised, has generated an analytical situation with few precedents in cancer epidemiology. This derives from the observation that HPV-DNA is almost always present in specimens from CC. In one large study it was shown that the HPV-DNA negative cases that were identified were largely false negative cases. HPV-DNA was initially undetected because of specimen inadequacy, a relative predominance of integrated HPV-18, or technological inadequacy (Walboomers et al., 1999). Consequently, some authors find it inappropriate to allow in the statistical analyses a comparison group of 'HPV negative' cases, even if in analytical terms this group existed as a small fraction (between 5 and 10% of the cases) in most studies. Now, it has become a standard procedure to restrict the analyses to HPV positive subjects when assessing the contribution of additional factors in cervical carcinogenesis.

The purpose of this paper is to summarise the evidence for the role of environmental co-factors in HPV carcinogenesis as assessed from selected studies that report associations within a well-defined HPV-positive group. These include: a case–control of CIN 1–3 from a cohort study conducted in Portland (USA) (Schiffman et al., 1993; a US case–control study of CIN3 and CC Lacey et al., 1999); a case–control study of ASCUS, LSIL and HSIL from a cohort study in Copenhagen (Kruger-Kjaer et al., 1998); a case–control study of CIN3 from a cohort study conducted in Manchester (Deacon et al., 2000) and a case–control study of HSIL and CC from a cohort study carried out in Costa Rica (Hildesheim et al., 2001). Results are also presented from the large pooled analyses of the International Agency for Research on Cancer (IARC) case–control studies conducted in Spain, Colombia, Brazil, The Philippines, Thailand, Morocco, Peru, and Paraguay (Moreno et al., 2002; Munoz et al., 2002; Plummer et al., submitted, Smith et al., 2002a,b; Castellsague et al., 2002). Given that the IARC multicentre case–control studies can be considered as the leading project in relation to environmental co-factors and invasive CC among HPV-positive women, the relevant results from the pooled analyses of these studies are summarised in Table 1.

2. Parity

High parity has consistently been found to be associated with both CC and carcinoma in situ (CIS) in most case–control studies. Furthermore, most of the major studies restricting the analysis to HPV-positive women report an increased risk for HSIL/CC with increasing number of pregnancies. In the large IARC study, women with seven or more full-term pregnancies had a four-fold increase in the risk of developing squamous-cell CC as compared with nulliparous women (OR = 3.82, 95% CI: 2.66–5.48) (Table 1) (Munoz et al., 2002). Risk of HSIL/CC significantly increased with increasing number of live births in the large Costa Rica study (P for trend, 0.04) (Hildesheim et al., 2001). A similar trend was also found among HPV-positive women in the Portland CIN study (Schiffman et al., 1993). A statistically borderline association with CIN3 was found in the Manchester study for women with three or more full-term pregnancies as compared with nulliparous women

Table 1
Summary results of the IARC pooled analyses assessing the association between selected environmental co-factors and risk of squamous-cell carcinoma of the cervix among HPV-positive women

Co-factor	Number of cases	Number of controls	OR (95% CI)
PARITY: number of full-term pregnancies			
Nulliparous	57	24	1.0
1–2	279	59	1.81 (1.31–2.52)
3–4	450	70	2.55 (1.95–3.34)
5–6	353	48	2.83 (2.02–3.96)
≥7	534	52	3.82 (2.66–5.48)
P for trend	–	–	<0.0001
OC USE: status			
Never	1071	163	1.0
Ever	605	92	1.42 (0.99–2.04)
OC USE: duration (years)			
Never	978	152	1.0
1	110	28	0.67 (0.41–1.08)
2–4	156	31	0.80 (0.51–1.24)
5–9	156	12	2.82 (1.46–5.42)
≥10	172	14	4.03 (2.09–7.79)
P for trend	–	–	<0.001
SMOKING: status			
Never	1265	218	1.0
Ever	409	36	2.08 (1.33–3.27)
Former	134	14	1.80 (0.95–3.44)
Current	275	22	2.30 (1.31–4.04)
SMOKING: amount (cigarettes per day)			
Never	1265	218	1.0
1–5	181	17	1.89 (1.05–3.41)
≥6	211	18	2.23 (1.18–4.20)
P for trend	–	–	NS
SMOKING: duration			
Never	1268	219	1.0
1–19	230	19	2.56 (1.39–4.71)
≥20	170	15	1.86 (0.98–3.53)
P for trend	–	–	NS
HSV-2: seropositvity status			
Negative	601	115	1.0
Positive	497	49	2.00 (1.33–3.00)
C. trachomatis: seropositvity status[a]			
Negative	225	45	1.0
Positive	205	16	2.1 (1.1–4.0)
C. trachomatis: seropositvity titer[a,b]			
Low	56	7	1.0
Intermediate	94	8	1.4 (0.6–3.3)
High	55	1	2.7 (1.2–5.9)
P for trend	–	–	0.01

NS denotes not statistically significant.

[a] Includes subjects from studies in Brazil and the Philippines.
[b] Restricted to seropositive subjects.

(OR = 1.9, 95% CI: 0.9–3.8) (Deacon et al., 2000). The Copenhagen case–control study did not detect an effect of parity on HSIL, but this could be due to the low parity of the study population (Kruger-Kjaer et al., 1998). A study in Honduras showed that the effects of parity were not significant among the HPV-positive cases and controls (Ferrera et al., 2000).

Hormonal, traumatic and immunological hypotheses have been put forward as biologically plausible mechanisms to explain the association between parity and cervical neoplasia, but because of the consistency of effects with OC use, hormonal influences are likely to play a role in HPV carcinogenesis. Furthermore, high parity may also increase the risk of CC because it maintains the transformation zone on the exocervix for many years (Autier et al., 1996), facilitating the direct exposure to HPV and possibly other co-factors. Hormonal changes induced by pregnancy may also modulate the immune response to HPV and influence risk of persistence or progression (Munoz et al., 2002; Sethi et al., 1998).

3. Oral contraceptives

Use of oral contraceptives (OC) has also been found to be associated with CC in many, but not all, epidemiological studies. Among HPV-positive subjects, the Manchester study found for CIN3 an OR of 1.5 (95% CI, 0.8–2.9) for 8 years or more of OC use as compared with never users (Deacon et al., 2000). The two US studies did not find an increased risk for CIN or CC (Schiffman et al., 1993; Lacey et al., 1999), but the study by Lacey found a strong association with current OC use only for cervical adenocarcinoma in situ (OR = 17.1, 95% CI: 1.5–188.2). In the Copenhagen study a pattern of a decreasing risk of ASCUS, LSIL and HSIL was seen with increasing number of years with OC use among HPV DNA positive women (Kruger-Kjaer et al., 1998). In the Costa Rica study of HSIL/CC, an increased risk for 5 or more years of use was found only among women with less than three pregnancies (OR = 3.1, 95%

CI: 1.1–9.1) (Hildesheim et al., 2001). In contrast, the pooled data from the IARC study among HPV-positive women showed that the risk increased for 5–9 years of use (OR = 2.8; 1.5–5.4), and for more than 10 years (OR = 4.0; 2.1–7.8) (Table 1). These findings suggest that use of OCs for 5 or more years is a cofactor that may increase up to four-fold the risk of CC among women who are carriers of HPV-DNA (Moreno et al., 2002).

Not much data are available concerning the mechanisms by which hormonal influences may modulate the risk of progression to advanced cervical disease among HPV infected women. Hormonal-related mechanisms may influence the progression from pre-malignant to malignant cervical lesions by promoting integration of HPV-DNA into the host genome, which results in deregulation of E6 and E7 expression (IARC, 1995). Experimental studies have shown that estradiol may stimulate the transcription of HPV16 E6 and E7 in cell lines that contain integrated HPV16 (Mitrani-Rosenbaum et al., 1989). Since the E6 and E7 open reading frames have been associated with the oncogenic potential of HPV-16, the effect of estrogen on the transcription of these viral genes may be of biological relevance in the malignant transformation of HPV-16 infected cervical cells. Data from experimental studies demonstrate a synergistic mechanism between chronic estrogen exposure and HPV16 oncogenes that modulates squamous carcinogenesis in the female reproductive tract of transgenic HPV16 expressing mice (Arbeit et al., 1996; Elson et al., 2000). Alternatively, OCs might facilitate HPV reactivation or persistence, although indirect evidence form several studies, does not suggest an association between OC use and HPV-positivity among control women (IARC, 1999; Moreno et al., 2002).

4. Smoking

Smoking has been related to CC since the late 1970s, based upon the correlations seen between CC incidence and the incidence of other tobacco related cancers (Winkelstein, 1977). The 1986 IARC monograph on smoking considered that the evidence available for CC was insufficient to rule out confounding with sexual behaviour traits known to be related to both smoking and CC (IARC, 1986). An extensive review of the relation between smoking and CC was published in 1998, including eight cohort and 44 case–control studies (Szarewski and Cuzick, 1998). The report concluded that the association was largely consistent in studies that adjusted for HPV-DNA or restricted analyses to HPV-positive women. The magnitude of the risk for current smokers was of the order of one- to three-fold; the ORs tended to be higher in more advanced preinvasive neoplasia, and in several studies a dose–response relation with the amount of tobacco consumed was seen. A recent review of the evidence conducted by the Surgeon General in the USA retained the hypothesis that a causal association between cigarette smoking and CC was plausible (Public Health Service 2, 2001). However, the report indicated that the extent to which cigarette smoking could be considered independent of HPV could not be definitively assessed.

These findings are consistent with those found among HPV-positive women in the IARC study (OR for ever vs. never, 2.2, 95% CI: 1.5–3.2) (Table 1) (Plummer et al., submitted), the Costa Rica study (OR for current vs. never, 2.3, 95% CI, 1.2–4.3) (Hildesheim et al., 2001), the Portland study (OR for ever vs. never, 2.7 95% CI, 1.1–6.5) (Schiffman et al., 1993), the Copenhagen study (OR for current vs. never, 1.9, 95% CI, 1.0–3.4) (Kruger-Kjaer et al., 1998), and the Manchester study (OR for ever vs. never, 2.2, 95% CI, 1.4–3.4) (Deacon et al., 2000).

Almost 60 years ago Rous reported the carcinogenic effect of tar on virus-induced rabbit papillomas (Rous and Friedwald, 1944). Further evidence using cigarette smoke condensate has more recently been proven in HPV 16-immortalized human endocervical cells (Yang et al., 1996). The fact that nicotine and tobacco-specific carcinogens have been detected in the cervical mucus of smokers (Prokopczyk et al., 1997) further strengthens the hypothesis of a synergistic action between cigarette smoking and HPV for the development of SIL and CC. Chemical tobacco-related carcinogens may exert a direct mitogenic

effect causing DNA damage. Some authors put forward that exposure to tobacco may affect the ability of the host to mount an effective local immune response against viral infections, as it has been shown that smoking may reduce the number of Langerhans cells and other markers of immune function (Poppe et al., 1995). The significant correlation found between the extent of smoking reduction and the reduction in lesion size in an intervention study of smoking cessation among women with minor-grade lesions, further strengthens the plausible role of tobacco smoking in HPV carcinogenesis (Szarewski et al., 1996).

5. Sexually transmitted infections

HPV infection with other sexually transmitted agents such as *C. trachomatis* and HSV-2, has inconsistently been associated with CC. In the IARC studies, HSV-2 seropositivity was significantly higher in women with invasive squamous cell carcinoma (44.4%) and adeno- or adenosquamous carcinoma (43.8%) than in control women (25.6%) (Smith et al., in press). Among HPV-DNA positive women, HSV-2 seropositivity was associated with an increased risk of both squamous CC (OR = 2.0; 95% CI: 1.3–3.0) (Table 1) and adeno or adeno-squamous carcinoma (OR = 2.6; 95% CI: 1.3–5.3). The authors postulate that HSV-2 infection may act in conjunction with HPV infection to increase the risk of invasive CC and that this effect is likely to be mediated by the induction of inflammatory responses.

Concerning *C. trachomatis*, a large, nested case–control study of CC, in which HPV exposure was assessed serologically, reported that presence of serum IgG antibodies to *C. trachomatis* serotype G was associated with a 6.6-fold increase in the risk of developing CC as compared with seronegative women (Anttila et al., 2001). The IARC multicentre study found a two-fold increased risk for the presence of antibodies to C. *trachomatis* (OR = 2.1; 95% CI, 1.1–4.0) (Table 1; Smith et al., 2002b).

HIV-positive women have consistently been shown to be at increased risk of cervical SIL when compared with their HIV-negative counterparts and the association appears to be stronger for women with low CD4+T-lymphocyte count. Women infected with both HIV and HPV are at a much higher risk of SILs than women infected with either of the two viruses separately (La Ruche et al., 1998). As HIV infection is related to an immunocompromised state, these findings underscore the importance of host's immunological cofactors in HPV carcinogenesis (see a detailed review on HIV and HPV carcinogenesis by de Sanjose and Palefsky in this issue).

6. Diet and nutritional factors

Little is known about the possible role of diet in HPV carcinogenesis. There are few published studies reporting associations between dietary factors and CC and most did not take into account the confounding effects potentially exerted by HPV infection.

Although the evidence is too limited to draw firm conclusions, a number of studies consistently show that higher intakes of fruits and vegetables are associated with reduced risk of cervical cancer. Evidence for dietary vitamin A and/or carotenoids and blood carotenoids is weakly consistent, whereas the evidence for dietary vitamin C and E is moderately consistent for a reduced risk of developing CC with higher intakes (reviewed in Working group on Diet and Cancer, 1998).

Epidemiological studies have also been inconsistent regarding a role for folates in the etiology of cervical neoplasia. Thus, there is moderately consistent evidence showing that higher intakes and blood levels of folates are associated with reduced risk of CC (Goodman et al., 2001). A recent study found that the dietary intakes of folate, vitamin B_6, and vitamin B_{12} were inversely related to the risk of developing cervical SIL after adjustment for HPV-DNA and other confounders (Goodman et al., 2001). Furthermore, the findings from this study suggest that certain genetic polymorphisms, in particular the methylenetetrahydrofolate reductase (MTHFR) T allele, may interact with dietary folate to influence the risk for cervical SIL.

Although these conclusions suggest that diet may play a role in HPV carcinogenesis, further

research using prospective designs and sensitive HPV markers is needed to draw firm conclusions on the relationship between diet, HPV infection and CC risk.

7. Other co-factors

The role of other potential co-factors is being actively investigated. The IARC studies have recently shown that male circumcision is associated with a reduced risk of genital HPV infection in men and with a reduced risk of CC in women with high-risk sexual partners (Castellsague et al., 2002). If confirmed in other studies, male circumcision could be considered an important co-factor in the natural history of HPV infection, since it may influence not only the risk of acquisition and transmission of HPVs but also the risk of CC.

Age at exposure has been shown to be a strong determinant of the prognosis of a carcinogenic viral infection in relation to cancer development. For example, HBV-induced liver cancer, and EBV-induced Burkitt's lymphoma are closely related to age at infection (Beasley et al., 1977; Coursaget et al., 1987; Evans and Niederman, 1989). Similarly, most studies have shown that the risk of CC is related to age at first sexual intercourse, generally used as a surrogate measure of age at first HPV exposure. However, definite evidence that CC progression is linked to age at first HPV exposure has not been provided. It has been proposed that the developing cervix (around peri-menarchy) or the healing cervix (as a consequence of deliveries, cervical trauma, or any other STD infection) are high risk situations for an HPV infection to reach the basal layer and establish a persistent infection (Bosch et al., 2002).

Non-specific inflammatory changes have also been related to modest increases in risk among HPV-positive women (Castle et al., 2001).

Finally, it is worth mentioning that few cofactors have been identified to distinguish invasive cancer from intraepithelial lesions or HSIL from LSIL. The IARC Spain–Colombia studies considered a large number of risk factors and found that, both CIN3 and CC cases, had very similar profiles of risk factors (Moreno et al., 1995). In contrast, a study comparing HPV-positive women with CIN3 to HPV-positive women with CIN1 found that cigarette smoking was significantly associated with CIN III, suggesting that HPV infected cells may require tobacco containing carcinogens for neoplastic progression (Ho et al., 1998). A recent study conducted in Thailand found that after controlling for HPV type, the risk of developing CC, as compared with the risk of developing intraepithelial lesions, was not related to any of the co-factors considered, except for two indices of socioeconomic status (Thomas et al., 2001). In fact, socioeconomic status is a potential co-factor currently under evaluation (de Sanjose et al., 1997).

8. Conclusion

Based on the evidence provided by the largest epidemiological studies that using sensitive detection methods allowed for the effects of HPV, it can be concluded that high parity, long-term OC use, smoking, and co-infection with other sexually transmitted agents are the most consistently identified environmental co-factors likely to influence the risk of progression from cervical HPV infection to HSIL and invasive CC (Fig. 1). In contrast, factors associated to the risk of HPV acquisition include a number of genital and sexual behaviour-related variables such as age at first HPV exposure, number of sexual partners, number of high-risk sexual partners, condom use, genital hygiene, and male circumcision. There is limited evidence for a role of dietary factors in HPV carcinogenesis, and in this regard, prospective studies that take into account HPV infection are still needed to properly explore whether nutritional factors act in conjunction with HPV to influence CC risk.

On-going epidemiological studies will shed more light into the role of these and other co-factors, but if confirmed, these conclusions may imply that multiparous women, women who are smokers, and women on long-term OC use, might need a closer cytological and HPV surveillance than women in the general population.

Large prospective and retrospective cohort studies of HSIL and CC in middle age women using

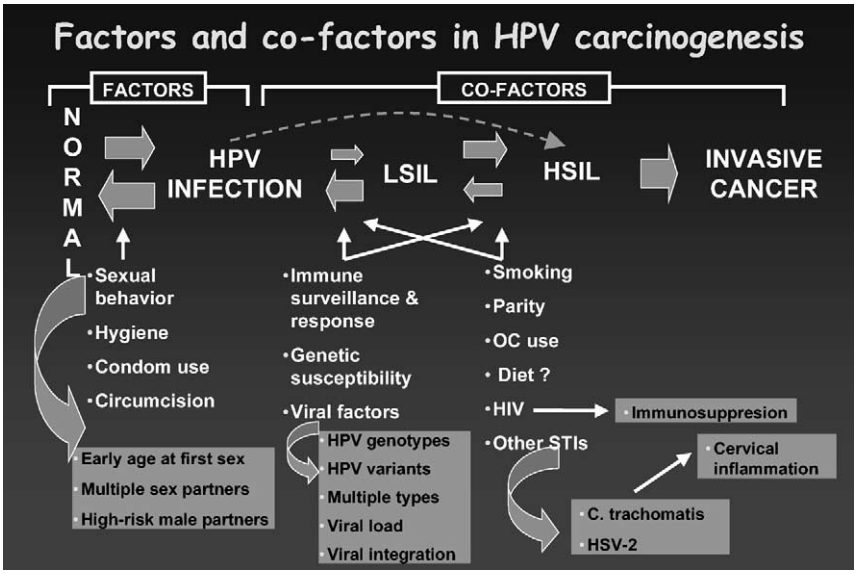

Fig. 1. Factors and co-factors in cervical carcinogenesis.

several markers of HPV exposure and documenting HPV persistence, are needed to better understand the role and impact of environmental co-factors in cervical carcinogenesis.

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