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CHAPTER 2

The epidemiology of human papillomavirus infection and its association with cervical cancer

F. Xavier Bosch*, You-Lin Qiao, Xavier Castellsagué

KEYWORDS
Cervical cancer;
Human papillomavirus;
Epidemiology

Abstract Cervical cancer has been recognized as a rare outcome of a common, sexually transmitted infection whose etiologic association is restricted to a few human papillomavirus (HPV) types. With optimal testing systems HPV DNA can be identified in nearly all specimens of invasive cervical cancer, and it is claimed that infection of the cervix with HPV is a necessary cause of cervical cancer. The evidence is consistent worldwide for squamous cell carcinomas (SCC), adenocarcinomas, and the vast majority (>95%) of the immediate cervical cancer precursors, namely high-grade squamous intraepithelial lesions (HSILs) — also known as cervical intraepithelial neoplasia 3 (CIN 3) or carcinoma in situ. Cofactors that modify the risk for HPV DNA-positive women include the use of oral contraceptives (OCs) for 5 or more years, smoking, high parity (5 or more full-term pregnancies), and previous exposure to other sexually transmitted diseases such as *Chlamydia trachomatis* and herpes simplex virus type 2 (HSV-2). Women exposed to the human immunodeficiency virus (HIV) are at high risk for HPV infection, HPV DNA persistence, and progression of HPV lesions to cervical cancer.

1. The global prevalence of HPV DNA

There have been studies on the prevalence of HPV DNA in cervical specimens from women with negative cytologic results. The studies' findings vary considerably, owing to the selection of study subjects (probabilistic population samples or opportunistic series from clinical settings) and to the tests used to detect HPV DNA. Two recent sources have provided estimates that may reflect global prevalence, age-specific prevalence, and type-specific prevalence, along with international variability of HPV. A multicenter, centrally coordinated international study conducted by the International Agency for Research on Cancer (IARC) has provided data from 15 areas in 4 continents regarding women aged from 15 to 74 years. The age-standardized prevalence ranged from less than 5% in some Mediterranean and South East Asian countries to more than 15% in several countries in Latin America and among a few African populations [1]. In a comprehensive review of studies that used standardized inclusion criteria and controlled for variables that may have challenged the comparability of the studies, prevalence estimates of HPV infection among women with

* Corresponding author.

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negative cytologic results ranged from 10% to 15%. Age-specific prevalence estimates showed HPV DNA to be more prevalent among very young women, with a decline in young adult women and a variable pattern afterwards. In some countries, notably in the Americas, the prevalence increased again in postmenopausal age groups. In Europe, a plateau in the middle-age groups was maintained whereas in other high-prevalence countries in Asia and Africa the prevalence remained fairly constant across all age groups [2].

2. Human papillomavirus epidemiology: HPV infection as a sexually transmitted disease (STD)

Several groups of studies have clearly shown that HPV is predominantly and largely transmitted through sexual intercourse. Other forms of transmission will be briefly outlined, but their implication in cervical cancer is probably marginal.

2.1. Behavioral determinants of HPV infection

Epidemiological studies investigating risk factors for HPV infection have clearly and consistently shown that the key determinants of infection in women are the number of sexual partners; the age at which sexual intercourse was initiated; and the likelihood that at least 1 sexual partner was an HPV carrier as estimated by his sexual behavior patterns [3,4].

The role of men in HPV infection of women was investigated in early epidemiological studies using questionnaires that addressed the sexual behavior of the husbands or sexual partners of women with and without cervical cancer. More recent studies had, in addition, been able to detect the presence of HPV DNA in exfoliated cells from the penile shaft, the coronal sulcus, and the distal urethra [4].

These and other studies have established that the risk of cervical cancer for a given woman is predictable from the sexual behavior of her husband or sexual partner as much as from her own sexual behavior. In populations where female monogamy is predominant, female sex workers play an important role in the maintenance and transmission of HPV infections. Moreover, the probability that a woman is an HPV carrier, as well as her risk of developing cervical cancer, have been shown to be related to the presence of HPV DNA on the penis or in the urethra of her husband or sexual partner [5]. These observations confirmed a scientific hypothesis formulated almost 30 years ago that male sexual behavior is a central determinant of the incidence of cervical cancer.

2.2. Follow-up studies with virgins initiating sexual intercourse

Because women who have not experienced sexual intercourse are not expected to harbor HPV on the cervix, studies with virgins should offer a unique opportunity to demonstrate the predominantly sexual nature of HPV transmission. However, HPV-positive specimens have been collected from the external genitalia of apparently virgin women.

Prevalence estimates of high-risk HPV DNA infection in virgins have ranged from 0% to 31% [6]. This wide range is due not only to differences in the type of samples used for HPV DNA detection (samples obtained from scrapings or lavage) and in the sites from which the samples were collected (cervix, vagina, or vulva), but also to the true virginal status of the study subjects. As the necessity for consistent HPV testing methods became understood, several studies in which HPV DNA was reliably measured by identical methods were conducted with closed cohorts. These studies concluded that HPV DNA is only detected in cervical specimens from sexually experienced women [3].

Two cross-sectional studies found no high-risk genital HPV DNA in women who had not experienced sexual intercourse. However, 2 of 154 samples from virgin women were found to be positive for HPV-6, suggesting that the transmission of low-risk types of HPV by nonsexual routes is possible but extremely rare in women who have not had sexual intercourse — even in those engaging in other forms of sexual activity such as digital penetration. Consistent with these results, a prospective cohort study of female Swedish students found that only the sexually experienced among these study subjects harbored HPV-DNA on the cervix, and that there was a positive correlation between presence of HPV DNA and number of coital partners [7].

The strongest evidence, however, that genital types of HPV are predominantly sexually transmitted is provided by longitudinal studies of virgin women who initiated sexual activity during the study period. A Danish population-based cohort study of 100 virgins and 105 monogamous women showed that all the women who remained virgins throughout the study period tested negative for both HPV DNA and serum HPV-16 antibodies at enrollment and at each visit thereafter [3]. As summarized in Table 1, only a fraction of those who initiated sexual activity during the study period tested positive for HPV DNA or serum HPV-16 antibodies. The most important determinant of HPV DNA acquisition in that study was the number of sexual partners between enrollment and subsequent visits, both among initially virgin women and initially

Table 1 Prevalence of cervical human papillomavirus (HPV) DNA and HPV-16 virus-like particle seropositivy in cohorts of virgin and monogamous women by number of sexual partners during follow-up*

Characteristic	No. of sexual partners during follow-up	HPV DNA No. positive/No. tested (% positive)		HPV-16 antibodies No. positive/No. tested (% positive)	
		Enrollment	Follow up	Enrollment	Follow-up
Virgin	0†	0/30 (0)	0/30 (0)	0/28 (0)	0/28 (0)
	1	1/67 (1.5)	23/65 (35.4)	1/67 (1.5)	10/67 (14.9)
Monogamous	1‡	2/78 (2.6)	4/78 (5.1)	7/78 (9.0)	7/77 (9.1)
	2	4/27 (14.8)	9/26 34.6)	4/27 (14.8)	6/26 (23.1)

* Adapted from reference [3].
† Virgins who remained virgin during follow-up.
‡ Monogamous women who remained monogamous during follow-up.

monogamous women. In that study, detection of serum HPV-16 antibodies and development of cervical lesions occurred only after HPV transmission, suggesting that sexual intercourse is a necessary step in both the acquisition of infection with genital HPV and the development of cervical neoplasia.

Another prospective study that included 105 HPV-negative women was carried out in the San Francisco bay area. It found that sexual behavior, specifically exposure to new partners, represented the strongest risk factor for incident HPV infection, and that there was a strong association between sexual behavior and incidence of HPV infection, as the risk increased nearly 10-fold for each new partner per month reported [8].

Taken together, these 2 prospective studies clearly demonstrate, from both HPV DNA detection and HPV serological testing, that the number of sexual partners is the key risk factor for HPV infection and that nonsexually transmitted HPV infections are rare or nonexistent among virgin adolescent girls and young women.

2.3. Studies relating HPV DNA prevalence in the genital tract to number of sexual partners in both sexes: studies in women practicing prostitution

Genital HPV infection, defined as the presence of HPV DNA in the genital tract, is the most prevalent sexually transmitted genital viral infection. Genital HPV infections are considered to occur predominantly, although not exclusively, through sexual transmission. Epidemiological studies have consistently shown that the sexual behavior of the individual and his or her partners are the most important risk factors in the acquisition of genital HPV. Specifically, the 3 most consistently identified determinants of HPV infection in the genital tract are number of sexual partners; age at which sexual intercourse was initiated; and recent partner change.

The results of 12 case-control studies of cervical cancer carried out with 2225 women and 1140 men by IARC in 10 countries (Colombia, Brazil, Paraguay, Peru, Spain, the Philippines, Thailand, India, Morocco, and Algeria) have shown that in both sexes, genital HPV DNA detection increased with increasing lifetime number of sexual partners and younger age at first sexual intercourse [5,9].

Data on groups known to be engaging or to have engaged in high-risk sexual behavior, such as female sex workers and individuals who attend STD clinics, have provided further evidence that the genital types of HPV are predominantly sexually transmitted. Two studies conducted in Spain and Denmark compared HPV DNA prevalence among women from the general population and women belonging to high-risk groups [10,11]. In all age groups HPV prevalence was the highest among female sex workers, followed by women attending STD clinics or who were incarcerated. Women from the general population had much lower age-specific HPV prevalence rates (Figure 1).

Many serologic studies have also found a very strong correlation between the presence of serum HPV antibodies and the lifetime number of sexual partners among women aged between 35 and 40 years. Some authors point out that the correlation between HPV antibodies and sexual behavior is actually stronger that the one observed in serologic studies for HSV-2 and *C. trachomatis*. Levels of HPV antibodies usually show long-term stability on follow-up, indicating that HPV seropositivity is a proxy marker of lifetime cumulative HPV exposure, as is the case for the seroepidemiology of almost any other STD.

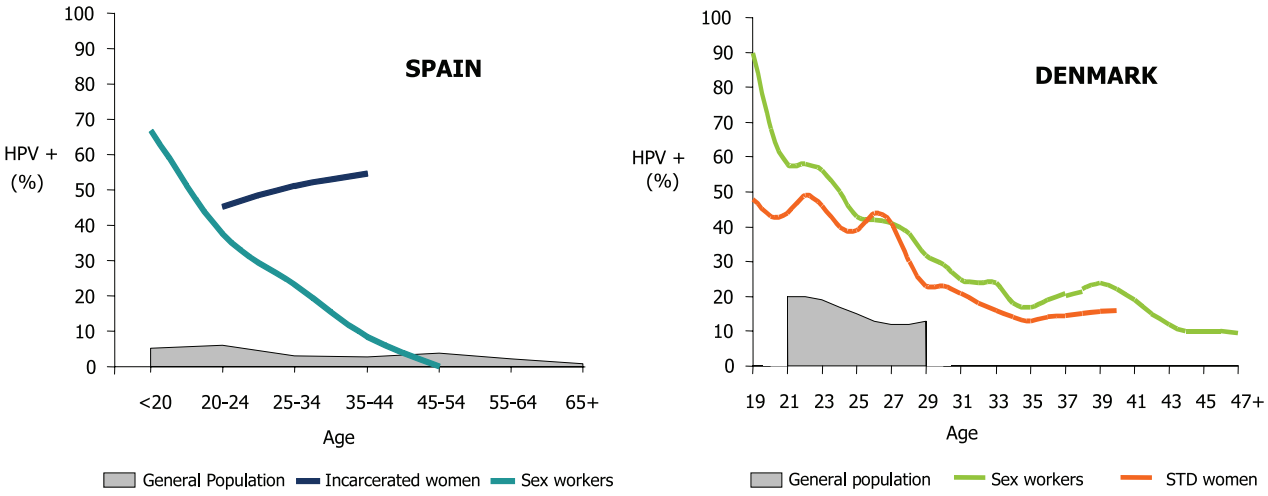


Figure 1 Prevalence of cervical human papillomavirus DNA in different risk groups in Spain and Denmark. Source of data: data from Spain is adapted from reference [10] and includes 187 female sex workers, 153 incarcerated women, and 1101 women from the general population. Data from Denmark is adapted from reference: [11] and includes 182 female sex workers, 187 female STD clinic attendees, and 1000 women from the general population.

2.4. Human papillomavirus DNA in the penis and cervical cancer in the spouse

After the identification of certain types of sexually transmitted HPV as agents etiologically linked to cervical cancer, firm evidence for a role of men as carriers and vectors of oncogenic types of HPV emerged from studies that introduced HPV DNA detection in penile samples.

The largest study to date exploring the role of men in cervical carcinogenesis using polymerase chain reaction (PCR) technology for the detection of penile HPV infection is the multicentrer case-control study coordinated by IARC. This study, which involved more than 1900 couples enrolled in 1 of 7 case-control studies of cervical carcinoma in situ and cervical cancer, was carried out in Spain, Colombia, Brazil, Thailand, and the Philippines [12]. Participating men answered a detailed risk-factor questionnaire and provided a specimen of exfoliated cells from the distal urethra, the glans, and the coronal sulcus for HPV DNA detection.

Figure 2 shows the correlation between penile infection and the sexual behavior of the couples enrolled in the studies conducted in Spain [5] and Colombia [9]. Whether their wives were monogamous or not, the prevalence of penile HPV infection clearly increased with the number of sexual partners that the men reported to have had, but it was systematically higher in the husbands of nonmonogamous women [4].

Findings from the IARC studies conducted in countries with low to intermediate risk such as Spain, Thailand, and the Philippines, indicate that

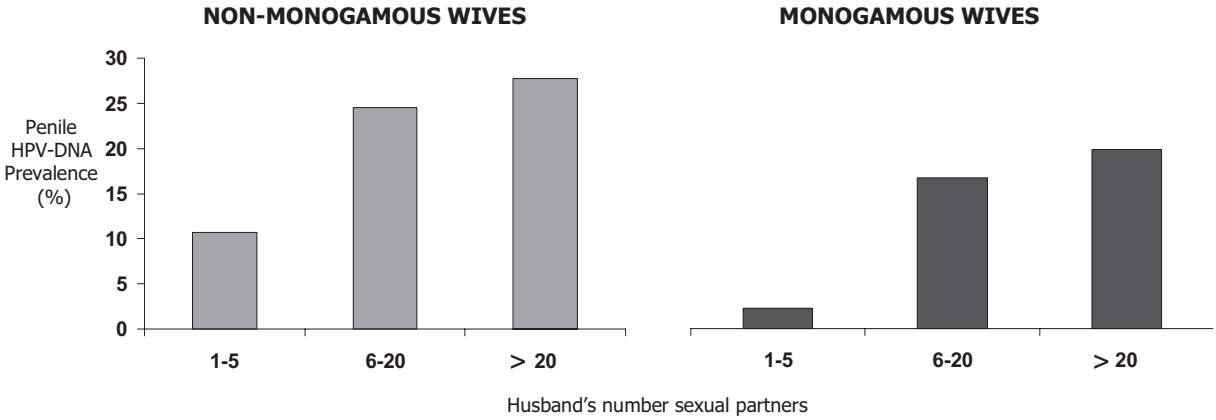


Figure 2 Prevalence of penile human papillomavirus DNA by number of sexual partners in husbands of monogamous and nonmonogamous women in Spain and Colombia. HPV: human papillomavirus. Source of data: adapted from reference [4]. Includes 595 men that were husbands or stable coital partners of women with and without cervical cancer.

the lifetime number of sexual partners of men, along with having prostitutes as sexual partners, are key determinants of cervical cancer risk for their wives. In Spain, the presence of HPV DNA in the husbands' penises conveyed a 5-fold increased risk of cervical cancer for their wives. The odds of cervical cancer among monogamous women increased 9- to 10-fold when high-risk HPV types were present in their husbands' penises, and the risk associated with HPV-16 was increased 6- to 9-fold compared with the controls. Furthermore, the prevalence of penile HPV infection showed a positive trend with both increasing number of sexual partners and number of sexual partners who were prostitutes [5]. In contrast, in high-risk countries such as Colombia and Brazil, no associations were found between cervical cancer risk and penile HPV DNA infection or with any other indicators of male sexual behavior [9].

The lack of association found between most variables related to male sexual behavior and risk of cervical cancer in high-risk countries could be explained by the hypothesis that, in these populations, HPV infection is so widespread that it reduces the ability of case-control studies to identify individuals at a higher risk. Cross-sectional HPV DNA detection in the penises of adult men, even if high, is still a poor reflection of lifetime exposure to HPV and reverse causality cannot be excluded. Other biological markers of lifetime sexual promiscuity in men, such as seropositivity to *C. trachomatis*, are consistent in distinguishing female partners who are at a high risk for cervical cancer within populations at both low and high risk for cervical cancer [5,9].

The role of men as occasional carriers of HPV DNA, and of subclinical lesions that are sources and routes of HPV transmission, are still being investigated, along with the role of penoscopy and the treatment of penile warts. However, the clinical implication of these investigations in protecting women from cervical cancer is still unclear.

2.5. Human papillomavirus concordance in couples

Several studies have investigated whether there was a concordance of genital HPV types in heterosexual couples, and most, but not all, found relatively poor correspondence of HPV positivity and HPV type between cervical and penile samples from the same couples [4]. This is particularly important in case-control studies in which women harbor cervical neoplasia, and thus are long-term carriers of type-specific HPV-DNA, whereas their husband are, or have been, transient HPV DNA carriers. Moreover, in some couples, the current partner may not be the relevant one in determining the woman's risk of HPV persistence and progression to cervical neoplasia. Agreement in HPV findings, however, was also modest in couples where both wife and husband reported only one lifetime sexual partner [13]. Among women with cervical neoplasia, the relevant infection may have occurred years earlier, and the relatively low prevalence of penile HPV infection in their husbands suggests that viral shedding of advanced cervical lesions is limited. Also, cross-sectional screening for penile HPV infection may detect relatively recent exposures to HPV that may be unrelated to the initiation of cervical neoplasia in the wife. Finally, the low agreement may be partly due to technical reasons, since a minute amount of exfoliated penile cells may be obtained in compared with the cellular yield obtained from the cervix.

2.6. Male circumcision, penile HPV infection, and cervical cancer

The IARC multicentric study on male circumcision and its association with cervical cancer compared the prevalence of HPV DNA in the penises of circumcised and uncircumcised men and estimated their wives' risks of cervical cancer [14]. The authors found that circumcised men were about 3 times less likely to harbor HPV in their penises than did uncircumcised men. Male circumcision also reduced both prevalence of genital HPV DNA and risk of cervical cancer in female partners, particularly and most strongly in women whose male partners had a promiscuous sexual history. Further, male circumcision also afforded protection from *C. trachomatis* infection. These findings, along with the literature on HIV transmission, also indicating a strong protection from carrying and transmitting HIV for circumcised men, underline the relevance of circumcision for the prevention of a series of sexually transmitted infections.

3. Other routes of HPV transmission

Despite the overwhelming evidence that genital types of HPV are predominantly sexually transmitted, some clinical and epidemiological observations have documented that they can also be transmitted in other ways, especially from mother to child. This is consistent with other microbial and viral infections that are predominantly or exclusively sexually transmitted in adults (e.g., HIV, hepatitis B virus, HSV-2, *C. trachomatis*, *Treponema pallidum*, and *Neisseria gonorrhoea*) but may be transmitted to the child during pregnancy or at the time of delivery.

The evidence for the nonsexual transmission of

genital types of HPV has been reviewed by several authors who concluded that (1) genital HPV infections, including genital warts, may occur in sexually naive populations such as infants, children, and virgin adolescents and adults; (2) there is some evidence of horizontal transmission of low-risk types of HPV; (3) vertical and perinatal transmission of HPV from mother to child exists, although rates are low; (4) high-risk genital types of HPV have been detected in nongenital mucosae, e.g., of the mouth and oropharynx as well as the conjunctiva, and they have been associated with some cancers of the oral cavity and oropharynx and with conjunctival squamous cell carcinoma.

3.1. Vertical and perinatal transmission HPV: laryngeal papillomatosis

Nonsexual transmission of HPV infection was first suggested in 1956, in a case report of a male child born to a mother with condylomas. He developed laryngeal papillomatosis 3 months and penile warts and 6 months after birth. Since then a large body of epidemiological data on perinatal transmission of HPV has been accumulating [6]. However, studies evaluating transmission of HPV from mother to child are conflicting.

In a carefully conducted study, 151 infants born to mothers with known HPV status were regularly checked for HPV infection from birth to the age of 3 years [15]. Samples from the infants' mouths, external genitalia, and anuses were taken and PCR was performed with HPV L1 consensus primers for hybridization of HPV types 6, 11, 16, 18, 31, 33, 35, 39, and 45, and a generic HPV probe. During pregnancy 112 (74%) of the 151 women had historic, clinical, or DNA evidence of genital HPV infection. After 479 infant visits, HPV DNA had been detected in 1.5%, 1.2%, and 0% of genital, anal, and nasopharyngeal specimens, respectively. Three (4%) of the 80 infants born to HPV-positive women and 5 (8%) of the 63 infants born to HPV-negative women were found to be HPV infected. All positive results in the infants were for unclassified HPV types, and specimens had been found to be HPV negative before or after obtaining all positive results. The study suggests that the few HPV infections detected in infants were probably caused by HPV contamination with, or horizontal transmission of, low-level genital or nongenital HPV types. The study also indicates that the risk of perinatal transmission of HPV, although present, is probably very low (<3%).

Perinatal HPV transmission is unequivocally demonstrated for recurrent laryngeal papillomatosis, a rare, potentially life-threatening condition associated with HPV-6 and HPV-11, the types of HPV the most commonly detected in genital warts. The disease can disseminate through the tracheobronchial tree and progress first to pulmonary papillomatosis and then to fatal chest infection. Since the disease has a bimodal age distribution, with the first peak among infants, it has been postulated that juvenile papillomatosis may be related to HPV infection acquired from a mother with genital warts or subclinical HPV infection. In one study, the risk for the juvenile form of laryngeal papillomatosis appeared to be the highest in first-born infants delivered vaginally to adolescent mothers. In contrast, the risk factors identified for the adult onset of the disease included lifetime number of sex partners and high frequency of oral sex, suggesting orogenital transmission [7].

3.2. Transplacental transmission

Several studies have shown some evidence of intrauterine HPV infection. In one of these studies, 24 of 37 samples of amniotic fluid from women harboring HPV DNA or with abnormal cytologic results were HPV positive by PCR. Another study detected HPV-16 DNA in cord blood specimens from neonates born to mothers who tested positive for HPV-16. Detection of HPV-6 DNA in infants born by cesarean section further suggests that prenatal HPV infection may occasionally occur, probably through ascending infection. Finally, a case report describing the detection of epidermodysplasia verruciformis (EV)-related HPV types in amniotic fluid, placenta, and cervical scrapes from patient with EV renders plausible a prenatal transmission of EV-related HPV types [16].

3.3. Horizontal transmission

Since the original case report on a 5 year old boy with HPV-2 positive warts on his anus and hand, a number of other case series have confirmed the possible horizontal transmission of HPV, particularly of the low-risk types. Human papillomavirus DNA has been detected in the nail brushes of 3 of 8 women with cervical HPV (the same type in 1 woman), and in the nail brushes of 9 of 13 men with penile HPV infection (the same types in 5 men). In total, 27% of patients had the same HPV type detected in both genital and hand samples. These findings raise the possibility that patients with genital warts may transfer genital HPV not only to their sexual partners by genital-finger transmission, but also horizontally to their children by touching them.

Finger-conjunctiva transmission has been suggested by studies reporting the presence of HPV DNA, predominantly type 16, in squamous neo-

plasias of the human ocular surface, including conjunctival carcinomas. A study in Uganda, a high-risk area for this tumor, found a statistically significant association between high titers of antibodies to HPV-16 and conjunctival squamous cell carcinoma.

Indirect transmission via HPV-contaminated fomites (e.g., clothing, sheets, towels, objects, and instruments) has also been suggested by several studies, but their role in passing and inducing active infections, if it exists, is most likely small [7].

3.4. Transmission via blood, breast milk, and sperm

No HPV has ever been detected in blood, and is very unlikely that the virus can be transmitted through blood because it is not known to have a viremic phase. Transmission of HPV to infants via breast-feeding has not been documented either. The possible role of sperm as a vector for HPV has been explored in several studies. One study using PCR primers targeting small gene regions detected HPV DNA sequences in 64% of sperm specimens. Another study found a correlation between high-risk types of HPV in the cervix of women and the semen of their sex partners. The source of HPV in semen was urethral epithelial cells, sloughed from the urethral epithelium during ejaculation and isolated on a Percoll gradient. A recent study using DNA amplification by nested PCR detected viral sequences in the sperm cells of 53% of subjects with past or current HPV infection and in 8% of healthy subjects. Evidence from these studies suggests that semen may be a transmitter of cell-associated HPV during ejaculation.

Although HPV DNA has been occasionally isolated from medical instruments or during medical examination or treatment (i.e., in the vaporization fumes at the time of laser ablation of CIN lesions), there is at present no evidence that viral DNA is able to initiate de novo from the patient, her sexual partner, or her health provider. The estimated relative importance of HPV transmission routes to the cervix is shown in Table 2.

4. Human papillomavirus and cervical neoplastic lesions

4.1. The prevalence of HPV DNA in cervical cancer specimens

The relation between HPV infection and cervical cancer has been recognized in a large body of studies, and determined as causal by international reviews since the early 1990s [12,17].

State-of-the art amplification techniques used in case-control studies, case-series, and prevalence surveys have unequivocally shown that HPV DNA can be detected in 90% to 100% of adequate specimens of cervical lesions. Figure 3 shows the prevalence of HPV DNA in specimens of invasive cervical cancer collected in 6 regions of the world and studied using PCR technology in a common research laboratory. After adjusting for sampling and testing variability, the results support the hypothesis of the universal presence of the same viral markers in established cancerous lesions. The prevalence is equally high in the more common squamous cell carcinomas and the rarer adenocarcinomas, although the distribution of HPV types varies slightly between these 2 histologic forms.

Further, detailed investigations of the few cervical cancer specimens in which no HPV DNA could be detected have been conducted in most series, with the conclusion that most of these negative results are probably false negatives. As a consequence, the claim has been made that the presence of HPV DNA in cervical neoplasias is the first necessary cause of a human cancer ever identified [18,19].

4.2. Risk estimates from case control studies

Cohort studies of preinvasive disease aiming at understanding the natural history of HPV infection have intrinsic limitations that prevent them from making inferences on the cause of cervical cancer: namely, disease progression is not allowed to continue beyond the stage of high-grade squamous

Table 2 Transmission routes of HPV to the cervix and estimated relative importance

Transmission	Relative importance
Sexual intercourse	Vast majority of cases (>99%)
Sexual contacts with incomplete penetration or no penetration	Has been described, probably rare
Mother to child	Established in cases of recurrent respiratory papillomatosis Transmission to skin or oral cavity has been documented but without clinical consequences
Fomites	Possible, not proven
Other	Possible, anecdotal

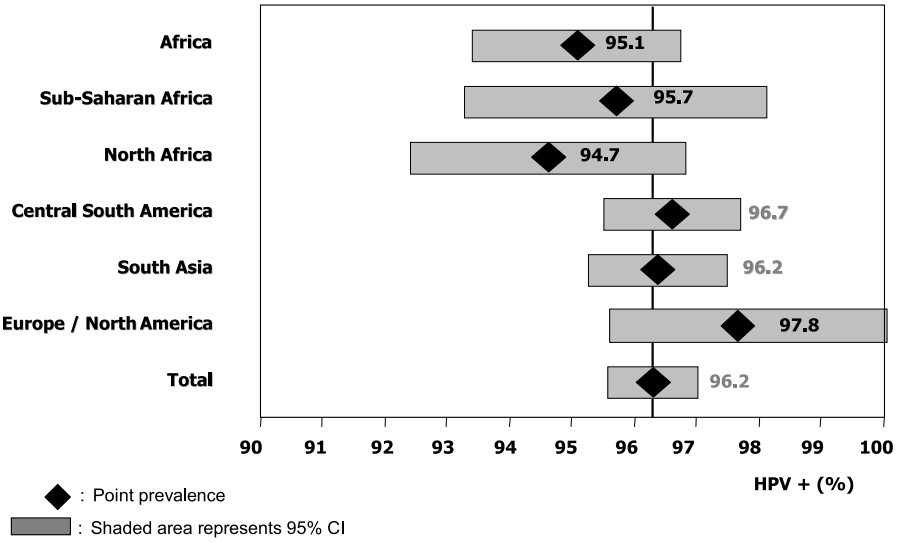
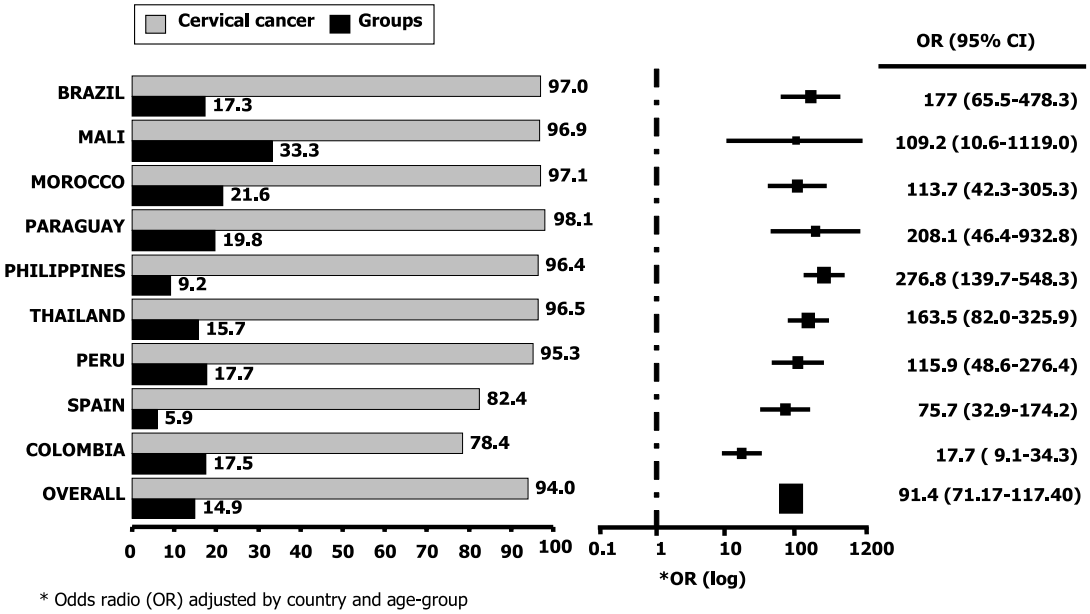


Figure 3 Prevalence of human papillomavirus DNA in cervical cancer specimens using GP5+/6+ polymerase chain reaction by world region. Source of data: adapted from reference [24].

intraepithelial lesions (HSIL/CIN 3) or carcinoma in situ. It is thus important to note that the information about cervical cancer comes primarily from case-control studies in which the target disease is indirectly investigated.

In an effort to simplify the vast literature pertaining to case-control studies, the results of the IARC multicenter case-control study on invasive cervical cancer will be used as example. In brief, this project included 9 case-control studies from different parts of the world, mostly from high-risk countries. A common protocol and questionnaire was used, and HPV DNA testing was done in 2 central research laboratories using the MYO9/11 and the general primer GP5+/6+ PCR testing systems. Figure 4 summarizes the results regarding the HPV DNA prevalence for case and control specimens, along with the risk estimates and confidence intervals for squamous cell carcinomas. The figure shows very high odds ratios (ORs), with estimates ranging from 50 to 150. These risk estimates allowed to calculate the proportion of cervical cancers attributable to HPV DNA, which was greater than 95% for the entire study [20].

Results are strikingly consistent in the literature concerning preinvasive lesions, squamous cell carcinomas, and adenocarcinomas, as well as in studies



* Odds radio (OR) adjusted by country and age-group

Figure 4 Prevalence of human papillomavirus DNA and odds ratios for squamous cell carcinoma of the cervix by country. Source of data: adapted from reference [20].

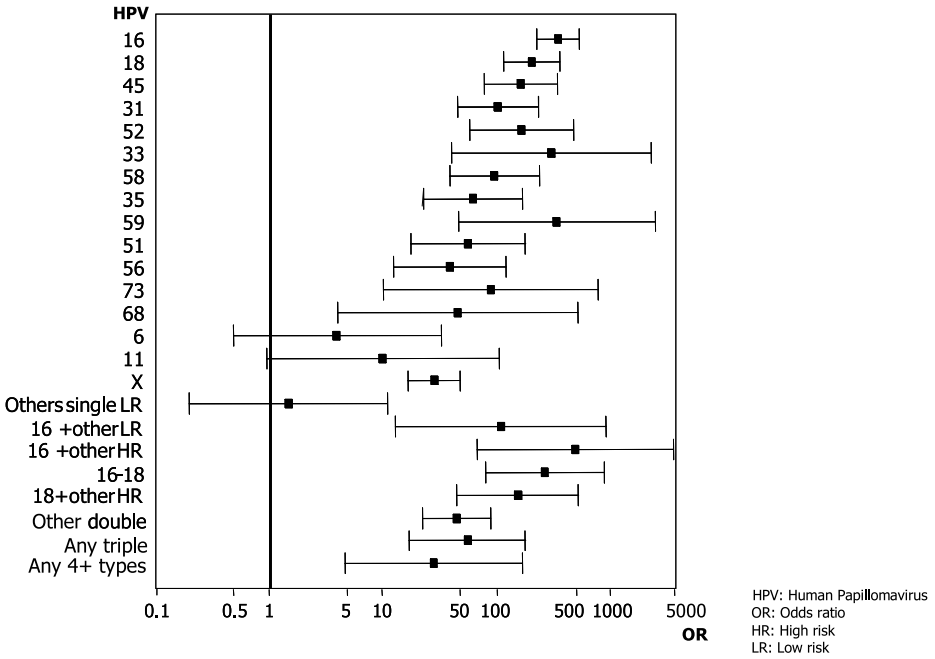


Figure 5 Human papillomavirus type-specific odds ratios and 95% confidence intervals for cervical cancer. Source of data: adapted from reference [20].

that tested for HPV DNA generically or in studies that tested for high-risk types of HPV. Studies that have compared risk factors for CIN 3 and invasive cancer have not reported any significant differences in these lesions' associations with HPV or in their epidemiological profile.

The pool of IARC studies was large enough to provide type-specific risk estimates for 18 types of HPV. Restricting the analyses to the studies that used the GP5+/6+ HPV detection system, the adjusted OR for HPV DNA detection (the factor by which the reference risk of cervical cancer is multiplied if HPV DNA is detected) was 158.2 (95% confidence interval [CI], 113.2—220.6) for squamous cell carcinomas and 81.3 (95% CI, 42.0—157.1) for adenocarcinomas [21]. Type-specific risk estimates for squamous cell carcinoma are shown in Figure 5. Odds ratios were 435 for HPV-16; 248 for HPV-18; 198 for HPV-45; 124 for HPV-31; 200 for HPV-52; 374 for HPV-33; 115 for HPV-58; 74 for HPV-35; 419 for HPV-59; 67 for HPV-51; 45 for HPV-56; infinite for HPV-39; and 54 for HPV-68. The risk for any given high-risk type was not statistically different from the risk reported for HPV-16. Likewise, the risk from the presence of multiple HPV types in the specimen was not different from the risk from a single HPV type.

These studies and a recent international review concluded that the evidence is now sufficient to consider HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 to be high-risk for carcinogenesis [12]. Other HPV types that are rarely found in neoplastic specimens have been classified as low-risk; they include HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108. From the IARC and other studies, HPV types 26, 53, 66 and perhaps others are considered of uncertain risk.

4.3. Cohort studies on HPV infection

Repeated sampling in women followed up for viral persistence and cervical abnormalities has shown that the median infection duration is about 8 months for high-risk HPV types, compared with 4.8 months for the low-risk types. In unrelated studies, the time estimates were fairly consistent. In one study in a high-risk population in Brazil, the mean duration of HPV detection was 13.5 months for high-risk HPV types and 8.2 months for the nononcogenic types. The HPV-16 type tended to persist longer than other high-risk types. Findings were remarkably similar in a student population from the United States and the United Kingdom. However, the observed time intervals may be imprecise because of inaccurate time of first exposure, different endpoints, and censoring due to treatment of early lesions.

Follow-up studies involving women with and without cervical abnormalities have indicated that the continuous presence of HR-HPV is necessary for disease development, maintenance, and progression to CIN. A substantial proportion (i.e., 15—30%) of women with HR-HPV DNA who are cytomorphologically healthy at recruitment will develop CIN 2 or CIN 3 within the following 4 years. Conversely, CIN

2/3 is unlikely to develop during a 2-year follow-up in women who tested negative for HR-HPV DNA but had a lesion cytologically identified as atypical squamous cells of undetermined significance (ASCUS), borderline, or mild dysplasia. In these women the cytologic results are likely to return to normal. Those who test positive for low-risk HPVs rarely become persistent carriers and the probability of their lesions to progress to CIN 2/3 is extremely low [12].

As study cohorts expand their follow-up time, more precise estimates are being provided on the predictive value of viral persistence (defined as repeated detection of viral types and variants). One of such cohorts in Sao Paulo has shown that the incidence of cervical lesions in women who were HPV negative on 2 separate occasions was 0.73 per 1000 women-months. The corresponding incidence among women with repeatedly positive results for HPV-16 or HPV-18 was 8.68, an incidence 12 times greater. The OR for HPV persistence among women who were twice found to be HPV positive for the same oncogenic type was 41.2 (95% CI, 10.7—158.3).

The follow-up studies in Costa Rica are beginning to assess the potential for progression to neoplasia of lesions caused by some of the most frequent high-risk HPV types. The study confirms that the risk of progression (given persistence) is significantly higher for carriers of HPV-16 and HPV-18 compared with carriers of any of the other high-risk types included in the cocktail test Hybrid Capture assay, version hc2 (Digene, Gaithersburg, Md, USA) [22].

Finally, the detection of HPV-DNA after treatment for CIN 2/3 is an accurate predictor of relapse, significantly more sensitive than repeated vaginal cytologic studies in the 24 months following surgery [23].

4.4. Relevance of HPV-16 and HPV-18 in cervical cancer and precancerous lesions

Of the more than 35 types of HPV found in the genital tract, HPV-16 accounts for 50% to 60% of all cervical cancer cases in most countries, followed by HPV-18 (10—20%), HPV-45 (4—8%), and HPV-31 (1—5%). Figure 6 [24] shows the cumulative distribution of 15 types of HPV in a series of nearly 3000 cervical cancers. Of these, the 5 most common HPV types, types 16, 18, 45, 31, and 33, are found in 80% of squamous cell carcinomas and 94% of adenocarcinomas. In most studies, HPV-18 predominates in adenocarcinomas in absolute or relative terms. The reasons for such specificity are unknown.

An interesting study among women with different degrees of HIV-induced immunosuppression pointed to a greater ability of HPV-16 to escape immunosurveillance compared with other HPV types as one possible mechanism of such advantage [25]. By inducing cervical cancer HPV-16 uses a biological advantage over other types of HPV to fully express its oncogenic capacity.

Table 3 [26] compares the distribution of HPV types in cervical cancer and in the preneoplastic precursor lesions HSIL/CIN 2/3. Based upon a review of the international literature, this table shows that HPV types 16, 18, and 45 are the only viral types that are more frequently found in invasive forms of neoplasia than in precursor lesions. The ability of

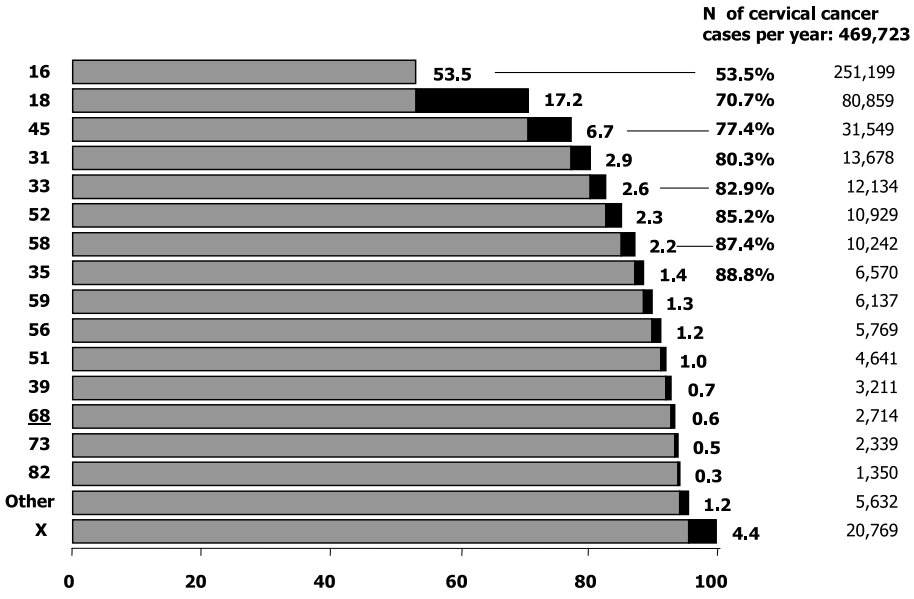


Figure 6 Human papillomavirus types in cervical cancer all world regions combined. Source of data: adapted from reference: [24].

Table 3 Comparison of overall and type-specific HPV prevalence between squamous cell carcinoma (SCC) and high-risk squamous intraepithelial lesion (HSIL)*

HPV type	SCC		HSIL		Prevalence ratio (95% confidence interval)
	No. of patients	HPV+ (%)	No. of patients	HPV+ (%)	
All	8550	87.6	4338	84.2	1.04 (1.03–1.26)
16	8594	54.3	4338	45.0	1.21 (1.16–1.26)
18	8502	12.6	4338	7.1	1.79 (1.56–2.10)
33	8449	4.3	4302	7.2	0.59 (0.53–0.68)
45	5174	4.2	2214	2.3	1.85 (1.35–2.91)
31	7204	4.2	4036	8.8	0.48 (0.43–0.54)
58	5646	3.0	2175	6.9	0.43 (0.37–0.52)
52	5304	2.5	2153	5.2	0.48 (0.40–0.60)

*Adapted from reference [26].

Table 4 Prevalence of the most common types of human papillomavirus (HPV) in cervical cancer by region*

Sub-Saharan Africa		Northern Africa		Central-South America		South Asia		Europe & North America	
HPV type	%	HPV type	%	HPV type	%	HPV type	%	HPV type	%
16	47.7	16	67.6	16	57.0	16	52.5	16	69.7
18	19.1	18	17.0	18	12.6	18	25.7	18	14.6
45	15.0	45	5.6	31	7.4	45	7.9	45	9.0
33	3.2	33	4.0	45	6.8	52	3.1	31	4.5
58	3.2	31	3.4	33	4.3	H 58	3.0	56	2.2

*Source: Adapted from reference [24]

HSILs to progress to invasive disease may therefore be due to the presence of one of these 3 types of HPV, compared with lesions induced by other types of HPV [27]. These findings are consistent with observations from cohort studies indicating that the probability of progression, given persistence, is significantly higher in women exposed to HPV-16 or HGPV-18 than to any other type of HPV.

The distribution of HPV types in cervical cancer in 5 world regions is shown in Table 4. It is clear that HPV-16 and HPV-18 are by far the most frequent types involved in all regions so far explored, and HPV-45 seems to deserve third place. The other types are much rarer in cervical lesions. The epidemiologic traits of viral infection and their associations have been described and are essentially universal for cervical cancer. On the other hand, cultural traits (e.g., age at initiation of sexual activity) may regionally affect the introduction and acceptability of the new HPV vaccines.

5. Other environmental risk factors for cervical cancer

Most of the sexual behaviors that had been linked to cervical cancer in the past are being re-evaluated in studies that consider the strong influence of the presence of HPV. Soon after the introduction of HPV testing in research protocols, it became clear that the key risk factors that reflected sexual behavior, such as the number of sexual partners, merely reflected the probability of HPV exposure. Because of the growing evidence that HPV was a necessary factor in cervical cancer, it soon became a standard procedure in case-control studies to include analyses restricted to HPV-positive patients and HPV-negative controls to properly assess the contribution of additional factors to the risk of disease. Such restricted IARC pooled analyses included 1768 HPV-positive patients and 262 controls, and the key findings regarding invasive cervical cancer and environmental risk factors are discussed briefly.

5.1. Long-term use of hormonal contraceptives

Women who ever used OCs had a significant increase in risk of cervical cancer (OR, 1.47; 95% CI, 1.02—2.12). Using an OC for less than 5 years was not related to cervical cancer (OR, 0.77; 95% CI, 0.46—1.29) but the risk increased significantly with a use of 5 to 9 years (OR, 2.72; 95% CI, 1.36—5.46) and with a use of 10 or more years (OR, 4.48; 95% CI, 2.24—9.36).

The evidence of an association between cervical cancer and the use of oral or other hormonal con-

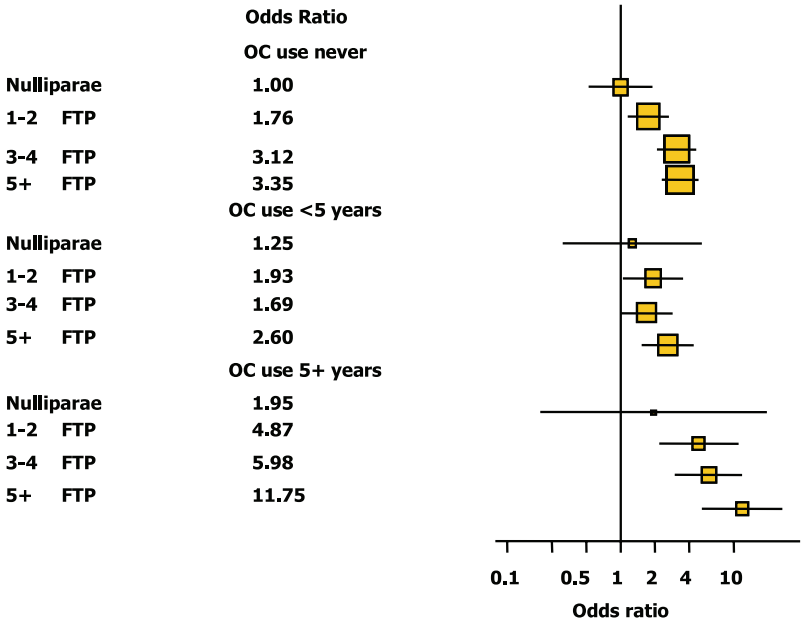


Figure 7 Combined effect of parity and oral contraceptive use in squamous cell carcinoma of the cervix. FTP: Full Term P, OC: oral contraceptive. Source of data: adapted from reference [29].

traceptives is not entirely consistent. A number of studies that investigated HPV-positive women found no association or only a weak association between the use of OCs and HSIL/CIN 3 in subgroup analyses. These apparently conflicting results may reflect the increased cytologic surveillance of women taking OCs in developed countries and the use of different lesion definitions (from ASCUS to HSIL/CIN3) or the vague *cervical cancer* in cohort studies.

Because of the potential public health importance of an interaction between long-term OC use and HPV infection in the development of cervical cancer, efforts are now being devoted to verify the results in different populations. A recent meta-analysis on the association between hormonal contraceptives and cervical cancer concludes that there is a linear dose-response relationship between the two, but that the relationship tends to disappear with time after OC cessation [28]. Relevant World Health Organization units examined the evidence with an international working group that recognized the significance of the association. However, the group concluded that, on balance, the benefits currently achieved by the use of OCs in developing countries (i.e., the avoidance of unwanted pregnancies) outweighed the increase in risk and should not force a change in current family planning strategies.

5.2. High parity

Women who reported 7 or more full-term pregnancies and were HPV positive had a 4-fold increase in risk of cervical cancer compared with nulliparous HPV-positive women with similar characteristics (OR, 3.8; 95% CI, 2.7–5.5). There was still a 2-fold increase in risk when women reporting 7 or more pregnancies were compared with HPV-positive women reporting 1 or 2 full-term pregnancies. Similar results were obtained in Costa Rica and Thailand as well as among women with preinvasive disease in the Portland Cohort Study. In Denmark and in the Manchester cohort study, 2 populations with low parity, the effects were less clear for preneoplastic lesions [12].

It has been speculated that the general reduction in the mean number of births in developed countries over the last decades may have contributed to the reduction in cervical cancer incidence, but formal proof of the hypothesis has not yet been produced.

Figure 7 shows the combined effect of parity and exposure to oral contraceptives on the risk of cervical cancer. There is an increased risk at each level of OC use, and a significant trend with the number of lifetime pregnancies. The trend is more marked as women report having used OCs for extended periods. Compared with the risk for nulliparous women who report not having used OCs, the risk for women who have used OCs longer that 5 years and had more than 5 full-term pregnancies is increased a significant 11-fold [29].

5.3. Cigarette smoking

The pooled results of the IARC studies found that "ever smoking" was associated with a significant 2-fold increase in risk of cervical cancer, with a defi-

nite dose-response curve. These findings regarding preneoplastic cervical lesions are consistent with those found for "current vs. never smoking" HPV-positive women in the Costa Rica study (OR, 2.3), the Portland study (OR, 2.7 for CIN 2/3), the Copenhagen study (OR, 1.9), and the Manchester study (OR, 2.2). These recent studies are providing growing evidence for a carcinogenic effect of cigarette smoking in women with persistent HPV infection. The IARC monograph program reviewed the evidence in 2002, and concluded that smoking was an independent risk factor for cervical cancer. However, the mechanisms by which cigarette smoking may affect cervical cancer (i.e., a direct effect of tobacco metabolites, or indirect effects related to tobacco-induced immunosupression or reduced intake of dietary antioxidants) remain elusive.

5.4. Coinfection with HIV

The evidence of a possible interaction between HPV and HIV at the origin of cervical cancer was formally recognized when cervical cancer was included as one of the criteria for acquired immune deficiency syndrome (AIDS) in HIV-positive women. The subsequent literature largely confirmed the evidence, although confounders of the epidemiologic association tend to obscure the results. In brief, the confounders refer to the powerful impact of screening in some populations, the medical surveillance of HIV carriers in developed countries, and the short survival time of patients with HIV/AIDS in many populations at high risk for cervical cancer compared with the long time needed between HPV infection and full-blown cervical cancer [30] (See also Chapter 4, Palefski J.).

5.5. Coinfection with other sexually transmitted infectious agents

Markers of exposure to other sexually transmitted infectious agents have been repeatedly associated with cervical cancer. Results from the IARC multicenter study found a 2-fold increase in risk of cervical cancer when antibodies to *C. trachomatis* (OR, 2.1; 95% CI, 1.1—4.0) or to HSV-2 were present. Nonspecific inflammatory changes have also been related to modest increases in risk for preneoplastic cervical lesions in HPV-positive women. The difficulty with the evaluation of such factors lies in the strong colinearity observed among all sexually transmitted infections, and the limitations of some of the biomarkers currently used to assess ever exposure or persistent exposure.

6. Conclusion

In the last 2 decades, etiologic studies of cervical cancer have identified several HPV types as necessary for the development of cervical cancer, coupled with a few additional intervening cofactors that promote the oncogenic potential of HPV infection. The association is universal. These findings have resulted in novel screening and vaccination strategies for the prevention of cervical cancer. Vaccines may soon overhaul cervical cancer prevention.

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