Cervical cancer and human papillomavirus: Epidemiological evidence and perspectives for prevention

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Abstract
Cervical cancer is a major public health problem, as it is the second most common cancer in women world-wide after breast cancer. About 80% of the half a million cases estimated to occur annually in the world, occur in developing countries. The epidemiological evidence linking human papillomavirus (HPV) to cervical cancer is reviewed. It is concluded that over 90% of cervical cancers can be attributed to certain HPV types. HPV 16 accounts for the highest proportion (50%) followed by HPV 18 (12%), HPV 45 (8%) and HPV 31 (5%). The associations with these HPV types are very strong and consistent with odds ratios over 15 in all case-control studies in high- and low-risk countries for cervical cancer. However, HPV is not a sufficient cause of this malignancy; certain cofactors are necessary for a proportion of HPV persistent infections to eventually progress to cancer. These include host factors such as histocompatibility types and immunological response, hormonal influences and infections with other sexually transmitted agents such as Chlamydia trachomatis. In addition, results from our studies carried out in Spain and Colombia support the hypothesis that male carriers of HPV play an important role in the development of cervical cancer in their wives. The recognition of the central role of HPV in cervical cancer has far-reaching implications for the primary and secondary prevention of this malignancy. Prophylactic and therapeutic HPV vaccines are now under development and HPV typing is being integrated into screening programmes in pilot studies in a few developed countries. In developing countries, well conducted conventional screening programmes remain Reprint requests to: Dra. Nubia Muñoz. International Agency for Research on Cancer. 150, Cours Albert Thomas, F-69372 Lyon Céde x 08, France.

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Reprint requests to: Dra. Nubia Muñoz. International Agency for Research on Cancer. 150, Cours Albert Thomas, F-69372 Lyon Céde x 08, France.
Cervical cancer is a major public health problem, as it is the second most common cancer in women worldwide after breast cancer. About 80% of the half a million cases estimated to occur annually in the world, occur in developing countries. The highest incidence rates are reported from African, South American and certain Asian countries and the lowest rates from southern and northern Europe, North America and the Middle-East. Until five years ago, critical assessments of the epidemiological evidence linking human papillomavirus (HPV) to cervical cancer concluded that it was highly suggestive but not conclusive of a causal association. Since then, a series of well-designed epidemiological studies using accurate hybridization assays to assess HPV exposure have been reported and recently reviewed. These studies were important contributors to the overall assessment of certain types of HPV as carcinogenic to humans carried out by an interdisciplinary expert group convened by the International Agency for Research on Cancer (IARC). They comprise an impressive and largely consistent set of case-series, case-control studies and some cohort investigations that will be reviewed below. Only those studies using accurate HPV DNA detection methods to assess exposure to HPV will be considered.

Case series

Prevalences of HPV DNA ranging from 22% to 100% have been reported in over 30 series of cervical intraepithelial neoplasia (CIN) and cervical cancer. This broad range in the HPV prevalence is due to various sources of variation: the hybridization method used (with or without amplification), the different tumour specimens (cervical swabs, lavages, biopsies or surgical specimens) and tissue preservation (fresh, frozen or fixed).

Case-control studies

High-grade CIN lesions (CIN II-III)

Considering that low-grade CIN or CIN I lesions are cytological or histologically indistinguishable from the morphological signs of HPV productive infection, CIN I could be regarded as an unsensitive marker of HPV exposure and not as a disease outcome. Thus,
only high-grade CIN lesions will be considered in this review.

Table I summarizes the results of six case-control studies combining a good epidemiological design and a PCR-based hybridization assay.7-11

In the IARC studies in Spain and Colombia, an early version of the L1 consensus primer system was used with a generic probe detecting a narrower spectrum of HPV types12 while in the studies in the US and Taiwan an improved version of the L1 consensus primer system with a more sensitive generic probe and 25 HPV type-specific probes were used.5,9,11 In the study in Norway nested general primers were used.10

Comparing the results of the six studies summarized in Table I, it is clear that HPV DNA prevalence among cases is higher (> 90%) in those studies using highly sensitive PCR-based assays than in those using the early versions of these assays (63-70%). In comparing the prevalence of HPV DNA among controls we shall take into account the age structure as well as the source of control patients, in addition to the accuracy of the hybridization techniques. Thus, the higher prevalences in Portland, USA and Norway than in Spain and Colombia are probably explained by the younger age of the study populations as well as the more sensitive PCR assays in the former studies, while the higher prevalence of HPV DNA in New Mexico than in the Portland study is probably determined by the nature of the control group (women referred to a colposcopy clinic). Thus, selection bias cannot be totally excluded in the study carried out in New Mexico.

As mentioned before, the PCR assay used in the studies in Spain and Colombia, was less sensitive than the PCR-based assay used in the other studies; thus, the odds ratios (ORs) and attributable fractions (AF) given in Table I for Spain and Colombia are probably underestimates of the true ORs and AFs. The adjusted OR for HPV DNA (any type) ranged from 16 in Co-

<table>
<thead>
<tr>
<th>Study area (author)</th>
<th>Cases</th>
<th>Controls</th>
<th>HPV prevalence</th>
<th>Adjusted OR (95% CI)</th>
<th>HPV AF (%)</th>
<th>Adjustment for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>157</td>
<td>193</td>
<td>Any HPV</td>
<td>70.7</td>
<td>56.9 (24.8-130.6)</td>
<td>72.4</td>
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<tr>
<td>Colombia</td>
<td>125</td>
<td>181</td>
<td>Any HPV</td>
<td>63.2</td>
<td>15.5 (8.2-29.4)</td>
<td>60.3</td>
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<tr>
<td>Portland, USA</td>
<td>50</td>
<td>433</td>
<td>Any HPV</td>
<td>90.0</td>
<td>42.0 (15.3-124.3)</td>
<td>87.9</td>
</tr>
<tr>
<td>N. Mexico, USA</td>
<td>176</td>
<td>311</td>
<td>Any HPV</td>
<td>93.8</td>
<td>20.8 (10.8-40.2)</td>
<td>89.0</td>
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<tr>
<td>Norway</td>
<td>98</td>
<td>221</td>
<td>Any HPV</td>
<td>90.8</td>
<td>72.8 (27.6-191.9)</td>
<td>92.0</td>
</tr>
<tr>
<td>Taiwan</td>
<td>39</td>
<td>261</td>
<td>Any HPV</td>
<td>91.7</td>
<td>122.3 (38.5-388.9)</td>
<td>91.0</td>
</tr>
</tbody>
</table>

NSP= number of sexual partners
AFSI= age at first sexual intercourse
E= education
OC= oral contraceptives
AF= attributable fraction
lombia to 122 in Taiwan and for HPV 16, from 10 in New Mexico to 296 in Spain. The OR for high-risk HPV types (HPV 16, 18, 31, 45) was 1.280 in Taiwan.

The fraction of high-risk CIN attributable to HPV ranged from 60% in Colombia to 92% in Norway.

**Invasive cervical cancer**

Table II summarizes four case-control studies fulfilling the inclusion criteria; in all of them PCR-based assays were used.

In Spain and Colombia, Muñoz et al. conducted two population-based case-control studies including women with invasive squamous cell cervical cancer and population controls randomly selected from the populations under study. HPV detection was done using PCR methods based on the L1 region consensus primers as described above.

In Brazil and China, hospital-based case-control studies were carried out and two different PCR assays were used. In Brazil, a PCR-based assay using a general primer which amplifies a small region of L1 gene and various type-specific probes, was employed. The PCR assay used in the Chinese study did not include a consensus primer that amplifies a broad spectrum of HPV types, but only primers for HPV 16 and 33; thus, it is not directly comparable with the other three studies.

The four case-control studies summarized in Table II give consistent results. The higher HPV DNA prevalence among cases from Brazil than among cases from Spain and Colombia is probably test-related. Among controls higher HPV prevalences are observed in the high-risk countries for cervical cancer (Brazil and Colombia) than in the low-risk countries (Spain and China). The adjusted ORs for HPV DNA (any type) ranged from 16 in Colombia to 46 in Spain and those for HPV 16 from 6 in Colombia to 75 in Brazil. The fraction of cervical cancer attributable to HPV ranged from 66% in Colombia to 86% in Brazil.

No formal case-control studies on cervical adenocarcinoma have been reported.

Case-control studies suffer from inherent temporal ambiguity concerning exposure and disease outcome. Thus, the higher prevalence of HPV DNA among cases than among controls could be interpreted in two ways:

1. If we assume that a single measurement of HPV DNA is a good marker of chronic persistent infection with HPVs, HPV DNA detected at recruitment of case and controls could be regarded as a marker of an HPV infection that preceded the cancer development.
2. HPV DNA could be more readily detected in tumoural cells than in normal cells or could be a marker of an opportunistic infection with HPV.

Direct evidence in support of the first possibility can only be derived from long-term follow-up studies.

### Table II

**PCR-BASED CASE-CONTROL STUDIES ON INVASIVE CERVICAL CANCER**

<table>
<thead>
<tr>
<th>Study area (author)</th>
<th>Cases No.</th>
<th>Controls No.</th>
<th>HPV prevalence</th>
<th>Adjusted OR (95% CI)</th>
<th>HPV AF (%)</th>
<th>Adjustment for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>250</td>
<td>238</td>
<td>Any HPV 69.0</td>
<td>4.6</td>
<td>46.2 (18.5-11.1)</td>
<td>67.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HPV 16 45.8</td>
<td></td>
<td>14.9 (5.0-49.5)</td>
<td>30.1</td>
</tr>
<tr>
<td>Colombia (Muñoz et al., 1992)</td>
<td>186</td>
<td>149</td>
<td>Any HPV 72.4</td>
<td>13.3</td>
<td>15.6 (6.9-34.7)</td>
<td>66.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HPV 16 50.6</td>
<td></td>
<td>5.5 (2.4-12.9)</td>
<td>29.3</td>
</tr>
<tr>
<td>Brazil (Eluf-Neto et al., 1994)</td>
<td>199</td>
<td>255</td>
<td>Any HPV 84.0</td>
<td>17.0</td>
<td>37.1 (19.6-70.4)</td>
<td>86.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HPV 16 53.8</td>
<td></td>
<td>74.9 (32.5-173.0)</td>
<td>79.7</td>
</tr>
<tr>
<td>China (Peng et al., 1991)</td>
<td>101</td>
<td>106</td>
<td>HPV 16/33 34.7</td>
<td>1.4</td>
<td>32.9 (7.7-141.1)</td>
<td>31.0</td>
</tr>
</tbody>
</table>

NSP= number of sexual partners
AFB= age at first birth
E= education
SES= socio-economic status
AF= attributable fraction
and a few of such studies will be reviewed below. However, indirect evidence may be obtained from the trend of HPV DNA prevalence by time since last sexual intercourse, because sexual transmission is the major route of transmission. Data from our studies in Spain and Colombia show a stable high rate of HPV DNA positivity both in women with cervical cancer who reported being sexually active at the time of the interview and in women who had their last sexual intercourse many years before entry into the study.7,13

The possibility of enhanced detectability in tumoural cells is unlikely because the HPV DNA prevalence in precursor lesions (CIN II-III) is as high as in invasive cervical cancer. Against the argument of HPV being an opportunistic infection there is a great deal of laboratory data indicating that DNA and transcripts of specific HPV types are usually detected in tissue specimens from cervical cancer and its precursor lesions, and that high-risk HPV are able to immortalize human cells and their oncoproteins interfere with the functions of negative cellular regulators.5

Cohort studies

Although several cohort or follow-up studies have been reported, only those having as end-point CIN II-III, using S. blot or PCR-based hybridization assays for HPV DNA detection and fulfilling basic design criteria will be considered here.

Three studies from the US have been reported. In the first one, a cohort of 241 cytologically normal women recruited from a STD clinic were followed every four months for an average of 25 months. HPV DNA was detected using dot blot and S. blot. HPV DNA positivity increased the risk of developing CIN II-III. The adjusted RR was 11.0 (95% CI= 3.7-31.0).16

In a second study, 206 women (173 with low-grade SIL and 33 with high-grade SIL) who participated in an intervention trial were followed every two months during six months. HPV DNA 16 was detected at study entry and at each follow-up examination by S. blot. By multivariate modelling and adjustment for age, race, smoking, oral contraceptive use and plasma levels of micronutrients, HPV 16 was found to be related to progression to high-grade SIL with a relative risk of 1.19 and 95% CI= 1.03-1.38.17

In the third study, 70 women with a histological diagnosis of dysplasia were followed at 3-months intervals during 15 months. These women were enrolled in a double-blind randomized trial to assess the efficacy of b-carotene for the treatment of CIN. HPV DNA was detected by both S. blot and a PCR-based assay. Persistent SIL was associated with persistent HPV infection and especially with persistent high viral load (OR= 4.1; 95% CI = 1.4-12.3), detected by S. blot. ORs were adjusted for randomized groups.18

In the Netherlands a cohort of 342 women with abnormal cytology (with Pap class 3b or lower, i.e. CIN III or lower) were followed-up every 3-4 months during an average follow-up period of 16 months.19 During the follow-up visits the following examinations were performed: cytology, colposcopy without biopsy and HPV DNA testing for 27 HPV types using an accurate PCR technique. Nine (3.0%) of the 298 women with an original cytological diagnosis of Pap 3a (CIN I-II) progressed to CIN III (diagnosed by colposcopy and histology) and all of them were HPV DNA positive for high-risk types at high-risk HPV types than among women with low-risk HPV or negative for HPV.

Two retrospective cohort studies based on archival cytological or histological slides have been reported. In the UK, a cohort of 93 untreated women with cervical abnormalities was identified from a randomized control trial undertaken some years ago. The patients were followed every four months by colposcopic and cytological examinations for a median period of 26 months. HPV 16 and 18 were detected in the baseline biopsy sections by a PCR-based assay. HPV 16 and/or 18 were detected in 47 women (51%) and their presence was associated with an increased risk of progression (OR= 2.3, 95% CI= 1.2-4.3).20

In Sweden, smears from 30 women with invasive cervical cancer (18 squamous cell carcinomas and 12 adenocarcinomas) and from 38 with in situ carcinoma positive for HPV DNA were compared with smears of a control group of women. For the cases, the smears were taken 1.5 to 7 years prior to the diagnosis of cancer. HPV DNA was detected with a nested PCR-based assay, in 67% of the smears preceding the cancer in case women and in 11% of control women (OR= 16, 95% CI= 6.8-3.8).21

Results from the above studies suggest that persistent infection with high-risk HPV types precedes the development of CIN II-III and predicts a high risk of developing it. The main limitation of this study design is that in most settings follow-up is interrupted at stages CIN II-III for treatment of these lesions and therefore the role of HPV in the progression to inva-
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Cervical cancer cannot be investigated. In addition, it is known that a certain proportion of CIN II-III lesions regress spontaneously.

Various other cohort studies are in progress in Colombia, Costa Rica, India, the US and UK but results have not yet been reported.

Conclusions on HPV and cervical cancer

The epidemiological data reviewed above indicate that the association between certain HPV types and cervical cancer fulfill the accepted criteria of causality proposed by Sir Bradford Hill:

1. It is very strong, with ORs over 15 in all methodologically sound case-control studies using reliable methods for HPV DNA detection. The strength of the association rules out the possibility that it can be explained by chance, bias or confounding.
2. It is consistent, as equally strong associations have been found both in high- and low-risk countries for cervical cancer.
3. There is a dose-response relationship with viral load. High levels of HPV DNA appear to carry a higher risk of cervical neoplasia than low levels.
4. Results from a few cohort studies indicate that infection with certain HPV types precedes the development of CIN II-III lesions.
5. The association is specific for certain HPV types called high-risk HPV types. Out of the 30 HPV types that infect the uterine cervix, HPV 16 accounts for the highest proportion of cervical cancer followed by HPV 18, 45 and 31.
6. The epidemiological evidence is supported by a great number of laboratory investigations indicating a carcinogenic potential of the HPV types implicated in cervical neoplasia.

These conclusions have been endorsed by an international multi-disciplinary group who met recently in Lyon to evaluate the carcinogenicity of HPV.

Results from the reviewed case-control studies and the IARC international prevalence survey of HPV DNA in invasive cervical cancer indicate that over 90% of these tumours can be attributed to certain HPV types.

Cofactors

The fact that only a small minority of the persistent HPV infections progress eventually to cancer indicates that there should be other factors or cofactors that increase the progression to malignancy. Thus, if we consider the small fraction of cervical cancers in which HPV DNA has not been detected as truly HPV-negative cases we shall conclude that HPV is neither a necessary nor a sufficient cause of cervical cancer. Three types of cofactors may be of importance:

- Viral types and variants: Results from the cohort studies referred to above and from on-going studies indicate that high-risk or oncogenic HPV types and perhaps certain variants of these types are associated with a higher risk of cervical neoplasia.
- Host factors that would modulate the effect of HPV, such as genetic factors (HLA or MHC haplotypes), genetic or induced immunosuppression, endogenous hormonal factors, reflected in the associations with high parity detected in our studies, as well as early age at first sexual intercourse that could be regarded as a surrogate of early age at first HPV infection.
- Exogenous factors. In our studies in Spain, Colombia and Brazil, only long-term use of oral contraceptives and infection with Ch. trachomatis emerged as cofactors among HPV-positive women. However, our observations need to be confirmed in other populations and in larger studies. Our ongoing multi-centre study in which a larger number of women with HPV-positive invasive cervical cancer will be compared with HPV-positive control women will produce valuable information on the role of cofactors, which are schematized in Figure 1.

Our studies also suggest that the above cofactors probably influence more the progression from persistent HPV infection to CIN III than from CIN III to invasive cervical cancer. In fact, a comparison of the risk factors identified for CIN III and invasive cancer in Spain and Colombia did not reveal any risk factor that

**Figure 1. The causes of cervical cancer**
was consistently different between CIN III and invasive cancer to suggest a role in the progression of CIN III to invasive cancer.25

Finally, the role of aetiological factors independent from HPV has not been considered as it is still uncertain whether the small proportion of cervical cancer negative for HPV DNA are truly negative or are false negative which might turn out to be HPV-positive when more sensitive methods of HPV DNA detection are available. In any case, if a subgroup of HPV-negative cervical cancer is finally identified, it would probably account for less than 5% of cervical cancers.

**Male role**

In 1982, a model was proposed whereby the high rates of cervical cancer in Latin America could be explained by a large number of sexual partners among males, including frequent contacts with prostitutes, coupled with monogamy or few sexual partners among women.26 Case-control studies assessing the contribution of male sexual behaviour and genital HPV DNA to the risk of developing cervical neoplasia have yielded inconsistent results. The effect of the number of husbands’ sexual partners was more apparent in countries at low risk of cervical cancer than in countries at high risk. The effect of the number of prostitutes was inconsistent and the early studies in which HPV DNA in the penis was detected by inaccurate hybridization assays were all negative.27

The IARC studies in Spain and Colombia were first in showing a strong relationship between HPV DNA in the male penis/urethra and the risk of cervical cancer in their wives. The prevalence of HPV DNA in the penis was strongly related to sexual behaviour and the number of sexual partners and use of prostitutes reported by the husbands was higher in Colombia than in Spain.28 In Spain, a country traditionally at low risk for cervical cancer, the presence of HPV DNA in the husband’s penis conveyed a 5 to 7-fold risk of cervical cancer to their wives. The risk was 9-fold for spouses of carriers of HPV 16. The risk of cervical cancer was strongly related to the number of extramarital sexual partners (OR= 11.0, 95% CI= 3.0-40.0, for 21 vs 1), and to the number of extramarital prostitutes as sexual partners (OR= 8.0, 95% CI= 2.9-22.2, for 10 vs none). The presence of antibodies to *Ch. trachomatis* and an early age at first sexual intercourse of the husband were both associated with a significant three-fold increased risk of cervical cancer in their wives.27

In Colombia, a high-risk country for cervical cancer, limited education and presence of antibodies to *Ch. trachomatis* were the only identified male risk factors for cervical neoplasia. The prevalence of HPV DNA in the penis was 25.7% among husbands of case women and 18.9% among husbands of control women (OR= 1.2, 95% CI= 0.6-2.3). Neither the lifetime number of sexual partners (OR= 1.0, 95% CI= 0.4-2.6, for > 50 partners vs 1 to 5), nor the lifetime number of prostitutes reported by the husbands (OR= 1.2, 95% CI= 0.7-2.0, for 21 prostitutes vs 1 to 5) were associated with the risk of cervical cancer in their wives.29

In Spain, the study supports the role of men as vectors of the HPV types that are related to cervical cancer. Lifetime number of sexual partners, number of prostitutes as sexual partners and detection of HPV DNA in the penis at the time of the study, are interpreted as surrogate markers of exposure to HPV during marriage. The results in Colombia are compatible with the hypothesis that in the high-risk population of Cali, exposure to HPV among young men is common and mediated by contacts with a high number of sexual partners and prostitutes. These widespread sexual practices limit the power of case-control studies to detect significant associations between men’s sexual behaviour and cervical cancer risk. In this population, HPV DNA detection in the penis of adult men is a poor reflection of lifetime or of the aetiolologically relevant exposure to HPV. The role of *Ch. trachomatis* in cervical carcinogenesis deserves further investigation.

The results of the studies describing the role of men in the epidemiology of cervical cancer strongly confirm that the HPV types related to cervical cancer are a widespread sexually transmitted disease. Furthermore, they suggest that men can operate as HPV carriers in the epidemiological chain. At present there is no obvious recommendation concerning clinical management of male HPV carriers. Detection requires testing for HPV DNA in exfoliated cells from the penis, not an easy task both technically and socially. Colposcopic inspection using acetic acid painting has been recommended and minute HPV-related lesions are often unveiled among partners of women with CIN or HPV infections. Finally, there is at present no reliable treatments for HPV and it has not been clearly shown that condoms would prevent HPV transmission. In spite of these difficulties, any comprehensive approach to HPV control should include research to further elucidate the male role in cervical carcinogenesis and to devise adequate intervention strategies.

**Implications for prevention**

The knowledge that certain types of HPV account for over 90% of cervical cancer has far-reaching implica-
tions for the primary and secondary prevention of this malignancy. Prophylactic and therapeutic HPV vaccines are now under development and a few phase I trials are under way with the latter ones. 30

The immunogens of choice for prophylactic vaccines appear to be the virus-like particles (VLPs) which have been produced for HPV 6, 11, 16, 18, 31, 33, 35, 39 and 45. Vaccines based on VLPs have been shown to be strongly immunogenic in animal models (rabbits, dogs and cattle), but have not yet been tested in humans. Several laboratories and companies are now proceeding to the large-scale production of VLP vaccines at the standards required for human trials. Phase I-II trials are being planned for 1997-1998 and phase III trials will probably not start before the year 2000.

Prophylactic HPV vaccines represent the most promising long-term strategy for control of cervical cancer, especially in developing countries where 80% of these cancers occur and where the screening programmes have largely failed. However, considering that it will take two or more decades before safe and effective HPV vaccines are introduced on a large scale into immunization programmes, prevention by other means (use of barrier contraceptives, safe sexual behaviour) and early detection by efficient screening programmes, should not be neglected.

Concerning screening, preliminary results suggest that HPV testing may be of great use in predicting high-grade CIN whenever the cytology fails. 31

A few trials to assess the value of HPV typing as an adjuvant to cytology in the triage of borderline and low-grade CIN lesions, are in progress in the US and Europe.

References