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ABSTRACT

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Etiological studies — human papillomavirus and cervical cancer

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Since zur Hausen (1976) drew attention to papilomavirus as a possible agent for genital cancer, impressive experimental data have been accumulated supporting an etiological role for human papillomavirus (HPV) in the pathogenesis of cervical cancer. Early epidemiological studies investigating the relation between HPV and cervical neoplasia found strong associations. These studies, however, presented important methodogical problems such as small sample size, biased selection of study subjects, different cervical sampling methods for cases and controls, and little or no control for confounding. Unexpcted findings reported from carefully designed studies were attributed to the use of filter in situ hybridization (FISH), now regarded as a technique with low accuracy. A reliable and sensitive HPV detection strategy based on polymerase chain reaction (PCR), recently developed, is now considered the technique of choice for epidemiological studies.

In developing countries cancer of the cervix is the leading cancer even when sites common to both sexes are combined. A hospital-based case-control study was undertaken to examine the etiological role of HPV in the development of invasive cervical cancer in São Paulo, Brazil, using PCR.

Women with a diagnosis of invasive cervical cancer and women selected as controls were recruited from seven hospitals of Sao Paulo city, between June 1990 and June 1991. The cases were women less than 80 years old, with diagnosis confirmed by biopsy and who had had no previous treatment for the disease. Controls enrolled from hospitals were the cases we recruited were age-frequency matched to the cases. Patients admitted for treatment of a gynecological condition or who had had a hysterectomy corisation were inelegible as controls as were those with diseases associated with known risk factors for cervical neoplasia. Evidence of a gynecological or cytological abnormality detected on examination after recruitment was not a criterion for exclusion.

Two hundred and six women with invasive cervical cancer and 238 controls were eligible for investigation; 199 (96.6%) and 225 (94.5%), respectively, were interviewed. The controls were selected from a wide range of diagnostic categories. Study subjects were interviewed privately in the hospital using a standardized questionnaire. All study had a pelvic examination performed by a gynacologist when exfoliated cells for cytological examination and for HPV analysis were collected. One control (0.5%) had cytologcal evidence of low-grade squamous intraepithelial lesion; she was retained in the study.

A PCR technique was used to analyse HPV DNA in cervical speciments collected with spatula and brush. During sample preparation care was taken to avoid contamination; the equipament used was disposable. When the study was completed, the material was sent to the Department of Pathology, Free University Hospital, Amsterdam. HPV detection was performed directly on crude cell suspensions without knowledge of the case/control status.

To estimate the risk of cervical cancer associated with selected factors, odds ratios (OR) and 95 per cent confidence intervals (95% Cl) were calculated using unconditional logistic regression analysis. Potential confounding variables and interactions with the factors of interest were examined. Statistical significance was assessed using the likelyhood ratio test. Tests for trend were made by categorizing the exposure variables and entering the scores as continuous.

HPV DNA was detected in 157 (84%) of the 186 cases and in 34 (17%) of the 196 controls with cervical speciments (exfoliated cells) in which the ß-globin gene was amplifed. Cervical speciments obtained by biopsy were tested in 16 of the 29 cases whose smear was negative for HPV DNA; in eight (50%) of them HPV DNA was detected. The odds ratios of cervical cancer associated with different types of HPV DNA hardly changed ofter allowing for number of sexual partners and age at first intercourse. The most common type of HPV among cases was HPV 16 whereas among controls, uni-

dentified HPV types were the most frequent. Grouping high-risk types (HPV 16/18/31/33), almost 66% of the cases were positive compared to only six-percent of the controls. The adjusted odds ratio of cervical cancer associated with these types was 69.7 (95% CI 28.7-169.6).

Interactions between detection of HPV DNA and several factors were investigated. Age was the only variable suggestng a possible interaction with HPV. The risk was higher among older women than among younger, but the term for interaction was not significant ($p = 0.09$). In the analisys of interaction entering HPV types (negative, unidentified and 16/18/31/33) instead of negative/positive, the enhanced effect in older women was essentialy restricted to unidentified HPV types and the term for interaction was statistically significant ($p = 0.007$).

The risk of cervical cancer associated with presence of the HPV types 16/18/31/33 was very high. Risks of such magnitude are rarely found in epidemiological studies, being very higher than those found with hepatitis B virus for hepatocellular carcinoma, The estimates remained practically unchanged after adjustment for measures of sexual behaviour, in accordance with the current proposed model for cancer of the cervix.

A higher risk of cervical cancer associated with HPV DNA detection was found among older women. The interaction was mainly between age and unidentified types. Since unidentified types should include HPVs associated with lower risk of cervical cancer than HPVs 16,18,31 and 33, it might suggest that unidentified types need longer to achieve malignant conversion. This finding deserves confirmation.

With a case-control design one cannot be sure whether the HPV infection preceded or postdated the disease. The higher prevalence of HPV in women withless advanced stages found in this study and by other suggests that HPV did not follow the malignant conversion. In addition, results from Colombia and Spain showed similar proportions of HPV-DNA positivity in women with cervical cancer regardless whether they were currently sexually active or whether their last sexual intercourse was more than 10 or even 20 years previously. It might be easier to detect HPV in infected cancer cells rather than in infected non-malignant cells. As density and sampling distribution for specimens of exfoliated cells may differ between cases and controls, it has been speculated that misclassification in detecting HPV DNA could be lower among cases than among controls. No clear evidence for increased susceptibility or higher detection of HPV DNA in malignant cells however, has so far been demonstrated.

Proving that a virus causes cancer presents some dificulties. Only a small proportion of individuals who carry the virus (often asyptomatic) will develop the disease. Latency between primary infection and cancer appearence usually takes several years of decades. A non-trivial proportion of women with normal cytology but with HPV detected indicates that HPV is not a sufficient cause. Conversely, a certain percentage, albeit small, of women with cervical cancer in whom HPV DNA cannot be detected suggests that this virus is not a necessary cause. It is a matter of concern that HPV types related to anogenital cancer have also been detected in many other diverse tumors such as laryngeal carcinoma, urinary bladder carcinoma, prostate carcinoma, esophageal squamous-cell carcinoma, oral cancer and Kaposi's sarcoma.

This case-control study was hospital-based. However, care was taken to select controls with a wide range of diagnoses and cases were restricted to invasive stages. The experimental evidence for the malignant potential of HPV and the very high risks found in this study, particularly in relation to HPV types 16,18,31 and 33, futher implicates this virus in the etiology of cervical cancer.