

HUMAN PAPILLOMAVIRUS INFECTION AND CERVICAL INTRAEPITHELIAL NEOPLASIA IN A COHORT OF LOW-RISK WOMEN

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SUMMARY:

Introduction: Nowadays it was discovered a relationship between some types of Human Papilloma Virus (HPV) and the uterine cervix lesions. Our paper is a prospective study of the incidence of HPV infection at women population with low risk - at whom the HPV typing is negative- and the role of HPV infection in determining and developing of LSIL lesions. (low-grade squamous intraepithelial lesions)

Method: We included in our study 561 women, sexual active, with age between 20-60 years. The criteria of inclusions were: non smokers, married or with a constant and single sexual partner, without history of pre-malign or malign lesions of the uterine cervix or surgical maneuvers performed on cervix, that accepted to participate to the study. We performed statistical analysis of the data using Kaplan-Meier method for estimating the HPV infection. We used also Berslow and Day tests for statistical comparisons.

Results: We observed 22 cases of HPV infection from the 561 women investigated. The study point also that the persistence of HPV infection is associated with an increased risk of LSIL lesions.

Key Words: human papillomavirus, intraepithelial neoplasia,

INFECȚIA CU PAPILOMA VIRUSUL UMAN ȘI NEOPLAZIA INTRAEPITELIALĂ CERVICALĂ LA FEMEILE CU RISC SCĂZUT

Rezumat:

Introducere. În ultima perioadă de timp s-a dovedit o legătură de cauzalitate între anumite tipuri de Papilloma Virus Uman (HPV) și leziunile cervicale. Lucrarea de față este de fapt un studiu prospectiv al incidentei infecției cu HPV la populația feminină cu risc scăzut la care tipajul HPV este negativ și a rolul infecției cu HPV în inițierea și dezvoltarea leziunilor de tip LSIL. **Metodă.** În studiu au fost incluse un număr de 561 de paciente active sexual, cu vârsta cuprinsă între 20-60ani. Criteriile de includere au fost: să fie nefumătoare, căsătorite sau cu un partener sexual stabil, care nu au avut un istoric de leziune precanceroasă sau cancrinoasă la nivelul colului sau manevre chirurgicale care să intereseze colul uterin, care și-au exprimat acordul să participe la studiu. S-a realizat analiza statistică a datelor culese,

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INTRODUCTION

Genital human papillomavirus infections are common in women who are sexually active. Papillomaviruses are characteristically epitheliotropic and cause proliferative lesions in infected epidermal or mucosal epithelia. They are commonly designated wart viruses, although many

members of the group induce only discrete lesions that differ histologically from common warts. Certain types may cause benign and certain types may cause malign tumours. Both experimental and epidemiological data support a causal role of high risk HPV types in the development of cervical cancer: specific viral genes (E6/E7) are transcribed in HPV-positive cancer biopsies. In

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approximately two-thirds of the biopsies viral DNA is integrated into the host cell genome, resulting in a prolonged lifespan of E6/E7 oncoproteins(1).

Infection of the uterine cervix with HPV usually occurs via sexual transmission; some subtypes of HPV infections are strongly related to the development of cervical cancer, its precursor lesions and of genital warts (2). However, most of the epidemiological evidence has come from retrospective studies which have focused on prevalence and factors associated with HPV infections(3,4). Although HPV infections are connected to the development of cervical cancer, more than 70% of HPV infections are transient.

A cytological manifestation, low-grade squamous intraepithelial lesions (LSIL), is thought to be a benign expression of HPV infection, and is also quite common in women. About 20% of these lesions progress to high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer(5).

The objective of this study was a prospective examination of the incidence of HPV infection in a low-risk female population who were HPV negative at enrollment, and of the role of HPV infection in the initiation of LSIL development.

METHODS

Study population and data collection

A total of 561 sexually active women aged 20-60 years, non-smokers, and married or living with a constant partner, who presented for cervical cancer screening at an outpatient clinic – were invited to participate in a prospective study of cervical HPV infection. Only those women were eligible who did not have a history of preneoplastic or neoplastic lesions of the cervix or of conization or hysterectomy, who were willing to participate and who signed an informed consent form. At study entry, participants responded to a questionnaire on the risk factors for cervical cancer and underwent a pelvic examination for the collection of cervical cells for cytological testing and for the detection of HPV DNA. Additionally, colposcopy was carried out in each case as part of the gynaecological examination. Only samples from women with normal cytological results at enrolment were included in the analysis. Follow-up visits were scheduled 3-monthly. At each follow-up visit, a pelvic examination was performed, and cervical specimens were collected for cytological testing and HPV DNA detection. The cervical smears were classified by use of the Bethesda system for cytological diagnoses. For the purpose of analysis, the following categories were used:

normal; atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), LSIL and HSIL or cancer. Histological examination results were reported as benign condyloma, cervical intraepithelial neoplasia (CIN) I, II, or III, depending on whether mild-, moderate-, or high-grade dysplasia was present, respectively.

HPV DNA determinations via HPV hybrid capture assay were carried out in accordance with the instructions of the manufacturer of the kit (DIGENE HPV hybrid capture 2).

Statistical analysis

Person-time methods were used to calculate the HPV incidence density. When interval-censored principles were used, a new infection was assumed to occur at the midpoint between the last negative and the first positive test result. Women continued to make follow-up visits until they developed a new infection or until the final visit, if they consistently gave negative test results. The age-specific incidence of HPV infections was calculated as the number of new cases of infection per woman-year observed at 5-years intervals.

The risk of post enrolment SIL in relation to the HPV infection status during the study period was modelled by Cox1 proportional hazards regression. The time to event was measured from enrolment to the first instance of a lesion event or to the last recorded visit date for censored subjects.

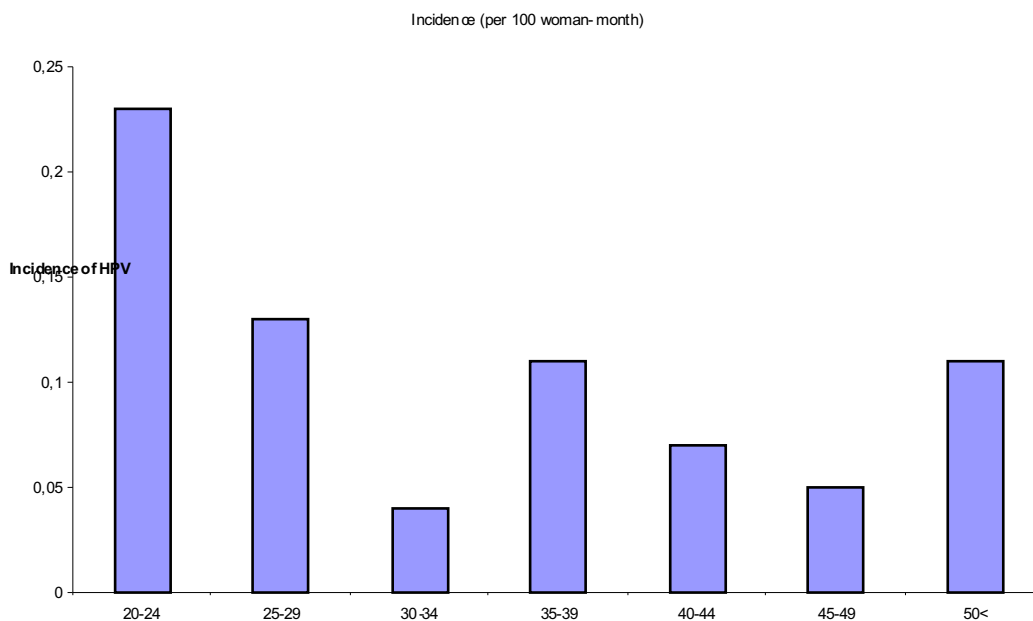
The cumulative risk of acquiring a new HPV infection was estimated by use of the Kaplan-Meier method, under the assumption that infections occurred at the midpoint between the last negative and the first positive test result. The time to infection was measured from the date of the study entry until the date of a new infection, with censoring at the visit with the last negative result. The Berslow and Day test was used for statistical comparison.

RESULTS

Of the 561 women who were invited to participate in the group study at the baseline, 27 women tested positive for HPV and 6 furnished abnormal cytological results, leaving 464 women who constituted the population of the study.

At baseline, the mean age was 33.7 years (SD: \pm 10.06 years). The age-specific HPV infection incidence are depicted on Figure 1. Of the 561 women at risk, 22 presented with HPV infections during the follow-up. Thus, the overall incidence rate of HPV infection was 0.13 cases/100 women-months. The age-specific incidence of

Figure 1. HPV the incidence according to age



HPV infection according to 5-years intervals from ages 20-24 to 45-49 years and over 50 years were 0.225/100 woman-month, 0.122/100 woman-month, 0.039/100 woman-month, 0.115/100 woman-month, 0.076/100 woman-month, 0.025/100 woman-month and 0.001/100 woman-month, respectively. For any HPV, the highest incidence was observed in the age group 20-24 years old. Thereafter, the incidence decreased harmonically with age.

An LSIL event developed in 18 women. Among these women, 13 were HPV-positive (10 high-risk and 3 low-risk types). The average duration of new LSIL was 21.8 (95%CI: 15.7-28.0) months and 55. months (95%CI: 54.7-55.5) in the HPV-positive and negative groups, respectively, these data being statistically different ($p < 0.001$ for Berslow-Day statistics).

With the use of Cox proportional hazard regression, we estimated the relative risk (RR) of a first instance of LSIL over the entire follow-up period among the 464 women free of lesions at study entry. The RR of LSIL was 107.7 (95%CI: 41.3–281.5) for women testing positive for HPV as compared with women testing negative for HPV.

No case of HSIL and CIN II+ occurred among the women in the study.

DISCUSSION

The results of this prospective study describe the incidence of HPV infection in a cohort of women of a broad age range and from a population at low risk of cervical cancer. The cohort comprised women 19–60 years old who were monitored on average for 3 years. We were able to estimate the age-specific incidence

rates of the various HPV types and estimated the relative risk of a first instance of LSIL over the entire period of follow-up for women testing positive for HPV as compared with women testing negative for HPV.

The traditional cross-sectional epidemiological study design does not allow an understanding of the role of HPV and the pattern of changes in the history of cervical dysplasia, whereas screening for cervical lesions on multiple occasions during follow-up does(6). The restriction to prevalence measures in studies produces similarly elevate risk associations for concurrent HPV infection and lesion development.

Our cohort study had several strengths, including the broad age range of the women enrolled, the very low proportion of those refusing to participate, the long follow-up period, and the use of sensitive and well-validated assays for the detection of HPV DNA. However, a limitation of this study is the lack of information on the sexual behaviour of the sexual partners of the women included in the cohort.

The family Papovaviridae contains 2 genera, Polyomavirus and Papillomavirus. They differ substantially in genomic organization and biological properties, although the morphology of the virions is similar. The HPVs are categorised into the Alpha-, Beta-, Gammapapilloma virus genuses regarding the taxonomy. In other generas there are the papillomaviruses of animals. In 2005 in the publication of Viral Taxonomy considered, until the issue more than 118 type of HPV had been sequentated and separated into the 16 genuses.

The HPV is a smaller, DNA virus without envelop and its diameter is 55 nm. It has ikozahedralic symmetry and 72 capsomers and a dial fibric DNA which contains

6800-8400 basis pair. The virus genome codes 8-10 protein in which there are structured (L1 and L2) and non-structured (E1; E2; E4; E5; E6 and E7) proteins, ether resistant and can bear 50 degrees until 1 hour. The viruses are not able to be detected in tissues cultures only on keratinocytes cultures and xenografts(7).

Approximately 80% of HPV infections is transient and asymptomatic(8, 9, 10). These infections do not produce epithelial abnormalities. Only 20% of high-risk HPV infections cause morphologic changes in the epithelium of the cervix without intervention(11). In our study, the 107.7 of RR of LSIL revealed similar relative risk to data in the literature for women testing positive for HPV as compared with women testing negative for HPV. However, progression of premalignant lesions is preceded by clearance of HPV (12). Similar to other reports we suggest that the cases who are HPV positive but have negative cytological test should be follow up more frequently. Nevertheless, the women who are HPV negative as well as cytologically negative and no inflammation might be screened at longer interval.

Further, more information about the natural history of HPV infection should be provided by appropriate education which will certainly increase participation in cervical cancer screening programs.

CONCLUSION

The study has provided evidence that persistent HPV infection is associated with an increased risk of incident LSIL. As far as we are aware, all cohort studies to date have investigated risk factors for HPV infection and cervical dysplasia simultaneously, especially in young women. In our study, however, we selected a low risk population in order to measure directly the relationship between HPV infection and the risk of LSIL. The incidence of HPV infection in a low-risk population could serve as basic information in cost-effective studies.(13)

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