

# Cervical intraepithelial neoplasia: risk factors for persistence and recurrence in adolescents

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## Summary

One of the most common sexually transmitted infections in adolescents is human papillomavirus. These infections can occur in one or multiple areas of the female genitalia but the vulva is usually the initial site of implantation for HPV.

We carried out a long-term follow-up study of adolescents to evaluate the incidence of single or multiple lesions in the lower genital tract, the correlation between sexual behaviour and their localisation and behavioural risk factors for persistence and recurrence of HPV lesions and cervical intraepithelial lesion (CIN).

We interviewed 268 women aged 12-21 years who had previously had cytology and/or physical examination suspicious for HPV infection. We asked them information about their lifestyle, sexual behaviour, work, personal or family history of genital warts and school attendance. Those who smoked more than five cigarettes a day were considered "smokers". We have no specific data about oral contraception although we know that none of the patients had used oral contraceptives for more than two years. Two hundred and thirty-four young women between the ages of 12 and 21 years were included in the study.

The diagnostic schedule for a complete evaluation included exo- and endocervical cytology, colposcopy and directed biopsy. We found that in 126 out of 234 (53.8%) adolescents using contraceptives, only 85 (36.3%) had used a condom.

The sites most frequently affected by lesions were the vulva, perianus and perineum (194/234; 82.9%), and the cervix (125/234; 53.4%). Vaginal lesions were detected in only 29/234 patients (12.3%). In 161 patients, sexual habits, age at first intercourse ( $p = 0.68$ ), frequency of intercourse ( $p = 0.49$ ) and number of lifetime partners ( $p = 0.27$ ) as well as age ( $p = 0.26$ ) played a role in transmission and incidence of HPV infection but not in the location of the lesions. This could be due to coexistent clinical and subclinical multiple infections as well as transmission via intercourse or from other sources, including tampons. HPV infection is detected by abnormal pap test, but the low correlation with colposcopic and histological findings in this study justify the support of other examinations such as colposcopy and punch biopsy for diagnosis.

Moreover more attention should be paid to the psychological aspect of diagnoses and treatment of adolescents compared to older women as there was a high rate of patients lost in our study: 75/234 (32.05%) before LASER surgery and 55/159 (34.59%) during follow-up. Only 12-45% of sexually active adolescent girls had obtained Pap smear screening. In our study we found no correlation between treatment failure and cigarette smoking or between the use of oral contraceptives and persistence/recurrence after LASER surgery.

*Key words:* Cervical intraepithelial neoplasia; HPV; Recurrence; Adolescents.

## Introduction

Genital HPV lesions are one of the most common sexually transmitted infections in adolescents [1]. The association between internal HPV lesions, cervical and/or vaginal, with external warts is probably due to transmission via intercourse or from other sources.

Mount *et al.* [2] showed that 15-19 year-olds had a rate of squamous intraepithelial neoplasia (SIL) slightly lower than 20-24 year-old women and that 10-14 year-olds had a rate of SIL higher than women over 30 years of age. Abnormal Pap smears and cervical dysplasia are also common among adolescents [2, 3] and rates of cervical cancer may be rising worldwide in adolescents [4, 5]. The behavioural risk factors linked to the acquisition of HPV in adolescents and young adult women include first sexual intercourse at an early age, number of lifetime male sexual partners and partner's number of sexual part-

ners [6-8]. In a CDC study conducted in 2000, 65% of high school seniors (aged 17-18 years) reported having had sexual intercourse, with 21% having had more than four partners. In the same survey, 39% of high school freshman (age 13-14 years) reported being sexually active, with 12% already having four or more partners. Biologic risk factors are less well defined, but several studies suggest that adolescents may be at higher risk for acquiring HPV due to the biologic vulnerability of the adolescent cervix [9, 10]. Moscicki *et al.* reported that most infections with high-risk HPV among adolescents and adult women resolve spontaneously [11]. This study confirms the transient nature of HPV infection among most adolescents. Persistent infection, particularly with high-risk HPV types, may be associated with cervical intraepithelial neoplasia (CIN), carcinoma-in-situ or carcinoma [12]. The aim of the study was to evaluate the incidence of the lesions and the behavioural risk factors for persistence and recurrence of HPV and CIN in a long-term follow-up.

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## Materials and Methods

Between 1990-2002, 268 adolescents attended the Department of Gynaecology, Perinatology and Childhealth, University of Rome 'La Sapienza'.

Patients aged 12-21 years who had previously had cytology and/or physical examination suspicious for HPV infection were included. They were asked questions about their lifestyle, sexual behaviour, work, personal or family history of genital warts and school attendance. Those who smoked more than five cigarettes a day were considered 'smokers'. No specific data was recorded about oral contraception (OC) and none of them had used OCs for more than two years.

Our diagnostic schedule for a complete evaluation included exo- and endocervical cytology, colposcopy, directed biopsy. Virgins underwent vaginoscopic examination where vulvar, perianal and perineal fields were also examined. An Ayre spatula was used for cervical scraping, while for endocervical sampling a cotton swab was used. Colposcopy was performed after a 5% acetic acid application; it was considered unsatisfactory when the squamocolumnar junction could not be seen. A microcolpohysteroscopic evaluation was then performed [14]. Colposcopic findings were classified according to the International Terminology of Colposcopy [13]. In case of abnormal colposcopic findings a colposcopically directed biopsy was performed. Cytological, colposcopic and histological findings were compared in order to plan ablative/excisional LASER surgery.

Adolescents included in the study were between the age of 12 and 21 years, had HVP lesions after entry examinations and had undergone no treatment for HPV lesions prior to entry. Other exclusion criteria relevant to this study included cervical excisional treatment prior to entry or later. The patients were treated with a LASER Coherent 400, model 451, with a Zeiss photo-colposcopy attachment.

Different methods were used depending on the site and diagnosis of the lesion. LASER surgery was always performed in the outpatient clinic and in cases of vulvar, vaginal, perineal and perianal lesions, 2% carbocaine was used for local anaesthesia. Patients underwent follow-up examinations one month after the initial treatment. A second follow-up examination was performed three months after the initial treatment and continued follow-up examinations were done every six months. Follow-up examinations consisted of exo- and endocervical cytology, colposcopy and microcolpohysteroscopy, and directed biopsy when necessary.

The Student's t-test was performed when suitable to analyse nominal data. We performed a chi-square (with previous Yates correction) or a Z-test for percentage. The conventional probability value  $p < 0.05$  was considered significant. The total number of samples satisfied the statistical power (0.8 at  $\alpha < 0.05$ ).

## Results

There was no cytological, colposcopic or histological evidence of HPV infections among 21 of our patients and they were therefore excluded from the study. Twelve patients had undergone previous treatment for HPV lesions. Only one case of CIN 3 was reported at entry examination and a conization was then planned. Two hundred and thirty-four adolescents were included in the study group. The average age of the 234 young women was 19 years (range 12-21). Three had attended primary

school (1.2%), 218 secondary school (93.1%) and 13 university (5.5%). Most of them were married (221/235). Information about work was also reported: 46 (19.5%) were unemployed, 131 (56.1%) were students, three (1.2%) housewives and 54 (22.9%) employed. Five patients (2.1%) were virgins, seven were pregnant at the time of examination (average gestation week: 29.1; range: 20-38). Two hundred and twenty (94.0%) had never been pregnant and 14 (5.9%) had been pregnant one or more times (average: 1.4; range: 1-3). The average age at first intercourse was 16.9 years (range 11-20) for 224 patients, average number of partners was 2.1 (range 1-15) for 222 patients and frequency of intercourse per week was 2.3 (range 1-7) for 173 patients. One hundred and eleven (47.2%) patients had been smoking more than five cigarettes/day and six (2.2%) were occasional cannabis users. Only 126/234 (53.8%) adolescents had used contraceptives: 85 (36.3%) had used a condom, 14 (5.9%) had used a diaphragm, three (1.2%) had used uterine devices and 76 (32.4%) had used OCs. Thus, only 85/234 (36.3%) of all the patients with HPV lesions used the only effective protection against viral infection. Punch biopsies were done in 98 cases. Comparing the results of cytology and colposcopy with those of histology, concordance occurred in 85 (86.7%) and 45 (45.9%) cases, respectively (Table 1). Cytology overestimated and underestimated lesions in 37 and 16 cases, respectively. Cytology underestimated all cases of CIN: two cytological normal cases corresponded to a histological case of CIN 1 and CIN 2; five cytological HPV lesions corresponded to four histological cases of CIN 1 and one case of CIN 2.

Table 1. — Correlation between cytology and colposcopy versus histology on colposcopically directed biopsy.

Histology	Colposcopy			Cytology		Total
	Normal	ANTZ1	Condyloma	Normal	HPV	
Negative	0	41	2a	7	37a	44
HPV/condyloma	3b	37	8a	9b	38	47
CIN 1	0	5	0	1b	4b	5
CIN 2	0	2b	0	1b	1b	2
CIN 3	0	0	0	0	0	0
Total	3	85	10	18	80	98

Histology/colposcopy:  $\chi^2 = 8.21$ ,  $p = 0.22$ ; Histology/colposcopy:  $\chi^2 = 1.5$ ,  $p = 0.67$ ; a = Overestimation; b = Underestimation.

Colposcopy overestimated and underestimated lesions in ten and five cases. Three colposcopic normal cases corresponded to histological HPV lesions and two cases of ANTZ1 to histological CIN 2.

In 128 patients, there were single lesions (30 cervical, five vaginal, 93 vulvar-perianal-perineal), while in the remaining 106 (45.2%) multiple lesions were found. The sites most frequently affected were the vulva, perianus and perineum (194/234), and cervix (125/234). In 161 patients, the age at first intercourse, frequency of intercourse and number of lifetime partners were traced and correlated with the localisation of the lesion as well as the age. Virgins, pregnant women, and women whose data about sexual habits were lacking were not taken into con-

Table 2. — LASER surgery of HPV lesions: correlation with smoking and oral contraception.

	Remissions			Persistence/recurrence			Lost		
	Within 1 year	After 1 year	Total	Within 1 year	After 1 year	Total	Never back	1 month exam	Total
Treatment	23	56	79	9	16	25	25	30	55
Smokers	12	23	35	5	8	13	—	—	—
Oral contraception	5	17	22	3	6	9	—	—	—

Smokers:  $\chi^2 = 0.04$ ;  $p = 0.84$ ; Oral contraception:  $\chi^2 = 0.10$ ;  $p = 0.744$ .

sideration. Only 159 of all 234 young women with HPV lesions underwent LASER CO<sub>2</sub> ablation treatment.

The average age of the treated patients was 19 years with a range of 12-21 years.

During the treatment sessions as well as during the follow-up complications occurred in 18 cases (11.3%). They included: itchiness, burning sensation, pain and fever. Follow-up was carried out in 104 (65.4%) of the treated patients. Twenty-five (15.7%) patients never came back to the offices after treatment and after one month 30 (18.8%) were lost after the first colposcopic examination from LASER treatment. Seventy-nine (49.6%) were cured, nine (5.6%) had persistence/recurrence within one year and 16 after one year from the treatment. The mean time of persistence/recurrence was 29 months (range: 3-108). A case of CIN 2 was detected by punch biopsy four months after LASER surgery for a cytological cervical condyloma and a case of CIN 1 after 108 months. The rate of remission and persistence/recurrence were correlated with smoking habits and use of OCs.

No correlation was found among smokers and non-smokers ( $\chi^2 = 0.04$ ;  $p = 0.84$ ) or between use of OCs and the rate of persistence/recurrence ( $\chi^2 = 0.10$ ;  $p = 0.744$ ) (Table 2).

Five cases of CIN (four CIN 1 and one CIN 2) also underwent LASER CO<sub>2</sub> surgery. Two cases (CIN 1) were lost at follow-up; two cases (CIN 1 and CIN 2) were normal after one year; one case (CIN 1) had a cytological cervical HPV lesion detected at follow-up and underwent a second treatment and no cervical lesion was detected during follow-up.

## Discussion

One hundred and twenty-six out of 234 (53.8%) adolescents used contraceptives; only 85 (36.3%) had used a condom, the only form of contraceptive to effectively protect against the virus, thus indicating a low awareness of the high risk of contracting HPV infection among young sexually active women. The areas most frequently affected in our study were the vulva, perianus and perineum (194/234, 82.9%), and cervix (125/234, 53.4%). Vaginal lesions were detected only in 29 out of 234 patients (12.3%). According to our data, in 161 patients, the age at first intercourse ( $p = 0.68$ ), the frequency of intercourse ( $p = 0.49$ ) and number of lifetime partners ( $p = 0.27$ ) as well as age ( $p = 0.26$ ) played a role in transmission and incidence of HPV infection but not in the location of the lesions. This could be due to coexistent

clinical and subclinical multiple infections as well as transmission via intercourse or from other sources, including tampons.

According to laboratory-based research there is definitely a link between HPV infection and cervical cancer [15].

Low-grade SIL was strongly associated with HPV; a period of latency occurs before low-grade SIL develops [16]. The reason for this latency and later up-regulation is not clear. In the same study, the majority of females with positive HPV results never developed LSIL within a median follow-up time of 60 months, which suggests that certain biological risks or conditions are required for LSIL to occur.

Cervical cancer rates have decreased dramatically since the implementation of widespread screening and follow-up procedures for women as well as adolescents, who generally have the same mirrored protocols [17]. However, a single cytological test has limited effectiveness in detecting cervical dysplasia, and current cervical cancer screening programmes are costly [18]. Cytologic screening is subject to sampling error, slide preparation error, interobserver variability or interpretation error and misclassification [12].

HPV infection can be detected in abnormal pap tests, but the low correlation with colposcopic and histological findings in this study justify the support of other examinations such as colposcopy and punch biopsy for diagnosis.

Cytology alone is not suitable to exclude CIN and a closer correspondence has been found between colposcopy and the result of histological sample after punch biopsy in CIN detection. Colposcopically directed biopsy is a safe and reliable method in adolescents [19]; no complications were observed following the biopsies in our study.

Among adolescents, high- and intermediate-risk type HPV is frequent, with a prevalence range of 9-16% [2, 12, 20]. However, distinguishing low-risk from high-risk HPV types might have only limited clinical utility [21].

In adolescents compared to older women, a lot of attention should be paid to the psychological aspect of diagnoses as a high rate of patients were lost in our study: 75/234 (32.05%) before LASER surgery and 55/159 (34.59%) during follow-up. International data show that only 12-45% of sexually active adolescent girls have obtained Pap smear screening [16, 21, 22], and although adolescents plan to return for screening and follow-up, many do not return for those procedures [23]. Smoking and use of oral contraceptives could play a role in HPV infection-CIN development as well as in the treatment, however it depends on the age of the woman. Cigarette smoking is a well known risk of LSIL. Cigarette smoking is closely linked to CIN treatment failure ( $p = 0.0013$ ); current smokers had a threefold increased risk of treatment failure of CIN compared to non-smokers (95% CI 1.65-5.91). In our study, we found no correlation between treatment failure and cigarette smoking or between the use of oral contraceptives and persistence/recurrence after LASER surgery.

Because of the small sample of our study group, other further analyses were required. HPV infection in adolescents indicate: the identification of behaviour and biologic risk factors for HPV; the use of specific screening protocols among adolescents, including Pap smear screening for those who are at risk of cervical dysplasia.

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