Low grade squamous intra-epithelial lesions and human papillomavirus infection in Colombian women

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Low grade squamous intra-epithelial lesions could be considered as a manifestation of human papillomavirus exposition, however the discrepancy between rates of infection with human papillomavirus and development of low grade squamous intra-epithelial lesions is notable. Here we report a cross-sectional three-armed case–control study in the Colombian population, to compare the risk factors of women with low grade squamous intra-epithelial lesions with that of human papillomavirus DNA-negative and positive women with normal cytology.

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Cervical cancer, the main cause of death from cancer among Colombian women, is known to be preceded by a series of squamous intra-epithelial lesions (SIL). Recently it has been established that human papillomavirus (HPV) is not only the central cause of cervical cancer, but also a necessary cause (Walboomers et al, 1999).

Natural history studies indicate that the chain of events leading to cervical cancer starts with HPV infection, followed by high-grade squamous intra-epithelial lesions (HSIL) and cancer. The category HPV infection could include a range of diagnoses from HPV DNA detectable only by PCR without cytological abnormalities (latent HPV infection) to CIN-1 lesions indistinguishable from koilocytic atypia, called low-grade squamous intra-epithelial lesions (LSIL) or productive HPV infection. LSIL should then be considered as a sign of exposure to HPV.

It is currently unclear if the determinants of HPV DNA detection in women with normal cytology are the same or similar to those of LSIL. A recent follow-up study in young women suggests that risk factors for HPV infection are different from those of LSIL (Moscicki et al, 2001).

In a previous study the risk factors for HPV DNA detection among Colombian women with normal cytology were identified (Molano et al, 2002). We report here a cross-sectional three-armed case-control study in the same population, to compare the risk profile of women with LSIL lesions with that of HPV DNA-negative and positive women with normal cytology.

MATERIALS AND METHODS

Study population

This study was conducted in Bogota, Colombia. Briefly, between 1993 and 1995 the Colombian Cancer Institute conducted a census in four health districts in Bogota. The first 2000 women aged 18–85 years identified in the census were invited to participate. In addition, 200 adolescents aged 13–17 years consulting an adolescent clinic giving contraceptive counselling were also invited to participate. At recruitment, all women were interviewed by specially trained interviewers using a structured questionnaire on socio-demographic characteristics, lifelong sexual behaviour, reproductive history, smoking and dietary habits. After interview all women underwent a pelvic examination during which cervical scrapes were collected for routine Pap smear and HPV DNA and Chlamydia trachomatis detection.

Out of the 2200 invited women, 53 refused to participate, eight were considered ineligible (history of hysterectomy or cervical cancer, mental illness), 14 did not fill in the epidemiological questionnaire, 29 did not provide cervical scrapes and 1010 had inadequate cervical specimens for amplification, as measured by failure to amplify a human β-globin fragment. Thus 1995 (90.7%) women with a cytological diagnosis and with adequate cervical samples for HPV DNA and C. trachomatis PCR testing constituted the sampling frame for the study. Informed consent was obtained from all women and the ethical committees of the National Cancer Institute of Colombia and of the IARC cleared the study protocol.

Case-control selection

The smears taken at recruitment were read by two expert cytopathologists as follows: normal cytology in 1845 women (92.5%), LSIL in 70 (3.5%) women, atypical squamous cells of undetermined significance (ASCUS) in 30 (1.5%), atypical glandular cells of undetermined significance (AGUS) in 14 (0.7%), HSIL in 8
of cytology controls. The second compared LSIL-positive for HPV DNA with two subgroups of normal cytology controls, one including only HPV DNA-negative controls, the other including only HPV DNA-positive controls.

The ORs estimates were adjusted for age (grouped as <20, 20–24, 25–29, 30–34, 35–39, 40–44, 45–54, ≥55).

RESULTS
The estimated overall HPV DNA prevalence in the total study population from which we selected our three study groups was 16.5%. Table 1 presents the distribution of study subjects, the prevalence of HPV DNA by diagnostic category and the age-adjusted ORs for any type of HPV. More severe lesions were associated with higher prevalence of HPV DNA, higher prevalence of high-risk HPV types and higher ORs. No association with HPV was observed for the group diagnosed as ASCUS/AGUS.

Table 2 shows the distribution of the HPV types detected in 70 women diagnosed with LSIL and in 557 control women. Eighteen different types were detected in LSIL and 30 among controls. HPV 6, 7, and CP6108 were exclusively found in LSIL and HPV types 11, 34, 40, 43, 44, 53, 54, 59, 68, 72, MM6&ISO39 and MM8 were exclusively detected among controls. The most common type detected in both cases and controls was HPV 16. All women with multiple infections (except one) had at least one high-risk type and the prevalence of these multiple infections among HPV-positive women was similar in LSIL (30.8%) and controls (27.6%).

The age-adjusted OR for LSIL associated with single HPV infections was 6.1 (95% CI=3.4–10.9) while that for multiple infections was 7.6 (95% CI=3.4–16.9). The median age of women with LSIL was 30.8. The prevalence of this diagnosis was higher in women younger than 25 years where it was 4.4% and then decreased to 1.7% in women over 55–64 years old and 0.0 in women over 65. The prevalence of C. trachomatis was 7.1% in the overall LSIL group and 5.2% in the control group (Table 3).

When all LSIL cases (HPV-positive and HPV-negative) were compared with all controls (HPV-positive and negative) and ORs adjusted for age and HPV were estimated, a significantly reduced risk appeared with ever use of OCs. Non-significant increased risk associated with the detection of C. trachomatis and non-significant decreased risks with number of partners, age at first sexual intercourse and smoking were observed (Table 3). When women with HPV DNA-positive LSIL were compared with HPV DNA-negative controls (Table 3), a significant increased risk for women attaining higher educational level was observed, and the reduced risk with number of partners, age at first sexual intercourse and with ever use of OCs persisted, however non-significantly. When HPV DNA-positive LSIL was compared with HPV DNA-positive controls, the reduced risk associated with the use of OCs was significant (OR=0.3; 95% CI=0.1-0.7), while the association with higher education lost statistical significance, and non-significant decreased risks with age at first sexual intercourse, number of part-

Table 1 Diagnosis, HPV and age-adjusted odds ratios

| Diagnosis         | Number | Prevalence of diagnosis | High-risk HPV % | Low-risk HPV % | Uncharac HPV % | Single infections % | Multiple infections % | Any HPV types % | Age-adjusted OR for any HP (95% CI) |
|-------------------|--------|------------------------|-----------------|---------------|---------------|--------------------|-----------------------|----------------|----------------------------------|
| Normal-total      | 1845   | 92.5%                  | 11.4            | 3.2           | 0.4           | 10.5               | 4.4                   | 14.9           | 1                                |
| ASCUS/AGUS        | 44     | 2.2%                   | 4.6             | 4.6           | 0.0           | 6.8                | 2.3                   | 9.1            | 0.7 (0.3–2.1)                    |
| LSIL              | 70     | 3.5%                   | 47.1            | 4.3           | 43            | 38.6               | 17.1                  | 55.7           | 7.4 (4.5–12.3)                   |
| HSIL/Cancer       | 10     | 0.5%                   | 70.0            | 10.0          | 0.0           | 70.0               | 10.0                  | 80.0           | 3.12 (6.4–150.7)                 |
| Inadequate smear  | 26     | 1.3%                   | 7.7             | 3.9           | 0.0           | 7.7                | 3.9                   | 11.5           | 1.2 (0.4–4.4)                    |
| Total Diagnosis   | 1995   | 100.0%                 | 12.7            | 3.3           | 0.5           | 11.7               | 4.8                   | 16.5           |                                  |
DISCUSSION

We report here the prevalence of cervical neoplasia and of HPV types in a Colombian high-risk population. The estimated overall prevalence of HPV was 16.5%. Among women with normal cytology it was 14.9%, while in LSIL it was 55.7%. Under the premise that HPV is a necessary cause of cervical cancer, we should expect that all true pre-neoplastic lesions contain HPV DNA. Thus, the 44.3% of HPV-negative LSIL could represent errors of diagnosis (false LSIL diagnosis), low sensitivity of the HPV assay or both. Considering that the PCR-based assays used in this study have shown excellent performance in previous investigations, the possibility of a misdiagnosis of LSIL is more likely. In line with this possibility is the fact that the prevalence of LSIL in this Colombian population (3.5%) was higher than the one reported in another high-risk population in Costa Rica (2.2%) using more strict diagnostic criteria (Herrero et al., 2000). The HPV DNA prevalence among women with normal cytology from the same study population. Molano et al. (2002) have previously reported that both number of sexual partners and OC use were associated with an increased risk of HPV detection in Colombian women with normal cytology. Our inconsistent results concerning number of sexual partners might be explained by the importance and the difficulty in assessing the role of male sexual behaviour in determining the risk of cervical neoplasia, as evidenced in previous studies in Colombia (Munoz et al., 1996b).

In relation to OC use, our results are in line with those reported by Schiffman et al. (1993) in a US population, that found a non-significantly reduced risk of CIN lesions among HPV-positive women associated with oral contraceptive (OC) use. These findings are in contrast to the risk factors for HPV DNA detection identified in women with normal cytology from the same study population. Molano et al. (2002) have previously reported that both number of sexual partners and OC use were associated with an increased risk of HPV DNA detection in Colombian women with normal cytology. Our inconsistent results concerning number of sexual partners might be explained by the importance and the difficulty in assessing the role of male sexual behaviour in determining the risk of cervical neoplasia, as evidenced in previous studies in Colombia (Muñoz et al., 1996b).

To assess the determinants of LSIL we carried out a three-armed case–control analysis in which all LSIL cases (HPV-positive or HPV-negative) were compared with a control group of normal cytology, independently of HPV status. Then LSIL HPV-positive cases were compared with two control groups: one composed by HPV-negative women with normal cytology and a second one of HPV-positive women with normal cytology.

Surprisingly, no association with the number of sexual partners was observed when LSIL cases were compared with HPV negative controls and a reduced risk of LSIL was associated with oral contraceptive (OC) use. These findings are in contrast to the risk factors for HPV DNA detection identified in women with normal cytology from the same study population. Molano et al. (2002) have previously reported that both number of sexual partners and OC use were associated with an increased risk of HPV DNA detection in Colombian women with normal cytology. Our inconsistent results concerning number of sexual partners might be explained by the importance and the difficulty in assessing the role of male sexual behaviour in determining the risk of cervical neoplasia, as evidenced in previous studies in Colombia (Muñoz et al., 1996b).

In relation to OC use, our results are in line with those reported by Schiffman et al. (1993) in a US population, that found a non-significantly reduced risk of CIN lesions among HPV-positive women associated with OC use, but not with those of a recent report by Moscicki et al. (2001). They found that OC use had a protective effect for incident HPV infection, but it was not a risk factor for incident LSIL.

Table 2  HPV types distribution in women with LSIL and control women

| HPV types | Single infection | Multiple infection | Single or multiple |
|-----------|------------------|--------------------|-------------------|
| 6         | 2                | 2                  |                   |
| 11        |                  |                    |                   |
| 16        | 4                | 3                  | 7                 |
| 18        | 5                | 5                  | 2                 |
| 31        | 1                | 2                  | 3                 |
| 33        | 3                | 3                  | 6                 |
| 34        |                  |                    |                   |
| 35        | 1                | 1                  | 2                 |
| 39        | 2                | 1                  | 3                 |
| 40        |                  |                    |                   |
| 42        | 1                |                    |                   |
| 43        |                  |                    |                   |
| 44        |                  |                    |                   |
| 45        |                  |                    |                   |
| 51        | 1                | 2                  | 3                 |
| 52        | 5                |                    | 5                 |
| 53        |                  |                    |                   |
| 54        |                  |                    |                   |
| 56        | 1                | 2                  | 3                 |
| 58        | 2                | 2                  | 4                 |
| 59        | 1                | 2                  |                   |
| 66        | 1                |                    |                   |
| 68        | 2                | 2                  |                   |
| 70        | 2                | 2                  | 1                 |
| 72        |                  |                    |                   |
| 73        |                  |                    |                   |
| CP6108    |                  |                    |                   |
| CP8304    | 1                | 1                  | 2                 |
| MM7       |                  |                    |                   |
| MM8       | 2                | 1                  | 3                 |
| ISO39     |                  |                    |                   |
| HR        | 4                | 1                  | 5                 |
| LR        | 3                | 3                  | 1                 |
| X         |                  |                    |                   |

High-risk types in bold.
In contrast to our results, tobacco smoking has been identified as a risk factor for LSIL in other populations. For example, Krüger-Kjær et al. (1998) found a positive association with smoking for both LSIL and HSIL among HPV-positive women in Denmark. Moscicki et al. (2001) also identified smoking as a risk factor for LSIL in a cohort study of young US women.

Although our study was limited, for the small number of cases, it indicates that with the possible exception of OC, we were unable to identify any other important co-factor for the development of LSIL. An additional major limitation of our study, as well as of the vast majority of the studies of LSIL is the inaccuracy of the cytological diagnosis of LSIL. We have attempted to circumvent this limitation by restricting the analysis to HPV-positive-LSIL, but this considerably limited the power of our study.

On the other hand, studies aimed to assess cofactors for HSIL and cervical cancer have identified parity, smoking and long-term OC use as important cofactors that modulate the risk of progression from HPV infection or LSIL to HSIL and cervical cancer (Deacon et al., 2000; Hildesheim et al., 2001; Lacey et al., 2001; Moreno et al., 2002; Muñoz et al., 2002).

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Table 3 Risk factors for LSIL

| Risk factors for LSIL (HPV+ and HPV−) | % cases (n=70) | % control (n=557) | OR adjusted for age and HPV (95% CI) | % cases (n=39) | % HPV-control (n=459) | OR adjusted for age (95% CI) | % cases (n=39) | % HPV+control (n=98) | OR adjusted for age (95% CI) |
|-------------------------------------|----------------|------------------|-------------------------------------|----------------|----------------------|-----------------------------|----------------|----------------------|-----------------------------|
| Education                           |                |                  |                                     |                |                      |                             |                |                      |                             |
| Primary or none                     | 27.1           | 30.2             | 1                                  | 15.4           | 32.0                 | 1                           | 15.4           | 21.4                 | 1                           |
| Secondary or higher                 | 72.9           | 69.8             | 1.1 (0.6 – 2.0)                    | 84.6           | 68.0                 | 2.5 (1.0 – 6.2)              | 84.6           | 78.6                 | 1.9 (0.6 – 5.8)            |
| Age at first intercourse            |                |                  |                                     |                |                      |                             |                |                      |                             |
| 20+                                 | 40.0           | 31.2             | 1                                  | 43.6           | 32.7                 | 1                           | 43.6           | 24.5                 | 1                           |
| 17 – 19                             | 32.9           | 36.5             | 0.7 (0.4 – 1.3)                    | 33.3           | 35.7                 | 0.6 (0.3 – 1.3)              | 33.3           | 39.8                 | 0.4 (0.2 – 1.1)            |
| <17                                 | 27.1           | 32.3             | 0.6 (0.3 – 1.2)                    | 23.1           | 31.6                 | 0.3 (0.1 – 0.87)             | 23.1           | 35.7                 | 0.3 (0.1 – 1.02)           |
| Number of regular sexual partners   |                |                  |                                     |                |                      |                             |                |                      |                             |
| 1                                   | 83.3           | 78.2             | 0.7 (0.3 – 1.3)                    | 86.5           | 78.8                 | 1.6 (0.2 – 1.7)              | 86.5           | 75.0                 | 1                           |
| 2+                                  | 16.7           | 21.8             | 1                                  | 13.5           | 21.2                 | 0.6 (0.2 – 1.7)              | 13.5           | 25.0                 | 0.5 (0.2 – 1.4)            |
| Chlamydia                           |                |                  |                                     |                |                      |                             |                |                      |                             |
| Trachomatis infection               |                |                  |                                     |                |                      |                             |                |                      |                             |
| No                                  | 92.9           | 94.8             | 1                                  | 97.4           | 95.4                 | 1                           | 97.4           | 91.8                 | 1                           |
| Yes                                 | 7.1            | 5.2              | 1.4 (0.5 – 4.1)                    | 2.6            | 4.6                  | 0.6 (0.07 – 4.3)             | 2.6            | 8.2                  | 0.3 (0.4 – 2.6)            |
| Parity                              |                |                  |                                     |                |                      |                             |                |                      |                             |
| 0 – 1                               | 35.7           | 37.7             | 1                                  | 46.2           | 35.5                 | 1                           | 46.2           | 48.0                 | 1                           |
| 2 – 3                               | 45.7           | 47.4             | 1.1 (0.6 – 2.3)                    | 43.6           | 48.4                 | 0.8 (0.3 – 1.8)              | 43.6           | 42.9                 | 0.9 (0.3 – 2.3)            |
| 4+                                  | 18.6           | 14.9             | 1.6 (0.7 – 4.1)                    | 10.3           | 16.1                 | 0.5 (0.2 – 2.0)              | 10.3           | 9.2                  | 0.9 (0.2 – 4.1)            |
| Oral contraception                  |                |                  |                                     |                |                      |                             |                |                      |                             |
| Never                               | 60.9           | 51.2             | 0.6 (0.3 – 0.99)                   | 68.4           | 52.8                 | 1                           | 68.4           | 43.8                 | 1                           |
| Ever                                | 39.1           | 48.8             | 0.6 (0.3 – 0.99)                   | 31.6           | 47.2                 | 0.5 (0.3 – 1.1)              | 31.6           | 56.2                 | 0.3 (0.1 – 0.7)            |
| Smoking                             |                |                  |                                     |                |                      |                             |                |                      |                             |
| Never                               | 74.3           | 71.6             | 0.7 (0.4 – 1.3)                    | 74.4           | 74.1                 | 1                           | 74.4           | 60.2                 | 1                           |
| Ever                                | 25.7           | 28.4             | 0.7 (0.4 – 1.3)                    | 25.6           | 25.9                 | 1.1 (0.5 – 2.4)              | 25.6           | 39.8                 | 0.5 (0.2 – 1.1)            |

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