Prevalence, genotype profile and risk factors for multiple human papillomavirus cervical infection in unimmunized female adolescents in Goiânia, Brazil: a community-based study

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Abstract

Background: The epidemiology of infection with multiple human papillomavirus (HPV) types in female adolescents is poorly understood. The purpose of this study was to explore the epidemiology of infection with multiple HPV types in adolescents and its association with demographic, behavioral and biological variables, as well as with cytological abnormalities.

Methods: This community-based study included 432 sexually active females between 15 and 19 years of age. Genotyping for 30 HPV types was performed using a reverse blot strip assay/restriction fragment length polymorphism. Unconditional multivariate logistic regression was performed to identify factors significantly associated with HPV infection. The association between HPV infection and cytological abnormalities was calculated using a prevalence ratio.

Results: The most common HPV types detected were 16, 51, 31, 52 and 18. Of the 121 HPV-positive women, 54 (44.6%) were infected with multiple HPV types. Having more than one lifetime sexual partner was associated with infection with any HPV infection, single HPV infection, and infection with multiple HPV types. The presence of cytological abnormalities was associated with infection with multiple HPV types.

Conclusions: Co-infecting HPV genotypes occur in a high proportion of sexually active adolescents. Socio-demographic or sexual behavior factors associated with single HPV infection were similar to those associated with multiple HPV types. The higher risk of cytological abnormalities conferred by infection with multiple HPV types suggests a potential role of co-infection in the natural history of HPV infection.

Keywords: Epidemiology, Multiple HPV infection, PCR-PGMY09/11/line blot hybridization/RFLP, Adolescents, female, Cytological abnormalities
Background

Human papillomavirus (HPV) cervical infection is one of the world's most common sexually transmitted infections, and is also a well-established cause of cervical cancer [1,2]. The highest prevalence of single or co-infection with multiple HPV types has been found in sexually active adolescents, young women [3-7], and women with impaired immune responses [8]. Studies of the dynamics of multiple HPV infections suggest that the acquisition of different HPV types occurs more often than expected by chance, and shared risk factors may explain the higher frequency of co-infections [9,10]. According to previous studies, the risk factors for cervical infection with multiple HPV types among HPV-infected women share several similarities with risk factors for HPV infection in general [6,7]. In addition, previous studies suggest that Chlamydia trachomatis infection can provide target host cells for the acquisition of HPV and may enable persistent HPV infection [6,11].

HPV infections and cytological abnormalities are common in adolescents, appearing shortly after the onset of sexual activity. More than 90% of HPV infections and cytological abnormalities regress within 3 years [12,13]. Co-infection with multiple HPV types has been associated with longer infection duration and a higher risk of cytological abnormalities and cervical neoplasia [3,14-16]. However, a consensus concerning these associations has not been reached [2,5,17].

Data on HPV infection and cytological abnormalities in adolescents derived from non-healthcare settings are scarce, especially in South America [18,19], and particularly in the mid-western region of Brazil [20]. The recent availability of a vaccine against HPV infection is a promising primary prevention measure to reduce the burden of cervical cancer. Therefore, a description of the genotype distribution of multiple HPV infections and the prevalence and degree of cytological abnormalities in sexually active adolescents is necessary for characterizing the target population for this vaccine as well as reinforcing the recommendation for cervical cancer screening in this age group.

The objectives of this study were to estimate the prevalence, describe the genotype profile and identify the risk factors for multiple HPV infections among healthy adolescents in the central part of Brazil, prior to the implementation of a large-scale HPV immunization program. Additionally, the study aimed to investigate the potential association between multiple HPV infections and cytological abnormalities among adolescents from a non-healthcare setting.

Methods

Study design and setting

This was a cross-sectional, community-based study. The design and population characteristics of this study have been described previously [21]. Briefly, this study was conducted between 2002 and 2003 in the Northwest sector of Goiânia, a city of 1,093,007 inhabitants in the central part of Brazil. The overall population of the Northwest sector was approximately 160,030 inhabitants. During the study period, a total of 4,091 females aged 15–19 years were registered at the local Public Family Health Program.

Study population and sampling

Households with potential participants were randomly selected from census information provided by the local health department using a systematic sampling scheme. All female adolescents in each selected household were invited by letter to attend the health center nearest to their residence. Among the 914 adolescents who accepted the invitation, 472 (51.6%) were sexually active. The adolescents were eligible if they were between 15 and 19 years of age, were not currently pregnant or postpartum, had not used oral or vaginal antimicrobial drugs in the previous 15 days and had not engaged in sexual intercourse in the previous 48 hours. In total, 432 adolescents fulfilled the requirements, answered an interviewer-administered questionnaire and had a gynecological examination.

All participants provided written informed consent to participate in the study. The study was reviewed and approved by the Ethics Committee on Human and Animal Medical Research of the University Hospital, Federal University of Goiás.

Data collection

The data were obtained through an interviewer-administered questionnaire addressing sociodemographic characteristics (age, education, marital status), tobacco smoking habits, as well as the subject’s sexual, gynecological and reproductive history, including age at menarche, age at first intercourse, interval in years between the age of first intercourse and the age of menarche, lifetime number of sexual partners, number of new sexual partners in the previous 3 months, frequency of condom use, history of full-term pregnancies and age of first pregnancy.

The cervical samples collected during the gynecological examinations were used for Papanicolaou tests, HPV and C. trachomatis DNA detection. Ectocervical and endocervical cells were collected with a cytobrush, which was then immersed in 10 mM Tris (pH 7.4 plus 1 mM EDTA), refrigerated and transported to the laboratory of Tropical Pathology and Public Health, Federal University of Goiás, and stored at −80°C until analysis to detect HPV and C. trachomatis.

C. trachomatis DNA detection was performed by PCR with primer pairs CP24 and CP27, which amplify a 207 nucleotide sequence of the cryptic plasmid. Positive and negative controls were included in each assay. An internal control was conducted in each amplification reaction.
to detect inhibitors. The assay was performed at the Department of Immunology, Tropical Pathology and Public Health Institute/Federal University of Goiás.

Papanicolaou smears were transported to and interpreted in the Pathology and Anatomy University Laboratory, in accordance with the 2001 Bethesda System [22]. Cytology assessments were conducted blind to the results of the exposure status.

Detection of specific HPV types by polymerase chain reaction (PCR)/reverse blot strip assay/restriction fragment length polymorphism (RFLP)

DNA was extracted using phenol chloroform and amplified using PGMY09/11 HPV specific primers that amplify a 450-bp fragment of the L1 open reading frame of genital HPV [23]. DNA typing with a reverse line blot assay (Roche Molecular Systems) permitted the identification of 27 individual genital HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83 and 84). The assay was performed following the manufacturer’s guidelines at the Virology Unit of the São Paulo Branch of the Ludwig Institute for Cancer Research. RFLP analysis of the same 450-bp PCR fragment was also undertaken for some specimens [24]. DNA isolated from HPV-16 positive SiHa cells was used as a positive control, while negative controls were included to monitor potential PCR contamination. To confirm the integrity of the DNA material extracted from the specimens, assays included an additional set of primers (GH20 and PC04) to amplify a 268-bp region of the β-globin gene.

Sample size and statistical analysis

A sample size of 430 participants was calculated to provide enough power to estimate a 10% prevalence of multiple HPV infections, with a precision of 3.0% and confidence interval of 95%, with a design effect of 1.2.

The prevalence of single HPV infection and multiple HPV infections (detection of more than one HPV type per sample) and their respective 95% confidence intervals (CIs) were calculated. Socio-demographic and sexual behaviors variables that were normally distributed were presented as mean and standard deviation (sd) and were categorized based on their mean values. Variables that were asymmetrically distributed were presented as median and interquartile range (IQR, Q1–Q3). Dichotomization of asymmetric variables was based on their median value. Years of schooling were dichotomized at 8 years, corresponding to the elementary Brazilian school period.

Potential risk factors for HPV infection were assessed using univariate and multivariate logistic regression analyses. Three different outcomes were analyzed: any HPV infection, single HPV infection, or infection with multiple HPV types. The three outcomes were each compared with non-infected participants, by calculating unadjusted and adjusted odds ratios (ORs) with respective 95% CIs. Variables with p < 0.20 in univariate analysis were included in multivariate models. Participants’ age was included in all models as a continuous variable, because age could be a confounding factor for HPV infection.

The association between HPV infection and cytological abnormalities was calculated using prevalence ratios and respective 95% CIs. All analyses were conducted with SPSS, version 16 for Windows. Differences were considered to be statistically significant when p < 0.05.

Results

Baseline characteristics

In total, 432 adolescents fulfilled the inclusion criteria and were interviewed. The participants’ mean age was 17.2 years, with a standard deviation of 1.3 years; approximately 88.0% were from low income families, 67.4% were unmarried, 57.3% reported 8 years of schooling or less, and 11.6% reported tobacco use. First sexual intercourse occurred at or before 15 years of age for 61.1% of the adolescents in this study. Almost 80.0% of participants reported inconsistent use of condoms by their partners and 46.0% reported more than one lifetime sexual partner. Furthermore, 41.7% of participants reported a previous pregnancy, with 19.0% conceiving before they were 15 years old (Table 1).

The prevalence of C. trachomatis infection was 13.9% (95% CI: 10.8–17.6). The prevalence of cytological abnormalities was 15.4% (95% CI: 12.3–19.4), with atypical squamous cells of undetermined significance occurring in 10.6% (95% CI: 8.0–14.0), low-grade squamous intraepithelial lesion occurring in 4.6% (95% CI: 2.9–7.2) and atypical squamous cells cannot exclude high-grade intraepithelial lesion occurring in 0.2% (95% CI: 0.0–1.5) of adolescents. No participant had high-grade squamous intraepithelial lesion or “atypical” glandular cells (Table 1).

Prevalence and classification of HPV infection

HPV DNA was detected in cervical specimens of 121 of the 432 adolescents, resulting in an overall prevalence of 28.0% (95% CI: 23.9–32.5; Table 2). The number of HPV types detected per specimen ranged from 0 to 5. Among the 121 HPV-infected participants, a single type of HPV infection was detected in 67 individuals, whereas multiple HPV types were detected in 54 adolescents (54/432; 12.5%), representing 44.6% of the positive samples (Table 2).

Thirty different HPV types were detected; nineteen types were high-risk (HR)-HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 54, 56, 58, 66, 68, 70, 73 and 82), and 11 were low-risk (LR)-HPV types (6, 11, 40, 42, 44, 54, 55, 61, 62, 84, 89), according to the International
Committee on the Taxonomy of Viruses [25]. Figure 1 shows the absolute frequency of the 30 different HPV types detected. These types were stratified by multiple (black bars) and by single (gray bars) infection (Figure 1). The prevalence of HR-HPV was 24.8% (CI: 20.8–29.2), which was higher than the LR-HPV type prevalence (Table 2).

The most prevalent HPV types were 16, 51, 31, 52 and 18, and the most common HPV types detected in multiple infections included 16, 51, 18, 31 and 52. The most common HPV type detected in single or multiple infections was HPV-16; HPV-6 was the most common LR-HPV detected (Figure 1). The prevalence of either HR-HPV type that is present in bivalent vaccines (HPV types 16 and 18) was 10.2%, while 12% of adolescents were infected with one of the types included in the quadrivalent vaccine (HPV types 6, 11, 16 and 18).

After using both HPV DNA detection assays, only one specimen had an unclassified HPV type.

**Risk factors for HPV infection**

Three different outcomes were analyzed: any HPV infection, infection with a single HPV type, and infection with multiple HPV types. The three outcomes were each compared to participants with no infections. The associations between baseline variables and HPV infection, as well as the results of the multivariate analyses for all variables retained in the final regression models, are presented in Tables 3, 4 and 5.

Having a number of lifetime sexual partners greater than one was associated with a higher risk of any HPV infection (adjusted OR: 4.9 [95% CI: 2.5–9.5]) (Table 3), single HPV infection (adjusted OR: 4.7 [95% CI: 2.4–9.1]) (Table 4), and multiple HPV infection (adjusted OR: 3.7 [95% CI: 1.8–9.5]) (Table 5).

**Risk factors for cytological abnormalities**

The prevalence ratio for cytological abnormalities between samples from adolescents infected with either one or multiple HPV types was calculated. Participants were categorized into either “negative for intraepithelial

| Characteristics                          | Values                             |
|------------------------------------------|------------------------------------|
| Age (years)                              | Range 15 to 19 Mean (sd) 17.2 (1.3) |
| Education (years)                        | ≤ 8 years (%) 247 (57.3) > 8 years (%) 184 (42.7) |
| Marital status                           | Unmarried (%) 291 (64.4) Married (%) 141 (35.6) |
| Family income (minimum wages)            | ≤ 4 (%) 359 (88.0) > 4 (%) 49 (12.0) |
| Age at menarche (years)                  | Range 9 to 17 Mean (sd) 12.5 (1.3) |
| Age at first intercourse (years)         | Range 10 to 19 Mean (sd) 15.1 (1.5) |
| Number of lifetime sexual partners       | Range 1 to 20 Median (IQR Q1-Q3) 1 (1–3) |
| New sexual partner in past 3 months      | Yes (%) 63 (14.6) |
| Number of sexual partner in past 3 months| Range 1 to 5 Median (IQR Q1-Q3) 1 (1–1) |
| Condom use                               | Never/occasional (%) 345 (79.9%) |
| History of full-term pregnancies         | History (%) 87 (20.1) |
| Age at first full-term pregnancy (years) | Range 12 to 19 Mean (sd) 15.6 (1.4) |
| Chlamydia trachomatis infection (%)      | 60 (13.9) |

Table 1 Characteristics of participants (Continued)

| Cytological abnormalities               | Values                             |
|------------------------------------------|------------------------------------|
| Overall (%)                              | 67 (15.4) |
| ASC-US (%)                               | 46 (10.6) |
| LGSIL (%)                                | 20 (4.6) |
| ASC-H (%)                                | 1 (0.2) |

sd: Standard deviation; IQR, Q1-Q3 interquartile range; * one adolescent without information; ** 24 adolescents without information; MW = 334 US$; † four adolescents without information; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells cannot exclude high-grade intraepithelial lesion; LGSIL: low-grade squamous intraepithelial lesion.
Participants with positive HPV results were then classified into non-overlapping categories, and the impact of multiple infections on cytological abnormalities (excluding HPV 16) was analyzed (Table 6). The prevalence ratio was 2.1 (95% CI: 1.1–4.0) for cytological abnormalities associated with a single HPV type, and was 6.4 (95% CI: 4.1–10.0) for multiple HPV types. The prevalence ratio when all adolescents infected with HPV-16 were excluded was 2.2 (95% CI: 1.3–4.3) for a single HPV type and 6.2 (95% CI: 3.7–7.1) for multiple HPV types.

Discussion
This community-based study investigated patterns of HPV infection in exfoliated cells from Brazilian adolescents.
according to HPV types. This study confirmed the high prevalence of HPV infection in sexually active adolescents. Moreover, it reaffirms the high rate of multiple HPV types, which agrees with previous studies involving sexually active adolescents and young women [4,6,14,26,27].

HR-HPV types were the most common HPV types detected (88.4%), and they were similar to the HPV types described in other studies [6,8,26,27]. HPV-16, in particular, was the most common type detected in cases of both single and multiple infections. The distribution of other HPV types differed from previous studies [4,26,27]. In the present study, HPV-18 was the fifth most prevalent type, behind types 16, 51, 31 and 52. More than one-third (36.4%) of HPV-positive specimens exhibited HPV-16/18 dual infection. Differences in the prevalence and distribution of the individual HPV types may be explained by the use of different methods used to detect HPV, variability in the clinical specimens used, the age of the participants and regional variation in the distribution of the types.

The HR-HPV types were detected in 80.6% of all single-type infections, and in 98% of all multiple-type infections. The high prevalence of multiple HPV types may be because high-risk types are more likely to cause persistent infections than low-risk types [4,12]. Furthermore, the high prevalence of multiple HPV types may be related to the increasing levels of estrogen exposure during pubertal development. Previous studies suggest that the increased levels of estrogen may modulate the immune response and facilitate cervical ectropion and squamous metaplasia, both of which add to the biological susceptibility of the uterine cervix [28,29].

The present study correlated HPV infection with the number of prior sexual partners, which produced results consistent with previously published reports [6,7,26], emphasizing once more the importance of sexual

Figure 1 Absolute frequency of the 30 HPV types detected as single and multiple HPV infections (HPV: human papillomavirus; *oncogenic types; dark bars represent multiple HPV types; dotted bars represent a single HPV type).
transmission. The highest prevalence of single or co-infection with multiple HPV types has been found in sexually active adolescents and young women [3-7]. However, it is uncertain whether the presence of multiple HPV types in adolescents is due to behavioral characteristics or whether adolescents with multiple HPV types possess certain intrinsic biological characteristics that increase susceptibility. The present

Table 3 Factors associated with HPV infection

| Variables                              | HPV infection | Total (%) | OR (IC 95%) | p       | Adjusted OR IC95% | p       |
|----------------------------------------|---------------|-----------|-------------|---------|-------------------|---------|
| Age (years)                            |               |           |             |         |                   |         |
| ≤ 17                                   | 69            | 237       | 29.1        | 1.1 (0.7 – 1.7) | 0.57    | -                  | -       |
| > 17                                   | 52            | 195       | 26.7        |         |                   |         |
| Education (years)a                     |               |           |             |         |                   |         |
| ≤ 8                                    | 79            | 247       | 32.0        | 1.6 (1.0-2.5) | 0.02    | 1.2 (0.6-2.2)      | 0.54    |
| > 8                                    | 41            | 184       | 22.3        |         |                   |         |
| Marital status                         |               |           |             |         |                   |         |
| Unmarried                              | 93            | 291       | 32.0        | 1.9 (1.2-3.0) | <0.01   | 1.1 (0.5-2.1)      | 0.76    |
| Married                                | 28            | 141       | 19.9        |         |                   |         |
| Smoking                                |               |           |             |         |                   |         |
| Yes                                    | 22            | 50        | 44.0        | 2.2 (1.4-4.1) | <0.01   | 1.7 (0.8-3.7)      | 0.15    |
| No                                     | 99            | 382       | 25.9        |         |                   |         |
| Age at first intercourse (years)       |               |           |             |         |                   |         |
| 10–15                                  | 80            | 264       | 30.3        | 1.3 (0.9-2.1) | 0.18    | 0.8 (0.4-1.6)      | 0.59    |
| 16-19                                  | 41            | 168       | 24.4        |         |                   |         |
| Age at menarche b (years)              |               |           |             |         |                   |         |
| 13–17                                  | 56            | 207       | 27.1        | 0.9 (0.6-1.4) | 0.70    | -                  | -       |
| 9-12                                   | 64            | 223       | 28.7        |         |                   |         |
| Condom use                             |               |           |             |         |                   |         |
| Never/occasional                       | 98            | 345       | 28.4        | 1.1 (0.6-1.8) | 0.71    | -                  | -       |
| Always                                 | 23            | 87        | 26.4        |         |                   |         |
| Number of lifetime sexual partners     |               |           |             |         |                   |         |
| > 1                                    | 88            | 199       | 44.2        | 4.8 (3.0-7.6) | <0.001  | 4.9 (2.5-9.5)      | <0.01   |
| 1                                      | 33            | 233       | 14.2        |         |                   |         |
| New sexual partner in past 3 months    |               |           |             |         |                   |         |
| Yes                                    | 21            | 63        | 33.3        | 1.3 (0.7-2.4) | 0.30    | -                  | -       |
| No                                     | 100           | 369       | 27.1        |         |                   |         |
| Number of sexual partner in past 3 months |           |           |             |         |                   |         |
| > 1                                    | 12            | 21        | 57.1        | 3.7 (1.5-9.0) | <0.01   | 1.2 (0.3-4.2)      | 0.80    |
| ≤ 1                                    | 109           | 411       | 26.5        |         |                   |         |
| Age at first full-term pregnancy (years) c |           |           |             |         |                   |         |
| No                                     | 70            | 252       | 27.8        | 1        | 0.31    | -                  | -       |
| < 15                                   | 25            | 82        | 30.5        | 1.1 (0.6-1.9) | 0.34    | -                  | -       |
| ≥ 15                                   | 24            | 94        | 25.5        | 0.9 (0.5-1.5) |         |         |
| C. trachomatis infection               |               |           |             |         |                   |         |
| Yes                                    | 23            | 60        | 38.3        | 1.7 (1.0-3.1) | 0.05    | 1.0 (0.5-2.2)      | 0.96    |
| No                                     | 98            | 372       | 26.3        |         |                   |         |

HPV: Human papillomavirus; OR: odds ratio; CI: confidence interval; C. trachomatis: Chlamydia trachomatis. a one without information; b two without information; c Four without information. The variables “Education”, “Marital status”, “Smoking”, “Age at first intercourse”, “Number of lifetime of sexual partners” and the “Number of sexual partners in the past 3 months” were included in the multivariate model. The odds ratios have been adjusted for age as a continuous variable.
study shows that the risk factors for multiple HPV infection were similar to the risk factors for any HPV infection and single HPV infection, which agrees with previous studies of sexually active adolescents and young women [6,7]. However, the cross-sectional design of this study will not be able to adequately answer that if the risk factors for multiple HPV infection were distinct from risk factors for any HPV infection. 

Table 4 Factors associated with single HPV infection

| Variables                  | Single HPV infection | Total (%) | OR (IC 95%) | p     | Adjusted OR IC95% | p     |
|----------------------------|----------------------|-----------|-------------|-------|-------------------|-------|
| Age (years)                |                      |           |             |       |                   |       |
| ≤ 17                       | 37                   | 205       | 18.0        | 1.0 (0.6-1.8) | 0.48  | -                 | -     |
| > 17                       | 30                   | 173       | 17.3        |       |                   |       |
| Education (years)a         |                      |           |             |       |                   |       |
| ≤ 8                        | 41                   | 209       | 19.6        | 1.4 (0.8-2.4) | 0.11  | 1.1 (0.6-2.1)     | 0.70  |
| > 8                        | 25                   | 168       | 14.8        |       |                   |       |
| Marital status             |                      |           |             |       |                   |       |
| Unmarried                  | 48                   | 246       | 19.5        | 1.4 (0.8-2.6) | 0.13  | 1.3 (0.6-2.8)     | 0.48  |
| Married                    | 19                   | 132       | 14.4        |       |                   |       |
| Smoking                    |                      |           |             |       |                   |       |
| Yes                        | 14                   | 42        | 33.3        | 2.7 (1.3-5.4) | <0.001 | 1.4 (0.6-3.2)     | 0.37  |
| No                         | 53                   | 336       | 15.8        |       |                   |       |
| Age at first intercourse (years) |                      |           |             |       |                   |       |
| 10–15                      | 44                   | 228       | 19.3        | 1.3 (0.7-2.3) | 0.19  | 0.8 (0.4-1.6)     | 0.53  |
| 16-19                      | 23                   | 150       | 15.3        |       |                   |       |
| Age at menarche b (years)  |                      |           |             |       |                   |       |
| 13–17                      | 29                   | 180       | 16.1        | 0.8 (0.5-1.4) | 0.21  | -                 | -     |
| 9-12                       | 38                   | 197       | 19.2        |       |                   |       |
| Condom use                 |                      |           |             |       |                   |       |
| Never/occasional           | 52                   | 299       | 17.4        | 0.9 (0.5-1.7) | 0.42  | -                 | -     |
| Always                     | 15                   | 79        | 19.0        |       |                   |       |
| Number of lifetime sexual partners |                    |           |             |       |                   |       |
| > 1                        | 18                   | 218       | 8.2         | 2.9 (1.6-5.5) | <0.001 | 4.7 (2.4-9.1)    | <0.001|
| 1                          | 49                   | 160       | 30.6        |       |                   |       |
| New sexual partner in past 3 months |                |           |             |       |                   |       |
| Yes                        | 8                    | 50        | 16.0        | 0.8 (0.4-1.9) | 0.37  | -                 | -     |
| No                         | 59                   | 328       | 18.0        |       |                   |       |
| Number of sexual partner in past 3 months |          |           |             |       |                   |       |
| > 1                        | 12                   | 61        | 7.4         | 1.2 (0.6-2.3) | 0.32  | -                 | -     |
| ≤ 1                        | 55                   | 317       | 17.3        |       |                   |       |
| Age at first full-term pregnancy (years) b |                |           |             |       |                   |       |
| No                         | 34                   | 216       | 15.7        | 1     | 0.12              | 1     | 0.81              |
| < 15                       | 19                   | 89        | 21.3        | 1.4 (0.7-2.7) | 0.36  | 0.8 (0.3-1.9)     | 0.63  |
| ≥ 15                       | 12                   | 69        | 17.4        | 1.1 (0.5-2.3) | 0.6   | 0.6 (0.2-1.4)     |       |
| C. trachomatis infection   |                      |           |             |       |                   |       |
| Yes                        | 11                   | 48        | 22.9        | 1.4 (0.7-3.0) | 0.27  | -                 | -     |
| No                         | 56                   | 330       | 16.9        |       |                   |       |

HPV: Human papillomavirus; OR: odds ratio; CI: confidence interval; C. trachomatis: Chlamydia trachomatis. a one without information; b Four without information. The variables “Education”, “Marital status”, “Smoking”, “Age at first intercourse”, “Number of lifetime of sexual partners” and the “Age at first full-term pregnancy” were included in the multivariate model. The odds ratios have been adjusted for age as a continuous variable.
infection. Indeed, HPV infections in sexually active teenagers come and go. Those classified as “single infection” during this study may well have had a multiple infections a few weeks prior, and vice versa. The role of multiple HPV infections in cervical carcinogenesis is still controversial. Some studies have reported an association between multiple HPV infections and a higher risk of cytological abnormalities and cervical
The US multicenter clinical trial, observer reproducibility in all cytological categories [30,31].

Screening interpretation presents known problems without corresponding biopsy results. The cytological reliance on cytological samples of cervical lesions grade cytological abnormalities found in this study persistence of HPV infections [15]. In contrast, the low- and/or progression of lesions, regardless of the per-
types confers an increased risk for the development there is some evidence that infection with multiple HPV present as persistent infections, representing the main prevalence of HR-HPV types detected in samples with infections with multiple HPV types, as reported in other studies [14,15].

HR-HPVs are often prevalent in samples with multiple infections in this study. HR-HPVs are often present as persistent infections, representing the main risk factor for the development of lesions [12]. However, there is some evidence that infection with multiple HPV types confers an increased risk for the development and/or progression of lesions, regardless of the persistence of HPV infections [15]. In contrast, the low-grade cytological abnormalities found in this study may reflect the viral load resulting from productive infections, many of which tend to regress over time, thereby resulting in less clearly identifiable lesions. Additionally, the excess risk of cytological abnormalities resulting from multiple infections remained even after excluding the adolescents who harbored HPV-16, a similar finding to the Ludwig-McGill cohort study [15]. The smaller sample size for multiple HPV infection, without those with HPV-16, may have limited the power of the study to detect large differences.

This association could be explained by the elevated prevalence of HR-HPV types detected in samples with multiple infections in this study. HR-HPVs are often present as persistent infections, representing the main risk factor for the development of lesions [12]. However, there is some evidence that infection with multiple HPV types confers an increased risk for the development and/or progression of lesions, regardless of the persistence of HPV infections [15]. In contrast, the low-grade cytological abnormalities found in this study may reflect the viral load resulting from productive infections, many of which tend to regress over time, thereby resulting in less clearly identifiable lesions. Additionally, the excess risk of cytological abnormalities resulting from multiple infections remained even after excluding the adolescents who harbored HPV-16, a similar finding to the Ludwig-McGill cohort study [15]. The smaller sample size for multiple HPV infection, without those with HPV-16, may have limited the power of the study to detect large differences.

A potential limitation of this investigation was the reliance on cytological samples of cervical lesions without corresponding biopsy results. The cytological screening interpretation presents known problems with specimen collection, smear preparation and interobserver reproducibility in all cytological categories [30,31]. The US multicenter clinical trial, “ASCUS-LSIL Triage Study” (ALTS), showed that inter-observer reproducibility of cytological interpretation of a large series of specimens was only moderate and the greatest source of disagreement involved interpretation of atypical squamous cells of undetermined significance (ASC-US), of which 37% were HPV positive [30]. In the present study, 61.0% of samples with cytological abnormalities and 47.8% of ASC-US samples were positive for HPV. This reflects careful cytological assessment in a reference laboratory following strict quality control guidelines. This strategy was adopted to avoid unnecessary biopsies for lesions that have a higher potential for spontaneous regression.

Another potential limitation of this study was the estimation of prevalence by cross-sectional detection of type-specific HPV infections, which may underestimate the cumulative cytological abnormalities resulting from exposure to different HPV types over time. Furthermore, this investigation relied on the accuracy of the memories of subjects for precise information about their sexual and reproductive history.

**Conclusions**

The detection of any, single or multiple HPV types in cervical specimens of adolescents was associated with the number of lifetime sexual partners. These findings also show that in adolescents, infection with multiple HPV types is associated with the development of more cervical cytological abnormalities than infection with a single HPV type.

By providing data on the epidemiology of HPV infection with multiple HPV types in adolescents, these results may inform the planning and the interpretation of models evaluating the impact and cost effectiveness of vaccines. These estimates, obtained prior to the introduction of HPV vaccination, provide a baseline for monitoring and surveillance of the effectiveness of this primary prophylactic intervention in vaccinated and unvaccinated adolescents. The high rate of HPV detection in adolescents emphasizes the advantages of early vaccination, prior to the onset of sexual activity. Furthermore, the high prevalence of minor cytological abnormalities, which tend to regress with time, reinforces the recommendation not to include teenagers in cervical cytology screening programs. Although most cytological abnormalities in adolescents may regress over time, there is a need for longitudinal studies to assess the cumulative influence of infection with multiple HPV types in the progression of cervical lesions with increasing age.

**Abbreviations**

AGC: Atypical glandular cells; ASC-US: Atypical squamous cells of undetermined significance; ASC-H: Atypical squamous cells, cannot exclude high-grade intraepithelial lesion; CI: Confidence interval; HGSIL: High-grade squamous intraepithelial lesion; HPV: Human papillomavirus; HR-HPV: High-risk HPV; LGSIL: Low-grade squamous intraepithelial lesion; LR-HPV: Low-risk HPV; OR: Odds ratio; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; sd: Standard deviation.

| Table 6 Association between cytological abnormalities and HPV infection (n = 432) |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Cytological     | PR (95% CI)     | p               |
|                                | abnormalities   |                 |                 |
|                                | Positive | Total    |                 |                 |
| HPV infection                  |          |          |                 |                 |
| Negative                        | 26      | 311     | -               | -               |
| One type                        | 12      | 67      | 2.1 (1.1-4.0)   | 0.02            |
| Multiple types                  | 29      | 54      | 6.4 (4.1-10.0)  | <0.01           |
| HPV infection without HPV-16   |          |          |                 |                 |
| Negative                        | 26      | 311     | -               | -               |
| One                            | 11      | 59      | 2.2 (1.2-4.3)   | 0.03            |
| Multiple types                  | 17      | 33      | 6.2 (3.7-10.1)  | <0.01           |

HPV: human papillomavirus; n: number; PR: prevalence ratios.
Competing interests
LLV is a board member of Merck & Co.

Authors’ contributions
EMBG and MFCA were responsible for the study design, coordination and data collection. LES, LLV and MCC carried out the molecular genetic studies. MARL performed cytological assessment. MMGD and MSCS participated in the data collection and the gynecological examinations. RFA and MDT performed the statistical analyses and wrote the manuscript. All authors have read and approved this manuscript.

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References
1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer C, Munoz N: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999, 189:12–19.
2. Munoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003, 348:118–127.
3. Schmitt M, Depuydt C, Renon I, Bogers J, Antoine J, Arbyn M, Pawlita M: Multiple HPV infections with high viral loads are associated with cervical lesions but do not differentiate grades of cervical abnormalities. JCM 2013, 51:1485–1464.
4. Brown DR, Shew ML, Qadadri B, Neptune N, Vargas M, Tu W, Juliar BE, Breen TE, Fortenberry JD: A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. J Infect Dis 2005, 191:89–192.
5. Chatuvedi AK, Khati HA, Hildebrandt K, Rodriguez AC, Quint W, Schiffman M, Van Doorn LJ, Porras C, Wacholder S, Gonzalez P, Sherman ME, Herrero R: Human papillomavirus infection with multiple types: pattern of coinfection and risk of cervical disease. J Infect Dis 2011, 203:910–920.
6. Nielsen A, Kjaer SK, Munk C, Efters T: Type-specific HPV infection and multiple HPV types: prevalence and risk factor profile in nearly 12,000 younger and older Danish women. Sex Transm Dis 2008, 35:276–282.
7. Rousseau MC, Abrahamowicz M, Vila LL, Costa MC, Rohan TE, Franco EL: Predictors of cervical coinfection with multiple human papillomavirus types. Cancer Epidemiol Biomarkers Prev 2003, 12:1029–1037.
8. Chatuvedi AK, Myers L, Hammons AF, Clark RA, Dunlap K, Kissingier PJ, Hagens EE: Prevalence and clustering patterns of human papillomavirus genotypes in multiple infections. Cancer Epidemiol Biomarkers Prev 2005, 14:2439–2445.
9. Rousseau MC, Pereira JS, Prado JC, Vila LL, Rohan TE, Franco EL: Cervical coinfection with human papillomavirus types as a predictor of acquisition and persistence of HPV infection. J Infect Dis 2002, 184:1508–1517.
10. Thomas KK, Hughes JP, Nuyens JM, Kivist NB, Lee SK, Adam ED, Koutsky LA: Concurrent and sequential acquisition of different genital human papillomavirus types. J Infect Dis 2000, 182:1097–1102.
11. Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, Papp JR, Black CM, Unger ER: Association of chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol 2005, 162:668–675.
12. Moscicki AB, Shiboski S, Bicerising J, Pawel K, Clayton L, Jay N, Darragh TM, Brescia R, Kanowtiz S, Miller S, Stone J, Hanson E, Palefsky J: The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 1998, 132:277–284.
13. Moscicki A-B, Cox JT: Practice improvement in cervical screening and management (PICSM): symposium on management of cervical abnormalities in adolescents and young women. J Low Genit Tract Dis 2010, 14:73–80.
14. Rousseau MC, Vila LL, Costa MC, Abrahamowicz M, Rohan TE, Franco EL: Occurrence of cervical infection with multiple human papillomavirus types is associated with age and cytologic abnormalities. Sex Transm Dis 2002, 29:581–587.
15. Trottier H, Mahmud S, Costa MC, Sebrinjo JP, Duarte-Franco E, Rohan TE, Ferenczy A, Vila LL, Franco EL: Human papillomavirus infections with multiple types and risk of cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2006, 15:1274–1280.
16. van der Graaf Y, Mollin A, Dooenewaard H, Quint W, van Doorn LJ, van den Tweel J: Human papillomavirus and the long-term risk of cervical neoplasia. Am J Epidemiol 2000, 156:158–164.
17. Campos NG, Rodrigues AC, Castellanos S, Herrero R, Hildesheim A, Kati H, Kim JJ, Wacholder S, Morales J, Burk RD, Schiffman M: Persistence of concurrent infections with multiple human papillomavirus types: a population-based cohort study. J Infect Dis 2011, 203:823–827.
18. Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S: Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010, 201:789–799.
19. de Sanjosé S, Diaz M, Castellsague X, Clifford G, Bruni L, Muñoz N, Bosch FX: Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007, 7:453–459.
20. Ayres ARG, Silva GA: Cervical HPV infection in Brazil: systematic review. Rev Saúde Pública 2010, 44:963–974.
21. Guiraud E, Guiraud ME, Veira M, Bontempo NM, Seixas MS, Garcia MD, David L, Côrtes R, Alves M: Lack of utility of risk score and gynecological examination for screening for sexually transmitted infections in sexually active adolescents. BMC Med 2009, 7:9–14.
22. Solomon D, Davey D, Kurman R, Moriarty A, O’Connor D, Prey M, Raab S, Sherman M, Willbur D, Wright T Jr, The Yen et al: Bethesda system: terminology for reporting results of cervical cytology. JAMA 2001, 2002:875–2114–2119.
23. G lizard PE, Peyton CL, Aliess TG, Wheeler CM, Courile F, Hildesheim A, Schiffman MH, Scott DR, Apple Rj: Improved amplification of genital human papillomaviruses. J ClinMicrobiol 2000, 38:357–361.
24. Bernard HU, Char SN, Manos MM, Ong CK, Vila LL, Delling H, Peyton CL, Bauer HM, Wheeler CM: Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. J Infect Dis 1994, 170:1077–1085.
25. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H: Classification of papillomaviruses, Virology 2004, 324:17–27.
26. Dunne EF, Unger ER, Stinner M, McQuillan G, Swan DC, Patel SS, Markowitz LE: Prevalence of HPV infection among females in the United States. JAMA 2007, 297:813–819.
27. Johnson AM, Mercle CH, Beddows S, de Silva N, Desai S, Howell-Jones R, Carder C, Sonnember P, Fenton KA, Lawwides C, Soldan K: Epidemiology of, and behavioural risk factors for, sexually transmitted human papillomavirus infection in men and women in Britain. Sex Transm Infect 2012, 88:212–217.
28. Kahn JA, Rosenthal SL, Succop PA, Ho GY, Burk RD: Mediators of the association between age of first sexual intercourse and subsequent human papillomavirus infection. *Pediatrics* 2002, 109:1–8.

29. Marks MA, Gravitt PE, Burk RD, Studentsov Y, Farzadegan H, Klein SL: Progesterone and 17beta-estradiol enhance regulatory responses to human papillomavirus type 16 virus-like particles in peripheral blood mononuclear cells from healthy women. *Clin Vaccine Immunol* 2010, 17:609–617.

30. Stoler M, Schiffman M: Interobserver reproducibility of cervical cytologic and histological interpretations: realistic estimates from the ASCUS-LSIL triage study. *JAMA* 2001, 285:1500–1505.

31. Bigras G, Wilson J, Russell L, Johnson G, Morel D, Saddik M: Interobserver concordance in the assessment of features used for the diagnosis of cervical atypical squamous cells and squamous intraepithelial lesions (ASC-US, ASC-H, LSIL and HSIL). *Cytopathology* 2013, 24:44–51.

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