

Human papillomavirus infection in Brazilian women with normal cervical cytology

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ABSTRACT. We examined the prevalence of human papillomavirus (HPV) infection in a sample of Brazilian women presenting normal cervical cytology. Possible interactions between patient characteristics and HPV infection were analyzed in order to provide background data to improve cervical cancer screening and prophylaxis. Cervical samples of 399 women, received for routine evaluation in the Health Department of Ouro Preto, MG, Brazil, were subjected to HPV-DNA testing by PCR with MY09/11 primers. HPV-positive specimens were typed by RFLP. A structured epidemiological questionnaire was administered to each woman. HPV prevalence among these cytologically normal women

was 11%. Twelve viral types were detected, the most common being HPV-16, -6, -61, -83, and -66. HPV was more prevalent in younger women; high-risk viral types were detected in 61% of the infected women and 27% of the infected women had multiple HPV infections. Significant associations of HPV infection were found with age, literacy, residence, marital status, lifetime number of sexual partners, and parity. We detected a great diversity of HPV types in women with normal cytology. This kind of information about local populations is useful for HPV prevention and vaccination strategies.

Key words: HPV; Human papillomavirus; Cervical cancer screening; Normal cytology; Epidemiology; Risk factors

INTRODUCTION

Human papillomavirus (HPV) is a DNA virus with oncogenic potential and is distributed worldwide. HPV has a causal relationship with cervical cancer depending on the virus type and load, the characteristics of the infection, and the virus physical status - integrated or episomal (Schiffman and Burk, 1997; zur Hausen, 2009). Persistent HPV infection is an important factor for the development of cervical neoplasia. The progression of cervical lesions is affected by additional co-factors such as multiple sex partners, early beginning of sexual activity, contraceptives, smoking, and other sexually transmitted diseases (Herrero et al., 1990; Ho et al., 1998; Atalah et al., 2001; zur Hausen, 2009).

Knowledge about the association of HPV with cervical cancer has improved public health programs by the introduction of virus screening using specific and sensitive diagnostic tests directed toward high-risk HPV types (Scarinci et al., 2010). These tests are included in routine gynecological evaluations and are essential for the identification of infections at a very precocious stage in women without cervical abnormalities. Data about HPV obtained from cytologically normal women revealed important aspects of virus prevalence, which can differ across regions and age groups. There is evidence that infections caused by different HPV types are not independent, but synergistic, and epidemiologic modeling predicts that vaccination should have a favorable effect on non-vaccine types (Bonnez, 2007). This information should be taken into account for the development of vaccine strategies (Cuzick et al., 2003).

According to the International Agency for Research Cancer (IARC), the HPV prevalence in women with normal cervical cytology ranges from 1.4% in Spain to 25.6% in Nigeria (Clifford et al., 2005). When age is considered, HPV infections have been found to be more frequent in younger women. However, some reports described a second peak of HPV infection in women over 55 years, possibly due to latent infection (Cuzick et al., 1999; Herrero et al., 2000). Similar data have been reported in specific regions (de Sanjosé et al., 2007).

We examined the prevalence of different HPV types in women with normal cervical cytology by conducting routine examinations in the City of Ouro Preto, Minas Gerais, Brazil. To our knowledge, this is the first study in this region, which was selected due to its particular characteristic traditional habits. In Ouro Preto, the bracken fern *Pteridium aquilinum* is included in the human diet. This bracken is considered as an important co-factor of bovine papillomavirus in the development of cancer in cattle and is related to gastric cancer in humans (Alonso-Amelot

et al., 2002; Marlière et al., 2002; Recouso et al., 2003). Our study is part of a larger one about regional HPV prevalence and co-factors, for comparison with other regions of Brazil, in order to provide relevant data for the development of vaccines appropriate for the different HPV types.

MATERIAL AND METHODS

Study population and collection of specimens

The study population included a sample of 399 women (age, 18-65 years) who visited the Municipal Health Department of Ouro Preto, MG, for routine gynecological evaluation. Preliminary exclusion criteria for participation were current pregnancy and mental incompetence. Women with cytological evidence of atypical squamous cells, atypical glandular cells, squamous intraepithelial lesions, *in situ* adenocarcinoma, or malignancy were also excluded from this study group. The project was reviewed and approved by the Institutional Ethics Committee, and informed consent was obtained from all subjects. Epidemiological data concerning social condition and reproductive aspects, as well as clinical signs and symptoms, were obtained from private interviews with the patients and recorded. These data included age, literacy, residence, marital status, age at first intercourse, lifetime number of sexual partners, parity, oral contraceptive use, smoking status, wood smoke exposition, alcohol consumption, and bracken fern diet. Speculum examination was performed by gynecologists; cervical samples were obtained using an extended tip "Ayre" spatula. After the conventional cervical smear was spread onto a glass slide, the tip of the spatula was broken off and placed in a container containing cytopreservation solution for HPV testing.

Cytology

The smears were processed for Papanicolaou staining and analyzed according to the Bethesda 2001 classification (Solomon and Nayar, 2005).

HPV DNA typing

For the extraction of DNA from cervical samples, we used a GenomicPrep Blood kit (GE Healthcare), with proteinase K digestion. HPV was detected by the amplification of DNA by using a standard polymerase chain reaction (PCR) protocol with L1 consensus primer pair MY09 and MY11, which promotes amplification of an approximately 450-bp product and can detect more than 40 distinct low- and high-risk genital HPV types. Positive and negative controls were used in all the tests. The amplification was performed according to the following protocol: 94°C for 30 s, 51.5°C for 30 s, 72°C for 30 s for 35 cycles, followed by a final step at 72°C for 7 min (Schiffman et al., 1991; Qu et al., 1997). The β -actin gene primers were used as internal controls. HPV-DNA-positive specimens were typed by restriction fragment length polymorphism (RFLP), according to Bernard et al. (1994).

Data analysis

For studying HPV prevalence and risk factors, a subset of 399 women with normal cytology reports and available HPV results was evaluated. Risk factor analysis was performed

using the chi-square test, and multivariate analysis was performed using multiple logistic regression and the SPSS software version 15.0.

RESULTS

The study included 399 women with normal cervical examination results: 44 of these women were positive for HPV-DNA (11%). Twelve HPV types were identified as shown in Figure 1. The most prevalent viral types identified in the patients were HPV-16 (46%), HPV-6 (16%), HPV-61 (12%), HPV-83 (7%), and HPV-66 (5%) (Figure 1). High-risk viral types were detected in most of the HPV-infected women (61%).

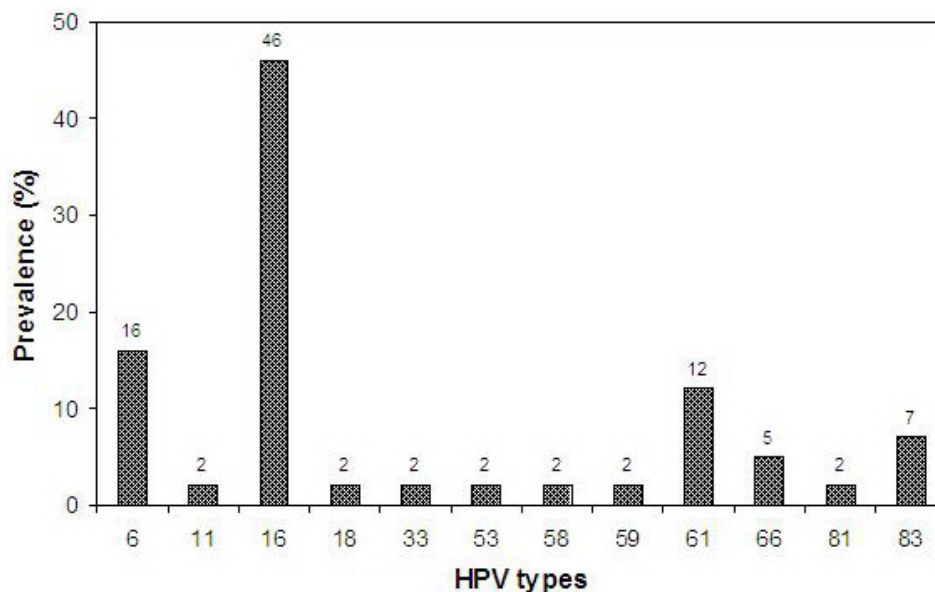


Figure 1. Prevalence of HPV types in women with normal cytology evaluated in Ouro Preto, Minas Gerais, Brazil.

Thirty-two women (73%) presented only one viral type and 12 women (27%) had multiple infection.

The occurrence of HPV in women of different ages and the prevalence of high- and low-risk HPV according to age are shown in Figure 2. HPV-DNA was more prevalent in younger women (Figure 2A); this group also had the highest frequency of high-risk HPV types (Figure 2B). Data on possible associations with age, literacy, type of residence, marital status, age at first intercourse, lifetime number of sexual partners, parity, oral contraceptive use, smoking status, alcohol consumption, and bracken fern diet are presented in Table 1. There was significant association ($P < 0.05$) between HPV infection and some factors, i.e., age, literacy, residence, marital status, lifetime number of sexual partners, and parity (Table 1). The final multivariate model showed that age of less than 30 years, literacy, type of residence, marital status, smoking status, and number of sexual partners were independently associated with HPV infection (Table 2). Parity did not show a significant association in the multivariate model.

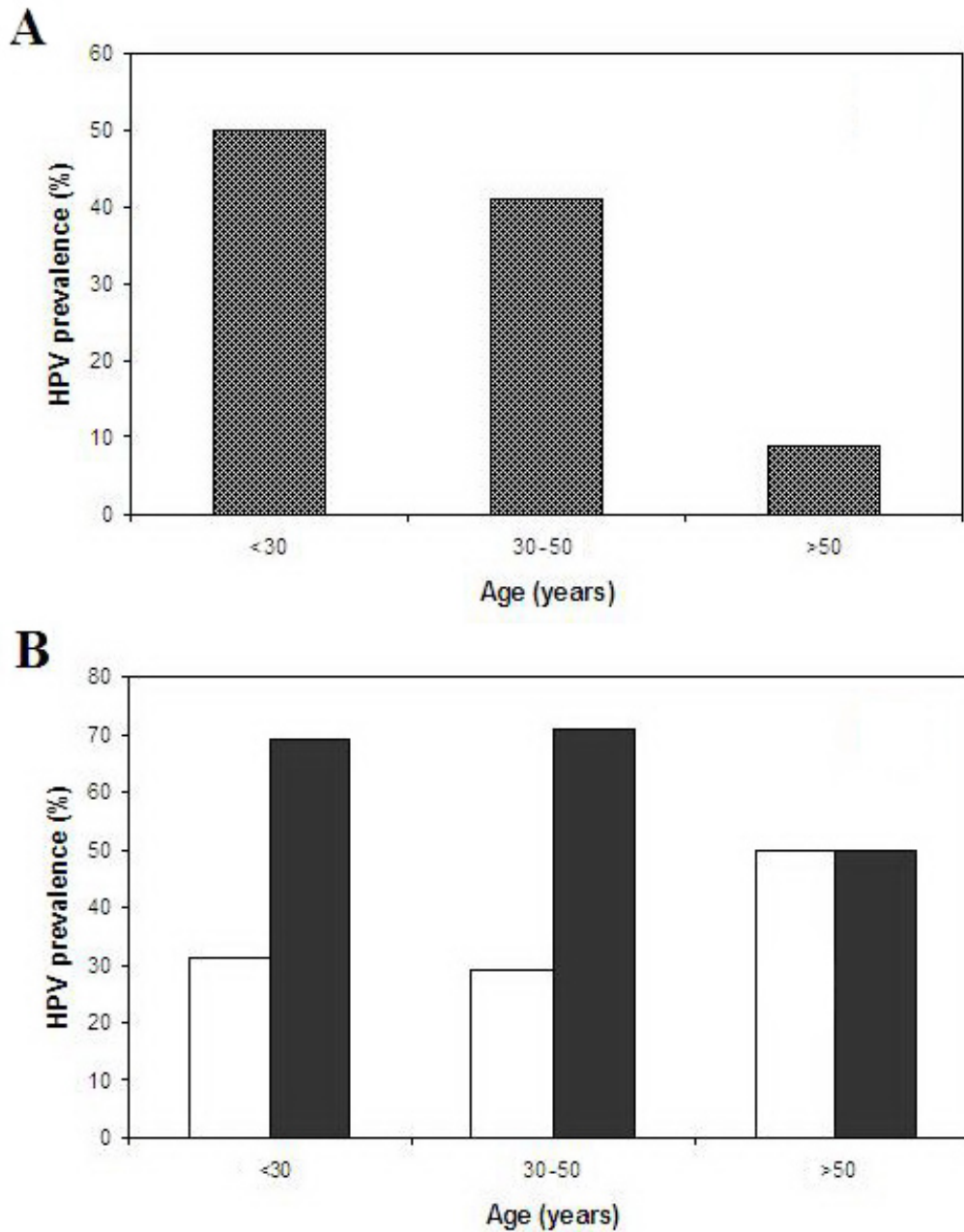


Figure 2. HPV prevalence in women with normal cytology according to age group (A) and risk type of HPV (B). Filled columns = high risk HPV; open columns = low risk HPV.

Table 1. HPV detection in women with normal cytology in Ouro Preto, MG, Brazil: association with co-factors.

	Normal cytology (N = 399)	HPV positive (N = 44)	P value*
Age (years)			
<30	128	22 (17.2%)	0.004
30-49	190	18 (9.5%)	
>50	81	4 (4.9%)	
Literacy			
Illiterate/primary	234	18 (7.7%)	0.011
Middle school	131	18 (13.7%)	
High school	34	8 (23.5%)	
Residence			
Rural	133	9 (6.8%)	0.037
Urban	266	35 (13.5%)	
Marital status			
Single	89	20 (22.5%)	0.01
Married	194	17 (8.8%)	
Others	116	7 (6.0%)	
Age at first intercourse (years)			
≤15	75	5 (6.7%)	0.126
>15	324	39 (12.0%)	
Lifetime number of sexual partners			
1	199	14 (7.0%)	0.03
2	69	9 (13.0%)	
≥3	131	21 (16.0%)	
Parity			
0	65	15 (23.1%)	0.003
1 a 3	239	21 (8.8%)	
≥4	95	8 (8.4%)	
Oral contraceptive use			
Yes	187	19 (10.2%)	0.361
No	212	25 (11.8%)	
Smoker			
No	265	24 (9.1%)	0.057
Yes	134	20 (14.9%)	
Alcohol consumption			
No	172	16 (9.3%)	0.214
Yes	227	28 (12.3%)	
Bracken fern consumption			
No	145	15 (10.3%)	0.440
Yes	254	29 (11.4%)	

*Wald χ^2 statistic.**Table 2.** Multivariate analysis of factors associated with HPV infection among women presenting normal cervical cytology in Ouro Preto, MG, Brazil.

	OR (95%CI)
Age (years)	
<30	3.99 (1.32-12.96)
30-49	2.01 (0.66-6.15)
>50	1 [reference]
Literacy	
High school	3.69 (1.46-9.33)
Middle school	1.91 (0.97-3.92)
Illiterate/primary	1 [reference]
Residence	
Urban	2.08 (0.97-4.48)
Rural	1 [reference]
Marital status	
Single	4.51 (1.81-11.23)
Married	1.49 (0.60-3.72)
Others	1 [reference]
Lifetime number of sexual partners	
≥3	2.52 (1.23-5.16)
2	1.98 (0.82-4.81)
1	1 [reference]
Smoker	
Yes	1.76 (0.93-3.32)
No	1 [reference]

OR = odds ratio; 95%CI = 95% confidence interval.

DISCUSSION

Several studies on HPV infection in women with normal cervical cytology have been reported; however, few of them have been performed in developing countries.

This study was conducted in Ouro Preto in the Southeast Region, of Brazil, and revealed an 11% HPV prevalence in women with normal cytology. This result is similar to that reported by de Sanjosé et al. (2007) in a meta-analysis of 78 data survey from many regions of the world, which suggested a prevalence of 10.4%. The frequency reported in our study was similar to that reported in North America (11.3%); lower than that found in Africa (22.1%), Central America, and Mexico (20.4%), and greater than that in Europe (8.1%) and Asia (8.0%). Higher frequencies of HPV infection have been found in developing countries (15.5%) (de Sanjosé et al., 2007). Several cultural factors could be related to this high viral prevalence, especially education levels, lifetime number of sexual partners, multiparity, and habit prevalence, conceptions, and beliefs that limit educational procedures and information about sexually transmitted diseases (Reeves et al., 1989; Melchers et al., 1994; Franco et al., 1999; Herrero et al., 2000; Cuschieri et al., 2004; Tabora et al., 2009).

Our analysis revealed that infections caused by high-risk HPV types were predominant (61%). HPV-16 and HPV-6 were the most prevalent among the high- and low-risk types, respectively. On the other hand, HPV-18 and HPV-11 were verified as less prevalent (2%) in the population. The second most common type of high- and low-risk HPV detected were HPV-66 (5%) and HPV-61 (12%), respectively. The most common viruses found in women with normal cytology were HPV-16, HPV-18, HPV-31, HPV-58, and HPV-52 (de Sanjosé et al., 2007). Data on the prevalence of HPV reported by IARC revealed that viral prevalence can differ almost 20 times between regions. HPV-16 is the most prevalent type, although large differences can be found in its distribution compared to that of other virus types in women with no cytological abnormalities. HPV-18 presents lower prevalence rates in various regions (Clifford et al., 2005). In a Colombian survey, infections with HPV-16 were the most frequent, followed by HPV-58 (Molano et al., 2002). Studies from the Netherlands, Mexico, and Great Britain reported HPV-31 as the second most frequent type. Herrero et al. (2000) described oncogenic types 58, 51, and 52 as relatively abundant in Costa Rica, followed by types 31 and 18. In Japan and Taiwan, HPV-52 was reported as the most prevalent type. HPV-53 has been detected frequently in Brazilian, British, African, and North American women with normal cytology (Jacobs et al., 2000; de Sanjosé et al., 2007).

Considering the commercial vaccines available, our data indicating high prevalence (15%) of different types of high-risk HPV are very relevant. The quadrivalent vaccines present some limitations: they are effective primarily against some HPV types, are expensive to produce, and need a cold chain. New vaccines targeting the minor capsid protein L2 have been proposed (Campo and Roden, 2010).

In this report, single and multiple infections were verified: 27% of the women were infected with more than one HPV type. This frequency was similar to that reported in the Netherlands (28%); higher than that described by IARC in Philippines (14.3%), Thailand (9.8%), Morocco (5.3%), and Paraguay (16.7%), and lower than that reported in Costa Rica (39%) (Chaouki et al., 1998; Chichareon et al., 1998; Ngelangel et al., 1998; Rolon et al., 2000; Lazcano-Ponce et al., 2001; de Sanjosé et al., 2007). Our data are similar to those obtained for normal cervical smears collected from Dutch women who were evaluated using the same laboratory techniques used in this study (Jacobs et al., 2000). The possible influence of the technical proce-

dures used (sensitivity, specificity, and type identification level) and regional characteristics on the described prevalence rates in different groups has been discussed.

Our evaluation suggested that infection with HPV was more prevalent in young women. Similar data have been reported in other studies of cytologically normal women (Lazcano-Ponce et al., 2001; de Sanjosé et al., 2007; Tabora et al., 2009). However, the second peak of infection was not found in older women, as reported in other studies (Lazcano-Ponce et al., 2001; de Sanjosé et al., 2007), possibly due to our limited sample size. A decrease in HPV prevalence with age has been shown in several studies involving younger women, irrespective of the cell collection technique and HPV detection method used (Moscicki et al., 1990; de Roda Husman et al., 1995; Figueroa et al., 1995; Burk et al., 1996; Kjaer et al., 1997). Another interesting observation was related to the association of multiple infections and age. Young women were found to have a greater tendency for multiple infections (58% <30 years). Similar results were obtained by Mejlhede et al. (2009) suggesting that 61% of the youngest age group of infected women had multiple infections.

There were significant differences between controls and HPV-positive women according to age, residence, marital status, lifetime number of sexual partners, schooling, and parity. Women in urban areas were more frequently infected, as expected, due to the behavioral characteristics of this group. Single women were more frequently infected with HPV, and these data reflect differences in the sexual behavior of younger women. Younger women also tended to have more sexual partners. The frequency of infection increased with the number of partners. A high number of pregnancies was found to be inversely associated with HPV infection. These data are consistent with the findings of previous reports (Bauer et al., 1993; Burk et al., 1996; Lazcano-Ponce et al., 2001) but different from those in which pregnancy was positively associated with HPV infection (Bauer et al., 1993).

Ingestion of fern sprouts (*P. aquilinum*), which contain mutagenic and carcinogenic compounds, in the form of diet as is used in some populations such as Ouro Preto, MG, Brazil, and Japan has been associated with neoplasms in chronic consumers (Marlière et al., 2002). There was no significant association of consumption of fern sprouts with HPV infection in women with normal cytology. Two possible hypotheses can be argued: the bracken fern does not increase the susceptibility to HPV or since only women with normal cytology have been included in this group, the patients consuming bracken should have included the group of women presenting cervical alterations.

In this study, smoking status, alcohol consumption, and oral contraceptive use did not show significant association with HPV infection. Consistent with the lack of association in this study, most other studies have shown that smoking is not an independent risk factor for HPV infection (Lazcano-Ponce et al., 2001).

Inclusion of HPV detection tests in cervical cancer screening programs along with cytological examinations, educational strategies, and risk factor studies can help in the prevention and vaccination strategies against HPV.

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