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AQ1 Seroprevalence of Human Papillomavirus 6, 11, 16, and 18 in Young Primiparous Women in Sao Paulo, Brazil

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Introduction: Data on epidemiology of HPV infection are needed for the development of human papillomavirus (HPV) vaccine recommendations, especially in countries where HPV vaccination is not yet included in public vaccination programs. The aim of this study was to determine the prevalence of serum antibodies to HPV types 6, 11, 16, and 18 and associated factors among young women after birth of the first child.

Methods: This cross-sectional study was carried out in a large public maternity hospital in Sao Paulo, Brazil. Three hundred one women aged 15 to 24 years who gave birth to their first child were recruited between 43 and 60 days after delivery. Seroprevalence was performed using a type-specific enzyme-linked immunosorbent assay based on HPV L1 viruslike particles. The association of seroreactivity with these 4 HPV types with selected demographic and behavioral factors was assessed by Generalized Linear Model analysis.

Results: Fifty-eight (19.3%) women (95% confidence interval, 15.0%–24.2%) had antibodies to any of the 4 viruslike particles tested. The overall seroprevalence rates of the HPV types were: HPV16, 9.0%; HPV18, 7.0%; and HPV6+11, 7.4%, which are targeted by the HPV prophylactic vaccines. In the multivariate analysis, only age (inversely, $P = 0.044$ for trend) and previous sexually transmitted disease ($P = 0.008$) were 2 factors independently associated with HPV seropositivity.

Conclusions: These data offer additional information on the epidemiology of HPV in a group of young Brazilian women after first delivery and contribute to establish a baseline of HPV seroprevalence against which post–HPV vaccine era seroprevalence can be compared.

Key Words: HPV serology, VLP antibodies, Cervical cancer, HPV immunization, Sexually transmitted disease

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Worldwide, approximately 80% of cervical cancer cases affect women in developing countries; in Brazil approximately 20,000 cervical cancer cases occur each year, and the number of cervical cancer deaths remains high.[1]

Genital infection by oncogenic human papillomavirus (HPV) is a necessary factor in the development of cervical cancer and HPV types 16 and 18 were identified in approximately 70% of all cervical cancer cases.[2]

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Epidemiological studies have shown that whereas detection of HPV DNA does not provide information on women who were infected but cleared the HPV, seroprevalence data can provide a better estimate of cumulative exposure to HPV. However, it could also be an underestimate because only approximately 50% of those infected with HPV have detectable antibodies.[3] Humoral anti-HPV immune responses measured by enzyme-linked immunosorbent assay (ELISA) using HPV type–specific viruslike particles (VLPs) as antigens have been used for seroprevalence studies in several countries.[4]

The principal application of anti-HPV serology is monitoring anti-HPV vaccination.[5] In addition, data on the epidemiology of HPV infection are needed for the development of vaccine recommendations. In countries where HPV vaccination is not yet included in public vaccination programs, estimates of seroprevalence can provide information on the natural history of HPV infections to establish recommendations on suitable age groups that can benefit from the vaccination.

Young women from low-income groups are at significantly higher risk than those from higher-income groups of infection with HPV and cervical cancer.[6] Importantly, young age at first delivery has been also identified as a risk factor for cervical cancer development.[7]

Therefore, the aim of this study was to determine the seroprevalence of specific HPV types and the risk factors associated with HPV seropositivity after the delivery of the first child among young women in a public maternity hospital in the city of São Paulo, Brazil that serves mostly low-income people from adjacent areas.

MATERIALS AND METHODS

This study was conducted at Hospital Maternidade Leonor Mendes de Barros, one of the largest public maternity hospitals in the city of Sao Paulo, Brazil. All primiparous women between 15 and 24 years who had been living in the metropolitan area of Sao Paulo for at least 6 months and gave birth at this hospital after more than 32 weeks of gestation were eligible for this study. The study protocol was submitted and approved by the National Ethical Committee (number 188/2006).

This study is part of a cross-sectional study carried out from June 2006 to February 2007 and previously published.[8] The following exclusion criteria were applied: non-Brazilian, inability or refusal to give informed consent, and immunodeficiency (including acquired immunodeficiency syndrome/human immunodeficiency virus infection checked in medical records).

Women were recruited to take part in this study during the postdelivery follow-up period in the hospital. Eligible women were contacted by a health professional (a nurse or a physician) and asked whether they wanted to know about a study on prevention of cervical cancer; those interested had a postnatal visit scheduled within 43 to 60 days after delivery to be enrolled in the study. Women had to attend the postnatal visit and sign the informed consent form before being included in the study (women younger than 18 years were also required a written consent from their parents or legal representative).

Data and Specimen Collection

During the routine postnatal visit, women were enrolled and interviewed using a structured epidemiologic questionnaire that included information about demographic characteristics, sexual behavior, reproductive history, contraceptive practice, and smoking habits. Variables known or suspected to be related to cervical cancer and HPV infection were included in the questionnaire, such as age, ethnicity, education, marital status, income (the approximate amount earned per month, summing up all the family wages), smoking habits (current smoker: a person who smokes at least one cigarette a day for 1 year or more; former smoker: a person who stopped to smoke at least 1 year before the interview), age at first sexual intercourse, lifetime number of sexual partners, previous sexually transmitted disease (any sexually transmitted disease referred by women in the interview), previous miscarriage/abortion, contraception use, condom use (male or female condom), and hormonal contraception use (oral or injectable).

After the interview, approximately 10 mL of blood sample was taken by a nursing assistant and processed by centrifugation at the collection site, and the serum specimen was frozen at −20°C and transferred to the Ludwig Institute for Cancer Research in Sao Paulo to perform serological assays to HPV.

HPV Serology

The presence of IgG antibodies to HPV6, HPV11, HPV16, and HPV18 in the serum samples was detected with a type-specific ELISA using L1 VLPs synthesized in *Saccharomyces cerevisiae*[9] provided by Dr Ian Frazer (University of Queensland, Woolongaba, Australia). Fifty microliters of a solution with 0.03 mg/mL of HPV VLPs in phosphate-buffered saline (PBS) were used to coat each well of the ELISA plates (96 Maxisorp ELISA plates, Nunc) overnight (14–20 hours) at 4°C. To every 2 wells coated with VLP, one was filled with PBS, pH 7.2. The plates were then washed once with PBST (0.02% Tween in PBS, pH 7.2) and blocked with 100 μL of bovine serum albumin (BSA) blocking solution (1.5% of BSA in PBS, pH 7.2) for 2 hours. After the blocking step, plates were washed twice with PBST. Sera and control samples, diluted 1:50 with 5% fat-free milk powder and 0.1% BSA in PBST, were tested in duplicates and incubated at 37°C for 1 hour. The plates were washed 5 times with PBST, pH 7.2 and tested with horseradish peroxidase conjugated with antihuman IgG (Tago Immunologicals). After 45 minutes of incubation at 37°C, the plates were washed 5 times with PBST pH 7.2 and 50 μL of ABTS solution (0.05% 2.2′-Azino-bis (ethylenebenzthiazoline-6-sulfonic acid, Sigma) was added to each well. The reaction was stopped after 4 minutes by adding 50 μL of 1% sodium dodecyl sulfate. Optical density (OD) was read at 415 nm in a microplate reader (Biorad). The OD value of the serum was calculated by subtracting the mean OD of the wells coated with HPV L1 VLP from the OD of the wells coated with PBS.

For quality control and cutoff evaluation, we used positive and negative serum sample controls provided by the

World Health Organization in the WHO Workshop 2003.[10] The mean and 2 SDs (standard deviations) of absorbance values for HPV6, HPV11, HPV16, and HPV18 were calculated, and values greater than the mean plus 2 SDs were excluded. The cutoff points for seropositivity to HPV6, HPV11, HPV16, and HPV18 were 0.54 or greater, 0.37 or greater, 0.13 or greater, and 0.1 or greater, respectively.

Statistical Analysis

Detection rates of HPV antibody using a VLP-based ELISA were described as percentages with 95% confidence intervals (95% CI). To estimate the association of HPV antibody detection with selected risk factors, prevalence ratios (PR) and 95% CI were calculated, with HPV antibody detection as the dependent variable and various exposure factors as independent variables. Most independent variables were grouped into 2 or more categories. For ordered (categorical) exposure, variables tests for linear trend (χ^2 trend) in the PR were conducted by categorizing the exposure variables and entering the continuous scores. Variables selected in univariate analysis at a 0.20 significance level were included in the multivariate analysis. Prevalence ratios and their 95% CI were estimated using a Generalized Linear Model with binomial distribution and log link function.[11] Statistical significance was assessed using the likelihood ratio test. A 2-sided $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using STATA version 8.2 (STATA).

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RESULTS

A total of 509 women were invited to participate: 24 refused, 163 women previously interested in participating did not return to the postnatal visit, and 322 attended the postnatal visit. However, 18 women were not eligible (11 attended the postnatal visit after 60 days from delivery, one had human immunodeficiency virus–positive serological finding, one had more than one delivery and 5 were older than 24 years). Three eligible women were not included in the study: one had an acute infectious disease and 2 were younger than 18 years, lived in a reformatory, and had difficulty obtaining a legal representative signature of the informed consent form within the study period. Therefore, 301 primiparous women were included in this analysis.

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We compared participants and no respondents by using medical hospital records. No statistically significant differences were found regarding age ($P = 0.205$), marital status ($P = 0.480$), smoking habits ($P = 0.183$), prenatal health care ($P = 0.436$), and number of prenatal health care visits ($P = 0.214$).

The mean age of the study participants was 20 years; more than 60% were white and more than 80% were married or living with a partner. Almost a quarter of the study participants reported first sexual intercourse earlier than 15 years of age, and 40% reported only one lifetime sexual partner.

Fifty-eight (19.3%) women had antibodies to any of the 4 VLPs tested (95% confidence interval [CI], 15.0%–24.2%). The seroprevalence of HPV16 was higher than those of the other 3 types: 18, 11, and 6.

Antibodies to HPV types 16 and 18, high-risk types present in both bivalent and quadrivalent HPV vaccines, were detected in 9.0% and 7.0% of the study participants, and antibodies to low-risk types HPV6 and/or 11, present in the quadrivalent HPV vaccine, were detected in 7.7% of the study participants (Table 1). T1

Forty-six (15.3%) women were positive for a single HPV type antibody, 11 (3.7%) women had antibody for 2 HPV types, and only one (0.3%) woman had antibody for 3 HPV types of the 4 VLPs tested. Three (1%) and two (0.7%) participants had evidence of concomitant antibody by both high-risk types (HPV16 and HPV18) and by both low-risk types (HPV6 and HPV11), respectively. No woman was seropositive for all the 4 types present in the quadrivalent vaccine as detected by our assay.

Prevalence ratios for HPV antibody detection according to sociodemographic characteristics and smoking habits are shown in Table 2. Human papillomavirus seroprevalence T2 decreased with increasing age ($P = 0.078$ for trend). Women with more years of schooling had lower prevalence than those with 7 or less years of schooling ($P = 0.034$).

Regarding sexual behavior, reproductive characteristics, and contraception history, only ever having a sexually transmitted disease (STD) was associated with HPV antibody detection (PR, 2.40; 95% CI, 1.11–5.19; Table 3). T3

Ethnic group, marital status, income, smoking habits, age at first sexual intercourse, number of lifetime sexual partners, previous miscarriage/abortion, use of contraception, condom, or hormonal contraception were not associated to HPV antibody detection positivity (Tables 2 and 3).

All selected variables (age, years of schooling, and previous STD) were included in the multivariate analysis model. However, when age and years of schooling were included, the model did not converge. Then, the variable years of schooling was taken out. The multivariate analysis

TABLE 1. Seroprevalence of 4 common HPV types among 301 young primiparous women, Sao Paulo, Brazil, 2006–2007

HPV Serology	N	%
Negative	243	80.7
Positive	58	19.3
One HPV antibody		
16	27	9.0
18	21	7.0
6	15	5.0
11	8	2.7
2 HPV antibodies		
11 and 16	4	1.3
16 and 18	3	1.0
6 and 11	2	0.7
6 and 18	2	0.7
3 HPV antibodies		
6, 16, and 18	1	0.3

revealed that only age (P =0.044 for trend) and STD (P = 0.008) were independently associated with HPV seroprevalence. The prevalence ratio of HPV seropositivity for women aged 19 to 21 years was 0.84 (95% CI, 0.50–1.42) and that for women 22 to 24 years old was 0.51 (95% CI, 0.27–0.99) as compared with the women aged 15 to 18 years. The women who reported ever having an STD were more likely to be HPV antibody positive (PR, 2.81; 95% CI, 1.31–6.05) compared with the women who never had a previous STD.

TABLE 2. Prevalence ratios and corresponding 95% CI for seroprevalence of any of HPV types 6, 11, 16, and 18 according to selected sociodemographic characteristics and smoking among 301 young primiparous women, Sao Paulo, Brazil, 2006–2007

	Total	HPV+ (%)	PR	95% CI	*P*
Age, yrs					0.078‡
15–18	90	23.3	1		
19–21	119	21.0	0.90	(0.54–1.50)	
22–24*	92	13.0	0.56	(0.29–1.07)	
Ethnic group					0.581
White	190	18.9	1		
Black	31	12.9	0.68	(0.67–1.33)	
Mulatto	77	23.4	1.23	(0.87–1.33)	
Indian	3	—			
Years of schooling					0.034‡
≤7	37	29.7	1		
8	67	19.4	0.65	(0.33–1.31)	
9–10	64	23.4	0.79	(0.41–1.53)	
11	124	15.3	0.52	(0.27–0.98)	
>11	9	—		—	
Marital status					0.937
Living with partner	245	19.2	1		
Single	56	19.6	1.02	(0.57–1.84)	
Income†					0.668‡
<1	13	38.5	1		
1–3	188	16.5	0.43	(0.20–0.92)	
4–6	80	21.3	0.55	(0.25–1.24)	
7–10	11	18.2	0.47	(0.11–1.97)	
>10	5	40.0	1.04	(0.29–3.72)	
Smoking habits					0.648
Never	231	18.6	1		
Former	20	15.0	0.81	(0.27–2.37)	
Current	50	24.0	1.29	(0.73–2.26)	

*Includes 6 women who were 24 years old when invited but were 25 years old when they answered the questionnaire and collected biological specimens.

†Number of minimum wages per month (one minimum wage, R$250.00 or US$119.80; US$1.00 = R$ 2.09; Feb/2007). Data for 4 participants were missing.

‡χ^2 for trend.

TABLE 3. Prevalence ratios and corresponding 95% CIs for seroprevalence of any of HPV types 6,11,16, and 18 according to selected sexual behavior, reproductive characteristics, and history of contraception among 301 young primiparous women, Sao Paulo, Brazil, 2006–2007

	Total	HPV+ (%)	PR	95% CI	*P*
Age at first sexual intercourse, yrs					0.465†
≤14	72	22.2	1		
15–18	188	18.6	0.84	(0.50–1.42)	
>18	41	17.1	0.77	(0.34–1.71)	
No. lifetime sexual partners					0.379†
1	120	20.0	1		
2–3	116	13.8	0.69	(0.39–1.23)	
≥4	65	27.7	1.38	(0.81–2.36)	
Previous STD					0.073
No	292	18.5	1		
Yes	9	44.4	2.40	(1.11–5.19)	
Previous miscarriage/abortion					0.769
0	282	19.1	1.00		
1–2	19	21.1	1.10	(0.45–2.71)	
Contraception					0.443
Yes	227	20.3	1		
No	74	16.2	0.80	(0.45–1.43)	
Condom use*					0.363
Yes	127	18.1	1		
No	100	23.0	1.27	(0.76–2.13)	
Hormonal contraception*					0.727
Yes	148	20.9	1		
No	79	19.0	0.91	(0.52–1.58)	

*Only women who reported ever use of contraception.

†χ^2 for trend.

DISCUSSION

The seroprevalence of any HPV types 6, 11, 16, and 18 found in the young primiparous women included in the present study was 19%. These results are within the range of seroprevalence for these 4 HPV types reported also in young women in other surveys, varying from 13% to 25%.[12–14] The HPV16 (9%), HPV18 (7%), and the combined HPV6+11 (8%) seroprevalence found in the present study were similar to those recently reported in 2 population-based surveys in women 14 to 24 years old in the United States[14] and Australia[15], and in England (women 10–29 years old)[12] varying from 6% to 12% for HPV16, 2% to 5% for HPV18, and 9% to 13% for HPV6+11 .

The prevalence of HPV infection in Latin America is the highest in the world. In female subjects (16–24 years old) from 5 Latin American countries (Brazil, Costa Rica, Guatemala, Mexico, and Peru), the overall seroprevalence (25%) and the baseline seroprevalence of the 4 HPV vaccine types (ie, HPV16, 14%; HPV18, 4%; HPV6+11, 13%) were slightly higher than the seroprevalence found in the present study, except for HPV18.[13]

However, the frequency of HPV18 seropositive individuals (7%) found in the present study is consistent with reports from Costa Rica (13% in women 18–24 years).[16] In addition, a higher HPV16 and/or HPV18 combined seroprevalence (28%) was previously reported in Brazilian women (15–25 years).[17]

In Brazil, another study revealed an even higher overall HPV6, HPV11, HPV16, and HPV18 seroprevalence (39%)[18] than that of the present study. However, that study included participants notably of different age groups, and the age of participants was substantially higher (15–70 years old)[18] than that in our study. Therefore, a plausible hypothesis to explain this difference in seroprevalence of HPV would be different exposures to HPV in successive Brazilian birth cohorts.

Women were enrolled in the present study within 43 to 60 days after delivery and thus were under the physiological processes established during pregnancy that modify the host-immune response and hormonal status. Interestingly, in a similar study conducted in primiparous women (≤30 years old) from Finland, the reported HPV16 seroprevalence was 24% for 2 periods of analysis (1983 and 1990).[19]

It is very important to stress that the accuracy of HPV serological measures are affected by the type of assay used and the associated seropositivity cutoff values. Therefore, it is often very difficult to compare results from different epidemiological studies.[4] There are current efforts by the World Health Organization to develop HPV type 16 and HPV type 18 serological reference standards, as well as an agreed definition of what level of response indicates effective seroreactivity.[10] Moreover, the diagnostic techniques used in different studies and the discrepancies in HPV seroprevalence results also depend on the age of participants, the clinical history of the women included in the study (immunodeficiency, abnormal cervical cytology), and on sexual patterns accepted in each population.

Approximately 4% of women in the present study were seropositive for at least 2 HPV types, 1% was seropositive for both HPV16 and HPV18, and 0.7% was seropositive for both HPV6 and HPV11, and none had simultaneous antibodies to all 4 HPV types. The latter observation suggests that most of these young women after first delivery could still draw benefit from catch-up HPV vaccination and protection against cervical cancer.

It is not clear whether naturally acquired HPV antibody responses to an HPV type provide protection against reinfection or subsequent infection due to related types.[15] In addition, serologic measurements may not identify all immune reactive persons because approximately 50% of HPV-infected women do not show evidence of seroconversion but may nevertheless be protected against subsequent infection.[3]

Serological results suggest that anti-VLP antibodies detection is strongly asynchronous with infection; they are found in approximately 10% to 20% of infected individuals at the time that HPV DNA is detectable, and they are detected primarily in association with persistent infections.[5] Similarly, low rates of concordance between the presence of HPV DNA in primiparous young women[8] and serological results in this study have been observed. In our previous publication, with this same population of study, 17.3% of the women were positive for any of the 4 HPV types (HPV6, HPV11, HPV16, or HPV18), and the overall prevalence of the HPV types was HPV16, 12.0%; HPV18, 2.3%; and HPV6+11, 4.3%.[8]

One limitation of this study is that 32% of eligible women, previously interested to participate, did not return to the postnatal visit. The likely reason for the relatively large number of women not attending the postnatal visit at our hospital is the availability of postnatal services in public health care centers near their homes, and therefore, women may have preferred those for practical reasons. However, participating and no participating women did not differ as analyzed according to several factors, and this limitation is unlikely to have affected our results significantly.

We also studied the relationship between HPV seropositivity and several risk factors for cervical cancer. The multivariate analysis highlights 2 associated factors with HPV seropositivity: the history of STDs and age (inversely associated with HPV seroprevalence). In this respect, Hagensee et al[20] reported that history of *Neisseria gonorrhoeae* infection was associated with an increased seroprevalence of HPV16. Other investigations reported that HPV seropositivity is strongly associated with markers of sexual activity, mainly with a woman's elevated number of lifetime sexual partners. This last factor is strongly associated with the presence of HPV16 antibodies[16,20] as much as any HPV6, HPV11, HPV16, or HPV18 [14,18] antibodies detection. However, we did not find association with the number of lifetime sexual partners and HPV seropositivity.

Age was associated with HPV seropositivity in several studies. We have observed a trend of decreasing HPV seroprevalence with increasing age specific to this group of young women, whereas other studies showed highest seroprevalence rates in older women.[12,14] These previous studies included participants who had a higher age range (10–29 years[12] and 14–59 years[14]) than the present study. However, in agreement with our findings, another study reported that antibody levels are likely to wane over time.[3]

Despite the highlighted limitations of this study, data presented here offer unique information on the epidemiology of HPV in young primiparous women in Brazil, a group of women at high risk of HPV infection, who belong to an age group eligible for catch-up HPV vaccination. Moreover, it may set a basis to establish a baseline of HPV seroprevalence against which post–vaccine era seroprevalence can be compared.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES

AQ1 = HPV was spelled out to Human Papillomavirus. Please check.

AQ2 = Please expand/spell out L1.

AQ3 = The word 'rates' was inserted in "The overall seroprevalence rates of the HPV types…" Please check if appropriate.

AQ4 = Please provide the current affiliation of Dr. Pagliusi. The former affiliation can be added as a footnote.

AQ5 = Please provide the city and state/country location of Nunc.

AQ6 = Please expand/spell out PBST.

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AQ10 = Please provide the city and state/country location of Biorad.

AQ11 = Please provide the city and state/country location of STATA.

AQ12 = The word serology, which is general and abstract (AMA style manual, p255) was changed to serological finding. Please check if appropriate.

AQ13 = Please check the changes made here: "…diagnostic techniques used in different studies and the discrepancies in HPV seroprevalence results also depend on the age of participants…"

AQ14 = Please provide the complete date the reference was accessed.

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