

Clinical Study

Prevalence of HPV in Adolescents Virgins and Sexually Active at a University Hospital in the City of Rio de Janeiro, Brazil

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Received 20 May 2013; Accepted 18 June 2013

Academic Editors: J.-M. Bart and M. Pourshafie

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Sexually transmitted diseases (STDs) are common worldwide, with especially alarming numbers in Brazil. Among the most common infections is human papillomavirus (HPV). The possibility of the nonsexual transmission of HPV is not well defined and is the subject of debate. This study aimed to identify the prevalence of HPV in adolescents with no history of sexual intercourse compared with a group of similar age with sexual activity. 100 adolescents were evaluated with at least two years after menarche, who attended from January 2007 to January 2009 at the University Hospital Pedro Ernesto, Rio de Janeiro, Brazil. Among the adolescents, 50 had intact hymen and 50 reported regular sexual activity. For patients without sexual intercourse (group 1) we collected material from vestibule and for patients with sexual activity (group 2) we collected material from vagina and endocervix. The search for HPV-DNA 2nd generation hybrid capture (hC2) was performed. In group 1 the test was positive in 3 cases (6%). In the second group, 33 cases (66%) were positive for at least one site. The positivity in girls with sexual activity is high. On the other hand, the HPV infection, although rare, may occur in girls without sexual intercourse.

1. Introduction

Sexually transmitted diseases (STDs) are common worldwide, with especially alarming numbers in Brazil. Among the most common infections is human papillomavirus (HPV), which has been shown to be associated with genital and anal neoplasms [1]. There are over 100 types of HPV that have been identified, and more than 40 types infect the genital tract [2].

Global estimates indicate that approximately 20% of asymptomatic individuals and individuals with normal cytology and colposcopy examinations are infected with HPV [3, 4]. Despite their high prevalence, many infections are transient (60%). These infections usually last for an average duration of 8 to 10 months. However, persistent infections are found in 5% to 10% of women 35 years of age or older, which

are associated with an increased risk of intraepithelial lesions [5].

In clinical practice, there are many questions regarding the possibility of reactivation of latent HPV and the subsequent formation of clinical or subclinical lesions. Moreover, puberty is a period in which disease can appear due to the physiological changes that occur at this stage that may predispose individuals to infection and, eventually, epithelial lesion [2, 5].

The possibility of the nonsexual transmission of HPV is not well defined and is the subject of debate. There has been a discussion about laryngeal papillomas and perinatal transmission [6–9], as well as transmission via the placenta and umbilical cord [10]. In head and neck cancers, some authors have considered the perinatal route of HPV transmission and

discussed the possible role of HPV in the development of these cancers [11]. In addition, transmission via fomites is suspect in reported cases of nonsexual transmission. Some reports demonstrated that HPV may maintain infectivity at room temperature and even when desiccated. This virus also was found on objects used for treatment of HPV lesions [12, 13].

The objective of this study was to identify the prevalence of HPV-DNA in nonsexually active adolescents who had an intact hymen upon clinical examination compared to sexually active adolescents.

2. Methods

A cross-sectional study was made from January 2007 to January 2009 in which we evaluated 100 patients aged 11–20 years who had their menarche at least 2 years previously. These patients attended at the outpatient gynecology clinic for children and adolescents at the University Hospital Pedro Ernesto (State University of Rio de Janeiro, Brazil). The patients were divided into two groups. The first one (group 1) consisted of 50 patients who reported not having had sexual intercourse and who had an intact hymen upon physical examination performed by an experienced gynecologist. After collecting sociodemographic data, vestibular samples were taken from these patients using a specific swab and placed into collection tubes to preserve the samples in specimen transportation medium [STM] (HC2; Qiagen, Gaithersburg, MD, USA).

The second group (group 2) was composed of 50 patients who reported sexual activity. After collecting socio-demographic data, vaginal and endocervical samples were taken from these patients using site-specific swabs and collection tubes containing STM.

The properly labeled tubes were sent to the molecular biology laboratory to test for high-risk and low-risk HPV-DNA using the 2nd generation Hybrid Capture version 2 (hC2v2), according to the manufacturer's specifications (HC2; Qiagen, Gaithersburg, MD, USA). The test results are reported in relative light units (RLUs) that result from reading the amplification signal by chemiluminescence. RLU values have a cutoff, and RLU/PC values greater than one were considered positive for HPV.

Sociodemographic data were analyzed for correlations among the variables and compared to the results of hC2v2 tests. Chi-squared and Fisher's exact tests were used to determine the statistical significance of the results to 95% confidence interval.

The informed consent was obtained from all research subjects and the study was reviewed and approved by institution's Ethics in Research Committee.

3. Results

In group 1 (no sexual intercourse), the ages of the participants varied from 11 to 20 years old (mean: 15.8 ± 2.0). The ages of patients in group 2 (sexually active) varied from 13 to 20 years old (mean: 16.5 ± 1.5). The majority of patients in both groups were students. The age at menarche in group 1 varied from 8 to 15 years old (mean: 11.5 ± 1.5). In the patients in

group 2, the age at menarche was 9 to 15 years old (mean: 11.8 ± 1.3), and sexual activity started at 12 to 18 years old (mean: 14.9 ± 1.5). The age of the group 2 patients' sexual partners varied from 15 to 37 years of age (mean: 23.4 ± 4.3), and the age difference between the patients and their partners varied from 2 years older than their partner to more than 20 years younger than their partner (mean: 6.4 ± 4.6). The average number of partners varied from 1 to 5 (mean: 2.1 ± 1.2) (Table 1).

The overall positivity for HPV DNA was 72% (72 cases) (Table 2). In group 1, positivity was observed in three cases (6%). Two with low-risk HPV DNA, and one had high-risk HPV DNA.

In group 2, 33 cases (66%) tested positive in at least one site. Vaginal specimens tested positive in 30 cases (60%), and cervical specimens tested positive in 27 cases (54%). Positivity for high-risk HPV DNA was detected in 21 cases (42%), low-risk HPV DNA was detected in three cases (6%), and concomitant high- and low-risk HPV DNA was detected in nine cases (18%) (Table 2).

In endocervical samples from group 2, HPV DNA was detected in 27 patients (54%), including 20 high-risk (40%), one low-risk (2%), and six concomitant high- and low-risk (12%) (Table 3).

The same HPV type was present in vagina and cervix in 22 cases, 17 of which for high-risk HPV and five for concomitant high- and low-risk HPV. Two positive vaginal cases of high- and low-risk HPV tested positive for only high-risk HPV in the cervix, whereas the other case tested positive for only low-risk HPV in the cervix. hC2v2 positivity was observed only in the vagina in four cases of high-risk HPV, in one case of low risk HPV and in one concomitant high- and low-risk case of HPV, and there were no cases of positivity in the cervix alone (Table 4).

Upon stratifying the sociodemographic data into HPV-positive and negative patients in group 2, the average age for the negative patients was higher, and the difference was just over one year of age. The age at menarche did not vary between the groups. The ages of onset of sexual activity were 14.8 and 15.2 years of age for the positive and negative groups, respectively. Among these, only the difference in age was statistically significant ($P = 0.02$).

The age of the sexual partners varied greatly in the positive group from 15 to 37 years old (mean = 22), while in the negative group varied from 18 to 25 years old (mean = 21.5). For both the adolescents with positive and negative samples, the male partners were older than the female partners (5.7 and 4.4 years older) ($P = 0.05$).

The number of sexual partners varied from one to five. The average was two for patients with positive samples and varied from one to two for patients with negative samples. The average was lower for the negative group compared to the positive group ($P = 0.02$) (Table 5).

4. Discussion

The nonsexual transmission of HPV is controversial, and its frequency is unclear. Studies are necessary to define the clinical approach in an understudied population.

TABLE 1: Sociodemographic data of children and adolescents with no sexual intercourse (group 1: $n = 50$) and with sexual activity (group 2: $n = 50$).

	Group 1 (mean \pm SD)	Group 2 (mean \pm SD)	<i>P</i>
Age	15.8 \pm 2.0	16.5 \pm 1.5	ns
Menarche	11.5 \pm 1.5	11.8 \pm 1.3	ns
First intercourse	—	14.9 \pm 1.5	*
Age of the partners	—	23.4 \pm 4.3	*
Partner age difference	—	6.4 \pm 4.6	*
Number of partners	—	2.1 \pm 1.1	*

ns: not significant, *: not estimated, SD: standard deviation.

TABLE 2: HPV DNA test (2nd generation Hybrid Capture version 2, Qiagen, Gaithersburg, MD, USA) in group 1 (children and adolescent with no sexual intercourse).

DNA-HPV	<i>N</i>	%
Negative	47	94
Low risk	2	4
High risk	1	2
Low and high risk	0	0
Total	50	100

TABLE 3: Sociodemographic data of children and adolescents with no sexual intercourse (group 1: $n = 50$).

Sociodemographic data	<i>N</i>	%
Age (years-old)		
11–14	14	28
15–17	24	48
18–20	12	24
Occupation		
Student	50	100
Menarche (years-old)		
<10	5	10
10–12	32	64
>12	13	26
Genital contact without penetration		
HPV Positive		
Yes	2	4
Not	1	2
HPV negative		
Not	47	94

The age at menarche observed in our study was 11.5 and 11.8 years for the groups of patients with no history of sexual intercourse (group 1) and those sexually active (group 2), respectively. These data are in accordance with other research [14], in which adolescents from 12 to 19 years of age in Rio de Janeiro reported menarche around 11.8 years.

The age of sexual debut was on average 14.9 years old, approximately the same of the patients in study mentioned [14], where the average was 14.7. Another study [15] on women from three different regions of Brazil reported the average

TABLE 4: HPV DNA detection of high and low risk vaginal collection in groups 1 (no sexual activity and intact hymen) and 2 (with sexual activity).

	Group 1 (<i>N</i> = 50) <i>N</i> (%)	Group 2 (<i>N</i> = 50) <i>N</i> (%)
HPV DNA		
High risk	1 (2%)	21 (42%)
Low risk	2 (4%)	3 (6%)
High and low risk	—	9 (18%)
Positive (total)*	3 (6%)	33 (66%)
Negative	47 (94%)	17 (34%)

* $P < 0.05$ (chi-square test), considering the difference of positives between groups 1 and 2.

TABLE 5: Presence of HPV DNA according to the classification and site in group 2 (children and adolescent with sexual activity).

HPV DNA	Vagina <i>N</i> (%)	Endocérvice <i>N</i> (%)
High and low risk positive	8 (16)	6 (12)
High risk positive/low risk negative	21 (42)	20 (40)
Low risk positive/high risk negative	1 (2)	1 (2)
High and low risk negative	20 (40)	23 (46)
Total	50 (100)	50 (100)

age of patient's first sexual intercourse of 18.5 years (± 4.0). However, in this study, younger women reported having had their sexual debut earlier than older women, suggesting that teenagers are starting their sexual lives at an earlier age than in previous generations.

Of all samples from patients 72% were positive for HPV DNA, and 6% (3 cases) of girls with no history of vaginal intercourse tested positive for HPV DNA. Previous studies suggested that HPV infection of women with an intact hymen may occur via genital contact without penetration [16]. The numbers vary between 4.9% in a study using anti-L1 IgG antibodies to detect HPV and 14.8% in a study using PCR to detect HPV [17, 18]. Conversely, in a study involving 251 patients from a psychiatric institution there was no case of positive HPV DNA by hybrid capture in virgins [19].

Two of the three patients (66%) in group 1 that tested positive for HPV DNA had identified low-risk HPV. A similar finding was also found, using ELISA-based methods to test 100 virgins [20]. They identified one case of HPV DNA that was weakly positive for type 6 HPV (low oncogenic risk HPV). Another report studied the transmission dynamics of HPV between family members for 24 months using PCR and observed that the most common profile was high-risk HPV present in all family members (29%), followed by the mother-child pairing (26%). The positivity of father-child pairings of HPV test was less frequent (11%), and in six families (8%), only the child was positive. In children, HPV DNA was detected in 15% of genital samples and 10% of oral samples at birth, which increased to 18% and 21% at 6 months of age and then decreased to 10% at 24 months [21]. These data suggest

the possibility that neonatal infections may be able to persist latently until adolescence.

The patients who reported having vaginal intercourse (group 2) were positive for HPV in 66% of patients, 58% of which for high-risk HPV. In Brazil, other researchers have reported HPV prevalence rates that vary from 27% using PCR to 50.1% using hybrid capture [22, 23]. Notably, differences in methodology and the number of sites surveyed may account for the variation in the percent positive. Although one study based on serology [5] showed that only 27.7% of cases were positive for HPV. This study surveyed only HPV types 16 and 18, whereas our study used hybrid capture probes that can identify 13 types of high-risk HPV and 5 types of low-risk HPV.

The same HPV types were observed at both sampling sites (vagina and cervix) in 71% of high-risk HPV cases and 21% of concomitant low- and high-risk HPV cases. More HPV-positive vaginal samples (60%) were observed compared to samples from the endocervix (54%), but this difference was not significant ($P = 0.4752$).

Factors associated with HPV infection in sexually active patients in this study were age, number of sexual partners, and age difference between the partners. The association between age and risk of HPV infection in the general population is well characterized and specifically in adolescents that is a particular risk group for HPV infection [24, 25]. The number of sexual partners was associated with HPV positivity, and it was estimated that each new partner per month increases the risk of infection 10-fold [25–27]. Other studies, have shown that the risk of acquiring HPV is five times higher for teenagers whose first sexual partner is over 10 years older [20].

This study has limitations such as the small number of cases studied and the approach to identify factors that might be associated with positivity of cases among girls without sexual activity. Best and most well designed studies should identify such conditions. However, it seems that the intercourse, especially the sexual debut, is an important facilitating factor in the transmission of HPV, even though it is not required [27].

5. Conclusion

HPV infection can occur in Brazilian girls that have not had sexual intercourse. However, the number of cases in this study was small, and additional population-based studies would be required to make more definitive conclusions. Despite the limitations of the study as the small number of cases and lack of viral genotyping, we may infer that sexual activity among Brazilian adolescents carries a high risk for infection, especially when taking into account the age of the first sexual intercourse, the age difference between the partners, and number of partners.

Acknowledgments

The authors thank investigators and abstractors at the University Hospital Pedro Ernesto, Rio de Janeiro, Brazil, and Professor Eleutério Laboratory, Fortaleza, Brazil.

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