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CLINICAL ARTICLE

Prevalence of HPV infection by cervical cytologic status in Brazil

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ABSTRACT

Objectives: To assess the prevalence of human papillomavirus (HPV) infection according to cervical cytologic status in northeastern Brazil; identify other risk factors for low- and high-grade squamous intraepithelial lesions (LSILs and HSILs); and identify the most prevalent HPV genotypes associated with the lesions. *Method:* Two cervical smears were collected from 250 women referred for cancer screening, one for cytologic examination and the other to test for the presence of HPV by PCR with genotyping by dot blot hybridization. *Result:* There were 110 healthy cervices, 82 LSILs, and 58 HSILs. The overall HPV prevalence was 48%, with higher rates for HSILs, and HPV-16 was the most prevalent type. Age, multiple sexual partners, type of HPV present, smoking, and early onset of sexual activity were risk factors for cervical lesions. *Conclusion:* Age, multiple sexual partners, and infection with HPV-16 increased the risk of having LSILs or HSILs. Early onset of sexual activity and smoking only increased the risk of having HSILs.

1. Introduction

Human papillomavirus (HPV) is a large group of epitheliotropic viruses of more than 120 different types. Of the 40 HPV types infecting the human body, more than a dozen are associated with probable or definite oncogenic risk [1,2]. Infection of the cervix is initiated when infectious particles reach and then bind to the basal layer of the cervical epithelium, where they enter cells through small tears. It has been suggested that for maintenance of the infection, the virus has to infect an epithelial stem cell [3].

The infection occurs mainly in young, sexually active women in the first years following their first sexual encounters [4,5]. The highest HPV prevalence is among women younger than 25 years. It decreases from age group to age group and is lowest among women aged between 45 and 50 years, but increases in the postmenopausal years [6]. Most HPV infections clear spontaneously, but cervical lesions can develop in women who have persistent infection with high-risk types of HPV [7]. A current epidemiologic challenge is to identify etiologic cofactors that lead to HPV persistence and progression to neoplastic lesions, and clarify how these cofactors contribute to carcinogenesis [8]. The high-risk types of HPV have a higher tendency to cause persistent infection and, in the presence of other environmental and host factors, are associated with an increased risk of cervical lesions that may progress to cervical cancer [8,9].

Various studies of screening testing for cervical cancer were recently realized in the south and southeast regions of Brazil as part of the *Latin America Screening Study*. These studies have analyzed the relations between the cytologic and histologic abnormalities present in cervical smears, and between HPV infection and the predictive factors of these anomalies [10–12], but so far have not included the Northeast region of Brazil. Moreover, the method used for HPV detection in these studies was Hybrid Capture 2, which does not identify HPV type.

The present study was conducted to analyze prevalence of HPV infection in women with healthy and abnormal uterine cervices in the Northeast region of Brazil, and to identify the HPV types prevalent in the local population, as well as risk factors for HPV infection.

2. Materials and methods

The cervical samples used were obtained between April 2001 and June 2002 at Luis Antonio Hospital in Natal, Rio Grande do Norte State, Northeastern Brazil, from 258 women referred for cancer screening because of a history of cytologic alterations. The women's age ranged from 15 to 65 years. All women were informed about the methods and objectives of the research. All signed an informed consent form and

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answered a standardized questionnaire about their marital status, ethnicity, sexual behavior and reproductive activities, oral contraceptive use, smoking habits, and family history of cancer. The patients' ethnicity was defined based on self-reports according to the criterion of the Instituto Brasileiro de Geografia e Estatística (IBGE) that classifies ethnicity into 5 categories: white, black, mulatto, yellow, and native. In this study, the black, mulatto, yellow, and native categories were combined into a nonwhite category. The study was approved by the Ethical Committee in Research of Federal University of Rio Grande do Norte.

The cervical smears were obtained using a cytobrush for optimal collection of exfoliated cells. A trained cytopathologist from the Department of Pathology of Federal University of Rio Grande do Norte stained the smears and analyzed them according to the Bethesda classification (available at: http://bethesda2001.cancer.gov/terminology.html). All abnormal smears were re-examined for result confirmation. Samples with scant squamous cellularity were considered unsatisfactory for evaluation and excluded from analysis. The women with abnormal cytologic results were followed up according to the guidelines of the National Cancer Program of Brazil. Based on the results of the cytologic evaluation, the 250 retained participants were allocated to 1 of 3 groups: a normal cytology group (n = 110); a low-grade squamous intraepithelial lesions (LSIL) group (n = 82); and a high-grade squamous intraepithelial lesions (HSIL) group (n = 58).

Another sample of exfoliated cervical cells was collected from each participant and the collection brush was placed in a tube containing PBS, vancomycin, and nystatin. The tube was kept at 4 °C until processing for DNA extraction. The content was centrifuged at 3000 g for 10 minutes following vigorous tube agitation and brush removal; the supernatant was removed; and the remaining pellet was processed for DNA extraction, using rapid isolation of DNA from mammals, with proteinase K [13].

To check the quality of the obtained DNA, aliquots with about 30 ng of DNA were submitted to polymerase chain reaction (PCR) using the primers PCO3+/PCO4+, specific for the amplification of a 110 bp fragment of the β-globin gene [14]. Only the 250 DNA samples positive to β-globin were checked for HPV DNA.

Of the 250 good-quality DNA samples 110 were from participants in the normal cytology group, 82 from participants in the LSIL group, and 58 from participants in the HSIL group. The presence or absence of HPV DNA was established using the degenerate primers MY09/M11, specific for the L1 open reading frame (ORF) of the HPV genome [15]. The products of PCR were submitted to electrophoresis on 7% polyacrilamide gel, followed by silver staining [16]. The specimens were considered positive for HPV DNA if they presented a band of 450 bp. The amplicons were submitted to HPV genotyping by Dot blot hybridization as described [17].

To check for associations between sociodemographic characteristics and cervical lesions, and for differences in the prevalences of HPV infection in the 3 groups, we used the Pearson χ^2 test for comparison of proportions. To identify variables that could be considered risk factors for cervical lesions we used all the considered variables in a multivariate logistic regression analysis, with the dependent variable assigned a value of 1 in the presence of low- or high-grade intraepithelial cervical lesions and a value of 0 in the absence of lesions. To check for associations between HPV type and health status we used univariate logistic regression. All analyses were performed using the software SPSS, version 13.0 (SPSS, Chicago, Illinois, USA). $P \leq 0.05$ was considered statistically significant.

3. Results

Statistical analysis by the χ^2 test showed an association between age and presence of HSIL ($P = 0.000$) and between ethnicity and presence of HSIL ($P = 0.002$). Considering separately married and single women, we did not observe an association between marital status and the presence of LSIL or HSIL ($P = 0.09$ and $P = 0.62$), respectively (Table 1).

We observed that most (81.5%) of the women with both a normal cytology result and HPV infection were younger than 34 years, with a lesser prevalence of HPV infection in the older age groups. Among women with LSILs, the prevalence of HPV infection increased as they reached the age of 34 years, decreased as they aged from 35 to 44 years, and increased again after they were 45 years old. Most (77.8%) of the women with HSILs were between 25 and 54 years old (Table 1). Differences in HPV prevalence between the normal cytology group (24.5% of the participants) and the LSIL (58.5%) and HSIL groups (77.6%) were statistically significant by comparison of the proportions, revealing that HPV infection increased the risk of developing both low-grade ($P = 0.000$) and high-grade ($P = 0.000$) premalignant lesions. Age was associated with the occurrence of HSIL ($P = 0.000$), as was nonwhite status ($P = 0.002$). We observed no associations between marital status and the presence of cervical lesions (Table 1).

The women older than 34 years were at greater risk for LSILs and those older than 45 years were at greater risk for HSILs. The women who had their first sexual intercourse between the ages of 14 and 17 years were at greater risk only for HSILs. Having had multiple sexual partners and being a smoker also increased the risk of having HSILs. There was no evidence that age at first pregnancy, number of pregnancies, use of oral contraceptives, and a family history of cancer increased the risk of either LSIL or HSIL (Table 2).

Cervical HPV infection increased the risk of both LSIL and HSIL. Fourteen different types of HPV were identified in the study population, and most had a high oncogenic potential. Infection was caused by 1 type of virus in 19.0% of the women in the normal cytology group, 40.2% of the women in the LSIL group, and 67.2% of the women in the HSIL group, and HPV-16 was the most prevalent type in all 3 groups. The second most common type was HPV-58 in the first 2 groups and HPV-45 in the HSIL group. Infection with 2 HPV types were detected in the 3 groups, the most frequent coinfection being with HPV-56 and HPV-57 in the first 2 groups, and with HPV-31 and HPV-33 or HPV-45 and HPV-59 in the HSIL group. With the probes we used, it was not possible to identify the HPV types carried by 2 participants in the normal cytology group, 1 participant in the LSIL group, and 1 participant in the HSIL group. Single infection by HPV-16 increased the

Table 1
Distribution of socio-demographic characteristics, according to health status

Variable	Normal cytology group		LSIL group		HSIL group	
	No. of patients	No. of patients with HPV (%)	No. of patients	No. of patients with HPV (%)	No. of patients	No. of patients with HPV (%)
Age at study entry[a]						
<25	37	9 (24.3)	19	13 (68.4)	4	4 (100.0)
25-34	44	13 (29.5)	30	17 (56.7)	14	11 (78.6)
35-44	23	5 (21.7)	24	11 (45.8)	18	13 (72.2)
45-54	4	0	5	3 (60.0)	13	11 (84.6)
≥55	2	0	4	4 (100.0)	9	6 (66.7)
Total	110	27 (24.5)	82	48 (58.5)	58	45 (77.6)
Ethnicity[b]						
White	87	19 (21.8)	64	40 (62.5)	33	24 (72.7)
Nonwhite	23	8 (34.8)	18	8 (44.4)	25	21 (84.0)
Marital status[c]						
Single	32	11 (34.4)	15	12 (80.0)	14	10 (71.4)
Married	78	16 (20.5)	67	36 (53.7)	41	32 (78.0)
Widowed	0	0	0	0	3	3 (100.00)

[a] Statistical analysis for age among the groups: Group I × Group II: $\chi^2 = 5.262$, = 0.261; Group I × Group III: $\chi^2 = 39.606$ $P = 0.000$.
[b] Statistical analysis for ethnicity among the groups: Group I × Group II: $\chi^2 = 0.030$, $P = 0.862$; Group I × Group III: $\chi^2 = 9.166$, $P = 0.002$.
[c] Statistical analysis for marital status among the groups: Group I × Group II: $\chi^2 = 2.963$, $P = 0.085$; Group I × Group III: $\chi^2 = 0.241$, $P = 0.623$.

Table 2
Distribution of patients by cytological status according to the considered variables, and associated odds ratio (OR) obtained by multivariate logistic regression model

Variable	Normal cytology group(n = 110)	LSIL group(n = 82)			HSIL group(n = 56)		
	No. of patients	No. of patients	OR	95% CI	No. of patients	OR	95% CI
Age at study entry, y							
<25	37	19	1	[Reference]	4	1	[Reference]
25–34	44	30	2.27	[0.91–5.64]	14	1.72	[0.32–9.25]
35–44	23	24	3.44	[1.17–10.14][a]	18	5.49	[0.94–32.04]
45–54	4	5	9.66	[1.30–71.56][a]	13	28.04	[2.24–350.29][a]
>54	2	4	30.18	[2.01–453.90][a]	9	332.80	[7.58–14621.26][a]
Age at 1st sexual intercourse, y							
14–17	49	52	2.34	[0.71–7.76]	49	26.09	[1.93–352.05][a]
18–21	38	20	1.19	[0.37–3.83]	8	9.68	[0.80–117.00]
≥22	23	10	1	[Reference]	1	1	[Reference]
Age at 1st pregnancy, y							
Never pregnant	17	11	NA	NA	1	0.90	[0.03–26.53]
14–17	14	18	2.43	[0.70–8.43]	27	0.61	[0.11–3.50]
18–21	43	36	1.69	[0.64–4.41]	21	0.50	[0.12–2.19]
≥22	36	17	1	[Reference]	9	1	[Reference]
No. of pregnancies							
0	17	10	1	[Reference]	2	1	[Reference]
1–3	76	54	101.44	[0.0–∞]	22	2.52	[0.23–28.11]
4–6	11	12	53.42	[0.0–∞]	22	6.32	[0.47–84.25]
>6	6	6	27.09	[0.0–∞]	12	NA	NA
Oral contraceptive use, y							
0	37	32	1	[Reference]	28	1	[Reference]
1–3	43	27	0.65	[0.29–1.47]	9	0.56	[0.12–2.58]
4–6	20	13	0.56	[0.20–1.53]	15	0.68	[0.15–3.06]
7–9	5	6	1.01	[0.23–4.48]	1	0.47	[0.03–8.31]
≥9	5	4	0.75	[0.14–4.09]	5	1.74	[0.23–13.41]
Multiple sexual partners							
Yes	29	46	3.56	[1.80–7.04][a]	45	10.50	[3.04–36.35][a]
No	81	36	1	[Reference]	13	1	[Reference]
Smoking							
Yes	12	13	1.44	[0.52–4.02]	32	4.80	[1.36–16.94][a]
No	98	69	1	[Reference]	26	1	[Reference]
Family history of cancer							
Yes	21	26	1.33	[0.58–3.05]	18	0.74	[0.21–2.66]
No	89	56	1	[Reference]	40	1	[Reference]

Abbreviations: CI, confidence interval; HSIL, High-grade lesion; LSIL, low-grade lesion; NA, not applicable; OR, odds ratio.
[a] Denotes statistical significance.

risk of both LSIL and HSIL, whereas single infection by HPV-58 and coinfection with HPV-56 and HPV-58 only increased the risk of LSIL (Table 3).

4. Discussion

The prevalence of HPV infection in women with normal cytology results was high in this study. Higher than in another study conducted in Recife, also in Northeast Brazil [18], it was similar to that estimated for Africa and higher than that estimated for South America in 2 large studies of meta-analyses [19,20]. This high prevalence of HPV infection in women with normal cytology results could be due to the high proportion of women whose sexual activity began before they were 18 years old, when they were biologically the most vulnerable to this virus. It could also be attributed to false-negative results, which commonly occur in cytologic studies [2,21]. Besides, most infections were caused by high-risk HPV types, mainly by HPV-16, which has a greater capacity than the other types to maintain infection without cytologic alterations.

The overall HPV prevalence among the participants with normal cytology results was 24.5%. It was highest in the 25 to 34 years age group and less in the 35 to 44 years age group, a finding similar to those reported for other World Regions [20]. Among women with LSILs, the

Table 3
Distribution of HPV types according to health status and associated odds ratio obtained by univariate logistic regression model

Variable	Normal cytology group(n = 110)	LSIL group(n = 82)			HSIL group(n = 56)		
	No. of patients	No. of patients	OR	95% CI	No. of patients	OR	95% CI
Only type							
HPV 16	14	15	2.62	[1.14–6.00][a]	29	13.68	[5.77–32.42][a]
HPV 18	1	2	4.88	[0.43–55.65]	2	6.38	[0.38–108.49]
HPV 58	3	7	5.70	[1.39–23.33][a]	2	4.26	[0.65–27.96]
HPV X	2	1	1.22	[0.11–13.91]	1	3.19	[0.7–37.76]
Other	1	8	19.53	[2.35–162.18][a]	5	31.92	[3.45–295.46][a]
Double Infection							
HPV 16+18	1	0	NA	NA	0	NA	NA
HPV 16+58	1	1	2.44	[0.15–40.16]	1	6.38	[0.38–108.49]
HPV 55+58	1	1	2.44	[0.15–40.16]	0	NA	NA
HPV 56+58	2	8	9.76	[1.97–48.37][a]	0	NA	NA
Other	1	5	12.21	[1.38–108.39][a]	5	31.92	[3.45–29.546][a]
HPV status							
Positive	27	48	4.34	[2.34–8.05][a]	45	10.64	[5.00–22.63][a]
Negative	83	34	1	[Reference]	13	1	[Reference]

Abbreviations and note: See Table 2.

prevalence was higher in this study than that reported for Porto Alegre, in Southern Brazil [22]; it was similar to that estimated for Africa [23]; and it was less than that reported for Recife [18], Brasilia [24], or estimated for Central and South America [23]. It was highest among women younger than 34 years, less in the 35 to 44 years age group, and higher again among women 45 years or older. Most of the women with HSILs were between 25 and 54 years old, and there was no linear relation between age group and HPV prevalence. The overall HPV prevalence in this study for women with HSILs was higher than that reported for Porto Alegre [22], but less than that reported for Recife or Brasilia [18,24]. These differences can be explained not only by variations usually observed between populations of different geographic regions, but also by differences in age and lesion grades among study populations and the sensitivity of the HPV DNA assays used in different studies [2,25].

An association between ethnicity and cervical lesion was observed only for HSILs, suggesting a trend for lesions to progress faster in nonwhite women. Most of the women in the 3 groups were married or had only 1 sexual partner. No associations were observed between HPV prevalence or development of cervical lesions and marital status. Compared with the participants with normal cytology results, HPV prevalence was significantly higher in participants with either lesion grade. The overall prevalence of HPV-16 in this study was similar to that estimated for Africa, but higher than that reported for Recife [18] or estimated for South America [19,20]. We found an association between HPV infection and the presence of LSILs among women infected with HPV-16 and HPV-58 as single infections as well as among those infected with both HPV-56 and HPV-58. We also found an association between HPV-16 infection and presence of HSILs.

We found both LSILs and HSILs to be associated with age and multiple sexual partners. On the other hand, we found only HSIL to be associated with early age at first sexual intercourse or with smoking, even when all variables were considered simultaneously in a logistic regression model. And we found no association between the presence of premalignant lesions of either grade and oral contraceptive use or family history of cancer. These results are concordant with those reported in studies conducted in São Paulo, Porto Alegre, and Buenos Aires [12,22].

In the 3 study groups, the most of the women testing positive for HPV were infected with 1 or 2 high-risk types. Among women with normal cytology results, the HPV-16 prevalence was very similar to that estimated for South America [19], but higher than that estimated for Africa and twice than that reported for Recife [18]. These results show that HPV-16 is highly prevalent in the female population of Natal, Brazil. And since other high-risk HPV types also infect the same population, these women in our region are at high risk for HPV infection and, consequently, for cervical cancer. The distribution of the HPV types in this study was similar to that described for women in Africa and in Central and South America.

The results of this study lead us to conclude that high-grade lesions are associated with age and ethnicity. Multiple lifetime sexual partners, single infection with HPV-16 or HPV-58, and double infection with HPV-56 and HPV-58 increased the risk of having LSILs. Early age at first sexual intercourse and multiple sexual partners, smoking, and infection with HPV-16 all increased the risk of having HSILs. The most prevalent virus type was HPV-16 in the 3 groups, followed by HPV-58 in the normal cytology and LSIL groups, and HPV-45 in the HSIL group.

Despite similarities in the order of prevalence of some HPV types found for Natal in this study and other regions of Brazil in different studies, there were important differences in the prevalence of HPV types 31, 33, 45, 56, 57, and 59. By virtue of the existence of multiple HPV types and variations in their distributions, even within the same geographic region, more local studies should be conducted to evaluate the cost-benefit and expected efficacy of a HPV vaccination campaign, if found justified.

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