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Human papillomaviruses in Amerindian women from Brazilian Amazonia

E. B. BRITO[1], S. J. MARTINS[2]* AND R. C. MENEZES[2]

[1] *Centre for Tropical Medicine, Federal University of Pará, Belém, Brazil*
[2] *Health Subprogram, Programa Parakanã, Brasília, Brazil*

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SUMMARY

We evaluated the prevalence of human papillomavirus (HPV) infection in Amerindian women from a tribe in Brazilian Amazonia. Demographic data, pap smears and cervical samples for HPV DNA detection by polymerase chain reaction (PCR) were obtained for women aged above 10 years old. In total, 79 (85·9%) out of 92 eligible women who lived there were interviewed; all women already had engaged in sexual activity. Seventy-eight and 49 women allowed collection of pap smears and PCR samples, respectively. Cytological signs of HPV infection were observed in 11 patients; 6 of these were probed for HPV infection and 1 shown to be HPV 16. Overall prevalence of HPV infection detected by PCR was 14·3%. Three patients presented high-risk HPV DNA types: two HPV 16 and one co-infection of HPV 16 and 58. Cervical infection by oncogenic HPV types occurs in Amerindian women and cervical cancer screening should be a priority in this setting.

INTRODUCTION

Papovaviruses have long been considered as examples of tumour-inducing agents and human papillomaviruses (HPV) are related to benign self-limiting cutaneous and mucosal proliferation in their natural hosts. Current biological and epidemiological data strongly relate certain HPV types to cervical oncogenesis [1], but their oncogenic potential and prevalence differ either geographically or according to the ethnic background [2].

During the past two decades, there has been an increase in the number of patients seen with HPV infections. This may represent a heightened awareness of several clinical and laboratory signs of HPV infection, but the raised incidence of condyloma acuminatum in developing countries, the second most frequent sexually transmitted disease (STD) in North Brazil women [3], as well alarming incidence rates of cervical cancer in those regions highlight the importance of this issue. The incidence rate of cervical cancer in Brazil is high. It varies from 23·7:100000 in Porto Alegre (south region) to 83·2:100000 in Recife (northern region) [4]. Comparable data about cancer incidence in Brazilian Amerindians are not currently available.

We have studied STD and cervical cancer in the Parakanã tribe, a remote people from Brazilian Amazonia. In a previous evaluation, we observed 16 (23%) out of 69 women with suggestive cytological findings of HPV-infection in cervical smears [5]. Now, we report the prevalence of selected HPV DNA types in those Amerindian women.

METHODS

Study group

Parakanã Amerindians are an ancient human tribe from the Southeastern Brazilian Amazonia. This people established contact with our society in 1980,

* Author for correspondence: Av Paulista 648, ap 2007, 01310-100, São Paulo, Brazil.

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and they continue to exist as an isolated tribe. A health care programme oriented by an epidemiologist (R.C.M.) has been attending Parakanã Amerindians since 1989, focusing on prevention, early diagnosis, and treatment of the common diseases and endemics. A Papanicolaou (Pap) cervical cytology screening procedure was implemented in 1991, when STD had been recognized as an emerging problem in this tribe. All women aged above 10 years were deemed eligible for this study. An educational team permanently working there informed the Amerindians about the relevance of HPV detection and invited women to participate in this study. Local Amerindian leaders, the Institutional Ethical Committee, and the National Indian Foundation cleared the study protocol.

Physical examination and cytology

Informed consent was obtained along with demographic and behavioural information by personal interview before examination, with assistance of local health professionals and interpreters. Endo- and ectocervical samples were obtained by one cytopathologist (E.B.B.) with a cervical brush and wooden spatula. The pap smears were stained with modified Papanicolaou stain and reviewed by one cytologist who had no knowledge of the clinical or laboratory findings. The cytological diagnosis of koilocytosis was based on the presence of characteristic 'halo' cells with cytoplasmic perinuclear clearing. The presence of dyskeratosis, binucleation, parakeratosis and other benign atypias that were not consistent with dysplasia was observed. According to the Bethesda cytology rating system, cytological changes were categorized into benign, including inflammation, and abnormal, including low- (LGSIL) and high (HGSIL) grade squamous intraepithelial lesions. These results were promptly conveyed to local health professionals; participating institutions provided further investigation (colposcopy and biopsy) and specific treatment on an as-needed basis.

Polymerase chain reaction (PCR)

Samples for HPV DNA detection obtained by cervical brushing were placed into specimen transport media, stored at 4 °C for 5 days at most, and brought to the HPV laboratory of the São Paulo Branch of the Ludwig Institute for Cancer Research, where they were kept frozen at –20 °C until testing. A PCR-based protocol was applied for HPV detection, amplifying the 450 bp segment of the L1 gene flanked by the MY09/MY11 consensus primer pair (Cetus/Perkin Elmer, Emmeryville, CA, USA), as described elsewhere [6]. As previously described [7], PCR product was hybridized with specific individual oligonucleotide probes for all 27 genital HPV types whose nucleotide sequences for probes within the MY09/11 fragment were known; PCR amplicons that hybridized with the generic probe but with none of the type-specific probes were considered positive for HPV of unknown type.

Data analysis

Statistical comparisons within the study group were limited to the age distribution because of design and sample size constraints. The Kruskal–Wallis test was chosen and a 95% significance level was stated for inference. Data management and calculations were performed with the SPSS software package, version 8.0.

RESULTS

Ninety-two women eligible to this study lived in the community at time of this study and 79 (85·9%) were asked to participate. The median age of enrolled women was 25 years, with a range of 10–73 years. These values contrasted with those observed for the 13 women who did not take part in the study: median age of 14 years, with range of 10–28 years ($P = 0{\cdot}0011$). Reasons found for non-inclusion in the study were refusal to participate (2) and unknown location (11).

Although interpreters were available during the interview, systematic collection of specimens or even individual data on risk factors for HPV infection were not obtained due to cultural differences. Genital inspection could not be performed in all cases; we did not observe genital warts. Table 1 shows some characteristics of the study group. Of note, all participating women already had engaged in sexual activity.

Permission for cervical sample collection was obtained from 78/79 women for Pap smear and 49/79 for HPV DNA detection (Table 2). No age bias was noted either in cytology or PCR results but old women often rejected specimen collection for HPV DNA detection. Fifty-five women had Pap smears with non-specific inflammatory changes. Koilocytosis

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Table 1. *Some characteristics of the study population*

Characteristics	No. (%)
Age (years)	
10–14	11 (13·9)
15–19	13 (16·5)
20–44	38 (48·1)
45+	17 (21·5)
Offspring	
0	7 (8·9)
1–3	25 (31·6)
4–6	14 (17·7)
7+	14 (17·7)
Unknown	19 (24·1)
Sexually active	79 (100)

Table 2. *Age (median and range) and test results*

Test	No. (%)	Age in years (range)
Papanicolaou smear		
Abnormal	11 (15·3)	19·0 (16·0–42·0)*
Benign	61 (84·7)	22·0 (10·0–70·7)
Inadequate sample	6	47·1 (37·0–73·1)
Refusal	1	69·4
Human papillomavirus detection		
Present	6 (14·3)	22·1 (12·8–45·0)†
Absent	36 (85·7)	19·0 12·5–60·7)
Inadequate sample	7	19·0 (12·7–22·0)
Refusal	30	39·5 (10·0–73·1)‡

* Variation of age distribution (normal *vs.* abnormal; Kruskal–Wallis test): $P = 0{\cdot}91$.
† Variation of age distribution (present *vs.* absent; Kruskal–Wallis test): $P = 0{\cdot}89$.
‡ Variation of age distribution (compliance *vs.* refusal; Kruskal–Wallis test): $P = 0{\cdot}00011$.

and other cytological signs of HPV infections were observed in 11 (15·3%) of 72 patients with adequate Pap smears and in these cases cytology was compatible with benign atypia in 8, LGSIL in 2, and HGSIL in 1. Six out of these 11 patients were probed for HPV infection and 1 was found to have HPV 16. Thus, in 42 women tested for HPV DNA, cytology had low sensitivity (16·7%) and intermediate specificity (83·3%) for HPV diagnosis. No case of cervical malignancy was found. Women with cytology findings compatible with LGSIL or HGSIL were referred for colposcopic-oriented biopsy, which did not reveal further abnormalities.

The overall prevalence of HPV infection as detected by PCR product hybridization was 14·3%. All but one HPV DNA positive women exhibited an inflammatory cytologic pattern (Table 3). We identified high-risk HPV DNA types in 3 women: 2 HPV DNA 16 and 1 co-infection of HPV DNA 16 and 58 (Table 4).

Table 3. *Pap smears and PCR status of 42 Amerindian women*

Cytologic findings	HPV DNA+ ($n = 6$)	HPV DNA− ($n = 36$)
Normal	—	2
Benign atypia (inflammatory)	5	27
Low-grade squamous intraepithelial lesion	1	5
Inadequate sample	—	2

DISCUSSION

Clinical HPV infection can be diagnosed by medical examination alone. Subclinical disease can be detected using colposcopy, cytology or histology. However, these morphological methods exhibit low specificity and poor inter- and intra-observer reproductibility [8]. Detection of HPV DNA or RNA, by PCR DNA amplification technology seems at present to be the most sensitive way of diagnosing subclinical disease [1, 9, 10].

No association between cytology and PCR results was noted and we could not detect HPV DNA in 4 out of 5 women with abnormal cytology (LGSIL). The low number of patients weakens the significance of these findings but further explanation can be drawn. HPV genital tract infection is usually a multifocal disease [9]; asymptomatic HPV infections are relatively common [11, 12]. A two-step amplification of HPV DNA, using the MY11/09 primer pair in combination with the GP5/6 primers, would increase the sensitivity of HPV detection [13]; nondifferencial misclassification of HPV status can occur when insensitive methods are applied [14].

Unfortunately, our communication skills were insufficient to override cultural differences and the compliance of Amerindians with specimen collection was lower for PCR analysis (62·0%) than for Pap test (98·7%), principally among old women. The prevalence of HPV DNA reported here (14·3%) may be age-biased and would probably have been lower if all women had taken part in the PCR study. Many HPV

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Table 4. *Selected characteristics of HPV-DNA positive Amerindian women*

Patient	Age (years)	Sexual activity	Actual pregnancy	Number of children	Cytology	HPV-DNA type
1	45	Yes	No	8	Inflammatory	Untypeable
2	42	Yes	No	10	Inflammatory	16/58
3	28	Yes	No	6	Inflammatory	Untypeable
4	16	Yes	No	2	LGSIL*	16
5	13	Yes	No	—	Inflammatory	53
6	13	Yes	Yes	—	Inflammatory	16

* Low-grade squamous intraepithelial lesion.

infections are transitory and the prevalence rate of infection generally is higher for young women [15, 16]. Even so, our observation is in line with the overall prevalence figures reported for three Amazonian Indian tribes (14·4%) [17], and for low-income urban residents in Brazil (13·8%) [16]. The absence of exophytic condyloma noticed in the study group may be matched with the failure to detect HPV-6 or HPV-11 infection, but this inference should be viewed cautiously because full genital examination could not be done in all women. Since oncogenic HPV-positive women are at high-risk for development of pre-malignant lesions [18] and cervical cancer [19], continued follow-up of these women is advisable.

Human papillomavirus infection appears to have a cosmopolitan distribution since there is growing evidence of co-evolution of HPV and human ancestries [17, 20–22], and epidemiological studies on HPV infection in Amerindian people, perhaps the utmost isolated human groups, offer a singular opportunity to resolve this issue. Furthermore, the actual clinical importance of the detection of oncogenic HPV in Amerindians should not be dismissed. Our study supports earlier finding that non-specific inflammatory atypia is common in cervical smears from Parakanã women [5], possibly due to their sexual conduct. They also share several behavioural attributes regarded as risk factors for cervical cancer [2] and HPV infection [1, 19]: matrimony occurs at childhood and husbands are middle-aged adults; they usually have sexual initiation at puberty, soon after menarche; at 11 or 12 years old, become pregnant shortly afterwards, and have children at regular intervals of 2 or 3 years; bigamy is allowed and multiplicity of sexual partners is tolerated.

In conclusion, the results of the present study indicate that cervical infection by oncogenic HPV types occurs in Amerindian women. Considering the additional hazard rendered by their socio-cultural behaviour, cervical cancer screening should be regarded as a priority for health care providers assisting these people.

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REFERENCES

1. Villa LL. Human papillomaviruses and cervical cancer. Adv Cancer Res 1997; **97**: 321–41.
2. Muñoz N, Bosch FX, de Sanjosé S, et al. The causal link between human papillomavirus and invasive cervical cancer: a population-based case control study in Colombia and Spain. Int J Cancer 1992; **52**: 743–9.
3. Silva D, Salgado U, Macedo C, Neves C. Donovanosis in Pará, Brazil. Rev Soc Bras Med Trop 1991; **24**: 251–2.
4. Neto AA. Epidemiological aspects of cervical cancer. Rev Saúde Públ 1991; **25**: 326–33.
5. Brito EB, Menezes RC, Martins SJ, Bastos MG, Sousa A. Preliminary study on low-trait genital infection and cervical epithelial dysplasia in women from the Parakana tribe of South America. Rev Assoc Med Bras 1996; **42**: 11–5.
6. Bauer HM, Ting Y, Greer CE, et al. Genital, human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 1991; **265**: 472–7.
7. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994; **169**: 235–40.
8. Nanda K, McCrory DC, Myers ER, et al. Accuracy of the Papanicolou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 2000; **132**: 810–9.

https://doi.org/10.1017/S0950268802006908 Published online by Cambridge University Press

9. Schneider, A, Koutsky, LA. Natural history and epidemiological features of genital HPV infection. In: Muñoz N, Bosch FX, Shah KV, Meheus A, eds. Epidemiology of cervical cancer and human papillomavirus infection. IARC Scientific Publication no. 119. Lyon: International Agency for Research on Cancer, 1992: 25–52.
10. Manos MM, Kinney WK, Hurley LB, et al. Identifying women with cervical neoplasia using human papillomavirus DNA testing for equivocal Papanicolaou results. JAMA 1999; **281**: 1605–10.
11. Nieminen P, Soares VRX, Aho M, et al. Cervical human papillomavirus deoxyribonucleic acid and cytologic evaluations in gynecologic outpatients. Am J Obstet Gynecol 1991; **164**: 1265–9.
12. Jenison SA, Yu X, Valentine JM, et al. Evidence of prevalent genital-type human papillomavirus infections in adults and children. J Infect Dis 1992; **162**: 60–9.
13. Evander M, Edlund K, Bodén E, et al. Comparison of a one-step and a two-step polymerase chain reaction with degenerate primers in a population-based study of human papillomavirus infection in young Swedish women. J Clin Microbiol 1992; **30**: 987–92.
14. Ritter DB, Kadish AS, Vermund SH, Rommey SL, Villari D, Burk RD. Detection of human papillomavirus deoxyribonucleic acid in exfoliated cervicovaginal cells as a predictor of cervical neoplasia in a high-risk population. Am J Obstet Gynecol 1998; **159**: 1517–25.
15. Moscicki AB, Palefsky JM, Gonzales J, Smith G, Schoolnik GK. Colposcopic and hystologic findings and human papillomavirus (HPV) DNA test variability in young women positive for HPV DNA. J Infect Dis 1992; **166**: 951–7.
16. Franco EL, Villa LL, Sobrinho JP, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999; **180**: 1415–23.
17. Ong CK, Bernard HU, Villa LL. Identification of genomic sequences of three novel human papillomavirus sequences in cervical smears of Amazonian Indians. J Infect Dis 1994; **170**: 1086–8.
18. Rozendaal L, Walboomers JMM, van der Linden JC, et al. PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. Int J Cancer 1996; **68**: 766–9.
19. Bosch FX, Manos MM, Muñoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995; **87**: 796–802.
20. Chan SY, Ho L, Ong CK, et al. Molecular variants of human papillomavirus type 16 from four continents suggest ancient pandemic spread of the virus and its coevolution with humankind. J Virol 1992; **66**: 2057–66.
21. Ong CK, Chan SY, Campo SM, et al. Evolution of human papillomavirus type 18: an ancient phylogenetic root in Africa and intratype diversity reflect coevolution with human ethnic groups. J Virol 1993; **67**: 6424–31.
22. Stewart AC, Eriksson AM, Manos MM, et al. Intratype variation in 12 human papillomavirus types: a worldwide perspective. J Virol 1996; **70**: 3127–36.

https://doi.org/10.1017/S0950268802006908 Published online by Cambridge University Press