Amerindian women of the Brazilian Amazon and STD

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Summary

Papanicolaou tests, PCR for HPV, C. *trachomatis,* HSV-1/2 and *N. gonorrhoea,* and Hybrid Capture II were performed for high- and low-risk HPV groups during screening for cervical cancer in 49 women of the Parakana tribe. Cytological diagnoses of HPV were suggested in three samples: PCR showed 12 (22.4%) cases of DNA positive HPV, 16 (1), 18 (2), 58 (3), 39 (1), 61 (1), 33 (1), 35 (1), unknown (2), and HCII analyzed 48 samples: 19 positive (39.58%) for the high-risk group and four (18.33%) for the low-risk group. The prevalence of HPV was 42.85% ($p = 0.001$) by molecular biology methods. The largest viral load was 1588.11 pg/ml for HPV 39 in a 16-year-old. PCR was positive for *C. trachomatis* and negative for HSV-1/2 and *N. gonorrhoea*. Parakana women present a high risk for the development of cervical cancer.

Key words: STD; High-risk HPV; Amerindian of the Amazonian; Prevention of cervical cancer.

Introduction

Amerindian Parakanã are called awaete - truth people. Today they inhabit an area of 351,697.41 Km^2, between the middle courses of the Tocantins and Xingu rivers in the municipal districts of Novo Repartimento and Itupiranga, State of Pará in the Brazilian Amazon. Rarely do the women and seniors speak nor do they understand the Portuguese language. The Paranatinga (PNT) and Maroxewara (MXW) villages gave origin to the Paranowaona (PNW), Itaigoa (ITG) and Inaxiganga (ING) villages in 2000 [1]. The Indigenous Health Initiative for Parakanã and the Nucleus of Tropical Medicine/UFPA began a prevention program for gynecological cancer in 1991 after the occurrence of sexually transmitted diseases (STDs), seeking early detection of alterations in uterine cervical neoplasia [2].

The present work evaluates the profile of HPV infection and other STDs in Parakanã women by molecular biology tests in the year 2000.

Methods

The project was approved by the Ethics Committee for Research at the São Paulo School of Medicine (EPM)/UNIFESP (no. 766/2000), NMT/UFPA (no. 011/2000) and CONEP (registration 1625/2000).

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A study on women of the Parakanã tribe for the prevention of uterine cervical cancer through the Indigenous Health Program in 2000 was carried out. Most of the women had gathered and already been informed regarding the collection of the specimens for the nursing team. Participants in the study included females above ten years of age having sexual activity, who followed the norms of the protocol and who spontaneously attended the health center, with the exception of pregnant women. Women who did not attend the health center and those that were menstruating were excluded.

Information was obtained by personal interview before examination with the assistance of local health professionals of the program and interpreters. Forty-nine women from the five villages were selected to be tested by PCR for HPV, *N. gonorrhoea, C. trachomatis* and HSV and the Hybrid Capture II (HC II) for high- and low-risk HPV groups together with the Papanicolaou (Pap) test. Endo and ectocervical samples were obtained by a cytologist, first for HC II, the "kit" denominated collector STM® (specimen transport medium) was used of Digene, and following for PCR in a tube containing 1 ml of DNAzol and last for the Pap test. The smears were obtained with a cervical brush and wooden spatula fastened with "spray". The samples were properly directed to specialized laboratories for processing.

The HCII test for HPV was carried out at the Digene Laboratory. For the probes for HPV A (low risk) and HPV B (high risk), 13 types of high risk for cervical cancer (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and five types of HPV of low risk (HPV 6, 11, 42, 43 and 44) were used. The final evaluation indicated the viral load for the RLU cut-off ≤ 1.0. Probes B and A were considered "positive" or "negative", respectively, for all types in the groups of high and low risk: viral load less than 1, negative; from 1 to 5, lower load; from 5 to 20, intermediate load and above 20, high load [3].

PCR tests were performed at the Laboratory of Molecular Biology EPM/UNIFESP. Oligonucleotides, MY09 and MY11, which amplify a conserved area of the genome of several types of HPV [4] and for HSV, N. gonorrhoea and C. trachomatis with specific "primers" were used.

The Pap test was done at the Laboratory of Cytopathology of

NMT-UFPA. Papanicolaou stains were read under a Nikon binocular microscope and diagnoses were in agreement with two pathologists according to the Bethesda system and norms of the Brazilian Health Department.

The statistical package MINITAB was used to evaluate molecular biology tests.

Results

The anamnesis data were somewhat prejudiced due to linguistic problems (cultural). The Parakanã female population in the year 2000 consisted of 142 women aged above ten years (Table 1). Cervical samples were obtained from 49 (34.5%) women from the five Parakanä villages.

Table 1. — *Female Parakanã population in 2000.*

Age	< 10	11-20	21-30	31-40	41-50	+50	Total
	109	55	45	13	13	16	251

Detection of HPV by PCR showed 12 (22.4%) of the 49 samples had the following types: HPV16 (1), 18 (2), 58(3), 39 (1), 61 (1), 33 (1), 35 (1), unknown type (2) and for HC II 48 samples were analyzed with 19 being positive (39.58%) in the high-risk group and four (8.33%) in the low-risk group (Table 2). The prevalence of HPV infection was 42.85%, and the analysis with the MINITAB statistical package showed a significant increase (p = 0.000), for the PCR/HCII procedures of 24.4% and 38.77%, respectively.

The largest viral load was in a 15-year-old adolescent with a value of 1588.11 pg/ml (HPV 39) and an 81-year-old senior with HPV 58; HCII had a viral load for a high degree of risk: 7.62 pg/ml and low degree 1.25 pg/ml, respectively. Nine PCR negative samples were HCII positive, two positive for the two groups. PCR for HSV and N. gonorrhoea was negative and four positive samples for C. trachomatis were associated with HPV35, HPV16, HPV58 and HPV18 (Table 2).

Table 2. — *Tests of PCR (HPV and CT) x HCII A and B (viral load) + Pap test of Parakanã women in 2000.*

n	Pregnancy	Age	Village	HCII B	HC II A	PCR HPV	PCR CT
1	No	16	ING	1588,11	1,02	39	N
2	No	21	MXW	586,81	–	58	P
3	No	14	PNT	80,97	–	35	P
4	No	79	PNW	47,02	–	58	N
5	No	15	PNT	35,17	–	16	P
6	No	55	PNT	18,86	–	N	N
7	No	23	ITG	18,45	–	33	N
8	No	27	PNT	17,32	–	N	N
9	No	82	PNT	7,62	1,25	58	N
10	No	14	PNW	6,56	–	N	N
11	No	24	PNW	6,50	–	?	P
12	No	22	PNT	6,43	–	18	N
13	No	17	PNW	5,29	–	61	N
14	No	22	PNW	4,52	–	N	N
15	No	26	PNT	2,29	–	N	N
16	No	30	ING	1,57	1,38	N	N
17	Yes	25	ING	1,38	–	N	N
18	No	29	MXW	1,29	–	N	N
19	No	18	ING	1,18	75,48	N	N
20	No	71	PNT	–	–	?	N
21	No	25	PNW	–	–	18	N

Source: Parakanã Health Program, Cytopathology Lab - NMT/UFPA, Molecular Biology Lab of the Department of Gynecology - EPM/UNIFESP and Digene Brasil, SP.
N = negative; P = positive; CT = Chlamydia trachomatis.
ING = Inaxiganga; MXW = Maroxewara; PNT = Paranatinga; ITG = Itaigoa; PNW = Paranowaona.

Evaluation of the 49 cytological exams resulted: one normal, 30 inflammatory, 17 abnormal, with four HGSIl, six LGSIL, four ASCUS and three AGUS. Only three samples had a suggestive diagnosis of HPV infection from the 12 HPV DNA positives by PCR (Table 3).

Table 3. — *Pap test + molecular biology (PCR and HCII) of 49 Parakanã women in 2000.*

Cytology	HPV DNA positive n = 21	HPV DNA negative n = 28
Normal	–	1
Inflammatory	9	21
LGSIL	4	2
HGSIL	3	1
ASCUS	4	–
AGUS	1	2
Unsatisfactory	–	1

Source: Cytopathology Lab - NMT/UFPA, Digene Lab, SP and Molecular Biology Lab of the Department of Gynecology - EPM/UNIFESP.

Discussion

Infection presence was demonstrated by HPV and C. trachomatis for the DNA tests in the female population of Parakanã. The existence of multiple infection reservoirs for *C trachomatis* and *N. gonorrhoea* has been demonstrated in Canada [5]. A high prevalence of HPV was found in women with several partners that used oral contraceptives in Arizona [6]. No differences among aboriginal women and non aboriginal women were seen in Canada [7]. In the cervical smears of Indians of the Amazonian genome structures of the new sequence tree of HPV and positive samples for HPV infection by PCR were identified [8].

We demonstrated a prevalence of HPV infection in 2000 of 42.85% which was considerably high when compared with a PCR test carried out in 1993 of 14.3% [9], a significant increase (p = 0.000) for the "middleman" types with a high risk of developing cervical carcinoma. The ages of the women in the DNA positive HPV group ranged from 16 to 32 years. The medium prevalence of high-risk HPV of 14.68% was higher in 25-29 year-old women and progressively reduced in the more advanced age group, arriving at 9% in women above 60 years [10].

The Parakanã community presents a risk of developing cervical cancer due to the presence of high-risk HPV infection in young women that have early sexual activity, precocious pregnancies and risky behavior with partner risk in the presence of abnormal cytology, ultimately HGSIL [11]. The data recounts the nature of the infection for high-risk HPV in young women and the long duration [12], conditions that favor development of CIN [13] and infection for cervical HPV to persist in assymptomatic women [14]. It is our intention to follow the Parakanã women with conventional methods and by molecular

biology according to preceding protocols [15 and 16] and immunocytochemical tests with P16 [17]. After specimens are obtained, visual inspection with acetic acid (VIA) is performed [18], in addition to the Shiller test which contributes to the diagnosis of HPV infection [19].

The presence of *C. trachomatis* infection in this community, in accordance with Koskela *et al.* [20], favors the development of cervical cancer, and in the long-term produces sequels due to pelvic inflammatory disease and induces infertility [21].

Multidisciplinary studies recount the nature of HPV infection, the viral cycle that can influence the permenance of the virus in the bearers and his/her influence on the progression of the infection [22]. It has been reported that the vaccine protects against persistent infection for HPV, preventing CIN 2-3 induced by HPV16 and promoting longer protection against cervical cancer [23].

Conclusion

Parakanã women present a risk for the development of cervical carcinoma due to the high prevalence of high-risk HPV and presence of *Chlamydia trachomatis.*

Conventional exams and molecular biology should be appraised in Parakanã women and their partners to know the real prevalence of STD and SIL in this population.

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