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Short Communication

Comparison of the prevalence and distribution of human papillomavirus infection and cervical lesions between urban and native habitants of an Amazonian region of Peru

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ABSTRACT. We made a study of the prevalence of human papillomavirus (HPV) and cervix lesions in an Amazonian Bora native population (Bn) and compared it with the prevalence in an urban population in Iquitos (Iq). We also examined the distribution of HPV types among abnormal cervical smears in the Iq population. Swabs and cytologies were collected from 472 females. DNA consensus PCR, followed by direct sequencing, were used to determinate the HPV types in the swabs. Cytologies were classified based on the lesion grade. HPV prevalence was 43.9%

in Iq and 35.4% in Bn. Cervix lesion prevalence was 20.0% in Iq and 0.3% in Bn. The frequency of high-risk HPV types among HPV+ females was 71.9% in Iq and 56.3% in Bn. The frequencies of low-risk and undetermined risk HPV types were 19.3/6.3% (Iq/Bn) and 12.3/37.5% (Iq/Bn), respectively. In lesIq (women seeking cytological services with a previous cervix lesion diagnosis) the prevalence of HPV, was 72.9%. The incidence of carcinoma and high-grade squamous intraepithelial cervix lesions in lesIq were 31.2 and 18.8%, respectively. The Bn population had a lower incidence of high-risk HPV and cervix lesions. The high-risk strain HPV16 was significantly more frequent in Iq compared with Bn. lesIq high-risk HPVs were more frequent in high-grade squamous intraepithelial lesions and carcinoma. High-risk HPV16 prevalence was significantly higher than the prevalence of the other high-risk HPVs, especially in the high-grade squamous intraepithelial lesions and carcinoma.

Key words: Human papillomavirus; Amazon population; Urban population; Cervical cancer; Peru

INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide; in developing countries it is the most common cause of death among females at the reproductive stage. Human papillomavirus (HPV) prevalence is high in South American countries (29.2%), see Molano et al. (2002), Matos et al. (2003) and Ferreccio et al. (2004) for Colombia, Argentina and Chile in specific studies with a mortality rate of 13.6 per 100,000 inhabitants. Those values are especially higher in some countries such as Peru where the prevalence is 48.2% and mortality rate is doubled [30.9 per 100,000 inhabitants (WHO/ICO, 2010)].

One of the major associated burdens of HPV is the cancer of the cervix uteri, representing the second most common cancer among women worldwide (in 2008, 529,409 new cases and 274,883 deaths were estimated). The ratio of mortality to incidence worldwide is 52% (Ferlay et al., 2010). The most common case is squamous cell carcinoma and adenocarcinoma is less common (Parkin et al., 2002; Parkin and Bray, 2006). Approximately 86% of the cases occur in developing countries, representing 13% of female cancers.

In Peru there are 9.51 million of women at risk (WHO/ICO, 2010); the number of annual cervical cancer cases was 4446 in 2010 and it is expected to grow from 2025 to 6882 (WHO/ICO, 2010). Furthermore, Peru represents a special case within the Latin-America community: nearly 45% of the population are native, mostly localized in the Andes. In the Amazonian region of Peru the population exceeds 330,000 individuals (INEI-Peru).

Other studies in the Peruvian Amazon have been carried out (Zavaleta et al., 2007; Bartlett et al., 2008) but, to the best of our knowledge, this is the first study that compares HPV prevalence and/or cervix lesion distribution between native and urban population in the Peruvian Amazon.

MATERIAL AND METHODS

Study design and specimen collection

This study was coordinated by the Department of Pathology (Medical School, University of Valencia, Spain). Two different female populations were considered: a Bora native settlement (Bn) from Loreto Department (Peru) and an urban population from Iquitos (Iq, Peru). All samples were collected between February and May 2009.

In order to obtain a representative number of samples at the Hospital III Essalud (Iquitos, Peru) we gathered cytologies and swabs of 202 women seeking cytological services for the first time and without previous diagnosis, and cytologies and swabs of 183 women seeking cytological services with a previous cervix lesion diagnosis (lesIq). A third set of samples contains the samples from the entire population of a prison for women (N = 37) as special case of urban female population (convicted, convIq). The final set of samples considered the samples from the total population of women older than 18 years old of the Bn tribe (N = 50). In the last two sets the collection was carried out in the jail and in the settlement, respectively.

In all cases, only women within risk age were considered. Written informed consent was obtained from all the participants including the health authority. For each individual "age", "pregnancy" and "anti-pregnancy measures" were obtained for the epidemiological study.

HPV genotype determination

Swab DNA isolation was performed by standard phenol-chloroform and proteinase-K protocols. The DNA of HPV was detected by nested polymerase chain reaction (PCR) using general consensus primers MY09/MY11 and GP5+/GP6+. Appropriate DNA was valued with β-globin primers as an internal control for sample amplification. The second amplification cycle was performed using the LightCycler (Roche Diagnostics GmbH, Mannheim, Germany). PCR products were purified and directly sequenced with Cy5 labeled GP6+, using an ALFexpress II automated sequencer (GE Healthcare) (Szuhai et al., 2001; Martorell et al., 2005). For typing, the single nucleotide sequences obtained were aligned with the GenBank database (at NCBI), and the multiple superimposed sequences were used with the data-base of Feoli-Fonseca et al. (1999).

Cytologic diagnosis

Cytologies with lesions were classified based on the lesion grade (LG) as: cytological high-grade squamous intraepithelial lesion (HSIL), cytological low-grade squamous intraepithelial lesion (LSIL), cytology that could not exclude HSIL, "cytological atypical squamous cells of undetermined significance" (ASCUS), and carcinoma (CARC). Spanish and Peruvian clinicians were involved in the diagnosis; the diagnostic process was as follows: first each clinician generated a separated diagnosis; next it was shown that the diagnosis had a high correlation. Finally, for cases with a different diagnosis both clinicians met to achieve a final consensus.

Statistical analysis

To examine the association between age and overall HPV prevalence, age was categorized into 2 intervals: ≤35 and >35 years. For 26 females age was not obtained and those individuals were excluded from the analysis where age was under consideration. In the cases were a confidence interval is given, it was considered to be a 95% confidence interval (95%CI). A P value of 0.05 indicated statistical significance. All statistical tests were performed in R (R Development Core Team, 2005) by well-tested packages and self-code scripts.

RESULTS

Description of the samples

A total of 489 females participated in the study. The study included representatives from: i) urban environment without previous lesion diagnosis (Iq, N = 202); ii) urban environment with previous lesion diagnosis (lesIq, N = 183); iii) Bn tribe (N = 50), and iv) convIq women (N = 37).

HPV experiments were non-conclusive in 22 cases: 6 in Iq, 13 in lesIq , 2 in convIq, and 1 in Bn; these samples were not considered in the study of prevalence.

HPV prevalence in different populations

The prevalence in Iq was 43.9% (95%CI = 36.8-51.1). convIq and Bn prevalence were 27.8% (95%CI = 14.20-45.19) and 35.4%, respectively. Table 1 summarizes these results.

Table 1. HPV prevalence in different populations.

Population	Prevalence	LB	UB	HPV+	N
Iq	43.9%	36.8%	51.1%	86	196
lesIq	66.8%	59.6%	73.5%	125	187
Bn	35.4%	-	-	17	48
convIq	27.8%	14.2%	45.2%	10	36

LB and UB = lower and upper bound respectively of the confidence interval ($\alpha = 0.05$). HPV+ = the number of HPV positive samples; N = the total number of samples. In Bns no confidence interval is provided because the full population has been studied. Iq = Iquitos; lesIq = previous cervix lesion diagnosis; Bn = Bora native; convIq = convicted.

Comparison between Iq and Bn female populations

Table 2 shows the distribution of HPV types according to the cytologic diagnosis in Iq and Bn populations. Bn had a lower incidence of cytologic lesions ($P < 0.001$). A higher prevalence of high-risk (HR) HPV in Iq ($P < 0.001$) was also observed. HPV16 is the HPV with higher prevalence in Iq; in the case of normal cytologies it is 58.5% compared to 12.2% of HPV18 and HPV31. However, this higher prevalence was not observed in the Bn population. Some HPV types have been uniquely observed in the Bn population: SIBx3a, HPV39, HPV71, and HPV96.

Table 2. Distribution of HPV types according to the cytologic diagnosis in Iquitos (Iq) and Bora native (Bn) populations.

	Iq								Bn			
	Normal	%	ASCH	%	ASCUS	%	LSIL	%	HSIL/CARC	%	Normal	%
N	156	79.6	2	1.0	17	8.7	13	6.6	8	4.1	47	97.9
HPV+	57	36.5	2	100.0	7	41.2	12	92.3	8	100.0	16	34.0
HR	41	71.9	2	100.0	6	85.7	11	91.7	7	87.5	9	56.3
HPV16	24	58.5	1	50.0	3	50.0	9	81.8	5	71.4	1	11.1
HPV18	5	12.2	0	0.0	0	0.0	1	9.1	1	14.3	2	22.2
HPV31	5	12.2	0	0.0	2	33.3	1	9.1	1	14.3	2	22.2
HPV33	1	2.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV39	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	11.1
HPV52	2	4.9	0	0.0	1	16.7	0	0.0	0	0.0	2	22.2
HPV53	2	4.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
HPV56	0	0.0	0	0.0	0	0.0	1	9.1	0	0.0	0	0.0
HPV58	3	7.3	1	50.0	0	0.0	1	9.1	0	0.0	2	22.2
HPV59	1	2.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
LR	11	19.3	0	0.0	1	14.3	2	16.7	1	12.5	1	6.3
HPV6	2	18.2	0	-	0	0.0	0	0.0	0	0.0	0	0.0
HPV11	3	27.3	0	-	0	0.0	2	100.0	0	0.0	0	0.0
HPV44	1	9.1	0	-	0	0.0	0	0.0	0	0.0	0	0.0
HPV70	5	45.5	0	-	0	0.0	0	0.0	0	0.0	0	0.0
HPV81	0	0.0	0	-	1	100.0	0	0.0	1	100.0	1	100.0
UR	7	12.3	0	0.0	1	14.3	0	0.0	0	0.0	6	37.5
HPV62	1	14.3	0	-	0	0.0	0	-	0	-	1	16.7
HPV67	2	28.6	0	-	0	0.0	0	-	0	-	0	0.0
HPV71	0	0.0	0	-	0	0.0	0	-	0	-	1	16.7
HPV83	2	28.6	0	-	1	100.0	0	-	0	-	0	0.0
HPV85	1	14.3	0	-	0	0.0	0	-	0	-	0	0.0
HPV90	1	14.3	0	-	0	0.0	0	-	0	-	0	0.0
HPV96	0	0.0	0	-	0	0.0	0	-	0	-	1	16.7
SIBx3a	0	0.0	0	-	0	0.0	0	-	0	-	3	50.0
SW1	1	14.3	0	-	0	0.0	0	-	0	-	0	0.0

HPV+ = HPV-positive samples. In Bn population only one ASCUS was observed; in this case the low-risk (LR) HPV81 was identified. % = the percentage computed within the population of relevance. For instance in normal 79.6% = the % within all the samples; 36.5% = the percentage of 57 HPV+ within 156 normal cytologies; high-risk (HR) = 71.9% = the percentage that 41 represents within 57 HPV+, and HPV16 58.5% represents the % of 24 HPV16 within 41 HR. UR = undetermined risk HPV; ASCH = atypical squamous cells - cannot exclude a high-grade lesion; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; CARC = carcinoma *in situ*.

Distribution of HPV types according to the cytologic diagnosis in lesIq

Table 3 summarizes the distribution of HR types among the different LG. HR HPV have a higher prevalence in HSIL and CARC lesions; however, they were higher in all LG. The incidence of HPV16 is especially higher in HSIL and CARC, and it is significantly higher than the rest of HR HPV ($P < 0.01$). Interestingly, the HR HPV prevalence is lower than in Iq (non-significant).

Lesion type-HPV type distribution by age intervals

Tables 4 and 5 summarize the analysis per age in all populations. In Bn no changes were observed by age; in Iq the prevalence of HSIL is higher in females older than 35, however, no statistical significance was found.

Interestingly, the population of convIq females shows incidence levels similar to those observed in the Bn population.

In the lesIq population, prevalence was similar among different age intervals, however it shows the same trend as that mentioned in Iq: the >35 group shows a higher incidence of CARC.

Table 3. Distribution of HPV types according to the cytologic diagnosis in previous cervix lesion diagnosis.

	ASCH		ASCUS		LSIL		HSIL/CARC		CARC	
	N	%	N	%	N	%	N	%	N	%
N	21	12.4	42	24.7	22	12.9	32	18.8	53	31.2
HPV+	16	76.2	10	23.8	13	59.1	32	100.0	53	100.0
HR	15	93.8	6	60.0	13	100.0	32	100.0	53	100.0
HPV16	6	40.0	5	83.3	5	38.5	16	50.0	27	50.9
HPV18	3	20.0	1	16.7	1	7.7	1	3.1	3	5.7
HPV31	4	26.7	0	0.0	3	23.1	5	15.6	7	13.2
HPV33	0	-	1	16.7	0	0.0	6	18.8	6	11.3
HPV51	1	6.7	0	0.0	0	0.0	0	0.0	0	-
HPV52	0	-	0	-	3	23.1	2	6.3	1	1.9
HPV53	0	-	0	-	2	15.4	0	0.0	0	-
HPV56	0	-	0	-	1	7.7	0	0.0	1	1.9
HPV58	3	20.0	1	16.7	2	15.4	5	15.6	7	13.2
HPV59	1	6.7	0	0.0	0	0.0	0	0.0	0	-
HPV66	0	0.0	1	16.7	0	0.0	0	0.0	1	1.9
LR	2	12.5	3	30.0	1	7.7	2	6.3	0	-
HPV6	0	0.0	1	33.3	0	0.0	0	0.0	0	-
HPV54	2	100.0	0	0.0	1	100.0	0	0.0	0	-
HPV72	0	-	0	0.0	0	0.0	1	50.0	0	-
HPV81	0	-	2	66.7	0	0.0	1	50.0	0	-
UR	0	-	1	10.0	0	0.0	0	0.0	0	-
HPV83	0	-	1	100.0	0	-	0	-	0	-

For abbreviations, see legend to Table 2.

Table 4. Lesion type *vs* age.

	Total	Normal	OR (95%CI)	ASCUS	OR (95%CI)	LSIL	OR (95%CI)	HSIL	OR (95%CI)
Iq									
≤35	58	45	77.6 (64.7-87.5)	7	12.1 (5.0-23.3)	4	6.9 (1.9-16.7)	1	1.7 (0.0-9.2)
>35	138	111	80.4 (72.8-86.7)	10	7.2 (3.5-12.9)	9	6.5 (3.0-12.0)	7	5.1 (2.1-10.2)
Bn									
≤35	30	30	100.0	-	-	-	-	-	-
>35	14	14	100.0	-	-	-	-	-	-
convIq									
≤35	15	14	93.3 (68.1-99.8)	1	6.7 (0.2-31.9)	0	-	0	-
>35	21	19	90.5 (69.6-98.8)	0	-	0	-	2	9.5 (1.2-30.4)

ASCH has been omitted because only two samples were found; one pertains to UB ≤35 and the second to UB ≥35. OR = odds ratio; 95%CI = confidence interval at 95%. For abbreviations, see legends to Tables 1 and 2.

Table 5. Lesion distribution per age in previous cervix lesion diagnosis population.

	≤35	>35
Total	52	118
ASCH	6	15
OR (95%CI)	11.5 (4.3-23.4)	12.7 (7.3-20.1)
ASCUS	15	27
OR (95%CI)	28.8 (17.1-43.1)	22.9 (15.7-31.5)
LSIL	9	13
OR (95%CI)	17.3 (8.2-30.3)	11.1 (6.0-18.1)
HSIL	10	22
OR (95%CI)	19.2 (9.6-32.5)	18.6 (12.1-26.9)
Carcinoma	12	41
OR (95%CI)	23.1 (12.5-36.8)	34.7 (26.2-44.0)

OR = odds ratio; 95%CI = confidence interval at 95%. For abbreviations, see legend to Table 2.

DISCUSSION

To the best of our knowledge, this is the first prevalence study in the Peru Amazonian-native population that compares both HPV prevalence and cervix lesion incidence with the urban population. Several conclusions can be extracted from the study.

The Bn population showed lower levels of HPV prevalence, and, specifically lower prevalence of HR HPV type. Furthermore, all cytologies within the Bn population were considered normal. This reflects the isolation of the community compared with the urban population. Interestingly the prevalence of HR HPV16 type was higher than the rest of HPV types only in the Iq population. This evidence shows the higher impact of HPV16 (prevalence 20.6% in Iq) within well-communicated populations. HPV16 has been targeted by vaccine, which was recently implemented and is considered one of the HPV types with higher risk of associated carcinoma (Martorell et al., 2010; Garcia-García et al., 2010). However, the prevalence of HPV16 is much higher that the prevalence found in developed countries.

Interestingly, the female convict population studied had a lower incidence of cervix lesions and lower HPV prevalence than the urban population; however, is it still slightly higher than the prevalence found in Bn. Further studies would be needed to validate this result in other populations.

Age showed a trend of higher risk of cervical lesions in older females; however, no statistical significance was observed.

This study provides an initial insight into the questions addressed in the introduction; however, a more detailed study with a larger number of samples would be necessary to provide stronger conclusions. A second study within the next few years will be able to provide insights into the HPV incidence (such as HPV growth rate within the population).

Nevertheless, it is necessary to maintain screening programs to ensure health control and to reduce HPV prevalence in Bn and Iq women; furthermore, it is necessary to strengthen the health programs in both populations.

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