HPV

Prevalence of high risk human papillomavirus types among Nicaraguan women with histological proved pre-neoplastic and neoplastic lesions of the cervix

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Objectives: To determine the prevalence of high risk human papillomavirus (HPV) types in Nicaraguan women with histological proved pre-neoplastic and neoplastic cervical lesions, and to assess its potential impact on preventive strategies.

Methods: 206 women with histopathological confirmed cervical lesions (CIN I or worse) were screened for HPV DNA on a liquid based cytology sample, using an HPV short fragment polymerase chain reaction based assay. HPV positive samples were genotyped with a reverse hybridisation line probe assay (Lipa). HPV negative samples were re-analysed using type specific real time polymerase chain reaction.

Results: Of all lesions CIN II or worse, 12% tested negative. Prevalence of high risk HPV increased from 48.1% in cervical intraepithelial neoplasia I (CIN I) to 94.7% in invasive squamous cervical carcinoma (SCC). The most prevalent high risk HPV types were, in order of prevalence rate, HPV 16, 58, 31 and 52. HPV 16 and/or HPV 31 were present in 63.2% of SCC cases.

Conclusion: Targeting HPV 16 and 31 with prophylactic vaccines could possibly have an important impact on the incidence of invasive cervical carcinoma in Nicaragua. Further research is needed to define the oncogenic potential of other high prevalent HPV genotypes. Meanwhile, primary prevention and cervical cancer screening programmes should be optimised.

In developing countries, cervical cancer is the main cause of cancer related mortality in women.[1] High risk human papillomavirus (HPV) genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) are accepted to be causal cervical carcinogens.[2] The distribution of high risk types may vary according to geographic and demographic factors.[3] The aim of this study was to determine the prevalence of high risk HPV types in Nicaraguan women with (pre)neoplastic cervical lesions.

MATERIAL AND METHODS

Study population and sample taking

We collected data of women, living in the district of Rivas, Nicaragua, who were referred to the clinic of ''Servicios Medicos Comunales'', San Juan del Sur because of an abnormal Papanicolaou result, over a period of 4 years (2001–5). Of all referred women, 206 actually attended the colposcopy clinic. Exclusion criteria were known HIV infection and hysterectomy. After informed consent, demographic data were recorded. Samples for HPV DNA detection were collected with a cervexbrush (Rovers, Oss, Netherlands), before biopsy taking. The brushes were put in a liquid base transport medium and stored at 5°C until further transport to Belgium, where the aliquotting procedure occurred.

Sample analysis and inclusion criteria

Abnormal biopsies were scored by two independent observers (one Nicaraguan and one Belgian pathologist). Interobserver agreement was approximately 75%. In case of disagreement, a consensus diagnosis was made by joint re-analysis of the samples by both observers. Only women with histological confirmed cervical intraepithelial neoplasia (CIN I, CIN II, CIN III) or invasive squamous cervical carcinoma (SCC) were included in this study.

Aliquots of the exfoliated cervical cell samples were analysed using a polymerase chain reaction (PCR) fragment (SPF 10) primer set for HPV DNA detection and a Inno-Lipa HPV research assay for genotyping of the HPV positive samples (Innogenetics, Ghent, Belgium).[4 5] Samples that were positive for a unknown genotype or did not reach the detection limit for genotyping were noted as ''X.''

Of the 47 HPV negative samples, 39 had enough aliquot left to be retested using real time PCR. The test was validated using fully characterised cell populations.[6] HPV testing, typing, and viral load determination were performed with an ABI Prism 7500 (Applied Biosystems). Specimens were assayed with E7 type specific primers for high risk HPV types 6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

Data analysis

The data were analysed using SPSS version 11.5 for Windows (SPSS, Inc, Chicago, IL, USA).

RESULTS

Cervical lesions

Of the 206 patients, 79 (38.3%) had a biopsy result of CIN I, 40 (19.4%) of CIN II, 68 (33.0%) of CIN III, and 19 (9.2%) of SCC.

Demographic data, sexual and screening history

The mean age of the study population was 36.7 years (range 17–69). Only 6.5% were smokers. Coitarche took place at a mean of 17.2 years. In lifetime, 56.2% of the women had had more than one partner.

Excluding the referral Pap test, of all women, 68.6% had ever had a Pap test in lifetime, and 39.6% had it done within the last 3 years. This was 52.6% and 31.6% for women with invasive cancer.

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; PCR, polymerase chain reaction; SCC, squamous cervical carcinoma

Table 1 High risk HPV type specific prevalence according to cervical lesion

HPV type	Cervical lesion on biopsy									
	CIN I (n = 79)		CIN II (n = 40)		CIN III (n = 68)		SCC (n = 19)		Total (n = 206)	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
16	6	(7.6)	5	(12.5)	24	(35.3)	10	(52.6)	45	(21.8)
18	0	(0.0)	3	(7.5)	4	(5.9)	1	(5.3)	8	(3.9)
31	6	(7.6)	6	(15.0)	7	(10.3)	2	(10.5)	21	(10.2)
33	2	(2.5)	3	(7.5)	3	(4.4)	1	(5.3)	9	(4.4)
35	2	(2.5)	2	(5.0)	1	(1.5)	0	(0.0)	5	(2.4)
39	3	(3.8)	2	(5.0)	3	(4.4)	0	(0.0)	8	(3.9)
45	2	(2.5)	1	(2.5)	3	(4.4)	1	(5.3)	7	(3.4)
51	5	(6.3)	4	(10.0)	3	(4.4)	1	(5.3)	13	(6.3)
52	8	(10.1)	5	(12.5)	7	(10.3)	1	(5.3)	21	(10.2)
53	2	(2.5)	1	(2.5)	1	(1.5)	1	(5.3)	5	(2.4)
56	3	(3.8)	0	(0.0)	2	(2.9)	2	(10.5)	7	(3.4)
58	5	(6.3)	4	(10.0)	12	(17.6)	1	(5.3)	22	(10.7)
59	2	(2.5)	1	(2.5)	2	(2.9)	0	(0.0)	5	(2.4)
66	5	(6.3)	0	(0.0)	1	(1.5)	0	(0.0)	6	(2.9)
68	3	(3.8)	2	(5.0)	3	(4.4)	1	(5.6)	9	(4.4)

Patients with invasive cancer were significantly older than the patients with preinvasive disease (mean 50.8 years versus 35.3 years, $p<0.001$) and had more children (mean parity 6.8 versus 3.9, $p<0.001$).

HPV findings

With the Inno-Lipa HPV research assay, 47 out of 206 samples resulted negative. An additional 10 positive samples were found by real time PCR.

In total, 37 samples (18.0%) were HPV negative, 10 (4.9%) had only low risk types, 142 (68.9%) were positive for high risk HPV, and 17 (8.3%) were classified as HPV ''X.'' High risk HPV was detected in 38 (48.1%) CIN I, 27 (67.5%) CIN II, 59 (86.8%) CIN III, and in 18 (94.7%) SCC. There was no correlation between the severity of the lesion and the number of HPV genotypes detected. HPV 16 was the most prevalent (21.8%), followed by type 58 (10.7%), 31 (10.2%), and 52 (10.2%). One or more of these HPV genotypes were detected in 73.5% of all CIN III lesions and in 73.7% of SCC (see table 1).

DISCUSSION

In this study, the HPV prevalence was 82.0%. Most HPV negative results were found in low grade cervical lesions (22/37 were CIN I), as in other studies.[7] One patient with SCC tested negative. This sample was not retested with real time PCR. False negative test results are previously described, with important clinical impact.[8]

The most prevalent genotypes were, respectively, HPV 16, 58, 31, and 52. The SCC:CIN III ratio were 1.49 for HPV 16, 1.02 for HPV 31, 0.51 for HPV 52, and 0.30 for HPV 58. Considering our findings, prophylactic vaccination against HPV 16 and 31 could possibly have the most important impact on the incidence of cervical cancer in this region in Nicaragua. Those findings are different from worldwide data.[3 9]

The prevalence of HPV 18 in our study population was surprisingly low. This could be because of geographical variations, but HPV 18 can also be missed by the SPF primers, being more susceptible for disruption in the L1 region than other genotypes.[10] However, no additional HPV 18 was detected using E7 type specific primers. It has also been described that cytological abnormalities detected after a rapidly progressive HPV 18 infection may easily understate the severity of the associated CIN.[11]

Further investigation is necessary to analyse the oncogenic potential of other high prevalent HPV genotypes such as HPV 52 and HPV 58, in order to establish the HPV types that future vaccines should target. Meanwhile, the public health authorities should optimise their screening programmes and promote correct and consistent condom use. This would not only have an impact on the incidence of HPV dependent neoplasia, but also on the prevalence of other sexually transmitted infections in the country.

In summary, this study confirms regional differences in occurrence of HPV types and indicates that HPV prophylactic vaccines may need to target different HPV types in various countries or regions.

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CONTRIBUTORS

PH, data analysis and elaboration of the draft manuscript; AG, colposcopy, sample collection and treatment of women; PC, study design, supervision of data collection and analysis, revision of manuscript; CG, data collection and revision of manuscript; RV, histological classification (first observer); JPB, real time PCR, revision of manuscript; LVR, HPV genotyping using Inno-Lippa technique; CC, supervision of the project, histological classification (second observer).

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The study was approved by the ethics committees of the UNAN-Managua and of the University Hospital of Ghent.

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