HPV detection and genotyping as an earlier approach in cervical cancer screening of the female genital tract

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Summary

Human papillomavirus (HPV) infection is the leading risk factor for cervical intraepithelial neoplasia (CIN) and cervical cancer. More than 100 virus genotypes have been identified so far, some of them strongly associated with the development of neoplasia. The aim of this study was to evaluate the prevalence of the different HPV genotypes in women presenting no cytological alterations in cervical cells, in women presenting light alterations, and in women presenting severe alterations at routine gynecological examination. We retrospectively analyzed 97 HPV results of women submitted to cervical cancer screening compared to their Papanicolaou and colposcopy examinations. Data were analyzed individually and within groups to correlate the HPV genotypes identified by polymerase chain reaction (PCR) and the respective alterations in cervical cells. Among the nine cases diagnosed as CIN I (9.3%), two were positive for low-risk HPV genotypes (22%), and the other seven were negative for HPV by PCR (78%). CIN II or CIN III diagnoses were associated with positive HPV results by PCR in four cases (36%), for high-risk as well as low-risk genotypes. There were two patients with severe cytological alterations in cervical cells, but with an indeterminate HPV genotype (18%), and one case with a negative HPV result (9%). Among the 57 cases without cytological alterations, seven were positive for low-risk HPV (12%) and two for high-risk HPV genotypes (3.5%). In the 48 remaining cases, we observed one with an indeterminate HPV genotype (2%), and the other 47 were negative for HPV by PCR (47%). Our study demonstrates an important prevalence of high- and low-risk HPV genotypes in our population, including those not present in the commercially available vaccine, even in patients with no evidence of cytological alterations in cervical cells. These results highlight the usefulness of HPV detection and typing as an early approach for cervical cancer screening and prevention.

Key words: HPV; Genotypes; CIN; Papanicolaou; PCR, cancer.

Introduction

The human papillomavirus (HPV) is an oncogenic microorganism which occurs naturally in humans, inducing epithelial proliferation during the course of a productive infection, and is known as being constantly associated with cervical cancer [1]. HPV invades germinative epithelial cells through micro lesions, and the resulting infection may be transient or persistent [2, 3]. Even if infection with oncogenic HPV genotypes is frequent among sexually active women, most cases are self limiting [4]. Development of cervical lesions occurs only in a small proportion of women who present persistent infection with oncogenic genotypes [5, 6]. Integration of the virus genome in malignant cells has been demonstrated in every case of cervical cancer, what is believed to be a necessary condition for the development of neoplasia [7].

HPV actually comprises a heterogeneous group of viruses with more than 100 different genotypes, from which about 40 are capable of infecting the anogenital region. Among the anogenital genotypes, the 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 73 and 82 are classified as high risk for development of neoplasia, for being clearly identified in patients with malignant lesions [5, 8].

Uterine cervical cancer is the second most frequent neoplasia in women, but in Latin America, prevalence rates may be about four times higher than those found in developed countries [9, 10]. Despite the fact that, in our country, data about the prevalence of different HPV genotypes are abundant [11, 12], in certain locations awareness about the importance of HPV detection in the prevention of cervical cancer is still scarce, such as the prevalence of HPV in women presenting light cytological alterations (CIN I, ASCUS or AGUS), and also the importance of monitoring HPV infection after therapeutic measures for severe alterations.

The simplest method for prevention of cervical cancer is the Papanicolaou smear or liquid cytology test, considered as an examination of excellence in evaluating the degree of cellular alterations in squamous cervical epithelium, that has contributed to drastically reduce the incidence of cervical cancer around the world. However, in the last decades, several studies have pointed out non-ideal rates of sensitivity of the conventional smear preparation, what may vary from 50% to 60% [13].

Today, two methods are widely used for HPV detection: Hybrid Capture (HC) and the polymerase chain reaction (PCR) with consensus primers. PCR is virtually capable of detecting every anogenital HPV genotype with greater sensitivity, also available in the form of standardized commercial kits [14]. The Hybrid Capture II test (HC II) is capable of detecting the DNA of 18 HPV genotypes among those commonly infecting the anogenital region (of males and females), and a positive result is reported as presence of high- (A) or low-risk (B) HPV [15].

Revised manuscript accepted for publication February 19, 2008

Table 1. — *Prevalence of HPV genotypes in relation to the level of cytological alterations in cervical cells.*

Genotype HPV	CIN I (n = 9) No	(%)	CIN II (n = 13) No	(%)	CIN III (n = 3) No	(%)	CIN I/NICII (n = 1) No	(%)	Class 2 (n = 1) No	(%)	[1]No Exam. (n = 13) No	(%)	[2]Negative (n = 57) No	(%)	Total (n = 97) No	(%)
6	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	1	2%	2	2%
11	0	0%	1	8%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1%
16	0	0%	2	15%	2	67%	0	0%	0	0%	0	0%	1	2%	5	5%
31	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	0	0%	1	1%
33	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	1	2%	2	2%
40	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	0	0%	1	1%
45	1	11%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1%
46	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	0	0%	1	1%
52	1	11%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1%
53	0	0%	0	0%	0	0%	0	0%	0	0%	1	8%	2	4%	3	3%
54	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	2%	1	1%
62	0	0%	1	8%	0	0%	1	100%	0	0%	0	0%	0	0%	2	2%
66	0	0%	1	8%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1%
72	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	4%	2	2%
CP4773	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	2%	1	1%
CP8304	0	0%	1	8%	0	0%	0	0%	0	0%	0	0%	0	0%	1	1%
[3]Undet.	0	0%	2	15%	0	0%	0	0%	0	0%	0	0%	1	2%	3	3%
[4]Neg.	7	78%	5	38%	1	33%	0	0%	1	100%	7	54%	47	82%	68	70%
Total	9	100%	13	100%	3	100%	1	100%	1	100%	13	100%	57	100%	97	100%

([1]No exam.: patients who did not undergo cytological examination; [2]Negative: no alterations in cytological examination; [3]Undet.: undetermined HPV genotype; [4]Neg.: negative result of PCR for HPV).

With this picture in mind, we have aimed to evaluate the prevalence of the different HPV genotypes in women undergoing cervical cancer screening in our population. We have also aimed to determine the prevalence of these genotypes in women presenting no cytological alterations in cervical cells, in those presenting light alterations, and in women already presenting severe alterations, to evaluate the feasibility of HPV detection and typing as an early approach in cervical cancer screening and prevention.

Materials and Methods

We have retrospectively evaluated the results of HPV detection and typing in the cervical samples of 97 women, aged between 15 and 60 years, attending gynecological clinics in our city from February 2005 to February 2006. The obtained results were correlated to the existent cytopathological and clinical data.

Gynecological and colposcopic examinations were performed upon routine consultation, with the techniques established in clinical practice. Papanicolaou smear examination was performed in a cytology laboratory upon medical requisition. HPV detection was performed in the same way in samples of cervical cells collected by the physician in a clinical laboratory using the consensus primers MY09 and MY11, and genotyping was performed by restriction fragment length polymorphism (RFLP) of PCR products, after digestion with the enzymes *BamH* I, *Dde* I, *Hae* III, *Hinf* I, *Pst* I, *Rsa* I, and *Sau3A* I [16]. This study had the approval of the Committee of Ethics in Research with Human Beings of our institution (Protocol n. 017/06). Statistical analysis was performed through the chi-square test with a one-sided p-value, with the aid of the software Instat ™ (San Diego, CA, USA).

Results

A total of 97 women were evaluated in this study, with a median age of 32.6 years (15 to 60). Thirteen patients (13%) did not undergo cytological examination and 57 (59%) presented a Papanicolaou smear without alterations. Among the 27 women with cytological alterations, nine presented CIN I (9%), 13 CIN II (13%) and three CIN III (3%) according to the Bethesda Classification. One patient presented an undifferentiated result between CIN I and CIN II, and another was recorded as Class II.

A total of 68 patients (70%) had a negative result for HPV by PCR in cervical samples, 16 (16.5%) presented infection by low-risk genotypes, and ten (10%) presented high-risk genotypes. Only three patients (3%) presented an indeterminate result in the RFLP pattern.

The most prevalent genotype was HPV 16 (5%), followed by 53 (3%), and the genotypes 6, 33, 62 and 72 (2% each) (Figure 1). The frequency of the different genotypes found in our population according to the respective Papanicolaou result is shown in Table 1.

HPV infection was detected in two patients among those with a CIN I cytopathological result (22%), in six with CIN II (61%), and in two of the women with CIN III alterations (67%). In our population, ten women among those with a positive HPV result by PCR (18%) presented a Papanicolaou test with no alterations.

Discussion and Conclusions

In our study the prevalence of HPV in women undergoing routine cervical cancer screening was 30%, lower than that found by other authors [5, 17]. This difference is probably due to the social and economical level of the studied population, originating almost entirely from private clinics, which in our country poses a remarkable difference, and also because of the specific geographic characteristics of our region compared to the heterogeneous population of the country.

Among the women already presenting cytological alterations, most of the cases were classified as cervical

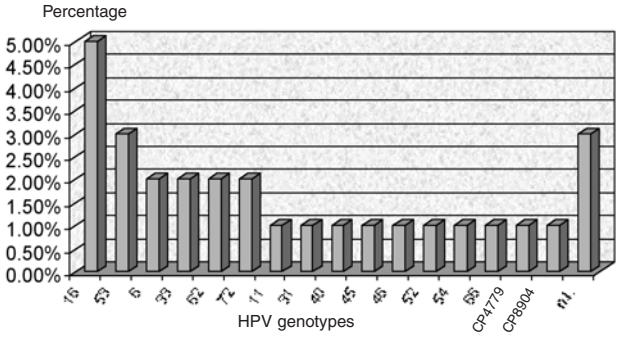

Figure 1. — Prevalence of HPV genotypes identified by PCR-RFLP, in cervical samples of women undergoing uterine cancer screening. (N.I.: indeterminate RFLP).

intraepithelial alterations of low degree (7/68 CIN I), which is in agreement with the results found in other studies [18, 19]. A remarkable finding was the occurrence of seven patients with negative a HPV result, but presenting moderate- to high-degree alterations at the cytopathological examination (5/68 NIC II, 1/68 Class II and 1/68 NIC III). After a review of these cases with the respective physicians, we could evaluate that, invariably, those were cases with cytological/colposcopic alterations treated before the specimen collection for HPV detection, with the aim of monitoring therapeutics.

Most of the cases with no alterations in the Papanicolaou test presented a negative HPV result. However, an important number of patients without cytological alterations presented HPV infection, being of low-risk (6/57, $p = 0.0084$) and high-risk genotypes (2/57, $p = 0.0804$), with one case with an indeterminate genotype. This indeterminate genotype, which was high-risk, would lead statistical analyses to a significant level of evidence ($p = 0.0436$) of infection in women with no alterations in colposcopic or cytological examinations in our population. These data highlight the importance of early HPV detection in the prevention of cervical cancer, once development of neoplasia is definitively associated with the presence of these viruses.

Another contribution of HPV detection and typing in cervical samples of women undergoing gynecological assistance is the identification of an eventual previous infection by the virus, and in monitoring the efficacy of HPV vaccination. As is known, the commercially available vaccine so far protects only against four genotypes: 6, 11, 16 and 18 [20]. Our results are in accordance with other previous studies which demonstrate an epidemiological HPV profile only reasonably defined, being observed among the high-risk genotypes, a higher prevalence of HPV 16, followed by HPV 52, 51, 31, and others, with scarce findings of HPV 18 [21]. However, some epidemiological differences are noticeable in relation to the distribution of HPV genotypes in different geographic locations around the globe [22, 23]. HPV 18 seems to be more prevalent only in certain populations [24]. This epidemiological profile typical of each population may have important implications in vaccination efficacy. A women eventually infected by an HPV genotype not included in the vaccine should be constantly monitored for the development of cervical intraepithelial neoplasia, such as in the cases of infection with the high-risk HPV genotypes 31, 33, 45 and 52 found in our study. It is also important to remember that even HPV genotypes considered of intermediary risk may be associated with high degree intraepithelial alterations [25].

In brief, these data indicate that HPV detection and typing may constitute a useful early approach in prevention of uterine cancer, once an important number of women without colposcopic or cytological alterations may be infected by HPV, such as the 15.8% found in our study, including high-risk genotypes (3.5%). These cases must be monitored much more carefully in comparison to women without colposcopic or cytological alterations and a negative HPV result.

Acknowledgements

We gratefully thank the physicians who collaborated with this study, especially Dr. S. D. Marchi for his kind help. W. M. Krambeck was supported by the program Pibic/CNPq.

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