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Relationship between smoking, HPV infection, and risk of cervical cancer

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Summary

Purpose of investigation: To document the relationship between smoking and HPV infection, and the risk of developing preinvasive lesions and cervical carcinoma. *Materials and Methods:* Prospective, cross-sectional descriptive study. A total of 1,007 patients were recruited among women seen at the cervical pathology clinic of Sant Joan de Déu University Hospital in Barcelona (Spain) between January 2003 and March 2011. Patients were asked specifically about their smoking habits. Statistical analyses were done with SPSS v.19 software. Differences between groups were considered statistically significant at $p < 0.05$. *Results:* In patients studied, 48.7% were smokers. The average number of cigarettes per day among smoking patients was 7.07 (1-40). In the of patients with HPV infection, 53% were smokers versus 37% of patients without HPV infection ($p < 0.05$). The average number of cigarettes per day among patients with HPV infection was 7.64 cigarettes/day versus 5.55 cigarettes/day among patients without HPV infection ($p < 0.05$). In the patients with high-risk HPV genotypes infection, 54.5% were smokers versus 43.2% of patients without high-risk HPV infection ($p < 0.05$). Risk of HPV infection increases 1.905 times among smoking patients versus no smoking patients (OR = 1.905, CI 95% (1.426 - 2.545), $p < 0.05$). Among patients with changes associated to HPV and atypical cells, there were 29.2% and 14.4% of smokers, respectively, versus 45.5%, 55.6%, and 48.6% of smokers among patients with grade 1 cervical intraepithelial neoplasia (CIN 1), CIN 2-3, and carcinoma, respectively ($p < 0.05$). Risk of CIN 2-3 or cervical carcinoma cervical increases 1.642 times among smoking patients versus no smoking ones (OR = 1.642, CI 95% (1.325 - 1.884), $p < 0.05$). *Conclusions:* Smoking interferes in the increase of HPV infection prevalence and in an increased risk of CIN and cervical carcinoma. Risk also increases with more cigars smoked per day.

Key words: Human papillomavirus infection; Cervical cancer; Cervical intraepithelial neoplasia Grade 1, 2, 3; Smoking.

Introduction

The infection by HPV is considered a necessary cause but not enough to develop cervical cancer. Most women infected by HPV will never present cervical cancer. Therefore, other cofactors are needed for the progression from cervical infection by HPV to cervical cancer. Cofactors of progression that contribute to the persistence of the infection are classified in: viral, genetics, and environmental.

Smoking is one of the environmental cofactor identified with the risk of suffer precarcinogenic lesions and cervical cancer. Among women infected by HPV, tobacco is the most important cofactor of progression, it increases the risk from two to four times compared to non-smoking women. This increase is also identified in passive smokers. Moreover, stopping smoking is associated with a decrease of the size of the lesion of cervical intraepithelial neoplasia (CIN) [1-5].

The aim of this study was to confirm the relationship between smoking habits and infection by HPV and the risk of presenting cervical intraepithelial lesions and cervical carcinoma among patients who smoke.

Materials and Methods

Study population

Prospective, cross-sectional descriptive study. A total of 1,007 patients were recruited among women who visited the cervical pathology clinic of Sant Joan de Déu University Hospital in Barcelona (Spain) between January 2003 and March 2011 [6]. Patients were asked specifically about their smoking habits. These patients were referred to the present hospital for presenting alterations in routine cytology.

All patients with some kind of anomaly in cervical cytology, who had visited the present unit of cervical pathology, were included. Patients who did not receive the correct follow-up according to their pathology or those who abandoned monitoring at the present centre, were excluded.

From each patient, information regarding the cytological and histological diagnosis and about the infection by different genotypes of HPV was collected. Moreover, demographic information about the patient was also collected, among which the authors included if they were smoking at the time of diagnosis or not and the intensity of smoking habits measured by number of cigarettes consumed per day. In this work, neither the smoking habit history prior to diagnosis, nor the duration of smoking were taken into account.

Data collection

In all patients, a cytological study was performed classifying the abnormalities according to Bethesda classification: glandular atypia of uncertain significance (AGUS), squamous atypia of un-

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certain significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). A colposcopy was also performed applying acetic acid solution 3-5%. The classification used was proposed by the International Federation of Cervical Pathology and Colposcopy in Barcelona 2002: normal findings (acetowhite epithelium, punctation, mosaicism, negative to iodine stain, atypical vessels), findings suggesting invasive cancer and non-satisfactory colposcopy.

Biopsy was performed in atypical zones observed in colposcopy. The sample was fixed with formaldehyde and analysed by the pathologist and classified as: negative, low-grade lesion, high-grade lesion, changes suggestive of infection by HPV, carcinoma, adenocarcinoma, vaginal intraepithelial neoplasia, and atypia.

Genotype of human papilloma virus by PCR

The cervical sample was obtained with a brush and was transported at room temperature to the Microbiology and Molecular Department. During the time of this study two techniques for genotype HPV were used consecutively (Linea Probe assay – LIPA – and microarray assay). In Linea Probe Assay (LIPA assay), for the extraction of cervical DNA the commercial kit Qiagen (QOAmp DNA Mini Kit) was used and samples were diluted in a final volume of 200 µl. For Microchip arrays Assay, the extraction of DNA was made using a lysis solution of proteinase (20 mgr/ml). Purified DNA extracts were kept at -80ºC in both cases.

Linea Probe assay (LiPA assay) is based on the principle of reverse hybridization and gives us specific information for 25 different genotypes of HPV simultaneously (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74). Amplification of HPV-DNA is based on "SPF10_PCR primer set", that amplify a piece of only 65 pairs of bases with the region "L1 open reading frame" (ORF). Part of the gene of human beta-globin (268pb) is amplified in each sample as a control. ESPF10-LIPA was used with ten µl of extracted DNA in a final volume of 100 µl.

Microchips arrays assay detects infections and co-infections up to 35 of most relevant genotypes of HPV (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 85, and 89). It is based on microarray of low density joined to the inferior part of a classic Eppendorf two-ml tube. DNA amplification is based on a mix reaction that amplifies an extract of 450 pairs of bases with "L1 open reading frame" (ORF) region. A piece of 892 pairs of bases of human gene CFTR was amplified in each sample as a control.

The authors considered HVP genotypes of high risk to be the genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 70 and 85 and of probable high risk 53 and 66, according to recent literature.

Statistical analysis

The statistical analysis of all data was processed by SPSS (v.19). Analysis with *t* Student was used in quantitative variables that follow a normality tendency and U Mann-Whitney if normality was not proven. Chi-squared test was used for qualitative variables. To analyse more than two samples, Anova test was used. The results were considered statistically significant with a p value < 0.05.

Results

In all patients the diagnosis was confirmed histologically. In almost half of patients (47.3%, 476) a CIN 2-3 was diagnosed, in 38.7% (389) CIN 1, in 6% (61) atypical cells, in 3.7% (37) a carcinoma, and in 3.6% (36) HPV-associated changes. Most patients (77.9%, 686) were submitted to surgical treatment, of these, 23% were treated with large loop excision of transformation zone (LLETZ) and up to 54.9% with conisation (cone biopsy). In this latter group, 9.3% eventually underwent hysterectomy. A total of 740 women (73.2%) had HPV infection, among whom 86.4% (639) had a high-risk HVP genotype.

Table 1. — *Smoking patients depending on HPV infection*

		HPV infection		
		Yes	No	
Number of patients		716	264	980
Cigars/day		7.64 cigars	5.55 cigars	$p<0.05$
Smoking patients	Yes	379 (53%)	98 (37%)	$p<0.05$
	No	337 (47%)	166 (63%)	

Table 2. — *Smoking patients depending on HR-HPV infection*

		HR-HPV infection		
		Yes	No	
Number of patients		619	97	716
Cigars/day		7.81 cigars	6.52 cigars	p>0.05
Smoking patients	Yes	337 (54.5%)	42 (43.2%)	p<0.05
	No	282 (45.5%)	55 (56.8%)	

Table 3. — *Smoking patients depending on cervical lesion*

Diagnosis	Cigars/Day	Smoking patients		Total	
		Yes	No		
CIN 1	6.33	146 (45.5%)	175 (54.5%)	321	
CIN 2-3	8.32	247 (55.6%)	197 (44.4%)	444	
Carcinoma	7.43	17 (48.6%)	18 (51.4%)	35	
HPV-associated changes	4.38	7 (29.2%)	17 (70.8%)	24	$p<0.05$
Atypical cells	3.10	6 (14.4%)	23 (79.3%)	29	
Total		423	430	853	

Of all patients 51.3% did not smoke and the remaining 48.7% smoked. The average number of cigarettes per day smoked by patients of the sample was 7.07 (1-40).

Of the patients with HPV infection, 53% were smokers compared to 37% of patients with no infection by HPV ($p < 0.05$). The average number of cigarettes per day among patients infected by HPV was 7.64 cigarettes/day, in contrast with an average of 5,55 cigarettes/day in patients with no HPV infection ($p < 0.01$) (Table 1). The risk of infection by HPV increased by 1.905 times among smoking women compared to non-smoking (OR = 1.905, IC 95% (1.426-2.545, $p < 0.05$).

Of the patients with infection by high-risk HPV genotypes (HPV-HR), 54.5% were smokers compared to 43.2% of patients with no infection by HPV-HR ($p < 0.05$). The risk of infection by HPV-HR increases by 1.886 times among smoking women in contrast with non-smoking (OR = 1.886, IC 95% (1.448 - 2.457), $p < 0.05$) (Table 2).

In those patients diagnosed with changes associated to HPV and atypia, the authors found 29.2% and 14.4% of

smokers, respectively ($p < 0.05$). Patients with changes associated to HPV and atypical cells smoked an average of 4.38 and 3.10 cigarettes per day, respectively. On the other hand, in patients diagnosed of CIN 1, CIN 2-3, and carcinoma, the average of cigarettes per day were 6.33, 8.32, and 7.43, respectively ($p < 0.05$) (Table 3). The risk of CIN 2-3 or cervical carcinoma increased 1.642 times among smoking women compared to non-smoking (OR = 1.642, IC 95% (1.325 - 1.884), $p = 0.05$)(Table 3).

Discussion

Different references in the literature show that multiple factors can take part in cervical carcinogenesis related with smoking, especially with a direct local effect as carcinogenic, because it increases the oxidative stress, and local immunosuppression caused by smoke, because it reduces the number of Langerhans cells, as well as the affectation of systemic immunity [7]. The fact that nicotine and specific tobacco carcinogens have been detected in cervical mucus of smokers supports the hypothesis that there is a synergic action between tobacco and HPV in the development of high-grade cervical intraepithelial lesions and cervical carcinoma. Tobacco increases cell proliferation and turnover in transformation zone. For example, tobacco has been related with ki67, a proliferation marker and metaplasia.

An in vitro study showed that the exposition of cervical cells to benzopyrene, the main carcinogen of tobacco, increased the number of HPV copies. This fact makes an increase of HPV infection persistence needed for the cervical lesion progression [2, 3].

Moreover, in some studies, it has been demonstrated that the infection by HPV in smoking women lasts longer and they have less probability of clearance of the infection [8], and a significant relationship between the reduction of lesion size and giving up smoking in patients with low-grade lesions has been proved.

In the present study, as in most information reviewed in the literature, it is proved that tobacco increases the prevalence of HPV infection [9-17]. The present authors also obtained a significant relationship between the risk of infection by high-risk HPV genotypes and being a smoker. OR for tobacco in women with positive HPV goes from 1,5 to 5. In the study by Yetimalar *et al*. [16], that included patients without cervical pathology, 63% of smokers had positive HPV compared to 40% of smoking patients with no infection by HPV. In the present results, although the authors only referred to patients with cervical pathology, they also observed similar results with 53% of smokers among those with HPV infection, compared to 37% of smokers among those with no infection by HPV. Giuliano *et al.* [8] proved that smoking increases the persistence of HPV infection and decreased the probability of clearance of infection. The average duration of the infection by HPV in smokers was 10.7 months compared to 8.5 months in patients who have never smoked.

In many case-control studies, as observed in the present study where patients with serious lesions smoked more cigarettes, it is demonstrated that tobacco increases the risk of developing high-grade intraepithelial lesions like cervical carcinoma. Moreover, most studies on the risks of tobacco, according to its intensity or its duration, show an increase of cervical carcinoma with a rise in tobacco exposition [18,19].

Kjellberg *et al.* [2] in his study reports an increased risk of three times of CIN 2-3 in smokers compared to patients that have never smoked. The present authors also found an increasing risk of CIN 2-3 and cervical carcinoma in smokers with OR 1.64. The longer the smoking history, and the higher the number of cigarettes smoked per day, increases the OR for CIN 2-3. Matsumo *et al.* [20], monitored 516 patients with CIN 1 with cytology and colposcopy every four months. The probability of regression in two years was significantly lower in smokers than in those that have never smoked (55% *vs.* 68.8%, $p = 0.004$), and risk of persistence increased with the higher intensity and duration of smoking ($p = 0.003$ and $p < 0.001$, respectively). Moreover, smokers presented an increase of persistence of infection (OR = 2.50, IC 95% (1.30 - 4.81), $p = 0.006$). Hildesheim *et al.* [3] also reported an increased risk of CIN 2-3 and cervical carcinoma in smokers compared to those that have never smoked (OR= 2.3, IC 95% (1.3 - 4.3), $p < 0.05$) that also increases the longer the smoking history and the higher the number of cigarettes smoked per day.

Smoking also affects the progression of cervical lesion. In Szarewski *et al.* [5] study, a significant relationship between giving up smoking and a reduction in size of cervical lesion was proved. Of those patients that stop smoking, 82% showed a reduction of 20% in lesion size compared to 28% of those that continued smoking. In the present study, the authors observed an increase of the incidence of cervical carcinoma in smokers but they have not followed the progression of cervical lesions through time.

Conclusion

Smoking interferes in the increase of HPV infection prevalence and in an increased risk of CIN and cervical carcinoma. Risk also increases with more cigars per day smoked. With the present results and data published in the literature, smoking women with cytological alterations should be monitored with more precaution than the general population.

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