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Risk Factors for Persistent Human Papillomavirus (HPV) Infection after Conization: A Retrospective Case-Control Study Showed No Significant Association with HPV Vaccination

Leia Peralta, Miguel García Blanco, Eduardo González-Bosquet*

Department of Obstetrics and Gynecology, Sant Joan de Deu Hospital, University of Barcelona, Pg. Sant Joan de Déu, 2 , 080950 Esplugues (Barcelona), Spain

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***Corresponding Author:**
Eduardo González-Bosquet
Sant Joan de Deu Hospital,
University of Barcelona
Passeig Sant Joan de Déu, 2
Esplugues 08950 (Barcelona)
Spain
Email: edugonzalez@hsjdbcn.org
Tel/Fax: +34 932852939/ +34 93 2033959

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ABSTRACT

Introduction: Despite conization, high-grade squamous intraepithelial lesion can recur. Persistent human papilloma virus infection is an important factor of recurrence. We analyse different situations that could favor the persistence of this infection. **Methods:** A retrospective case-control study of 256 patients who underwent conization between 2015 and 2020 was conducted. Depending on positive or negative result of HPV test after the first 6 months post conization, two groups were defined and compared: 1) control group HPV-negative patients, 2) case group HPV-positive patients. Age, parity, smoking habit, menopause, body mass index (BMI), vaccination, HPV genotypes and exo and endocervical margin status were analysed. **Results:** 63 of the 256 patients studied, (24.6%) persisted positive for HPV after conization, while 193 (75.4%) became negative for HPV. Patients over 35 years old had a significantly higher risk of persistence of HPV after conization (OR 1.9). Being menopausal was also significantly associated with the persistence of HPV (OR 2.5). The presence of affected resection margins in cone specimen proved to be a risk factor for the persistence of HPV (OR 2.3). The coexistence of multiple HPV genotypes before conization seemed to be a protective factor for HPV persistence (OR 0.3). The rest of clinical characteristics studied failed to demonstrate statistical significance; therefore, it was not possible to classify them as risk and/or protective factors. **Conclusions:** Age over 35, menopause and affected resection margins of conization were significantly associated ($p< 0.05$) with the persistence of HPV infection; however, HPV vaccination was not among the risk factors.

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INTRODUCTION

The prevalence of human papillomavirus (HPV) infection for sexually active women is greater than 70% [1,2]. The Immune system spontaneously clears approximately 85-90% of all infections, however, 10-15% of infections persist [1,3,4]. The persistence of HPV infection can cause a squamous intraepithelial lesion that can eventually progress to invasive carcinoma if left untreated [5,6]. Conization is intented both for diagnosis and treatment for high-grade squamous intraepithelial lesion (HSIL) while it preserves functional integrity of the uterine cervix [1,7]. Despite conization, HSIL can recur in some patients even when the lesion is completely removed. Patients with persistent HPV infection have a higher risk of recurrence, which can ultimatey progress to cervical cancer [1,7].

Thus, determining risk factors for HPV persistence after conization may help to identify women with a higher risk of recurrence. Several studies have suggested different risk factors that predict the persistence of HPV after conization; however, their results are variable [3,5]. The aim of this study was to analyse the factors that are potentially associated with the persistence of HPV in patients treated with conization.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Institutional Review Board of Sant Joan de Deu Hospital, University of Barcelona. All recruited patients gave written consent to participate in the study.

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Study Design and Population

A retrospective case-control study was conducted analysing the medical history of all patients who underwent conization between 2015 and 2020 in our centre (Sant Joan de Déu Hospital, Barcelona, Spain) and who presented the following inclusion criteria:

a) Patients with a diagnosis of HSIL before treatment (based on the 2001 Bethesda system) [8].

b) Patients who had an HPV test before and after treatment.

c) Patients who had completed at least one-year follow-up.

A total of 256 patients satisfied the inclusion criteria. Depending on positive or negative results of their HPV test in the first 6 months after the intervention, patients were divided into two groups: 1) control group with 193 HPV-negative patients, 2) case group with 63 HPV-positive patients. Age, parity, smoking habit, menopause, body mass index (BMI), vaccination status, exo and endocervical margin status and HPV genotypes before and after conization, high and low risk genotypes, and subclassifying those with HPV 16, 18 or others HR-HPV, were analysed.

Based on International Agency for Research on Cancer (IARC) Monograph 100B, A Review of Human Carcinogens, published in 2012 [9], where previous classification of 2007 is revised, HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are considered to be high risk. For the study of exo and endocervical margin status, patients were classified as affected if they presented affected conization edges, affected endocervical curettage, or both.

Surgical Technique and Histological Examination

All patients underwent conservative treatment with loop electrosurgical excision procedure (LEEP), delineating the abnormal epithelium with Lugol's iodine solution. Conization was carried out with the objective of removing the lesion and transformation zone entirely, as one single piece. After resection, an endocervical curettage was performed and the base of the wound was cauterized with a ball electrode. Specimens were then submitted for histopathologic examination and definitive diagnosis and resection margins' affectation was determined.

Follow-Up

All patients were followed-up in our centre for at least one year and HPV test was performed in subsequent visits.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Subscription compilation 1.0.0.1447 (SPSS Inc., Chicago, IL, USA). Categorical variables were indicated as numbers and percentage, while continuous variables as mean with their standard deviation (SD). Student's t test and Chi-square test were used for continuous and categorical variables respectively. A logistic regression model was used to estimate odds ratio (OR) and 95% confidence interval (CI) to characterize the associations between HPV infection and clinical characteristics. All P values in both tests were considered statistically significant when $P < 0.05$.

RESULTS

Table 1 summarizes patient clinical characteristics and the subseqüent analysis of the possible risk factors for HPV persistence after treatment with conization.

Data collection for some analysed factors was incomplete, notably the BMI of 48 patients (18.75% of the total study population). Furthermore, the histopathological study of resection margins of 10.1% of cone pieces was not assessable. Out of 256 patients studied, 63 (24.6%) persisted positive for HPV after conization, while 193 (75.4%) became negative for HPV.

The mean age of the patients was 38.3 ± 9.1 years (range: 20 – 82), while the mean BMI was 24.4 ± 4.4 (range: 16 – 39). A large proportion of the patients had had children at the age of diagnosis (162, 63.3%) and most of them were premenopausal (230, 89.8%). A vast majority had been vaccinated against HPV (199, 77.7%).

Regarding the smoking habit, there was a similar proportion of smokers and non-smokers (115, 44.9% and 116, 45.3% respectively) with a small percentage of ex-smokers (9%). Affected resection margins, including cone specimen, endocervical curettage or both, were detected in 150 patients (58.6%). Regarding HPV genotypes, slightly more than half of the patients (146, 57%) had a single genotype before surgery, while the percentage of multiple HPV was lower (77, 30.1%). After conization, this difference was increased between multiple (16, 25.4%) and single HPV genotype (47, 74.6%). In most cases, high-risk HPV genotype was detected before conization (220, 85.9%), and if we look at those at high risk, the majority (138, 62.7%) had HPV-16, while, after surgery, others HR-HPVs were found to be more frequent than HPV-16/18 (66.7% vs 30.1/3.2%).

Subsequently, an analysis of the possible risk factors for HPV persistence after treatment with conization in both groups was performed: HPV-positive patients (n = 63) and HPV-negative patients (n = 193) as shown in Table 1. The mean age was slightly older, but not significantly different, in the group of patients with persistence of HPV (40.5 ± 10.5 years vs 37.6 ± 8.6 years, P = 0.054). However, the number of menopausal patients was significantly higher in the HPV-positive group (P = 0.048). It should also be highlighted that affected resection margins in cone specimen, endocervical curettage or both were significantly related to the persistence of HPV (P = 0.049). The presence of multiple or single HPV genotypes was found to be significative too (P = 0.002), but this did not occur when dividing into high-risk and low-risk genotypes. We also found no significance when analysing the high-risk subgroup. No significant association was demonstrated with parity, vaccination status or smoking habit.

A univariate logistic regression was performed to determine odds ratio (OR) of each risk factor individually. The results are presented in Table 2.

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Table 1. Clinical characteristics and Analysis results of risk factors associated with persistence of HPV after conization.

	Total patients, n=256, (%)	**HPV-positive subgroup, n=63, (%)**	**HPV-negative subgroup, n=193, (%)**	***P* value**
Age, mean ± SE (years, range)	38.3 ± 9.1 (20 – 82)	40.5 ± 10.5	37.6 ± 8.6	0.054
BMI, mean ± SE (range)	24.4 ± 4.4 (16 – 39)	23.7 ± 4.0	24.6 ± 4.5	0.205
Parity				
Yes	162 (63.3)	40 (63.5)	122 (64.6)	0.895
No	90 (35.1)	23 (36.5)	67 (35.4)	
Unknown	4 (1.6)			
Menopause				
Yes	26 (10.2)	11 (17.5)	15 (7.8)	0.048
No	230 (89.8)	52 (82.5)	178 (92.2)	
Vaccination				
No	54 (21.1)	8 (12.7)	46 (24.2)	0.098
Yes	199 (77.7)	55 (87.3)	144 (75.8)	
Unknown	3 (1.2)			
Smoking habit				
Smoker	115 (44.9)	32 (50.8)	83 (43.5)	0.378
Non smoker	116 (45.3)	27 (42.9)	89 (46.6)	
Ex-smoker	23 (9.0)	4 (6.3)	19 (9.9)	
Unknown	2 (0.8)			
Affected resection margins				
Yes	150 (58.6)	43 (78.2)	107 (61.1)	0.049
No	80 (31.3)	12 (21.8)	68 (38.9)	
Unknown	26 (10.1)			
Multiple or single HPV before conization				
Multiple	77 (30.1)	31 (49.2)	46 (23.8)	0.002
Single	146 (57.0)	27 (42.9)	119 (61.7)	
Negative	33 (12.9)	5 (7.9)	28 (14.5)	
Multiple or single HPV after conization				
Multiple		16 (25.4)	-	
Single		47 (74.6)		
HPV genotype before conization				
High-risk	220 (85.9)	56 (88.9)	164 (85.0)	0.176
HPV 16	138 (62.7)	34 (60.7)	104 (63.4)	0.366
HPV 18	17 (7.7)	2 (3.6)	15 (9.2)	
Other HR HPVs	65 (29.6)	20 (35.7)	45 (27.4)	
Low-risk	3 (1.2)	2 (3.2)	1 (0.5)	
Negative	33 (12.9)	5 (7.9)	28 (14.5)	
HPV genotype after conization				
High-risk		63 (100)		
HPV 16		19 (30.1)		
HPV 18		2 (3.2)		
Other HR HPVs		42 (66.7)		
Low-risk		0 (0)		

Patients over 35 years old had a significantly higher risk of persistence of HPV after conization (OR 1.9; 95% CI 1.02 - 3.49; P = 0.042). Moreover, the fact of having menopause was also significantly associated with the persistence of HPV (OR 2.5; 95% CI 1.09 - 5.80; P = 0.027). The presence of affected resection margins in cone specimen proved to be a risk factor for the persistence of HPV (OR 2.3; 95% CI 1.12 - 4.63; P = 0.021). A curious finding was that having multiple HPV genotypes before conization was found to be a protective factor for HPV persistence (OR 0.3; 95% CI 0.18 – 0.63; P ≤ 0.001). The rest of studied clinical characteristics failed to demonstrate statistical significance, therefore unsuitable to be considered as risk and/or protective factors. Although not being vaccinated was close to being a risk factor for recurrence (OR 2.2, 95% CI 0.98 - 4.95, P = 0.053).

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Table 2. Analysis results of univariate logistic regression.

Category	Odds ratio (95% IC)	*P* value
Age (years)		
≥35	1.9 (1.02 – 3.49)	0.042
<35	1	
BMI		
Obese	0.5 (0.13 – 1.64)	0.223
Overweight	1.2 (0.57 – 2.38)	0.679
Normal weight	1.2 (0.59 – 2.23)	0.681
Underweight	1	
Parity		
Yes	1.0 (0.53 – 1.73)	0.879
No	1	
Menopause		
Yes	2.5 (1.09 – 5.80)	0.027
No	1	
Vaccination		
No	2.2 (0.98 – 4.95)	0.053
Yes	1	
Smoking habit		
Smoker	0.7 (0.42 – 1.32)	0.310
Non smoker	1.2 (0.66 – 2.07)	0.605
Ex-smoker	1	
Affected resection margins		
Yes	2.3 (1.12 – 4.63)	0.021
No	1	
Multiple or single HPV before conization		
Multiple	0.3 (0.18 – 0.63)	<0.001
Single	1	
HPV genotype before conization		
High risk	1.4 (1.26 – 1.47)	0.570
HPV 16	1.1 (0.60 – 2.09)	0.718
HPV 18	0.4 (0.08 – 1.66)	0.177
Other HR HPVs	1	
Low risk	1	

DISCUSSION

Out of 256 patients included in our study (n=), 63 presented persistence of infection 6 months after the treatment (24.6%). Previous studies have shown HPV persistence rates from 14.2% to 33.0% (1,3,5,7,10–13). Comparing clinical features of our population with previous studies, it was shown that mean age of our patients was 38.3 ± 9.1, similar to So et al. (38.1 ± 11.5) (1) and slightly higher than Costa et al (35.8) (3). In our study, postmenopausal status was found in 10.2% of women, a low percentage compared to other studies (15%-25.2%) (1,14,15). The percentage of smoking patients was higher in our study (44.9%) compared with previous publications (30.5% - 40.5%) (3,6,15). Finally, only 85.9% of our patients presented HR-HPV before conization, lower than the 92.5% (1) or 91% (16) described by other authors.

In our study, the univariate logistic regression of all the analysed factors showed that age of 35 or more, menopause, and affection of the resection margins were associated with a significantly higher risk of persistent HPV infection, while the presence of multiple HPV genotypes pre-surgery was found to be a protective factor.

Sarian et al. (17) already described that women older than 35 years had a significantly higher risk of persistent HPV infection after conization. Similar results were obtained by other authors (1,3,15), finding, all of them, a relationship between the persistence of infection and the age of the patients. In contrast, Nobbenhuis et al. (18), Song et al. (5) and Kim et al. (10) describe that the clearance of HPV is not influenced by age. Likewise, our results also support those obtained by So et al. (1) and Kilic et al. (15) regarding the significant relationship between menopause and persistence of infection.

The negative effect of the affectation of the conization resection margins on the persistence of HPV is observed in all the revised studies (1,3,7,17,19,20). It is worth highlighting the results obtained by Costa et al. (3) where a statistically significant relationship is described between the persistence of HPV and the affectation of the endocervical margins specifically.

In this study, parity was not shown to affect the persistence of HPV infection, consistent with the results by other previous studies (1,5,10,15). The influence of smoking in the persistence of HPV infection was observed by Sarian et al. (17); however, not confirmed by Kilic et al. (15) and our study. In addition, no association was found between HPV genotype before conization (high-risk, low-risk or negative) and the persistence of the infection. These observations support those obtained by Kilic et al. (15), So et al. (1) or Kudoh et al. (21), other authors no confirm this results (14,16,19,22). Thus, Moore et al. (22) describe a higher clearance rate for HPV 16 and HPV 18. Ouh et al. (14) report that HPV 53 was the type most likely to

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persist, while Lindroth et al. (16) state a greater persistence of low-risk HPV genotypes compared to high-risk HPV genotypes. Our finding regarding the possible protective effect of presenting multiple HPV genotypes may be due to the fact that multiple infection is more common in younger women.
Finally, HPV persistence was not influenced by BMI or vaccination. BMI has not been analysed in the different studies carried out previously. However, our results of vaccination were similar to Bogani et al., patients having vaccination experience a slightly lower risk of recurrence than women who had not, although not as statistically significant (23).

This study has several limitations. Data collection was incomplete, especially regarding some factors. BMI was only obtained from 81.25% of patients, highlighting potential loss of information. Likewise, 10.1% of the patients had non-assessable resection margins and no differentiation was made between involvement of the exo and endocervical margin. Furthermore, the two study groups were carried out taking into account the HPV determination made 6 months after conization. Alonso and cols (24) have observed a clearance of HPV infection of 70% at 6 months in patients with complete excision of intraepithelial lesion. In view of the results obtained by other groups, HPV negativity increases with longer follow-up time (3,19), it would have been interesting to study the risk factors for HPV persistence beyond 6 months. In conclusion, age of 35 years or older, menopause and affected resection margins of conization were associated with a significantly higher risk of persistent HPV infection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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