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Anal study in immunocompetent women with human papillomavirus related lower genital tract pathology

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ABSTRACT

Objetive: To estimate the prevalence of anal dysplasia in immunocompetent women with cervical intraepithelial dysplasia.
Study design: We did a prospective cohort study, in which we enrolled 166 women with gynecological pathology related to human papilloma virus (HPV) infection. All patients underwent an anal cytology and HPV detection. Statistical analysis with a 95% confidence interval was used for prevalence calculations. A X2 test and Fisher's exact one were used to determine differences between groups of qualitative variables. Differences between normally distributed and non-normally distributed groups in quantitative variables were accounted for using Student's *t*-test or Mann-Whitney's *U* test, respectively.
Results: Out of the 166 patients studied, high risk HPV in the anal canal was detected in 107 (64.46%) cases. The most prevalent genotype observed was non 16/18 high risk HPV, present in 54 (50.47%) patients. There was no a significant association with smoking, use of condom, anal intercourse, or anal benign pathology. However, a significant correlation between the presence of high risk HPV in the anal canal and the antecedent of condylomas was observed (p = 0.047) (CI95%: 1.00%–12.58%). Women with cervical intraepithelial neoplasia (CIN) grade 1 had a significantly increased presence of high risk HPV in the anal canal (p = 0.044). Out of the 166 women, 6 (3.61%) had abnormal anal cytology results, and were referred to high-resolution anoscopy. Anal biopsy was performed in these six cases. In 2 patients the biopsy reported low-grade Anal Intraepithelial Neoplasia: 1.20% (0.15%–4.28%).
Conclusions: Women with cervical intraepithelial dysplasia have 1.20% prevalence of anal intraepithelial neoplasia, so that it does not seem necessary to screen this population.

Introduction

The Human Papillomavirus infection plays an important role in the etiopathogenesis of anal cancer. Most of these, 90%–96%, are HPV dependent [1–3]. HPV 16 is the most prevalent, present in 65.6% of the cases according to Darragh [4] and 81% according to Ouhoummane [3].

Anal cancer is relative rare in general population [5], but the incidence and mortality have experimented a worldwide progressive increment since 1975 [6] rising up to 1–2 per 100.000 [7]. In women, the prevalence is 2.06 per 100.000 [8]. According to the American Society of Cancer in 2014, some 7210 new cases of anal cancer will occur in USA, including 4550 (63%) women [9]. The risk factors for development of anal cancer include [5,10]: HIV disease, men who have sex with men, receptive anal intercourse [11,12], number of sexual partners, history of genital warts, smoking, prolonged immunosuppressive therapy [13,14], anal fissures and fistulas [15] and in women, the antecedent of lower genital tract squamous intraepithelial neoplasia [16–20].

Regarding immunocompetent women with cervical dysplasia, there are very few publications available: Park [21], Santoso [22], Jacyntho [23], Lamme [6], and Scholefield [24], which published prevalence rates of 9%, 12.2%, 17.4%, 17.6% and 19% respectively.

Patients with high-grade cervical dysplasia and cervical carcinoma are more likely to develop anal cancer and anal intraepithelial neoplasia (AIN) [19,20,25]. For some authors, CIN 3 involves an incidence of anal cancer 4 or 5 times higher than in the general population [26,27].

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There are no universally standardized guidelines for screening program for precancerous anal lesions [28]. Palefsky's group [29] was the first to introduce anal cytology in this screening. They proposed an algorithm of action based on screening for cervical cancer [27,29,30]. Currently, the anal Pap test is the primary screening for anal cancer.

High Resolution Anoscopy (HRA) is the gold standard for diagnosis of high-grade AIN after abnormal cytology [31,32].

The majority of studies have focused on homosexual men and in HIV disease [23] and not in immunocompetent women with cervical dysplasia.

The aim of the present study is to determine the prevalence of anal dysplasia in immunocompetent women being studied and treated for cervical dysplasia in our department.

Materials and methods

Study design

This was a prospective cohort study. Participants were recruited from July 2013 to March 2015, out of women who were treated at the Hospital's Gynecological Lower Genital Tract and Colposcopy Unit. A total of 166 immunocompetent women with gynecological pathology related to HPV infection were enrolled in this anal lesions study. Women were eligible for inclusion if they had either persistent viral infection, or ASCUS (atypical squamous cells of undetermined significance) cytology, or ASC-H (atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion) or LSIL (low-grade intraepithelial lesion) or HSIL (high-grade intraepithelial lesion), or histologically confirmed CIN grade 1, 2 or 3.

This study excluded pregnant women, as well as HIV-positive, non-HIV immunocompromised, and those previously treated for CIN.

At the first visit, all patients signed an informed consent form and were interviewed in person about their medical history and their sexual habits. Participants underwent colposcopy examination and screening of anal dysplasia, which included HPV typing and anal liquid based cytology. The sample taken for anal cytology and HPV tests was performed with cytobrush and preserved in liquid medium, using the ThinPrep preparation (Hologic, Inc., Marlborough, MA). The anal cytology results were classified according to the Bethesda System 2001 as normal, ASCUS, LSIL, ASC—H, HSIL or cervical carcinoma.

Sample collected material was tested for HPV with HC2 HPV DNA assay (Digene®) and Cobas® HPV test. Patients with anal positive cytology (ASCUS or more) were sent to the specific Colorectal Surgery Unit, for performing HRA.

Sample size

The sample size was calculated to achieve the main objective of the study, knowledge of the prevalence of anal dysplasia in women with HPV infection related lesions of the lower genital tract. According to literature, the prevalence range oscillates between 9% and 19%, so we assumed the prevalence to be around 10%. This prevalence was estimated with a 95% confidence interval, with a $P < 0.05$ significance level and a probability of obtaining favorable results of 80%. Using Ene 2.0 (GlaxoSmithKline, Barcelona, Spain) the required sample size to reach significance was at least 150.

Data analysis

Statistical analysis with a 95% confidence interval was used for prevalence calculations. A X2 test and Fisher's exact one were used to determine differences between groups of qualitative variables. Differences between normally distributed and non-normally distributed groups in quantitative variables were accounted for using Student's *t*-test or Mann-Whitney's *U* test, respectively.

A p-value < 0.05 was considered statistically significant with the use of a two-sided test. The statistical analysis was carried out using SPSS 20.0 (Statistical Package for the Social Sciences, SPSS Inc, Chicago, IL, USA).

The study protocol was approved by the Research Ethics Committee of Hospital General Universitario of Alicante.

Results

The final study cohort included a total of 166 immunocompetent women with an average age at recruitment of 38 years (range 22–71). All of them underwent an anal citology and an HPV test. Table 1 shows the demographic characteristics of the study population.

Among the 166 women studied, HR HPV in the anal canal was detected in 107 (64.46%) cases. Of these, the most prevalent genotype observed was non 16/18 HR HPV, present in 54 (50.47%) cases, followed by 16/18 HR HPV in 51(30.72%) cases, unspecified HR HPV in 36 (21.69%) cases and not determined in 16 (9.64%) cases.

Table 2 shows the risk factors for anal HPV infection.

Anal cytologic abnormalities were detected only in 6/166 samples (3.61%). Four patients had ASCUS and there were 2 diagnoses of LSIL. In five of the six cases, the HR HPV was detected in the anal canal (in the other one we identified HPV genotype 6) (Table 3). The age of the 6 patients was between 24 and 49 years. None of them smoked or had a history of genital herpes. One had an antecedent of anal fissure, and two women had a history of perianal warts. The age of first intercourse was between 17 and 22 years. Five patients practiced unprotected vaginal coitus. Four of them were non-oral contraceptive users. Only one had no regular sexual partner, lasting their relationships between 1 and 17 years. Regarding the practice of anal intercourse, three claimed not to have, and the other three had only sporadic anal intercourse (between 1 and 2 times a year).

Table 1
Demographic characteristics of the study population.

Characteristics	N [Range]	% (95% CI)
Mean Age [Range]	38 [22–71]	
Age at first intercourse	18 [13–26]	
Current Smokers	70	42.17% (34.56–50.07)
Condom users		
Never	80	48.19% (40.38–56.07)
User	57	34.34% (27.15–42.09)
Ocasionally	29	17.47% (12.02–24.12)
Oral Contraceptives	24	14.46% (9.49–20.74)
Number of sexual partners		
1	8	4.82% (2.10–9.27)
2–5	117	70.48% (62.92–100)
>5–10	29	17.47% (12.02–24.12)
>10–20	12	7.23% (3.79–12.29)
Regular sexual partner	96	57.83% (49.93–65.44)
Receptive anal Intercourse. Yearly frecuency		
Never	115	69.28% (61.66–76.19)
1–10	34	20.48% (14.62–27.43)
>10–365	17	10.24% (6.08–15.89)
Without Anal Benign Pathology	105	63.25% (54.43–70.59)
History of Herpes genitalis	9	5.42% (2.51–10.04)

Table 2
Variables associated with anal canal high risk HPV.

Variable	No HPV	HPV	P value	RR	95% CI	N
Tobacco						166
Positive	28	42	0.65	1.39	0.74–2.65	
Negative	31	65				
Condom						166
Positive	25	55	0.41			
Ocasionally	13	16				
Negative	21	36				
Anal coitus						166
Positive	13	38	0.08	1.94	0.93–4.05	
Negative	46	69				
Anal condyloma history						166
Positive	3	17	**0.047**	4.94	1.0–12.6	
Negative	56	90				
Benign anal pathology						166
Negative	33	72	0.18	0.61	0.31–1.17	
Positive	26	35				
Cervical Intraepithelial Neoplasia (Biopsy)						117
Positive	33	68	0.406			
Negative	7	9				
CIN I						117
CIN I	9	32	**0.044**	2.37	0.96–5.84	
CIN 2/3	31	45				

P-values close to, but not fully significant, in bold face.

Table 3
Anal citology and high resolution anoscopy biopsy according to anal HPV

	Abnormal Smear	Normal Smear	N 166	P value	RR	95% CI	Biopsy AIN 1
Non High Risk HPV	1 (LSIL)	58	59	0.42	0.35	0.04–3.08	1
High Risk HPV	5 (1 LSIL-4 ASCUS)	102	107				1

All 6 women with abnormal anal cytology were referred to HRA. Anal biopsy was performed in these six cases. In 4 patients the histological result was normal and there were two patients whose biopsy reported LSIL: 1.20% (0.15–4.28) (Fig. 1).

Comment

Anal cancer is rare, but it is increasing worldwide, probably because there are no standardized guidelines for screening and

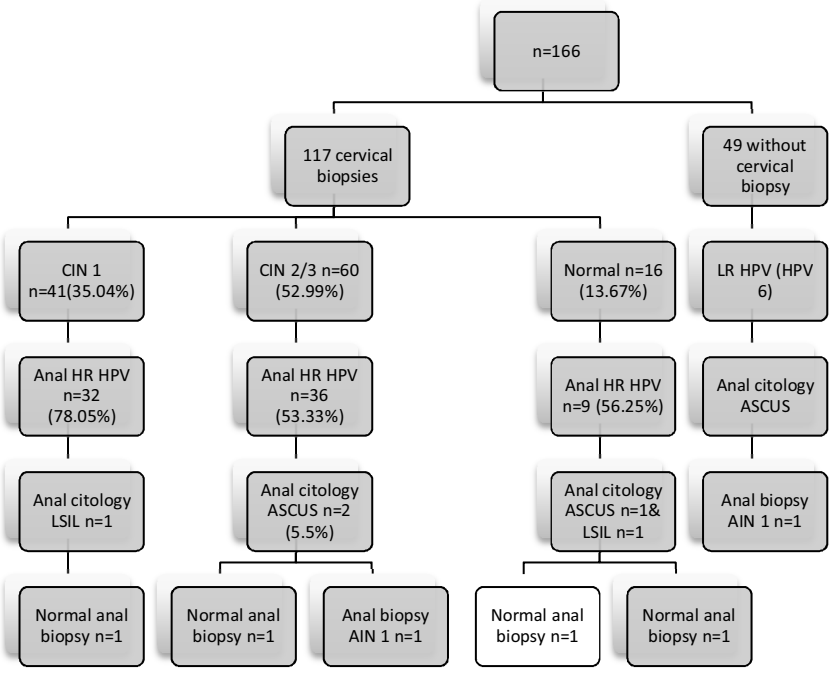

Fig. 1. Summary of the distribution of the results of the study.

treatment for anal disease, especially in HIV negative women [33]. Women with HPV related lesions of the lower genital tract are a group of high risk for anal cancer [15,18,19,22,34] and the prevalence of anal intraepithelial neoplasia in this population is not well known.

In the present study, the prevalence of anal HR HPV infection was 64.46%. This finding suggests that anal HPV infection is very common among women with HPV related lower genital tract pathology. The presence of the virus in the anal canal was much higher than previously published. Valari [35], in a population homologous to ours, reported prevalence rates of 30%; Lammé 32.5% [36]; Palefsky 42% [37]; Sehnal 48,3% [25]; Stier 23 to 36% [38].

We detected 107 cases of HR HPV in the anal canal. We also found a high degree of agreement when comparing the HPV type detected in the cervix and the HPV type detected in the anal canal. Out of 51 cases of cervical non 16/18 HR HPV, we found the same genotype in the anal canal in 24 cases, which accounts for 47.06% (CI 95% 32.93%–61.54%) of all cases. Out of 63 women with cervical 16/18 HPV, we found the same genotype in the anal canal in 24 cases (38.10%) (CI 95% 26.15%–51.20%).

It is peculiar that when non 16/18 HR HPV was present in the cervix (51 cases), we found 10 cases of 16/18 HR HPV in the anal canal. In a similar manner, when taking into account our 63 cases of cervical 16/18 HR HPV, we found 13 not expected anal non 16/18 HR HPV. Our findings suggest that the lower genital tract would serve as a reservoir for anal infection, and the HPV would be transmitted through vaginal secretions and digital autoinoculation [27,83].

Goodman [39] suggested that both the cervix and the anus can act as a reservoir for HPV infection.

We did not find any association between smoking status or anal sexual habits and HPV infection in the anus. Our results are similar to other studies published [21,35]. Neither behavioural risk factors like the number of sexual partners, or the use of condom, nor the antecedent of history of benign anal disease (hemorrhoids or fissure) and nor genital herpes was associated with significant presence of anal HPV.

In the current study, we found the frequency of HPV infection in the anal canal to be significantly increased among women who had a history of genital warts. Our findings are consistent with those published by Daling et al. [40], and it is probably due to the increased exposure to the virus.

In our study there was a higher prevalence rates of anal HPV infection in the group with cervix intraepithelial neoplasia (67.33%), in agreement with other previously published studies [5,13,22]. Interestingly, we found a higher rate of anal infection only in women with CIN 1 (78.05% of cases), with an odds ratio of 2.37 (CI 95%: 0.96%–5.84%; p = 0.04) and not in those cases in which the diagnosis was CIN 2–3. This rise questions about the possible role of a primoinfection in the genesis of infections at both locations.

According to Valari [35] the HPV test is unsuitable for anal cancer screening due to the high rate of anal infection. We agree with his group and we don't think anal HPV screening is an option with our population.

We only screened cases with altered anal cytology. The cytology sensibility is just 50%, so it is reasonable to expect that more cases would have been detected if an HRA had been used as a primary screening tool. Anyway, even if we take this into account, still a low incidence of AIN should be expected.

In our study, the rate of abnormal anal cytology was lower than previously published: 3.61% (CI 95%: 1.34%–7.70%). Santoso et al. [22] described a prevalence of abnormal anal cytology in a homologous population to ours of 5.9%. Even in healthy women, Holly et al. [41] described rates of abnormal anal cytology of 8% and Moscicki et al. [34] of 3.9%. Probably, we would have observed a higher rate of anal lesions if we had submitted all our patients to HRA. Some authors describe an infradiagnosis of AIN when the anal cytology is the only method of screening, detecting only 35–63% of the histological alterations compared with anoscopy-guided biopsy [42–44]. In our view, submit to HRA all the patients with CIN, seems to be an unnecessary overtreatment and probably inefficient.

Pollution and poor cellularity has been mentioned as factors in the low sensibility of the cytology, but in our study only in a sample insufficient material was reported, which means 0.68% of the total (CI95%: 0.02%–3.76%). In anal citology, a 9%–17% of samples insufficient for diagnosis is considered appropriate.

In the current study we were not able to find any association between the presence of abnormal anal cytology and tobacco (none of the subjects was a smoker), or the use of condom, or anal coitus.

In our population, only 2 out of the 166 patients had AIN 1 (1.20%; CI95%: 0.15%–4.28%), a prevalence lower than others authors published previously, as Park (9%), Santoso (12%), Jacyntho (17.4%), Lamme (17.6%), or Scholefield (19%) [6,21–23,45]. The group of Scholefield [20], Jacyntho [23] and Santoso [22], submitted all their patients to HRA. Lammé [6] and Park [21] like ourselves, derived to anoscopia only those women whose cytological results had been abnormal.

We believe that the low prevalence of AIN in our cohort, even having high rate of viral infection, could mainly be explained by the less aggressive nature of the European variant of 16 HPV compared to the African-American or the Asian ones (up to 5 times less progression to CIN) [46], as well as the ephemeral resulting infection in the anal canal. Valari et al. [35] found a lower expression of E6 and E7 in the anal epithelium compared to the cervix and this data might explain the lower prevalence of high AIN, suspecting that the zone of anal transformation is not as susceptible to carcinogenesis as the cervix zone. Although we found a high rate of anal infection for HR HPV, the most common HPV type detected was "high-risk no 16/18" (50.47%), and not HPV-16, as described in the literature [4,47–49], and it is well known that the first group is a more benign genotype than HPV -16.

Furthermore, we believe that the low prevalence of AIN found in our patients could be due to a synergy of both factors (anal resistance and less aggressive HPV). In addition, external factors such as dietetic habits, which might exercise a protective effect. There are publications that mention the beneficial effects of a diet rich in folates and vegetables in cervical cancer, and we could extrapolate to the anus [50–53].

Another possible cause of anal dysplasia protection could be the characteristics of the anal mucosa's ecosystem, which would act as a barrier against the carcinogenic effects of HPV.

In our study, the two histologically proven anal lesions were preceded by an anal cytology result of ASCUS (Fig. 1). Moscicki et al. [54] published that ASCUS anal citology results were usually associated with intraepithelial lesions confirmed by biopsy. We think that, since we found a high prevalence of HPV in the anal canal and a very low prevalence of anal dysplasia, dysplastic transformation is greatly diminished in our cohort of women, either by factors that reduce the pathogenesis of HPV or by local protective factors that are able to slow down the viral oncogenic activity.

Currently there are no screening guidelines for anal cancer in the HIV negative female population [22]. They do exist in HIV-positive women, who should undergo an annual anal cytology. Some authors have advocated this line of action for immunocompetent women with cervical dysplasia [27].

Conclusions

Women with cervical HPV disease have high presence of HR HPV in the anal canal but, contrary to expected, a very low rate of AIN.

We believe that anal screening in women with HPV caused cervical lesion is unsuitable, due to their low prevalence of AIN.

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