Human Papillomavirus Type Distribution in Cervical Intraepithelial Neoplasia Grade 2/3 and Cervical Cancer in Portugal

A CLEOPATRE II Study

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Objective: Cervical cancer is the third most frequent cancer in women, worldwide and etiologically associated with infection by human papillomavirus (HPV). Following the results of the first epidemiologic population-based CLEOPATRE study in Portugal, it was important to understand the HPV type-specific distribution in women with cervical intraepithelial neoplasia (CIN) grades 2 and 3 and invasive cervical cancer (ICC).

Methods: This was an observational, multicenter, cross-sectional study with retrospective data collection. Between January 2008 and May 2009, paraffin-embedded samples of histologically confirmed cases of CIN2, CIN3, and ICC were collected from the 5 regional health administrations in mainland Portugal. Eligible samples were sent to 2 central laboratories for histological reassessment and HPV genotyping. Prevalence estimates were calculated together with 95% confidence intervals.

Results: A total of 582 samples, 177 cases of CIN2, 341 of CIN3, and 64 of ICC, were included. The mean age of participants was 41.8 years (range, 20–88 years). The overall HPV prevalence was 97.9% with a higher prevalence of high-risk genotypes, particularly HPV 16. Multiple infections were observed in 11.2% of the cases. Human papillomavirus prevalence was 95.5% in CIN2, 99.4% in CIN3, and 96.9% in ICC. The 8 more frequent genotypes in order of decreasing frequency were HPV 16, 31, 58, 33, 51, 52, 18, and 35 in CIN2 and HPV 16, 31, 33, 58, 52, 35, 18, and 51 in CIN3. In ICC cases, the 12 detected HPV genotypes were HPV 16, 18, 31, 33, 45, 51, 52, 53, 56, 58, 59, and 73. However, HPV 53 and 73 were always associated to other high-risk genotypes. Human papillomavirus types 31, 51, 52, 56, and 59 were detected in 1 case each.

Conclusions: Human papillomavirus prevalence and patterns of type-specific HPV positivity were comparable with other studies. Current HPV vaccines should protect against HPV genotypes responsible for 77.4% of ICC in Portugal.

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Cervical cancer is the third most frequent cancer among women, worldwide.[1] The highest incidence rate in 2008, adjusted to age, was observed in Eastern Africa (34.5 per 100,000 women), whereas the lowest was found in Western Asia (4.5 per 100,000 women).[1] In Europe, incidence rates in 2008 varied between 2.1 per 100,000 women in Malta and 23.9 per 100,000 women in Romania.[2]

In Portugal, cervical cancer is the second most frequent cancer in women aged 15 to 44 years, with an incidence rate of 12.2 per 100,000 women (949 cases) and a mortality rate of 3.6 per 100,000 women (346 deaths).[2]

Human papillomavirus (HPV) is the most commonly sexually transmitted virus, and some genotypes are etiologically associated with the development of cervical cancer.[3–5] To date, more than 120 HPV genotypes have been identified, and about 40 of them are known to infect the genital tract.[6] These HPV genotypes are classified as high-risk (HR) or low-risk (LR) based on their association with cancer development. High-risk HPV genotypes are usually associated with cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC).[7] Most HPV infections regress spontaneously without clinical consequences, regardless of the oncogenic potential of the genotype.[8] However, HR-HPV may also integrate the genome of the infected cells, which may lead to carcinogenesis.[9]

The purpose of the Cervical Lesions Observed by Papillomavirus Types—A Research in Europe (CLEOPATRE) studies is to provide a better understanding of the epidemiology of HPV burden across Europe. The first CLEOPATRE study carried out in Portugal[10] was an observational population-based study that included 2326 women, aged 18 to 64 years. The overall HPV prevalence was 19.4%. In abnormal cytologies, HPV was detected in 81.1% of low-grade squamous intraepithelial lesions and 100% in high-grade squamous intraepithelial lesions. At least 1 HR-HPV was identified in 57% of HPV-positive women, and the most prevalent HR genotypes were HPV 16 (19.7%), 31 (11.8%), 53 (11.8%), and 51 (9.8%). Human papillomavirus type 16 was the most common genotype in all abnormal cytological results, except for atypical glandular cells lesions.[10]

Following these results, and because of scarce epidemiologic data, it was necessary to understand the distribution of the different HPV genotypes in cases of CIN and ICC in mainland Portugal. The primary objective of this study was to estimate the HPV type-specific prevalence in histological samples with CIN2, CIN3, and ICC diagnoses. The present study provides useful information on the burden of HPV cervical disease and can be important to predict the potential benefits and the impact of HPV vaccination.

MATERIALS AND METHODS

Study Design

This was an observational, multicenter, cross-sectional study with retrospective data collection. Paraffin-fixed biological samples of CIN2, CIN3, and ICC (squamous cell carcinoma and adenocarcinoma) were collected from January 2008 to May 2009 across the 5 regional health administrations in mainland Portugal: North, Center, Lisbon, Alentejo, and Algarve. Each health administration had at least 1 center involved, and samples were obtained during the same recruitment period and from the same centers that were involved previously in the CLEOPATRE study.[10] Samples were collected from biopsy and/or hysterectomy specimens fixed and included in paraffin blocks. For eligibility purposes, available hematoxylin and eosin–stained sections of each case were reviewed to confirm the lesions. Correspondent paraffin blocks were then selected and used for histological review and molecular detection of HPV DNA. In cases with more than 1 block, the chosen one was the most representative of the primary diagnosis or the block containing the most severe lesion.

All paraffin blocks were sent to the pathological anatomy laboratory in Abel Salazar Institute for Biomedical Sciences and were processed under rigorous conditions to avoid contamination. Five sections of 3 to 5 μm were obtained from each block (sandwich method). The first and last sections were used for histological assessment.

For cases where histopathologic reassessment confirmed the initial histological diagnosis, the remaining sections were sent to the National Institute of Health Doutor Ricardo Jorge for genotyping. Negative or discordant results led to reassessment of the sample, which included slides review by both the pathologist of the center and the reference pathologist. If an agreement was obtained in the identification of the lesion, the correspondent block was selected for study; otherwise, the case was rejected. Detection and genotyping were carried out using the commercial system CLART Human Papillomavirus 2 (Genomica, Madrid, Spain) according to the manufacturer's instructions. This assay amplifies a fragment of 450 base pairs in the L1 region of HPV and posterior detection, toward hybridization with specific capture probes, in a microarray platform, and allows the identification of 35 genotypes (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, 84, 85, and 89). For negative HPV tests, results were confirmed by an alternative genotyping method using MY11/09 e GP5+/6+ primers, followed by DNA sequencing.

Along with histological and genotyping information obtained by the analysis of the biological sample, data on demographics, sexual activity, obstetric history, past episodes of sexually transmitted infections (STIs), and the last cytology performed before the histological diagnosis was collected in a clinical report form. A unique identification code was assigned to each eligible case and used for all laboratory analyses, data management, and statistical analysis.

Data collection was authorized by the Portuguese Data Protection Authority. Informed consent was obtained from all patients, except in case of death, a scenario previously considered and agreed with this authority. Study procedures were previously approved by the study centers' ethics committees and carried out in accordance with the Good Epidemiological Practice guideline[11] and study protocol.

Statistical Analysis

A planned sample size of 515 histological samples of CIN2 and CIN3 and ICC was defined by protocol. This sample allowed the computation of 95% confidence intervals (95% CIs) for HPV prevalence with a maximum margin of error of 5.5%.

For statistical purposes, HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59 66, 68, 70, 73, and 82 were considered as HR-HPV (includes HR and probably HR genotypes), and HPV 6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 85, and 89 were considered as LR-HPV (includes LR and undetermined genotypes). Type-specific HPV positivity is presented as the proportion of HPV-positive cases in which the particular HPV genotype was detected and includes the contribution of multiple HPV infections. The HPV type-specific prevalence (overall and by histological diagnosis) is presented together with 2-sided 95% CI.

Qualitative variables were summarized by counts and percentages and quantitative variables by mean and SD. Pearson χ^2 test or Fisher exact test was used to assess the distribution of HPV and HPV genotypes within histological diagnosis and age group. These tests were also performed to assess statistical differences regarding age, sexual activity, obstetric history, previous history of STI and CIN, use of hormone contraceptives, and smoking habits between histological diagnoses.

All statistical tests were 2-tailed considering a significance level of 5%. Missing values were not replaced. Statistical analysis was performed using IBM SPSS Statistics 18.

RESULTS

A total of 582 blocks of archived paraffin-embedded tissues diagnosed between January 2008 and May 2009 were considered eligible for this analysis. The mean age of participants was 41.8 years (range, 20–88 years). Characteristics of the study population are shown in Table 1.

The most common histological diagnosis was CIN3 (58.6%; 341/582), followed by CIN2 (30.4%; 177/582), squamous cell carcinoma (9.8%; 57/582), and adenocarcinoma

TABLE 1. Characteristics of study population

	Total (n = 582)
Age, mean (SD), y	41.8 (12.2)
Age group, n (%)	
<30 y	60 (10.3%)
30–39 y	240 (41.2%)
40–49 y	164 (28.2%)
50–59 y	67 (11.5%)
≥60 y	51 (8.8%)
Age at first sexual intercourse in years, mean (SD), y	18.1 (2.8)
Data missing*	118
Age at first pregnancy, mean (SD), y	23.3 (5.1)
No pregnancies	86
Data missing*	182
Lifetime no. sexual partners, n (%)	
1	121 (33.5%)
2–4	181 (50.1%)
5–10	50 (13.9%)
>10	9 (2.5%)
Data missing*	221
No. pregnancies, n (%)	
0	86 (15.9%)
1	131 (24.3%)
2	140 (25.9%)
3	91 (16.9%)
≥4	92 (17.0%)
Data missing*	42
No. births, n (%)	
0	104 (19.2%)
1	177 (32.7%)
2	152 (28.1%)
3	66 (12.2%)
≥4	42 (7.8%)
Data missing*	41
Previous STI, n (%)	
Yes	91 (21.6%)
No	331 (78.4%)
Data missing*	160
Oral contraceptive use, n (%)	
Yes	370 (70.6%)
No	154 (29.4%)
Data missing*	58
Smoking habits, n (%)	
Yes	217 (44.9%)
No	266 (55.1%)
Data missing*	99
Type of histological samples	
Conization	371 (63.7%)
Other biopsy	204 (35.1%)
Hysterectomy	7 (1.2%)
Previous history of CIN, n (%)	
Yes	31 (7.7%)
No	373 (92.3%)
Data missing*	178
Histological diagnosis, n (%)	
CIN2	177 (30.4%)
CIN3	341 (58.6%)
ADC	7 (1.2%)
SCC	57 (9.8%)

*Not determined or unknown.

ADC, adenocarcinoma; SCC, squamous cell carcinoma.

TABLE 2. HPV prevalence by cervical disease grade

	Total (n = 582)	CIN2 (n = 177)	CIN3 (n = 341)	ICC			
				Total (n = 64)	ADC (n = 7)	SCC (n = 57)	*P**
HPV							
Positive	570 (97.9%)	169 (95.5%)	339 (99.4%)	62 (96.9%)	6 (85.7%)	56 (98.2%)	NA
Negative	12 (2.1%)	8 (4.5%)	2 (0.6%)	2 (3.1%)	1 (14.3%)	1 (1.8%)	
Single HPV†	506 (88.8%)	144 (85.2%)	305 (90.0%)	57 (89.1%)	6 (100%)	51 (91.1%)	0.234
Multiple HPV†	64 (11.2%)	25 (14.8%)	34 (10.0%)	5 (7.8%)	0 (0.0%)	5 (8.9%)	

*χ^2 Test.

†Denominators are number of HPV-positive cases.

NA, not applicable; ADC, adenocarcinoma; SCC, squamous cell carcinoma; NA, not applicable.

(1.2%; 7/582) (Table 1).The overall HPV prevalence was 97.9% (570/582; 95% CI, 96.7%–99.1%), of which 88.8% (560/570) were single infections (Table 2). The presence of at least 1 HR-HPV was observed in 96.1% (548/570) of the HPV-positive cases (Table 2).

Overall, 26 different genotypes were identified, with a proportion ranging from 58.4% to 0.2% (333/570 to 1/570). The 8 most common genotypes in decreasing order were HPV 16 (58.4%; 95% CI, 54.4%–62.4%), 31 (10.4%; 95% CI, 7.9%–12.9%), 33 (8.1%; 95% CI, 5.9%–10.3%), 58 (7.7%; 95% CI, 5.5%–9.9%), 52 (5.1%; 95% CI, 3.3%–6.9%), 18 (3.5%; 95% CI, 2.0%–5.0%), 51 (3.5%; 95% CI, 2.0%–5.0%), and 35 (3.3%; 95% CI, 1.8%–4.8%) (Table 3, Fig. 1), and their combined contribution was 89.3% (95% CI, 86.9%–91.7%). Regarding LR-HPV, 7 genotypes were detected, with HPV 6 being the most common (0.7%; 95% CI, 0.0%–1.4%) (Table 3).

Multiple infections were observed in 11.2% (64/570) of the HPV-positive cases, decreasing in proportion with increasing cervical disease grade (14.8% in CIN2, 10.0% in CIN3, and 7.8% in ICC) (Table 2). Human papillomavirus type 16 (56.2%; 36/64) was the most common genotype observed among these infections, followed by HPV 33 (21.9%; 14/64), 31 (20.3%; 13/64), 52 (12.5%; 8/64), 70 (12.5%; 8/64), 51 (10.9%; 7/64), 58 (10.9%; 7/64), and 18 (7.8%; 5/64) (data not shown).

According to cervical disease grade, HPV was found in 95.5% (169/177; 95% CI, 92.4%–98.6%) of the CIN2 (22 different genotypes) and 99.4% (339/341; 95% CI, 98.6%–100.0%) of the CIN3 cases (21 different genotypes) (Table 2). Human papillomavirus type 16 was the most common g, Fig 1).enotype in CIN2 (49.7%; 84/169) and CIN3 (60.2%; 204/341), followed in decreasing order by HPV 31, 58, 33, 51, 52, 18, and 35 and HPV 31, 33, 58, 52, 35, 18, and 51, respectively (Table 3, Fig. 1). In ICC, HPV was detected in 96.9% (62/64; 95% CI, 92.7%–100.0%) of the cases, with a detection rate of 98.2% (56/57; 95% CI, 94.7%–100.0%) in squamous cell carcinoma (12 different genotypes) and 85.7% (6/7; 95% CI, 53.3%–100.0%) in adenocarcinoma (1 genotype) (Tables 2 and 3, Fig 1.). Human papillomavirus type 16 was the most common genotype in ICC (72.6%; 45/62), followed in decreasing order by HPV 33, 18, 58, 45, 53, 73, 31, 51, 52, 56, and 59. The probably HR-HPV 53 and 73 were never detected as single agents, and no LR-HPV genotypes were identified in this histological category. Human papillomavirus types 31, 51, 52, 56, and 59 were detected in only 1 case each (Table 3).

The mean age of study patients at the time of histological diagnosis is presented in Figure 2. Overall, mean age of HPV-positive ICC patients was 52.8 years, whereas in CIN2 and CIN3, mean ages were 39.4 and 41.2 years, respectively. Statistically significant differences were found between age groups regarding cervical disease grade ($P < 0.001$). Cervical intraepithelial neoplasia grades 2 and 3 were more frequent in women aged 30 to 39 years, whereas ICC was more frequent in women aged 40 to 49 years and older than 60 years (data not shown). Mean age was lower for women with CIN2 associated with single HPV 18 infection (n = 7) than for women with CIN2 that were positive for HPV 16 (n = 70) or any other HR-HPV (n = 58; HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) as a single infection (36.1 vs 39.6 or 39.3 years). In CIN3 cases, mean age of women with single HPV 16 (n = 184) was lower than the age of women with single infections by HPV 18 (n = 5) or any other HR-HPV genotype (n = 107) (40.1 vs 53.8 or 42.3 years). The mean ages of women with ICC related to single infection by HPV 16 (n = 43) and 18 (n = 3) were 50.6 and 49.3 years, respectively, which were lower than the mean age of women with ICC related to single infection by any other HR-HPV (n = 9; 60.1 years) (Fig. 2).

Differences in parity within histological categories were statistically significant, with higher number of pregnancies and births in women with ICC ($P = 0.010$ and $P = 0.001$, respectively; data not shown).

Women with a diagnosis of CIN3 showed a significantly higher use of hormone contraceptives, whereas smoking habits were more common in CIN2 ($P = 0.005$ and $P = 0.044$, respectively; data not shown). Differences in medical history of STI within histological categories were also statistically significant ($P = 0.034$), with higher proportions observed in women with CIN2 (data not shown).

DISCUSSION

The purpose of this epidemiologic, retrospective, multicenter study conducted in mainland Portugal was to estimate the HPV type-specific prevalence in histological samples with CIN2, CIN3, and ICC diagnoses.

TABLE 3. HPV type-specific prevalence in HPV-positive cases by cervical disease grade

	All Infections (Single or Multiple)			
	Total (n = 570)	CIN2 (n = 169)	CIN3 (n = 339)	ICC (n = 62)
HR types				
HPV 16	333 (58.4%)	**84 (49.7%)**	**204 (60.2%)**	**45 (72.6%)***
HPV 18	20 (3.5%)	8 (4.7%)	9 (2.7%)	3 (4.8%)
HPV 26	3 (0.5%)	1 (0.6%)	2 (0.6%)	—
HPV 31	59 (10.4%)	20 (11.8%)	38 (11.2%)	1 (1.6%)
HPV 33	46 (8.1%)	10 (5.9%)	31 (9.1%)	5 (8.1%)
HPV 35	19 (3.3%)	8 (4.7%)	11 (3.2%)	—
HPV 39	2 (0.4%)	1 (0.6%)	1 (0.3%)	—
HPV 45	5 (0.9%)	—	3 (0.9%)	2 (3.2%)
HPV 51	20 (3.5%)	10 (5.9%)	9 (2.7%)	1 (1.6%)
HPV 52	29 (5.1%)	9 (5.3%)	19 (5.6%)	1 (1.6%)
HPV 53	8 (1.4%)	3 (1.8%)	3 (0.9%)	2 (3.2%)†
HPV 56	5 (0.9%)	4 (2.4%)	—	1 (1.6%)
HPV 58	44 (7.7%)	19 (11.2%)	22 (6.5%)	3 (4.8%)
HPV 59	6 (1.1%)	1 (0.6%)	4 (1.2%)	1 (1.6%)
HPV 66	4 (0.7%)	1 (0.6%)	3 (0.9%)	—
HPV 68	4 (0.7%)	2 (1.2%)	2 (0.6%)	—
HPV 70	10 (1.8%)	7 (4.1%)	3 (0.9%)	—
HPV 73	2 (0.4%)	—	—	2 (3.2%)†
HPV 82	8 (1.4%)	4 (2.4 %)	4 (1.2%)	—
LR types				
HPV 6	4 (0.7%)	1 (0.6%)	3 (0.9%)	—
HPV 42	2 (0.4%)	1 (0.6%)	1 (0.3%)	—
HPV 72	1 (0.2%)	—	1 (0.3%)	—
HPV 81	1 (0.2%)	1 (0.6%)	—	—
HPV 83	1 (0.2%)	—	1 (0.3%)	—
HPV 84	1 (0.2%)	1 (0.6%)	—	—
HPV 85	1 (0.2%)	1 (0.6%)	—	—

Bold font indicates statistical significance ($P < 0.050$). Because some cases contained more than 1 HPV type, the total number of genotypes is greater than the number of cases. As a result, the columns do not add to 100%.

*Includes 6 cases of adenocarcinoma.

†HPV 53 and 73 were always associated to other HR-HPV genotypes.

Overall HPV prevalence was 97.9%, and all histological categories showed an HPV prevalence higher than 95%, which is in agreement with data from other European studies.[12,13] At least 1 HR-HPV genotype was identified in 96.1% of the HPV-positive cases, and as expected, HPV 16 was the most common genotype in all histological groups (58.4%).[12–19]

As reported by other studies, multiple infections were more frequent in CIN2 (14.8%) than in CIN3 (10.0%) and ICC (7.8%).[12,18,19] However, this difference was not statistically significant.

The 8 more frequent genotypes identified in this study showed different distributions within histological diagnosis groups. In CIN2, the most frequent genotypes in order of decreasing frequency were HPV 16, 31, 58, 33, 51, 52, 18, and 35, whereas in CIN3 were HPV 16, 31, 33, 58, 52, 35, 18, and 51. The higher prevalence of these genotypes in these histological groups was also observed in several European studies,[12,13] although Coutlée et al[19] reported that in Canada HPV 39 and 42 were also found among the most common in these groups.

The prevalence of HPV in ICC was 96.9%, which is higher than the proportions reported by others,[12,16,18,19] probably because of variations in polymerase chain reaction–based HPV detection methods. Only 12 HPV genotypes were detected in ICC cases. The 7 most frequent genotypes in order of decreasing frequency were HPV 16, 33, 18, 58, 45, 53, and 73, accounting for 92.5% of the HPV-positive ICC cases. However, HPV 53 and 73 were always associated to other HR-HPV genotypes. Human papillomavirus types 31, 51, 52, 56, and 59 were detected in only 1 case each. No LR-HPV was detected in this diagnosis, which confirms that the presence of these genotypes in ICC is a rare event.[17]

Human papillomavirus type 16 was significantly more prevalent in ICC (72.6%) than in CIN2 (49.7%) and CIN3 (60.2%), a trend also observed by others.[12,14] According to several studies, HPV 18 and 45 were among the most frequent genotypes identified in ICC.[12,14,16–19] In the present study, although HPV 18 and 45 were more frequent in cases of ICC (HPV 18: 4.8% in ICC vs 2.7% in CIN3; HPV 45: 3.2% in ICC vs 0.9% in CIN3), the prevalence was not so evident, and the difference was not statistically significant. No cases of HPV 45 infection were identified in patients with CIN2, and the proportion of this genotype is enriched in ICC compared with CIN3, suggesting a higher carcinogenic potential relative to other non–HPV 16 genotypes.[18] Human papillomavirus types 35 and 39 were not detected in cases of ICC. Human papillomavirus type 31 showed similar frequency in CIN2 and CIN3 as observed by Coutlée et al.[19] Although not statistically significant, HPV 35, 51, 56 and 58 were more frequent in CIN2, and HPV 33 was more frequent in CIN3, as observed in others studies.[12,19] The different size of histological diagnosis groups (CIN3 represented 58.6% of the study sample, whereas CIN2 accounted for 30.4% and ICC for 11.0%) might have had some impact in the inferences obtained, regarding the prevalence of certain HPV genotypes.

As expected, mean age at histological diagnosis was higher in women with ICC than that of those with CIN3 and CIN2.[12,17] Regarding CIN2 cases, in all HPV subgroups, mean age at diagnosis was less than 40 years, which is in agreement with data from Italy.[12] Our findings showed that CIN3 associated to HPV 16 arises at a younger age (40 years) and that ICC related to HPV 16 or 18 arises early than ICC related to infection by other HR-HPV (51 vs 60 years). The mean ages at histological diagnosis for ICC cases were 52.8 years, 50.6 years for ICC associated with HPV 16, and 49.3 years for ICC associated with HPV 18. These results were higher than those presented by de Sanjose et al.[17] Some of these HPV subgroups presented low sample sizes; therefore, no strong conclusions can be drawn regarding patients' age by histological diagnosis and HPV genotype.

Differences in parity within histological categories were statistically significant, with higher number of pregnancies

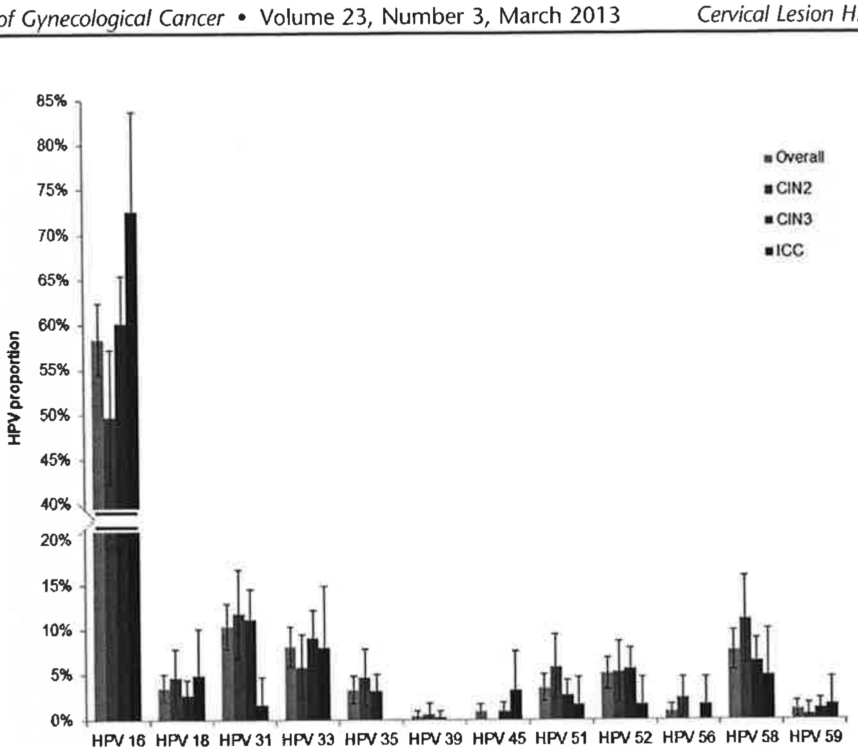

FIGURE 1. The 12 most common HR-HPV genotypes, by total of HPV-positive cases with CIN and ICC (bars correspond to 95% CIs).

and births in women with ICC. Women with a diagnosis of CIN3 showed a significantly higher use of hormone contraceptives, whereas smoking habits and medical history of STI were more common in women with CIN2.

This study has several strengths; namely, the women were recruited from several cities in mainland Portugal, the grade of lesion was assessed by histology, the HPV genotyping method used can detect a wide spectrum of HPV genital genotypes, all samples were tested with the same methodology, and prevalence rates were provided separately for CIN2 and CIN3, which is the true precursor lesion of invasive squamous cell carcinoma. However, there were also some limitations. The different size of histological diagnosis groups and the limited number of squamous cell carcinoma and adenocarcinoma cases included in the study might have had some impact in the inferences obtained and in the prevalence of certain HPV genotypes, particularly in ICC.

In conclusion, this study showed a high HPV prevalence in histological samples with CIN2, CIN3, and ICC in mainland Portugal. Type-specific HPV prevalence, overall and by histological diagnosis, was similar to data from other studies, showing a high proportion of HR-HPV genotypes, particularly HPV 16, and confirms the universal contribution of the 8 most common genotypes (HPV 16, 18, 31, 33, 45, 51, 52, and 58) in ICC, with some exceptions. Our results suggest that vaccination against HPV 16 and 18 has the potential to prevent more than two thirds of ICC (77.4%) and more than half of CIN2 (54.4%) or CIN3 (62.9%) cases in Portugal. Additional studies in squamous cell carcinoma and adenocarcinoma are needed to confirm the HPV-type specific prevalence achieved.

This study is important to identify variations of the different HPV genotypes by cervical lesions grade in Portugal. Such information is useful for identifying genotypes that differ largely in their carcinogenic potential, for the differential management in HPV-based screening programs,

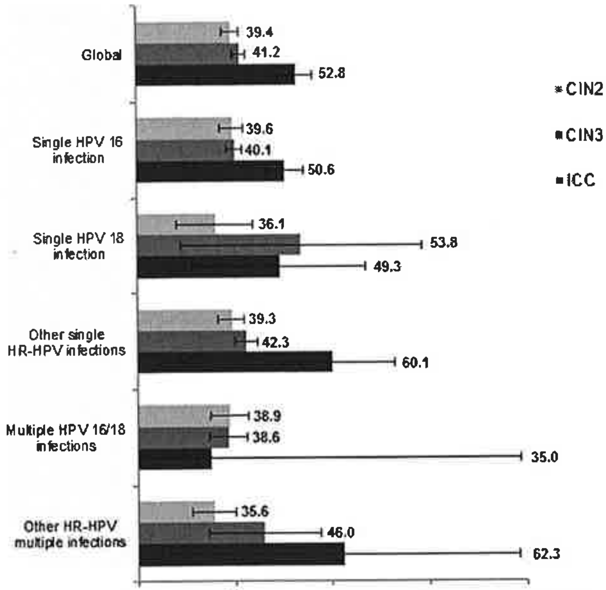

FIGURE 2. Mean age at diagnosis of CIN grades 2 and 3 or ICC (horizontal bars correspond to 95% CIs). Other HR-HPV includes HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

and it may be an important tool for evaluation of the HPV vaccination impact.

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