Papillomavirus Research 8 (2019) 100179

Contents lists available at ScienceDirect

Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr

High-Risk human papillomavirus genotype distribution in the Northern region of Portugal: Data from regional cervical cancer screening program

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ARTICLE INFO

Keywords:
Human papillomavirus (HPV)
Cervical cancer
Screening
Genotyping
Prevalence
Epidemiology

ABSTRACT

High-Risk Human papillomavirus (HR-HPV) full genotyping methods have been described as of great potential use in epidemiology and preventive strategies, including cervical cancer screening and HPV vaccination. We characterized the prevalence and distribution of HR-HPV genotypes in cervico-vaginal samples obtained from the Regional Cervical Cancer Screening Program from the Northern Region of Portugal. HR-HPV genotyping was performed using Anyplex™ II HPV-HR Detection kit in 105,458 women enrolled between August 2016 and December 2017. HR-HPVs were detected in 10,665 women (10.2%) with a prevalence ranging from 6.2 to 17.1% depending on age, and from 8.7 to 10.7% depending on geographical location. Multiple infections with two or more HR-HPVs were detected in 2736 (25.7%) of HR-HPV women ranging from 16.5 to 31.0% depending on age. Amongst HR-HPV positive women, HPV-16 (17.5%), HPV-39 (16.7%), HPV-31 (15.0%), HPV-68 (13.2%), HPV-52 (10.7%) and HPV-51 (10.6%) were the most common genotypes in our population, being HPV-16 more frequent in women aged from 30 to 45 years and HPV-39 in 50–65 years. Results also show that HPV16/18 are present in 22.1% and HPV16/18/31/33/45/52/58 in 47.6% of HR-HPV positive women. This is the largest study on HR-HPV genotyping for Cervical Cancer Screening in European populations and provides critical data for program management and vaccine policy.

1. Introduction

Worldwide, invasive cervical cancer (ICC) is one of the most important cancers in women, being responsible for 569,847 new cases and 311,365 related deaths in 2018, according to Globocan [1]. In Portugal, in 2018 it was estimated a total of 750 new cases and 350 deaths, with an Age-Standardized incidence and mortality rates of 8.9 and 2.8 per 100,000 women, respectively [1].

Since the 1970s that Human Papillomavirus (HPV) was identified as the etiological factor of cervical cancer, and since 1995 that many authors have described the different HPV genotypes according to the risk of carcinogenesis [2–6]. There are over 150 different HPV genotypes [3], nevertheless, HPV-16 and HPV-18 are responsible for the majority of cervical cancer cases worldwide, and together with HPV-31, 33, 45, 52 and 58 represent over 90% of all cases [7–11]. The persistent infection by High-Risk-HPVs (HR-HPV) is the crucial event for the

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https://doi.org/10.1016/j.pvr.2019.100179
Received 19 May 2019; Received in revised form 29 July 2019; Accepted 31 July 2019
Available online 01 August 2019

development of high-grade cervical lesions that can evolve to ICC [5,7]. There are 14 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) included in the majority of HPV-tests commercialized and its identification may be useful for the development of future HPV vaccines or cervical cancer prevention strategies [12–15].

Cervical cancer is actually a preventable cancer since there are both primary prevention measures with the implementation of vaccines against human papillomavirus (HPV), and with secondary prevention by cervical cancer screening strategies [7,16–18]. In Portugal, cervical cancer screening was introduced back in 1978 as an opportunistic strategy and in 1990 it started to be partially-organized in the Centre region of the country [19]. Due to the division of the country in different regional health administrations, the cervical cancer screening program was progressively implemented in the Alentejo, Algarve, Azores and the Northern region of Portugal [20]. In the Northern region of Portugal, cervical cancer screening started in 2009 and progressively extended to the whole region, using liquid-based cytology and HPV-testing as a reflex in cases of atypical squamous cells of undetermined significance (ASC-US) [20]. In 2016, a pilot study was implemented in the North Region of Portugal using HR-HPV full genotyping as the primary method for cervical cancer screening [20].

In this study, we report the results of that initiative which encompassed HR-HPV full genotyping in women attending the Regional Cervical Cancer Screening Program in the Northern region of Portugal and discuss its potential impact in the definition of and monitoring of the HPV vaccination program.

2. Material and methods

2.1. Study population

The study was performed based on women that attended the Regional Cervical Cancer Screening Program in the Northern Region of Portugal between August 2016 and December 2017. The Regional Cervical Cancer Screening Program is an organized screening performed in all women aged 25–60 years old (with possible extension up to 64 years of age) from the North region of Portugal [20]. Briefly, the screening is performed by general practitioners from the community-based health centers using liquid-based cytology samples in all women at illegible ages, nevertheless, women outside the illegible ages without at least one cervical cytology in the last 3 years can also be included.

2.2. HPV genotyping

High-Risk HPV genotyping was performed in liquid-based cytology samples with Anyplex™ II HPV HR Detection (Seegene®, Seoul, Korea) according to the manufacturer's instructions and according to the validation for use in cervical cancer screening [21]. This kit comprises an automated system for DNA isolation (Nimbus IVD or STARlet from Hamilton), a real-time PCR system (CFX96 PCR from Bio-Rad) and a data analysis software (Seegene Viewer™ from Seegene®). The multiplex real-time PCR allows for simultaneous detection and genotyping of 14 high-risk (HR) HPV types, including HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66, and −68 plus an internal control (human beta-globin) in a single reaction. All reactions include positive and negative controls provided in the kit.

2.3. Statistical analysis

The statistical analysis was performed with IBM SPSS Statistics for Mac, Version 24.0 (Armonk, NY: IBM Corp) using Chi-Square (χ^2) or Fisher exact-test to compare the categorical variables with a 5% significance level. The overall prevalence was described by frequencies and percentages, and age (continuous variable) using means and standard deviations (SD). The description of results was performed considering the total number of women positive for HR-HPV, multiple infections, or specific HPV genotype. Type-specific HPV-positivity was estimated as the proportion cases positive for HPV regardless of being with or without co-infection with other HPV genotypes. Results were stratified considering the different age groups 25 (24–27 y.o.), 30 (28–32 y.o), 35 (33–37 y.o.), 40 (38–42 y.o.), 45 (43–47 y.o.), 50 (48–52 y.o.), 55 (53–57 y.o.), 60 (58–62 y.o.) and 64 (63–66 y.o.); and also the geographic location considering the districts (Aveiro, Braga, Bragança, Porto, Viana do Castelo, Vila Real, and Viseu).

3. Results

A total of 105,458 women (mean age 43.8 ± 10.6 years old; median age 45; range 24–66) were enrolled, of which 465 (0.44%) had an insufficient sampling. From the 104,993 cases included, 48 cases were inconclusive (0.04%) and 10,665 (10.2%) were HR-HPV positive, with multiple infections (by two or more HR-HPVs) being detected in 25.7% (n = 2736) of HR-HPV positive women – Table 1. Within the HR-HPV positive women, the most common genotypes found in our population were HPV-16 (17.5%), HPV-39 (16.7%), HPV-31 (15.0%), HPV-68 (13.2%), HPV-52 (10.7%) and HPV-51 (10.6%) – Fig. 1 and Table 1.

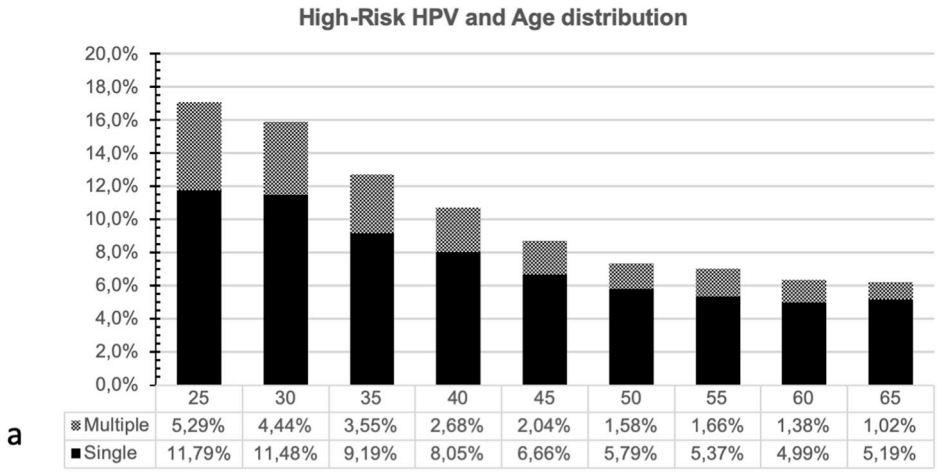
HR-HPV infection varied according to age ranging from a maximum of 17.1% at age 25 to a minimum of 6.2% at age 64 ($p < 0.001$); while regarding the geographical location of women, HR-HPV prevalence ranges from 8.7% to 10.8% ($p < 0.001$) – Fig. 2 and Table 2. Multiple infections ranged from 16.5% at the age of 64 to 31.0% at the age of 25 ($p < 0.001$). The analysis also revealed that there was no significant variation of multiple infections prevalence regarding geographical location (range 21.5%–26.9%, $p = 0.769$) – Fig. 2 and Table 2.

HR-HPV genotype distribution according to age revealed that HPV-16 predominated in women from age groups of 30, 35, 40 and 45 years old, whereas HPV-39 predominated in women from age groups of 50, 55, 60 and 64 years – Fig. 3a and Table 2. The four most common HR-

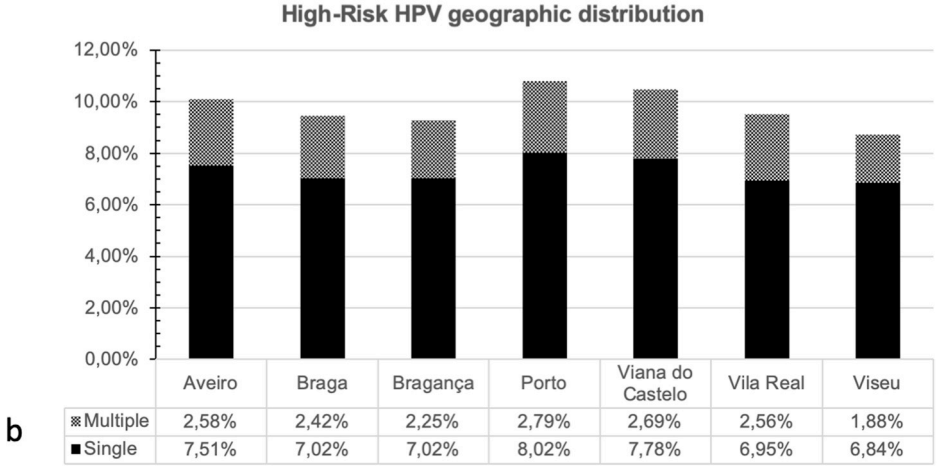
Table 1
High-Risk HPV genotypes distribution.

	HR-HPV Positive n (%)[a]	Single Infection n (%)	Multiple Infection (%)
All genotypes	10665 (10.2)	7929 (74.3)	2736 (25.6)
HPV-16	1864 (17.5)	1121 (60.1)	743 (39.9)
HPV-18	546 (5.12)	245 (44.9)	301 (55.1)
HPV-31	1598 (14.98)	850 (53.2)	7481 (46.8)
HPV-33	464 (4.35)	237 (51.1)	227 (48.9)
HPV-35	546 (5.12)	227 (41.6)	319 (58.4)
HPV-39	1780 (16.7)	1220 (68.5)	560 (31.5)
HPV-45	405 (3.80)	215 (53.1)	190 (46.9)
HPV-51	1126 (10.6)	609 (54.1)	517 (45.9)
HPV-52	1139 (10.7)	600 (52.7)	539 (47.3)
HPV-56	998 (9.36)	512 (51.3)	486 (48.7)
HPV-58	891 (8.35)	461 (51.7)	430 (48.3)
HPV-59	669 (6.27)	315 (47.1)	354 (53.9)
HPV-66	956 (8.96)	487 (51.0)	468 (49.0)
HPV-68	1411 (13.2)	830 (58.8)	581 (51.2)
HPV Vaccine genotypes			
HPV 4-valent vaccine			
HPV-16/18 [b]	2353 (22.1)	1364 (58.0)	989 (42.0)
Non-vaccine genotypes[b]	8312 (77.9)	6565 (79.0)	1741 (21.0)
HPV 9-valent vaccine			
HPV16/18/31/33/45/52/58[b]	5073 (47.6)	3129 (61.7)	1944 (38.3)
Non-vaccine genotypes[b]	5592 (52.4)	4800 (85.8)	792 (14.2)

HR-HPV, High-Risk Human Papillomavirus; n, number of women.
[a] Total percentage is not equal to 100.0% due to rounding.
[b] Includes and/or specific genotypes, including multiple infecions.



a



b

Fig. 1. High-Risk HPV genotypes distribution in population.

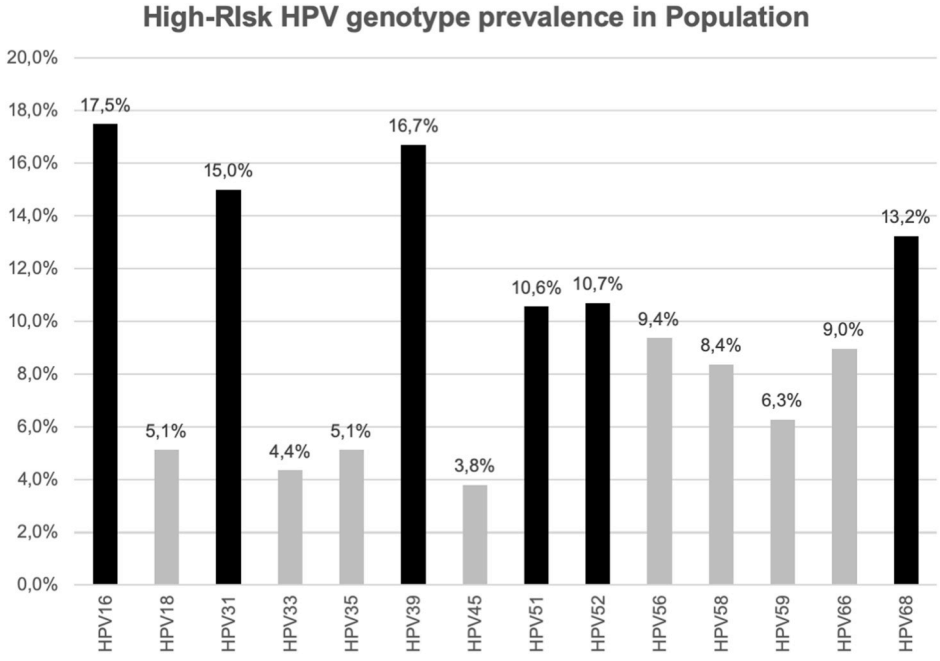


Fig. 2. High-Risk HPV prevalence according to age distribution (a) and geographic location (b).

Table 2
High-Risk HPV genotypes distribution according to age and geographical location.

	Age groups (y), n									Geographical location, n							
	25	30	35	40	45	50	55	60	64	Aveiro	Braga	Bragança	Porto	Viana do Castelo	Vila Real	Viseu	Aveiro
ALL cases, n = 104993	8109	10426	12716	15573	16194	15712	13811	11084	1368	9294	30134	2264	46638	7664	5695	3304	9294
HPV Positive, n = 10665	1385	1660	1620	1670	1409	1159	971	706	85	938	2844	210	5041	802	542	288	938
Single Infection, n	956	1197	1169	1253	1078	910	742	553	71	698	2114	159	3740	596	396	226	698
Multiple Infection, n	429	463	451	417	331	249	229	153	14	240	730	51	1301	206	146	62	240
HR-HPV genotypes [a]																	
HPV-16, n = 1864	175	357	349	304	246	176	139	106	12	159	525	31	900	110	83	56	159
HPV-18, n = 546	49	95	91	92	63	74	50	27	5	56	145	8	274	32	23	8	56
HPV-31, n = 1598	185	300	300	264	188	152	123	77	9	141	404	33	760	128	95	37	141
HPV-33, n = 464	60	73	69	74	51	48	54	30	5	38	120	10	229	32	26	9	38
HPV-35, n = 546	86	73	68	92	64	56	54	46	7	41	163	7	251	39	30	15	41
HPV-39, n = 1780	229	233	251	272	239	214	179	149	14	161	482	31	820	132	103	51	161
HPV-45, n = 405	48	59	50	79	55	49	35	25	5	35	110	7	187	39	22	5	35
HPV-51, n = 1126	197	184	164	170	141	101	87	73	9	106	314	21	519	93	54	19	106
HPV-52, n = 1139	207	169	195	151	146	112	95	54	10	95	297	19	541	88	64	35	95
HPV-56, n = 998	152	170	136	142	128	90	100	72	8	96	254	26	467	74	53	28	96
HPV-58, n = 891	134	137	138	131	105	85	88	68	5	79	251	22	415	58	47	19	79
HPV-59, n = 669	120	109	105	101	87	56	53	34	4	64	181	17	305	52	29	21	64
HPV-66, n = 956	176	143	135	158	123	96	75	46	4	90	255	18	456	64	40	33	90
HPV-68, n = 1411	187	206	182	206	200	177	144	99	10	113	348	28	686	121	78	37	113
Multiple genotypes																	
HPV16 + 18, n = 21	2	5	2	2	5	4	1	0	0	3	2	1	11	4	0	0	3
HPV16 + 18+other, n = 35	6	6	3	4	6	6	3	0	1	4	10	1	18	1	0	1	4
HPV16 + other, n = 688	78	136	125	109	88	58	50	42	2	50	185	12	345	44	37	15	50
HPV18 + other, n = 245	25	43	52	38	21	31	23	11	1	25	68	3	116	19	11	3	25
HPV other, n = 1747	318	273	269	264	211	150	152	100	10	158	465	34	811	138	98	43	158

HR-HPV, High-Risk Human Papillomavirus; n, number of women; age groups 25 (24–27), 30 (28–32), 35 (33–37), 40 (38–42), 45 (43–47), 50 (48–52), 55 (53–57), 60 (58–62) and 64 (63–66); y, years of age;

[a] The sum of the number of women positive for each HR-HPV positive women is higher than the total number of women positive for HR-HPV due to rounding caused by multiple infections.

HPV genotypes (HPV-16, -39, −31 and −68) were the same in all geographic locations – Fig. 3b and Table 2. Results also show that HPV16/18, which are present in HPV 4-valent vaccine, represent 22.1% of the overall HPV genotypes found in women, ranging from 15.6 to 26.8% according to age and from 17.1% to 23.2% according to geographic location (Fig. 4a and Fig. 4c). The HPV genotypes present in the 9-valent vaccine (HPV16/18/31/33/45/52/58) represent in 47.6% of all positive cases, ranging from 40.9% to 53.7% according to age and from 42.7% to 48.1% according to geographic location (Fig. 4b and Fig. 4d).

4. Discussion

The infection by HR-HPV is extremely common in sexually active women, however, only a small percentage of infected women will develop premalignant lesions that may evolve into ICC [22]. The evolution from persistent infection to low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs) and ultimately ICC is slow and takes years or even decades [22]. This slow evolution provides a great opportunity for screening and detection of early lesions and therefore to a high probability of cure, decreasing the incidence and mortality rates of ICC.

In the past 10 years, the literature clearly describes that HPV DNA testing is more sensitive for identifying women with CIN 2 + compared with cytology, despite having a lower specificity [18,23–25]. Indeed, studies have shown that in women aged 30–69 years the sensitivity of the HPV test is around 95% compared with 55% for cytology [23]. The new strategies for cervical cancer screening support the use of HPV test as the primary test (higher sensitivity) followed by a triage of HPV-positive results with cytology (higher specificity) [26–29].

Portugal has a high incidence of cervical cancer when compared with the majority of European countries and therefore Portuguese governments have started with vaccination and more effective screening strategies since 2008 [1,20]. While HPV vaccination was included in the National Vaccination Program, cervical cancer screening was applied differently by the different regional health administrations. However, since September 2017, the Portuguese Ministry of Health has decided that cervical cancer screening should be performed using the HPV test as the primary screening method. Actually, there are over 200 different tests for HPV detection but only a few have been validated to be used in cervical cancer screening [30–32]. HR-HPV full genotyping has been used more often since it is considered to provide additional epidemiological data which can ultimately improve vaccination and screening strategies. In this study, we report HR-HPV genotypes detected in samples from women attending the cervical cancer screening from the Northern region of Portugal using the Anyplex™ II HPV HR Detection from Seegene, which was validated for use in screening [21,30,33].

Our results report an overall prevalence of HR-HPV of 10.2% which is significantly different from the reported value (19.4%) in the CLEOPATRE study from Pista et al. [34] and the reported (17.8%) in the ICO/IARC Information Centre on HPV and Cancer [35]. The higher HPV prevalence rate found in the study by Pista et al. [34] may be explained by the higher number of samples collected in women from 18 to 24 years of age, while we have only women from 24 to 66 years of age, and in addition, the test used in that study allowed the identification of 35 different HPV genotypes including possible high-risk HPVs (HPV-26, -53, −70, −73, and −82). However, the prevalence we

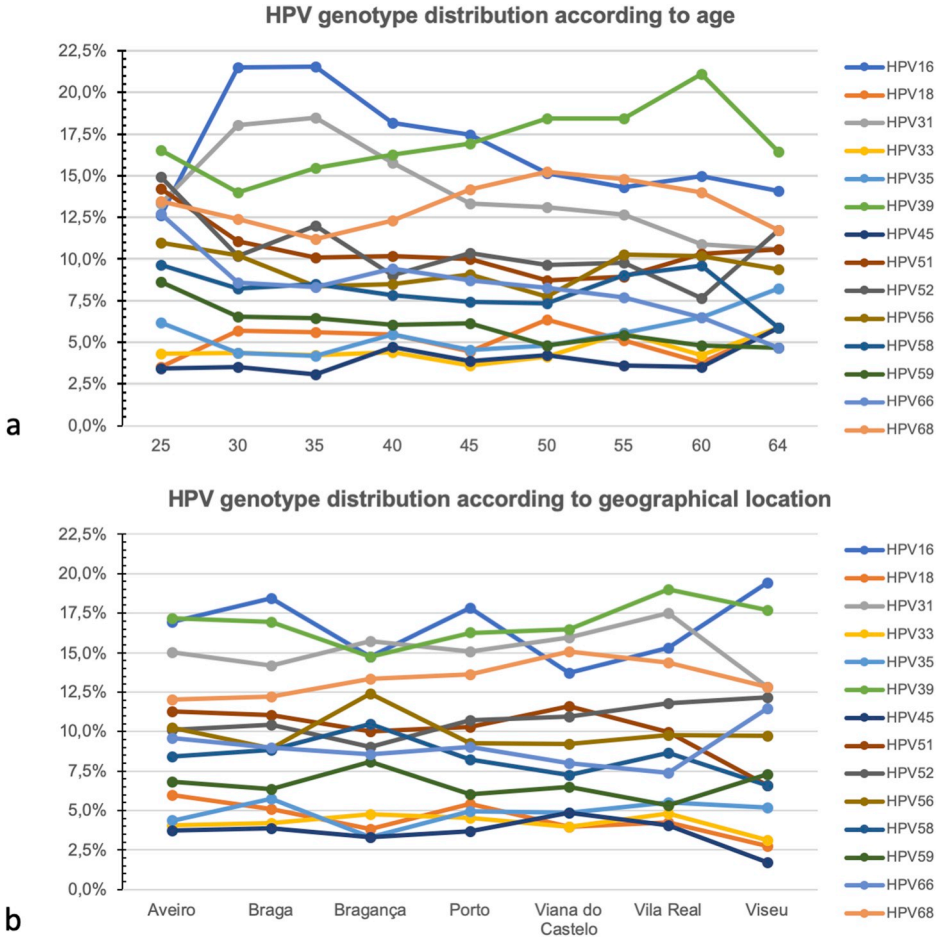


Fig. 3. High-Risk HPV genotypes distribution according to age distribution (a) and geographic location (b).

found is similar to the expected prevalence in the majority of European countries [36] and the preliminary analysis of data from the screening program from 2018 discloses a prevalence of approximately 14% (unpublished data). Moreover, we observed that HR-HPV infection varied according to age, ranging from a maximum of 17.1% at age 25 to a minimum of 6.2% at age 64, as expected. Another interesting data from our study was the prevalence of simultaneous infections by two or more HR-HPVs (25.7%), which ranged from 31.0% at age of 25 to 16.5% at age of 64. These data corroborate published evidence that HPV infections are extremely more frequent in young women and tend to become much lower after the age of 45 [6,36,37]. Furthermore, it is important to analyze the potential impact of this data since it is unclear what is the biological behavior of multiple infections and its outcome.

Regarding the HPV genotypes, we found that the most prevalent were HPV-16 (17.5%), HPV-39 (16.7%), HPV-31 (15.0%), HPV-68 (13.2%), HPV-52 (10.7%) and HPV-51 (10.6%). These data are distinct from those reported by Pista et al. in the CLEOPATRE study which disclosed that the most common HPVs in Portuguese population were HPV-16 (19.7%), HPV-31 (11.8%), HPV-53 (11.8%), HPV-51 (9.8%) and HPV-66 (8.6%) [34]. These differences are likely due to the fact that populations analyzed were different in their characteristics, such as age distribution and especially because there is a contribution of cases from all over the country (especially bigger cities) while in our study we have women from all over the North region of Portugal. Furthermore, it should be emphasized that the number of cases tested in our study largely exceeds that of Pista et al. and derives from a community-based, organized, screening program and not from selected women. Felix et al. developed a study to identify the HR-HPVs that were associated with cervical cancer between 1928 and 2005 in Portugal, showing that in our population the most common HPVs in ICC cases were HPV-16 (58.2%), HPV-18 (9.2%), HPV-33 (6.2%), HPV-45 (4.7%) and HPV-31 (4.4%) [38]. The most recent report regarding HPV-genotype distribution in women with normal cytology shows that worldwide, the five most prevalent types are HPV-16, followed by HPV-52, HPV-31, HPV-53 and HPV-18, and in developed countries are HPV-16, HPV-53, HPV-31, HPV-52, HPV-51, in this specific order [36]. An important remark is that despite HPV-53 is assuming a significative prevalence worldwide and in developed countries, as we have also shown in one study [39], nevertheless, this HPV genotype is not included in our HPV test and therefore, we cannot compare its results. Interestingly, we found that in our population, HPV-18 is relatively infrequent, and the high prevalence of HPV-39 was surprising, despite similar results in the literature [28]. Our study also showed that HPV-16 predominated in women 30–45 years, while others predominate in women from 50 to 64 years of age.

Despite HR-HPV test is starting to be used in cervical cancer screening, a wide number of countries are using tests that identify the HR-HPVs but only genotype HPV-16 and HPV-18. The strategy of specifically identifying any and all HR-HPV genotypes in a single sample is, in our viewpoint, advantageous: on one hand, it may improve the management of cases in which HR-HPV other than HPV-16/18 are detected; and it generates data by extended genotyping which provides invaluable information concerning the relative frequencies and dynamics of specific HR-HPV infection in the target population, enabling improved assessment of the actual and future efficacy of vaccination programs and forecast changes in infection patterns.

This is the largest study on HR-HPV genotyping performed as the first-line test in an organized Cervical Cancer Screening in European

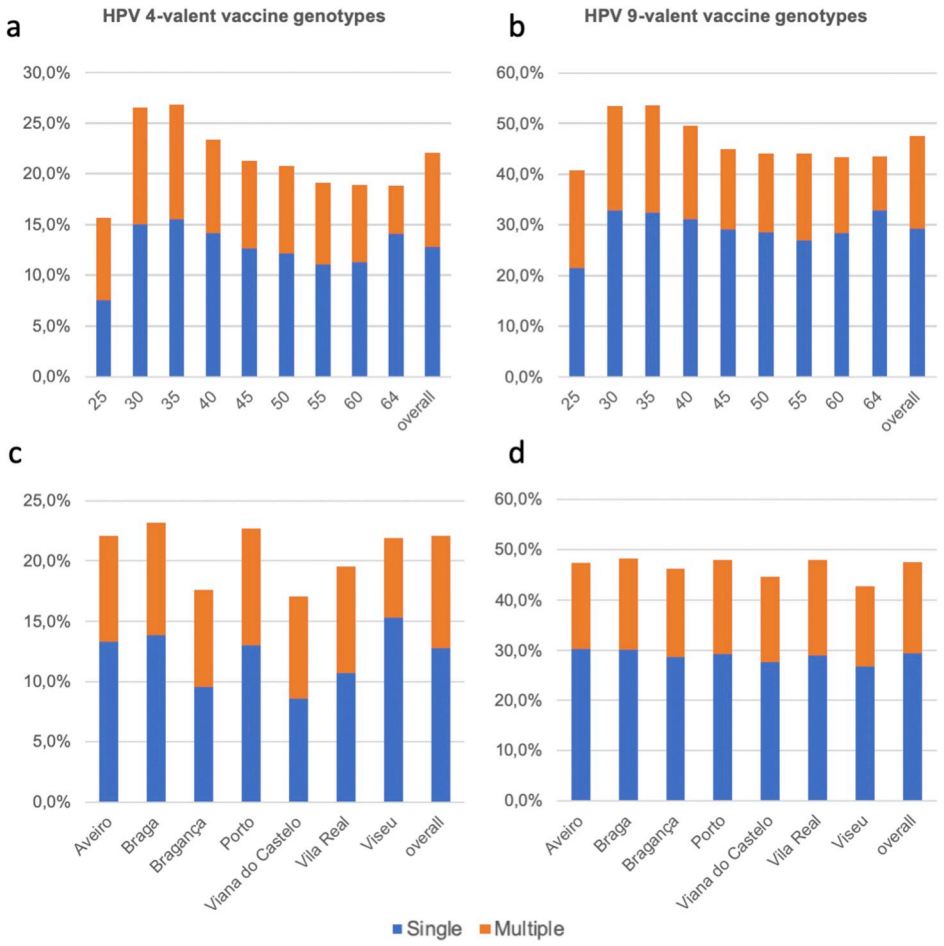


Fig. 4. High-Risk HPV genotypes in vaccines and age distribution (a and b) and geographic location (c and d).

populations including a total of 105,458 women over a period of 17 months. In our study, the overall prevalence of HR-HPVs in women from the Northern region of Portugal was of 10.2%, and the most common HR-HPVs genotypes are HPV-16, HPV-39, HPV-31, HPV-68, HPV-52, and HPV-51. Age-related HR-HPV genotype distribution shows that HPV-16 predominated in women from 30 to 45 years whereas HPV-39 predominated in women from 50 to 65 years. Our study shows that HPV16/18 which are included in the 4-valent HPV vaccine are present in only 22.1% of HR-HPV positive women, furthermore, HPV16/18/31/33/45/52/58, which are the genotypes present in 9-valent HPV vaccine also represent only 47.6% of HR-HPV positive women. These results estimate HR-HPV infection dynamics and provide critical data with obvious implications regarding cervical cancer screening, especially in vaccine women, and HPV-vaccination policies.

Finantial disclosure

All authors declare that they have no competing financial interests.

Conflicts of interest

All authors declare no conflict of interest.

Acknowledgements

Authors would like to acknowledge all the collaborators of the Regional Cervical Cancer Screening Program of Northern Portugal.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pvr.2019.100179.

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