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 positive 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...
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 (Seegen 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 (χ²) 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 3.

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-HPV genotypes were HPV-16, HPV-39, HPV-31 and HPV-52, representing 89.8% of all genotypes detected.

Table 1: High-Risk HPV genotypes distribution.

| HPV 9-valent vaccine |
|----------------------|
| HPV4/6/11/16/18/31/33/35/45/52/58
| Non-vaccine genotypes | 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 infections.
Fig. 1. High-Risk HPV genotypes distribution in population.

Fig. 2. High-Risk HPV prevalence according to age distribution (a) and geographic location (b).
as the primary test (higher sensitivity) followed by a triage of HPV-

new strategies for cervical cancer screening support the use of HPV test

the HPV test is around 95% compared with 55% for cytology [23]. The

studies have shown that in women aged 30–69 years the sensitivity of

with cytology, despite having a lower specificity [18,23–25]. Indeed,

testing is more sensitive for identifying women with CIN 2 + compared

ultimately ICC is slow and takes years or even decades [22]. This slow

tion from persistent infection to low-grade squamous intraepithelial

velop premalignant lesions that may evolve into ICC [22]. The evolu-

women, however, only a small percentage of infected women will de-

4. Discussion

HPV genotypes (HPV-16, -39, −31 and −68) were the same in all

gEOGRAPHICAL locations – Fig. 3b and Table 2. Results also show that

HPV16/18, which are present in HPV 4-valent vaccine, represent 22.1%

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 geo-

HPV genotypes present in the

50, 55–60, 60–62, and 64–66); age groups 25 (24–27), 30 (28–32), 35 (33–37), 40 (38–42), 45 (43–47), 50 (48–52), 55 (53–57),

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),

HR-HPV other, n = 688

HPV18 + other,

HPV-39, n = 1411

HPV-59, n = 669

HPV-58, n = 891

HPV-57, n = 1139

HPV-56, n = 998

HPV-55, n = 10665

HPV-54, n = 405

HPV-53, n = 1199

HPV-52, n = 268

HPV-51, n = 1126

HPV-50, n = 25

HPV-49, n = 25

HPV-48, n = 25

HPV-47, n = 25

HPV-46, n = 25

HPV-45, n = 25

HPV-44, n = 25

HPV-43, n = 25

HPV-42, n = 25

HPV-41, n = 25

HPV-40, n = 25

HPV-39, n = 25

HPV-38, n = 25

HPV-37, n = 25

HPV-36, n = 25

HPV-35, n = 25

HPV-34, n = 25

HPV-33, n = 25

HPV-32, n = 25

HPV-31, n = 25

HPV-30, n = 25

HPV-29, n = 25

HPV-28, n = 25

HPV-27, n = 25

HPV-26, n = 25

HPV-25, n = 25

HPV-24, n = 25

HPV-23, n = 25

HPV-22, n = 25

HPV-21, n = 25

HPV-20, n = 25

HPV-19, n = 25

HPV-18, n = 25

HPV-17, n = 25

HPV-16, n = 25

HPV-15, n = 25

HPV-14, n = 25

HPV-13, n = 25

HPV-12, n = 25

HPV-11, n = 25

HPV-10, n = 25

HPV-9, n = 25

HPV-8, n = 25

HPV-7, n = 25

HPV-6, n = 25

HPV-5, n = 25

HPV-4, n = 25

HPV-3, n = 25

HPV-2, n = 25

HPV-1, n = 25

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),

4. Discussion

The infection by HR-HPV is extremely common in sexually active

women, however, only a small percentage of infected women will de-

velop premalignant lesions that may evolve into ICC [22]. The evolu-

tion 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-
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 predominated 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
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 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.

Financial 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.

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

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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