CIN 2+ High-Risk HPV in a portuguese unit: the importance of single and multiple infections

HPV de alto risco em CIN 2+ numa unidade portuguesa: a importância das infeções simples e múltiplas

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Abstract

Overview and Aims: Cervical cancer is one of the most prevalent and deadly cancer, caused by the persistent infection of High-Risk Human papillomavirus (HR-HPV), mostly HPV 16 and 18. In Portugal, the CLEOPATRE group observed an HPV prevalence in Cervical Intraepithelial Neoplasia grade 2 or worse (CIN 2+) of 97.9%, being HPV 16 the most frequent. We aim to add information about HR-HPV distribution in the Portuguese population and analyse the histological outcome with simple and multiple HR-HPV infections. Study design: Retrospective observational study from the clinical database of our Unit. Population: Patients observed in the Colposcopy and Laser Unit of our hospital from September 2009 to June 2013.

Methods: All patients with CIN2+ and HR-HPV genotype detection were selected. The collected data included histological and cervical citology results, HR-HPV type(s), age and parity. The HPV genotype test used was AmpliSens® HPV HCR genotype-FRT and if performed after the procedure with CIN 2+ result, the patient was excluded.

Results: Of the 122 women selected, eleven were excluded. From the remaining 111 cases CIN 2 and CIN 3 had similar prevalence (52 and 54 cases), and there were 5 cases worse than CIN 3 (microinvasive or invasive cervical cancer and adenocarcinoma in situ). Women in the CIN 3 group were older than those in the CIN 2 group (p=0.001). The most frequent HR-HPV types detected were 16, 58, 31, 51 and 52. Multiple infections were more frequent in CIN 2 and single infection in CIN 3 (p=0.02).

Conclusions: HPV 16 was more prevalent in our study than previous reported in Portugal, and HPV 18 was detected less frequently than expected. The inverse relation between the increase in HR-HPV types (multiple infections) and the increasing cervical disease grade was significant.

Keywords: Cervical cancer; Cervical intraepithelial neoplasia; Human papillomavirus; Portugal; HPV genotypes.

OVERVIEW AND AIMS

Cervical cancer is the fourth most prevalent cancer in women worldwide and, in 2012, motivated about 266,000 deaths[1,2]. In Portugal[3], cervical cancer represents the second most frequent and deadly cancer among women aged 15-44 years, with an annual incidence rate of 17.2/100,000.

It is globally assumed that all cervical cancers are caused by human papillomavirus (HPV) persistent infections with high-risk (oncogenic) types[4], with HPV 16 and 18 being responsible for 70% of the cervical cancers[5]. The HPV types are classified according to their ability of generate benign or malignant lesions, in low or high oncogenic risk. Among the high risk HPV (HR-HPV), beyond 16 and 18, are at least other 13 types, strongly associated with the evolution of the cervical lesions to high-grade and invasive cancer[6].

The HR-HPV prevalence in invasive cervical cancers has been established, with the identification of HPV 16, 18, 31, 33, 35, 45, 52 and 58 as the main HPV types involved[7]. This characterization has an important role in preventive strategies, such as HPV vaccination, and in the selection of HR-HPV tests[7]. The CLEOPATRE group, in the Portuguese HPV epidemiologic characterization study, found an infection

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prevalence of 19.4%, 76.5% of which were HR-HPV[8]. In cervical intraepithelial neoplasia (CIN) 2/3 and invasive cervical cancer (ICC), the HPV prevalence was 97.9% (96.1% HR-HPV), with HPV 16 as the most common HPV identified[9].

The studies that analysed single and multiple HR-HPV infections report a decreasing trend in multiples infections as the lesion grade increases, although without statistical significance[9,10]. In the Portuguese CLEOPATRE study, multiple infections were present in 11.2% of the HPV positive group, decreasing with the increase of the cervical disease grade (14.8% in CIN 2, 10.0% in CIN 3 and 7.8% in ICC)[9].

The aim of this study is to contribute to the knowledge of HR-HPV present in the Portuguese population with cervical dysplasia, referred to a specialised colposcopy unit. Additionally, we aimed to analyse the relation of single and multiple HR-HPV infections and histological outcome.

METHODS

This retrospective work was developed considering data from the clinical database of our Colposcopy and Laser Unit, collected between September 2009 (when the Unit was created) and June 2013 (46 months). The patients gave an oral consent to be included in the database (with the ensuing studies), and to perform all the complementary diagnostic exams, as total confidentiality of all personal data retrieved was ensured. All patients with histological diagnosis of CIN 2 or superior cervical disease grade (CIN2+) and HR-HPV genotype analysis were selected.

All histological samples were evaluated by the hospital staff pathologists. When there was a histological discrepancy in the same patient (e.g. cervical biopsy vs. excisional procedure) the patient was classified according to the worse result. When the HR-HPV genotype test was not made shortly before or at the time of the procedure that conducted to a histological diagnosis, the patient was excluded (e.g. HR-HPV tests in the follow-up period after an excisional or ablative procedure).

Besides histological diagnosis and HR-HPV genotype results, demographic data (age, parity) and cervical cytology results, classified according to the 2001 Bethesda system[11], were collected. The abnormal cervical cytology results were the main referral cause, so their results came also from other institutions.

A) HPV genotype analysis

The commercially available AmpliSens® HPV HCR genotype-FRT (InterLabService, Russia) kit was used for HPV DNA genotyping by an external lab (Genomed, Lisbon, Portugal). This PCR kit detects and differentiates high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59.

B) Statistical analysis

Continuous variables were analysed considering mean±standard deviation, minimum and maximum. Categorical variables are described by the absolute and relative frequencies of its variables. Differences between independent categorical variables were tested using Pearson's

Chi-Squared Test. Student's t-Test for independent samples was used to assess the statistical significance of differences between two independent continuous variables.

All analyses were performed with a 95% significance level.

RESULTS

From the 1098 women observed in the Unit in the selected period, 122 had CIN 2+ and positive HR-HPV genotype test. From these, 11 were excluded due to HR-HPV test done in the follow-up.

A total of 111 women were selected, aged 21-57 years old, characterised in Table I. CIN 2 and CIN 3 had approximately the same representation in the study group (52 vs. 54 cases). The reduced number of other histological diagnosis (ICC, Microinvasive Cervical cancer and Adenocarcinoma in situ) made it impossible to establish correlations with other factors, so the statistical analysis was only performed considering CIN 2 and 3.

The CIN 3 group was older than the CIN 2 group (mean age 37.83±8.38 vs 32.59±7.37 years, p=0.001), while the CIN 2 group had a larger proportion of women below 35 years and the CIN 3 group a larger proportion of women with 35 years or more (p=0.001).

There were no statistical differences between women aged below 35 years old and those with 35 years old or more concerning HR-HPV single infections (46.4% vs. 63.6%) and multiple HR-HPV infections (53.6% vs. 36.4%) (p=0.068).

Women with CIN 2 had a low-risk alteration in cervical cytology (ASC-US, LSIL) in 69.3% of cases (36/52) and, in the CIN 3 group, 57.4% (31/54) had a

TABLE I. CHARACTERIZATION OF THE STUDY SAMPLE (N=111)

Age, years, mean (SD)	35.31 (8.19)	
Age group, n (%)	< 35 years	56 (50.5)
	≥ 35 years	55 (49.5)
Parity, n (%)	Nuliparous	50 (45.0)
	Multiparous	59 (53.2)
	Data missing	2 (1.8)
Histological samples, n (%)	Cervical biopsy	35 (31.5)
	Excisional procedure (conization)	74 (66.7)
	Histerectomy	2 (1.8)
Previous cervical citology* , n (%)	NILM	2 (1.8)
	ASC-US	10 (9.0)
	ASC-H	9 (8.1)
	LSIL	40 (36.0)
	HSIL	44 (39.6)
	AGC	2 (1.8)
	AIS	1 (0.9)
	Data missing	3 (2.7)
Histological diagnosis, n (%)	CIN 2	52 (46.8)
	CIN 3	54 (48.6)
	Microinvasive Cervical Cancer	3 (2.7)
	ICC	1 (0.9)
	Cervical Adenocarcinoma in situ	1 (0.9)

SD: Standard Deviation; CIN: Cervical Intraepithelial Neoplasia; ICC: Invasive Cervical Cancer ; *- Cervical citology classified by Bethesda classification of cervical citology[11]

previous HSIL (p<0.001).

The most frequent HR-HPV types were 16 (58 cases), 58 (25 cases), 31 (23 cases), 51 (18 cases) and 52 (16 cases) (Table II).

Multiple HR-HPV infections were observed in 45% (50/111) of the study population, which had the mean number of HR-HPV of 1.71 types (±1.01). In CIN 2 group 57.7% (30/52) had multiple HR-HPV infections, while the CIN 3 group had mainly single infections (64.8%, 35/54), settling a statistically significant difference (p=0.02). The distribution of the study population by number of HR-HPV is presented in Table III.

The analysis of each HR-HPV type (Figure 1) shows that some HR-HPV types were only present in co-infections, like HPV 39, 56 e 59.

TABLE II. HR-HPV DISTRIBUTION

HR-HPR genotype	n
HPV 16	58
HPV 18	8
HPV 31	23
HPV 33	11
HPV 35	11
HPV 39	10
HPV 45	6
HPV 51	18
HPV 52	16
HPV 56	2
HPV 58	25
HPV 59	2

DISCUSSION

The knowledge of HR-HPV epidemiology in a certain geographical area ideally needs a designed prospective study and a uniform process that is difficult to obtain without well-developed health systems, as in the Scan-

TABLE III. HR-HPV

Number of HR-HPV	n (%)
1	61 (55.0)
2	32 (28.8)
3	11 (9.9)
4	4 (3.6)
5	2 (1.8)
6	1 (0.9)

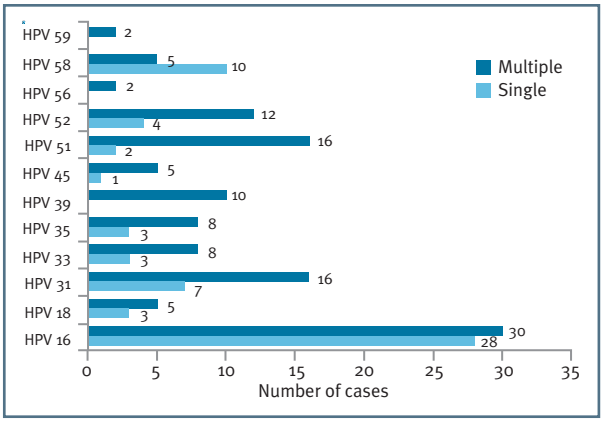

FIGURE 1. Simple and multiple infections by HR-HPV type

dinavian countries. However, it is also possible to obtain reliable data merging different studies performed in an area or country, to establish the prevalence of virus genotypes. In our study we aimed to contribute with information from a specific Portuguese population to the knowledge of Portugal HPV genotype distribution.

HPV 16, 58, 31, 51, 52, 33 and 35 were the most frequent HR-HPV genotypes involved in high-grade CIN and cervical cancer, and HPV 16 stands out, present in more than half of the situations. This predominance was previous described in Portuguese studies[9,12-14], only exceeded in the Medeiros et al study[12], where HPV 16 rate was 76%. Worldwide data[15] are consistent with the majority of the Portuguese studies, but the HPV 16 proportion found in our study is slightly larger. Conversely to Nobre *et al*[13], HPV 18 was not one of the most frequent types in our population, a difference that may be explained by the high-grade lesions group dimension of each study.

Multiple infections were more frequent in CIN 2 than in CIN 3 (57.7% vs. 35.2%), as expected from the results of two studies conducted in Portugal[9,16], that demonstrated a decrease in multiple infections with increasing cervical disease grade. However, our results had a statistical significant difference, like the relation between the increasing cervical grade disease with increasing age, exposed in previous Portuguese studies[13,14]. Beca *et al*[14] researched the HPV genotypes prevalence in HSIL, and observed a rate of multiple infections closer to ours (36.9%); accordingly Pista *et al*[16], although considering multiple infections independently of being HR or Low Risk HPV types, found multiple infections in 37.6% of CIN 2+ cases.

Our work main limitation came from a selection bias: the data came only from one center, and the Unit is part of a private hospital, although it is well-recognised in cervical disease. Other limitation came from the lack of systematic request of HR-HPV-genotype that could enlarge our sample but, as this was a retrospective study, there is no clinical applicability of HPV genotyping every high-grade cervical lesion, and it was not our aim to evaluate the prevalence of different HPV-genotypes, the error could be minimized. Finally, the execution of HR-HPV genotype test by an external institution can increases potential bias of the results.

Further studies with representative samples of the Portuguese population can optimize the characterization of HPV genotype distribution in our country. The main advantage of this knowledge is the improvement of accuracy in preventive strategies, such as HPV vaccination as Nobre et al study[13] results suggested HPV 16/18 vaccines could prevent about 70% of cervical cancer cases in Portuguese populations.

In conclusion, this study demonstrated that HPV 16 can be even more relevant than previously described in high-grade cervical lesions, with HPV 18 playing a minor oncogenic role in our group. We also confirmed the decreasing trend in multiple infections with increasing cervical disease grade, and this information can help, in the future, to understand the role of multiple infections in the oncogenic process. Ethnicity, smoking habits and immunosuppression, as pointed out by Pista et al [17], are important risk factors that should be aimed in futures studies.

HPV-genotyping tests were frequently used in the clinical practice in the period considered, although the current state of art privileges the HPV 16/18 detection tests.

ACKNOWLEDGMENTS

The authors thank GlaxoSmithKline for their financial support for

the conduction of the statistical analysis of this article.

We thank the other Unit clinicians, doctors Adelaide Vitorino and Bárbara Bettencourt, for their care in the clinical database data-entry.

We thank doctors Ana Catarino, Pedro Oliveira and Eveline Mendonça, from the Pathology Department of our hospital, for the careful histological and citological analysis of our patients.

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