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HPV distribution in cervical cancer in Portugal. A retrospective study from 1928 to 2005

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ABSTRACT

Objectives: To determine human papillomavirus (HPV) types in invasive cervical cancer in Portugal.
Methods: Cases diagnosed at the Instituto Português de Oncologia de Lisboa de Francisco Gentil from the year 1928 to 2005 were selected for HPV DNA detection and genotyping using SPF10/DEIA/LiPA25 system.
Results: Of the 1214 samples that were considered appropriate for HPV detection, 714 (58.8%; 95% CI: 56.0–61.6%) were positive for HPV DNA. This detection rate varied being lower in the first 3 decades (31.3%; 50.1%; 46.5%) and higher in the last decades (77.4–95.1%). This difference was due probably to the fixative used in the first three decades. The five most common types identified among HPV positive cases were HPV16 (58.2%), HPV18 (9.2%), HPV33 (6.2%), HPV45 (4.7%) and HPV31 (4.4%). Multiple infections were detected in 2.8% of the cases. HPV16 and 18 accounted for 67.4% of infections. There were no statistically significant changes of these types over the studied period. An increase at patient's age at diagnosis was observed in the last decades ($p < 0.001$).
Conclusion: HPV16 and 18 accounts for almost 70% of cervical cancers in all 9 decades studied and support data that effective vaccination against these 2 types will reduce the cervical burden in Portuguese women.

1. Introduction

Human Papillomavirus (HPV) infection is causally linked to invasive cervical cancer (ICC) [1]. In Portugal the incidence of ICC is high (age-standardized rate of 10.8 cases per 100,000 women), and mortality is estimated to be 4.9 deaths per 100,000 women [2]. Accordingly with the results of the CLEOPATRE Portuguese study, overall HPV prevalence was estimated to be 19.4%, in the general female population [3]. This was the first large study on HPV infection in the general population and the extrapolated prevalence for HPV infection was higher than the one obtained in other neighbor countries such as Spain, with HPV prevalence in general female of a 14.3% [4]. The higher incidence and mortality of cervical carcinoma in Portugal could be related to a higher prevalence of HPV infection and most probably related with the absence of a National Cervical Cancer Screening Programme, although planed, is still not implemented. In Portugal, in 2008, quadrivalent HPV vaccination was included in the National Immunization Plan for girls aged 13, having an overall coverage rate of 88 and 93% [5].

There is scarce data regarding HPV distribution in ICC in the Portuguese population [6,7]. Large international series demonstrate that, although a small number of different types are more prevalent than others, there are some geographical differences regarding HPV type distribution [8]. Our aim was to describe the HPV genotype distribution in ICC in Portugal, to analyze differences in along nine decades (from 1928 to 2005). This study is part of a larger study, RIS HPV TT study [9], whose objective was to describe the HPV genotype distribution in invasive cervical cancer worldwide.

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2. Material and methods

1309 cases with the diagnosis of ICC and treated in the Instituto Português de Oncologia de Lisboa de Francisco Gentil (IPOLFG) were identified. Between 1928 until 1939, all available cases were retrieved, and from the year 1940 to the year 2005, consecutive cases diagnosed during the months of March and April on even-years of the decade were selected. All tumor samples were fixed and included in paraffin blocks. The fixative fluid varied along the decades. In the period from 1928 to 1944, almost all cases were fixed in Bouin. After that year of 1944, all cases were fixed in formalin, being buffered formalin used beyond 2000. After histological verification at Catalan Institut of Oncology-ICO (Spain), a total of 1214 cases were finally selected for HPV detection and genotyping.

All clinical charts were reviewed to confirm tumor site and patient age at the time of diagnosis.

2.1. Pathology and HPV detection protocol

Tumor samples were re-embedded in paraffin blocks (when it was needed) and tested at Catalan Institute of Oncology-ICO (Spain) for HPV DNA. All the methodology has been previously described in de Sanjosé et al. [9]. Briefly, four sections were done (first and last were stained with haematoxylin and eosin (H&E) and used to confirm the diagnosis and to evaluate the adequacy of the tissue to retrieve DNA and the two in-between collected to an eppendorf tube for HPV detection with the adequate precautions of contamination. As control, blocks from all years corresponding to those of tumor samples from other locations not HPV related (appendix, breast, and thyroid) were used.

Proteinase K (Sigma) digestion for 16 h at 56 °C temperature was used to obtain a tissue lysate containing DNA from the paraffin inner 5 μm sections. SPF-10 PCR was performed using 10 μl of a 1:10 dilution with water of the tissue digest in a final reaction volume of 50 μl. The amplified PCR products were tested using a hybridization probe with a cocktail of conservative probes recognizing at least 54 mucosal HPV types in a microtitre plate format for the detection of HPV DNA. Optical densities (OD_{450}) were read on a microtitre plate reader. HPV DNA positive samples were subsequently analyzed by the reverse hybridization line probe assay ($LiPA_{25}$) (version 1; produced at Labo Biomedical Products Rijswijk, The Netherlands), a technique that detects 25 high risk and low risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, 74). The sequence variation within the SPF-10 primers allows the recognition of the different HPV types, except for the types 68 and 73 as theirs interprimer regions are identical and cannot be distinguished on this test. The positive hybridization on the strips is visualized as a purple band by means of a precipitating color substrate on the probe site. Specimens that were HPV DNA positive by DEIA (DNA-Elisa) but did not hybridize with any of the 28 probes in the $LiPA_{25}$ were further analyzed by sequencing. Further, specimens that were HPV 68 or 73 or HPV 39 or HPV 68 or HPV 73 were also sequenced to discriminate the specific type as described in de Sanjosé et al. [9]. HPV detection was analyzed by histological classification (squamous cell carcinoma, adenocarcinoma or other), patient age, and time at diagnosis. Multiple infections were counted as weighted proportional attribution model as described in de Sanjosé et al. [9]. Linear trend test was used to evaluate changes over time. Statistical significance cut point was set at 2-sided 0.05.

3. Results

A total of 1309 cases with the diagnosis of ICC were retrieved and 1214 cases were finally selected for HPV detection and genotyping. 95 cases were discarded for further analysis due to high proportion of necrosis, non-invasive tumor confirmed on the H&E slides and poorly preserved paraffin block. Table 1 shows the distribution of ICC cases by time at diagnosis and age and histological characteristics of the cases included in the HPV analysis. The distribution of tumor samples by decades showed that approximately half of the cases (56.3%) were from the 1930-39 decade and the remaining decades had similar representation showing proportions from 3% to 9%. Patient's mean age at diagnosis was 53 years old and ranged between 20 and 94 years. In 54 cases, age was unknown (not registered in the clinical files). Mean age of patients at the date of diagnosis showed significant differences between decades. Mean age between 1920 and 1959 was statistically lower than in more recent decades (7 years; p-value < 0.001).

Regarding the morphological features, squamous cell carcinoma (SCC) was the most common histological type corresponding to 1126 cases (92.8%), followed by adenocarcinoma (63 cases; 5.2%), adenosquamous carcinoma (7 cases; 0.6%) and the remaining 18 (1.5%) were tumors classified as other types (10 undifferentiated and 8 neuroendocrine carcinomas). SCC was the most common histological type over all decades (more than 90%).

The presence of HPV DNA in cervical carcinomas was detected in 714 cases (overall detection rate was 58.8%; 95% confidence interval-CI: 56.0–61.6%). The higher detection rate was obtained in last decade (95.4%) and lower rate from older cases of 1920–1030 (35.3%). A low detection rate of HPV DNA was also obtained in cases diagnosed between 1930 and 1949 (around 50%). In the subsequent decades, HPV DNA detection was higher ranging from 77.4% to 95.1%.

Single, multiple and unknown types were identified in a 95.7%, 2.8% and 1.5%, respectively. Table 2 shows the detection of HPV by decade, HPV detection increased with the year of diagnosis (p trend test < 0.05). Table 3 shows HPV type distribution by decades, among HPV positive cases. The most common DNA viruses were HPV16 (58.2%) and 18 (9.2%) with an overall prevalence of 67.4%, followed by HPV33 (6.2%), HPV45 (4.7%), HPV31

Table 1
Clinical data by decade (number of cases, age and histological diagnosis).

Decades	No. of cases (%)	Mean age (range) [age registry missing]	Histology SCC/ non SCC
1928–29	112 (9.2%)	48 (24–74) [2]	106/6
1930–39	684 (56.3%)	50 (25–85) [34]	623/61
1940–49	86 (7.1%)	50 (29–76) [4]	83/3
1950–59	88 (7.2%)	50 (28–77) [6]	83 /5
1960–69	66 (5.4%)	57 (31–85) [7]	65/1
1970–79	50 (4.1%)	57 (20–85) [0]	47/3
1980–89	56 (4.6%)	56 (29–84) [1]	52/4
1990–99	31 (2.6%)	59 (31–94) [0]	26/5
2000–05	41 (3.4%)	57 (32–88) [0]	41/0
Total	**1214 (100%)**	**53 (24–94) [54]**	**1126/88**

Legends: "SCC" – Squamous Cell Carcinoma; "non SCC" – non Squamous Cell Carcinoma.

Table 2
HPV detection by decade.

	1928–1929	1930–1939	1940–1949	1950– 1959	1960– 1969	1970–1979	1980– 1989	1990– 1999	2000–2005	Total
No. of cases	112	684	86	88	66	50	56	31	41	1214
HPV DNA positive (N;%)*	35 (31.3%)	343 (50.1%)	40 (46.5%)	82 (93.2%)	58 (87.9%)	46 (92.0%)	47 (83.9%)	24 (77.4%)	39 (95.1%)	714 (58.8%)
HPV DNA positive (95% CI)	22.8–40.7	46.3–54.0	35.7–57.6	85.7–97.5	77.5–94.6	80.8–97.8	71.7–92.4	58.9–90.4	83.5–99.4	56.0–61.6
No of Single HPV	34	325	40	80	55	44	46	22	37	683
No Multiple HPV	1	8	0	2	3	2	1	1	2	20
No of HPV Undetermined	0	10	0	0	0	0	0	1	0	11

Legend: "*": N - number of cases; % percent; "95%CI": 95% confidence interval.

Table 3
Distribution of HPV DNA types by decades.

HPV type*	1928–29		1930–39		1940–49		1950–59		1960–69		1970–79		1980–89		1990–99		2000–05		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
11	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**1**	**0.1**
16	24	68.6	188	54.8	24	60.0	48	58.5	36	61.8	30	65.2	26	55.0	16	66.7	24	61.5	**415**	**58.2**
18	2	5.7	39	11.4	8	20.0	4	4.9	4	6.7	3	6.5	4	8.8	0	0.0	1	2.6	**66**	**9.2**
30	0	0.0	2	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.6	**3**	**0.4**
31	1	2.9	15	4.4	2	5.0	3	4.0	3	5.2	2	4.3	1	2.1	2	8.3	2	5.1	**31**	**4.4**
33	0	0.0	19	5.5	1	2.5	9	10.6	7	12.1	3	6.5	3	6.4	2	8.3	1	2.6	**45**	**6.2**
35	2	5.7	4	1.3	2	5.0	2	2.4	0	0.0	1	2.2	2	4.3	2	8.3	4	10.3	**19**	**2.6**
39	0	0.0	7	2.0	0	0.0	2	2.4	1	2.2	0	0.0	1	2.1	0	0.0	0	0.0	**11**	**1.6**
*39**	0	0.0	2	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**2**	**0.3**
42	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.6	**1**	**0.1**
45	3	8.6	19	5.5	0	0.0	5	6.1	3	5.2	0	0.0	2	4.3	0	0.0	2	5.1	**34**	**4.7**
51	0	0.0	4	1.2	1	2.5	2	2.4	0	0.0	1	2.2	0	0.0	1	4.2	0	0.0	**9**	**1.3**
52	0	0.0	9	2.5	0	0.0	4	4.9	0	0.0	2	4.3	1	2.1	0	0.0	2	5.1	**17**	**2.4**
53	0	0.0	1	0.3	0	0.0	0	0.0	1	1.7	0	0.0	0	0.0	0	0.0	0	0.0	**2**	**0.3**
56	0	0.0	4	1.2	2	5.0	0	0.0	0	0.0	1	2.2	1	2.1	0	0.0	0	0.0	**8**	**1.1**
58	2	5.7	5	1.5	0	0.0	2	2.4	1	1.7	1	2.2	0	0.0	0	0.0	0	0.0	**11**	**1.5**
59	0	0.0	5	1.5	0	0.0	0	0.0	0	0.0	1	2.2	4	8.5	0	0.0	0	0.0	**10**	**1.4**
66	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	1	2.1	0	0.0	0	0.0	**2**	**0.3**
67	0	0.0	1	0.3	0	0.0	0	0.0	1	1.7	0	0.0	0	0.0	0	0.0	0	0.0	**2**	**0.3**
68	0	0.0	1	0.3	0	0.0	1	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**2**	**0.3**
*68**	1	2.9	3	0.9	0	0.0	0	0.0	0	0.0	0	0.0	1	2.1	0	0.0	0	0.0	**5**	**0.8**
69	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**1**	**0.1**
70	0	0.0	2	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	**2**	**0.3**
73	0	0.0	0	0.0	0	0.0	0	0.0	1	1.7	1	2.2	0	0.0	0	0.0	0	0.0	**2**	**0.3**
91	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.6	**1**	**0.1**
Undetermined	0	0.0	10	2.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	4.2	0	0.0	**11**	**1.5**

Legend: "*": single infections and multiple infections counted following a weighting algorithm, "%" are estimated among HPV DNA positive cases; 39* – 39 or 68 or 73 HPV types; 68* – 68 or 73 HPV types; "Undetermined" – unknown HPV type or types.

(4.4%), HPV35 (2.6%), HPV52 (2.4%), and HPV39 (1.6%). In 10 cases a low risk HPV type was detected, but only in 4 cases there was a low risk HPV type detected as single infection (one HPV42, one HPV11 and two HPV70), and in the rest, these types were found as part of co-infection with other types, most of them high risk types (HPV6 and HPV16 in two cases; and HPV74 and HPV16 in four other cases). Regarding time at diagnosis, no trends neither for HPV16 nor HPV18 over time were observed. The average age at diagnosis showed differences by HPV type. Mean age of patients with HPV18 and HPV45, as single infection was 49.1 and 47.1 years old, respectively. In contrast, mean age of patients infected by HPV16, HPV33 and HPV31 was 51.2, 57.0 and 55.6 years old, respectively. Patients, in this last group, were significantly older than patients harboring HPV types 18 and 45.

Table 4 shows the HPV type distribution by histological type. In adenocarcinoma cases, the second most common histological type (5.2%) after squamous cell carcinoma, HPV detection rate (31.7%) was very low compared to the previous type (60.5%). The most frequently HPV types detected in the 20 HPV positive adenocarcinomas, were HPV16 (55.0%), HPV18 (35.0%), and HPV45 (10.0%). The five most common types identified in SCC were, HPV16 (58.1%), HPV18 (8.2%), HPV33 (6.5%), HPV45 (4.7%), and HPV31 (4.6%).

Table 4
Distribution of the more common HPV types by histology.

	SCC		ADC		ADSCC		OTHER		Total	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
*Cases analyzed**	1126	92.8	63	5.2	7	0.6	18	1.5	1214	100
*HPV DNA positive***	681	60.5	20	31.7	2	28.6	11	61.1	714	58.8
HPV type***										
16	395	58.1	11	55.0	1	50.0	8	72.7	415	58.2
18	56	8.2	7	35.0	1	50.0	2	18.2	66	9.2
33	44	6.5	0	0.0	0	0.0	0	0.0	45	6.2
45	32	4.7	2	10.0	0	0.0	0	0.0	34	4.7
31	31	4.6	0	0.0	0	0.0	0	0.0	31	4.4
35	18	2.6	0	0.0	0	0.0	1	9.1	19	2.6
52	17	2.5	0	0.0	0	0.0	0	0.0	17	2.4
39	11	1.6	0	0.0	0	0.0	0	0.0	11	1.6
58	11	1.6	0	0.0	0	0.0	0	0.0	11	1.5
Undetermined	11	1.6	0	0.0	0	0.0	0	0.0	11	1.5

Legend: "SCC" – Squamous Cell Carcinoma; "ADC" – Adenocarcinoma; "ADSCC" – Adenosquamous Cell Carcinoma; "OTHER" – Other histological diagnosis; "Undetermined" – unknown HPV type or types.

* Percent among all cases.
** Percent among HPV analyzed cases by histological diagnosis.
*** Percent among HPV DNA positive cases by histological diagnosis.

4. Discussion

This is a historical retrospective analysis along nine decades of ICC diagnosed and treated in just one cancer institution covering the southern part of Portugal. This data was not disturbed by external factors, such as organized national cervical cancer screening, as it was not implemented in southern part of Portugal.

In this cohort, age at diagnosis of ICC was 53.3 years old. This value is within the range of values in other countries [10]. In the evaluation of mean age and age range along the 90 years of this study, we found a significant difference between the first and last decades of the 20th century. Patients diagnosed between 1928 and 1959 had a lower mean age at diagnosis than patients diagnosed in more recent decades. In the first 40 years of this study, patient's mean age at diagnosis was 50.4 years old (SD 10.3) and in the subsequent years the mean age was 7 years significantly higher (57.7 years old with SD 14.1; p-value < 0.001). This increased age at diagnosis by decades was also observed in Sweden, where a small increase (4.3 years) on incidence age at diagnosis was reported [11], although in their study, no statistically difference was reported. Many factors can be held responsible for this incidence change, such as better health care system and changes in known risk factors. During these nine decades modifications have occurred regarding protective effects and risk factors. For instance, a better health care system and an improved education of the population were developed; and at same time risks factors such as use of contraceptive pills and cigarette smoking were introduced or increased. Organized screening cannot be held as a contribute factor to this change of the delayed age of presentation in these last decades, as a no organized cervical screening practices existed in this region of Portugal.

Clinical data from the 1214 cases showed a wide variation of tumor staging at the time of diagnosis, treatment and patient survival (data not showed) reflecting the very large span of the study.

In this study, we obtained an overall detection rate of HPV DNA in ICC of 58.8% (95% CI: 56.0-61.6%). This rate was very low compared to series reporting a prevalence of HPV DNA detection of almost a 100% [1]. The low overall rate of this series is due to the very low HPV DNA detection in the first two decades. In 112 samples from 1928 to 1929, HPV DNA was found in 35 (31.3%), being slightly more elevated (around 50%) between 1930 and 1949. HPV DNA detection range was from 77.4% to 95.1% in the subsequent decades. The explanation of this low rate of HPV DNA detection was due to the fixative used for tissue preservation. Till 1944, in our department, Bouin was used as the main fixative for specimen preservation. This fixative, although known by its ability to preserve nuclear details, was also associated with poorly DNA and RNA preservation for molecular studies [12,13] and a special retrieval protocol is needed to obtain better results but it was not used in this study. In the following decades specimens were fixed in formalin resulting in higher rates of HPV DNA detection. A special recovery protocol should have been applied to cases from 1944 and before in order to verify if the prevalence rate of HPV DNA obtained with the used protocol in this study is real or underestimated.

Regarding HPV DNA types, the large majority of cases had just one genotype of HPV DNA. Only in 20 cases (2.8%) multiple HPV types were identified. In 11 cases (1.5%), although HPV DNA was identified, the types were not recognized by the SPF10/DEIA/LiPA kit and subsequent sequencing analysis. In our series, we were able to establish the profile of HPV genotype in invasive carcinomas in Portugal. The most common HPV types in cervical carcinoma of Portuguese women were HPV16, 18, 33, 45, 31, 35, 52, 39 and 58 by order of frequency. Invasive cervical tumors were associated with the presence of the most common high-risk HPV types, with a similar but not identical distribution to the rest of the European countries [9]. The third more common type in our series was HPV33 and not HPV45. This last type ranked in fourth place. Data on the distribution of HPV types in ICC in Portugal is very scarce, but the results of our series were slightly different from previous results reported [5]. Medeiros et al. [5] found HPV16 and HPV18 in 80% and 15%, respectively in their series of 60 cases of ICC. Both results are higher than the obtained in our cohort and differences might be explained by the larger sample of our study or differences in methodological criteria.

We did not observed statistically significant variations regarding HPV DNA types 16–18 in cancer over the decades, being HPV16 the most frequent type, in all decades (58.2%), of all viruses detected followed by HPV18 (9.2%).

We, as other did previously, also observed a lower age at diagnosis of cervical cancer attributable to HPV16, HPV18 and HPV45 [14–16]. This finding might be important regarding the beginning of screening of HPV vaccinated cohorts, as HPV45 type is not included in quadrivalent vaccine.

The overall detection of HPV DNA was different accordingly to the histological type. In squamous cell carcinoma cases HPV DNA detection was almost the double than in adenocarcinoma type. Several explanations can be advance to explain our results such as non-HPV related cervical adenocarcinomas, contamination of the series with endometrial primary adenocarcinomas, worse DNA retrieval in adenocarcinoma cases due to fixation or other factors. A higher prevalence rate of HPV DNA in adenocarcinomas diagnosed at IPOLFG, using a similar HPV detection protocol, from the 2000-10 decade in a small series of cervical adenocarcinomas was obtained. In that series, 89% of cases (33/37) were HPV DNA positive [17]. The explanation of this finding maybe is related with the small number of adenocarcinomas cases studied in this series after 1980 (6 out 63 cases).

In our series HPV33, the third more prevalent genotype in squamous cell carcinomas (6.2%) was not found in any other histological types. This association between HPV types and histology has been suggested in many reports, as for example, in adenocarcinomas, HPV18 and HPV45 are more common than in squamous cell carcinoma. This last association is also found in this study as well as the previous one [17]. Given the excellent vaccination coverage rate achieved in Portugal, a special program in a near future can be organized in order to study the presence and the HPV types in vaccinated women population, as cross protection is low with quadrivalent HPV vaccine that is currently implemented in Portugal.

In conclusion, in this historical retrospective analysis of invasive carcinoma of the cervix, diagnosed and treated in one cancer institution in Portugal, we characterized HPV genotype profile of invasive carcinomas of the cervix along the XX century. As in other countries HPV16 and HPV18 were the most common types representing 67.4% of all cases. We were able to verify that there were no changes of the most prevalent HPV types throughout the nine decades that comprised this study. We also found that in the Portuguese population the most prevalent HPV types in cervical carcinoma are not the same types responsible for HPV infection. This information can be important in the evaluation of the results of the Immunization Plan and in the development of second-generation HPV vaccines. Moreover, as in other countries, the implementation of the 9vHPV vaccine will allow to cover around 85% of HPV types in invasive cervical carcinomas in Portugal.

Conflict of interest statement

F. Xavier Bosch has received occasional lecture fees from GlaxoSmithKline, Merck, Sanofi Pasteur MSD, and Qiagen, and

unrestricted grants through the institution to conduct epidemiologic and HPV vaccine studies from GlaxoSmithKline, Merck, Sanofi Pasteur MSD, Qiagen, and Roche.

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Other authors declare that no conflict of interest.

References

[1] J.M. Walboomers, M.V. Jacobs, M.M. Manos, F.X. Bosch, J.A. Kummer, K.V. Shah, et al., Human papillomavirus is a necessary cause of invasive cervical cancer worldwide, J. Pathol. 189 (1) (1999) 12–19.

[2] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W.W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer. 49 (6) (2013) 1374–1403.

[3] A. Pista, C.F. de Oliveira, M.J. Cunha, M.T. Paixao, O. Real, CLEOPATRE Portugal Study Group, Prevalence of human papillomavirus infection in women in Portugal: the CLEOPATRE Portugal study, Int. J. Gynecol. Cancer. 21 (6) (2011) 1150–1158.

[4] X. Castellsague, T. Iftner, E. Roura, J.A. Vidart, S.K. Kjaer, F.X. Bosch, et al., Prevalence and genotype distribution 1 of human papillomavirus infection of the cervix in spain: the CLEOPATRE study, J. Med. Virol. 84 (6) (2012) 947–956.

[5] Boletim Vacinação – edição especial. Abril 2014, n° 08, Direção Geral da Saude Ministério da Saude, Portugal

[6] R. Medeiros, H. Prazeres, D. Pinto, I. Macedo-Pinto, M. Lacerda, C. Lopes, E. Cruz, Characterization of HPV genotype profile in squamous cervical lesions in Portugal, a southern European population at high risk of cervical cancer, Eur. J. Cancer Prev. 14 (5) (2005) 467–471.

[7] J. Silva, J. Ribeiro, H. Sousa, F. Cerqueira, A.L. Teixeira, I. Baldaque, T. Osório, R. Medeiros, Oncogenic HPV types infection in adolescents and university women from North Portugal: from self-sampling to cancer prevention, J Oncol. 2011 (2011) 953469.

[8] N. Li, S. Franceschi, R. Howell-Jones, P.J. Snijders, G.M. Clifford, Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication, Int. J. Cancer. 128 (4) (2011) 927–935.

[9] S. de Sanjose, W. Quint, L. Alemany, D.T. Geraets, J. Klaustermeier, B. Lloveras, et al., Human papillomavirus genotype attribution in a restrospective cross-sectional study on 10,575 cases of invasive cervical cancer worldwide, Lancet Oncol. 11 (11) (2010) 1048–1056.

[10] Marisa R. Nucci, Esther Oliva, Diagnostic Pathology. Gynecological, First edition, Amirsys Publishing, Inc., Salt Lake City, Utah, 2014.

[11] K. Hellman, A.-C. Hellström, B.F. Pettersson, Uterine cervix cancer treatment at Radiumhemmet: 90 years′ experience. Time trends of age, stage, and histopathology distribution, Cancer Med. 3 (2) (2014) 284–292.

[12] S. Bonin, F. Petrera, J. Rosai, G. Stanta, DNA and RNA obtained from Bouin's fixed tissues, J. Clin. Pathol. 58 (3) (2005) 313–316.

[13] G. Baloglu, A. Haholu, Z. Kucukodaci, I. Yilmaz, S. Yildirim, H. Baloglu, The effects of tissue fixation alternatives on DNA content: a study on normal colon tissue, Appl. Immunohistochem. Mol. Morphol. 16 (5) (2008) 485–492.

[14] L. Alemany, S. de Sanjosé, S. Tous, W. Quint, C. Vallejos, H.R. Shin, L.E. Bravo, P. Alonso, M.A. Lima, N. Guimerà, J. Klaustermeier, A. Llombart-Bosch, E. Kasamatsu, S.A. Tatti, A. Felix, C. Molina, J. Velasco, B. Lloveras, O. Clavero, E. Lerma, J. Laco, I.G. Bravo, R. Guarch, A. Pelayo, J. Ordi, M. Andújar, G.I. Sanchez, X. Castellsagué, N. Muñoz, F.X. Bosch, RIS HPV TT Study Group, Time trends of human papillomavirus types in invasive cervical cancer, from 1940 to 2007, Int. J. Cancer. 135 (1) (2014) 88–95.

[15] C.M. Wheeler, W.C. Hunt, N.E. Joste, C.R. Key, W.G. Quint, P.E. Castle, Human papillomavirus genotype distributions: implications for vaccination and cancer screening in the United States, J. Natl. Cancer Inst. 101 (7) (2009) 475–487.

[16] S. Vinokurova, N. Wentzensen, I. Kraus, R. Klaes, C. Driesch, P. Melsheimer, F. Kisseljov, M. Dürst, A. Schneider, M. von Knebel Doeberitz, Type-dependent integration frequency of human papillomavirus genomes in cervical lesions, Cancer Res. 68 (1) (2008) 307–313.

[17] A.C. Policiano, P. Alves, L. Martins, A. Félix, Notch1 and HPV genotyping in cervical adenocarcinomas, Mod. Pathol. 23 (supp1) (2010) S1162.