EJGO European Journal of Gynaecological Oncology

Risk of cervical cancer and squamous intraepithelial lesions according to human papillomavirus high-risk serotypes detected by qualitative real-time in vitro PCR

R.J. Martinez-Portilla[1], M. Rial-Crestelo[1], M.A. Mejia-Ugarte[2], J.L. Lopez-Velazquez[2]

[1] Fetal Medicine Research Centre, BCNatal | Barcelona Centre for Maternal Fetal and Neonatal Medicine Hospital Clinic of Barcelona, Barcelona University, Barcelona (Spain)
[2] Colposcopy Department, Adolfo Lopez Mateos Hospital, ISSSTE, Mexico City (Mexico)

Summary

Purpose of investigation: To establish the relationship between high-risk human papillomavirus (hrHPV) serotypes and the odds of developing cervical intraepithelial lesions. *Material and Methods:* Retrospective cohort study using altered cervical cytology results and hrHPV test obtained by PCR. Groups were divided according to low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and squamous cervical cancer (SCC) results, while hrHPV was divided according to polymerase chain reaction (PCR) results in "other HR-HPV, HPV-16, and HPV-18. Logistic regression analysis was performed to estimate the odds of each hrHPV serotype in developing squamous intraepithelial lesions or SCC. *Results:* A total of 252 samples were analysed, mean age was 42 ± 12.65 years. Prevalence of hrHPV infection was 60% (n=152). "Other HR-HPV" group was the most common infection in our population (39%); nonetheless, there were no increased odds for any HPV group and the risk for developing LSIL. The odds for HSIL were 4.58 and 7 for "other HR-HPV" and HPV-16, respectively. Odds for SCC were 11.29 and 7.35 for HPV-18 and HPV-16. There were no significant odds in HPV-18 for HSIL and "other HR-HPV" SCC. *Conclusion:* The present authors found a greater prevalence for "other HR-HPV" group in this population. Nonetheless, HPV-16 and HPV-18 contribute the highest probability of developing HSIL and SCC.

Key words: Human papillomavirus; HPV; Prevalence; LSIL; HSIL; Cervical carcinoma.

Introduction

Cervical carcinoma is one of the leading causes of cancer among women in developing countries [1]. Worldwide, an estimated 400,000 cases of invasive cervical carcinoma are diagnosed annually [2]. In Mexico, cervical cancer (CC) shows the highest incidence among women in reproductive age and is the second leading cause of death in the same group [3]. Human papillomavirus (HPV) infection is recognised as the most important factor implicated in the pathogenesis of cervical cancer [4], about 99.7% of squamous cervical cancers (SCCs) underlies an HPV infection [5]. Papillomaviruses are double-stranded DNA viruses that constitute the papillomavirus genus of the papillomaviridae family [6]. There are more than 200 types of HPV, and approximately 30 to 40 HPV serotypes infecting the genital tract mucosa; of which 15 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73) are classified as high-risk HPVs serotypes (hrHPV) [7]. HPV-16 and -18 are responsible for about 70% of cervical cancer in women worldwide [8] and the persistence of HPV infection has demonstrated to play an important role in carcinogenesis [9], displaying the importance of HPV-DNA testing as a screening test. Meanwhile, studies analysing DNA testing for HR-HPV serotypes, found a test sensitivity ranging from 96% to 99%, compared to the low sensitivity of cervical smear itself [10, 11]; this shows the importance of co-testing as a screening method for detection of cervical cancer.

The present study aims to determine the relationship between hrHPV serotypes and the risk of developing high-grade squamous lesions (HSIL) and SCC.

Material and Methods

This retrospective cohort study was part of an open-population campaign aiming to reduce cervical cancer prevalence designed by Institute of Social Security and Services of State Workers (ISSSTE) in Mexico City. During a three-year period from December 2013 to October 2016, cervical cytology and HPV samples were obtained from all women attending Lopez Mateos hospital's colposcopy centre. Eligibility criteria were patients with complete record and results for Pap smear, HPV testing. and age. Patients with abnormal results were followed according to The American Society for Colposcopy and Cervical Pathology

Revised manuscript accepted for publication June 26, 2017

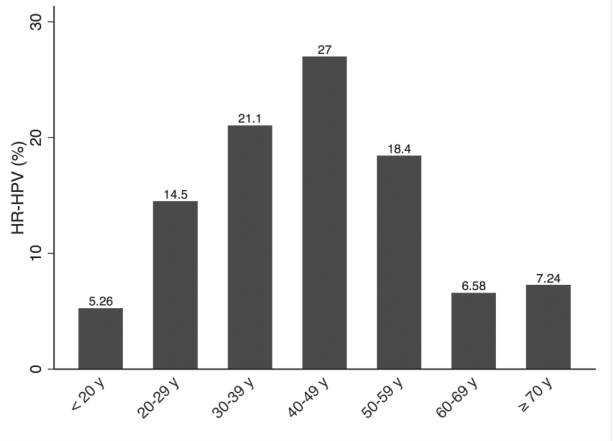

Figure 1. — HPV distribution according to age group.

(ASCCP) [12]. Results from Pap smear, HPV screening, and age were recorded for analysis. The study protocol was approved by the Hospital's review board.

Cytology samples were obtained according to World's Health Organization guidelines [13] using standard smearing of the specimen on a glass slide. Cytology report was made based on 2001 Bethesda system for cervical cytology [14]. HPV sampling was obtained using ThinPrep endocervical brush [15], and storage on PreservCyt solution component, that serves as an alternative transport medium for biological specimens [16].

HPV was analysed using Cobas 4800 HPV Test, an FDA-approved qualitative real-time in vitro test for the detection of HPV in patients specimens. The test uses DNA amplification using polymerase chain reaction (PCR) and nucleic acid hybridization. This test detects 14 high-risk (HR) HPV types in a single analysis. The test identifies HPV 16, HPV 18 and 12 more high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using a generic label called "other HR-HPV". Cobas test uses primers to define a sequence of 200 nucleotides within the polymeric L1 region of the HPV genome. HPV primers employed in the master mix are designed to amplify the 14-targeted HPV types; a fluorescent oligonucleotide probe binds to polymorphic regions within the sequence defined by these primers. Real-time PCR amplification is used for the particularly linked regions. [17]

HPV and cytology were considered positive or negative and then were divided into three groups 1) "other HR-HPV", 2) "HPV-16", and 3) "HPV-18". Type-specific cytology results were reported as low-grade squamous intraepithelial lesion (LSIL), HSIL, and SCC. Age was presented by mean, standard deviation and then divided by groups. HPV prevalence was described according to abnormal cytology results and age. Comparison between type-specific HPV and cytology results was made. Differences between groups were calculated by X^2 or Fisher's exact test for categorical variables and *t*-test for continues variables. Univariate logistic regression was performed for each LSIL, HSIL, and SCC outcomes in order to obtain specific OR for each HPV serotype. *P*-value < 0.05 was considered significant. Data was analysed using STATA v.14.1.

Table 1. — *HR-HPV results according to squamous epithelial lesion*

Characteristic	LSIL n=190	HSIL n=45	SCC n=17	*p*-value[b]
No HPV, n (%)	99 (39)	1 (0.4)	0	<0.001
Other HR-HPV[a], n (%)	67 (27)	25 (10)	7 (3)	
HPV 16, n (%)	10 (4)	12 (5)	5 (2)	
HPV 18, n (%)	3 (1)	2 (1)	1 (0.4)	
HPV HR/16, n (%)	7 (3)	5 (2)	0	
HPV HR/18, n (%)	4 (2)	0	0	
HPV 16/18, n (%)	0	0	4 (2)	

[a] *"Other HR-HPV" (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).*
[b] *p-value calculated by Fisher's exact test.*

Results

A total of 3,274 cervical and HPV samples were obtained, and 3,022 samples were excluded because cytology results were other than LSIL, HSIL. and SCC. A total of 252 samples were used for this analysis.

The mean age was 42 ± 12.65 years. hrHPV distribution according to age group is described in Figure 1. HPV infection occurred in 60% (n=152) of the samples, and according to each squamous cervical lesion, HPV was present in 48% (n=91) of the LSIL samples, a 98% (n=44) in HSIL group, and 100% (n=17) in the case of SCC. The most common infection in the present population was "other HR-HPV" group, infecting 46% (n=115) of the samples, followed by HPV-16 (17%, n=43) and HPV0-18 (6%, n=14). Co-infection occurred in 8% of cytology results. Description HPV test results according to squamous epithelial lesion are depicted in Table 1.

Logistic regression analysis for LSIL did not show higher odds for any HPV group. While in the HSIL group, "other HR-HPV" odds were 4.58 (2-10; $p < 0.001$) and 7 (3-16.6; $p < 0.001$) for HPV16, while HPV-18 infection had no statistical significance. In the squamous cervical cancer group, odds were 7.35 (2.39-22.6; $p < 0001$) for HPV-16 and 11.29 (0.87-44.4; $p = 0.001$) for HPV-18 "other HR-HPV" infection showed no statistical significance. (Table 2)

Discussion

HPV infection exhibits a contrasted prevalence according to continents, countries, and even among regions. In the present study, HPV infection was more prevalent between 30 and 59 years of age, similarly to age-distribution patterns between South-eastern Asia and Eastern Africa results [18]. In women with HPV infection and abnormal cytology, the present data depicted comparable results to those reported by Mu-Mu-Shwe *et al.* [19] who tested 1,771 women for HPV infection and cytology, reporting a 41.4% prevalence of HPV in patients with abnormal cytology result. Meanwhile, in Algeria, Hammouda *et al.* reported a 33.3% prevalence of HPV infection in women with abnormal cytology result [20], showing an even lower prevalence

Table 2. — *Odds ratio for cervical squamous lesion according to HPV group.*

HPV Group	LSIL		HSIL		SCC	
	OR 95% CI [b]	*p*-value	OR 95% CI [b]	*p*-value	OR 95% CI [b]	*p*-value
Other HR-HPV[a]	0.22 (0.1-0.48)	< 0.001	4.58 (2-10)	< 0.001	1.54 (0.49-4.76)	0.452
HPV 16	0.08 (0.35-0.18	< 0.001	7 (3-16.6)	< 0.001	7.35 (2.39-22.6)	< 0.001
HPV 18	0.23 (0.06-0.83)	0.025	0.79 (0.16-3.9)	0.778	11.29 (2.87-44.41)	0.001

[a] *"Other HR-HPV" (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).* [b] *Odds ratio with 95% confidence interval.*

compared to the results.

Type-specific serotype studies have shown a higher prevalence for HPV-16 and HPV-18, being HPV-16 the most common infection among all cervical cytology results [21]. Iwasaki using the same qualitative assay for HPV testing found "other HR-HPV" group serotype to have the highest prevalence (28.08%) *vs*. HPV-16 (10.77%) and HPV-18 (2.0%). Meanwhile, the present results also found a higher prevalence for "other HR-HPV" group, similarly to other studies in this population [22].

In this investigation, the authors found that odds for HSIL were higher for HPV-16, and had no statistical significance for "other HR-HPV group"; this differs from other populations were "other HR-HPV" have higher odds for HSIL [23]. In case is squamous cervical lesions, the present authors found HPV-18 to present the highest odds for cervical cancer, unlike other studies that show HPV-16 as the most common infection in case of cervical neoplasia [24].

Conclusions

HPV infection shows a very different distribution and prevalence according to regions, continents, and countries. Overall prevalence according to the present data is very similar to global HPV prevalence, but distribution for type-specific group of infection shows important differences from other studies, being "other HR-HPV" serotypes the most common infection among women with normal and abnormal cytology result. Despite this, odds ratio for HSIL and SCC is higher for HPV-16 and HPV-18, reiterating HPV-16 and -18 as the most important factor for developing high-grade squamous cervical lesions and SCC. Heterogeneity of HPV results requires continued epidemiological studies by country and region; therefore, this investigation may contribute to the continued epidemiological mapping of HPV among regions.

References

[1] Mukakalisa I., Bindler R., Allen C., Dotson J.: "Cervical cancer in developing countries: effective screening and preventive strategies with an application in Rwanda". *Health. Care. Women*. Int., 2014, *35*, 1065.

[2] Soler M.E., Gaffikin L., Blumenthal P.D.: "Cervical cancer screening in developing countries". *Prim. Care. Update. Ob. Gyns*., 2000, *7*, 118.

[3] Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., *et al.*: "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012". *Int. J. Cancer*, 2015, *136*, E359.

[4] Plummer M., de Martel C., Vignat J., Ferlay J., Bray F., Franceschi S.: "Global burden of cancers attributable to infections in 2012: a synthetic analysis". *Lancet. Glob. Health*, 2016, *4*, e609.

[5] Walboomers J.M., Jacobs M.V., Manos M.M., Bosch F.X., Kummer J.A., Shah K.V., *et al*.: "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide". *J. Pathol*., 1999, *189*, 12.

[6] Orth G., Favre M., Croissant O.: "Characterization of a new type of human papillomavirus that causes skin warts". *J. Virol*., 1977, *24*, 108.

[7] Munoz N., Bosch F.X., de Sanjose S., Herrero R., Castellsague X., Shah K.V., *et al.*: "Epidemiologic classification of human papillomavirus types associated with cervical cancer". *N. Engl. J. Med*., 2003, *348*, 518.

[8] Clifford G.M., Smith J.S., Aguado T., Franceschi S.: "Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis". *Br. J. Cancer*, 2003, *89*, 101.

[9] Coser J., Boeira Tda.R., Wolf J.M., Cerbaro K., Simon D., Lunge V.R.: "Cervical human papillomavirus infection and persistence: a clinic-based study in the countryside from South Brazil". *Braz. J. Infect. Dis*., 2016, *20*, 61.

[10] Longatto-Filho A., Erzen M., Branca M., Roteli-Martins C., Naud P., Derchain S.F., *et al.*: "Human papillomavirus testing as an optional screening tool in low-resource settings of Latin America: experience from the Latin American Screening study". *Int. J. Gynecol. Cancer*, 2006, *16*, 955.

[11] Mayrand M.H., Duarte-Franco E., Rodrigues I., Walter S.D., Hanley J., Ferenczy A., *et al.*: "Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer". *N. Engl. J. Med*., 2007, *357*, 1579.

[12] Massad L.S., Einstein M.H., Huh W.K., Katki H.A., Kinney W.K., Schiffman M., *et al.*: "2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors". *Obstet. Gynecol*., 2013, *121*, 829.

[13] WHO Guidelines Approved by the Guidelines Review Committee.: "WHO Guidelines for Screening and Treatment of Precancerous Lesions for Cervical Cancer Prevention-2". Geneva, 2013.

[14] Solomon D., Davey D., Kurman R., Moriarty A., O'Connor D., Prey M., *et al.*: "The 2001 Bethesda System: terminology for reporting results of cervical cytology". *JAMA*, 2002, *287*, 2114.

[15] "Cervical cancer screening: quick reference guide, endocervical brush/spatula protocol". Available at: http://wwwthinprepcom/hcp/indexhtml.

[16] "Hologic, Inc. ThinPrep® 2000 system". Avaliable at: http://wwwthinprepcom/pdfs/thinprep_package_insertpdf

[17] "Roche Molecular Systems, Inc". Available at:http://wwwaccessdatafdagov/cdrh_docs/pdf10/p100020cpdf

[18] Bruni L., Diaz M., Castellsague X., Ferrer E., Bosch F.X., de Sanjose S.: "Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings". *J. Infect. Dis*., 2010, *202*, 1789.

[19] Mu Mu S., Harano T., Okada S., Aye Aye W., Khin Saw A., Hlaing Myat T., *et al.*: "Prevalence of high-risk human papillomavirus (HR-HPV) infection among women with normal and abnormal cervical cytology in Myanmar". *Acta. Med. Okayama*, 2014, *68*, 79.

[20] Hammouda D., Clifford G.M., Pallardy S., Ayyach G., Chekiri A.,

Boudrich A., *et al.*: "Human papillomavirus infection in a population-based sample of women in Algiers, Algeria". *Int. J. Cancer*., 2011, *128*, 2224.

[21] Smith J.S., Lindsay L., Hoots B., Keys J., Franceschi S., Winer R., *et al.*: "Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update". *Int. J. Cancer*, 2007, *121*, 621.

[22] Martinez-Portilla R.J., Lopez-Velazquez J.L., Martinez-Rojas G.C., Aguilar-Villagomez M.I., De la Torre-Rendon F.E, Villafan-Bernal J.R.: "Prevalence of HPV high-risk serotypes detected by PCR in patients with normal cervical cytology at the Hospital Regional Adolfo Lopez Mateos, ISSSTE". *Ginecol. Obstet. Mex*., 2016, *84*, 556.

[23] Xu H., Lin A., Shao X., Shi W., Zhang Y., Yan W.: "Diagnostic accuracy of high-risk HPV genotyping in women with high-grade cervical lesions: evidence for improving the cervical cancer screening strategy in China". *Oncotarget*, 2016, *7*, 83775.

[24] Kay P., Soeters R., Nevin J., Denny L., Dehaeck C.M., Williamson A.L.: "High prevalence of HPV 16 in South African women with cancer of the cervix and cervical intraepithelial neoplasia". *J. Med. Virol*., 2003, *71*, 265.

[25] Ghosh A.K., Smith N.K., Stacey S.N., Glew S.S., Connor M.E., Arrand J.R., *et al.*: "Serological response to HPV 16 in cervical dysplasia and neoplasia: correlation of antibodies to E6 with cervical cancer". *Int. J. Cancer*., 1993, *53*, 591.

Corresponding Author:
R.J. MARTINEZ PORTILLA, M.D.
Sabino Arana 1
08028 Ellios II, Barcelona (Spain)
e-mail. raifet@hotmail.com