Genetics and Molecular Research

Prevalence of human papillomavirus (HPV), distribution of HPV types, and risk factors for infection in HPV-positive women

M.V.C. Santos Filho[1], A.P.A.D. Gurgel[2], C.D.P. Lobo[1], A.C.F. Freitas[2], J.C. Silva-Neto[3] and L.A.F. Silva[1]

[1]Laboratório de DNA Forense, Departamento de Biologia, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, Maceió, AL, Brasil
[2]Laboratório de Estudos Moleculares e Terapia Experimental, Departamento de Genética, Universidade Federal de Pernambuco, Recife, PE, Brasil
[3]Laboratório de Pesquisas Citológica e Molecular, Departamento de Histologia e Embriologia, Universidade Federal de Pernambuco, Recife, PE, Brasil

Corresponding author: J.C.S. Neto
E-mail: jacinto.costa@ufpe.br

Genet. Mol. Res. 15 (1): gmr.15028315
Received August 8, 2015
Accepted November 26, 2015
Published July 15, 2016
DOI http://dx.doi.org/10.4238/gmr.15028315

ABSTRACT. The aim of this study was to describe the prevalence of human papillomavirus (HPV), the distribution of different HPV types, and the putative risk factors for infection among HPV-positive women from the State of Alagoas, Northeast Brazil. We analyzed data from 515 patients attending public and private health centers. HPV DNA from cervical samples was extracted and HPV genotyping was performed by polymerase chain reaction using MY09/11 consensus primers followed by direct sequencing. The chi-squared test for independence was used to assess statistical differences between the HPV groups. HPV DNA was found in 111 (21.55%) cervical samples. Twenty genotypes were

detected: HPV6, 11, 16, 31, 33, 35, 39, 52, 53, 54, 58, 61, 62, 66, 70, 72, 81, 82, 83, and 84. In addition, multiple sexual partners (P = 0.002) and the use of oral contraceptives (P = 0.015) were associated with the presence of HPV. These findings may be relevant to the design of screening and vaccination strategies targeting specific groups of women in Northeast Brazil.

Key words: Human papillomavirus; Human papillomavirus prevalence; Risk factors; Northeast Brazil

INTRODUCTION

Cervical cancer is the third most common cause of female cancer worldwide (WHO, 2015). The most important etiological factor for cervical cancer is persistent infection by the human papillomavirus (HPV) (zur Hausen, 1996, 2002). HPV belongs to the Papillomaviridae family, which comprises 29 genera of 189 papillomaviruses (PVs), of which 120 are human PVs (Bernard et al., 2010). Fifteen of these HPV types are considered high-risk (HR) HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) (zur Hausen, 2002). Among these, HPV16 and HPV18 are responsible for 70% of invasive cervical cancer cases worldwide. Moreover, genotypes 31, 33, 35, 45, 52, and 58 are involved in 18% of squamous cell carcinoma cases. Although HR HPV prevalence suggests that HPV genotypes 16, 18, 31, 33, and 45 are the five most frequent types involved in cervical cancer, there is evidence of geographical heterogeneity in the extent of HPV genotype distribution, as well as the degree of cervical lesions (de Sanjose et al., 2010).

Despite the large number of women infected with HPV, few develop cervical cancer (Woodman et al., 2007). Hence, apart from HPV infection, other factors such as the individual's lifestyle seem to have an impact on the disease (de Freitas et al., 2012). Moreover, certain aspects of sexual behavior, such as multiple sexual partners, are associated with cervical cancer progression (Finan et al., 2002; Au 2004). Some studies have also shown a link between oral contraceptive use and persistent HPV infection (Efird et al., 2011; Ghanem et al., 2011). Women infected by multiple HPV types can be more susceptible to cervical cancer (Baldez da Silva et al., 2009; Soto-De Leon et al., 2011). Furthermore, co-infection with other multiple HPV types is associated with cervical lesions (de Freitas et al., 2012; Chagas et al., 2015). However, despite the prevalence of HPV infection and its potential impact on health, there is a paucity of data on the risk factors related to cervical lesions/cervical cancer among women living in Northeast Brazil (Baldez da Silva et al., 2009; Chagas et al., 2015). Therefore, the aim of this study was to describe the prevalence of HPV, the distribution of different HPV types, and the putative risk factors for infection among HPV-positive women residing in the State of Alagoas, Northeast Brazil.

MATERIAL AND METHODS

Population

Cervical smear samples were obtained between February and July 2010 from private and public cytology and colposcopy services located in Maceió, Alagoas, Brazil. The public

health centers furnished 339 samples, and 176 came from private clinics. A total of 515 randomly selected sexually active women signed medical ethics consent forms and were included in the study. They all received clear information on the research objectives and completed a questionnaire with socio-demographic and lifestyle questions about putative risk factors for HPV infection [use of oral contraceptives, previous sexually transmitted diseases (STD), number of sexual partners, and nulliparous status]. The study was approved by the Ethics Committee of the Federal University of Alagoas (RG. 004650/2010-55).

Cytology and colposcopy assessment

Cervical samples were collected using an Ayre's spatula and an endocervical brush (cytobrush) for cytology (Papanicolaou smear). Two samples for each patient were collected: one fixed with aerosol spray for cytological smears (alcohol and polyethylene glycol) and the other collected using a Digene female swab specimen collection kit (Digene Corporation, Gaithersburg, MD, USA). Cytological evaluations were carried out by a trained pathologist at the Federal University of Alagoas in accordance with Bethesda 2001 Classification System guidelines (Solomon et al., 2002). All abnormal cervical samples were re-examined for confirmation of the results. Cytological findings were categorized into three groups: 1) normal cytology [all cervical samples without intraepithelial lesions, inflammation, or atypical squamous cells of undetermined significance (ASCUS)]; 2) abnormal cytology [patients with low squamous intraepithelial lesions (LSILs), high squamous intraepithelial lesions (HSILs), or ASCUS]; and 3) negative for intraepithelial lesions (patients with cervical inflammation).

Gynecologists conducted the colposcopy evaluations. These analyses were carried out using 3% acetic acid and iodine. The presence of acetowhite and iodine-negative areas were considered abnormal colposcopy results.

DNA extraction and HPV detection

Exfoliated cervical cells were collected using a Digene female swab specimen collection kit (Digene Corporation) and subsequently sent to the Laboratory of Forensic Genetics at the Federal University of Alagoas. After collection, samples were kept at 4°C until the whole procedure had been completed. HPV DNA was isolated using the phenol-chloroform method (Sambrook and Russell, 2000). DNA integrity control was measured by beta-globin gene amplification, using the following pair of primers: PC04 (CAACTTCATCCACGTTCACC) and GH20 (GAAGAGCCAAGGACAGGTAC). HPV DNA detection was performed by polymerase chain reaction (PCR) with consensus and degenerated primer MY09/11, which amplifies a fragment of the L1 gene of several HPV types. The PCR was performed in a final volume of 20 µL containing 150 ng DNA, 1 mM $MgCl_2$, 50 µM each dNTP, 0.65 µM specific primers, 1 U Taq DNA Polymerase and 1X buffer. The target DNA sequence was amplified by initial denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 30 s, and a final extension at 72°C for 4 min. The final PCR product was run through a 2.0% agarose gel.

HPV genotyping

The PCR products were purified using the isopropyl/alcohol method (Sambrook and

Russell, 2000). Positive HPV DNA samples were sequenced using a BigDye™ Terminator kit and an ABI PRISM™ 310 Genetic Analyzer version 3.1 (Applied Biosystems, Foster City, CA, USA), according to the manufacturer recommendation. HPV genotyping was classified based on sequence homology compared with sequences included in the GenBank database. Sequence homology was determined with BLAST and ClustalW (Tamura et al., 2011) software packages.

Statistical analysis

The chi-squared test of independency was used to assess correlation between putative risk factors (age, use of contraceptive, previous STD, number of sexual partners, and nulliparous status), and clinical cytology findings. P values less than or equal to 0.05 were considered statistically significant. All statistical analyses were performed using PASW statistics 18.

RESULTS

Characteristics of the study population

We analyzed data from 515 women, of which 132 presented normal cytology (25.63%), 100 had abnormal cytology (ASCUS, LSIL, HSIL; 19.41%) and 283 showed cervical inflammation (54.95%). The mean ages of women in the normal, abnormal, and negative for intraepithelial lesions groups were 32.5, 31.5, and 32.2 years, respectively. Within the abnormal cytology group, 86 women (16.69%) were treated in a public health center, while 14 patients (2.71%) were treated in a private health center. Thirty-one patients with abnormal cytology (31%) were diagnosed with ASCUS, 17 (17%) with HSIL, and 52 (52%) with LSIL. Within the negative for intraepithelial lesions group, 202 (39.22%) patients were treated in a public health center and 81 (15.73%) were treated in a private health center. In the normal cytology group, 51 women (9.90%) were treated in a public health center and 81 (15.72%) in a private health center.

Distribution of HPV types

PCR followed by direct sequencing identified HPV DNA in 111 (21.55%) patients. The prevalence of HR HPV infection in the study population was 10%, while 8.5% were infected with low-risk HPV. We identified 20 HPV genotypes: 6, 11, 16, 31, 33, 35, 39, 52, 53, 54, 58, 61, 62, 66, 70, 72, 81, 82, 83, and 84 (Figure 1). HPV genotypes 6, 16, 58, and 70 were the four most common types detected in the cervical samples.

Risk factors, cytological findings, and HPV DNA

There was no association between a patient's age and the cytology group (P = 0.563; Table 1). However, previous STD (P = 0.0002), contraceptive use (P = 0.032), and number of sexual partners (P = 0.00006) were significant within the abnormal cytology group (Table 1). Stratified analyses showed no association between the presence of HPV infection and age (P = 0.060), previous STD (P = 0.091), or nulliparous status (P = 0.677; Table 2). However,

associations were observed between the positive HPV group and the use of contraceptives (P = 0.015), the number of sexual partners (P = 0.002), colposcopy (P = 0.0001), and cytological findings (P = 0.008; Table 2).

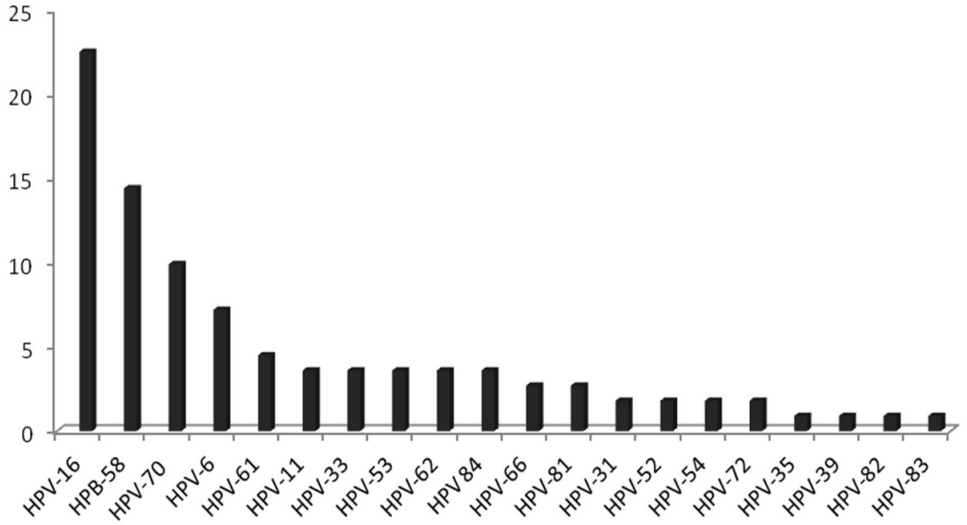

Figure 1. Percentage of human papillomavirus (HPV) in normal, abnormal, and negative for intraepithelial lesions in smears of women from Alagoas State, Northeast Brazil. The figure shows the high frequency of HPV16 (22.52%), followed by HPV58 (14.41%), HPV70 (9.91%), HPV6 (7.21%), HPV61 (4.50%), HPV11 (3.60%), HPV33 (3.60%), HPV53 (3.60%), HPV62 (3.60%), HPV84 (3.60%), HPV66 (2.70%), HPV81 (2.70%), HPV31 (1.80%), HPV52 (1.80%), HPV54 (1.80%), HPV72 (1.80%), HPV35 (0.90%), HPV39 (0.90%), HPV82 (0.90%), and HPV83 (0.90%) genotypes.

Table 1. Distribution and risk factors of normal, abnormal, and negative for intraepithelial cytology associated with age, contraceptive use, previous sexually transmitted diseases (STDs), number of sexual partners, and nulliparous status in women from Alagoas State, Brazil.

Risk factors	Normal cytology group (N = 132)	Negative for intraepithelial lesions (N = 283)	Abnormal cytology group (N = 100)	P value
Age (years)	25	69	27	0.563
15-25	88	169	57	
26-40	19	45	16	
>40				
Contraceptive use	96	214	62	0.032*
No	36	69	38	
Yes				
Previous STD	84	173	39	0.0002*
No	48	110	61	
Yes				
Number of sexual partners	81	147	35	0.00006*
1	46	92	44	
2-3	5	44	21	
>4				
Nulliparous	130	275	98	0.691
No	2	8	2	
Yes				

*Statistically significant.

Table 2. Association between positive human papillomavirus (HPV) patients and age, contraceptive use, previous sexually transmitted diseases (STDs), number of sexual partners, and nulliparous status in women from Alagoas State, Brazil.

Cofactors	Positive HPV group (N = 111)	Negative HPV group (N = 404)	P value
Age (years)	35	86	0.060
15-25	63	251	
26-40	13	67	
>40			
Contraceptive use	70	302	0.015*
No	41	102	
Yes			
Previous STD	56	240	0.091
No	55	164	
Yes			
Number of sexual partners	42	221	0.002*
1	45	137	
2-3	24	46	
>4			
Nulliparous	109	394	0.677
No	2	10	
Yes			
Colposcopy	24	171	0.0001*
Normal	87	233	
Abnormal			
Cytology	20	112	0.008*
Normal	59	224	
Negative for intraepithelial lesions	32	68	
Abnormal			

*Statistically significant.

DISCUSSION

In this study, we provided information on HPV genotype distribution among women who underwent cervical cancer screening for abnormal smears in the state of Alagoas, Northeast Brazil. A quarter of the study population were infected with HPV DNA, and 10% were infected with HR HPV. The most prevalent HR HPV genotypes detected were 16, 31, 33, and 58, which is consistent with the global data (de Sanjose et al., 2010).

Although many sexually active women are infected with HPV, few develop cervical cancer (Woodman et al., 2007). This study showed an association between abnormal cytology and oral contraceptive use, number of sexual partners, and previous STDs. When patients were stratified by HPV samples, we observed an association between HPV positivity, the use of contraceptives, and the number of sexual partners. The results of this study corroborate previous findings from other studies that have shown a link between the use of contraceptives and HR HPV infection (Finan et al., 2002; Au, 2004; Chagas et al., 2013; Amaral et al., 2014). Therefore, our results suggest that behavioral factors might have an impact on the status of cervical cytology in our study population.

Several investigations have been carried out on the prevalence and distribution of HPV in different regions of Brazil (Villa et al., 2000; Sichero et al., 2007; de Medeiros Fernandes et al., 2008; Baldez da Silva et al., 2009; Fernandes et al., 2009, 2010; Castro et al., 2011; Oliveira-Silva et al., 2011; Freitas et al., 2014; Chagas et al., 2015; Gurgel et al., 2013, 2015). The results of these studies have shown divergent HR HPV genotype distribution, although most have pointed to HPV16 as the most frequent type (with the exception of the study developed by Chagas et al., 2015). The results of the present study suggest that HPV genotypes 16, 31, 33, and 58 are the most widespread types in the State of Alagoas. The

HR HPV types reported in this study are alpha-9 species. It should be noted that a recent study showed that bivalent vaccines (Cervarix, GlaxoSmithKline Biologicals, HPV16/18) and quadrivalent vaccines (Gardasil, Merck, HPV/6/11/16/18) can confer cross-protection against non-targeted HPV types such as HPV genotypes 31, 33, and 45 (Hildesheim et al., 2001; Combita et al., 2002; Khan et al., 2008; Lehtinen et al., 2012; Malagón et al., 2012; Goldstone et al., 2013; Kavanagh et al., 2014; Tabrizi et al., 2014). Therefore, it is reasonable to suppose that bivalent and quadrivalent vaccines would protect women from the three most frequent HPV types found in the State of Alagoas. Indeed, the alpha-9 HPV species are phylogenetically related to HPV16, and for that reason, it could share epitopes that induce cross-reactivity. However, there is no cross-protection in bivalent or quadrivalent vaccines against other alpha-9 HPV species, notably HPV58 (Wheeler et al., 2012). Recently, the U.S. Food and Drug Administration approved a 9-valent vaccine (Gardasil 9), which can protect against HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58. Therefore, Gardasil 9 could be a useful option for women from Northeast Brazil requiring protection against HPV58.

In conclusion, this study provides data on the prevalence of HPV genotypes, as well as risk factors among women with abnormal cytology in Northeast Brazil. These findings may be relevant to the design of screening and vaccination strategies targeting specific groups of women.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Brazilian Federal Agency for the Support of Post-Graduate Education (CAPES) and the Science and Technology Support Foundation of Pernambuco State (FACEPE).

REFERENCES

Amaral CM, Cetkovská K, Gurgel AP, Cardoso MV, et al. (2014). MDM2 polymorphism associated with the development of cervical lesions in women infected with Human papillomavirus and using of oral contraceptives. *Infect. Agent. Cancer* 9: 24. http://dx.doi.org/10.1186/1750-9378-9-24

Au WW (2004). Life style, environmental and genetic susceptibility to cervical cancer. *Toxicology* 198: 117-120. http://dx.doi.org/10.1016/j.tox.2004.01.022

Baldez da Silva MF, Chagas BS, Guimarães V, Katz LM, et al. (2009). HPV31 and HPV33 incidence in cervical samples from women in Recife, Brazil. *Genet. Mol. Res.* 8: 1437-1443. http://dx.doi.org/10.4238/vol8-4gmr677

Bernard HU, Burk RD, Chen Z, van Doorslaer K, et al. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401: 70-79. http://dx.doi.org/10.1016/j.virol.2010.02.002

Castro MM, Farias IP, Borborema-Santos CM, Correia G, et al. (2011). Prevalence of human papillomavirus (HPV) type 16 variants and rare HPV types in the central Amazon region. *Genet. Mol. Res.* 10: 186-196. http://dx.doi.org/10.4238/vol10-1gmr992

Chagas BS, Gurgel AP, da Cruz HL, Amaral CM, et al. (2013). An interleukin-10 gene polymorphism associated with the development of cervical lesions in women infected with Human Papillomavirus and using oral contraceptives. *Infect. Genet. Evol.* 19: 32-37. http://dx.doi.org/10.1016/j.meegid.2013.06.016

Chagas BS, Comar M, Gurgel AP, Paiva S, et al. (2015). Association study between cervical lesions and single or multiple vaccine-target and non-vaccine target human papillomavirus (HPV) types in women from Northeastern Brazil. *PLoS One* 10: e0132570. http://dx.doi.org/10.1371/journal.pone.0132570

Combita AL, Touzé A, Bousarghin L, Christensen ND, et al. (2002). Identification of two cross-neutralizing linear epitopes within the L1 major capsid protein of human papillomaviruses. *J. Virol.* 76: 6480-6486. http://dx.doi.org/10.1128/JVI.76.13.6480-6486.2002

de Freitas AC, Gurgel AP, Chagas BS, Coimbra EC, et al. (2012). Susceptibility to cervical cancer: an overview. *Gynecol. Oncol.* 126: 304-311. http://dx.doi.org/10.1016/j.ygyno.2012.03.047

de Medeiros Fernandes TA, de Vasconcellos Meissner R, Bezerra LF, de Azevedo PR, et al. (2008). Human papillomavirus infection in women attended at a cervical cancer screening service in Natal, Brazil. *Braz. J. Microbiol.* 39: 573-578. http://dx.doi.org/10.1590/S1517-83822008000300031

de Sanjose S, Quint WG, Alemany L, Geraets DT, et al.; Retrospective International Survey and HPV Time Trends Study Group (2010). Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* 11: 1048-1056. http://dx.doi.org/10.1016/S1470-2045(10)70230-8

Efird JT, Toland AE, Lea CS and Phillips CJ (2011). The combined influence of oral contraceptives and human papillomavirus virus on cutaneous squamous cell carcinoma. *Clin. Med. Insights Oncol.* 5: 55-75. http://dx.doi.org/10.4137/CMO.S6905

Fernandes JV, Meissner RdeV, de Carvalho MG, Fernandes TA, et al. (2009). Prevalence of HPV infection by cervical cytologic status in Brazil. *Int. J. Gynaecol. Obstet.* 105: 21-24. http://dx.doi.org/10.1016/j.ijgo.2008.12.004

Fernandes JV, Meissner RV, Carvalho MG, Fernandes TA, et al. (2010). Prevalence of human papillomavirus in archival samples obtained from patients with cervical pre-malignant and malignant lesions from Northeast Brazil. *BMC Res. Notes* 3: 96. http://dx.doi.org/10.1186/1756-0500-3-96

Finan RR, Tamim H and Almawi WY (2002). Identification of Chlamydia trachomatis DNA in human papillomavirus (HPV) positive women with normal and abnormal cytology. *Arch. Gynecol. Obstet.* 266: 168-171. http://dx.doi.org/10.1007/s00404-001-0261-8

Freitas LB, Chen Z, Muqui EF, Boldrini NA, et al. (2014). Human papillomavirus 16 non-European variants are preferentially associated with high-grade cervical lesions. *PLoS One* 9: e100746. http://dx.doi.org/10.1371/journal.pone.0100746

Ghanem KG, Datta SD, Unger ER, Hagensee M, et al. (2011). The association of current hormonal contraceptive use with type-specific HPV detection. *Sex. Transm. Infect.* 87: 385-388. http://dx.doi.org/10.1136/sextrans-2011-050005

Goldstone SE, Jessen H, Palefsky JM, Giuliano AR, et al. (2013). Quadrivalent HPV vaccine efficacy against disease related to vaccine and non-vaccine HPV types in males. *Vaccine* 31: 3849-3855. http://dx.doi.org/10.1016/j.vaccine.2013.06.057

Gurgel AP, Chagas BS, do Amaral CM, Albuquerque EM, et al. (2013). Prevalence and genetic variability in capsid L1 gene of rare human papillomaviruses (HPV) found in cervical lesions of women from North-East Brazil. *BioMed Res. Int.* 2013: 546354. http://dx.doi.org/10.1155/2013/546354

Gurgel AP, Chagas BS, do Amaral CM, Nascimento KC, et al. (2015). Prevalence of human papillomavirus variants and genetic diversity in the L1 gene and long control region of HPV16, HPV31, and HPV58 found in North-East Brazil. *BioMed Res. Int.* 2015: 130828. http://dx.doi.org/10.1155/2015/130828

Hildesheim A, Herrero R, Castle PE, Wacholder S, et al. (2001). HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa Rica. *Br. J. Cancer* 84: 1219-1226. http://dx.doi.org/10.1054/bjoc.2001.1779

Kavanagh K, Pollock KG, Potts A, Love J, et al. (2014). Introduction and sustained high coverage of the HPV bivalent vaccine leads to a reduction in prevalence of HPV 16/18 and closely related HPV types. *Br. J. Cancer* 110: 2804-2811. http://dx.doi.org/10.1038/bjc.2014.198

Khan NA, Castillo A, Koriyama C, Kijima Y, et al. (2008). Human papillomavirus detected in female breast carcinomas in Japan. *Br. J. Cancer* 99: 408-414. http://dx.doi.org/10.1038/sj.bjc.6604502

Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, et al.; HPV PATRICIA Study Group (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol.* 13: 89-99. http://dx.doi.org/10.1016/S1470-2045(11)70286-8

Malagón T, Drolet M, Boily MC, Franco EL, et al. (2012). Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect. Dis.* 12: 781-789. http://dx.doi.org/10.1016/S1473-3099(12)70187-1

Oliveira-Silva M, Lordello CX, Zardo LM, Bonvicino CR, et al. (2011). Human Papillomavirus in Brazilian women with and without cervical lesions. *Virol. J.* 8: 4. http://dx.doi.org/10.1186/1743-422X-8-4

Sambrook J and Russell D (2000). Molecular Cloning: A Laboratory Manual. 3rd edn. Cold Spring Harbor Laboratory Press, New York.

Sichero L, Ferreira S, Trottier H, Duarte-Franco E, et al. (2007). High grade cervical lesions are caused preferentially by non-European variants of HPVs 16 and 18. *Int. J. Cancer* 120: 1763-1768. http://dx.doi.org/10.1002/ijc.22481

Solomon D, Davey D, Kurman R, Moriarty A, et al.; Forum Group Members; Bethesda 2001 Workshop (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 287: 2114-2119. http://dx.doi.org/10.1001/jama.287.16.2114

Soto-De Leon S, Camargo M, Sanchez R, Munoz M, et al. (2011). Distribution patterns of infection with multiple types of human papillomaviruses and their association with risk factors. *PLoS One* 6: e14705. http://dx.doi.org/10.1371/journal.pone.0014705

Tabrizi SN, Brotherton JM, Kaldor JM, Skinner SR, et al. (2014). Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: a repeat cross-sectional study. *Lancet Infect. Dis.* 14: 958-966. http://dx.doi.org/10.1016/S1473-3099(14)70841-2

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739. http://dx.doi.org/10.1093/molbev/msr121

Villa LL, Sichero L, Rahal P, Caballero O, et al. (2000). Molecular variants of human papillomavirus types 16 and 18 preferentially associated with cervical neoplasia. *J. Gen. Virol.* 81: 2959-2968. http://dx.doi.org/10.1099/0022-1317-81-12-2959

Wheeler CM, Castellsagué X, Garland SM, Szarewski A, et al.; HPV PATRICIA Study Group (2012). Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol.* 13: 100-110. http://dx.doi.org/10.1016/S1470-2045(11)70287-X

WHO (2015). GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012. Available at http://globocan.iarc.fr/Default.aspx. Accessed January 22, 2016.

Woodman CB, Collins SI and Young LS (2007). The natural history of cervical HPV infection: unresolved issues. *Nat. Rev. Cancer* 7: 11-22. http://dx.doi.org/10.1038/nrc2050

zur Hausen H (1996). Papillomavirus infections--a major cause of human cancers. *Biochim. Biophys. Acta* 1288: F55-F78.

zur Hausen H (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat. Rev. Cancer* 2: 342-350. http://dx.doi.org/10.1038/nrc798