RESEARCH ARTICLE

Detection of Human Papillomavirus among Women with Atypical Squamous Cells of Undetermined Significance Referred to Colposcopy: Implications for Clinical Management in Low- and Middle-Income Countries

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Abstract

To determine the prevalence of human papillomavirus (HPV) among women with atypical squamous cells of undetermined significance (ASC-US) referred to colposcopy and the implications for clinical management in low- and middle-income countries (LMIC), the present study was conducted. We included 200 women living in Maringá/Brazil referred to colposcopy service between August 2012 and March 2013 due to an abnormal cytology from ASC-US until high-grade intraepithelial lesion (HSIL). HPV was detected and genotyped by polymerase chain reaction (PCR). The mean age was 36.8±10.5 years, and women with and without ASC-US had similar mean ages (37.4±11.5 and 36.4±9.96 years, respectively). The highest prevalence of ASC-US occurred at 20-24 years (40%). HPV-DNA was positive in 164 (82.0%) women.Of the 57 women with ASC-US, 30 (52.6%) were HPV-DNA-positive and 21 (70%) were high-risk HPV-positive (HR-HPV); the latter was similar to women without ASC-US (76.9%) but with other abnormal cytological findings present. Our data demonstrated that performing tests for HR-HPV can be used for management of women with ASC-US to support the decision of which women should be referred for an immediate or later colposcopy. The same conclusions can be applied to other LMICs for which HPV testing for primary screening has not been adopted.

Keywords: Cervical cancer - atypical squamous cells of undetermined significance - cervical cytology

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Introduction

At present, cervical cancer (CC) is the fourth leading cause of cancer among women worldwide despite the existence of highly effective prevention and screening methods (Koh et al., 2015; INCA, 2016). Its incidence varies between different regions, but over 85% of the disease burden occurs in developing countries as a result of the weakness of screening programs (Ferlay et al., 2010; Sudenga et al., 2014). In Brazil, CC is the third most common type of cancer in the female population, preceded by breast and colorectal cancers, and CC is the fourth most common cause of death from cancer in females. In 2016, an estimated 16.340 new cases of CC were determined in the country, with approximately 5.430 deaths (Globocan, 2015; INCA, 2016).

Implementation of screening examinations by cervical cytology can reduce the incidence of CC and its associated mortality, but such reductions are dependent on the age of the patients, the quality of the service and the coverage of programs (Solomon et al., 2002; Davey et al., 2004; Solomon et al., 2007). An atypical squamous cells of undetermined significance (ASC-US) result is the most common non-normal cytology finding in cytological screening, affecting 2% to 5% of women screened or well over 1 million women annually in the United States (Davey et al., 2004; Solomon et al., 2007). An ASC-US result is not a true biological entity that progresses or regresses, but it represents indeterminate cellular changes that do not meet the morphological criteria for classification of normal or premalignant lesion (Solomon et al., 2002; Ferlay et al., 2010). As such, the interpretation by its nature is highly variable across readers (Stoler et al., 2001; Wright et al., 2014).

Women with a cytology result of atypical squamous cells cannot exclude HSIL (ASC-H) or worse and should be immediately referred for colposcopy because of a sufficient risk of having a HSIL (Massad et al., 2013). However, the clinical management of women with ASC-US results is particularly problematic and may be variable in different institutions or regions ranging from follow-up cytology to referral for colposcopy, which is

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particularly preferred in the areas with high CC incidence (Kietpeerakool et al., 2014). A cytologic follow-up is a triage strategy for ASC-US, but this could result in a delayed diagnosis in some patients (Arbyn et al., 2013). As human Papillomavirus (HPV) infection is a necessary cause of almost all CC, testing for high-risk (HR) HPV-DNA can be used for triage and management of women with ASC-US to decide who should be referred for immediate versus delayed colposcopy (Tota et al., 2011; Arbyn et al., 2013; Massad et al., 2013).

Although Brazil is considered a low- and middle-income country (LMIC), and it is on the cusp of becoming a high-income country, it is a country with great socioeconomic disparities. Still, HPV testing for primary screening has not been adopted in Brazil. Therefore, Brazil is the ideal setting to evaluate the impact of HPV tests in the clinical management of women with ASC-US; Brazil serves as a model of LMICs and low-resource settings. Thus, our study aims to assess the HPV prevalence and genotype distribution in women with ASC-US results referred forcolposcopy in a region with high CC incidence.

Materials and Methods

Study population

The study included a series of 200 women living in Maringa, Parana State, Brazil referred to the reference service for colposcopy (Zona Sul Clinic) between August 2012 and March 2013 due to an abnormal Papanicolaou findings (ASC-US, ASC-H, low-grade SIL-LSIL and HSIL). Women were excluded from the study if (a) they had a previous history of cervical intraepithelial neoplasia (CIN) or cervical, vaginal, or vulvar cancer; (b) they were referred because of a cervical cancer cytological finding; (c) they had immunosuppression; or (d) they were pregnant. All participants voluntarily agreed to provide a sample for HPV-DNA detection and signed an informed consent form before enrollment. All study procedures were approved, and the project was monitored by the Committee for Ethics in Research Involving Humans/ COPEP at the State University of Maringa/UEM/Brazil. The study was recorded in the National Commission for Research Ethics (CONEP)/Health Ministry of Brazil (n° 489/2010 and n° 083/2011).

Study procedures and sampling

In all patients, colposcopy was performed after application of 3% acetic acid. Colposcopically targeted biopsies were taken from the most abnormal area of the cervix. An endocervical curettage was performed if the transformation zone was not entirely visible. Women with a suspicious image penetrating the cervical canal and those in whom colposcopy was unsatisfactory were submitted to cervical conization. The material was fixed in formaldehyde.

At colposcopy, HPV-DNA samples were obtained with a cervical cytobrush then immediately suspended in 1 ml of sterile 0.9% NaCl solution and frozen at -80°C until they were analyzed for HPV-DNA.

Histopathology

Histopathology results were determined by a panel of 3 pathologists blinded to all participants in accordance with the World Health Organization criteria (Scully et al., 2004), and were classified as CIN 1, CIN 2, CIN 3, invasive squamous cell carcinoma or invasive adenocarcinoma. According to the Health Ministry of Brazil, all cases with cytological findings of ASC-H, HSIL and CC should be analyzed by colposcopy and histology and properly treated and/or have a follow-up.

HPV detection and genotyping

An AxyPrepTm Body Fluid Viral DNA/RNA Miniprep Kit (Axygen, CA, USA) was used for DNA extraction according to the manufacturer's instructions. The quality and quantity of purified DNA were measured by spectrophotometry (NanoDrop 2000 Spectrophotometer, Thermo Scientific, Wilmington, USA).

A single-target PCR (sPCR) method has been in use in our laboratory for several years for HPV detection and consists of HPV-PCR amplification carried out using primers MY09 (5'CGTCCMAARGGAWACTGATC-3') and MY11 (5'-GCMCAGGGWCATAAYAATGG-3'). The reaction consisted of 2.5 mM of each dNTP, 1 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), 0.6 mM of MgCl2, 25 mM of each primer and 50 ng of extracted DNA for a final volume of 15 μL. Co-amplification of the human β-globin gene was performed as an internal control using primers GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') and PC04 (5'-CAACTTCATCCACGTTCACC-3') under the same conditions as the HPV-PCR. Two types of controls were also included in each reaction: 'no-DNA' (negative control) and 'HPV-positive DNA' (positive control). PCR products were electrophoresed in 1.0% agarose gel, stained with 1.0 μg/mL ethidium bromide, and photodocumented under UV light (De Abreu et al., 2012a; De Abreu et al., 2012b; Gimenes et al., 2014; Rocha et al., 2014).

HPV-positive samples were genotyped by PCR-RFLP (Restriction Fragment Length Polymorphism) as described previously (Santiago et al., 2006; Chen et al., 2013). For initial RFLP, 10 μL of each sample were digested in a final volume of 15 μL with the restriction enzyme HpyCH4V (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions (Santiago et al., 2006). To better distinguish some HPV genotypes, such as HPV 11/30, 18/68, 44/55, and 61/83/84, which present similar RFLP patterns, the same protocol was used with a second enzyme NlaIII (New England Biolabs, Ipswich, MA, USA) (Chen et al., 2013).Restriction fragments were resolved in 8% polyacrylamide gels. HPV genotypes were determined by analyzing each band with LabImage 1D software (Loccus Biotechnology, São Paulo, Brazil), and the molecular weights were compared for HPV genotype determination. A total of 39 individual HPV-DNA genotypes (17 genotypes considered to be either high-risk or potentially high-risk, 22 low-risk genotypes not associated with carcinogenesis, and 1 genotype with undetermined-risk for carcinogenesis) was determined by the PCR-RFLP method as follows: high-risk (HR) (genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58,

59, 66, 68, 73 and 82); low-risk (LR) (6, 11, 30, 34, 40, 42, 43, 44, 54, 55, 61, 62, 64, 67, 69, 70, 72, 74, 81, 83, 84 and 91); and undetermined-risk (UR) (26) (Santiago et al., 2006; Monsonego et al., 2015).

Statistical analysis

All data were analyzed using the GraphPad Prism software version 6 (Graph-Pad Software Inc., San Diego, CA). Data were compared by Fisher's exact test as appropriate. The significance level for the tests (P) was set at 0.05.

Results

The mean age of the 200 women included in the study was 36.7±10.4 years. Women with and without ASC-US had a similar mean age (37.4±11.4 and 36.3±9.9 years, respectively) (P>0.05). Overall, 12 (6.0%) women were aged <20 years, 25 (12.5%) were 20-24 years, 64 (32.0%) were 25-34 years, 48 (24.0%) were 35-44 years, 40 (20%) were 45-60 years and 11 (5.5%) >60 years. ASC-US was recorded in 57 (28.5%) women. The highest prevalence of ASC-US was recorded among women aged 20-24 years (40%) but the association between age and ASC-US was not significant (P>0.05). Among the 143 women without ASC-US, 4 (2.8%) had AGC, 26 (18.2%) had ASC-H, 41 (28.7%) had LSIL and 72 (50.3%) had HSIL.

The HPV-DNA test showed that 164 (82.0%) women had HPV infection. Among 57 women with ASC-US, 30 (52.6%) were HPV-DNA-positive. However, the association between HPV positivity and ASC-US was not significant (P>0.05). Among 143 women without ASC-US, 134 (93.7%) were HPV-DNA-positive (Table 2). The association between HPV positivity and women without ASC-US was significant (P=0.0012). Considering the different cytological findings of women without ASC-US, only HSIL was associated with HPV positivity (P=0.0008).

Molecular analysis showed that 117 (58.5%) of the 164 women with positive HPV samples had single infections, and 47 (23.5%) had multiple genotype infections. Among the 30 HPV-DNA-positive women with ASC-US, 10 (33.3%) had multiple infections. There was no significant association of ASC-US with single or multiple HPV infections (P>0.05). Women with and without ASC-US had similar prevalence of single (66.7% and 72.4%, respectively) and multiple (33.3% and 27.6%, respectively) HPV infections (Table 3).

HR-HPV genotypes were recorded in 21 (70%) of the 30 HPV-positive women with ASC-US and 103 (76.9%) in the 134 without ASC-US. LR-HPV genotypes were

Table 1. Prevalence of ASC-US by Age[a]

Age groups (Years)	With ASC-US (n=57)	Without ASC-US (n=143)	Total (n=200)
	n %	n %	n %
< 20 (n = 12)	04 (33.3)	08 (66.7)	12 (6)
20-24 (n = 25)	10 (40.0)	15 (60.0)	25 (12.5)
25-34 (n = 64)	12 (18.7)	52 (81.3)	64 (32)
35-44 (n = 48)	14 (29.2)	34 (70.8)	48 (24)
45-60 (n = 40)	13 (32.5)	27 (67.5)	40 (20)
> 60 (n = 11)	04 (36.4)	07 (63.6)	11 (55)
Total	57 (28.5)	143 (71.5)	200 (100)

ASC-US, atypical squamous cells of undetermined significance; [a]Values are given as number (percentage).

Table 2. Frequency of HPV-DNA in Cervical Samples from Women with and without ASC-US

HPV-DNA	With ASC-US (n=57)	Without ASC-US (n=143)	Total (n=200)
	n %	n %	n %
Positive	30 (52.6)	134 (93.7)	164 (82)
Negative	27 (47.4)	9 (6.3)	36 (18)

*HPV, human Papillomavirus; ASC-US, atypical squamous cells of undetermined significance; [a]Values are given as number (percentage).

Table 3. Frequency of Single, Multiple and Oncogenic Risk Infections in HPV Positive Women with and without ASC-US[a]

HPV infection	With ASC-US	Without ASC-US	Total
	(n = 30)	(n = 134)	(n = 164)
	n %	n %	n %
Single	20 (66.7)	97 (72.4)	117 (58.5)
Multiple	10 (33.3)	37 (27.6)	47 (23.5)
Low only	06 (20)	08 (5.9)	14 (8.5)
High only	21 (70)	103 (76.8)	124 (75.6)
Low and high	03(10)	23 (13.1)	26 (15.8)

HPV, human Papillomavirus; ASC-US, atypical squamous cells of undetermined significance; [a]Values are given as number (percentage).

Table 4. Prevalence of HPV Genotypes in Cervical Samples[a]

Genotype	With ASC-US	Without ASC-US	Total
	(n = 30)	(n = 134)	(n = 164)
	n %	n %	n %
High-Risk			
16	11 (23.3)	64 (35.8)	75 (33.5)
18	4(3.3)	4 (2.2)	8 (2.4)
31	1(3.3)	13 (1)	14 (6.7)
33	0 (0.0)	11 (5.2)	11 (4.3)
45	0 (0.0)	3 (1.5)	3 (1.2)
51	0 (0.0)	1 (0.7)	1 (0.6)
52	0 (0.0)	3 (0.7)	3 (0.6)
56	0 (0.0)	2 (0.7)	2 (0.6)
58	4 (6.7)	15 (5.2)	19 (5.5)
59	0 (0.0)	3 (0.7)	3 (0.6)
66	3(3.3)	11 (4.5)	14 (4.3)
82	2 (6.7)	3 (0.7)	4 (1.8)
Low-Risk			
6	2 (3.3)	2 (0.7)	4 (1.2)
11	2(3.3)	3 (0.0)	5 (0.6)
13	1(3.3)	3 (1.5)	4 (1.8)
53	2 (6.7)	2 (0.0)	4 (1.2)
54	0 (0.0)	4 (0.7)	4 (0.6)
70	1(3.3)	4 (0.0)	5 (0.6)
72	0 (0.0)	5 (0.7)	5 (0.6)
81	1 (0.0)	3 (0.7)	4 (0.6)
None	27	9	36 (21.9)
Multiple-Infections	20	6	26 (15.8)

HPV, human Papillomavirus; ASC-US, atypical squamous cells of undetermined significance.

found in 6 (20%) of HPV-DNA-positive women with ASC-US and in 8 (5.9%) of those without ASC-US (Table 3). ASC-US was not associated with HR or LR-HPV genotypes (P>0.05).

Thirty-six different HPV genotypes were detected in the samples using PCR-RFLP (Table 4). The most prevalent HPV genotypes in women with and without ASC-US were HPV 16 (33.5%), HPV 31 (6.7%), and HPV 58 (5.5%).

Discussion

In the current study, we evaluated the HPV prevalence and genotype distribution in women with ASC-US results referred for colposcopy in a region with high CC incidence and in which HPV testing for primary screening has not been adopted. Despite the fact that the association between HPV positivity and ASC-US was not significant, these women had a high prevalence of HPV-DNA (52.6%). Still, HR-HPV positivity in women with ASC-US (70%) was also high, which was similar to women without ASC-US (76.9%) but who had other abnormal cytology (AGC, ASC-H, LSIL or HSIL). Thus, we demonstrated that performinga HPV molecular test in women with ASC-USwill helptheir clinical management, similar to what is already well established for women with cervical lesions.

In the overall samples, HPV-DNA was detected in 82% of women that were referred for colposcopy, highlighting the importance of Papanicolaou screening in cervical cancer prevention. The HPV-DNA prevalence was higher but at the same time consistent with previous studies performed in different countries around the world, which found HPV prevalence ranging from 35% to 80% among women with some cervical abnormalities (Gargiulo et al., 2007; Abreu et al., 2012b; Alameda et al., 2012; Balbi et al., 2012).

Our study showed that approximately 16% of women with ASC-US were 20-60 years old, which is higher than the 10% of women aged 20-60 years with ACS-US found in another Brazilian study (Paesi et al., 2009).

Women with ASC-US findings usually don't receive further diagnostic tests and treatments, which show and confirm that most cases can be LSIL or HSIL (Fernandes et al., 2009). The first cytological exam that identifies ASC-US is important because it segregates the normal patients from those patients needing to undergo more investigation (Lee et al., 2013). It is well known that sometimes, cytological conditions found in ASC-US women may not be sufficient to diagnose high or low lesions, however, they are enough to support the fact that patients should no longer be treated as normal (Meloni et al., 2014). In our study, HPV-DNA prevalence was 52.6% in women with ASC-US and 93.7% among those without ASC-US. These data may explain at least in part the small number of ASC-US cases who progress to cervical lesion as described by other authors (Lee et al., 2013; Hou et al., 2012; Kasamatsu et al., 2012). In this study, 23.5% of women with positive HPV-DNA had multiple infections. Other researchers had reported similar frequency of HPV-DNA multiple infections,including 21% in Europe, 24% in Africa and 14% in a different area of Brazil. However, these studies did not include only women referred for colposcopy, which was different from our study (Hou et al., 2012; Kasamatsu et al., 2012; Lee et al., 2013). Another Brazilian study reported a rate of 80.8% of HPV multiple infections (Paesi et al., 2009), which highlights the fact that data differences can be due to epidemiologic characteristics of each population studied. Serious and adequate analysis of HPV-DNA multiple infection has its importance in the clinic because it has been found that women with multiple viral genotypes have a greater chance to progress to low and or high lesions (Meloni et al., 2014).

In this study, 36 different HR and LR-HPV genotypes were identified, with HPV 16, HPV 58 and HPV 31 being the most prevalent. It is well known that HPV 16 is the commonest genotype in the world, including in Brazil (Klug et al., 2008; De Abreu et al., 2012a; De Abreu et al., 2012b; Rocha-Brischiliari et al., 2014; Discassiati et al., 2015). Regarding the association between HPV genotypes and cytological findings, HR-HPV genotypes were recorded in 21 (66.7%) of the 30 HPV-DNA-positive women with ASC-US and in 103 (76.9%) of the 134 without ASC-US, representing 75.61% of HPV-DNA-positive women. Thus, the rate of HR-HPV positivity was high in women referred for colposcopy but not significantly associated with ASC-US. Hou et al. (2012) found a much lower incidence of HR-HPV (26.4%) in Chinese women with ASC-US.

In conclusions, We observed that women with ASC-US had a high prevalence of HPV-DNA. Additionally, these women had high HR-HPV positivity, which is similar to women without ASC-US but that had other abnormal cytology (AGC, ASC-H, LSIL or HSIL). Taken together, our data demonstrated that performing tests for HR-HPV can be used to triage and manage women with ASC-US to support the decision of who should be referred for an immediate or late colposcopy. Considering that Brazil is the ideal setting to evaluate the impact of HPV tests in the clinical management of women with ASC-US and serves as a model of LMICs and low-resource settings due its great socioeconomic disparities, our data can be important to other LMICs as well.

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References

Alameda F, Bellosillo B, Lloveras B, et al (2012). PCR study of a series of ASCUS cases HPV-positive by HCII. *Diagn Cytopathol*, **40**, 1043-6.

Arbyn M, Roelens J, Simoens C, et al (2013). Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. *Cochrane Database Syst Rev*, **28**, 3.

Balbi G, Napolitano A, Giordano F, et al (2012). Role of the association of high-risk HPV identified by real-time PCR in cervical preneoplastic lesions. *Eur J Gynaecol Oncol*, **33**, 467-71.

Chen L, Watanabe K, Haruyama T, et al. (2013). Simple and rapid human papillomavirus genotyping method by restriction fragment length polymorphism analysis with two restriction enzymes. *J Med Virol*, **85**, 1229-34.

Davey DD, Neal MH, Wilbur DC, et al (2004). Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the college of american pathologists interlaboratory comparison program in cervicovaginal cytology. *Arch Pathol Lab Med*, **128**, 1224-9.

De Abreu ALP, Gimenes F, Nogara PRB, et al. (2012a). A review of methods for detect human Papillomavirus infection. *Virol J*, **9**, 262.

De Abreu ALP, Gimenes F, Nogara PRB, et al (2012b). Molecular detection of HPV and Chlamydia trachomatis infections in Brazilian women with abnormal cervical cytology. The Amer *J Trop Med and Hyg*, **87**, 1149-51.

Discacciati MG; Gimenes F, Pennacchi PC, et al. (2015). MMP-9/RECK imbalance: a mechanism associated with high-grade cervical lesions and genital infection by Human Papillomavirus (HPV) and Chlamydia trachomatis. *Cancer Epid Biom Prev*, **24**, 1539-47.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **15**, 2893-917.

Fernandes JV, Meissner R de V, de Carvalho MG, et al (2009). Prevalence of HPV infection by cervical cytologic status in Brazil. *Int J Gynecol Obstet*, **105**, 21-4.

Gargiulo F, De Francesco MA, Schreiber C, et al (2007) .Prevalence and distribution of single and multiple HPV infection in cytological abnormal samples from Italian women. *Vir Res*, **125**, 176-82.

Gimenes F, Medina FS, Abreu ALP, et al (2014). Sensitive simultaneous detection of seven sexually transmitted agents in semen by multiplex-PCR and of HPV by Single PCR. *Plos One*, **9**, 98862.

GLOBOCAN. Cancer Fact Sheet- Globocan (2015). Available at: [http://www.iarc.fr/en/media-centre/pr/2015/pdfs]. Acessed 10 Dec 2015.

Hou R, Xu C, Zhang S, et al (2012). Distribution of human papillomavirus genotype and cervical neoplasia among women with abnormal cytology in Beijing, China. *Int J Gynecol Obstet*, **119**, 257-61.

INCA. Instituto Nacional de Câncer. Available at: [http://www.inca.gov.br/wcm/dncc/2015/por-tipos.asp]. Accessed 10 Jan 2016.

Kietpeerakool C, Tangjitgamol S, Srisomboon J (2014). Histopathological outcomes of women with abnormal cervical cytology: a review of literature in Thailand. Asian Pac J Cancer Prev, 15, 6489-94.

Kasamatsu E, Cubilla AL, Alemany L, et al (2012). Type-specific human papillomavirus distribution in invasive cervical carcinomas in Paraguay. A study of 432 cases. *J Med Virol*, **84**, 1628-35.

Klug SJ, Hukelmann M, Blettner M. (2008). Knowledge about infection with human papillomavirus: a systematic review. *Prev Med*, **46**, 87-98.

Koh WJ, Greer BE, Abu-Rustum NR, et al (2015). Cervical Cancer, Version 2. 2015. *J Nat Comp Canc Netw*, **13**, 395-405.

Lee WC, Lee SY, Koo YJ, et al (2013). Establishment of a Korea HPV cohort study. *J Gynecol Oncol*, **24**, 59-65.

Massad LS, Pierce CB, Minkoff H, et al. (2013). Long-term cumulative incidence of cervical intraepithelial neoplasia grade 3 or worse after abnormal cytology: impact of HIV infection. *Int J Cancer*, **134**, 1854-61.

Meloni A, Pilia R, Campagna M, et al. (2014). Prevalence and molecular epidemiology of human papillomavirus Infection in Italian women with cervical cytological abnormalities. *J Pub Health Res*, **3**, 157.

Monsonego J, Cox JT, Behrens C, et al. (2015). Prevalence of high-risk human papillomavirus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: Data from the ATHENA trial. *Gynecol Oncol*, **137**, 47-54.

Paesi S, Serafini EP, Echeverrigaray S (2009). High prevalence of Human Papillomavirus type 58 in patients with cervical pre-malignant lesions in Southern Brazil. *J Med Virol*, **81**, 1270-5.

Rocha-Brischiliari SC, Gimenes F, De Abreu AL, et al (2014). Risk factors for cervical HPV infection and genotypes distribution in HIV-infected South Brazilian women. *Inf Ag Cancer*, **9**, 6.

Santiago E, Camacho L, Junqueira ML, et al. (2006). Full HPV typing by a single restriction enzyme. *J Clin Virol*, **37**, 38-46.

Scully RE, Bonfiglio TA, Kurman RJ, et al. (1994) Histological typing of female genital tract tumors. 2ed. Springer.I SBN (online): 978-3-642-85014-1.

Solomon D, Davey D, Kurman R, et al. (2002). The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*, **287**, 2114-9.

Solomon D, Breen N, Mc Neel, T. (2007). Cervical cancer screening rates in the United States and the potential impact of implementation of screening guidelines. *Cancer J Clin*, **57**, 105-111.

Stoler MH, Schiffman M (2001). Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA*, **285**, 1500-5.

Sudenga SL, Rositch AF, Oitieno WA, et al (2014). Brief Report: knowledge, attitudes, practices and perceived risk of cervical cancer among Kenyan women. *Int J Gynecol Cancer*, **23**, 895-9.

Tota JE, Chevarie-Davis M, Richardson LA, et al (2011) .Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med*, **53**, 12-21.

Wright TC Jr, Stoler MH, Behrens CM, et al (2014). Interlaboratory variation in the performance of liquid based cytology: insights from the ATHENA trial. *Int J Cancer*, **134**, 1835-43.