Invest Clin 51(1): 27 - 35, 2010

Presence of human papillomavirus infection determined by hybrid capture assay in cervical lesions in a Venezuelan population.

Maria Correnti[1], María Eugenia Cavazza[2], Odalis Herrera[3] and Armando Rodríguez[3].

[1]Instituto de Oncología y Hematología MPPS, Universidad Central de Venezuela,
[2]Instituto de Biomedicina y [3] Laboratorio Citomed. Caracas, Venezuela.

Key words: Human papillomavirus, LSIL, HSIL, cervical cancer, HCA II, Venezuela.

Abstract. The aim of this study was to establish the presence of HPV infection in cervical lesions in a Venezuelan population, by Hybrid Capture Assay II (HCA II), and its association with cytological diagnosis. The study included 1483 cervical samples analysed at Laboratorios CITOMED, Caracas, Venezuela, from 2005 to 2007. The woman age range was between 20 and 58 years, and the mean age was 28.8. HPV infection was determined using HCA II. The cytological diagnosis of the smears showed LSIL in 1120/1483 samples (75.5%), HSIL in 354/1483 (23.9%) and ASC-US, in 9/1483 (0.6%). The positivity of HPV DNA detected by HCA II was 54.6% (811/1483). Of the positives cases, 138/811 (17%) presented HPV DNA of low oncogenic risk and 673/811 (82.9%) had high-risk HPV. There were significant differences in the low and high oncogenic HPV type frequencies of the evaluated samples ($p>0.0001$). Low risk HPV types were detected in 127 cases of LSIL, 9 of HSIL and 2 of ASC-US. High-risk HPV was detected in most of the cases: 361 LSIL, 308 HSIL and 4 ASC-US. Our study showed a high presence of cervical infection by human papillomavirus of a high risk genotype. Our results contribute to the epidemiological data that report diversity in the prevalence rates in different countries.

Corresponding author: Maria Correnti. Instituto de Oncología y Hematología, Laboratorio de Genética Molecular, Universidad Central de Venezuela. Caracas, Venezuela. Av. Minerva. Urb. Los Chaguaramos. Teléfono 0212.605.06.47. Correo electrónico: mcorrentip@yahoo.com.

Presencia de la infección por virus papiloma humano determinada por ensayo de captura híbrida II en lesiones de cuello uterino en una población venezolana.

Invest Clin 2010; 51(1): 27 - 35

Palabras clave: Papilomavirus humano, LSIL, HSIL, cancer cervical, ECH II, Venezuela.

Resumen. El objetivo de este estudio fue establecer la presencia de la infección por Virus Papiloma Humano en lesiones cervicales de un grupo de pacientes venezolanos, mediante el Ensayo de Captura Híbrida II (ECH II), y su asociación con el diagnóstico citológico. Se analizaron 1483 muestras de citología cervical procesadas en el Laboratorio CITOMED, Caracas, Venezuela desde 2005 hasta 2007. El rango de edad de las mujeres fue entre 20 y 58 años, siendo la edad promedio 28,8 años. Se determinó la presencia de la infección por VPH empleando el método de Ensayo de Captura Híbrida II (ECH II). El diagnóstico citológico de las muestras reveló la presencia de lesiones intraepiteliales de bajo grado (LIE BG) en 1120/1483 (75,5%) pacientes, lesiones intraepiteliales de alto grado (LIE AG) en 354/1483 (23,9%) y lesiones con células atípicas de significado indeterminado (ASCUS) en 9/1483 (0,6%). La positividad de la infección por VPH detectada por ECH II fue de 54,6% (811/1483). De los casos positivos, 138/811 (17%) tenían ADN-VPH de bajo riesgo oncogénico y 673/811 (82,9%) presentaron tipos de VPH de alto riesgo. Las diferencias observadas en la frecuencia de los tipos de VPH de alto y bajo riesgo oncogénicos detectados en las muestras fueron estadísticamente significativas ($p<0,0001$). VPH de bajo riesgo fue detectado en 127 casos de LIE BG, 9 de LIE AG y 2 de ASC-US, mientras que VPH de alto riesgo fue detectado en la mayoría de los casos: 361 de LIE BG, 308 de LIE AG y 4 de ASC-US. Nuestro estudio demostró una alta presencia de infección por genotipos de VPH de alto riesgo oncogénico. Estos datos contribuyen a establecer el patrón epidemiológico que reporta una gran diversidad en las tasas de prevalencia en diferentes países.

Received: 11-10-2008. Accepted: 08-07-2009.

INTRODUCTION

Human Papillomavirus (HPV) is the most common sexually transmitted infection worldwide, being an important public health challenge (1). Although the majority of infections cause no symptoms and are self-limited, persistent genital HPV infection can cause cervical cancer in women, men and other types of anogenital and oropharyngeal cancers (2, 3). Approximately 100 HPV types have been recognized, over 40 of which infect the genital area (4). Genital HPV types are categorized according to their epidemiological association with cervical cancer. Infections with low risk types can cause benign or low-grade cervical cell changes, recurrent respiratory papillomatosis and genital warts (5, 6). High-risk HPV types (16, 18, 31, 33,

35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 73, 82) can cause low and high grade cervical cell abnormalities and act as carcinogens in the development of cervical cancer and other anogenital cancers (5, 7-9). The role of specific HPV types in the pathogenesis of cervical cancer and in other anogenital cancers has been well established (10-12). HPV is recognized as being a necessary, but not sufficient cause of cervical cancer (9). The association of genital types of HPV with nongenital cancer is less well established, but studies support a role in oral cavity and pharyngeal cancers (13).

In Venezuela, Liuzzi *et al.* (14) showed the presence of HPV infection in 34% of squamous cell carcinomas of head and neck. Correnti *et al.* (15) found high risk HPV infection in 50% of oral squamous cell carcinoma. Additionally, Jimenez *et al.* (16) reported the presence of the HPV genome in 55% of oral bening lesions. A number of cofactors that modify the risk among HPV DNA positive women have been suggested, including the use of oral contraceptives for five or more years, tobacco smoking, high parity (five or more full term pregnancies) and genital tract infections such as *Chlamydia trachomatis,* Human Immunodeficiency Virus type 1 (HIV-1) and Herpes Simples Virus Type 2 (HSV2) (11, 17).

Assays for HPV detection differ considerably in their sensitivity and specificity. The anatomic region sampled and the method of specimen collection will impact detection (18). Only the Digene Hybrid Capture II is approved by the US. Food and Drug Administration (FDA) for clinical use. The test uses liquid nucleic acid hibridization and detects 13 high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 and five of low risk (HPV 6,11, 42, 43, 44).

Recently developed, the second generation of the Hybrid Capture System HPV DNA detection test from Digene Diagnosis (Digene Diagnostics Inc, Silver Spring, Maryland, USA) is a non radioactive, relatively rapid, hibridization assay designed to detect 18 HPV types divided into high and low risk groups (19-21). As an advantage compared with other methods, the HCA II provides a semiquantitative viral load, which can correlate with the grade and the natural history of cervical pathology (22).

The aim of this study was to evaluate the prevalence of HPV infection in cervical lesions in a Venezuelan population, determined by HCA II, correlating it with its respective cytological diagnosis.

MATERIAL AND METHODS

Population and sample collection

The study included 1483 female cervical samples refered to Laboratorio CITOMED, Caracas, Venezuela, from 2005 to 2007. Laboratorio CITOMED is a private service that works cooperatively with the Universidad Central de Venezuela (UCV). The study was approved by the ethical commite of Institute of Biomedicine-UCV, implying informed written consent. The smears were collected with a cervical cytobrush and transported in Digene Specimen Transport Medium (Digene Diag, Md). Cytological diagnoses were reported according to Bethesda System, consensus guidelines 2006 (23) as Normal, for normal epithelium; LSIL for low grade squamous intraepithelial lesion, HSIL for high grade squamous intraepithelial lesion and ASC-US for atypical squamous cells of undetermined significance.

HPV testing

HPV testing was done using HCA-II (Digene Diagnostics Inc, Silver Spring, Maryland, USA). Following the kit protocol, specimens were treated with sodium hydroxide to hydrolyse specimen RNA and to denature the DNA. The liberated sin-

gle-strand DNA was hybridized in solution with an RNA probe mix consisting of high-risk or low-risk HPV types. Each reaction mixture, containing any RNA-DNA hybrids that formed, was transferred to a capture tube coated with antibodies to the hybrids, immobilizing them. Bound RNA-DNA was then reacted with an alkaline phosphatase-conjugated antibody directed against the hybrids. Unreacted material was removed by washing, and a dioxetane-based chemiluminescent compound, Lumi-Phos 530, was added as a substrate for alkaline phosphatase. The Light produced by the reaction was measured with a luminometer. The result of this test was expressed as relative light unit (RLU), which was converted into a ratio of specimen RLU to the positive cutt-off simple RLU. A ratio of >1 was taken as cutt-of value for positive.

Statistical analisis

The statistical evaluation of the results was realized with a contrast of the hypothesis, using the Chi square Test, using GraphPad InStat.software.

RESULTS

The presence of HPV infection was evaluated in 1483 female cervical samples. The mean age of female population was 28.8 years. The cytological diagnosis of smears distribution was: 1120 (75.5%) were diagnosed as LSIL, 354 (23.9%) were HSIL and 9 (0.6%) ASC-US (Table I).

The positivity of HPV DNA detected by HCA II was 54.6% (811/1483). The presence of the different HPV types among the women with HPV infection is observed in the Table II, 138 (17%) had HPV DNA of low risk for cancer and 673 (82.9%) presented high-risk HPV types. There were significant differences in the frequency of HPV types of the cervix of the evaluated patients (p=0.0001).

The presence of the different HPV types and the cytological diagnosis were compared (Table III). Low risk HPV types were detected in 127 cases of LSIL, 9 of HSIL and 2 of ASC-US, while, high-risk HPV was detected in most of the cases: 361 LSIL, 308 HSIL and 4 ASC-US.

TABLE I
DETECTION OF LESIONS ACCORDING TO CYTOLOGICAL DIAGNOSIS IN VENEZUELAN FEMALE PATIENTS

Citology	Number of samples	Prevalence (%)
LSIL (Low grade squamous intraepithelial lesion)	1120	75.5%
HSIL (High grade squamous intraepithelial lesion)	354	23.9%
ASC-US (Atypical squamous cells of undetermined significance)	9	0.6%
Total	1483	100%

TABLE II
HPV TYPES AMONG VENEZUELAN FEMALES ACCORDING TO HYBRID CAPTURE ASSAY II

HPV type	Number of positives	Prevalence (%)
Low risk	138	17%
High risk	673	82.9%
Total	811	100%

TABLE III
HPV TYPES DETECTED BY HYBRID CAPTURE ASSAY II AND ITS RELATION TO THE CYTOLOGICAL DIAGNOSIS

Cytological diagnosis	Low Risk	High Risk
LSIL	127/488-26%	361/488-74%
HSIL	9/317-2.8%	308/317-97%
ASC-US	2/6-33%	4/6-67%

DISCUSSION

Latin America is one of the regions of the world where the incidence of cervical cancer is high (24). The World Health Organization reported that cervical cancer is the second cause of malignant neoplasia and death in women worldwide (8). In poor countries, cervical cancer is the most frequent cause of death from cancer. During the last two decades this disease is presenting in early ages of the female population (25).

Epidemiological studies conducted worldwide clearly indicate that HPV infection is the cause of cervical cancer (9, 11). The distribution of specific types of HPV is known to vary in different regions in the world, as do the cofactors that may inhibite or promote carcinogenesis (26).

Since HPV persistence plays a central role in the etiology of cervical cancer, the evaluation of HPV among the general population is important for surveillance of cervical cancer specially in rural populations and cities of Latin American countries, where screening coverage is reduced or sometimes inexistent and little information regarding the prevalence of HPV is available (27).

Consequently, there has been a strong motivation to develop HPV testing for improvements and standarization of HPV DNA testing methods. HCA II has proved to be an accurate, and cost-effective method for HPV testing in routine clinical practice (28).

We used this method to detect the presence of HPV DNA in 1483 cervical smears from a group of Venezuelan women with cytological diagnosis of LSIL 75.5%, HSIL 23.9% and ASC-US, in 0.6%.

The presence of HPV infection was 54.6%. These findings are consistent with previous reports among Latin American women, where the prevalences for Brazilian and Argentinian women were similar than the observed in our study (19, 22, 29, 30). However, the prevalence of HPV among women appears to vary by countries within Latin America, where the frequency range is between 15% and 16% in Mexico, Costa Rica and Colombia (26, 31-34).

In Venezuela, Mendoza *et al.* (35), reported 10% of prevalence using hybrid capture, lower rates of HPV DNA prevalence (23% and 10%) compared with our results. Also, Contreras *et al.* (36) reported 27% of HPV infection in women in the age range between 45 to 54 years and 20% in women under 25 years.

Latin American countries are among those with highest incidences of cervical cancer rates in the World, together with countries from África, South and East Asiacentral, America (30.6%) and South America (28.6%). Variation in incidence among countries is large. Very high rates are found in Haiti, Nicaragua, and Bolivia. Latin Caribbean has the lowest and the highest risks in Latin America represented by Puerto Rico (10.3%) and Haiti 93.9%) (27).

It is very difficult to establish whether variations in incidence of cervical cancer

observed among Latin American countries (Central America, 30.6%; South America, 28.6%; Haiti 93.9%) (27), are due to differences in prevalences of HPV infection or by the using of different methods employed for the HPV-DNA detection. Some of the geographical variation may be responsable of the differences in the prevalence of different subtypes of HPV and host related factors (9).

Aditionally, demographic, cultural, socioeconomic variables, high parity, smoking, oral contracepcion, young age at first coitus, elevated number of partners, low socioeconomic status, low education level, poor genital hygiene, and genital tract infections such as *Chlamydia trachomatis,* Human Immunodeficiency Virus type 1 (HIV-1) and Herpes Simples Virus Type 2 (HSV2) among others, are probable co-factors that increases the risk of cervical cancer in women with papillomavirus infection (27, 37, 38).

The differences in the incidence of cervical cancer in Latin America may be explained too as the result of screening activities, inadequate collection and reading cytological samplings, as well as incomplete follow-up of the women afther the test (27).

In our study, among the 811 HPV positive cases, 138 (17%) were infected with low risk HPV types and 673 (82.9%) had high-risk HPV types. Results are similar to others reported in the literature, which indicate that about 50% of all women are infected by HPV, characterizing a worldwide public health problem (29, 39). In the present study, we associated the cytological diagnosis with the hybrid capture assay. We observed HPV DNA in 488 cases of the 1120 smears (43.5%) diagnosed as a LSIL and 632 (56.4%) were negative. Nearly 10% of HSIL presented negative results in HCA II. These observations are different from those reported by others investigators that found a very good correlation between cytology and HCA II (19, 30), but are consistent with a previous report in Venezuela (35).

Disagreement between DNA testing and cytology could be due to error in the cytological diagnosis, a low copy number of the HPV genome, infection by untested types or unidentified reasons such as DNA testing errors (40).

We found also that high-risk HPV was strongly associated with HSIL (97%) and with LSIL (74%). The Relative Risk (RR= 24) indicated that in Venezuela the most common types circulating are high-risk HPV types associated with LSIL as well as with HSIL. Since the persistence of the viral types has been associated with high risk HPV, is very important the follow up of the patients that could progress to cancer. Similar results have been reported (25).

The variability in oncogenic HPV type distribution will significantly impact vaccine efficacy in Latin America. More studies determining HPV types distribution and risk factors need to be conducted (26).

Molecular screnings based on Hybrid Capture Assay showed that HPV types are not always consistent with the clinical type of HPV-associated genital lesion. Ours results contribute to the epidemiological data that report diversity in the prevalence rates in different countries.

ACKNOWLEDGEMENT

To Dr. Maríanella Perrone, Facultad de Odontología, Universidad Central de Venezuela for her valuable review and comments.

REFERENCIAS

1. **Skerlev M, Giri M, Skerlev HS.** Human Papillomavirus male genital infections: Clinical variations and the significance of DNA typing. Clinics Dermatol 2002; 20:173-178.

2. **Weinstock H, Berman S, Cates W Jr.** Sexually transmitted diseases among American youth: incident and prevalence estimates, 2000. Perspect Sex Reprod Health 2004; 36: 6-10.
3. **Van Houten VM, Snijders PJ, van den Brekel MW, Kummer JA, Meijer CJ, Denkers F, Smeele LE, Snow GB, Brakenhoff RH.** Biological evidence that human papillomavirus are etiologically envolved in a subgroup of head and neck squamous cell carcinomas. Int J Cancer 2001; 93:232-235.
4. **Koutsky LA, Kiviat NB.** Genital human papillomavirus. In: Holmes K, Sparling, P. Mardh P, Lemon S, Stamm W, Piot P, Wasserheit J. Sexually Transmitted Diseases, 3rd edition. New York: McGraw-Hill, 1999, p. 347-359.
5. **World Health Organization.** IARC monograph on the evaluation of carcinogenic risk to humans: human papillomavirus. 1995. Lyons, France, IARC; 2000.
6. **Hong D, Ye F, Chen H, Lu W, Cheng O, Hu Y, Xie X.** Distribution of human papillomavirus genotypes in the patients with cervical carcinoma and its precursors in Zhejiang Province, China. Int J Gynecol Cancer 2008; 18(1):104-109.
7. **NIH Consensus Statement Cervical Cancer.** NIH Consensus Statement 1996; 1-38.
8. **Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ.** Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518-527.
9. **Bosch FX, Manos MM, Muñoz N, Sherman M, JansenAM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV.** Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995; 87:796-802.
10. **Bosch FX, de Sanjosé S.** The epidemiology of human papillomavirus infection and cervical cancer. Dis Markers 2007; 23:213-227.
11. **Walboomers JM, Jacobs MV, Manos MM, Bosch X, Kunmmer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N.** Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189:12-19.
12. **Pereira CR, Rosa ML, Vasconcelos GA, Faria PC, Cavalcanti SM, Oliveira LH.** Human papillomavirus prevalence and predictors for cervical cancer among high-risk women from Rio de Janeiro, Brazil. Int J Gynecol Cancer 2007; 17(3):651-660.
13. **Kreimer AR, Clifford GM, Boyle P, Franceski S.** Human Papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005; 14:467-475.
14. **Liuzzi J, Estanga N, Castillo L, Correnti M, Mijares A, Gardie J.** Tipificación del virus del papiloma humano en carcinoma de células escamosas de cabeza y cuello. Rev Venez Oncol 2007; 19:210-218.
15. **Correnti M, Rivera H, Cavazza ME.** Detection of human Papillomavirus of high oncogenic potential in oral squamous cell carcinoma in a Venezuelan population. Oral Dis 2004; 10:163-166.
16. **Jimenez C, Correnti M, Salma N, Cavazza ME, Perrone M.** Detection of human papillomavirus DNA in benign oral squamous epithelial lesions in Venezuela. J Oral Pathol Med 2001; 30:385-388.
17. **Marais D, Constant D, Allan B, Carrara H, Hoffman M, Shapiro S, Morroni C, Williamson A.** Cervical Human Papillomavirus (HPV) Infection and HPV Type 16 Antibodies in South African Women. J Clin Microbiol 2008; 732-739.
18. **Markowitz LE, Dunne EF, Saraiya M, Lawson H, Unger ER.** Centres for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Pep 2007; 56:1-24.
19. **Carestiato F, Silva K, Dimetz T, Oliveira L, Cavalcanti S.** Prevalence of human papillomavirus infection in the genital tract determined by hybrid capture assay. Bras J Infect Dis 2006; 10(5):331-336.
20. **Carozzi F, Bisanzi S, Sani C, Zappa M, Cechini S, Ciatto S, Confortini M.** Agreement between the AMPLICOR human papillomavirus test and the hybrid

capture 2 assay in detection of High-Risk Human Papillomavirus and Diagnosis of Biopsy-Confirmed High Grade Cervical Disease. J Clin Microbiol 2007; 45: 364-369.

21. **Knoepp SM, Kuebler DL, Wilbur DC.** Resolution of equivocal resultas with Hybrid Capture II high-risk HPV DNA test: a cytologic/histologic review of 191 cases. Diag Mol Pathol 2007; 16:125-129.
22. **Cox JT, Lorincz AT, Schiffman MH, Sherman ME, Cullen A, Kurman RJ.** Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologist diagnosis of ASCUS. Am J Obstet Gynecol 1995; 172: 946-954.
23. **Wright TC Jr, Stewart-Massad L, Dunton Ch J, Spitzer M, Wilkinson EJ, Solomon D.** Consensus guidelines for the management of women with abnormal cervical cancer screening tests. AJOF 2006; 197(4):346-355.
24. **Parkin DM, Bray FI, Devessa SS**. Cancer Burden in the year 2000. The global Picture. Eur J Cancer 2001; 37 (Suppl 8): s4-s66.
25. **Camara GL, Cerqueira D, Oliveira A, Silva E, Carvalho L, Martins C.** Prevalence of human papillomavirus types in women with pre-neoplastic and neoplastic cervical lesions in the Federal District of Brazil. Mem Inst Oswaldo Cruz 2003; 98:879-883.
26. **Giuliano A, Papenfuss M, Abrahamsen M, Denman C, de Zapien J, Navarro JL, Ortega L, Brown E, Stephan J, Feng J, Baldwin S, Garcia F, Hatch K.** Human Papillomavirus Infection at the United Status-Mexico Border: Implications for Cervical Cancer Prevention and Control. Cancer Epidemiol Biomarkers Prev 2001; 1129-1136.
27. **Arrosi S, Sankaranayanan R, Maxwell D.** Incidente and mortality of cervical cancer in Latin America. Salud Pública Mex 2003; 45:S306-S314.
28. **Schiffman MH, Brinton LA.** The epidemiology of cervical carcinogenesis. Cancer 1995; 76:1888-1901.
29. **Carvalho MO, Carestiato FN, Perdigao PH, Xavier MPPT, Silva KC, Botelho MO, Oliveira LHS, Cavalcanti SMB.** Human Papillomavirus infection in Rio de Janeiro, Brazil: a Retrospective Study. Braz J Infect Dis 2005; 9: 398-404.
30. **Tonon SA, Picconi MA, Zinovich JB, Nardari W, Mampaey M, Galuppo JA, Bos PD, Badano I, Di Lello F, Basiletti J, González JV, Alonio LV, Teyssie AR.** Prevalence of cervical infection by human papillomavirus 8HPV) in the Caucasian and Guarani populations residing in the Provence of Misiones, Argentina. Rev Argent Microbiol 2003; 35:205-213.
31. **Molano M, Posso H, Weiderpass E, van der Brule AJ, Ronderos M, Franceschi S, Meijer CJ, Arslan A, Munoz N.** HPV Study Group HPV Study. Prevalence and determinants of HPV infection among Colombian women with normal cytology. Int J Cancer 2001; 91:412-420.
32. **Lazcano E, Herrero R, Muñoz N, Cruz A, Shah K, Alonso P, Hernandez, Salmerón J, Hernandez M.** Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer 2001; 91:412-420.
33. **Herrero R, Hildesheim A, Bratty C, Sherman M, Hutchinson M, Morales J, Balmaceda L, Greenberg L, Alfaro M, Burk R, Wacholder S, Plummer M, Schiffman M.** Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst (Bethesda) 2000; 92: 464-474.
34. **Bosch XJ, Munoz N, de Sanjose S, Izarzugaza L, Pili M, Viladiu P, Tormo M, Moreo P, Aschunce N, Gonzalez LC, Tafur L, Kaldor JM, Guerrero E, Aristizabal N, Santamaria M, Alonso-Ruiz P, Shah K.** Risk factors for cervical cancer in Colombia and Spain. Int Cancer 1992; 32:750-758.
35. **Mendoza JA, Muñoz M, Vielma S, Noguera ME, López M, Toro M.** Infección cervical por el virus del papiloma humano: diagnóstico por citología y por captura de híbridos del ADN viral. Rev Obst Ginecol Venez 2000; 60:103-107.
36. **Contreras-Irrazabal L, Correnti M, Ávila M, Guerrero A, León A.** Virus Papiloma Humano (VPH) en contexto ecológico ve-

nezolano (I): Diagnóstico citológico y molecular. Salus 2008, 12, 3:30-44.

37. **Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith IS, Shah KV, Meijer CJ, Bosch FX.** Role of parity and human papillomavirus in cervical cancer: The IARC multicentric case-control study. Lancet 2002; 359:1093-1101.
38. **Moreno V, Bosch FX, Muñoz N, Meijer CJ, Shah KV, Walboomers IM, Herrero R, Franceschi S.** Effect of oral contraceptives on risk of cervical cancer in women with human Papillomavirus infection: The IARC multicentric case-control study. Lancet 2002; 359:1085-1092.
39. **Callaghan J, Karim S, Mortlock S, Winter M, Woodward N.** Hybrid capture as a mean of detecting human papillomavirus DNA from liquid-based cytology specimens: a preliminary evaluation. Brit. J Biomed 2001; 58; 184-189.
40. **Morrison H.** Human Papillomavirus absence predicts normal cervical histopathologic findings with abnormal Papanicolau smears. J Hum Virol 1993; 4: 283-287.
41. **Cavalcanti SMB, Zardo LG, Pasos MRL, Oliveira LHS.** Epidemiological aspects of Human papillomavirus infection and cervical cancer in Brazil. J Infection 2000; 40:80-87.