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Prevalence and risk factors of human papillomavirus infection in asymptomatic women in a Venezuelan urban area.

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Key words: Prevalence, Human Papillomavirus, hybrid capture 2, Venezuela.

Abstract. The purpose of this study was to investigate the prevalence and risk factors of genital human papillomavirus (HPV) infection in asymptomatic women, using the HPV DNA Hybrid Capture 2 (HC2) test. Three hundred and two women who attended the Out-Patient Gynecological Clinic of a tertiary level hospital, in a Venezuelan urban area, were selected for the study. A pap smear, a cervical swab for HC2 and gynecological exam were performed to each patient. The HC2 testing showed that 47 samples (15.6%) were positive to HPV. Forty patients (13.2%) were positive to high risk-HPV (HR-HPV) and 11 (3.6%) were positive to low-risk-HPV (LR-HPV). The prevalence of HPV infections was higher for women under 35 years (51.1%; $p < 0.02$), and decreased to 6.4% for women ≥ 65 years old. Women who had not finished high school had a higher prevalence of HPV infection ($p < 0.035$). Twenty six (42.6%) of 61 pathological Pap smears were positives to HPV infection. A statistically significant difference was found when HPV infection was compared in normal and abnormal Pap smear (HSIL+LSIL; $p<0.0001$). Twenty four of 56 (43%) women with diagnosis of LSIL, and 2(40%) of 5 with diagnosis of HSIL were positive for HPV infection. A statistically significant difference was found when we compared HPV infection in negative Pap smears and those with LSIL ($p<0.001$). The present study found that the prevalence of HPV infection in asymptomatic Venezuelan women who attended a tertiary level hospital was 15.6%. HPV infection was more frequent in young adult, and in women with low educational level.

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Prevalencia y factores de riesgo en la infección del virus del papiloma humano en mujeres asintomáticas en un área urbana venezolana.

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Palabras clave: Prevalencia, virus del papiloma humano, captura de Híbridos 2, Venezuela

Resumen. El propósito de este estudio fue investigar la prevalencia y factores de riesgo que influencia la presencia de la infección por el virus del papiloma humano (VPH) en pacientes asintomáticas que asistieron a un hospital nivel 3 en un área urbana venezolana. Se estudiaron las pacientes que acudieron a la Consulta de Patología del Cuello Uterino del Hospital Manuel Noriega Trigo. A cada paciente se le realizó una historia clínica, toma de citología cervico-vaginal y una muestra del cérvix para captura de híbridos 2(CH2). Se incluyeron 302 pacientes. La CH2 mostró 47 muestras (15,6%) positivas al VPH. Cuarenta mujeres (13,2%) fueron positivas a VPH de alto riesgo (VPH-AR) y 11 (3,6%) a VPH de bajo riesgo (VPH-BR). La prevalencia de la infección por VPH fue más alta en mujeres ≤ 35 años (51,1%; $p < 0,02$) y disminuyó a un 6,4% en mujeres ≥ 65 años. Las pacientes que no habían terminado los estudios de bachillerato presentaron un prevalencia más elevada del VPH ($p < 0,035$). Veinte y seis (42,6%) de 61 CCV patológicas fueron positivas a la infección del VPH. Una diferencia estadísticamente significativa fue encontrada cuando se comparó la presencia del VPH en las CCV normales con las CCV anormales (Lesión Intraepitelial Escamosa de Alto y Bajo Grado-LIE-AG y LIE-BG; $p < 0,0001$). Veinte y cuatro de 56 (43%) mujeres con diagnostico de LIE-BG, y 2(40%) de 5 con diagnóstico de LIE-AG fueron positivos a la presencia del VPH. Se encontró una diferencia estadísticamente significativa cuando se comparó la presencia de infección por el VPH en CCV normales y CCV con LIE-BG ($p < 0,001$). El presente estudio encontró una prevalencia de la infección por el VPH en mujeres asintomáticas que asisten a un hospital nivel 3 de 15,6% en área urbana venezolana. Fue más frecuente en mujeres jóvenes y de bajo nivel educacional.

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INTRODUCTION

Cervical cancer (CC) is the second most common cancer in women worldwide (1). Eighty percent of cases are diagnosed in developing countries (2). In most developing countries, CC is the leading female malignancy and the leading cause of death by cancer, especially among middle-aged women (3).

A number of epidemiological risk factors have been identified as contributing to the development of cervical carcinoma (4). Infection with human papillomavirus (HPV) is one of them. HPV infection of the uterine cervix is the most common viral sexually transmitted disease (5), which is usually acquired around the time sexual activity begins. HPV infection is more common in

young women and adolescents than older women (6-8) and it frequently resolves spontaneously (6). Several types of HPV are now recognized as one of the main causes of cervical cancer and its precursors (9). The prevalence of genital HPV has steadily increased over the past twenty years and varies among different countries and even between different populations within countries (3). Prevalence of HPV infection in African countries such as Zimbabwe and Mozambique is over 40% (3). Some European countries, Spain, Italy, United Kingdom, Netherlands, report prevalence below 10% (3, 10). Bosch et al. (3) reported 22.5% and 13.3% of HPV infection in USA and Canada, respectively. In Latin-America, Clifford et al. (10) reported that the prevalence of HPV was 14.3% in cytologically normal women. In Venezuela, there are few reports about the prevalence of HPV infection using DNA testing (11, 12).

The recognition that HPV infection is necessary for the development of CC has been important (13). This knowledge has led to the development of new CC screening strategies which incorporate HPV de-oxyribonucleic acid (DNA) testing. One of these tests is Hybrid Capture® 2 HPV DNA (HC2) testing.

This study was carried out to determine the prevalence and risk factors of genital HPV infection in asymptomatic women who attended a tertiary level hospital in a Venezuelan urban area using HPV DNA HC2.

MATERIALS AND METHODS

Study population

A total of 302 women who attended the Out-Patient Gynecologic Clinic for annual Pap smear check-up, were studied during the period of August 2 through August 19, 2005. Patients with previous hysterectomy and treatment of premalignant or malignant lesions of the cervix were excluded from the study.

This study was performed at the Manuel Noriega-Trigo Hospital, which is a tertiary and urban referral hospital, serving middle and low socio-economic classes, in the southern part of the city of Maracaibo, Venezuela.

The study was approved by the ethics committees of the Manuel Noriega Hospital and Faculty of Medicine, University of Zulia. All participants read and signed an informed consent agreement before enrolment in the study. Patients were also informed of the anonymity and confidentiality of the study.

Each patient provided a medical history including obstetric and gynecological information, and then had a Pap smear, a cervical swab for Hybrid Capture 2 and gynecological examination. Pap smear was taken and processed by the conventional way.

The DNA testing, HC2 (Digene Co., Gaithersburg, MD, USA) was processed at Viral Oncology Section (VOS) Core Laboratory, National Cancer Institute, Frederick, MD, USA. Each cervical swab sample was studied for High Risk probe (HR-HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and Low Risk probe (LR-HPV types: 6, 11, 42, 43, 44). Both HC2 probes were performed in order to know the prevalence of High and Low Risk HPV types.

Two pathologists (MD, JG) studied the Pap smears. The 2001 Bethesda System (TBS) for reporting cervical cytological diagnosis was used in the cytological analysis.

Statistical analysis

Mean values and Standard Deviation (SD) of all continuous variables were calculated. Categorical variables were expressed as percentages of each group, and the simple frequencies were used for the categorical variables. To determine statistical rele-

vance of the various parameters of the study Chi Square test, Fisher Exact test and logistic regression were performed. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

The mean age was 39.3±11.2 years old (mean ± SD) (range: 17-72). One hundred twenty seven women (42.1%) were married. One hundred thirty one (43.4%) were housewives. Two hundred seventy eight (92.1%) had pregnancies with 90.4% (n=273) reporting deliveries. Sexual and reproductive data are shown in Table I.

The HC2 testing showed that 47 samples (15.6%) were positive for HPV. Forty patients (13.2%) were positive for High Risk-HPV (HR-HPV) and 11 (3.6%) were positive for Low-Risk-HPV (LR-HPV). Four cases (1.3%) were positive for both. Table II shows that the prevalence of HPV infections was higher for women under 35 years (51.1%; p< 0.027; Odds Ratio, OR: 0.497; 95% Confidence Interval, CI: 0.265-0.930), and decreased to 6.4% for women ≥ 65 years old. HR-HPV and LR-HPV prevalence was 47.5% (p=NS) and 72.8% (p<0.018; OR: 4.453; 95% CI: 1.157-17.141) for women under 35 years, respectively. HR/LR-HPV infection decreased as women got older.

Women who were ≤ 30 years old (p < 0.02) and that had not finished high school had a higher prevalence of HPV infection (p < 0.035). Other risk factors for having HPV infection are summarized in Table III. Table IV shows that higher number of sexual partners was associated with a higher HPV infection in different age groups. However, there was no statistically significant difference in the number of sexual partners when the different groups of age were compared. The average number of sexual partners was 1.57 for women younger than 30 years of age, 2.01 for women 30

TABLE I
SEXUAL AND REPRODUCTIVE VARIABLES

Variables	Mean	SD	Range
1st SI*	19	3.8	13-37
Partners	1.72	0.96	1-8
No Pregnancies	3.16	1.85	1-10
No Deliveries	2.9	1.6	1-9

*Age of 1st SI: Sexual Intercourse. SD: Standard Deviation.

TABLE II
PREVALENCE OF HPV INFECTION ACCORDING TO AGE

Age group (yrs)	Women Nº (%)	Any type HPV Nº (%)	HR-HPV Nº (%)	LR-HPV Nº (%)
17-24	28(9.3)	10(21.3)	9(22.5)	3(27.3)
25-34	86(28.5)	14(29.8)	10(25)	5(45.5)
35-44	82(27.2)	12(25.5)	11(27.5)	2(18.2)
45-54	81(26.8)	5(10.6)	5(12.5)	0
55>	25(8.27)	6(12.8)	5(12.5)	1(9.1)
Total	302(100)	47(15.6)	40(13.2)	11(3.6)

TABLE III
RISK FACTORS ASSOCIATED WITH HPV INFECTION

Risk	Nº of cases	HPV Infected	%HPV	p	OR	95%CI
Age						
≤30	80	19	23.8			
≥31	222	28	12.6	0.02	2.076	1.055-4.088
Civil Status						
Married	127	14	11.0			
Unmarried	175	33	18.9	NS	-	-
Educational[a] Level						
<11	117	24	20.5			
>11	185	23	12.4	0.035	2.562	1.161-5.649
Age First Intercourse						
≤18	175	28	16			
≥19	127	19	15	NS	-	-
No Partners						
1	155	23	14.8			
≥2	147	15	10.2	NS	-	-
No Pregnancies						
≤1	76	14	18.4			
≥2	226	33	14.6	NS	-	-
No Deliveries						
≤1	273	40	14.7			
≥2	29	7	24.1	NS	-	-

[a]Years. p: significance. OR: Odds Ratio. CI: Confidence Interval.

TABLE IV
COMPARISON AMONG HPV, AGE AND NUMBER OF PARTNERS

Age(years)	Nº of Partners	Nº	HPV	
			Nº	%
<30	1	48	11	22.9
	≥<2	32	8	25
31-39	1	29	4	13.7
	≥2	47	7	14.8
≥40	1	78	8	10.3
	≥2	68	9	13.2
Total	1	155	23	14.8
	≥2	147	15	10.2

to 39 years, 1.4 for women older than 40 years (p=NS).

Pap smear findings showed that 241 (79.8%) patients were negative for intraepithelial lesion or malignancy, 56 (18.5%) patients with low-grade squamous intraepithelial lesion (LSIL), and 5 (1.7%) with high-grade squamous intraepithelial lesion (HSIL). The median age of patients with cytological diagnosis of LSIL was 38 years (37.16 ± 9.65) (mean ± SD). The prevalence of this diagnosis was higher in

women between 35 and 44 (33.9%) years old. The median age of patients with cytological diagnosis of HSIL was 32 years old (34.4 ± 10.6). Three of 5 (60%) women with a diagnosis of HSIL were between 25 and 38 years.

Twenty six (42.6%) of 61 pathological Pap smears were positive for HPV infection detected by HC2. Twenty-one patients (8.7%) of 241 with Pap smear negative for intraepithelial neoplasia or malignancy were positive for HPV infection. A statistically significant difference was found when HPV infection was compared between normal and abnormal Pap smear (HSIL + LSIL; p < 0.0001; OR: 7.782; 95%CI: 3.956-15.310). Twenty four of 56 (43%) women with diagnosis of LSIL, and 2(40%) of 5 with a diagnosis of HSIL diagnosis were positive for HPV infection, respectively, as shown in Table V. A statistically significant difference was found when we compared the presence of HPV infection in negative Pap smear and LSIL (p<0.001; OR: 7.857; 95% CI: 3.938- 15.715) and in normal Pap smear and HSIL was not statistically significant. (p < 0.07; OR: 0.1; 95% CI: 0.015-0.642).

Women with cytological diagnosis of LSIL, LR-HPV infection were statistically significant different (p < 0.037; OR: 0.260; 95% CI: 0.077-0.886).

HR-HPV infection was statistically significant in patients with Pap smear diagnosis of LSIL (p<0.0001; OR: 0.095; 95% CI: 0.045-0.201) and with diagnosis of HSIL (p<0.04; OR: 0.100; 95% CI: 0.15-0.642).

DISCUSSION

Human papillomavirus is a necessary cause for the development of carcinoma of the cervix and its precursors (13-15). The prevalence of genital HPV infection has steadily increased over the past 20 years, which varies among different countries and even between different populations within countries (16, 17). We investigated the prevalence of HPV infection in a population of 302 urban, asymptomatic, sexually active women and its relationship to a variety of biologic, demographic, and behavioral risk factors.

Among asymptomatic women in the general population, the prevalence of HPV infection ranges from 2 to 44% (5). The worldwide prevalence of HPV infection is estimated to be 10.41% (95% CI: 10.2-10.7) (5). The HPV prevalences estimated by continents are: Africa: 21.12% (95% CI: 20.87-23.43), America: 12.95% (95% CI: 12.41-13.51), Europe: 8.08% (95 CI: 7.77-8.41) and Asia: 7.95% (95% CI: 7.53-8.4) (3, 5, 10). Burchell et al. (5) mentioned that the number of women who harbor HPV-DNA worldwide is estimated in 291 million, and around 105 million will

TABLE V
PAP SMEAR AND HUMAN PAPILLOMAVIRUS INFECTION

	Total Cases		Any Type HPV		HR-HPV		LR-HPV	
	Nº	%	Nº	%	Nº	%	Nº	%
All diagnoses	302	100	47	15,6	40	13,2	11	3,6
Normall	241*	79,8	21	8,7	15	6,2	6	2,5
LSIL	56	18,5	24	43,0	23	41,1	5	8,9
HSIL	5	1,7	2	40.0	2	40,0	-	-

LSIL: Low-risk Squamous Intraepithelial Lesion. HSIL: High-risk Squamous Intraepithelial Lesion * HPV infection/Normal Pap Smear vs. LSIL+HSIL: p<0.0001(OR: 7.782; 95%CI: 3.956-15.310).

have a HPV 16 or 18 infections (16). Clifford et al. (10) found a worldwide HPV prevalence of 9.2%. Trottier et al. (18) reported that there are 6.2 million new cases of HPV infection cases each year in USA, and approximately 20 million Americans are infected with HPV at any one time.

This study found that the overall prevalence of HPV infection in asymptomatic women was 15.6%. This prevalence was similar to the prevalence reported in several Latin-America countries for different authors (3, 19). Herrero et al. (19) reported a prevalence of HPV infection of 16% in Costa Rica. Bosch and de Sanjosé (3) reported that HPV infection prevalence in the general population in Canada, Mexico and USA was 13.3%, 14.5% and 22.5%, respectively. In South America countries, such as Argentina and Colombia, the prevalence was 16.6% and 14.9%, respectively (3). In 2000, Mendoza et al. (11) found a prevalence of HPV infection of 9.9% in 101 patients using HC in another Venezuelan city. Scucces et al. (12) found a prevalence of 51% of HPV infection using PCR in Venezuela in 2001. Theses authors studied 39 patients.

The present study found that the HR-HPV infection (13.2%) in asymptomatic women was more common than the infection produced by LR-HPV (3.6%). Mendoza et al. (11) reported 7 cases of HR-HPV infection, 2 cases of LR-HPV infection and 2 cases positive for both types of infection. Scucces et al. (13) found 12.8% of prevalence of HR-HPV infection. Nyári et al. (16) reported 16% of HR-HPV type infection. Herrero et al. (19) found a prevalence of HR-HPV infection of 7.6% in more than 8,000 women. Clifford et al. (10) reported HR-HPV and LR-HPV prevalences of 6.1% and 2.5% in 15,613 sexually active women, respectively. Other studies (20, 21) have also shown HR-HPV prevalences ranging from 5 to 15%.

The present study found that women younger than 35 years of age ($p < 0.02$) had a higher prevalence of HPV infection. Other authors (16, 19, 20, 22, 23) have reported similar results. Different authors (19, 23, 24) have found an age-related decline in HPV prevalence. The decreasing prevalence of HPV infection with increasing age, even after adjustment for sexual behavior, could happen because the genital HPV infection is cleared by local cell mediated immunity and protection against a further infection by the same genotype of HPV (22, 25). Herrero et al. (19) found an increase of HPV infection to almost 20% for women 65 years old or older. Burchell et al. (5) reported that the prevalence of HPV infection is highest for young women, decreases in the middle age groups and increases at age 65. A two peak pattern is observed in many studies all over the world, with the exception of Asia, where the age-specific curves decrease smoothly with increasing age. The reasons for the second peak and its geographic variation are unclear, but may be influenced by one or more non-mutually exclusive mechanisms, for example, reactivation of previously undetectable infections acquired earlier in life could occur due to a gradual loss of type-specific immunity or to a sudden loss due to hormonal influences during the post-menopausal years(5, 18, 26). The second peak could also originate from acquisition of new infections due to sexual contacts with new partners later in life (5, 18, 26). Because the changes in sexual morals or patterns over the last several decades have affected some cultural groups more than others, this explanation cannot be ruled out. Further, other cofactors that may affect HPV progression or clearance (e.g., smoking, parity, oral contraceptives) and competing risks (e.g., mortality due to other causes) could also be involved (5). Trottier et al. reported (18) that the decrease in risk of HPV infection with increas-

ing age seems to be independent of changes in sexual behavior, suggesting a role for immune response. No second peak was observed in this investigation and found that the number of partners declined with increasing age.

Risk determinants for HPV infection that have been identified in various cross-sectional and prospective cohort studies include number of sexual partners (lifetime and recent), age at first intercourse, smoking, long-term use of oral contraceptive, other STDs (e.g. *Chlamydia* and herpes simplex virus), multiparity, vaginal infection, malnutrition, chronic inflammation, immunosuppressive conditions including HIV infection, and parity(5, 10, 18, 26-28). Results have been inconsistent partly owing to the fact that different populations have been studied. Furthermore, risk factor profiles have been found to differ depending on whether HR or LR-HPV types were considered (29). The present study did not find any correlation between civil status, number of partners, age of first intercourse, number of pregnancies and deliveries, and HPV infection. Other authors have found the civil status (unmarried) and nulliparous woman or few pregnancies as statistically significant predictor factors of the presence of HPV infection (5, 10). This investigation found a significant difference according to the educational level of women ($p > 0.035$, Table III). Most of the women studied were from low and middle low socioeconomic level. Low socioeconomic level is usually associated to low educational level and promiscuous sexual behavior (30).

The median age of patients with LSIL and HSIL were 38 and 32 years old in this study, respectively. Herrero et al. (19) reported a prevalence of LSIL highest among the youngest patients, with a median age of 29 years old. The same authors (19) found the median age of 34 years in patients with HSIL.

The present study found that the presence of HPV infection in women with cytological findings of SIL was statistically significant when compared with patients with normal Pap smears. Different authors (16, 18, 22) reported similar results. HPV infection was statistically significant when LSIL was compared with normal Pap smear but not HSIL and normal Pap smear. The low number of patients with HSIL could explain this result, and this is a limitation of this investigation. Forty three percent of women with LSIL Pap smear had HPV infection as observed in Table V. Twenty three of 24 (95.8%) patients with LSIL on Pap smear were positive for HR-HPV types. Herrero et al. (19) found cancer associated HPV types present in more than a 50% of LSILs. They (19) reported that LSILs having cancer associated HPV types are probably the most likely to persist and progress. Clifford et al. (31) reported that overall HPV positivity in LSIL varied from 29 to 100%.

Our results indicated that the prevalence of HPV infection in asymptomatic Venezuelan women was 15.6%, similar to other Latin-American countries. This study confirmed that HPV infection was more frequent in young adults, and was found more commonly in women with a low educational level. Also, HPV infection was statistically significantly associated with SIL, and more than 40% of the LSIL were positive to HR-HPV.

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