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# Human papillomavirus false positive cytological diagnosis in low grade squamous intraepithelial lesion.

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**Key words:** Human Papillomavirus, false positive, low-grade squamous intraepithelial lesion, pap smear, hybrid capture 2.

**Abstract.** The purpose of this study was to investigate the number of Human Papillomavirus false positive cytological diagnosis in low grade squamous intraepithelial lesions (LSIL). Three hundred and two women who assisted to an Out-Patient Gynecologic Clinic in Maracaibo, Venezuela, were recruited for this study. Each patient had the Pap smear and a cervical swab for Hybrid Capture 2 (HC2). Three cytotechnologists reviewed the Pap smears and two pathologists rescreened all of them. The cytotechnologists reported 161 (53.3%) Pap smears negatives for intraepithelial lesion (IL) or malignancy, and 141 cases (46.7%) with epithelial abnormalities. They reported 46% of 302 patients with HPV infection in Pap smear slides. The pathologists found that 241 (79.8%) Pap smears were negatives for IL or malignancy and 61 (20.2%), with abnormal Pap smears. They found 14.6% HPV infection in all Pap smears ($p<0.0001$; 46% vs 14.6%). The HC2 study showed that 47 samples (15.6%) were positive for HPV. The study found that 114 Pap smears (False Positive: 85%) of 134 reported by the cytotechnologists and 24 (False Positive: 43%) of 56 cytologies reported by the pathologists as LSIL, were negative for HPV infection determined by HC2 ($p<0.00003$). The present study suggests that the cytotechnologists overdiagnosed cellular changes associated with HPV infection in the Pap smear, increasing the FP cytological diagnosis of LSIL.

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**Falsos positivos en el diagnóstico citológico del virus del papiloma humano en lesiones intraepiteliales cervicales de bajo grado.**

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**Palabras clave:** Virus del Papiloma Humano, falsos positivos, lesión intraepitelial cervical de bajo grado, citología cervico-vaginal, captura de híbridos.

**Resumen.** El presente trabajo tuvo por objeto el investigar el número de falsos positivos reportados en la citología cervicovaginal (CCV) de la presencia del Virus del Papiloma Humano (VPH) con diagnóstico de Lesión Intraepitelial Escamosa de bajo grado (LIE-BG). Se estudiaron 302 mujeres que asistieron a la Consulta de Patología de Cuello Uterino del Hospital Manuel Noriega Trigo, en Maracaibo, Venezuela. A cada paciente se le practicaron una CCV y muestra para la captura de híbridos 2 (CH2). Tres citotecnólogos y 2 patólogos estudiaron las CCV. Los citotecnólogos reportaron 161(53,3%) de CCV negativas para lesión intraepitelial o malignidad y 141 casos (46,7%) con anomalías epiteliales. Éstos encontraron 46% de presencia de VPH en las 302 CCV. Los patólogos reportaron 241 CCV (79,8%) negativas y 61 CCV (20,2%) anormales. Estos encontraron en 14,6% de las CCV, la presencia de VPH ($p < 0, 0001$; 46% vs 14,6%). La CH2 mostró que 47 muestras (15, 6%) fueron positivas a VPH. Esta investigación mostró que 112 CCV de 134 (Falso Positivo: 85%) reportados por los citotecnólogos y 24 de 56 CCV (Falso Positivo: 43%) reportados por los patólogos como LIE-BG, fueron negativos a la infección del VPH determinados por la CH2 ($p < 0,00003$). La investigación sugiere un sobrediagnóstico de la presencia de cambios celulares debidos al VPH en la CCV, por parte de los citotecnólogos, incrementando los falsos positivos de la presencia del VPH en CCV con diagnóstico de LIE-BG.

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## INTRODUCTION

To date, the detection of premalignant and malignant lesions of the cervix by Papanicolaou (Pap) smear is widely recognized as the most effective method to screen and to prevent cervical carcinoma (CC) (1, 2). Since the 50`s, the implementation of the Pap smear as a screening test has led to a major reduction in the annual mortality rate on a worldwide basis by CC, especially in developed countries (2).

It has been established that the infection by Human Papillomavirus (HPV), especially high-risk types, is the primary cause of almost all CC (3-5). Ho *et al.* (6) have reported that the most important factor in CC development is long-term HPV persistence in combination with a weak immune response of the host.

There are different methods to diagnose the HPV infection: Pap smear, colposcopy (7), histological study, immunohistochemical stain (7), and HPV-deoxyribonucleic acid (DNA) technologies.

Cervical HPV infection can be observed by the Papanicolaou smear. It is a superficial or intermediate mature squamous cell that it is characterized by a large peri-

nuclear cavity associated with a peripheral rim of thickened cytoplasm. The peripheral cytoplasm is very dense and stains irregularly, exhibiting a brownish green color or a dense fuchsia reaction. The nuclei may become quite dense, hyperchromatic, and pyknotic. Binucleation is frequent, and multinucleation may be seen (6, 7). In 2001, the Bethesda system (TBS) encompassed HPV infection known as koilocytotic atypia and mild dysplasia/cervical intraepithelial neoplasia 1 as low-grade squamous intraepithelial lesion (LSIL) (8)

A questionable aspect of TBS is the inclusion of koilocytosis within the category of low grade-squamous intraepithelial lesion (LSIL) to indicate cellular changes associated with HPV infection (9). Some could contend that koilocytosis is indistinguishable from mild dysplasia/CIN 1. This could increase the HPV and/or LSIL false positive diagnosis.

Presently, the two technologies most widely used for HPV-DNA detection are the Polymerase Chain Reaction™ (PCR) using generic or consensus primers, and Hybrid Capture™-2 (HC2, Digene Co., Gaithersburg, MD, USA) (10).

Authors did not find any previous publication about the presence of HPV infection in LSIL in our country. The objective of this study was to evaluate the real incidence of HPV infection in LSIL in a Venezuelan urban area.

## MATERIALS AND METHODS

### Study population

A total of 302 women who assisted to the Out-Patient Gynecologic Clinic at the Manuel Noriega-Trigo Hospital, Maracaibo, Venezuela, for their annual Pap smear check-up, were studied during the period of august 2 and august 19, 2005. Patients with previous hysterectomy and treatment of premalignant or malignant lesions of the cervix were excluded from the study.

The Manuel Noriega-Trigo Hospital is a tertiary urban referral hospital serving middle and low socio-economic classes in the south part of the city of Maracaibo, Venezuela.

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

Each patient provided a medical history including obstetrics and gynecological information before she had the Pap smear, a cervical swab for Hybrid Capture 2 (HC2) and gynecological examination. Pap smear was taken by the conventional way.

HC2 (Digene Co., Gaithersburg, MD, USA) was performed by the Viral Oncology Section (VOS) Core Laboratory, National Cancer Institute, Frederick, MD, USA. Each cervical swab sample was studied for High Risk probe (HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and Low Risk probe (HPV types: 6, 11, 42-44).

Three experienced cytotechnologists reviewed all Pap smears. Once they finished, two of them reviewed the abnormal Pap smear slides again. The two pathologists (MD and JG) began to rescreen the slides when the cytotechnologists finished the second rescreening. The pathologists' studies were blind. Each pathologist reviewed half of the 302 Pap smears. The TBS 2001 was used in the cytological analysis.

### Statistical analysis

The means and standard deviations were calculated for the continuous variables, and the simple frequencies were used for the categorical variables. To determine the statistical relevance of the various parameters of the study, Chi Square test was 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%) reported previous pregnancies with 90.4% (n=273) reporting deliveries. Sexual and reproductive data are shown in Tab1e I.

Three hundred and two Pap smears were studied by the cytotechnologists, 161 (53.3%) Pap smears were negative for intraepithelial lesion (IL) or malignancy, and 141 cases (46.7%) presented cellular abnormalities. One hundred thirty four (95%) were LSIL and 7 (5%) high-grade squamous intraepithelial lesions (HSIL). The cytotechnologists found cytological findings suggesting Human Papillomavirus (HPV) infection in 139 (98.6%) of 141 abnormal cervical Pap smears: 132 (95%) LSIL and 7 (5%) HSIL. They reported 46% of 302 patients with HPV infection in Pap smear slides.

The pathologists (MD and JG) reviewed all 302 Pap smears after the cytotechnologists did. They reported: 241 (79.8%) Pap smears were negative for IL or malignancy and 61 (20.2%) were abnormal Pap smears. Fifty six (91.8%) were reported as LSIL and 5 (8.2%) as HSIL. Seventy two percent (n=44) of 61 abnormal Pap smears were reported having cellular changes associated with HPV infection: 42 cases (95.5%) LSIL and 2 (4.5%) HSIL. The pathologists found 14.6% HPV infection in all Pap smears.

Cellular changes by HPV infection reported in Pap smears by cytotechnologists and pathologists were compared, a statistically significant difference ($p<0.0001$; 46% vs 14.6%) was found.

The HC2 testing showed that 47 samples (15.6%) were positive for 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). Four cases (1.3%) were positive to both probes.

**TABLE I**
SEXUAL AND REPRODUCTIVE VARIABLES

| Variables | No. | 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 for 1st SI: Sexual Intercourse. SD: Standard Deviation.

The study found that 114 Pap smears (False Positive-FP: 85%) of 134 reported by the cytotechnologists as LSIL were negative for HPV infection determined by HC2 and 22 (False Negative-FN: 13.7%) of 161 Pap smears negative for IL or malignancy were positive for HPV-DNA HC2. Twenty (True Positive-TP: 15%) of 134 women with LSIL were positive to HPV-DNA HC2.

Twenty four Pap smears (FP: 43%) of 56 reported by the pathologists as LGSIL were negative to HPV-DNA HC2; 21(FN: 8.7%) of 241 negatives for IL or malignancy were positive to HPV-DNA HC2. Thirty two (TP: 57%) of 56 Pap smears with LSIL diagnosis were positive to HPV-DNA HC2.

A statistically significant difference was found when the results of the cytotechnologists' FP and the pathologists' FP were compared ($p < 0.00003$). When the FN reported by cytotechnologists and pathologists were compared, no statistically significant difference was found ($p < 0.115$). When TP between cytotechnologists' and pathologists' results were compared, a significant difference was found ($p < 0.00003$).

## DISCUSSION

Cervical screening based on conventional cytology is far from perfect as screening method, but the detection of cervical cancer (CC) and its precursors by Pap

smear is widely recognized as the most effective method for preventing CC (1, 11).

Descriptive epidemiological studies have demonstrated a remarkable decrease the incidence and mortality rates attributable to squamous cell carcinoma of the cervix subsequent to the introduction of cytological screening in developed countries over the last 4 decades (2, 11-13). Despite its success, Pap smear has failed to reduce CC rates in developing countries. There are several reasons to explain this failure, such as low coverage and attendance rate as well as technical limitations regarding sampling and laboratory errors in screening and interpretations (11). Pap smear has a low sensitivity, high specificity (14, 15), limited reproductibility, high FN and FP (11, 16).

Since 1989, TBS has been established as the method to study and report cervical cytology. In 1990, the American College of Obstetrician and Gynecologists (9) reported that TBS`elevation of koilocytosis to LSIL could introduce potential problems: 1.- overdiagnosis, 2.- increase of LSIL FP, 3.- unnecessary treatment (9) and 4.- patients with an elevated anxiety level (13). False-positive cytology results lead to unnecessary and frequently invasive procedures (11).

The discovery of HPV as the etiological agent of CC and its precursors, has allowed the development of tests to detect HPV-DNA in cervical cells and has had significant implications for strategies to prevent CC (17). HC2 is one of the technologies used to detect low and high-risk of HPV-DNA using signal amplification. It is now clear that HPV testing is substantially more sensitive than cytology at detecting high-grade cervical intraepithelial neoplasia (CIN) (9,17); however, HC2 testing is less specific than Pap smear (9, 17), although cytology has had a major impact on the detection rates of CC and its precursors (9,18). HC2 has been approved by the FDA (USA), for clinical proposes. HC2 detects 13 High Risk HPV types (HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and 5 Low Risk HPV types (HPV types: 6, 11, 42, 43, 44). HC2 does not provide individual typing information, so that patients infected by a different HPV type will have a HC2 testing negative.

The prevalence of HPV infection in the general population ranges from 2-42.8% (19). In Latin-America, the prevalence is about 14.5-16.6% (19). The prevalence of HPV infection in the current study was 15.6%. This investigation showed that the prevalence of HPV infection in this asymptomatic population to this viral infection who assist to the Out-Patients Gynecologic Clinic, Manuel Noriega Trigo Hospital, Venezuela, is similar to the prevalence in other Latin-American countries (19, 20). Although the population studied in this investigation is not a risk group for HPV infection, we noted the high number of LSIL reported by cytotechnologists. How do we know a LSIL has a HPV infection? Our cytotechnologists and pathologists have to write down the presence of HPV infection in the report when it is present in the Pap smear slide. This investigation found a statistically significant difference ($p < 0.0001$/ 46% vs 14.6%) when cellular changes associated with HPV infection reported in Pap smears (LSIL/HSIL) by cytotechnologists and pathologists were compared. The high percentage of LSIL reported by the cytotechnologist is explained by the high number of HPV cytological FP diagnosed. The pathologists reported cellular changes by HPV infection in Pap smear (14.6%), a close rate to the HPV-DNA HC2 findings (15.6%). Kornya *et al.* (21) found morphological changes associated with HPV in 117 cases (10.6%) of 1100 Pap smears.

Allan *et al.* (22) reported 10.9% of FN, Kornya *et al.* (21) and Venturoli *et al.* (23) found 18.3% and 21.3% FN, respectively. Agorastos *et al.* (11) reported FN of 2.31%

in Greek women. Other studies (16, 24) have reported from 10% to 14% of HPV infection among women with negative Pap smear. This study found 13.7% (cytotechnologist) and 8.7% (pathologist) HPV infection in women with normal Pap smears. Schiffman *et al.* (25) mentioned that a third of women with HPV infections detected by DNA testing have recognized cytopathology in the Pap smear slide, so that cytological abnormalities are less sensitive for detection of HPV infection than molecular testing.

The rate of LSIL has increased in the United States in the last decade (26). In 1998, a College of American Pathologists' study reported a LSIL median rate of 1.6% (27), and in 2003 the mean LSIL reporting rate was 2.9% for liquid-based specimens (26). According to Wright *et al.* (28), a result of LSIL is a good indicator of HPV infection. In a recent metaanalysis, Arbyn *et al.* (29) reported that the pooled estimate of HR-HPV DNA positivity among women with LGSIL was 76.6%. Clifford *et al.* (10) reported an overall HPV positivity in LSIL from 29 to 100% using PCR. The present study found 15% TP HPV infection in LSIL reported by the cytotechnologist and 57% reported by the pathologist, a difference statistically significant was found when these results were compared ($p < 0.00003$). The cytotechnologist reported 85% of FP HPV infection in LSIL and the pathologist found 43%. The difference between there two reports was statistically significant ($p<0.00003$). Kornya *et al.* (21) reported 35% TP and 65% FP. Agorastos *et al.* (11) reported TP of 0.54% and FP of 1.15%.

Fifty two (17%) of the women studied were ≥ 50 years old. Ten (19.2%) and 5 (9.6%) had the diagnosis of LGSIL by cytotechnologists and pathologists, respectively. The observers could have interpreted the cytological features that mimic koilocytes such as the pseudo-koilocytosis, that is present in atrophic smears (30). This misinterpretation could have increased the FP.

In Venezuela, as in most developing countries, the Cervical Cancer Screening Programs is based on Pap smear using the conventional way. The Venezuelan Public Health Services hire cytotechnologists part time and establish that each cytotechnologist must review 5-6 Pap slides/hour. Most of them have 2-3 part time jobs. Maybe, cytotechnologists have a high number of FP HPV infections because they could not look for all the cytological criteria to make the HPV infection diagnosis. Franco *et al.* (30) mentioned that Pap smear is a highly subjective interpretation of morphological changes present in cervical slides. The repetitive nature of the Pap smear screening leads to fatigue, which can cause interpretation errors.

This study has limitations: 1. each observer did not review all Pap smears so that we could not analyze the interobserver variability among the cytotechnologists and the pathologists; 2. the number of HSIL was low in order to analyze HPV FP; 3. we could not know if there were women infected by other HPV types, because of HC2 is able to detect the most common 18 HPV types.

In conclusion, the present study suggests that the cytotechnologists overdiagnosed cellular changes associated with HPV infection in Pap smears, increasing the FP LGSIL diagnosis rate at the Manuel Noriega Trigo Hospital. The pathologists diagnosed HPV infection in Pap smears at a similar rate to the detection rate of HPV by HC2. This investigation recommends improving the hiring conditions of cytotechnologists by Venezuelan health authorities. In addition, any Pap smear diagnosed with cellular changes associated with HPV infection should be reviewed by a pathologist. The screening program would also benefit from workshops to refresh, discuss

and upgrade knowledge in cytology and HPV infection.

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