UNIVERSIDAD AUTÓNOMA DE GUERRERO

UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS
UNIDAD ACADÉMICA DE MEDICINA
UNIDAD DE INVESTIGACIÓN ESPECIALIZADA EN MICROBIOLOGÍA

MAESTRÍA EN CIENCIAS BIOMÉDICAS

"EXPRESIÓN DE CICLINA A, D Y E EN LESIONES TEMPRANAS DEL CÉRVIX UTERINO ASOCIADAS AL VPH-AR EN TRABAJADORAS UNIVERSITARIAS GUERRERENSES"

T E S I S

QUE PARA OBTENER EL GRADO DE

MAESTRÍA EN CIENCIAS BIOMÉDICAS

PRESENTA:

MA. ISABEL ZUBILLAGA GUERRERO

DIRECTORA DE TESIS: **Dra. LUZ DEL CARMEN ALARCÓN ROMERO**

CHILPANCINGO, GRO., NOVIEMBRE DE 2011

UNIVERSIDAD AUTÓNOMA DE GUERRERO
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APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 1 días del mes de julio de dos mil once, se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado de la Maestría en Ciencias Biomédicas, para examinar la tesis titulada "**Expresión de ciclina A, D y E en lesiones tempranas del cérvix uterino asociadas al VPH-AR en trabajadoras universitarias guerrerenses**", presentada por la alumna Ma. Isabel Zubillaga Guerrero, para obtener el Grado de Maestría en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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Dra. Berenice Illades Aguiar
Codirección de tesis

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Dr. Marco Antonio Leyva Vázquez

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Dra. Berenice Illades Aguiar
Directora de la Unidad Académica de Ciencias Químico Biológicas

Este trabajo se realizó en el Laboratorio de Investigación en Citopatología e Histoquímica de la Unidad Académica de Ciencias Químico Biológicas de la Universidad Autónoma de Guerrero, en la ciudad de Chilpancingo, Gro, Mexico.

Bajo la dirección de:

Dra. Luz del Carmen Alarcón Romero

Y Codirección de:

Dra. Berenice Illades Aguiar

Y la asesoría de:

Dra. Eugenia Flores Alfaro

Dr. Marco Antonio Leyva Vázquez

Dra. Esther Ivonne López Bayghen Patiño

Con la colaboración de:

Dr. Eduardo Castañeda Saucedo

Jefe del Laboratorio de Biología Celular del Cáncer de la Unidad Académica de Ciencias Químico Biológicas de la U.A.G.

Dr. José Francisco Muñoz-Valle

Jefe del Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud (CUCS) de la U.D.G.

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Muchas veces nos preguntamos cual será nuestra misión en esta tierra, cual será aquella tarea que Dios ha encomendado a nosotros; cada persona en este mundo tiene un misión, tenemos tantos dones que a veces ni cuenta nos damos de cuál es, algunos escriben, otros componen, otras cantan, son tantos que la lista sería infinita y aun así no vemos que tan maravillosos podemos ser. Todo pasa por algo en la vida, nuestro deber es entender el porqué de que sucediera.

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DEDICATORIAS

Papá: Te quiero, porque entre más humano te veo más te quiero y te admiro. Saber que eres tan humano como yo eso si es de importancia, porque sé cuánto sufres. Sé que a veces tienes problemas pero lo guardas en tu silencio con mi madre para no preocuparnos.

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El poder y la persona misma desaparecerán, pero la virtud de unos grandes padres vivirá para siempre.

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“THE EXPRESSION OF CYCLINS A, D1 AND E AND THE ASSOCIATION WITH THE INTEGRATED STATE OF HR-HPV IN CYTOLOGIES WITH AND WITHOUT LOW-GRADE LESIONS”

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IGC Submission Confirmation for The expression of cyclins A, D1 and E and the association with the integrated state of HR-HPV in cytologies with and without low-grade lesions

11/11/2011

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International Journal of Gynecological Cancer

The expression of cyclins A, D1 and E and the association with the integrated state of HR-HPV in cytologies with and without low-grade lesions

Authors:

Ma. Isabel Zubillaga-Guerrero, MS[1]; Berenice Illades-Aguiar, Ph.D[2]; Marco Antonio Leyva-Vázquez, Ph.D[2]; Eugenia Flores-Alfaro, Ph.D[3]; Esther López-Bayghen, Ph.D[4]; José Francisco Muñoz-Valle, Ph.D[5]; Eduardo Castañeda-Saucedo, Ph.D[6]; Luz del Carmen Alarcón-Romero, Ph.D[1*]

Affiliations:

[1]Laboratory for Research in Cytopathology and Histoquemical, Academic Unit for Chemical Biological Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero, Mexico.

[2]Laboratory for Molecular Biomedicine, Academic Unit for Chemical Biological Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero, Mexico.

[3]Laboratory for Chronic-Degenerative Diseases, Academic Unit for Chemical Biological Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero, Mexico.

[4]Department of Genetics and Molecular Biology, CINVESTAV (Center for Research and Advanced Studies of the National Polytechnic Institute), DF, Mexico.

[5]Department of Molecular Biology and Genomics, University Center for Health Sciences (CUCS), University of Guadalajara, Jalisco, Mexico.

[6]Laboratory for Cancer Cellular Biology, Academic Unit for Chemical Biological Sciences, Autonomous University of Guerrero, Chilpancingo, Guerrero, Mexico.

***Corresponding author:**

Luz del Carmen Alarcón-Romero

Laboratory for Research in Cytopathology and Histoquemical, Academic Unit for Chemical Biological Sciences, Autonomous University of Guerrero.

Av. Lázaro Cárdenas, Ciudad Universitaria, Chilpancingo, Guerrero. México. CP. 39090

Email: luzdelcarmen14@gmail.com

Phone/Fax: +52 (747) 471-0901

ABSTRACT

Objective: To evaluate the association of the physical state of DNA and types of high risk human papillomavirus (HR-HPV) with the expression of cyclins A, D1 and E in cytologies with the diagnosis of low-grade squamous intraepithelial lesion (LSIL). **Methods/Materials:** 115 cytological specimens in liquid base (liquid-PREP™) were analyzed. 25 specimens were with no signs of SIL or HPV (NSIL without HPV); 30, with no signs of SIL (NSIL) but with low risk HPV (NSIL with LR-HPV); 30, with no signs of SIL but with HR-HPV (NSIL with HR-HPV); and 30, with both LSIL and HR-HPV. The expression of cyclins was evaluated by immunocytochemistry; the detection of viral DNA, by PCR (polymerase chain reaction) and RFLPs (restriction fragment length polymorphism) for genotyping or sequencing of high risk HPV. The physical state of HPV was evaluated by *in situ* hybridization with amplification with tyramide. **Results:** In the cytologies NSIL with LR-HPV, the expression of cyclins A, D1 and E was found respectively in 23.3%, 76.7% and 33.3% of the specimens. Among the specimens NSIL with HR-HPV, 33.3% expressed cyclin A; 90%, cyclin D1; and 40%, cyclin E while 100% of the LSILs with HR-HPV expressed the 3 cyclins. On the other hand, 100% of the samples NSIL with LR-HPV presented an episomal pattern. Of the specimens NSIL with HR-HPV, 56.6% exhibited an episomal pattern; 23.3%, an integrated one; and 20%, a mixed pattern. Among the LSILs, 90% of the patterns were mixed and 10%, integrated. **Conclusions:** The integrated and mixed state of HR-HPV had a significant effect in the greater expression of the cyclins A, D1 and E within LSIL specimens.

Keywords: low-grade squamous intraepithelial lesion (LSIL), high-risk human papillomavirus (HR-HPV), cyclin-A, cyclin-D1 and cyclin-E

INTRODUCTION

Invasive carcinoma of the uterine cervix involves precursory stages known as squamous intraepithelial lesions (SIL).[1] Cytologically SILs are divided into low-grade SIL (LSIL) and high-grade SIL (HSIL). LSIL represents an earlier diagnosis in cervical carcinogenesis. 80% of these lesions are associated with high-risk human papillomavirus (HR-HPV).[2] In the state of Guerrero, 10 different types having been encountered: 16, 18, 31, 33, 35, 39, 45, 52, 58 and 59. HPV-16 is the most frequently found in cervical carcinoma (68.1%) and in HSIL (27.4%).[3]

SIL emerges after a long period of viral persistence, as a result of viral genome integration into the host cell's genome, provoking E2 function loss and overexpression of E6 and E7, prerequisites for development of HSIL and invasive carcinoma.[4] It has been proposed that *in situ* hybridization (ISH) may detect the presence and physical state of HR-HPV DNA. The diffuse signal of viral DNA indicates an episomal state while the punctate signal indicates integration into the cellular genome.[5,6] In LSIL with HPV-16, the episomal state has been encountered in 15.4% of the cases; the integrated state, in 7.7%; and the mixed state, in 76.9%.[7]

Cyclins participate in various phases of the cellular cycle. Cyclin D1 is involved in beginning the G_1 phase and is essential for phase S initiation. Overexpression of cyclin D1 has been associated with a bad prognosis in cases of cervical carcinoma[8] and CIN-1 (cervical intraepithelial neoplasia-1), related to the HR-HPV oncoproteins E6 and E7[2,9,10] For its part, cyclin E is synthesized in the late G_1 phase and is indispensable for moving into phase S. In normal cells, the cyclin E expression diminishes rapidly as the cell enter into phase S. In premalignant and malignant lesions of the uterine cervix with expression of HR-HPV E7, the levels of cyclin E/cdk2 are found increased.[11] It has been reported that expression of HPV-16 E7 induces transcription of the promoter of cyclin A through the binding site to E2F. This observation indicates that the activation is implicated in cyclin A levels and this association is necessary for cellular transformation.[12] In cervical cancer, the overexpression of cyclin A, D1 and E associated with HPV-16 may note a worse prognosis.[13,14]

The objective of this work is to evaluate the relationship of expression of cyclins A, D1 and E with the physical state of HR-HPV DNA in cytologies with and without

LSIL, with the end to identify early cellular and viral biomarkers that may be utilized in identification of women with potential risk of progression to a greater lesion.

MATERIALS AND METHODS

Study Subjects

115 female residents of the state of Guerrero, Mexico were participants in this study approved by the Ethics Committee of the Autonomous University of Guerrero. Each one of the participants signed informed consent and responded to a questionnaire with the purpose of obtaining sociodemographic, clinic and obstetrical information.

Specimen Collection

From each participant, two endo/exocervical samples were taken. The first was used for the cytological study in a liquid base and for the Papanicolaou technique in the cytological diagnosis (Bethesda cytological classification);[15] the second, for the detection and typification of HPV. Subsequently the specimens were classified into four groups for the cytological study: (a) with no signs of SIL (NSIL) and HPV (that is, NSIL without HPV) (25), (b) NSIL with LR-HPV (30), (c) NSIL with HR-HPV (30), and (d) with LSIL and HR-HPV (30).

HPV Detection and Typification

The DNA was extracted in accordance with the standard SDS-proteinase K-phenol-chloroform method.[16] DNA amplification was done in a 2400 GeneAmp PCR system (Applied Biosystems, Foster City, CA, US). The products of PCR were analyzed by electrophoresis and were displayed on 1.5% agarose gels dyed with ethidium bromide. 1 pg and 1 ng HPV-6 recombinant plasmid DNA were used as positive controls; sterile water was used as the negative control. The products of PCR were subjected to digestion with restriction enzymes *BamHI, DdeI, HaeIII, HinfI, PstI, RsaI y Sau3AI* (Invitrogen, Carlsbad, CA, US). The viral type was determined by RFLPs.[17] When specimens were being analyzed with the GP5+/6+ system, they were subjected to sequencing in an automated system (310 ABI-PRISM GeneticAnalyzer, Applied Biosystems, Foster City, CA, US). The obtained

sequences were compared with HPV types' known sequences available on the NCBI website (http://www.ncbi.nim.nih.gov).[18]

Expression of Cyclins A, D1 and E by Immunocytochemistry

The expression of cyclins was determined by the streptavidin biotin peroxidase immunocytochemical method, utilizing Cytoscan HRP/DAB Cell detection system (Cell Marque Corporation, Hot Springs, AR, US). The monoclonal antibodies used were: cyclin A (6E6; 1:100; Novocastra, Newcastle-Upon-Tyne, UK), cyclin D1 (SP4; 1:100; Dako, Envision System, Carpinteria, CA, US) and cyclin E (13A3; 1:30; Novocastra, Newcastle-Upon-Tyne, UK). The cytology slides in liquid base were subjected to antigen retrieval (Immuno DNA Retriever with citrate, Bio SB Inc., Santa Barbara, CA, US) for 6 minutes at 120° C. The primary antibody was added for 1 hour, then the secondary antibody coupled with biotin was added. Streptavidin peroxidase was also added. For development, the chromogen DAB was used and Mayer's haematoxylin was used as a contrast dye. The cell line HeLA (HPV-18), which overexpresses cyclins, was used as positive controls. The same line, but omitting the primary antibody, was used as negative controls.

Expression of Cyclins A, D1 and E

Expression was determined by percentage of positive nuclei (≥10%, ≥30% and ≥50%[19]) and intensity as slight (1+), moderate (2+) and intense (3+).[20]

Evaluation criteria for cyclin positive cells were as follows. Cells not showing positive for the proteins had a score of 0. Those that showed reactivity to the cyclins but showed no nuclear alteration received 1 point. When the cells positive for these proteins had slight nuclear abnormalities and presented only one of the previously mentioned characteristics, the score was 2. Cells with an increased nucleus-cytoplasm ratio >50% and an additional positive criterion received 3 points, while the cells with an increased nucleus-cytoplasm ratio and more than one additional positive criterion were given a score of 4.[21]

In situ Hybridization (ISH)

Detection of the viral genome was done with a system of tyramide signal amplification (GenPoint Dako Cytomation, Carpinteria, CA, US). The monolayer smears were submitted to digestion for 1 minute with proteinase K (1:1000). A

drop of test reagent (biotinylated viral DNA) with probes for 13 HR-HPV genotypes (16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59 and 68) and with individual probes directed at HPV-6 and -11 was applied afterwards. The slides were denaturalized for 10 minutes and subjected to hybridization for 20 hours (Hybridizer Dako, Carpinteria, CA, US). They were then placed in an astringent solution; primary streptavidin peroxidase was added, afterwards biotinyl-tyramide, then secondary streptavidin. DAB was added and finally Mayer's haematoxylin (Merck). Positive reaction was visualized with a brown color inside of the nucleus and according to the type of signal was classified as diffuse (episomal state), punctate (integrated state) or mixed (diffuse and punctate)[6]. SiHa cell lines (HPV-16) were used as positive controls that showed an integrated state; the same cell lines without the probe were used as negative controls.

Statistical Analysis

Fisher's exact test was used for comparison of frequencies. To determine the relationship of cyclin expression and cervical lesion or physical state of DNA, multinomial logistic regression models were evaluated. The statistical analysis was done with the software STATA, version 11.1. A value of $p < 0.05$ was considered significant.

RESULTS

The age of the women was between 20-67 years old, with a median age of 44. The majority of women with LSIL and HR-HPV were between 51 and 60 years old (80%) and have had 3 or more sexual partners ($p<0.001$) (data not shown).

Frequency of HPV Genotypes and Viral DNA Physical States

9 HR-HPV genotypes were identified: 16, 18, 31, 35, 39, 45, 52, 58 and 59. HPV-16 was most frequent in NSIL (60%) and LSIL (53%) cytologies ($p<0.001$). 90% of the LR-HPV specimens exhibited HPV-6 and only 10% corresponded to type 11 (data not shown). In 100% of the specimens NSIL with LR-HPV, viral DNA was found in episomal state in a scant number of cells while, for the specimens NSIL with HR-HPV, the episomal state was observed in 56.6% of the cases (Figure 1a); mixed, in 20%; and integrated, 23.3%. For the specimens with LSIL and HR-HPV, 90% presented a mixed pattern and 10%, an integrated pattern (Figure 1b).

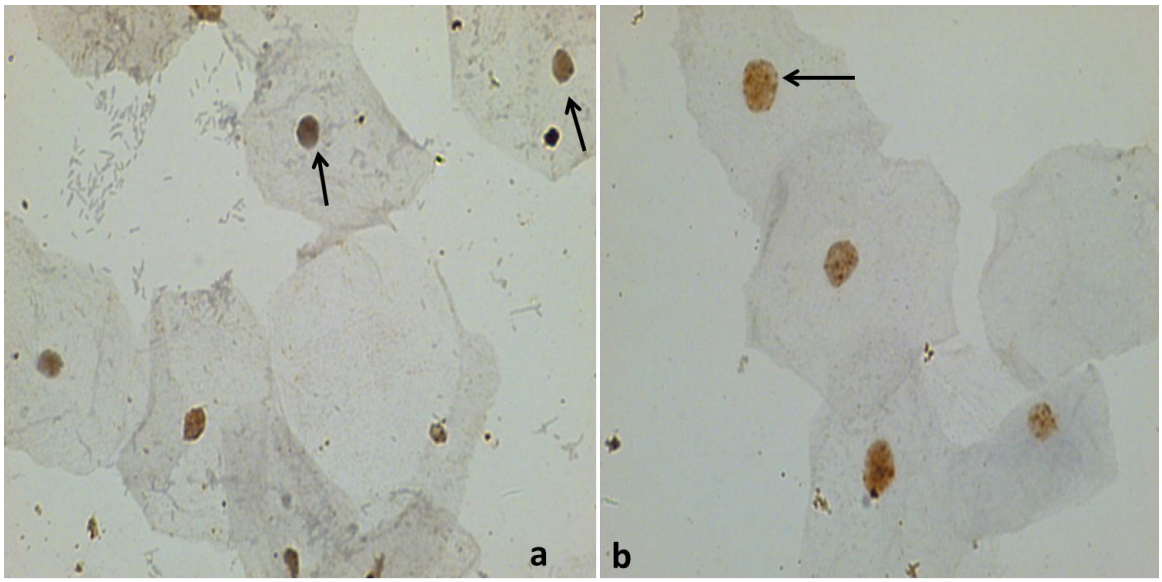

Figure 1. Physical state of HPV-16 DNA. **a)** Specimen NSIL with HR-HPV presented a episomal pattern (black arrows). **b)** Specimen LSIL with HR-HPV presented a integrated pattern (black arrow). Technique: In situ hybridization with tyramide amplification. 40X

Expression of Cyclins A, D1 and E and their Relation with Cytological Diagnosis

Expression of cyclins A, D1 and E increased gradually within the study groups. In 100% of the LSIL specimens, these cyclins' expression was found ($p<0.001$) (Table 1). Also in the LSILs, the percentage of nuclei positive for expression of the different cyclins was ≥10% and intensity was moderate, compared with the cytologies NSIL with HR-HPV where expression of these proteins was found in ≥30% of the nuclei and the intensity slight for cyclin A (33.3%) and moderate for cyclin D1 and E (80% and 36.7% respectively) (data not shown).

Nuclear Evaluation of Cyclins A, D1 and E and their Relationship with Cytological Diagnosis

LSILs positive for cyclins A, D1 and E correlated with the presence of cells with koilocytosis characterized by binucleation, karyomegaly and discrete perinuclear halos (Figure 2a-d). The nuclear score is correlated with the cytological diagnosis. Of the 30 cytologies with LSIL diagnosis, 28 (93.7%) showed positive to the cyclins with a score of 3. In 2 (6.7%), the nuclear criteria showed a score of 4, indicating a nuclear abnormality, in comparison with the rest of the groups that expressed these proteins and did not shown any nuclear alteration (score of 1) (data not shown).

Table 1. Expression of cyclin A, D1 and E and their relationship with the cytological diagnosis.

	Study groups				
	NSIL without HPV n = 25 (%) n	**NSIL with LR- HPV n = 30 (%) n**	**NSIL with HR- HPV n = 30 (%) n**	**With both LSIL and HR- HPV n = 30 (%) n**	**p trend***
Expression of cyclins					
Cyclin A					
Positive	16 (4)	23.3 (7)	33.3 (10)	100 (30)†	< 0.001
Negative	84 (21)	76.7 (23)	66.7 (20)	0	
Cyclin D1					
Positive	60 (15)	76.7 (23)	90 (27)‡	100 (30)‡§	< 0.001
Negative	40 (10)	23.3 (7)	10 (3)	0	
Cyclin E					
Positive	16 (4)	33.3 (10)	40 (12)	100 (30)†	< 0.001
Negative	84 (21)	66.7 (20)	60 (18)	0	

HR-HPV: Human papillomavirus risk-high
LR-HPV: Human papillomavirus risk-low
NSIL: No sign of intraepithelial lesion
LSIL: Low grade squamous intraepithelial lesion
* Rank sum test for trend
† p <0.001 compared with NSIL without HPV, NSIL with LR-HPV and NSIL with HR-HPV
‡ p <0.001 compared with NSIL without HPV
§ p <0.001 compared with NSIL with LR-HPV

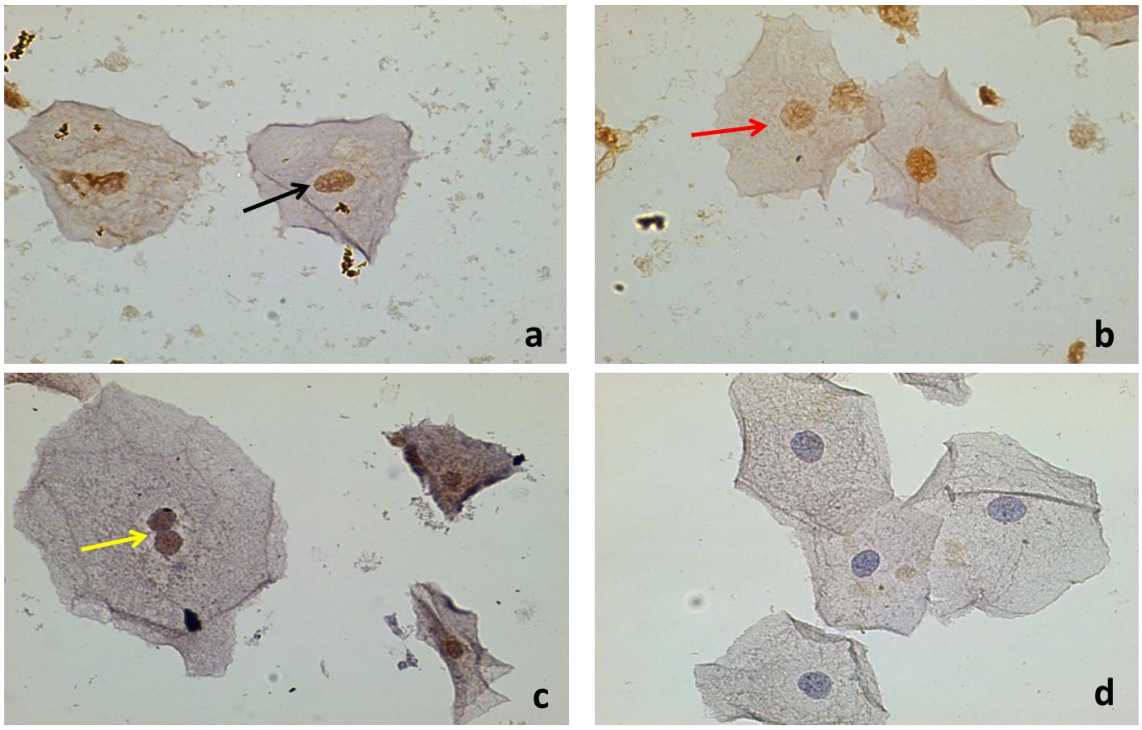

Figure 2. Expression of cyclin A, D1 and E in LSIL HPV-16. **a)** Positive for cyclin A expression, cells intermediate karyomegaly (black arrow). **b)** Expression of cyclin D1 positive, karyomegaly intermediate cells (red arrow). **c)** Expression of cyclin E positive, intermediate binucleation cell (yellow arrow). **d)** Expression of cyclin E negative, cytologies NSIL without HPV. Streptavidin biotin peroxidase technique. 40X.

Association of Expression of Cyclins A, D1 and E with Cytological Diagnosis

Expression of cyclins A and E is strongly associated with LSIL (OR: 393 and 188.3 respectively, p<0.001) in comparison with the women NSIL without HPV, while expression of cyclin D1 was 17.4 times more (p=0.010) (Table 2) in the same comparison. In the specimens NSIL with HR-HPV, this association was lesser for the cyclins A, D1 and E, with ORs of 5.9, 8.8 and 5.8 respectively in comparison with women NSIL without HPV (Table 2).

Table 2. Expression of cyclin A, D1 and E with the cytological diagnosis.

Diagnosis	Cyclin A OR (IC 95%)	Value *p*	Cyclin D1 OR (IC 95%)	Value *p*	Cyclin E OR (IC 95%)	Value *p*
NSIL without HPV*	1.0		1.0		1.0	
NSIL with LR-HPV	1.4 (0.3-6.9)	0.655	2.3 (0.7-7.6)	0.187	2.7 (0.7-10.8)	0.165
NSIL with HR-HPV	5.9 (1.2-28.3)	0.028	8.8 (1.9-41.0)	0.006	5.8 (1.4-23.8)	0.016
With both LSIL and HR- HPV	393 (28.1- ∞)	<0.001	17.4 (2.0-∞)	0.010	188.3 (17.8-∞)	<0.001

HR-HPV: Human papillomavirus risk-high
LR-HPV: Human papillomavirus risk-low
NSIL: No sign of intraepithelial lesion
LSIL: Low grade squamous intraepithelial lesion
*Reference category
OR: odds ratio adjusted for age
CI: confidence interval

Analysis of HR-HPV DNA State and its Relationship with Expression of Cyclins A, D1 and E for Diagnosis

Specimens from those women who exhibited integrated pattern and LSIL were related with greater frequency with the expression of cyclins A, D1 and E in comparison with the cytologies NSIL with HR-HPV (p<0.001). In addition, the cytologies NSIL with HR-HPV and the integrated pattern was related with the expression of cyclin D1 (p=0.001) (Table 3).

Analysis of HR-HPV DNA State and its Relationship with Expression of Cyclins A, D1 and E and HPV Type

The integrated state and presence of HPV-16 are related more frequently with the expression of cyclins A (33.3%), D1 (30%) and E (42.9%) (p<0.001) (data not shown). HPV-6 and -11 with episomal pattern were related with expression of these proteins. Nevertheless, this positivity was found in ≥10% of the cells (data not shown).

Table 3. Status physical of HR-HPV DNA and its relationship with the expression of cyclin A, D1 and E for cytological diagnosis.

Physical state	NSIL with LR-HPV % (n)	Value p*	NSIL with HR-HPV % (n)	Value p**	With both LSIL and HR-HPV % (n)
			Cyclin A		
Episomal	100 (7)		70 (7)		0
Integrated	0	0.228	30 (3)	<0.001	10 (3)
Mixed	0		0		90 (27)
			Cyclin D1		
Episomal	100 (23)		59.3 (16)		0
Integrated	0	0.001	25.9 (7)	<0.001	10 (3)
Mixed	0		14.8 (4)		90 (27)
			Cyclin E		
Episomal	100 (10)		58.3 (7)		0
Integrated	0	0.059	33.3 (4)	<0.001	10 (3)
Mixed	0		8.3 (1)		90 (27)

HR-HPV: Human papillomavirus risk-high
LR-HPV: Human papillomavirus risk-low
NSIL: No sign of intraepithelial lesion
LSIL: Low grade squamous intraepithelial lesion
*Compared NSIL with LR-HPV
** Compared NSIL with HR-HPV

DISCUSSION

The routine technique in diagnosis of early lesions of uterine cervix is the Papanicolaou method. Nevertheless, it lacks sensitivity and specificity due to the variability in observer interpretations of results.[22] This has led to ambiguous results that require colposcopic evaluation and finally cervical biopsy to rule out HSIL or cancer.[23] Non-invasive methods as cytology and biomarker expression may be of great utility in evaluation of progression of these lesions.[24]

Our data show viral HR-HPV integration (10%) in LSIL. Furthermore, these lesions exhibited HPV-16 with greater frequency. In a cervical scraping study of 40 LSIL cases with HPV-16, the integrated pattern was found in 33.3% of them.[25] This suggests that viral integration is an early event in the progression of the disease. We found mixed viral DNA (90%) with greater frequency in LSIL. This observation indicates a low number of integrated and episomal copies that sustain the latent state of the virus. These findings are similar to a study in which we found with greater frequency (76.9%) mixed forms in LSILs associated with HPV-16.[7]

In cytologies NSIL with HR-HPV, 23.3% showed the integrated pattern and presence of genotype 16 principally (60%). This suggests that these women have a high probability of progressing to LSILs. These results approach reports which found the integrated pattern associated with HPV-16 in 11% and 20% of the

cytologies NSIL,[7,25] unlike that reported in cases with LSIL and HPV-16 where the researchers did not observe viral integration.[26]

In our study, women NSIL with LR-HPV presented only the viral episomal state. This agrees with another study in which only the episomal pattern in cases NSIL with HPV-6 was observed, as well as a low viral load, suggesting that the low number of copies of this virus maintains E2 intact, by which the cellular cycle is not altered and morphological alterations are not observed.[27]

In the LSIL group with HR-HPV, we encountered an increase in expression of cyclins A, D1 and E, compared with the other study groups. Expression of cyclin D1 was found in all the cases. It is considered that the HR-HPV oncoproteins and cyclin D1 will act synergistically, permitting tumor cells a multiplication independent of growth factors, evading the cell cycle's control mechanisms.[28] These results resemble other studies that found expression of cyclin D1 in 80%, 87% and 90% of the CIN-1 specimens with HR-HPV[2,9,10] respectively, explaining that in LSILs with HR-HPV, the oncoprotein E7 is combined with pRb and deactivates it directly through ubiquitination. In these conditions, cyclin D1 is not necessary for entering into the S phase. Nevertheless, the incapacity of $p16^{INK4A}$ to deactivate cdk4/6/cyclin D1 may sustain the molecular basis for growth in cyclin D1 expression in the progression of cervical cancer.[2,10]

In the LSIL group associated with HR-HPV, the expression of cyclin E was found in 100% of the cases, results that concur with the findings in LSILs in which 96.7% of the expression of this cyclin was associated with HR-HPV.[11] Described in the literature is that coupling of E7 with E2F results in prolongation of phase S, a condition which prevents inhibition of the cyclin E/cdk complex.[12]

Our results show that in LSILs, activation of cyclin A by HR-HPV oncoproteins E6 and E7 seem altered by degradation of p53 by E6, a condition that prevents transcription of protein inhibitors of the cdk-cyclin complex, such as $p21^{Waf1/Cip1}$, being initiated, and consequently the increase in expression of cyclin A as well. On the other hand, E7 in conjunction with cdk2/cyclin A is important in cellular transformation.[12]

We also observed that cytologies NSIL with HR-HPV were positive for cyclins A, D1 and E in a minor percentage, in comparison with cases with LSIL and HR-HPV,

a result that has not been previously reported. These findings show that despite the fact that these cells do not present any morphological alterations, the elevated expression of these proteins permits on a molecular level prior detection of deregulation of the cell cycle induced by HR-HPV. The expression may be due to the cyclin E/cdk2 and cyclin A/cdk2 complex being augmented in phase S. It is probable that cells found positive were encountered in this cell cycle phase stimulated by presence of the HR-HPV oncoprotein E7. As for cyclin D1, it is known that upon inhibiting E6, the function of p53 does not activate inhibitors of kinases as $p21^{Waf1/Cip1}$, a situation which permits accumulation of the cyclin D1/cdk4, cdk6 complex in the nucleus and the cell's passing from phase G_1 to phase S.[29] To date, there are no reports which have evaluated these proteins in women NSIL with LR-HPV. It has been shown that LR-HPV oncoproteins possess low affinity for cellular proteins pRb and p53, LR-HPV specimens having low expression of cyclins and therefore a low number of copies in episomal form.[30]

There are no reports of joint evaluation of expression of cyclins A, D1 and E in cytological specimens NSIL without HPV. In one study, researchers found expression of cyclins A and E >1%[11,31] and expression of cyclin D1 100%, restricted to the cells of the basal and parabasal levels in normal tissue.[8,9] In the group NSIL without HPV, we found a high percentage of cyclin D1 expression in intermediate cells, with presence of atypical inflammatory changes. The biological process of the influence of inflammation in cyclin D1 expression is not recognized.

In a monitoring study on biopsies of premalignant and malignant uterine cervix lesions with HR-HPV,[14] in which expression of cyclins A and E was evaluated, researchers observed expression of these proteins as the lesion grade increased, suggesting that upon these proteins being overexpressed in early lesions, they may be indicators of bad prognosis.[14] We encountered a relationship highly significant between LSIL and the expression of the cyclins A and E.

In the women we studied with both LSIL and HR-HPV, 100% were positive for cyclin A, D1 and E. Besides, they had a nuclear score of 3 or 4, which correlated with the alterations characteristic of infection by HPV (koilocytosis, karyomegaly and perinuclear halos) while the other study groups exhibited a nuclear score of 1, indicating that the nuclear score may be of use in the interpretation of the degree of cellular abnormality.[21]

In our study, we found that presence of the HR-HPV DNA integrated state and LSIL were related with expression of cyclins A, D1 and E, emphasizing the importance of oncoproteins E6 and E7 in deregulation of the cell cycle, inhibition of the function of cdk inhibitors and activation of positive cell cycle regulators, such as cyclins A, D1 and E,[12] a condition that leads to continuing overexpression of these proteins and therefore, the passing across the cell cycle phases of transformed cells and finally cellular immortalization.

In summary, the relationship of the expression of cyclins A, D1 and E with LSIL and the presence of HR-HPV DNA integrated pattern permits the evidencing of early stages with the potential progression to HSIL, an observation which suggest a check, the cytological and colposcopic tracking of these patients.

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Competing interests

The authors state that no conflict of interest.

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