

*Full Length Research Paper*

# Frequency of episomal and/or Integrated HPV in Mexican women with varying grades of dysplasia

**María E. Cortez-López<sup>\*1</sup>, Fernando Hernández-Terán<sup>2</sup>, Pedro Cano-Ríos<sup>3</sup>, Mireya Sánchez-Garza<sup>4</sup>, Ricardo M. Cerda-Flores<sup>5</sup> and Ramón Román-Gómez<sup>1</sup>, Jesús R. Arguello-Astorga<sup>1</sup>**

<sup>1</sup>Departamento de Inmunobiología Molecular. Centro de Investigación Biomédica. Facultad de Medicina-Unidad Torreón. Universidad Autónoma de Coahuila. Torreón, Coahuila.

<sup>2</sup>Instituto de Medicina Genómica. Departamento de Biología Molecular. Torreón, Coahuila.

<sup>3</sup>Universidad Autónoma Agraria Antonio Narro. Torreón, Coahuila.

<sup>4</sup>Facultad de Medicina. IPICYT. Universidad Autónoma de San Luis Potosí.

<sup>5</sup>Facultad de Enfermería y Centro de Investigación y Desarrollo de Ciencias de la Salud, UANL. Monterrey, Nuevo León.

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**To determine the frequency of episomal and/or integrated HPV forms in women with varying grades of dysplasia and determine the risk in the various grades of dysplasia. The 53 cervical biopsies of women were previously diagnosed. All samples underwent subtype characterization and identification of the high oncogenic risk viruses. The control group consisted of 24 women without HPV. Frequencies for CIN I, CIN II, CIN III and Cervical Cancer were higher in the episomal form than in the integrated form. The control group showed neither episomal nor integrated forms. Women with CIN I showed OR of 0.151 ( $p = 0.049$ ) and CC of 26.4 ( $p = 0.006$ ). Results from this research show a trend towards decrease in the episomal form and towards increase in the integrated form in relation to the varying grades of dysplasia as it has been reported by the literature.**

**Keywords:** PCR, episomal, integrated forms, HPV, dysplasia.

## INTRODUCTION

Several studies have demonstrated epidemiologically and biochemically that persistent infection with an oncogenic human papillomavirus (high-risk HPV) leads to the manifestation of varying grades of cervical dysplasia (Zwerschke, 2006; Arends, 1990, 1991, 1993; Stanley, 1990; Donaldson, 1993) This is because during HPV infection, oncoproteins E6 and E7 are expressed, which participate in a complex cellular and viral regulation control mechanism that prevents apoptosis by inhibiting the role of p53 and generating dysplasia (Cuzick, 1994; Dyson, 1989; Hawley-Nelson, 1989; Ishiji, 2000; Lewis, 1999; Munger, 1989; Watanabe, 1989; Werness, 1990; Schneider, 1996; Smith-McCune, 1999). Some molecular studies have pointed out the relevance of the episomal and/or integrated HPV form within the host cell and its

association with the varying grades of dysplasia (Manavi, 2008).

The identification of the HPV integration in cervical samples proved to be a suitable tool for large-scale research with prognostic and clinical implications for cervical cancer treatment (De Marco, 2007), It has been recently suggested that the episomal and integrated forms of the HPV in relation to CC have high prognostic value (Nambaru, 2009).

The objectives of this study were: 1) to determine the episomal and/or integrated form of the HPV within the host cell; and 2) to associate the episomal and/or integrated forms of the HPV in Mexican women with varying grades of dysplasia using molecular methods with the aim of characterizing subtypes and identifying viruses of high oncogenic risk.

Our results reveal that the episomal and/or integrated forms of the HPV provide clear evidence that at the molecular level the integration process activates viral replication and hence the number of virus copies in-

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\*Corresponding Author E-mail: [marielpa@yahoo.com](mailto:marielpa@yahoo.com)

**Table 1.** Description of high and low risk oncogenic HPV based on their episomal and integrated form within the host cell

HPV type	F o r m						Total
	Integrated		Episomal		Episomal- Integrated Co-infection		
	No	%	No	%	No	%	
High Risk:							
16	1	12.50	6	16.66	4	44.44	11
18	1	12.50	0	0.00	0	0.00	1
31,35	3	37.50	10	27.77	4	44.44	17
33,58	2	25.00	0	0.00	0	0.00	2
52 B	0	0.00	6	16.66	1	11.1	7
NC	1	12.50	8	22.22	0	0.00	9
Low Risk:							
Negative Non Oncogenic	0	0.00	6	11.32	0	0.00	6
Total	8		36		9		53

NC = Type not classified by this method

creases and its association with the dysplasia grade make it an important risk predictor for CC.

## MATERIAL AND METHODS

Women population was recruited after they were to the Cytology department in a hospital unit (in and out the IMSS) in order to have their PAP test in the city of San Luis Potosi. City located to 22° 09' of North 04 latitude and 100° 58' 34" of west longitude to 363 km to the north-northwest of the City of Mexico. It practiced examination by routine and those to them that were diagnosed with diverse degrees of dysplasia. All of them were interviewed and a questioner was applied in order to obtain its anthropometric measures of each it gives the participants like stature, weight, index of corporal mass (IMC). Other variables to qualitative nature like beginning of sexual life were included in addition active civil state, monthly wage received. The number of sexual pairs, if they were smokers and/or they ingested spirits, use of contraceptives, each when they practiced the test of Papanicolaou of routine, if they counted on medical service of government IMSS (Mexican Institute of the social insurance) and his nutritional state.

After they voluntary consent to participate, they accepted to give a cervical exudate sample directly of the uterine neck. The samples were placed in means of denominated transport solution phosphate buffer (PBS) to come to realize the extraction of the DNA and to realize the molecular techniques necessary to obtain the physical form in which was the virus of the HPV within the genome of the cell guest. This study was reviewed, evaluated and approved by the Ethical Committee of the Faculty of Medicine in the Universidad Autónoma de Coahuila.

Fifty-three cervical biopsies were obtained from cases with varying grades of dysplasia: intracervical neoplasia I,

II and III, (CIN I, CIN II and CIN III) and with CC (Cricca, 2009). In addition healthy controls without HPV were supplied by the Institute for Scientific and Technological Research of San Luis Potosi (IPICYT).

Genomic DNA extraction was carried out using the DNAzol Method (Cat. 127 Maxim Biotech). HPV detection and typing was carried out by PCR for L1 and LCR to identify HPV genotypes of high oncogenic risk. The analysis of the viral integration was carried out by semi-automated high-resolution electrophoresis in polyacrylamide gel under denaturalizing conditions using the Amersham-Pharmacia Alflexpress instrument. The beta-globin gene was amplified as an internal control.

## Statistical analysis

Information was captured in the SPSS V.17 statistical package. The association of the episomal and/or integrated form of HPV in groups of women with and without HPV was determined using the  $\chi^2$  and OR calculation and its significance was determined using the Epimax Table Calculator program (<http://www.healthstrategy.com/epiperl/epiperl.htm>). The level of significance considered was  $p < 0.05$ .

## RESULTS

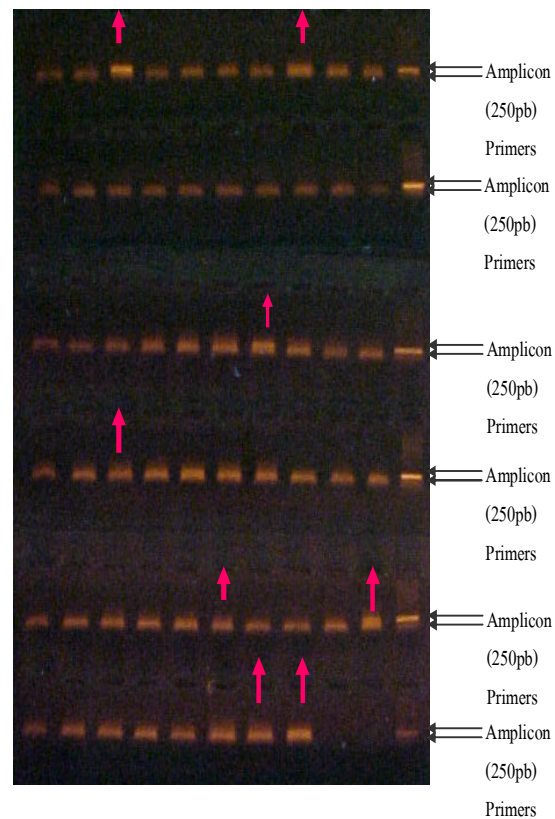
There were a total of 77 samples of which 53 were cases and 24 were controls. The 53 cases had diagnoses of CIN I, II and III and the remainder of CC. Of these, 45 cases presented an episomal physical state and in 8 the HPV state was integrated into the host cell (Table 1).

Regarding the physical state of the virus, the frequency found of the integrated form was 37.50% for HPV-31, 35 (37.50%), of the episomal form was 27.77% for the same HPV subtypes and for the co-infection forms

**Table 2.** Association of varying grades of dysplasia with episomal and/or integrated forms of HPV

Grades of dysplasia	Form				Total	$\chi^2$	Probability	OR (IC95%)
	Integrated		Episomal					
	No.	%	No.	%				
CIN I	2	25.00	31	68.89	33	3.857	0.049	0.151 (0.018-0.997)
CIN II	2	25.00	7	15.56	9	0.021	0.885	1.810 (0.203-13.748)
CIN III	1	12.50	6	13.33	7	0.000	1.000	0.929 (0.037-10.476)
UCC	3	37.50	1	2.22	4	7.587	0.006	26.400 (1.803-814.643)
Total		8		45	53			

P = 0.006 (RxC with 50,000 simulations): CIN I, CIN II, CIN III, and UCC



Electroforesis 100volts 1% Agarosa

LCR directa

Electrophoresis 100 volts 1% Agarose Direct LCR

**Figure 1.** Description of amplified products of the LCR region to determine the physical status within the host cell

(integrated + episomal) was 44.44% for HPV-16, 31 and 35 as shown in Table 1. The 24 controls (healthy without HPV) presented neither episomal nor integrated form.

Table 2 shows the association of the different grades of dysplasia with the episomal and/or integrated forms of HPV; in turn, the integrated form coincides with grades particularly to CIN II and in situ CC. This allows inferring the direct relationship between the physical state of the

virus and the progression of the disease. On application of the RxC test, a highly significant heterogeneity ( $p = 0.006$ ) can be observed of the percentage profile of the integrated form in relation to the episomal form according to the grade of dysplasia. These significant differences are mainly due to the high percentage of the episomal form found in CIN I (68.89%) and the high percentage of the integrated form found in women with CC (37.50%)

(Figure 1). These results are confirmed upon calculation of the OR, obtaining a value of 0.151 ( $p = 0.049$ ) for the former and 26.40 ( $p = 0.006$ ) for the latter.

## DISCUSSION

In this study, episomal and integrated forms of the HPV were found within the host cell. At the molecular level, these findings provide evidence that due to the integration process, the replication of the virus is activated and the number of viral copies increases. This turns it into a predictor of risk.

The episomal form of HPV-52 was found in cases diagnosed with varying grades of dysplasia, as previously reported in the literature (Ho, 2006). Some studies have found the HPV-16, independently of the severity of lesion and a mix of episomal and integrated forms (ALTS group, 2000; Bulk, 2006; Gravitt, 2003). These results confirmed the above finding that both the episomal and integrated forms can coexist in a single sample. At the same time it is possible to find a co-infection generated by two or three different subtypes. On the other hand, the importance of the E2 region in identifying the integration of the HPV-16 in patients diagnosed with high-grade lesions has been established. This approach also coincides with these findings, as this region is lost during progression of the lesions and integration of the virus. This was also confirmed with the results of HPV integration in the in situ CC analyzed (Cricca, 2009). Other studies have demonstrated the direct relationship with the progression grade of the lesion and persistence of the HPV-16 infection (Szostek, 2008). Our results coincide with this, as we observed that in diagnosis with a higher progression grade of lesions, the physical state of the virus is found integrated into the host cell.

With regard to frequencies of subtypes 16, 18 and 58 in the CC cases analyzed by PCR and reported in the literature (Zheng, 2006), our results differ both in relation to subtypes and to the physical form of the virus within the host cell. In this study, the most frequent subtypes for the episomal form were 31, 35 and 52 B and for the integrated form were 16, 31 and 35.

The frequency of the integrated or episomal form of HPV reported in relation to diagnosis (Arias-Pulido, 2006) coincides with findings of high association between diagnosis and the physical state of the virus in the cellular DNA.

RT-PCR and PCR-Dot Blot were used to determine the physical state of the HPV. However, they present certain limitations. This methodological proposal involves the best of both methods for the detection of episomal and integrated DNA by making the most of the high sensitivity and specificity of PCR (Nambaru, 2009). This confirms the position that early detection of the physical state of the virus may help to practice a timely approach to patients.

The results of this study show a trend toward a decrease in the episomal form and an increase in the integrated form in relation to the varying grades of dysplasia. These findings in the Mexican population support results reported in the literature, but it will be necessary to increase the sample size in order to strengthen the results found herein.

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