

Importance of p16 and Ki67. Immunohistochemical Expression and in a Typical Immature Metaplasia (AIM)

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Abstract

Introduction: The term AIM was coined in 1983 to describe a squamous proliferation of the cervical transformation zone and glands associated with abnormal cytology and colposcopic findings. This condition may be a precursor of HPV integration. This subject is controversial because its biological and clinical significance are not well defined. Colposcopy suffers from the same diagnostic difficulties than Cytology and Pathology. The effect of gene inactivation in the cervical epithelium was investigated for the overexpression of p16 protein by Immunohistochemistry (IHC), which results in the loss of activation of Rb by the E7 protein of high-risk HPV.

Objective: Investigate the use of biological markers, such as p16 and Ki67, which can be useful when diagnosing lesions with AIM.

Material and Methods: A descriptive study of the IHC expression of p16 and Ki67 in 60 formalin-fixed paraffin-embedded cervical biopsies obtained from the private archive of a Pathology Laboratory, was conducted.

Results: Negative cases for both p16 and Ki67 represented 69% of HPV lesions without dysplasia, whereas high-grade lesions (CIN III) were 100% positive for both p16 as Ki67. CIN I lesions were positive in 64% of the cases for both markers, and the rest were negative. CIN II lesions scored higher for p16 positivity, yielding positive results in 54% of the cases and 14% for Ki67.

Conclusion: AIM is a complex entity can be associated with HSIL. The similarities between the AIM and LSIL can be consider a form of LSIL. p16 is a marker for HPV-induced dysplasia. We suggest cautious behavior, for the sake of diagnostic accuracy. Considering the increased incidence of cervical carcinoma and its relationship to HPV, we deem useful to use biological markers such as p16 and Ki67, that may allow to determine the possible progression of SIL to invasive carcinoma as more economical tool that may be more in tune with the socio-economic reality of Latin America and cost-effective, when compared to other more expensive techniques.

Keywords: AIM (Atypical Immature Metaplasia), HPV, Colposcopy, Biological Markers (Immunohistochemistry), Latin America, Socio Economical Reality.

Introduction

The term AIM was coin in 1983 to describe a squamous proliferation

of the cervical transformation zone and the glands associated with abnormal cytology and colposcopy findings. This condition can be a precursor to HPV integration. This topic is controversial due to its biological and clinical importance are not well defined. Colposcopy suffers the same difficulties of diagnosis of cytology and pathology. The effect of gene inactivation on the cervical

epithelium was investigated by overexpression of p16 protein by immunohistochemistry (IHC), which results in the loss of pRb activation by the high-risk HPV E7 protein [1-4].

We know that HPV 16 is the virus that most frequently infects the uterine cervix and is associated with a wide range of cervical lesions from intraepithelial neoplasms to invasive carcinoma or end cervical glandular neoplasms. The other type of HPV associated with cancer is 18, which although involved in the genesis of squamous neoplasms, is related mainly to glandular neoplasms [5,6].

Virus 6 and 11 are the cause of more than 90% of genital warts or condylaracuminate. They are also associated with low-grade intraepithelial lesions (LIEBG) and only in rare cases with high-grade intraepithelial lesions (LIEAG) [5].

The HPV virus is a circular double-stranded DNA virus with 6 early genes (E) that regulate viral replication and 2 late genes (L). We know that in the E6 and E7 oncogenes present in the viral genome lies the neoplastic of the different viral subtypes. The oncoproteins encoding these E6 and E7 genes bind to host regulatory proteins, such as p53 that is degraded by E6 and the retinoblastoma gene protein (pRb) whose phosphorylation is inactivated by E7. The entry of HPV into the cell is related to integrin [7].

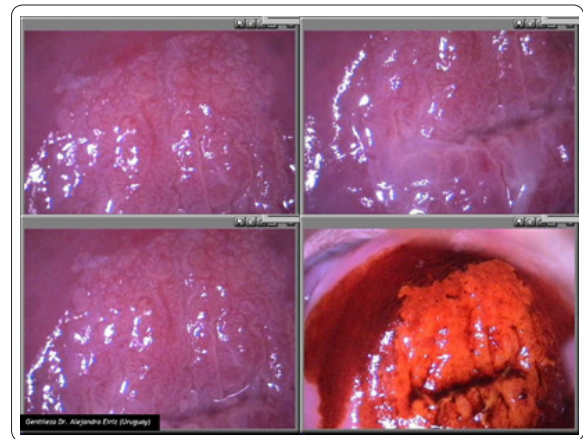
The effect of inactivation on the cervical epithelial genes were investigated through the expression of the p16 protein, which is a kinase that inhibits the cyclins involved in the "check point" G1 of the cell cycle, together with pRb and cyclin D1. p16 is overexpressed in cervical lesions associated with infections by high-risk strains of HPV. Instead of overexpressed upon inactivation of Rb by the HPV E7 protein. Nowadays we consider that p16, Cyclin D1 and Ki67 can be complementary biological markers that help to examine the relationship of HPV with the initial invasion capacity of cervical cancer. The expression of p16 in immunohistochemically stained is observed in the nucleus and cytoplasm of the affected cells.

The immunohistochemical detection of p16 can also be applied in cervical cytology, to clarify the diagnosis of the uncertain lesions of immature metaplasia. The p16 immunoreactivity could have the same predictive value of squamous lesions as the viral studies with Hybrid Capture [8].

Objectives

To investigate the use of biological markers, such as p16 and Ki67, which may be useful in the diagnosis of AIM lesions.

- CRUM1983:
– AIM ¿? CIN.
- PARK 1998:
– Unknown biological evolution.
- DUGGAN 2000:
– AIM represents HPV in metaplasia
- MIYATAKE 2007:
– True precursor.



(COLPOSCOPIC FINDINGS)

Material and Methods

For the expression of p16 and Ki67, we analyzed 60 tissues obtained from the private archives of a Pathology Laboratory, fixed in neutral buffered formalin 10% and embedded in paraffin. Cervical biopsies were included with MIA diagnoses with HPV and HPV + CIN In Situ Carcinoma and CIN I (Low-grade Intraepithelial Lesion), CIN II and CIN III (High-grade Intraepithelial Lesion). In it, there were lesions of immature metaplasia, with atypia (reactive?)

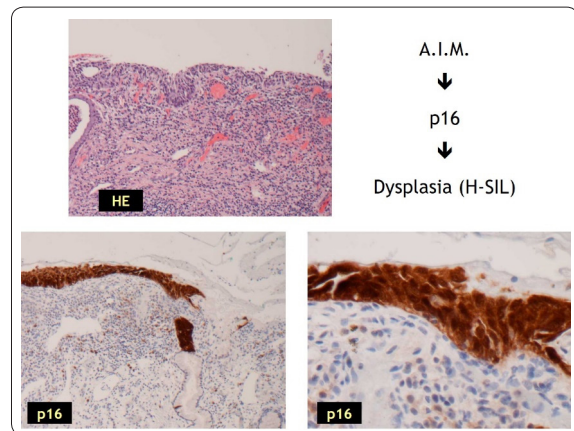


Figure 1

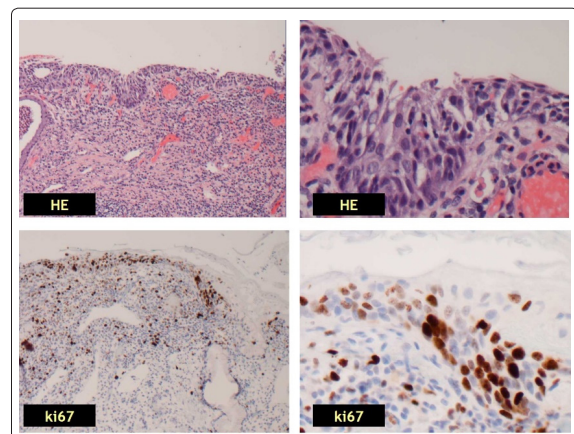


Figure 2

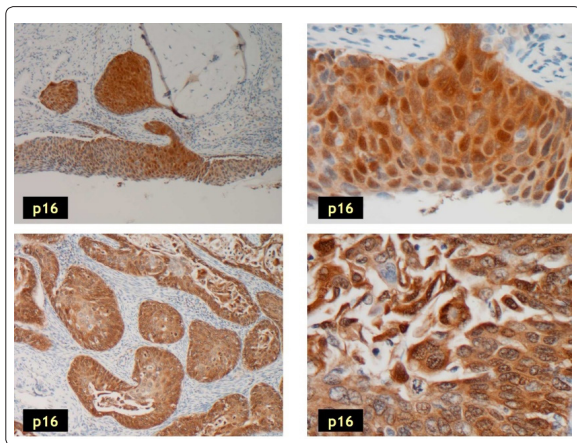


Figure 3

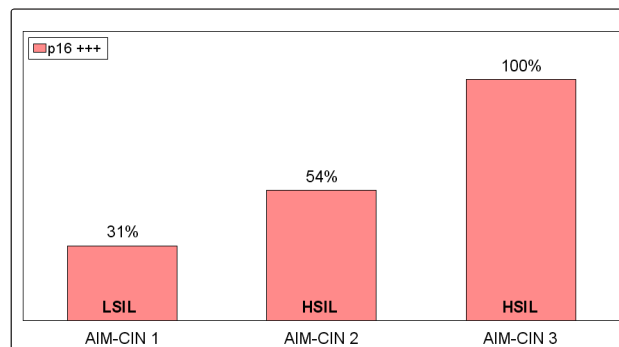


Figure 4: Findings on p16

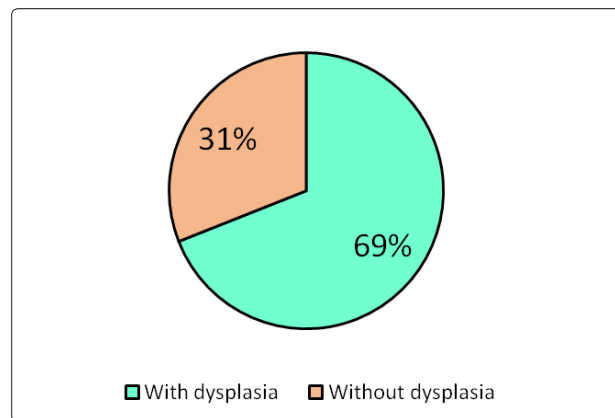


Figure 5: Findings: HPV lesions AIM, both p16 as Ki67

Protocol Used For IHC
STEP & TIME (ImmunoDetector HRP/DAB, Bio SB, CA, USA)
Dewaxing 2 x 3 min. Xylol
Dehydrated 2 x 3 min. Alcohol
Thermal treatment 1 x 15 min. Pressure cooker
Rest 1 x 15 min. Room Temperature
Peroxidase Block 1 x 5 min. Room Temperature
Washing in TBS 1 x 2 min. Room Temperature
Primary Antibody 1 x 30 min. Room Temperature
Washing TBS 1 x 2 min. Room Temperature
Secondary-Biotin 1 x 10 min. Room Temperature
Washing TBS 1 x 2 min. Room Temperature
Streptavidin HRP 1 x 10 min. Room Temperature
Washing TBS 1 x 2 min. Room Temperature
DAB 1 x 5 min. Room Temperature
Washing TBS 1 x 2 min. Room Temperature
Counterstain Hematoxylin 1 x 1 min
Wash with tap water 1 x 10 min.
Dehydrate 2 x 3 min. Alcohol
Clear 2 x 3 min. Xylol

Interpretation of the results: the degree of immunoreactivity for p16 is interpret as follows:

- 0 (negative): less than 1% of positive cells.
- Positive 1+: weak in nucleus and absent in cytoplasm.
- Positive 2+: moderate in the nucleus and weak to moderate in the cytoplasm.
- Positive 3+: strong marking in the nucleus and cytoplasm.

Results

Negative cases for both p16 and Ki67 accounted for 69% of HPV lesions without dysplasia, while high-grade lesions (CIN III) were 100% positive for both p16 and Ki67. Lesions (CIN II) were positive in 64% of the cases for both markers, and the rest were negative, CIN II scored higher for positivity for p16, giving intensely positive results in 54% of the cases and 14% for Ki67 (see figure 1-3).

Conclusion

AIM is a complex entity usually associated with HSIL. The similarities between the AIM and LSIL we can be considered a form of LSIL. P 16 is a marker of dysplasia induced by HPV. We suggest prudent behavior, for the sake of diagnostic precision. Considering the higher incidence of cervical carcinoma and its relationship with HPV, we find it is useful to use biological markers such as p16 and Ki 67, which allow determining the possible progression of SIL to invasive carcinoma as a more economical tool that can be more in tune with the socio-economic reality of Latin America [9-13].

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