Prognostic markers of low-grade squamous intraepithelial lesions: the role of topoisomerase IIα and active caspase-3

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Summary

Purpose: To study the relationship between topoisomerase IIα, active caspase-3 expressions and HPV DNA in uterine cervices with low-grade squamous intraepithelial lesions (LSIL). *Methods:* Forty women with LSIL and 32 without cervical neoplasia diagnosed through cytologic and histopathologic examination were evaluated regarding topoisomerase IIα and active caspase-3 expressions and HPV DNA detection using PCR (GP5/GP6) in cervicovaginal smears. *Results:* The mean percentage of cells immunomarked by topoisomerase in the group with LSIL was 11.62% while in the control it was 4.13% ($p < 0.0001$). In the presence of HPV DNA, topoisomerase expression was higher in the group with productive viral infection than in nonneoplastic tissue ($p = 0.004$). Caspase-3 expression was observed in 17 patients with LSIL (42.5%) and in five without cervical neoplasia (15.63%). *Conclusion:* The use of topoisomerase IIα and active caspase-3 in cervical biopsies may help to define the prognosis of HPV cervical infection.

Key words: Topoisomerase II alpha; Caspase-3; HPV; Neoplasia.

Introduction

Human papillomavirus is highly prevalent in sexually active women. Persistence of the viral infection is associated with a higher risk for development of intraepithelial lesions, mainly in the presence of high-risk viral types [1, 2]. The "ideal" screening test should initially target persistent high-risk HPV infections with superimposed cellular dysregulation, also detecting higher-grade cervical intraepithelial neoplasia (CIN) or invasive cancer, searching for the real potentially progressive oncogenic lesions [3].

Viral infection stimulates cell proliferation, while the host generates defense mechanisms against multiplication of viral particles through activation of apoptosis. DNA-topoisomerases I and II are nuclear enzymes present in all cells that act on condensation, chromosomal segregation and genic expression [4, 5]. Apoptosis involves caspases, pro-apoptotic proteases which destroy essential proteins or activate toxic proteins. Some are initiators and others, effectors, activated by other caspases [6-8]. Once activated, caspase-3 generates lysis of cell proteins, such as polymerases and epidermal growth factor receptor [9, 10]. Among the identified caspases in humans, caspase-3 is the one best related to apoptosis.

The present study was aimed to evaluate topoisomerase IIα and active caspase-3 immunohistochemical expressions in addition to HPV DNA presence in patients with low-grade squamous intraepithelial lesions (LSIL) and without cervical neoplasia, in the search for defining markers of productive viral infection and the potentiality for clinical progression.

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Materials and Methods

Seventy-two patients examined in the Gynecology Department of the Federal University of São Paulo (UNIFESP-EPM) were selected: 40 with LSIL, diagnosed through cytologic, colposcopic and histopathologic examination, and 32 without HPV-induced cytohistopathologic lesions. Women with previous treatment of the lower genital tract, immunosuppressed, or pregnant were excluded. The project was approved by the Ethical Committee of the Institution.

Patients were submitted to collection of material for cervicovaginal smears and for detection of HPV DNA, colposcopic evaluation, and tissue sampling of anormal findings. Both groups included only women with concordant cytohistopathologic diagnoses. Biomolecular HPV detection was performed by PCR (polymerase chain reaction) with Gp5/Gp6 primer.

Immunohistochemical reactions for topoisomerase IIα and active caspase-3 determination were performed with the biotin-streptavidine-peroxidase method. After hydration and blockade, incubation with monoclonal human anti-topoisomerase II alpha antibody (DakoCytomation S/A Denmark, code M7186, 1:50 titer) and polyclonal rabbit anti-active caspase antibody (Chemicon International Inc., 1:25 titer) was carried out. The slides were incubated overnight at a temperature of 2° to 8°C and then washed in PBS and incubated using the Kit System-HPR (DakoCytomation K0690). After this step, revelation with the chromogen 3,3-diaminobenzidine (DAB) (Sigma Chemical Co., St. Louis, MO, USA) and staining with Harris hematoxylin (Merck, Darmstadt, Germany) followed.

The staining for topoisomerase IIα was considered positive in the cells whose nuclear staining was brownish, and negative in the absence of staining or in those weekly stained. Active caspase-3 expression was considered positive in the cells whose nuclear and cytoplasmic staining was brownish, and negative in its absence. Counting was manual by two independent observers, with a minimum of near 200 cells, as described by HAFIAN *et al.* [11].

Comparison of the quantitative variable means between the case and control group was performed with the Student's *t*-test for two independent samples. Fisher's exact test was utilized for the analysis of homogeneity and of association in contingency tables. In both tests, the values $p \leq 0.05$ were regarded as statistically significant.

Results

Seventy-two patients, 40 with LSIL and 32 controls without neoplasia, were evaluated using immunohistochemistry together with HPV testing. Analyzing topoisomerase IIα, a mean of 11.62% immunomarked cells was observed in the case group and 4.13% in the control, with epithelial distribution of stained cells specially in the basal and parabasal layers ($p < 0.0001$) (Table 1). Immunohistochemical evaluation of active caspase-3 revealed nuclear and cytoplasmic marking by brownish poorly delimited staining, more evident in the epithelial layers near the basal layer, of difficult quantification. Active caspase-3 expression was evident in 17 (42.5%) patients with LSIL and in five (15.63%) control patients ($p = 0.020$) (Table 1). Topoisomerase IIα expression in patients with positive caspase-3 was 12.05%, while it was 6.71% in those negative for caspase-3 ($p = 0.0024$).

Table 1. — *Distribution of patients in the case and control groups according to topoisomerase IIα and active caspase-3 expression.*

Group	Topoisomerase IIa			Active caspase-3+			Total
	Mean %	SD	p	N	%	p	
LSIL	11.62	7.31	< 0.0001[b]	17	42.50	0,020[a]	40
control	4.13	3.48		5	15.63		32

[a] = Fisher's exact test; [b] = Student's t-test; SD = standard deviation; LSIL = low-grade squamous intraepithelial lesions; N = number of patients.

The test for presence of HPV DNA using PCR was performed in all study patients at the time of the first visit, or maximally 30 days afterwards. HPV DNA was detected in 26 (65%) patients with LSIL, and in 19 (59.38%) without LSIL. In the presence of HPV DNA ($n = 45$), the mean percentage of cells immunomarked by topoisomerase in the group with productive viral infection was 11.94% while in normal-appearing tissue it was 4.31% ($p = 0.004$). In those patients HPV DNA-positive, caspase-3 expression was observed in nine (34.6%) patients with LSIL and in three (15.8%) patients without LSIL ($p = 0.1911$) (Table 2).

Table 2. — *Distribution of HPV infected patients in the case and control groups according to topoisomerase IIα and active caspase-3 expression.*

Group	LSIL, HPV+	Control, HPV+	p
Topoisomerase IIα	11.94%	4.31%	0.004[b]
Active caspase-3	9 (34.6%)	3 (15.8%)	0.1911[a]
Total	26	19	45

[a] = Fisher's exact test; [b] = Student's t-test; LSIL = low-grade squamous intraepithelial lesions.

Discussion

The use of molecular markers in cervical intraepithelial lesions would offer a better analysis of risk for productive HPV infection. In this study a significant difference between mean topoisomerase IIα and active caspase-3 positivity indices was observed for patients with and without cytohistologic LSIL, which may suggest an increase in apoptosis in answer to atypias. There seems to be an association between increased proliferative activity and degree of apoptosis.

Several studies are concordant with our results regarding presence of HPV DNA [12-18]. The high prevalence of HPV DNA in our control group may be explained by the fact of dealing with a specialized outpatient clinic. Possible explanations for the high proportion of LSIL cases on cytohistopathologic examination with a negative result for HPV DNA by PCR in this study are: 1) viral clearance in the interval between the reference cytohistopathologic examination and sample collection for PCR; 2) false-positive diagnosis of LSIL, not very probably due to confirmation of the cases by an experienced pathologist; 3) LSIL cases without HPV, constituting a real biologic entity, as suggested by the study of Burger *et al.* [4] small amount of cells collected for PCR, because it was performed after collection of material for cytopathologic examination, with frequent bleeding and local inflammatory processes [19, 20].

In the presence of HPV DNA, the expression of topoisomerase IIα in the group with productive viral infection was higher than in nonneoplastic tissue. Samples with a higher mean topoisomerase IIα index expressed more active caspase-3, and were more evident in the case group. The use of topoisomerase IIα may help define which patients with viral infection will produce cervical lesions. There is a need for prospective studies with numerous LSIL to associate this finding with real progressive lesions and even to establish new guidelines for the management of these patients.

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