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RESEARCH

Expression of BAG-1 and PARP-1 in Precursor Lesions and Invasive Cervical Cancer Associated with Human Papillomavirus (HPV)

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Abstract Cervical cancer remains persistently the second most common malignancies among women worldwide, responsible for 500,000 new cases annually. Only in Brazil, the estimate is for 18,430 new cases in 2011. Several types of molecular markers have been studied in carcinogenesis including proteins associated with apoptosis such as BAG-1 and PARP-1. This study aims to demonstrate the expression of BAG-1 and PARP-1 in patients with low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs) and invasive squamous cell carcinomas (SCCs) of the uterine cervix and to verify a possible association with HPV infection. Fifty samples of LSILs, 50 samples of HSILs and 50 samples of invasive SCCs of the uterine cervix were analyzed by immunohistochemistry for BAG-1 and PARP-1 expression. PCR was performed to detect and type HPV DNA. BAG-1 expression levels were significantly different between LSILs and HSILs (p=0,014) and between LSILs and SCCs (p=0,014). In regards to PARP-1 expression, we found significant differences between the expression levels in HSILs and SCCs (p=0,022). No association was found between BAG-1 expression and the presence of HPV. However, a significant association was found between PARP-1 expression and HPV positivity in the HSILs group (p=0,021). In conclusion our research suggests that BAG-1 expression could contribute to the differentiation between LSIL and HSIL/SCC whereas PARP-1 could be useful to the differentiation between HSIL HPV-related and SCC. Further studies are needed to clarify the molecular aspects of the relationship between PARP-1 expression and HPV infection, with potential applications for cervical cancer prediction.

Keywords BAG-1 · PARP-1 · HPV · Cervix · PCR · Immunohistochemistry.

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Introduction

Cervical cancer remains persistently as the second most common malignancy among women worldwide, responsible for 500,000 new cases annually. Nearly 80% of these cases occur in women between 15 and 45 years of age who live in developing nations [1]. In addition, the HPV virus, which has over 100 subtypes, is found in 99.7% of women with cervical cancer [2]. Persistent HPV infection leads to proliferation of cervical epithelial cells, which may progress to various grades of cervical dysplasia and malignant transformation.

Programmed cellular death, or apoptosis, plays an important role in the homeostatic mechanism in multicellular organisms and is regulated by a large number of proteins. BAG-1 is among those regulating proteins and has antiapoptotic activities [3]. BAG-1 is a multifunctional protein that interacts with several cellular targets and affects important control pathways in both normal and malignant cells [4]. Several clinical studies have described BAG-1 expression in a variety of human cancers, including breast, prostate, endometrial, oral and lung cancers [5–9].

Another protein involved in regulating the apoptotic program is the poly(adenosine diphosphate-ribose) polymerase-1 (PARP-1) [10]. PARP-1 is 113 kD protein composed of

1,014 amino acids and plays a primary role in the process of poly(ADPribosyl)ation. This poly(ADP-rybosyl)ation consists of the synthesis of ADP-ribose polymers on target proteins and represents one of the immediate cellular responses to DNA strand breaks [11–13]. The cleavage of PARP-1 by caspases activated early in apoptosis provides strong evidence of the role of poly(ADP-ribosyl)ation in cell death [14]. Overexpression of PARP-1 has been reported in various human malignancies, such as malignant lymphoma, breast carcinoma, Ewing's sarcoma, hepatocellular carcinoma, endometrial carcinoma and melanomas [15–20].

The roles of BAG-1 and PARP-1 expression in squamous neoplastic changes or cervical carcinogenesis are not well established. Our study aims to investigate the expression profiles of BAG-1 and PARP-1 in a series of premalignant and malignant cervical lesions.

Materials and Methods

Subjects

The study protocol was approved by the Local Ethics Committee on Human Experimentation (11097/2008). Formalin-fixed and paraffin-embedded cervical biopsies diagnosed between 2003 and 2008 were retrieved from 50 patients with low-grade squamous intraepithelial lesions (LSILs), 50 patients with high-grade squamous intraepithelial lesions (HSILs), and 50 patients with invasive squamous cell carcinomas (SCCs) of the uterine cervix. All samples were collected from the archives of the Pathology Department, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. Clinicopathological information, such as age, history of smoking and alcohol consumption, parity and use of oral contraceptives was obtained from the patients' medical records. All biopsies were reviewed and evaluated by an experienced pathologist (EGS). For each patient, thin (5 μm) sections were cut from representative areas of the paraffin blocks, placed on organosilane-pretreated slides, and submitted to immunohistochemical assays for BAG-1 and PARP-1. Additionally, a 10 μm section was cut for DNA extraction and HPV typing.

Immunohistochemistry Assay for BAG-1 and PARP-1

Sections of 5 μm were cut, placed on organosilane pretreated slides, and subjected to immunohistochemical assay for BAG-1 and PARP-1 using MACH 4 Universal HRP Polymer (Biocare Medical, Concord, USA) and the streptavidin-biotin system (Vectastain, Vector Lab., CA, USA), respectively. The cervical specimens were dewaxed in xylene, rehydrated in a graded alcohol series, and rinsed in water. For antigen retrieval, the sections were immersed in 10 mM sodium citrate buffer, pH 6.0 and submitted to steam heating for 40 min at 95°C. Endogenous peroxidase blocking was performed with 3% hydrogen peroxide for 20 min. Nonspecific binding was blocked with horse serum diluted 1:50 in PBS. Slides were incubated with primary monoclonal antibody specific to BAG-1 (RAP46, diluted 1:100; Novocastra, Newcastle upon Tyne, United Kingdom) and PARP-1 (sc-8007, diluted 1:80) in a humidified chamber at 4°C overnight. For BAG-1 detection, slides were incubated with Mouse Probe for 20 min. Then, the slides were incubated with MR HRP Polymer for 30 min. For PARP-1 detection, slides were incubated with biotinylated anti-rabbit antibody and the streptavidin–peroxidase complex at 37°C for 30 min. Finally, samples were incubated with 3,3-diaminobenzidine (DAB, GIBCO, Gaithersburg, MD, USA) diluted to 0.01% in H_2O_2 for 10 min, lightly counterstained with Harri's hematoxylin for 60 s, exhaustively rewashed with tap water, air dried, and mounted with Permount mounting medium (MERCK; Darmstadt, Germany).

Immunohistochemical Evaluation

Evaluation of BAG-1 and PARP-1 included the staining intensity and the proportion of positive cells within the tumors. The intensity score represents the estimated staining intensity (0: negative, 1: weak, 2: moderate, and 3: strong reaction intensity). We considered the expression of BAG-1 and PARP-1 to be low when no signal or weak immunostaining was observed. BAG-1 and PARP-1 expression was classified as high when it was observed moderate or strong immunostaining.

For BAG-1 expression, the estimated fraction of positive cells was performed using the following grading system: 0, negative; 1, 1–30% positive cells, 2, 31–70% positive cells and 3, 71–100% positive cells [21]. For PARP-1 expression, the estimated fraction of positive cells was performed using the following grading system: 0, ≤5% positive cells; 1, 5–25% positive cells; 2, 26%–50% positive cells and 3, >50% positive cells [20]. For statistical purposes, in the present study, the scores 0 and 1 were considered low expression, and the scores 2 and 3 were considered high expression.

All scoring and interpretation of immunohistochemical results were conducted by an experienced pathologist (EGS).

DNA Isolation

DNA was isolated from formalin-fixed paraffin-embedded tissue specimens using the Promega Tissue Kit according to the manufacturer's instructions (MagneSilR Genomic, Fixed Tissue System, Promega Corp., Madison, WI, USA). The DNA solutions were stored at −20°C.

HPV DNA Detection and Typing

Amplifications of generic HPV genes and β-globin DNA were performed in the same reaction. The primers GP5+ and GP6+, which amplify small DNA fragments (150 bp) were used for generic HPV amplification. The generic HPV positive samples were amplified with primers specific for HPV16 (HPV16E7.667/HPV16E7.774) and HPV18

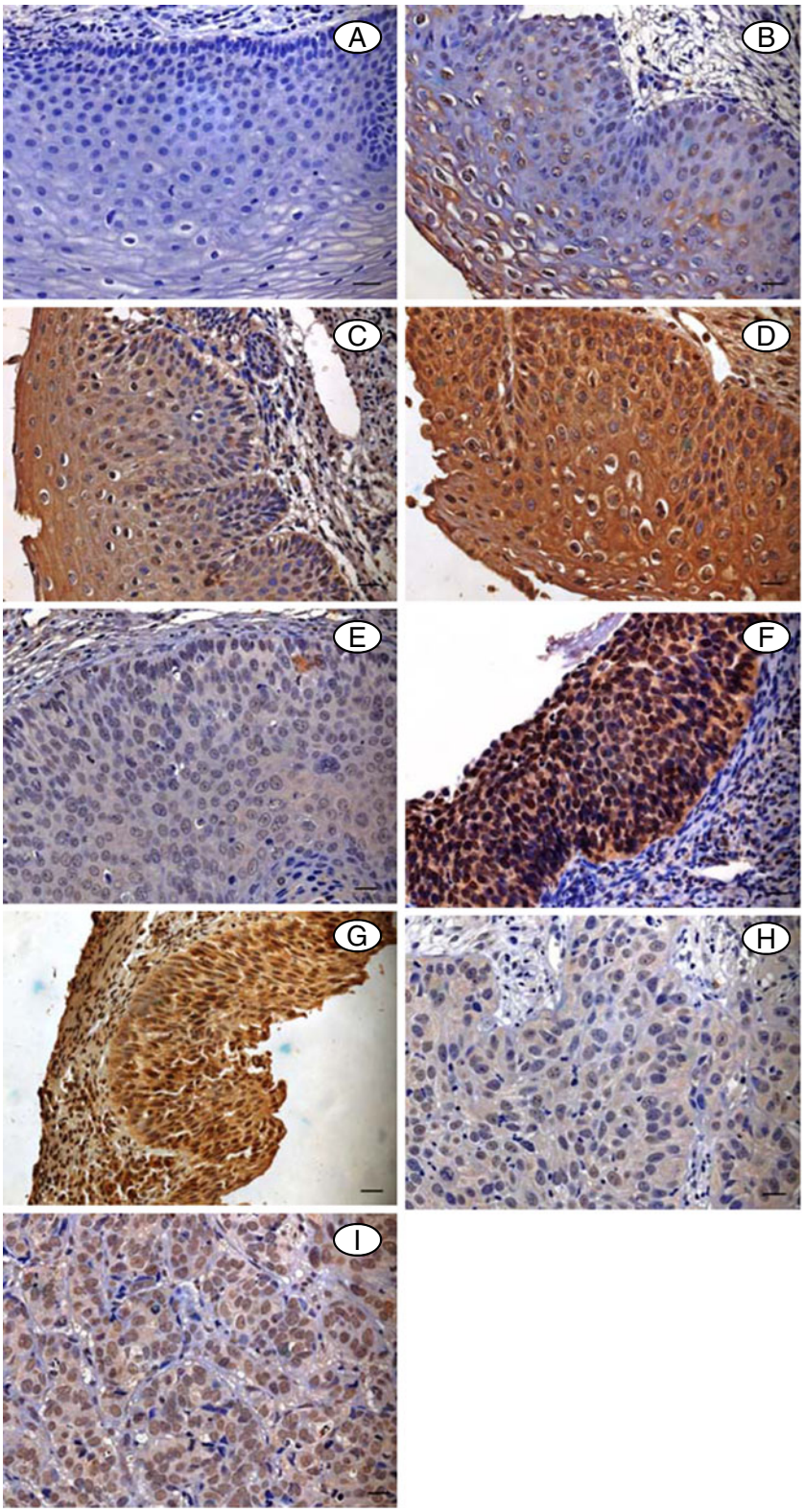

Fig. 1 BAG-1 expression determined by immunohistochemistry. **a** Absence of BAG-1 in normal cervix; **b** Weak expression of BAG-1 in LSIL; **c** Moderate expression of BAG-1 in LSIL; **d** Strong expression of BAG-1 in LSIL; **e** Weak expression of BAG-1 in HSIL; **f** Moderate expression of BAG-1 in HSIL; **g** Strong expression of BAG-1 in HSIL; **h** Weak expression of BAG-1 in SCC; **i** Moderate expression of BAG-1 in SCC. Scale bar =20 μm

(HPV18E7.696/HPV18E7. 799). A set of primers for the β-globin gene (PCO3 and PCO4) (PCO3+ CTT CTG ACA CAA CTG TGT TCA CTA GC and PCO4+ TCA CCA CAA CTT CAT CCA CGT TCA CC) were used as an internal control for amplification. A PCR test was performed on each microtube, which contained 4 μl of extracted genomic DNA and a mixture reaction containing 12.5 μl PCR Master Mix (Promega Corp.), 1.2 μl PC03/PC04 primer, 4.3 μl GP5+/GP6+ primer and 3.0 μl sterile water, for a total volume of 25 μl. The primers were added to a final concentration of 50 μM. In the detection of specific HPV subtypes, the PCR mixture contained 2 μl DNA sample, 12.5 μl PCR Master Mix (Promega Corp.), 2 μl HPV-16E7 or HPV-18E7 primers and 8.5 μl sterile water. For general HPV and β-globin genes, PCR was performed with 41 amplification cycles in a thermocycler (MyCycler Thermal Cycler, Bio-Rad Laboratories, Hercules, CA, USA) as follows: 1 cycle at 95°C×5 min for initial denaturation, annealing at 53°C×2 min, chain elongation at 72°C×2 min, and 39 subsequent cycles at 94°C×1 min for denaturation, annealing at 51°C×2 min and chain elongation at 72°C×2 min. A final extension of 72°C for 10 min followed the last cycle. For HPV-specific genotype detection, PCR was performed with 41 amplification cycles as follows: 1 cycle at 95°C×1 min for initial denaturation, annealing at 53°C×1 min, chain elongation at 72°C×1 min, and 39 subsequent cycles at 94°C×

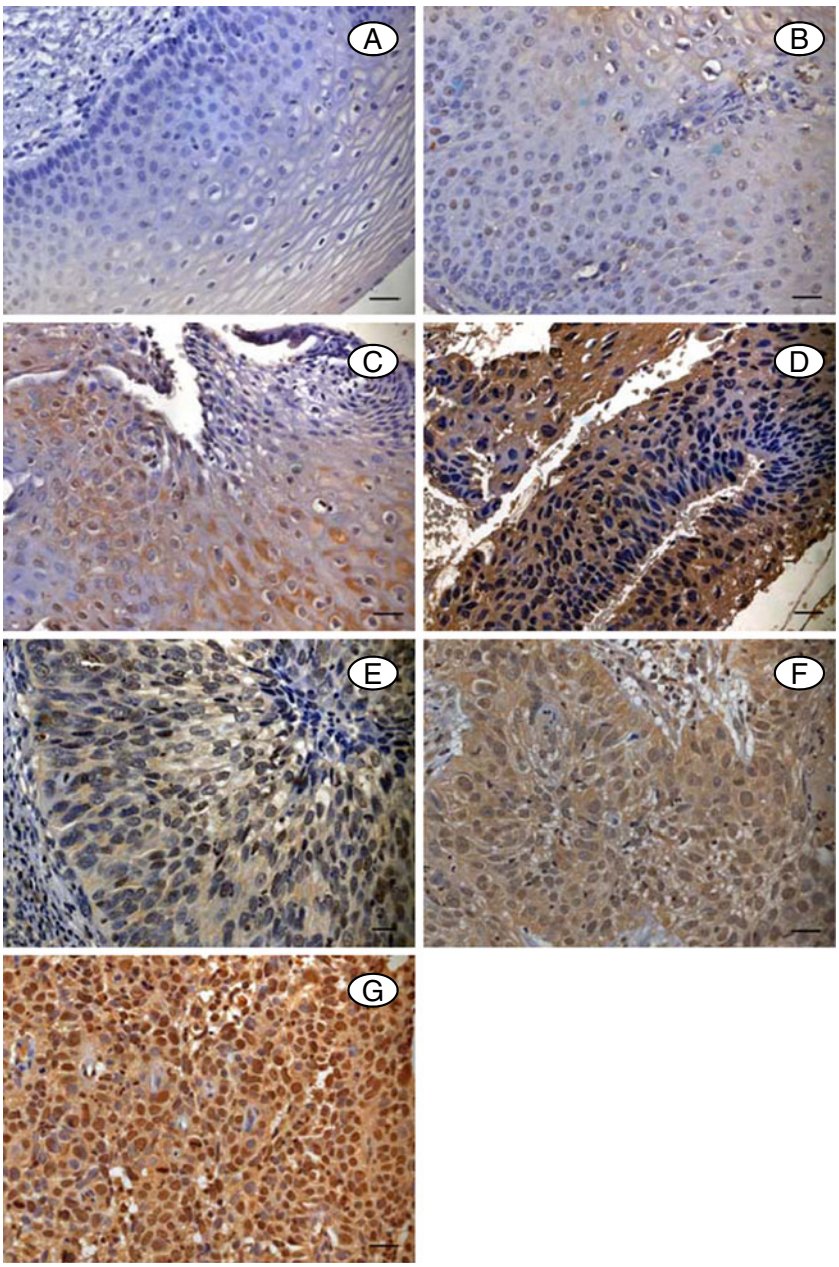

Fig. 2 PARP-1 expression determined by immunohistochemistry. **a** Absence of PARP-1 in normal cervix; **b** Weak expression of PARP-1 in LSIL; **c** Moderate expression of PARP-1 in LSIL; D) Moderate/Strong expression of PARP-1 in HSIL; **e** Weak expression of PARP-1 in SCC; **f** Moderate expression of PARP-1 in SCC; **g** Strong expression of PARP-1 in SCC. Scale bar =20 μm

Table 1 Intensity of expression of BAG-1 in squamous intra-epithelial lesions (SILs) and squamous cell carcinoma (SCC)

	Low expression		High expression	
	Negative n(%)	Weak n(%)	Moderate n(%)	Strong n(%)
LSIL n=50	5(10)	25(50)	17(34)	3(6)
HSIL n=49	2(4,1)	30(61,2)	14(28,6)	3(6,1)
SCC n=50	5(10)	32(64)	13(26)	0(0)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra-epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL versus HSIL (p=0,679)

[b] Fisher's exact test LSIL versus SCC (p=0,201)

[c] Fisher's exact test HSIL versus SCC (p=0,387)

Table 3 Intensity of expression of PARP-1 in squamous intra-epithelial lesions (SILs) and squamous cell carcinoma (SCC)

	Low expression		High expression	
	Negative n (%)	Weak n (%)	Moderate n (%)	Strong n (%)
LSIL n=50	7 (14)	22 (44)	20 (40)	1 (2)
HSIL n=49	11 (23.4)	29 (57.5)	9 (19.1)	0 (0)
SCC n=50	3 (6)	22 (44)	16 (32)	9 (18)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra-epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL versus HSIL (p=0,015)

[b] Fisher's exact test LSIL versus SCC (p=0,547)

[c] Fisher's exact test HSIL versus SCC (p=0,001)

1 min for denaturation, annealing at 51°C×1 min and chain elongation at 72°C×1 min. A final extension of 72°C for 10 min followed the last cycle. For HPV16 and HPV18 controls, DNA was extracted from SiHa and HeLa cell lines, respectively. For general HPV detection, a dilution mixture of both positive controls was used. As a negative control, sterile water was utilized instead of DNA. PCR products were electrophoresed in 10% polyacrylamide gels at 200 V for 1 h 45 min and stained with AgNO3 [22]. A 100 bp DNA ladder (Fermentas) was used as size marker.

Statistical Analysis

The relationship between low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs) and invasive squamous cell carcinomas (SCCs) of the uterine cervix according to BAG-1 and PARP-1 staining intensity and the proportion of positive cells was tested by Fisher's exact test.

Fisher's exact test was also used to compare BAG-1 and PARP- 1 expression between clinical parameters or HPV infection in the different groups of cervical lesions.

Statistical analyses were performed using the GraphPad Instat 3.05 software. A p-value of <0.05 was considered statistically significant.

Results

Expression of BAG-1 and PARP-1 in Cervical Lesions

Normal cervical specimens obtained from healthy patients at autopsy showed BAG-1 and PARP-1 negative staining or only light staining in the areas of the basal and parabasal layers cells of the ectocervical epithelium. In the patient samples, BAG-1 expression was detected in cytoplasm, nucleus or both. PARP-1 expression was detected primarily in the nucleus of cervical lesions (Figs. 1 and 2). One sample

Table 2 Quantitative expression of BAG-1 positive cells in in squamous intra-epithelial lesions (SILs) and squamous cell carcinoma (SCC)

	Low expression		High expression	
	0 (Negative) n (%)	1 (1-30%) n (%)	2 (31–70%) n (%)	3 (71–100%) n (%)
LSIL n=50	5 (10)	30 (60)	12 (24)	3 (6)
HSIL n=49	2 (4,1)	20 (40,8)	15 (30,6)	12 (24,5)
SCC n=50	5 (10)	17 (34)	14 (28)	13 (26)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra-epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL versus HSIL (p=0,014)

[b] Fisher's exact test LSIL versus SCC (p=0,014)

[c] Fisher's exact test HSIL versus SCC (p=1,000)

Table 4 Quantitative expression of PARP-1 positive cells in squamous intra-epithelial lesions (SILs) and squamous cell carcinoma (SCC)

	Low expression		High expression	
	0 (<5%) n (%)	1 (6–25%) n (%)	2 (26 a 50%) n (%)	3 (>50%) n (%)
LSIL n=50	7 (14)	14 (28)	17 (34)	12 (24)
HSIL n=49	11 (23.4)	13 (23.4)	14 (29.7)	11 (23.4)
SCC n=50	3 (6)	10 (20)	14 (28)	23 (46)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra-epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL versus HSIL (p=0,840;OR=0,888)

[b] Fisher's exact test LSIL versus SCC (p=0,138; OR=2,061)

[c] Fisher's exact test HSIL versus SCC (p=0,022; OR=2,732)

Table 5 BAG-1 immunoexpression and clinical parameters in cervical lesions

BAG-1	LSIL $n=50$		p	HSIL $n=49$		p	SCC $n=50$		p
	n (%)			n (%)			n (%)		
	+	−		+	−		+	−	
Age									
<45	43 (86)	5 (10)	1,000	40 (81,6)	2 (4,1)	1,000	14 (28)	1 (2)	1,000
>45	2 (4)	0 (0)		7 (14,3)	0 (0)		31 (62)	4 (8)	
Smoke									
No	30 (60)	4 (8)	1,000	33 (67,3)	1 (2,1)	0,523	32 (64)	3 (6)	0,629
Yes	15 (30)	1 (2)		14 (28,5)	1 (2,1)		13 (26)	2 (4)	
Alcohol									
No	37 (74)	5 (10)	0,577	45 (91,8)	2 (4,1)	1,000	43 (86)	4 (8)	0,276
Yes	8 (16)	0 (0)		2 (4,1)	0 (0)		2 (4)	1 (2)	
Parity									
No	10 (20)	0 (0)	0,568	4 (8,1)	0 (0)	1,000	1 (2)	1 (2)	0,191
Yes	35 (70)	5 (10)		43 (87,8)	2 (4,1)		44 (88)	4 (8)	
Contraceptive use									
No	27 (54)	3 (6)	1,000	35 (71,4)	2 (4,1)	1,000	45 (90)	4 (8)	0,100
Yes	18 (36)	2(4)		12 (24,5)	0 (0)		0 (0)	1 (2)	

from HSIL group was lost during antigen retrieval step, leading to its exclusion from immunohistochemical evaluation.

In LSIL and SCC patients, BAG-1 staining was observed in 45 out of 50 (90%) cervical specimens. In the HSIL group, this protein was detected in 47 out of 49 (95,9%) cervical specimens. BAG-1 staining intensity was found to be low in all groups. No significance was found between the intensity of BAG-1 expression and cervical lesions (Table 1). In terms of the proportion of BAG-1 positive cells (Table 2), we observed significantly higher expression in the HSIL and SCC groups when compared with the LSIL group.

PARP-1 staining was observed in 43 out of 50 (86%) LSIL specimens, 38 out of 49 (77,5%) HSIL specimens and 47 out of 50 (94%) SCC specimens. The intensity of PARP-1 expression was significantly different in the HSIL group (Table 3). In terms of the proportion of PARP-1 positive cells (Table 4), we observed a significantly higher expression in SCC group when compared with HSIL group.

Table 6 PARP-1 immunoexpression and clinical parameters in cervical lesions

PARP-1	LSIL $n=50$		p	HSIL $n=49$		p	SCC $n=50$		p
	n (%)			n (%)			n (%)		
	+	−		+	−		+	−	
Age									
<45	41 (82)	7 (14)	1,000	32 (65,3)	10 (20,4)	1,000	14 (28)	1 (2)	1,000
>45	2 (4)	0 (0)		6 (12,2)	1 (2,1)		33 (66)	2 (4)	
Smoke									
No	30 (60)	4 (8)	0,659	24 (48,9)	10 (20,4)	0,136	32 (64)	3 (6)	0,544
Yes	13 (26)	3 (6)		14 (28,6)	1 (2,1)		15 (30)	0 (0)	
Alcohol									
No	36 (72)	6 (12)	1,000	36 (73,5)	11 (22,4)	1,000	44 (88)	3 (6)	1,000
Yes	7 (14)	1 (2)		2 (4,1)	0 (0)		3 (6)	0 (0)	
Parity									
No	9 (18)	1 (2)	1,000	3 (6,1)	1 (2,1)	1,000	2 (4)	0 (0)	1,000
Yes	34 (68)	6 (12)		35 (71,4)	10 (20,4)		45 (90)	3 (6)	
Contraceptive use									
No	24 (48)	6 (12)	0,219	28 (57,1)	9 (18,4)	0,708	46 (92)	3 (6)	1,000
Yes	19 (38)	1 (2)		10 (20,4)	2 (4,1)		1 (2)	0 (0)	

Table 7 BAG-1 expression and HPV infection

BAG-1	HPV positive n (%)	HPV negative n (%)	HPV16 n (%)	HPV18 n (%)	HPV 16 & 18 n (%)	Other types n (%)
[a]LSIL *n*=45						
+	14 (31,1)	27 (60)	4 (26,7)	2 (13,3)	1 (6,7)	7 (46,6)
−	1 (2,2)	3 (6,6)	1 (6,7)	0 (0)	0 (0)	0 (0)
[b]HSIL *n*=49						
+	26 (53)	22 (44,9)	22 (81,5)	1 (3,7)	0 (0)	3 (11,1)
−	1 (2)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)
[c]SCC *n*=46						
+	29 (63)	13(28,3)	20 (62,5)	2 (6,2)	4 (12,5)	2 (6,2)
−	3 (6,5)	1 (2,2)	2 (6,2)	1 (3,1)	1 (3,1)	0 (0)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra- epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL: HPV+ versus HPV- (p=1,000;OR=1,556)

[b] Fisher's exact test HSIL: HPV+ versus HPV- (p=1,000; OR=0,392)

[c] Fisher's exact test SCC: HPV+ versus HPV- (p=1,000; OR=0,743)

BAG-1 and PARP-1 Expression and Clinical Parameters

No significant associations were observed regarding BAG-1 and PARP1 expression in relation to age, alcohol use, smoke exposure, parity and oral contraceptive use (Tables 5 and 6).

Human Papillomavirus Detection and Correlation with BAG-1 and PARP-1 Expression

Among 150 patients, 140 (93,3%) were positive to beta-globin (internal control) and 10 (6,4%) were beta-globin negative, indicating that the DNA from paraffin sections was degraded. Therefore, they were excluded from HPV evaluation. In the LSIL group, HPV virus was positive in 15 out of 45 (33.3%). In the HSIL group, the virus was detected in 27 out of 49 (55.1%), and in the SCC group, HPV virus was detected in 32 out of 46 (69.6%) of patients. There was no correlation between BAG-1 expression and HPV infection. However, there was a significantly higher expression of PARP-1 in HPV-positive patients than in HPV-negative patients in the HSIL group (Tables 7 and 8).

Table 8 PARP-1 expression and HPV infection

PARP-1	HPV positive n (%)	HPV negative n (%)	HPV16 n (%)	HPV18 n (%)	HPV 16 & 18 n (%)	Other types n (%)
[a]LSIL *n*=45						
+	13 (28,8)	26 (57,7)	5 (33,3)	1 (6,6)	1 (6,6)	6 (40)
−	2 (4,4)	4 (8,8)	0 (0)	1 (6,6)	0 (0)	1 (6,6)
[b]HSIL *n*=49						
+	24 (49)	13 (26,5)	20 (74)	1 (3,7)	0 (0)	3 (11,1)
−	3 (6,1)	9 (18,4)	3 (11,1)	0 (0)	0 (0)	0 (0)
[c]SCC *n*=46						
+	29 (63)	14 (30,4)	20 (62,5)	3 (9,4)	4 (12,5)	2 (6,2)
−	3 (6,5)	0 (0)	2 (6,3)	0 (0)	1 (3,1)	0 (0)

LSIL low-grade squamous intra-epithelial lesion; *HSIL* high-grade squamous intra-epithelial lesion; *SCC* squamous cell carcinoma

[a] Fisher's exact test LSIL: HPV+ versus HPV- (p=1,000;OR=1,000)

[b] Fisher's exact test HSIL: HPV+versus HPV- (p=0,021; OR=5,538)

[c] Fisher's exact test SCC: HPV+ versus HPV- (p=0,542; OR=0,290)

Discussion

Alterations in apoptotic mechanisms that promote excessive or deficient programmed cell death have been linked to a wide array of pathological conditions [23]. The analysis of genes involved in the regulation of apoptosis in cervical cancer is considered an important pathway into understanding the process of tumorigenesis [24].

The multi-functional prosurvival BAG-1 protein has pleiotropic effects on a large range of cellular responses including apoptosis, transcription, proliferation and signaling [25]. BAG-1 exhibits positive expression in many malignant tumors, such as breast carcinoma, lung cancer, prostatic carcinoma and gastrointestinal cancer. The protein shows minimal expression in normal tissue [6, 9, 26, 27]. In the present study, we evaluated the staining intensity as well as the percentage of positively stained cells. Our evidence indicates that there is a significantly higher expression of BAG-1 in HSIL and SCC samples, relative to LSIL, with a predominance of low intensity staining. Similar results have been reported in an article by Chang et al. (2003), which showed an increased percentage of cells expressing BAG-1 in SCC and HSIL when compared with LSILs and normal tissues [28]. These investigators suggested that BAG-1 has the potential to be a novel diagnostic molecular tumor biomarker and a determining factor in the progression of cervical carcinogenesis. Yang et al. (1999) used Western blot analysis to find overexpression of the antiapoptotic gene BAG-1 in human cervical cancer cell lines and tissues and provided the evidence that overexpression may partially contribute to enhanced resistance to apoptosis induced by DNA-damaging reagents [29]. BAG-1 is a multifunctional binding protein whose promoter domain is highly homologous with Bcl-2. It can bind to Bcl-2 and act as a positive Bcl-2 apoptosis regulator to enhance the antiapoptotic effect, possibly by affecting the stability of the protein [30].

Immunohistochemical analysis of PARP-1 has been performed in other types of primary malignancies, including endometrial and colon cancers, as well as cutaneous malignant melanomas [18–20]. PARP-1 dysfunction possibly affects genomic and epigenetic stabilities through effects on DNA repair and cell cycle regulation, which accompanies NAD depletion, and it may modulate tumor cells to confer malignant and invasive potential [31]. Staibano et al. (2005) found significant expression of PARP-1 in malignant melanomas and suggested that the neoplastic progression toward the invasive (vertical) growth phase of melanocytes in cutaneous malignant melanoma is characterized by the loss of cleavage of PARP-1, likely signaling an imbalance of the apoptotic process in these cells and therefore predisposing them to acquire alteration(s) of other gatekeeping genes, leading to more aggressive growth [32]. To the best of our knowledge, the present study is the first attempt at describing PARP-1 immunohistochemical expression in cervical lesions. In our study, we found significantly higher expression of PARP-1 in SCC samples when compared with HSIL samples. These results indicate that PARP-1 expression is potentially relevant to the development of cervical carcinomas, reiterating the importance of deregulation of apoptosis as a critical pathogenetic component of tumor progression.

It has been demonstrated that PARP-1 gene is up-regulated in HPV-infected cancer cells [33]. In our study, PARP-1 was more highly expressed in the samples of HPV-positive HSIL. D Lee et al. (2002) demonstrated that PARP-1 physically associates with the HPV-18 E2 protein and activates E2-dependent transcription. Moreover, they demonstrated that the NH2-terminal domain of PARP-1 is the sole requirement for transcriptional co-activation of E2 by PARP-1. The identification of PARP-1 as a potential co-activator of E2-dependent transcription is an important extension of the cellular functions of PARP-1, raising questions regarding its role in viral transcription [34]. In contrast, no correlations were found between HPV infection and BAG-1 expression.

In conclusion, our research suggests that BAG-1 expression could be an important marker for the diagnosis of early stage cervical lesions, contributing to the differentiation between LSIL and HSIL/SCC, whereas PARP-1 expression could be an important marker for the diagnosis of cervical cancer, contributing to the differentiation between HSIL and SCC. Therefore, both proteins can be complementary in the diagnosis of uterine lesions. Overall, overexpression and deregulation of BAG-1 and PARP-1 may play an important role in cervical carcinogenesis during different stages of primary carcinoma development. Further studies are needed to clarify the molecular aspects of the relationship between PARP-1 expression and HPV infection, with potential applications for cervical cancer prediction.

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Conflict of Interest We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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