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ORIGINAL ARTICLE

The possible role of Bcl-2 expression of tumors of the uterine cervix

Z. Protrka[1], J. Djurić[1], O. Protrka[2], S. Arsenijević[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Kragujevac, Kragujevac; [2]Health Center, Kragujevac, Serbia

Summary

***Purpose*:** *To assess the expression of Bcl-2 protooncogene in premalignant and malignant uterine cervix lesions.*

***Methods*:** *To establish the role of this protooncogene in uterine cervix carcinogenesis, we examined 69 tissue samples of low grade cervical squamous intraepithelial lesions (SIL) (n=16), high grade SIL (n=11), portio vaginalis uteri (PVU) carcinoma in situ (n=11) and PVU invasive carcinoma, stage IA-IIA (n=13) (study group) and 18 samples without SIL or malignancy (control group). The expression of Bcl-2 was detected immunohistochemically using a monoclonal antibody. Fisher's exact test ($p < 0.05$) was used to assess statistical significance. By establishing the sensitivity and specificity of the test, the level of reliability of these analyses was determined as a possible screening method for early detection of changes in the uterine cervix.*

***Results*:** *Overexpression of Bcl-2 was found to increase in direct relation to the grade of the cervical lesions. Statistically significant difference was found in the frequency of overexpression in patients with high grade SIL (6/11, p=0.006), PVU carcinoma in situ (5/11, p=0.018) and PVU invasive carcinoma (6/13, p=0.012), in relation to the control group. High sensitivity was of great diagnostic significance for the detection of these types of changes in the uterine cervix. On the basis of high predictive values it can be said that in patients with Bcl-2 overexpression there is a great possibility that they have premalignant or malignant changes in the uterine cervix.*

Conclusion: *Our results indicate that overexpression of Bcl-2 may play an important role in cervical carcinogenesis. However, more extensive series of samples is required to establish the prognostic significance of Bcl-2 in cervical carcinogenesis.*

Key words: Bcl-2, carcinogenesis, cervical neoplasia, immunohistochemistry

Introduction

Squamous cell carcinoma of the uterine cervix is currently one of the most common malignancies in women worldwide.

In searching ways of prevention, early diagnosis and effective treatment of premalignant lesions and malignant tumors of the uterine cervix, molecular-genetic researches play a significant role in the last few years.

Alterations at the genetic level or protein level of the protooncogenes result in oncogenic conversion and impaired cellular growth control mechanisms causing tumor development [1].

Analysis of oncogenes' expression in human cancer is increasingly important to gain a better insight in the process of tumorigenesis and to identify new markers for early diagnosis of malignant transformation. Abnormal expression of different cellular oncogenes in various cancers assessed by hybridization and immunological techniques has been previously reported [2]. The results of these analyses do not appear to be of any early diagnostic value since oncogene expression is, in general, only demonstrable in tumors that can already be classified as malignant.

The key difference between normal and malignant cell is in a subtle change of specific genes that control, by their products, the processes of growth, division and differentiation of a cell and are called pro-

Correspondence to: Zoran Protrka, MD, PhD. Department of Obstetrics and Gynecology, Kragujevac. Stevana Visokog 28, 340 00 Kragujevac, Serbia. Tel: +38 134 34 24 82, E-mail: protrka@eunet.yu

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tooncogenes. These genes that are present in the genotype of every normal cell possess oncogenic potential because their disturbed expression may lead to malignant transformation.

The Bcl-2 family of related proteins is one of the key regulators of the apoptotic process. It consists of two opposing groups of proteins: death antagonists (Bcl-2, bcl-XL, Mcl-1) and death agonists (Bax, Bak, Bcl-XS) [3]. Apoptosis occurs through competing dimerization between the two protein groups, the relative proportions of which ultimately control the sensitivity or resistance of cells to apoptotic stimuli. Overexpression of Bcl-2 and Bax and their prognostic significance have been reported in several epithelial cancers [4]. However, the data on squamous cell carcinoma of the uterine cervix are limited and the results are conflicting [5]. *In vitro* studies have also shown that Bcl-2 overexpression prolonged cell survival in cell culture studies [6]. Therefore, an *in vivo* study on the association between these apoptosis regulatory proteins may be a prerequisite for the complete understanding of the molecular pathogenesis underlying cervical cancer development.

Mitochondrial and cell-surface death receptor-mediated apoptosis are the two principal pathways leading to programmed cell death. The mitochondrial pathway is thought to play a major role in response to cancer treatments and is mediated by the Bcl-2 family proteins [7]. More than 20 members of this family have been described thus far in humans. A positive ratio between pro- and antiapoptotic Bcl-2 family members leads to cytochrome-*C* release from mitochondria, which triggers the final execution of cell death by the caspase cascade.

Many studies have shown that poor prognosis is in positive correlation with the degree of expression of this oncogene (ovary, uterine cervix, lung, prostate, breast, colon) [8-10].

The purpose of our study was to assess the expression and clinical significance of Bcl-2 oncogene in the uterine cervix carcinogenesis.

Methods

This prospective study was carried out during 2007 and 2008, at the Department of Obstetrics and Gynecology, Faculty of Medicine and the experimental part was performed at the Laboratory for Experimental and Clinical Immunology of the Faculty of Medicine and at the Immunological Laboratory of the Public Health Institute, Kragujevac.

From patients operated at the Department of Obstetrics and Gynecology because of premalignant and/or malignant changes of the uterine cervix, some tissue sections were taken from the operative material (hysterectomy, punch biopsy or conization) for pathological verification and used for this research.

The control group consisted of 18 females in whom ambulatory biopsy of the uterine cervix was performed (Papanicolaou test was indicated) and where malignant changes or SIL were not found by histopathology (cervicitis chronica of mild to moderate degree).

The study group consisted of 16 patients with pathological diagnosis of low grade SIL, 11 with high grade SIL, 11 with PVU carcinoma *in situ* and 13 with PVU invasive carcinoma, stage IA-IIA.

Bcl-2 oncogene expression followed in patients in the study and the control group.

Cryostat sections were sent for intraoperative diagnosis. Extra sections of cervical lesions were snap-frozen in liquid nitrogen and stored at -70^{o} C until used for immunohistochemistry.

Bcl-2 (clone 100, Ab-1, Calbiochem, Oncogene Research, Cambridge, USA) monoclonal antibody was used, which was diluted with phosphate buffered saline (PBS, pH 7.2).

Four-micrometer frozen sections were fixed in 100% acetone for 5 min and the endogenous peroxidase activity was quenched by 10 min incubation in 0.5% hydrogen peroxide. For monoclonal antibody Bcl-2 (concentration of 1 μg/ml) incubation with primary antibody was carried out overnight at 4^{o} C.

Sections of Bcl-2 positive lymphoid tissue were used as positive controls. For negative controls, the samples were taken through the procedure with omission of the primary antibody.

Slides were evaluated by two of the authors, unaware of immunohistochemical or clinical data using a semiquantitative method on a Zeiss AXIOSKOP 2 light microscope. The percentage of immunopositive cells in representative areas of the sections was assessed. The intensity of immunostaining was divided into 4 categories, namely: negative (0-5%); 1+ (5-25%); and positive 2+ (25-50%), 3+ (50-100%).

Tissue sections from the operative material or material taken by biopsy were taken after obtaining informed consent of patients in accordance with the Declaration of Helsinki and recommendations of the World Health Organization (WHO) for experiments on human material and after getting approval from the Ethics Committee.

Statistics

Based on the frequency of the lesions found, patients were split into 4 subgroups and 2×2 contingency tables were formed and specificity and sensitivity were

calculated. Also, based on the ROC (receiver operating characteristic) curve, which represents the relation between specificity and sensitivity, the discrimination power of the test was determined.

Differences between groups were considered significant (Fisher's exact test) at $p < 0.05$. By establishing sensitivity and specificity of the test, the level of reliability of these analyses was determined as a possible screening method for early detection of changes in the uterine cervix.

Results

Table 1 and Figure 1 show the expression levels of Bcl-2 oncogene in the study and control group. Overexpression of Bcl-2 was present in 5.6% of women in the control group and in 25.0% of patients with low grade SIL. Analysis of frequency of patients with positive findings of Bcl-2 oncogene revealed no statistical differences when control and low grade SIL groups were evaluated (Fisher's exact test, p=0.164; Photo 1).

On the basis of the frequency of patients with Bcl-2 overexpression, a 2×2 table of contingency was formed (Table 2), in which sensitivity and specificity were calculated. The sensitivity of determining Bcl-2 as a method for detection of low grade SIL was 25.0% and specificity was 94.4% (Figure 2).

The positive predictive value was 80.27%, which means that this percentage of patients with Bcl-2 overexpression may be expected to harbor low grade SIL in the cervix. The negative predictive value was 94.8%, meaning that this percentage of patients with Bcl-2 negative expression existence of this type of change is not expected.

Table 1 and Figure 1 show that Bcl-2 overexpression was present in 5.6% of women in the control group and in 54.6% of patients with high grade SIL (Fisher's exact test, p = 0.006; Photo 2).

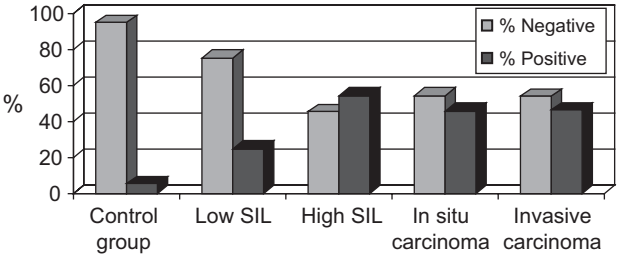

Figure 1. Percentage of positive and negative Bcl-2 expression.

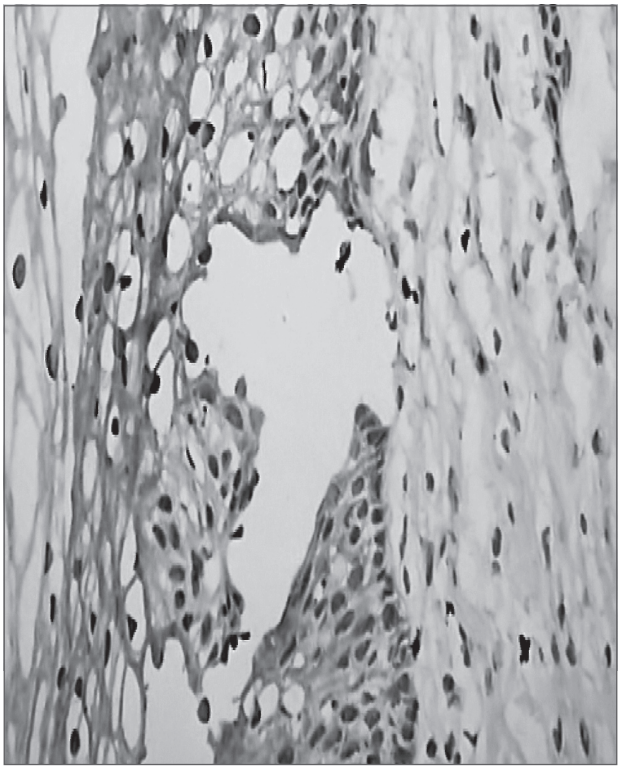

Photo 1. Bcl-2 overexpression (3+) in low grade SIL (×40).

Table 2. Table of contingency (low grade SIL)

Test	*Disease present (low grade SIL)*	*Disease absent (control group)*	*Total*
Bcl-2 overexpression*	4	1	5
Bcl-2 negative	12	17	29
Total	16	18	34

*p=0.164
For abbreviations see text

Table 1. Expression of Bcl-2 in the control and study groups

Bcl-2 expression	*Control group n (%)*	*Low grade SIL, n (%)*	*High grade SIL, n (%)*	*PVU carcinoma in situ n (%)*	*PVU invasive carcinoma n (%)*
Negative (–)	10/18 (55.5)	8/16 (50)	4/11 (36.3)	4/11 (36.3)	4/13 (30.8)
Negative (1+)	7/18 (38.9)	4/16 (25.0)	1/11 (9.1)	2/11 (18.2)	3/13 (23.1)
Positive (2+)	1/18 (5.6)	1/16 (6.2)	4/11 (36.3)	3/11 (27.3)	4/13 (30.8)
Positive (3+)	0/18 (0)	3/16 (18.8)	2/11 (18.2)	2/11 (18.2)	2/13 (15.3)

For abbreviations see text

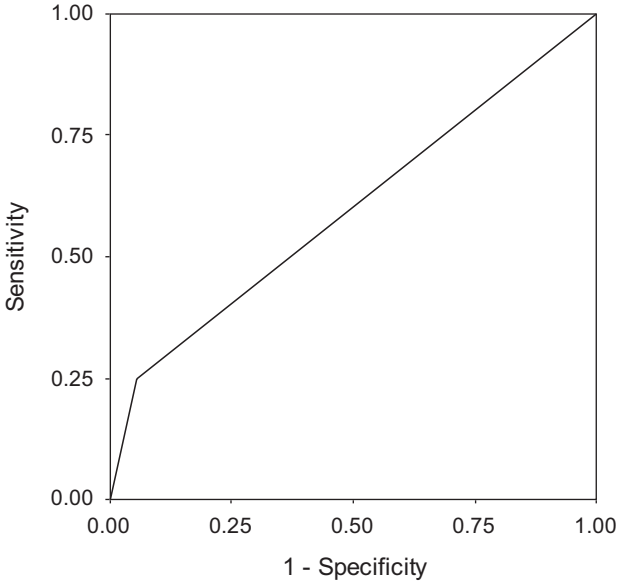

Figure 2. ROC (receiver operating characteristic) curve for Bcl-2 overexpression in patients with low grade SIL.

The sensitivity of determining Bcl-2 for detecting high grade SIL changes was 54.6% and specificity was 94.4% (Table 3 and Figure 3).

Regarding the obtained values of sensitivity and specificity, the discrimination power of overexpression of this oncogene with the aim of determining the existence of changes of this type was greater than the discrimination power of negative expression in the control group.

Table 3. Table of contingency (high grade SIL)

Test	*Disease present (high grade SIL)*	*Disease absent (control group)*	*Total*
Bcl-2 overexpression*	6	1	7
Bcl-2 negative	5	17	22
Total	11	18	29

*p=0.006
For abbreviations see text

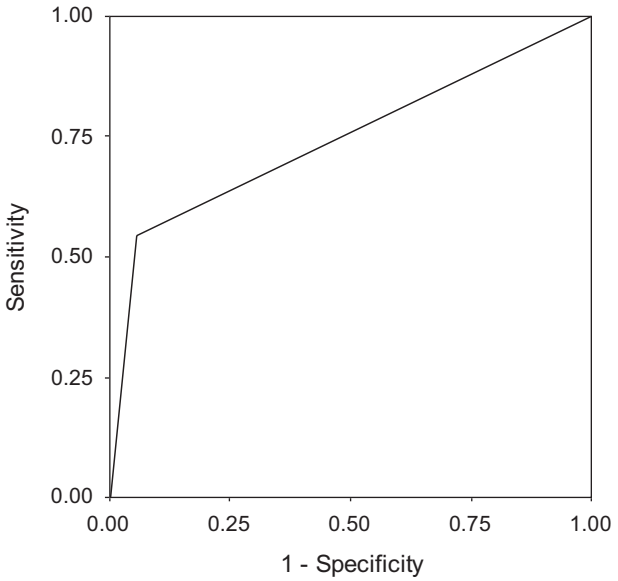

Figure 3. ROC (receiver operating characteristic) curve for Bcl-2 overexpression in patients with high grade SIL.

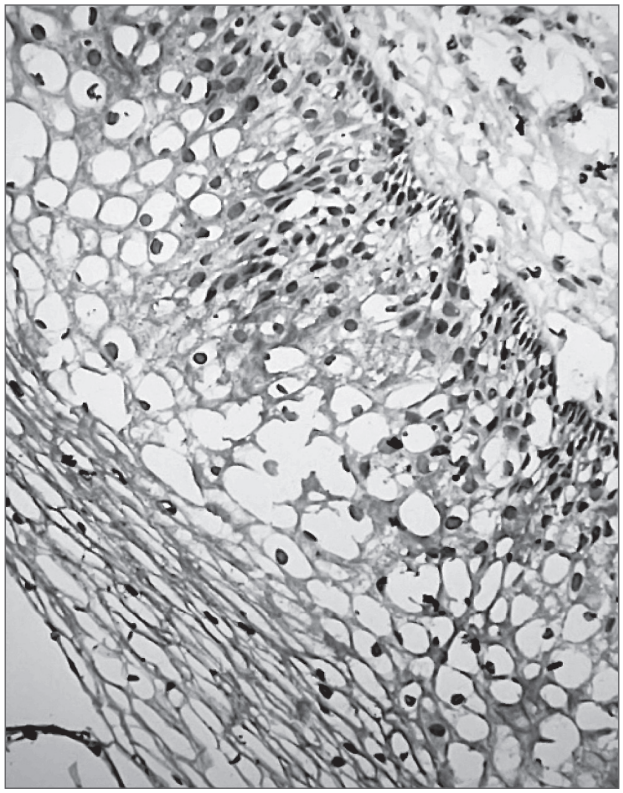

Photo 2. Bcl-2 overexpression (3+) in high grade SIL (×40).

The positive predictive value in high grade SIL changes was 85.89% and the negative predictive value was 77.21%.

Table 1 and Figure 1 show that Bcl-2 overexpression was present in 5.6% of patients in the control group and in 45.5% of patients with PVU carcinoma *in situ*. (Fisher's exact test, p=0.018; Photo 3).

The sensitivity of this oncogene for diagnosing this type of lesions was 45.5% and specificity was 94.2% (Table 4 and Figure 4).

The positive predictive value was 74.64% and the negative predictive value was 67.98%.

Table 1 and Figure 1 show that Bcl-2 overexpression was present in 5.6% of patients in the control group and in 46.1% of patients with PVU invasive carcinoma (Fisher's exact test, p = 0.012; Photo 4).

The sensitivity and specificity of the test were 46.2% and 94.4%, respectively (Table 5 and Figure 5), and the positive and negative predictive values were 85.84% and 70.80%, respectively.

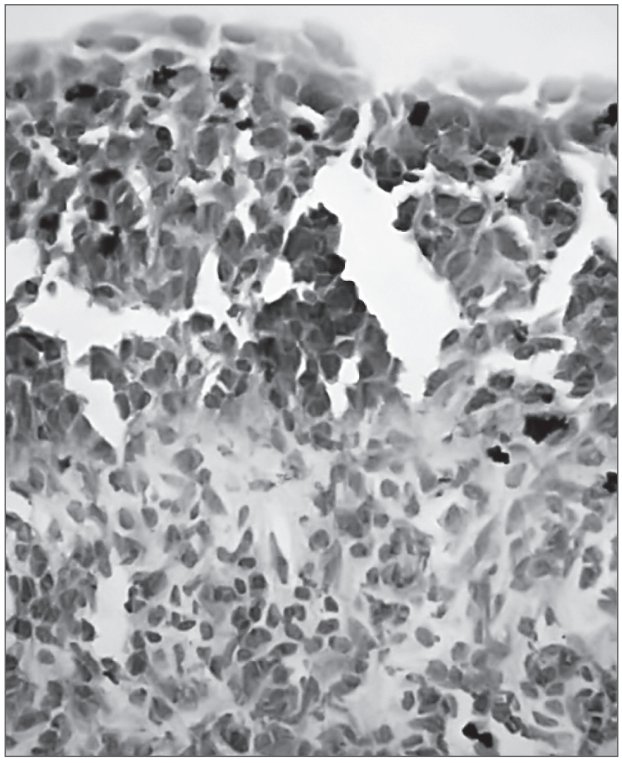

Photo 3. Bcl-2 overexpression (3+) in PVU carcinoma *in situ* (×40).

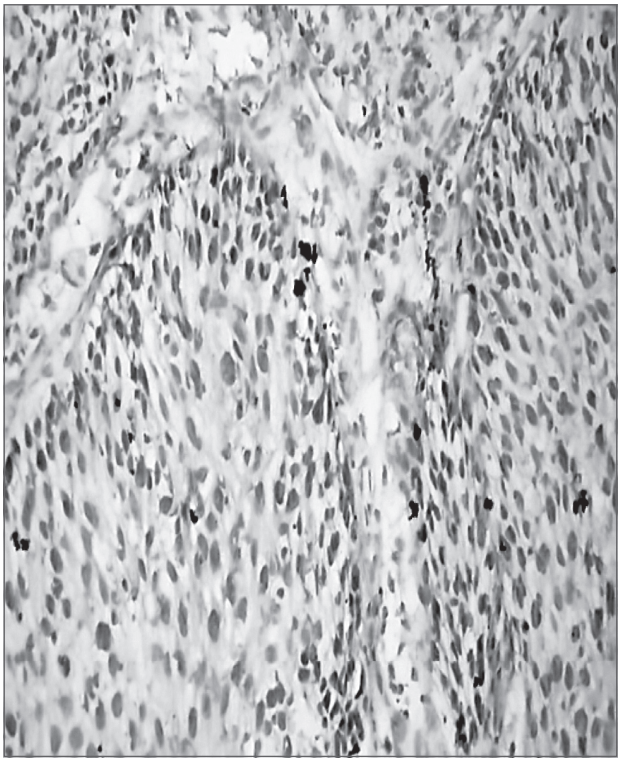

Photo 4. Bcl-2 overexpression (3+) in PVU invasive carcinoma (×40).

Table 4. Table of contingency (PVU carcinoma *in situ*)

Test	*Disease present (PVU carcinoma in situ)*	*Disease absent (control group)*	*Total*
Bcl-2 overexpression*	5	1	6
Bcl-2 negative	6	17	23
Total	11	18	29

*p=0.018
For abbreviations see text

Table 5. Table of contingency (PVU invasive carcinoma)

Test	*Disease present (PVU invasive carcinoma)*	*Disease absent (control group)*	*Total*
Bcl-2 overexpression*	6	1	7
Bcl-2 negative	7	17	24
Total	13	18	31

*p=0.012
For abbreviations see text

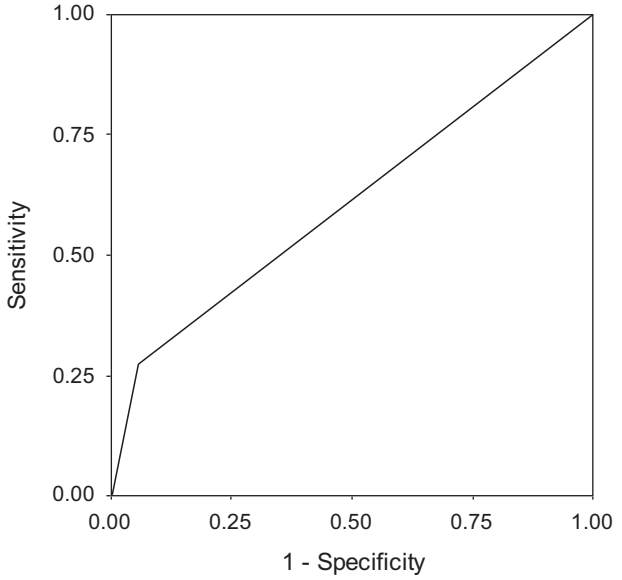

Figure 4. ROC (receiver operating characteristic) curve for Bcl-2 overexpression in patients with PVU carcinoma in situ.

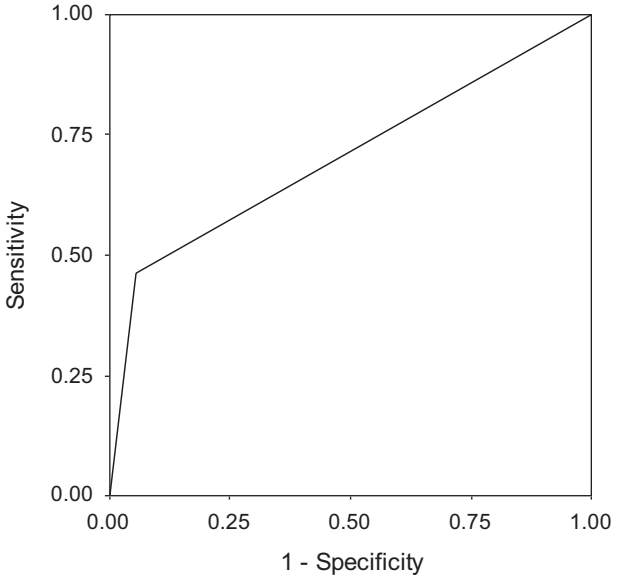

Figure 5. ROC (receiver operating characteristic) curve for Bcl-2 overexpression in patients with PVU invasive carcinoma.

Discussion

This study evaluated the expression of Bcl-2 oncogene in a range of tissues obtained from normal, dysplastic and neoplastic conditions of the cervix, in an attempt to elucidate the expression of this oncoprotein in uterine cervix premalignant and malignant lesions.

The expression of Bcl-2 in fetal tissues is thought to play an important role during the developmental morphogenesis through protection from programmed cell death [11]. In adult tissues, this protein is thought to play a role in maintaining the structural homeostasis of various organs [12].

Bcl-2 is an oncogene that has been investigated for prognostic significance in various malignancies, including carcinoma of the cervix [13-15]. Up to now, conflicting results have been obtained.

Bcl-2 has been shown in some studies to be an independent predictor of poor prognosis in carcinoma of the cervix [16]. On the other hand, other studies have revealed no statistically significant correlation with adverse outcome [17].

Contradictory results have also been published on the prognostic significance of this oncogene in other gynecological sites [18]. The conflicting results may be due to differences in institutional treatment standards and to varied subjective interpretations of staining intensity and distribution between centers. Because staining is judged on a continuum, differences in institutional "cut-off" determinations for positive staining may also affect correlation with clinicopathological results.

Hove et al. [19] found that Bcl-2 overexpression was a favorable prognostic factor on univariate analysis for both overall and disease-free survival, but was not significant on multivariate analysis.

Cheung et al. [20] in their analysis of 44 patients with cervical adenocarcinoma found that increased expression of Bcl-2 correlated with a poorer prognosis.

Absence of correlation between clinical prognosis and Bcl-2 expression was described by Jain et al. [21].

Fonseca et al. [22] suggest that coexpression of estrogen receptor, progesterone receptor and Bcl-2 may be a useful tool in identifying the CIN III lesions with low risk of progression to cervical cancer.

Leng and Ming [23] found that benign cervical squamous epithelium, low grade SIL, high grade SIL and SCC showed a generally diffuse Bax expression. Thus, Bcl-2 and Bax appeared to be upregulated at different stages of cervical carcinogenesis, Bcl-2 in HSIL and Bax after invasion.

In our study, no statistically significant difference was found in the frequency of overexpression of Bcl-2 between the control group and patients with low grade SIL. However, statistically significant difference was found in the frequency of overexpression in patients with high grade SIL, PVU carcinoma *in situ* and PVU invasive carcinoma in relation to the control group. High sensitivity values speak in favor of great diagnostic significance for the detection of these types of changes in the uterine cervix. On the basis of high predictive values, it can be said that in patients with overexpression of Bcl-2 oncogene, there is a great possibility that they have premalignant or malignant changes in the uterine cervix.

Conclusion

The findings presented in this study indicate that the evaluation of Bcl-2 expression may provide additional and independent prognostic information to predict the clinical course of cervical cancer.

Positive expression of this oncogene suggests with great certainty that there are premalignant or malignant changes in the uterine cervix.

However, studies with greater number of samples are required to establish the prognostic significance of Bcl-2 in cervical carcinogenesis.

References

1. Zha J, Harada H, Osipov K et al. BH3 domain of BAD is required for heterodimerization with Bcl-X(L) and pro-apoptotic activity. J Biol Chem 1997; 272: 24101-24104.
2. Protrka Z, Mitrovic S, Arsenijevic N et al. Her-2 expression in uterine cervix carcinogenesis. J BUON 2007; 12: 91-97.
3. Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev 1999; 13:1899-1911.
4. Schlesinger PH, Gross A, Yin XM et al. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc Natl Acad Sci USA 1997; 94: 1135-1139.
5. Tjalma WA, Weyler JJ, Bogers JJ et al. The importance of biological factors (bcl-2, bax, p53, PCNA, MI, HPV and angiogenesis) in invasive cervical cancer. Eur J Obstet Gynecol Reprod Biol 2001; 97: 223-230.
6. Boise LH, Gonzalez-Garcia M, Postema CE et al. Bcl-x, a Bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993; 74: 597-608.
7. Van der Heiden MG, Chandel NS, Schumacker PT et al. Bcl-x(L) prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. Mol Cell 1999; 3: 159-167.
8. Packham G, White EL, Eischen CM et al. Selective regulation of Bcl-XL by a jak kinase-dependent pathway is bypassed in murine hematopoietic malignancies. Genes Dev 1998; 12: 2475-2487.

9. Zha J, Harada H, Yang E et al. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-L(L). Cell 1996; 87: 619-628.
10. Strasser A, Harris AW, Bath ML et al. Novel primitive lymphoid tumors induced in transgenic mice by cooperation between myc and bcl-2. Nature 1990; 348: 331-333.
11. Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993; 74: 609-619.
12. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998; 281: 1305-1308.
13. Miyashita T, Krajewski S, Krajewska M et al. Tumor suppressor p53 is a regulator of Bcl-2 and bax gene expression in vitro and in vivo. Oncogene 1994; 9: 1799-1805.
14. Kihana T, Tsuda H, Teshima S et al. Prognostic significance of the overexpression of Bcl-2 protein in adenocarcinoma of the uterine cervix. Cancer 1994, 73: 148-153.
15. Chung TK, Cheung TH, Lo WK et al. Expression of apoptotic regulators and their significance in cervical cancer. Cancer Lett 2002; 180: 63-68.
16. Cheng EHY, Levine B, Boise LH et al. Bax- independent inhibition of apoptosis by Bcl-X(L). Nature 1996; 379: 554-556.
17. Nunez G, London L, Hockenberry D et al. Deregulated Bcl-2 expression selectively promotes survival of growth factor-deprived hematopoietic cell lines. J Immunol 1990; 144: 3602-3610.
18. Skirnisdottir I, Seidal T, Gerdin E et al. The prognostic importance of p53, Bcl-2, and bax in early stage epithelial ovarian carcinoma treated with adjuvant chemotherapy. Int J Gynecol Cancer 2002; 12: 265-276.
19. Hove MG, Dinh TV, Hannigan EV et al. Oncogene expression and microvessel count in recurrent and nonrecurrent stage Ib squamous cell carcinoma of the cervix. J Reprod Med 1999; 44: 493-496.
20. Cheung TH, Chung TK, Lo KW et al. Apoptosis-related proteins in cervical intraepithelial neoplasia and squamous cell carcinoma of the cervix. Gynecol Oncol 2002; 86: 14-18.
21. Jain D, Srinivasan R, Patel FD, Gupta SK. Evaluation of p53 and Bcl-2 expression as prognostic markers in invasive cervical carcinoma stage IIb/III patients treated by radiotherapy. Gynecol Oncol 2003; 88: 22-28.
22. Fonseca-Moutinho JA, Cruz E, Carvalho L et al. Estrogen receptor, progesterone receptor, and Bcl-2 are markers with prognostic significance in CIN III. Int J Gynecol Cancer 2004; 14: 911-920.
23. Leng CP, Ming LL. Significance of Bcl-2 and Bax proteins in cervical carcinogenesis. Pathology 2006; 28: 1-5.