Bcl-2 expression in preinvasive and invasive cervical lesions

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Summary

Purpose: To determine the correlation between bcl-2 expression and clinicopathological findings in cervical intraepithelial neoplasias (CIN I, II, III) and invasive cervical lesions, and its effect on overall survival rate.

Methods: Fifty specimens derived from 22 preinvasive and invasive cervical cancer cases up to surgical stage IIb (21 squamous cervical carcinoma and 7 adenocarcinoma cases) were preserved in paraffin blocks from primary surgery and constituted the study group. Tissues were processed and stained by immunohistochemical methods to assess the degree of bcl-2 expression.

Results: Positive bcl-2 expression was detected in 54% (13/21) of invasive lesions, while negative in 46% of cases. In CIN's, overall bcl-2 positivity was detected in 68% of cases. Bcl-2 expression was highly relevant between low grade (CIN I) and high grade (CIN II-III) lesions ($p < 0.05$). As regards degree of tissue staining for bcl-2 in CIN III cases, a statistically relevant difference was detected in comparison with low-grade preinvasive lesions ($p < 0.05$). In invasive cervical cancer cases, patients with bcl-2 positivity had a longer survival rate. By using the Cox regression model, univariate analysis did not show any specific prognostic factor to be important for survival rate, whereas, on multivariate analysis, histopathologic subtypes ($p = 0.0390$) and stage of tumor ($p = 0.0451$) had a statistically significant impact on overall survival rate.

Conclusions: Bcl-2 expression, especially in preinvasive lesions, may play a role in the apoptotic process and be regarded as a marker for disease progression. In invasive cervical carcinomas, bcl-2 expression has not been shown to be effective in overall survival rates.

Key words: Cervical intraepithelial neoplasia; Invasive cervical cancer; Bcl-2 espression.

Introduction

A precise balance between both spontaneous signals and radiotherapy, chemotherapy or heat-induced apoptopic signals or incessant cell growth initiators is of paramount importance during the cell growth cycle [1]. In cervical neoplasms, this balance has also been the topic of several researches based on the presence of various proto-oncogenes and oncogenes and their degrees of distribution in tumoral and eutopic tissue systems [2, 3].

Bcl-2, which is known to be a physiologic inhibitor of programmed cell death and a member of the mitochondrial protein family, is reponsible for the control of outer mitochondrial membrane permeability in response to apoptotic signals through intramembranous pore formation and a decline in transmembrane electrical potential. All these apoptotic changes lead to a unique result: intracellular cytochrome C accumulation and activation of endogenous caspase activity [3, 4]. Bcl-2 blocks both the pore and channel formation effects of apoptosis inducing genes including bax, bad or bcl-XI [3]. In recent years, the importance of tissue bcl-2 expression in cervical neoplastic processes has been discussed and tumors with a high bcl-2 expression rate have been claimed to have a better prognosis [4].

This retrospective analysis was designed to provide insights into the association between preinvasive and invasive cervical lesions and bcl-2 expression and degree of staining, in regard to several clinicopathological variables and overall survival in invasive lesions.

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Materials and Methods

Fifty cases diagnosed with cervical intraepithelial neoplasias (CIN I, II, III) and invasive cervical cancer surgically staged up to stage IIb at the Gynecologic Oncology Unit, Department of Obstetrics and Gynecology of Osmangazi University Faculty of Medicine from 1991 to 1998 were enrolled in this study. All CIN I and CIN II cases underwent the loop electroelectrical surgical procedure (LEEP) or cervical conisation. Cases with CIN III were treated with cervical conisation and hysterectomy if fertility was not a concern. Following a thorough clinical assessment, all cases underwent a primary surgical procedure by a unique team, including type III radical hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic and paraaortic lymphadenectomy, partial omentectomy and peritoneal washing.

Bcl-2 expression in preinvasive and invasive cervical lesions was evaluated in an aim to elucidate any association between the degree of bcl-2 tissue expression and tumor histopathology, stage, grade, tumor volume, lymph node involvement and mean overall survival time. All specimens were evaluated by an experienced co-pathologist to minimize the inter-observer and intra-observer variabilities. Paraffinized blocks derived from tissue specimens were processed into 4μ slides. Then, they were incubated at 30°C for 60 min and deparaffinized in xylol solution and treated with 96% alcohol solution for 3 min. Preparates were then added with EDTA-enriched buffer solution (Neo Markers, USA) and incubated at 121°C for 20 min. Following cooling, they were washed with buffer solutions for 3 min and incubated at room temperature for 20 min. Having being processed at room temperature with $3\%N_2O_2$ for 10 min and washed with buffer solution for 3 min, monoclonal mouse antibody against bcl-2 (Ab-3, Clone 8C8, Cat# MS-597-R7, 0.7 mm) was added to the specimens. Through a step-wise procedure the specimens were treated with buffer solutions, Biotiny-

lated Goat Anti-Mouse solution (Labvision, USA), and streptavidine peroxidase and incubated. Following serial washings and 1% NH_3 application, the specimens were covered with 22 x 22 mm glass slides. Under light microscope (x400), the percentage of staining areas to total slide areas was calculated. Tumor-stained areas < 5% of total slide surfaces were regarded as negatively stained, 5-50% of staining percentage as poorly stained, and 51-90% stained areas were regarded as positively stained. Statistical analysis was comprised of Fisher's exact test for nonparametric distributions of categorical data. Independent variables such as histopathology, stage, grade, tumor volume, lymph node involvement and bcl-2 expression were analysed in regard to unique dependent variables, and overall survival by the Cox regression model (univariate and multivariate analysis). Data were processed with the SPSS-PC program 10.0 (SPSS Inc, Chicago, IL, USA); $p < 0.05$ was considered significant for all two-tailed tests. Values are espressed as mean ± standard error of mean.

Results

Mean ages of patients with preinvasive and invasive cervical cancers were 47.2 ± 2.9 and 38.3 ± 2.5, respectively. Of all CIN cases, seven cases were diagnosed as CIN I, six cases as CIN II and nine as CIN III. Meanwhile, for invasive cervical cancers there were 21 squamous cell cancers, (13 large cell keratinizing and 8 large cell nonkeratinizing cases) and seven adenocarcinomas. According to stage and grade distribution for invasive lesions, the distribution was as follows: 14 cases were stage I (50%) and 14 cases were stage IIa (50%); in terms of grade, 17 cases were grade I, nine cases were grade II and two cases were grade III, respectively. Thirteen invasive cervical cancer cases (46%) were stained negatively for bcl-2, whereas 15 cases (54%) stained bcl-2 positive. Among CIN lesions, bcl-2 positivity was 68%. Degrees of staining, marked as none, poorly, moderately and strongly stained, were 32.0%, 31.8%, 45.5% and 18.2% for preinvasive lesions and for invasive lesions these figures were 46.0%, 14.2%, 32.2% and 7.2%, respectively.

Bcl-2 expression in preinvasive cervical lesions is shown in Table 1. Due to the small number of cases in each group, CIN I and CIN II, III cases were grouped as low-grade and high-grade cervical lesions. As shown in Table 1, as the degree of neoplastic process reaches the surface epithelium, bcl-2 expression gets higher ($p < 0.05$). In invasive lesions, of all studied clinicopathological variables, only histopathologic tumor subtypes and tumor volume had a statistically relevant effect on overall survival (Table 2). Out of 28 invasive cervical cancer cases detected during the study period, seven patients died due to progression or persistence of disease, of whom five cases with bcl-2 positivity had a mean overall survival of 48.5 months. This figure for cases with bcl-2 negativity (n = 2) was 56.7 months.

Table 1. — *Degree of bcl-2 expression in preinvasive cervical lesions (parentheses are percentages).*

Histopathology	bcl-2 expression Negative	Positive	Total
CIN I (low grade)	3 (43)	4 (55)	7 (100)
CIN II, III (high grade)	4 (27)	11 (73)*	15 (100)
Total	7	15	22

* $p < 0.05$

Table 2. — *Clinicopathologic variables and bcl-2 expression and degree (parentheses are percentages).*

Clinicopathological findings	bcl-2 expression		bcl-2 degree of staining	
Stage	+	–	+	–
stage I	6 (43)	8 (57)	9 (64)	5 (36)
stage IIa	7 (50)	7 (50)	8 (57)	6 (43)
Grade				
GI	8 (47)	9 (53)	9 (53)	8 (47)
GII	5 (45)	6 (55)	8 (73)	3 (27)
Histopathology*				
Squamous	7 (33)	14 (67)	11 (53)	10 (47)
Adenocarcinoma	6 (86)	1 (14)	6 (86)	1 (14)
Lymph node involvement				
LN (–)	8 (42)	11 (58)	11 (58)	8 (42)
LN (+)	5 (55)	4 (45)	6 (67)	3 (33)
Tumor volume*				
< 2 cm	5 (26)	14 (74)	9 (47)	10 (53)
> 2 cm	8 (89)	1 (11)	8 (89)	1 (11)

* $p < 0.05$

Table 3. — *Results of the Cox regression analysis of various clinicopathological findings in relation to overall survival.*

Prognostic Factors	Univariate Analysis p-value	Multivariate Analysis p-value
Histopathology	–	0.0390*
Stage	0.0567◊	0.0451*
Grade	0.3016◊	0.3996◊
Tumor volume	0.1312◊	0.4904◊
Lymph node metastasis	0.4780◊	0.5574◊
bcl-2 expression	0.1664◊	0.1698◊

◊: not significant
*: $p < 0.05$

Using the Cox regression model, although the total number of cases was small, univariate analysis demonstrated that none of the analysed variables (histopathology, stage, grade, tumor volume, lymph node metastasis, bcl-2 expression) was relevant. On multivariate analysis, only tumor histopathology and tumor stage were relevant in terms of overall survival (Table 3).

Discussion

There appear to be various studies in the literature concerning the presence of bcl-2 expression in cervical lesions pointing out a wide range in percentage of bcl-2 expression rate (4-75%) which is summarized in Table 4 [2, 4-17]. In our study, although the number of cases is small, the prevalence rate was found to be 54%. This variety of results could be partially explained by population homogenity under study (number in each category) and methods used to detect bcl-2. Although not totally

Table 4. — *Prevalent studies of bcl-2 expression in preinvasive and invasive cervical lesions.*

	Antibody used	n	bcl-2 positivity (%)
Preinvasive lesions			
Harmsel *et al.* [2]	Dako	31	100
Kurvinen *et al.* [5]	Dako	98	88
Dellas *et al.* [6]	Dako	73	64
Daikomanolise *et al.* [7]	Dako	63	47.6
Nakamura *et al.* [8]	Dako	31	6.5
Saegusa *et al.* [9]	Dako	121	50.4
Ozalp *et al.* [index study]	Dako	22	68
Invasive cancer			
Crawford *et al.* [4]	Dako	44	34
Harmsel *et al.* [2]	Dako	13	75
Ohno *et al.* [10]	Dako	20	15
Kokawa *et al.* [11]	Dako	28	18
McCluggage *et al.* [12]	Dako	33	27
Tjalma *et al.* [13]	Dako	76	63
Tjalma *et al.* [14]	Dako	137	61
Daikomanolis *et al.* [7]	Dako	81	8.4
Nakamura *et al.* [8]	Dako	21	4.7
Saegusa *et al.* [9]	Dako	60	20
Raikumar *et al.* [15]	Dako	28	54
Ozalp *et al.* [index study]	NeoMerkers	28	54

confirmed, a high bcl-2 expression rate in CIN III lesions compared to low grade CIN lesions may result in the conclusion that, in low grade CIN, due to this low bcl-2 expression rate, a very small percentage of CIN I lesions could progress or persist. However, high bcl-2 expression in CIN III lesions allows the pathology to persist or progress to invasive stages [16]. In our study, CIN III lesions had a statistically remarkable bcl-2 tissue expression rate compared to CIN I and II, which might partially explain the above hypothesis.

Saegusa *et al.* [9] found that the bcl-2 expression rate was higher in invasive cervical lesions compared to CIN III. As regards survival analysis, Crawford *et al.* [4] noted that 4-year survival rates in bcl-2 (+) and (–) invasive cervical cancers were 53% abd 84%, respectively. These results are consistent with those of Tjalma *et al.* [13] who found 34% and 71% 5-year survival rates. In another study, bcl-2 expression rate for in situ cervical lesions was reported to be 61% and for invasive lesions (stage I-IV) 82% [14]. The same study concluded that, on multivariate analysis, only bcl-2 expression, tumor stage and lymphovascular space involvement had an impact on overall survival, with 5-year survival rates being 74% for bcl-2 positive tumors and 18% for bcl-2 negative tumors. The authors also pointed out the fact that the cases that had responded to chemoradiotherapy were the cases with high bcl-2 expression. Contradictory to these results, there are also studies pointing out that bcl-2 expression had no effect on overall survival, including our series [16].

Although there appears to be conflicting data [2, 11, 13, 14, 17, 18] about the correlation between tumor histopathology and bcl-2 expression, in our series no relevant association was depicted. Adenocarcinoma cases in our study did show a bcl-2 expression prevalence of 14% compared to the squamous counterpart (67%), a finding that supports the poor prognostic tendancy for the former type of tumor group. Due to the small number of cases in the adenocarcinoma group, the life table analysis could not have been constructed with regard to low bcl-2 expression rate and overall survival in comparison to invasive squamous cervical carcinoma cases. In this study, only tumor histopathology and stage were important factors in predicting overall survival. Although there was a trend for longer survival in cases with bcl-2 expressing tumors, due to the small number of enrolled cases in this study, no obvious statistically important difference was detected between the two groups with or without bcl-2 expression regarding overall survival.

Finally, it may be concluded that any deviation in the delicate balance between bcl-2 and other related proto-oncogenes and apoptotic signals facilitate the cell to grow incessantly.

Detection of these recently discovered markers in tumoral tissues will allow the physician to choose more efficient therapeutic approaches and enable the gynecological oncologist to better understand the underlying pathophysiological process.

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