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# Bcl – 2 Expression in Carcinoma of the Uterine Cervix and its Relationship with Prognostic Variables

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**Abstract: Objective:** The aim of this study was to evaluate the importance of the apoptotic activity of squamous cell carcinoma of the cervix and Bcl-2 expression.

**Materials and Methods:** Twenty-seven women with cervical squamous cell carcinoma, admitted to our unit between 1996 and 1998, were reviewed. Patients were classified according to staging and grading systems. Bcl-2 protein material was investigated by immunohistochemistry (streptoavidin, biotine, alkaline phosphatase technique).

**Results:** After immunohistochemical staining, 12 of the 27 cases stained positively (44%). There was a correlation between the grade and staining percentage. We found no significant relationship with stage and staining intensity.

**Conclusions:** Bcl-2 could be used as a marker for blocking well-programmed cell death in low grade cases.

**Key Words:** Cervical carcinoma, genital tract tumors, Bcl-2

## Introduction

Cervical carcinomas are significant female genital tumors. Squamous cell carcinomas are the most frequent cervical cancers, while adenocarcinoma and other types are less frequent (1).

There are keratinised and non-keratinised large-cell and non-keratinised small-cell squamous cell carcinomas. Stage, grade, invasion depth and differentiation degree are factors that affect the prognosis (1,2).

Early sexual activity, race, changing partners frequently, smoking and HPV 16 and 18 are also significant (1,2).

Bcl-2 is a protooncogene situated in the inner mitochondrial membrane. It is a 24 kilodalton protein with 239 amino acids which protects the cells from apoptosis and is localised on the long arm of the 18th chromosome (3).

It is found in the endoplasmic reticulum and some parts of the nuclear membrane (4).

Bcl-2 oncoprotein inhibits programmed cell death. Some data supports the theory that they control the cell growth by the redox system (5,6).

We studied the prognostic importance of Bcl-2 protein as a protooncogene in cervical carcinomas.

## Materials And Methods

Our study group was composed of 27 patients diagnosed with squamous cell carcinomas in 1996-1998 in Zekai Tahir Burak Women's Hospital. Three patients with other types of cervical carcinomas were not considered (none of the cases in our study had radiotherapy). Extrafascial hysterectomy was adequate treatment of stage-one patients, when preservation of fertility was not desired. In stage II and more advanced disease, radical surgery (class III hysterectomy) was the choice of treatment. The reasons for the selection of radical surgery over radiation include bowel problems, fibrosis and ovarian function. All patients in stage II and III were premenopausal.

Materials were fixed in 10% formalin solution and embedded in paraffin blocks after routine tissue follow-up processes. Haematoxylen stain was applied to sections which were prepared from these blocks and evaluated again.

We considered the cases according to FIGO staging and grading systems. Sections 4 mm in thickness were made from the same blocks. The presence of Bcl-2 protein (antibcl-2 124 DAKO) antimouse antibody was investigated by using streptoavidin, biotine, alkaline phosphatase technique and immunohistochemical

methods. Bcl-2 antibody was expressed in cyctoplasma. Fast red was used as the chromogen. Staining intensity was evaluated according to the arbitrary grading system as follows: (+), weak; (++), moderate; (+++), strong positivity.

The Fisher Exact test and the Chi-Square test were used for statistical analysis and $P< 0.05$ was considered significant.

## Results

The youngest patient was 18 and the oldest was 63. The median age was 40.8, 12 (44%) of the 27 cases (diagnosed as cervical squamous cell cancer) were stage I, 7 (26%) were stage II, and 8 (30%) were stage III; there were no stage IV cases.

Nine cases (33%) were grade I (well differentiated), 11 cases (41%) were grade II (moderately differentiated) and 7 cases (26%) were grade III (poorly differentiated). After immunohistochemical staining, 12 of the 27 cases stained positively (44%).

Six of these 12 cases were grade I, 4 were grade II and 2 were grade III. There is a positive correlation between the grade and staining percentage. When we consider the staining intensity, 4 cases were (+++), 3 were (++) and 5 were (+).

Three of the 4 (+++) cases were grade I and 1 of the (+++) cases were grade III. All 3 of the (++) cases were grade II.

Three of the 5 (+) cases were grade I, and 2 of them were grade III.

We found no statistically significant relationship between the staining intensity and the grade.

Five of the 12 stage I cases, 3 of the 7 stage II cases and 4 of the 8 stage III cases were positively stained with Bcl-2.

We found no statistically significant relationship between the staining intensity and the stage (Chi-square test). The distribution of the cases according to their grade, stage, Bcl-2 positivity and staining intensity are shown in the Table.

We found no relationship between the stage and the Bcl-2 staining intensity.

Five cases had lymph node metastasis (grade I: 1 case, grade II: 1 case, grade III: 3 cases).

Ten cases had vascular invasion and 8 cases had necrosis.

None of the cases with lymph node metastasis stained positively with Bcl-2 (grade II: 1 case, grade III: 7 cases).

Two cases with vascular invasion (grade III) and 3 cases with necrosis (grade III) stained positively with Bcl-2 (Fischer Exact test).

## Discussion

Squamous cell carcinomas are the most common cervical carcinomas. They are studied in 3 different

Table: The distribution of the cases according to their grade stage Bcl-2 positivity and staining intensity.

| | | | | | Staining intensity Bcl-2 | | |
|---|---|---|---|---|---|---|---|
| | n | % | Bcl-2+ | % | + | ++ | +++ |
| Grade | | | | | | | |
| I | 9 | 33 | 6 | 50 | 3 | - | 3 |
| II | 11 | 41 | 4 | 33 | - | 3 | - |
| III | 7 | 26 | 2 | 17 | 2 | - | 1 |
| Total | 27 | 100 | 12 | 100 | 5 | 3 | 4 |
| Stage | | | | | | | |
| I | 12 | 44 | 5 | 42 | | | |
| II | 7 | 26 | 3 | 25 | | | |
| III | 8 | 30 | 4 | 33 | | | |
| Total | 27 | 100 | 12 | 100 | | | |

categories (large cell keratinised, non-keratinised and small cell non keratinised ).

The best prognosis is seen in the large-cell keratinised type, while the small-cell non-keratinised type has the worst prognosis (1).

Early sexual activity and multiple sexual partners increase the cancer incidence (2).

Low socio-economic level, smoking and HPV 16 and 18 are important etiologic factors.

Tumor markers (oncogene, protooncogene, and suppressor genes) are also studied, together with lymph node invasion, stage, vascular invasion, grade, metastasis and age, which are factors that affect the prognosis.

Bcl-2 is an inhibitor gene for apoptosis (4,5). Bcl-2 was recognised for the first time in Bcl lymphoma and non-Hodgkin's follicular lymphoma (with +(14-15) (q32:q31) translocation) in 1984 (6,7).

It is the most common chromosomal translocation in human lymphoid tissue tumors (8).

The Bcl-2 gene family includes a variety of genes: a-Cell death suppressor Bcl-2; b-Cell death promotors Bax, Bcl-xs, Bak, Bad (5).

Bcl-2 expression is higher in foetal tissues than in adults. It is thought that it may be related to morphogenesis (7,8).

There are 3 main groups of Bcl-2 in adult tissues (7):

1- In ductus cells of all exocrine glands (pancreas, etc.)

2- In proliferating cells and stem cells (basal keratinocytes, etc.)

3- In epithelial cells that react to hormonal stimulus (breast endometrium, prostate tissues) (7).

This protooncogene, which is expressed in the normal cell, is also shown frequently in lymphoma, endometrial carcinoma and prostate carcinoma studies (7,8).

Prostate adenocarcinomas, which have Bcl-2 expression, react better to treatment than non-Hodgkin's lymphomas which do not have Bcl-2 expression, which in turn react better to treatment than non-Hodgkin's lymphomas which have Bcl-2 expression (4).

Synovial sarcoma has 100% Bcl-2 expression while leiomyosarcoma has no Bcl-2 expression immunohistochemically in tissues (9).

Rajkumar et al. studied Bcl-2 and P53 expression as immunohistochemical techniques in 40 cases. Bcl-2 was expressed in 65% of cervix squamous cell carcinomas while P53 was stained positively in only 10% (10). Our Bcl-2 positivity was 44%.

Tjalma et al. studied Bcl-2 oncoprotein in in-situ carcinomas of the cervix and uterine cervical carcinomas. They supported the idea that Bcl-2 was a strong prognostic marker in in-situ carcinomas and invasive carcinomas (11,12).

Kokowa et al. studied Bax protein with the in-situ hybridisation technique in cervical squamous cell carcinomas and adenocarcinomas and they found a high expression for Bax protein and a low expression for Bcl-2 (13).

Havima et al. using similar markers (Bax, Bcl-2), indicated that Bax and Bcl-2 may be good prognostic markers in post-radiotherapy cervical carcinomas (14).

Pillai et al. studied Bcl-2 and P53 positively immunohistochemically in post-radiotherapy cervical carcinomas. They found that reduced Bcl-2 expressions correlated positively with DNA damage while there was no correlation with P53 (15). In our study, we found significantly reduced Bcl-2 rates related with increased grade. This may help to explain our result.

Crafford et al. showed that Bcl-2, Mcl-11, Bax and P53 were not good markers in cervical squamous cell carcinomas (16).

Waggoner et al. studied Bcl-2 and P53 expression in clear-cell adenocarcinomas of the cervix. P53 expression increases with the degree of malignancy while Bcl-2 expression decreases as in the recent study (17).

McCluggue et al. studied P53 and Bcl-2 protein in non-neoplastic and neoplastic cases. They found P53 expression less frequently in non-neoplastic tissues while Bcl-2 expression was higher in non-neoplastic cases (18).

Cooper et al. attempted to show Bcl-2 protein expression in HPV positive cervical intraepithelial neoplasias, but they could find no relationship between HPV positivity and Bcl-2 low-grade and high-grade CINs (19,20).

We found significantly reduced Bcl-2 rates related with increased grade in our study (general staining 44%). In the literature there are some studies supporting our results related with this correlation. However, we found no statistically significant relationship between stage and Bcl-2 expression. We observed the highest staining percentage in stage 1 cases, which were more numerous

(relatively higher positively). There are numerous studies indicating that Bcl-2 is a good prognostic parameter after radiation therapy, and some of them support the idea that it is a strong parameter without any relationship with radiotherapy. And of course there are reports stating just the opposite.

In the literature we found no reports on that subject that included age or lymph node invasion.

We found no significant relationship between the staining intensity, stage and grade, or between lymph node invasion, vascular invasion, necrosis, age and Bcl-2, which are the other prognostic parameters.

Some of these features correlated to some reports in the literature. We found no significant relationship between Bcl-2 and the other prognostic parameters, but Bcl-2 could be used as a marker for blocking well-programmed cell death in low-grade cases. We consider that more cases could be studied, the age range could be narrowed and an equal number of cases could be chosen to evaluate the relationship more accurately and specifically.

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## References

1. Blaustein A. Blaustein pathology of the female genital tract. Fourth edition Springer Verlag. New York 439-486, 1999.
2. Comoel et al. Bcl-2 expression in normal endometrium during the menstrual cycle. Am J Pathol 144(6): 1195-1202, 1994.
3. Hockenbery DM, Nunez G, Milliman C et al. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature; 348: 334-346, 1990.
4. Hockenbery DM, Zutter M, Hickey W et al. Bcl-2 protein is topograpically restricted in tissues characterized by apoptotic cell death. Proc. Nat Acad. Sci. USA; 88: 6961-6965, 1991.
5. Pezzela F, Tse AGD, Cordell JL et al. Expression of Bcl-2 oncogene protein is not specific for the 14,18 choromosomal translocation Am J Pathol; 137(2): 225-232, 1990.
6. Monaghan et al. Ultrastructural localization of Bcl-2 protein J Histochem: Cytochem. 10(12): 1819-1825, 1992.
7. Le Brun DP, Warnke RA, Clearly ML. Expression of Bcl-2 in fetal tissues suggests a role in morphogenesis Am. J. Pathol; 169: 431-437, 1993.
8. Lu Long Ql, Poulsorn R, Wang L, Hanby AM. Bcl-Expression in adult and embryonic non haemotopoietic tissues J Pathol; 169: 431-437, 1993.
9. Zeng W, Cao P, Zheng M et al. P53 over-expression and Bcl-2 persistence in endometrial carcinoma: Comparison of papillary, serous and endometrioid subtypes. Gynecol Oncol; 61: 167-174, 1996.
10. Rajkumar T, Rajan S, Baruch RK et al. Prognostic significance of Bcl-2 and P53 protein expression in stage IIB and IIIB squamous cell carcinoma of the cervix Eur J Gynaecol Oncol. 19(6): 556-60, 1998.
11. Tjalma W, De Cuyper E, Weyler J et al. Expression of Bcl-2 in invasive and in situ carcinoma of the uterine cervix; Am J Obstet Gynecol; 178 (1 pt 1): 133-7, 1998.
12. Tjalma W, Van Marck E, De Rooter C et al. Prognostic value of Bcl-2 expression in patients with operable carcinoma of the uterine cervix J Clin Pathol; 50(1): 33-6, 1997.
13. Kokawa S, Shikone T, Otani T, Naknof. Apoptosis and the expression of Bax and Bcl-2 in squamous cell carcinoma and adenocarcinoma of the uterine cervix. Cancer, 85(8): 1799-809, 1999.
14. Harima Y, Nagata K, Harima K et al. Bax and bcl-expressions predict response to radiotherapy in human cervical cancer. J Cancer Res Clin Oncol. 124 (9): 503-510, 1998.
15. Pillai MR, Jayaprakash PG, Nair MK. Bcl-2 immuno reactivity but not p53 accumulation associated with tumor response to radiotherapy in cervical carcinoma J Cancer Res Clin Oncol; 125(1): 55-60, 1999.
16. Crawford RA et al. Prognostic significance of the Bcl-2 apoptotic family of proteins in primary and recurrent cervical cancer. Br J Cancer. 78(2): 210-214, 1998.
17. Waggoner SF, Baunoch DA, Anderson SA et al. Bcl-2 protein expression associated with resistance to apoptosis in clear cell adenocarcinomas of the vagina and cervix expressing wild type p53. Am Surg Oncol, 5(6): 544-547, 1998.
18. McCluggage GI, McBride H, Maxwell P, Bhaurcha N. Immunohistochemical detection of P53 and Bcl-2 proteins in neoplastic and non-neoplastic endocervical glandular lesions. Int J Gynecol Pathol, 16(1): 22-7, 1997.
19. Dellas A et al. Investigation of the Bcl-2 and C myc expression in relationship to the KI-367 gene grown in cervical intraepithelial neoplasia. Int J Gynecol Pathol. 16 (3): 212-218, 1997.
20. Cooper K, Haffajen Z, Taylor L. Bcl-2 immunoreactivity, human papillomavirus DNA, and cervical intraepithelial neoplasia. Mod Pathol. 12(6): 612-617, 1999.