BCL-2 EXPRESSION IN ENDOMETRIAL CARCINOMA IN RELATION TO DIFFERENT HISTOPATHOLOGIC PROGNOSTIC FACTORS

SUMMARY:
The bcl-2 protooncogene inhibits programmed cell death (apoptosis) and over-expression of bcl-2 might play a role in carcinogenesis and malignant progression of endometrial carcinoma. The aim of this study was to determine bcl-2 immunohistochemical expression in patients with endometrial carcinoma and to correlate it with different histological parameters including histological type, tumor grade, depth of myometrial invasion, lymph-node involvement and stage. Fifty-four patients with endometrial carcinoma who were treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy were assessed. The immunohistological staining was performed on formalin-fixed paraffin-embedded tissue, and haematoxylin and eosin-stained sections were used to evaluated the conventional prognostic markers. Bcl-2 expression was positive in 63.41% of endometrioid carcinomas and in 53.84% of non-endometrioid carcinoma (p = 0.5374 NS). Statistically significant correlation was found between the surgical staging, depth of myometrial invasion, lymph node involvement, tumor grade and the bcl-2 expression (p < 0.001, p = 0.0019, p = 0.0138 and p = 0.0136 respectively).

Key-words: bcl-2, endometrial carcinoma, prognosis

REZUMAT:
Protooncogena bcl-2 inhibă moartea celulară programată (apoptoză), supraexpresia bcl-2 putând juca un rol în carcinogeneza și evoluția carcinomului endometrial. Scopul acestui studiu este de a determina expresia imunohistochimică a anticorpului bcl-2 la paciente cu carcinom endometrial și corelarea acesteia cu diferiți parametri histologici incluzând tipul histologic, gradul de diferențiere, adâncimea invaziei miometriale, statusul limfonodulilor și stadiul bolii. Au fost luate în studiu cincizeci și patru de paciente cu carcinom endometrial tratați prin histerectomie totală cu anexectomie bilaterală. Imunocolorarea s-a realizat pe țesuturi fixate în formaldehidă, incluse la parafină, iar markerii convenționali de prognostic au fost evaluați pe secțiuni colorate cu hematoxilină–eosină. Expresia bcl-2 a fost pozitivă în 63.41% din carcinoamele endometrioide și în 53.84% din carcinoamele non-endometrioide (p = 0.5374 NS). Între expresia bcl-2 și stadiul bolii, adâncimea invaziei miometriale, statusul limfonodulilor și gradul de diferențiere al tumori s-a obținut o corelație semnificativă statistică (p < 0.001, respectiv p = 0.0019, p = 0.0138 și p = 0.0136).

Cuvinte-cheie: bcl-2, carcinom endometrial, prognostic

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1. Obstetrics and Gynecology Department, Clinical Hospital, Arad
2. Service of Pathology, Emergency Clinical Hospital, Timisoara
3. Department of Pathology
4. Department of Medical Informatics and Biostatistics, „Victor Babes” University of Medicine and Pharmacy, Timisoara
5. Department of Pathology, „Vasile Goldis” Western University, Arad

Correspondence to: G Gușet, MD, Obstetrics and Gynecology Department, Clinical Hospital, Arad, 3 Cornel Radu Street, 310329 Arad, Romania; Phone +40744607047, E-mail gusetgrig@yahoo.com
INTRODUCTION

The bcl-2 gene encodes a protein of 25-26 kDa (Bcl-2), which is located in the mitochondrial membrane, endoplasmic reticulum and nuclear membrane.\(^{(1)}\)

In the endometrium, bcl-2 expression assessed by immunohistochemistry varies during the menstrual cycle and is highly expressed in the proliferative phase, with downregulation during the secretory phase.\(^{(2)}\) Many studies support the concept that bcl-2 protein expression persists at high levels in simple hyperplasia but progressively diminishes in atypical hyperplasia and with decreasing differentiation in invasive endometrial adenocarcinoma.\(^{(3,4,5,6,7,8,9)}\)

The aim of this study was to determine bcl-2 immunohistochemical expression in patients who have been surgically treated for endometrial carcinoma, and to correlate it with conventional histological markers. Conventional histological and prognostic markers used to determine the clinical course of endometrial carcinoma include histological type, tumor grade, depth of myometrial invasion, lymph-node involvement and stage.

MATERIALS AND METHODS

In order to investigate bcl-2 expression in endometrial neoplasia, a total of fifty-four endometrial carcinoma cases surgically resected at Obstetrics and Gynecology Department of Clinical Hospital Arad were examined. The mean age of patients was 57 years with a minimum of 38 and maximum of 81 years. All patients had been treated surgically with total abdominal hysterectomy and bilateral salpingo-oophorectomy. Of these, 25 patients were stage I, and 29 patients were stage II and III. N staging was based on pre-operative lymphangiography or pelvic and abdominal tomography (CT) scan. The cases investigated comprised 24 grade 1 (G1), 21 grade 2 (G2), and 9 grade 3 (G3) endometrial carcinomas (Table I). Histologic typing of the endometrial tumours, tumor grade and depth of myometrial invasion (< \(\frac{1}{2}\) vs \(>\frac{1}{2}\)) were evaluated based on the criteria of the World Health Organization.

Tissue samples were fixed in 10% formalin and embedded in paraffin wax for routine histopathology.

Immunohistochemical Analysis: The deparaffinized tissue sections were submitted to routine immunohistochemical analysis using as positive controls for bcl-2 expression lymph nodes. The antibody used was mouse monoclonal anti-human bcl-2 antibody (clone 124, Dako) through LSAB 2 detection system. A variable intensity of bcl-2 immunoreactivity was observed with cytoplasmic brown staining; the visualization system used diaminobenzidine tetrahydrochloride (DAB, Dako).

Statistical Analysis: The correlation between bcl-2 expression and histopathologic markers were analyzed using Chi-square test. A p value less than 0.05 was considered significant.

RESULTS

On the basis of the percentages of bcl-2 immunopositive cells in the endometrial carcinomas, the lesion were included into four categories as follows:

- 0 = negative: no or fewer than 5% positive cells;
- 1+ = positive immunoreaction in 5-20% of tumor cells;
- 2+ = positive immunoreaction in 30-50% of tumor cells;
- 3+ = positive immunoreaction in more than 50% of tumor cells;

A variable intensity of a bcl-2 immunoreactivity (weak, moderate or strong) and a heterogenous distribution of cytoplasmic staining were observed.

The correlations of bcl-2 expression with histological parameters are listed in Table II. In our study, we detected cytoplasmic bcl-2 expression in 26 endometrioid carcinomas (63.41%) and 7 non-endometrioid carcinomas (53.84%); differences were not statistically significant (\(p = 0.537467\) NS). Statistically significant correlation was found between the tumor differentiation grade and bcl-2 expression: 21 (87.50%) of G1 endometrial carcinoma were bcl-2 positive, while bcl-2 expression was positive in only 12 (57.14 %) of G2 and G3 cases (\(p = 0.013676\) S).

Statistically significant correlation was found between depth of myometrial invasion and bcl-2 expression (\(p = 0.001916\)) and between surgical staging and bcl-2 expression (\(p < 0.001\) ES). By comparing bcl-2 expression and the status of lymph nodes we obtained positive bcl-2 reactions in 67.34% of cases without metastasis while the lymph nodes with metastasis were not positive (\(p = 0.013852\) S).

DISCUSSION

Several studies are focused on the genetic and molecular changes that are observed in endometrial carcinomas.

The fact that bcl-2 expression was correlated with favorable clinicopathological markers in our study is in line with other studies (Saegusa et al, Saegusa et Okayasu, Erlich et al, Creasman et al, Sivridis et al) that demonstrated a close association between bcl-2 and
progesterone receptor immunoreactivity, since it is widely accepted that the later is also linked to a favorable outcome (10,11,12,13,14). In many studies (Geisler et al, Taskin et al, Saegusa et al, Coppola et al, Yamauchi et al)) bcl-2 positivity has been directly correlated with a favorable clinical outcome and good histopathologic pro...
Several other studies do not reveal any correlation between bcl-2 expression and known prognostic factors. Giatromanolaki et al (20) performed a study on 133 patients with endometrial carcinoma and did not find any relationship between bcl-2 and tumor grade, depth of myometrial invasion and lymphovascular invasion. Sakuragi et al (8) did not demonstrate any significant differences in bcl-2 expression when lymph node involvement and tumor grade were analyzed, but this expression was linked inversely proportional to myometrial invasion ($p=0.025$). Kounelis et al (21) performed a study on 61 patients and did not obtained any significant difference between bcl-2 expression and histopathological type, tumor grade and staging. Appel et al (22) studied 48 Brazilian patients with a histological diagnosis of endometrial carcinoma and did not demonstrate any correlation between bcl-2 expression and age, histological type, tumor grade, depth of myometrial invasion and survival.

**CONCLUSIONS**

In conclusion, the present study provided strong evidence that, in endometrial carcinoma, bcl-2 expression is associated with favorable prognostic factors and may play important roles in tumor progression from early to advanced stages.

References: