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# Expression of topoisomerase II, BCL-2, and Ki-67 in ovarian cancer tissue and their effects on the development and prognosis of ovarian cancers

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**Summary**

*Purpose:* The objective of this study was to evaluate the role of the expression of topoisomerase II, BCL-2, and Ki-67 in an ovarian tissue and their effects on the development and the prognosis of ovarian cancers. *Materials and Methods:* The study consisted of 38 female patients with ovarian cancers. Patients' characteristics, tumor type, stage, and the grade of the disease, and topoisomerase II, Bcl-2, and Ki-67 values in ovarian tissue were compared. *Results:* Extensivenesses of topoisomerase II and Ki-67 staining in early-stage disease were found statistically different from those in the advanced stage diseases (for topoisomerase II, 60.38 ± 33.87% *vs* 85 ± 8.5%, $p = 0.017$, for Ki-67; 25.07 ± 25.04% *vs* 51±25.29%, $p = 0.012$). Differences of Bcl-2 staining between two groups were not significant (30.38 ± 38.82 % *vs* 18.2 ± 30.58%, $p = 0.377$). *Conclusions:* Expressions of topoisomerase II and Ki-67 in ovarian cancer tissues were associated with advanced stage diseases and expression of Bcl-2 refers to good histological grade in patients.

*Key words:* Ovarian cancer; Topoisomerase II; Bcl-2; Ki-67; Cancer tissue.

## Introduction

Ovarian cancer is the fourth most common cause of cancer deaths in women after lung, breast, and colorectal cancer, and life time risk of developing this disease is approximately 1.7% [1]. Incidence of ovarian cancer among women is 4%, as it accounts for 23% of gynecological malignancies [2]. Ovarian cancer is responsible for 47% of deaths related to genital tract cancers and it is the leading cause of death from gynecological malignancies in the world [3]. Majority of cases (70%) present with advanced disease at the time of diagnosis. Since the disease is completely asymptomatic during the early stage, and the pathogenesis has not been completely clarified; the overall five-year survival rate for ovarian cancer does not exceed 37% [4]. The most important prognostic factors for ovarian cancer patients are histological types and the stage of the disease at the time of diagnosis. B-cell lymphoma 2 (Bcl-2) is a 26 kDa weighted regulator protein described as proto-oncogene in chromosomal translocation involving chromosomes 18. It is located in the mitochondria and the cell membrane. The main function of this proto-oncogene is to inhibit the apoptosis and arrest the cell cycle in G0/G1 phase [5]. In addition, it is believed that Bcl-2 has an effect on the early phase of cell proliferation. It is well known that expression of Bcl-2 oncoprotein in malignant tumors such as lung, colon, breast cancer or neuroblastoma causes different clinical manifestations [6]. Bcl-2 expression in premalignant lesions is much higher than that in malignant lesions [7]. In addition, Bcl-2 expression can be seen in the long-lived B and T lymphocytes, various glandular and complex epithelial cells, and in non-regenerative cells such as neurons.

Topoisomerase II, a nuclear enzyme, plays a pivotal role in regulating DNA replication by introducing transient double-strand breaks in the super-stranded DNA. This ATP dependent process is catalyzed by topoisomerase II which is the only one protein providing the segregation of replicated molecules; moreover this 170-180 kD protein participates in many of the nuclear processes that generate topological problems. Topoisomerase II is expressed during S, G1, G2, and M phases of the cell cycle [8]. The activity of this enzyme differs in malignant lesions compared to benign or borderline tumors. Topoisomerase II expression is detected in formalin fixed, paraffin wax embedded tissues by using monoclonal antibody and immunohistochemical staining.

The Ki-67 protein with the apparent molecular weights of 345 and 395 kDa is a nuclear, non-histone protein involved in cell proliferation. The Ki-67 antigen is expressed during all active phases of the cell cycle (G1, S, G2, and M-phases). A number of studies have shown that the expression of this protein is significantly related to prognosis of malignant tumors [8-16].

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In this study, the authors examined the relationship between the level of topoisomerase II, Bcl-2, and Ki-67 expression in paraffin blocks of cancerous ovarian tissue samples and histological type and stage of ovarian cancer. The objective of this study was to investigate the influence of aforementioned proteins, which are known to be related to malignant transformation, on the pathogenesis and prognosis of ovarian cancer.

## Materials and Methods

The study consisted of 38 patients who underwent surgical intervention due to adnexal masses, and the diagnosis of ovarian cancer was made based on the pathological investigation. Informed consent was obtained from all individual participants included in the study. All procedures performed in the study were in accordance with the ethical standards of the institutional research committee of Yildirim Beyazit University and with the 1964 Helsinki declaration. Patient demographics (e.g. age, menopausal status, parity) and clinicopathologic characteristics, such as preoperative tumor markers (CA–125, CA 19.9, and CA 15.3), histological type and the stage of ovarian cancer, ultrasound imaging results, and postoperative pathology results were recorded for further analyses.

Formalin fixed, paraffin embedded tissue blocks were cut into three- to four-micron thick sections and mounted on poly-L-lysine coated slides. Sections were deparaffinized for 12 hours at 37°C and for 35 minutes at 65°C. After being deparaffinized with xylene, they were rehydrated with graded alcohol solutions. Standard immunohistochemical techniques were applied for Ki–67 (clone: 7B11, mouse anti-ki–67), Bcl–2 (mouse anti- Bcl–2–100), and topoisomerase II beta (anti Top-2B pAb). Sections were immersed in antigen retrieval solution (ten mM. citrate buffer, pH 6.0, and 0.1% NP-40) and heated in a autoclave at 120°C for ten minutes. Endogenous peroxidase was quenched in 3% hydrogen peroxide phosphate oidel phosphate buffered saline for ten minutes. Slides were then washed with phosphate buffered saline (PBS), 0.01 mol/L, pH 7.4 and water. Tissue sections for Bcl-2 (1/50 dilution), topoisomerase IIb (1/250 dilution), and Ki-67 (ready to use) staining were then incubated with the primary antibody for one hour at room temperature using optimized protocols. Antibody dilutions in phosphate buffered saline were those recommended by the manufacturer. After washing with PBS, tissue sections were incubated with a biotinylated secondary antibody for 20 minutes and with streptavidin-peroxidase for 20 minutes, and followed by washing with PBS. Immunohistochemistry was performed with liquid diaminobenzidine (DAB) plus substrate-chromogen system for ten minutes according to the procedures provided by the manufacturer. Slides were developed in substrate chromogen and counterstained with hematoxylin, dehydrated, and mounted. Cytoplasmic staining with Bcl-2 and nuclear staining with Ki-67 of lymphocytes in the lymph node served as an control of Bcl-2 and Ki-67. Cytoplasmic staining with topoisomerase II beta of glandular cells of the breast tissue was accepted as positive control. Both staining intensity and extensiveness were evaluated for Bcl-2, Ki-67, and topoisomerase II. The staining intensity was graded as negative (0), weak (1+), moderate (2+), and strong (3+). The staining extensiveness was the percentage of tumor cells positively stained with a range from 0% to 100%. Staining intensity (0-3+) and extensiveness (0%-100%) were equalized as the following: 0-10 % staining: negative staining, 11-30% staining: 1+ staining, 31-60% staining: 2+ staining, and > 61% staining: 3+ staining.

Data were analyzed using the SPSS 16.0 statistical software. The patients divided into two groups as early-stage ovarian cancer group (Stages 1 and 2), and advanced-stage group (Stages 3 and 4). Differences of extensiveness of topoisomerase II and Bcl-2 staining between groups were analyzed by using Mann Whitney-U test. Differences of Ki-67 staining were analyzed by student *t*-test. The correlation between tumor grade and the extensiveness of topoisomerase II and Bcl-2 staining was analyzed by using Kruskar Wallis test. Mann Whitney-U test was used as Post Hoc analysis. For all analyses, $p < 0.016$ was considered statistically significant. The correlation between tumor grade and the extensiveness of Ki-67 staining was analyzed by using Analysis of Variance (ANOVA) with Post Hoc analysis of Tukey test. Differences of menopausal status of patients between early-stage and advanced-stage ovarian cancer groups were evaluated by using chi-square test. Correlations between variables were analyzed by using Spearman correlation coefficient test.

## Results

The study consisted of 38 ovarian cancer patients aged 31- 90 (56 ± 13.43) years with parity of 3.79 ± 2.99 (range from 0 to 13). Twelve patients (31.6%) were diagnosed with ovarian cancer in the premenopausal period and 26 patients (68.4%) were in the postmenopausal period. Out of 38, 36 women (94.8%) were diagnosed with epithelial ovarian cancer, one patient (2.6%) had germ cell tumor, and another had non-specific mesenchymal cell tumor. Based on the histological type, 14 patients (36.8%) had serous carcinoma, six patients (15.8%) had mixed epithelial carcinoma containing different range of endometrioid and serous components, four patients (10.5%) had undifferentiated carcinoma, three patients (7.9%) had transitional cell carcinoma, one patient (2.6 %) had carcinosarcoma, one patient (2.6%) had fibrosarcoma, and one patient (2.6%) had teratoma with malignant transformation.

The mean CA-125 level was 923.21 ± 1520.57 kU/L (range 10.6–5230), the mean CA 19.9 was 42 ± 144.2 kU/L (range 0.8–895), and mean CA 15.3 levels was 54.19 ± 73.96 (range 0.8–333). The mean CA-125 levels was not statistically significant between early-stage ovarian cancer group and advanced-stage ovarian cancer group (662.7 ± 1327.6 kU/L (10.6–4986) *vs* 1058.68 ± 1620.93 kU/L (17.7–5230, $p = 0329$).

The patients in the study were followed up for 12–55 months and 11 patients died during this period. The period between the time of diagnosis and the death was observed as 14.90 ± 11.12 (1–36) months. While 71.1% of patients received chemotherapy treatment after the surgery and 11 patients had not due to the inconvenient clinical status.

In the study, 15.8 % of the patients were in Stage 1, 17.9% were in Stage 2, 55.3 % were in Stage 3, and 11% of patients were in Stage 4 of ovarian cancer. While 66.6% of premenopausal patients were diagnosed as having early-stage disease, only 19.2% of postmenopausal women in the study had early-stage ovarian cancer. There was a significant difference of the stage of the disease between premenopausal and postmenopausal women in the study ($p = 0.004$). At the time of diagnosis, the stage of the disease in premenopausal

Table 1. — *The extensiveness of staining of variables in early and advanced stage ovarian cancer patients.*

| Extensiveness of staining | Early stage (n=13) | Advanced stage (n=25) | *p* |
|---|---|---|---|
| Topoisomerase II (%) | 60.38 ± 33.88 | 85.52± 8.49 | 0.017 |
| Bcl-2 (%) | 30.38 ±38.32 | 18.20± 30.58 | 0.377 |
| Ki-67 (%) | 28.08±25.04 | 18.20± 30.58 | 0.012 |

Table 2. — *The staining properties based on nuclear grade.*

| | Grade I (n=5) | Grade II (n=8) | Grade III (n=25) | *p* |
|---|---|---|---|---|
| Topoisomerase II (%) | 29±32.48 | 83.13±15.1 | 84.52±8.80 | 0.003 |
| Bcl-2 (%) | 67±29.92 | 10.6±21.78 | 17.2±30.76 | 0.003 |
| Ki-67 (%) | 12±7.58 | 38.13±26.18 | 51±25.62 | 0.008 |

women was observed to be earlier than that in the postmenopausal women. When the patients were classified based on their pathological results, 13.2 % of patients had grade 1, 21.1% of patients had grade 2, and 65.8 % of patients had grade 3 diseases.

The mean extensiveness of topoisomerase II staining was 79 ± 23.77 % (0–95) in all cases; 5.3% of the patients had 1+ staining, 36.8% of the patients had 2+ staining, and 55.3% of the patients had 3+ staining, with topoisomerase II. One patient had no staining with this enzyme.

The mean extensiveness of Bcl-2 staining was 22.36 ± 33.42% (0–95) in the study; 63.2% of the patients did not display any staining with Bcl-2 and 13.2% had 1+ staining, 15.8% had 2+ staining, and 7.9% had 3+ staining with Bcl-2. The mean extensiveness of Ki-67 staining was calculated as 43.15 ± 27.19% (5–90%) in the study.

In both early and advanced stage ovarian cancer groups, the intensity and the extensiveness of the staining of topoisomerase II and Bcl-2 were evaluated. For the Ki-67 staining, only the extensiveness was evaluated in the groups. The extensiveness of the staining of topoisomerase II and Ki-67 in early stage ovarian cancer group was statistically different from those in the advanced stage ovarian cancer group (for topoisomerase II, 60.38 ± 33.87 % (0–95%) *vs* 85 ± 8.5% (60–95%) $p = 0.017$, for Ki-67; 25.07 ± 25.04% (5–70%) *vs* 51 ± 25.29% (5–90%), $p = 0.012$). Differences of the extensiveness of Bcl-2 staining between early and advanced stage disease groups were not statistically significant in the study (30.38 ± 38.82% (0–95%) *vs* 18.2 ± 30.58% (0–80%), $p = 0377$). Table 1. shows the extensiveness of staining of aforementioned variables in different stages of the disease.

In early stage ovarian cancer group, 7.7% of the patients had negative staining for Topoisomerase II, 15.4% had 1+, 30.8% had 2+, and 46.2% had 3+. These results ranged as 53.8% for negative staining for Bcl-2, 15.4% for 1+, 7.7% for 2+, and 23.3% for 3+ for intensity of Bcl-2 staining. In advanced stage ovarian cancer group, 40% of the patients had 2+ staining intensity for topoisomerase II and the remaining had 3+ intensity. In the same group, 12% had 1+ staining and 20% had 2+ staining in the study. Patients were divided into three groups based on the nuclear gradient of the cancer cells. There were significant differences of topoisomerase II and Bcl-2 staining among three groups ($p = 0.003$ and $p = 0.003$). The significant differences for topoisomerase II and Bcl-2 staining between grades 1 and 2 and grades 1 and 3 were observed in the study. There were no differences in topoisomerase II and Bcl-2 staining between grade 2 and 3 groups. The significant differences of Ki-67 staining were also observed among three groups too ($p = 0.008$). Table 2 shows the extensiveness of staining of variables in the different nuclear grades.

## Discussion

In this study, the expressions of topoisomerase II, Bcl-2 and Ki-67 in ovarian cancer tissues, and their effects on the prognosis and the development of ovarian cancer were investigated. The intensity and the extensiveness of Bcl-2 staining were higher in low grade tumors, however this result could not be related to the stage of the disease.The increases in the intensity and the extensiveness of topoisomerase II and Ki-67 staining were accompanied by the increases in the histological type and the stage of the tumor.

The most prognostic factor in ovarian cancer is the stage of the disease. The survival rates can vary depend on the stage of the disease, the degree of differentiation, findings during the surgery, residual cancer tissue after surgery, and postoperative medical treatment. Although clinical recuperation can be seen in advanced stage ovarian cancer, recurrence and relapse rates are extremely high in the disease. It is a common knowledge that the disease will progress even in patients with complete remission. The most common finding related to recurrence of the disease is the increase in the serum CA-125 levels without any physical or radiological findings [17]. In order to prevent the relapse of the disease in postoperative period, different treatment modalities such as extended usage of chemotherapic agents, hormonal treatment, immunotherapeutics, vaccines, and matrix metalloproteinase inhibitors, *etc.* were suggested [18]. However, no treatment regarding the increase the survival rate has been developed so far [18]. The mechanism of action of chemotherapeutic agents targeting the DNA topoisomerase II enzymes is the stabilization of mediators used in the topoisomerase II reactions. The task of these mediators is primarily to detect the protein-bound DNA and double-stranded DNA breaks.

The study evaluating topoisomerase-targeted drugs in sensitive and resistant human ovarian cancer cells, has found that the alterations in the topoisomerase II results in

the resistance to the chemotherapeutic agents [19]. Bcl-2 retards the cell cycle entry of resting cells and factor-dependent hemapoietic cells in to G1 phases. It can affect the progression of cells through G1 phase of the cell-cycle by using p130 proteins. It delays entry of fibroblasts into the cell cycle induced by Myc. Bcl-2 mediated growth inhibition is affected by deregulated expression of Myc which is common in cancer cells [20]. The study investigating the effect of Bcl-2 on the proliferation and apoptosis of ovarian cancer cells [21] has shown that overexpression of Bcl-2 in ovarian cancer cells is associated with decreased cell proliferation; however ovarian cancer cells with low Bcl-2 expression is found to be more malignant both in vivo and in vitro.This study has also demonstrated that the loss of expression of Bcl-2 in ovarian carcinomas is related to more aggressive phenotype. Although Bcl-2 affects the cell-cycle, it does not alter the therapeutic response to cytotoxic chemotherapy. Bcl-2 independently of its anti-apoptotic function has an inhibitory effect on the cell growth in ovarian cancer cells. Another study has shown that 97% of benign and 30% of malignant epithelial ovarian tumors in the study have Bcl-2 expression in ovarian tissues [21]. In this current study, the authors could not reveal any significant differences of Bcl-2 staining between early and advanced stage diseases, however they did find significant correlation between Bcl-2 expression and histological type of the tumor as low grade (grade1) tumors had an increased Bcl-2 staining compared to grade 2 and 3 tumors and this finding complied with the literature. The study of Gotlieb *et al* [22], investigated the prognostic effects of topoisomerase II and Ki-67 on the ovarian cancer and they found that patients with short survival time (less than three years) had increased topoisomerase II and Ki-67 expressions when comparing to those with long survival duration (more than five years). Since the increase in the topoisomerase II expression was more prominent, they accepted the level of topoisomerase II as prognostic factor of ovarian cancer. In the current study, the percentage of the extensiveness of topoisomerase II and Ki-67 staining in the advanced ovarian cancers were higher than those in the early stage disease. However, no correlation between Bcl-2 and the tumor grade was observed in the study. The study of Brustmann *et al.* [23] evaluated 41 patients with serous ovarian cancer and found that the percentage of topoisomerase II alpha staining was much more higher in the serous carcinomas compared to benign serous tumors [23]. This result was correlated with the increased mitotic activity in serous ovarian cancers [23]. In contrast to topoisomerase II alpha, Bcl-2 expression was found to be low in serous ovarian cancer patients. These results complied with the findings of Chan *et al.* [24]. In their study, borderline or malignant ovarian cancers displayed high apoptotic rates despite the low rate of apoptosis in benign ovarian tumors. The highest apoptotic rate was observed in grade 3 ovarian tumors. However no correlation was observed between Bcl-2 and apoptosis in their study. This finding was supported by the study of Belanger *et al.* [25]. In the present study, only the early and the advanced stage diseases were compared due to exclusion of benign lesions. Although there were no differences in the extensiveness of Bcl-2 staining between early and advanced stage ovarian cancers, significant inverse correlation between histological type of the tumors and the expression of Bcl-2 was observed and these results were consistent with the literature.

In conclusion, Bcl-2 expression is related to better histological grade in ovarian cancer tissues whereas, the high expressions of topoisomerase II and Ki-67 are found to be associated with poor grade or advanced diseases. Due to small study population, differences of aforementioned variables in histological types of tumors were not assessed appropriately. Although the enzyme and proteins subject to this study have possible effects on the development and progression of ovarian cancers, large population-based studies should be conducted in order to achieve accurate results. Thus, the effectiveness of chemotherapeutic agents targeting the DNA topoisomerase II enzymes can be increased and the patients may be referred to appropriate treatment options by predicting the prognosis of the disease.

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