Bcl-2 Expression and Its Correlation with Histopathological Features in Ovarian Surface Epithelial Tumours

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ABSTRACT

BACKGROUND

Bcl-2 gene is an apoptotic protein that blocks apoptosis and thereby its over expression contributes to neoplastic transformation and decreased tumour survival. So, it is necessary to find out the relationship of *Bcl-2* expression with histological types and tumour grade in ovarian surface epithelial tumours, which may predict the prognosis.

METHODS

The objective was to study the expression of *Bcl-2* in ovarian surface epithelial tumours and to correlate *Bcl-2* expression with histopathological features and tumour grade in ovarian surface epithelial tumours. Histological types and tumour differentiation for each case is determined from the routine H and E sections. Immunohistochemical stain for *Bcl-2* was done. Then intensity and extent of staining for *Bcl-2* was compared with the age, histological type and tumour grade.

RESULTS

Out of the 47 cases studied, 66% were in <55 years of age category and 34% in >55 years of age group. There was statistically significant associations of *Bcl-2* expression with various histological types (P<0.001) and tumour differentiation (P<0.001). In the case of extent of *Bcl-2* staining, statistically significant associations were present with various histological types (P=0.004) and tumour differentiation (P<0.001).

CONCLUSIONS

Bcl-2 expression decreases with tumour progression. Poorly differentiated tumours with decreased *Bcl-2* expression may be helpful in predicting disease progression. Further studies are warranted since, *Bcl-2* expression may be important for prognostic outcome or provide useful targets for therapeutic intervention in patients with surface epithelial ovarian cancers.

KEY WORDS

Bcl-2, Immunohistochemistry, Tumour Grade, Epithelial Tumours.

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BACKGROUND

Ovarian cancer is the third most common type among gynaecological cancers as per the recent estimate of incidence and mortality of cancers worldwide.[1] The fact sheet also shows ovarian cancer as the leading cause of death among Indian women. Early diagnosis of ovarian cancer (stage I) shows an increased 5-year survival rate of 94%, while advanced stages (which make up 62% of the diagnosed cases) show dismal prognosis with 5-year survival of 28%.[2] Poor prognosis of ovarian cancer in the later stages may be partly due to the lack of symptoms in the earlier stages as well as lack of an effective screening marker and method. Majority of ovarian cancers arise from ovarian surface epithelial tumours. Ovarian cancer cells are known to avoid immune response and also carry mechanisms to survive and to evade therapeutic treatments. This cancer shows a very good response to first line chemotherapy but also shows increased recurrence and development of resistance to treatment.

Like many other solid tumours, one of the most important characteristics of tumorigenesis in ovaries is the dysfunction of programmed cell death or apoptosis.[3] Alterations in regulation of apoptosis lead to the formation of immortal cell phenotypes and also tumour progression. Several proteins are involved in the regulation of apoptotic pathway in cells including P-53, *Bcl-2*, A1, and Mc11.[4] B-cell lymphoma 2 (*Bcl-2*), an anti-apoptotic protein, is overexpressed in a number of solid tumours including ovarian cancer. These cells are significant regulators of programmed cell death and facilitate neoplastic transformation in cells by inhibiting apoptosis.[5] Additionally, *Bcl-2* expression may confer chemotherapeutic resistance to cancer cells by evading apoptosis.

Bcl-2 being one the most commonly altered functional protein in ovarian tumours and understanding the association between its expression and staining with histological subtypes and other clinicopathological characteristics is imperative. Moreover, studies have shown that response to chemotherapy decreased with increased expression of this protein in ovarian cancer.[6] While another study reports that expression of *Bcl-2* decreases with tumour progression.[7] Combination of and *Bcl-2* with another protein p53 (tumour suppressor protein) is claimed as independent prognostic factor in ovarian tumours, where tumours are *Bcl*2-positive but p53 negative, in a specific study conducted by Geisler.[8]

Conventional treatment of epithelial ovarian cancer includes surgical removal followed by platinum-based chemotherapy. Neoplastic cells evade apoptotic effects of platinum drugs through increased expression of *Bcl-2* proteins that are inhibitors of apoptotic proteins.[9] Thus understanding the expression of these proteins as well as drugs that target *Bcl-2* proteins is of great clinical significance. Studies on this line is very less in an Indian cohort, especially Kerala. Hence the need to study the expression and staining of this protein considering all other technical factors.

The purpose was to study the expression of *Bcl-2* in ovarian surface epithelial tumours and to see its correlation with histopathological features and tumour grades. The objectives of this study are-

1. To study the expression of *Bcl-2* in ovarian surface epithelial tumours.
2. To correlate *Bcl-2* expression with histopathological features and tumour grade in ovarian surface epithelial tumours.

METHODS

This cross-sectional study was conducted for a one and half year period from January 2020 to June 2021 at the Department of Pathology of a tertiary care institution in Kerala. All specimens diagnosed histologically as primary surface epithelial tumours of ovary, received in the department was included in the study. Those specimens insufficient for performing immunohistochemical studies were excluded from the study. Approval for the study was obtained from Institute Ethics Review Board.

Clinicopathological characteristics of the patients were gathered from pathology reports and medical records submitted in the department.

All neoplasm specimens were formalin-fixed and paraffin-embedded. Four micrometre thick sections were cut from each of the tissue blocks and stained with haematoxylin and eosin (H & E) for diagnostic confirmation and histologic grading. For immunohistochemical studies, formalin-fixed, paraffin-embedded tissue blocks were cut at 3 micrometres and dried overnight at room temperature. These specimens were then deparaffinized and rehydrated. Heat-mediated antigen retrieval was done using a phosphate buffer of pH6. Specimens were immersed in hydrogen peroxide to block endogenous peroxidase activity and then washed in phosphate-buffered saline. These sections were treated with protein blocking reagent and incubated for 20 min at 37°C. The treated sections were placed in a refrigerator overnight, at 4°C in a humid chamber. The bound antibodies were detected using streptavidin-biotin complex method, after an immunoreaction.

Immunohistochemical staining was evaluated independently by two pathologists. The pattern of staining was evaluated as cytoplasmic or nuclear. Amount of epithelial staining was assessed as percent staining from each section and scored as having either <50% or >50% positive cells. Staining intensity was also evaluated and classified as negative, weak, moderate or intense. The diagnosis of histological type and grade were performed according to the classification of tumours of ovary.[10] Immunohistochemical expressions of *Bcl*2 were then compared with age, histological types and grades of primary epithelial ovarian tumours.

Statistical Analysis

The collected data was coded and entered into the IBM SPSS statistics, version 20.0 for statistical analysis. Continuous variables are presented as Mean ± Standard deviation, while categorical variables are presented in the form of number and percentage. Associations in the epithelial staining between tumour types and staining intensity between tissue types were tested using Fisher's exact test. Significance was assessed at 5% significance level.

RESULTS

Immunohistochemical analysis was performed on 47 samples collected during the study period. Maximum number of participants were in the age group of 41 – 50 years with a mean age of 50.6 (19, 40.4%) years (50.6 ±12.51; range = 18 – 83 years). A total of 31 participants (66%) belonged to <55 years, while 34% were above 55 years of age. Among the different histological types of ovarian cancer, more than half of the samples belonged to the serous type (n=24) followed by mucinous type (n=16, 34%). Five samples belonged to endometroid category, and two (4.3%) were Brenner type of ovarian cancer.

Majority of the tissue samples under study were well differentiated (n=25, 53.2%). Poorly differentiated cases accounted to 42.6% of the samples, while 2 (4.3%) of the tissue sections were moderately differentiated. All tissues in this study showed some degree of epithelial *Bcl*2 staining. Weak staining was noted in 46.8% (n=22), moderate in 38.3%, and intense in 14.9% of the specimens collected. Extent of epithelial *Bcl-2* staining was >50% in 66%, and <50% in the rest of the sample ovarian tumours.

Comparing the *Bcl*-2 expression in the two age groups (less than and more than 55 years), majority of the tumours (63.3%) with weak expression was found in the <55 years age group (Table 1, Figure 1). More than 80% of the intensely stained tumours also belonged to the <55 years group. There was no statistically significant association between the two age groups with respect to *Bcl-2* expression (P=0.589).

More than half of the tumours (57.1%) with intense staining of *Bcl*-2 belonged to the serous type. Similarly, majority of the weakly stained tumours (72.7%) belonged to the serous type. Thirteen (72.2%) of moderately stained tumours had mucinous type of ovarian tumour. The relation between *Bcl-2* expression and different histological types of primary epithelial ovarian tumours was statistically significant (P=<0.001).

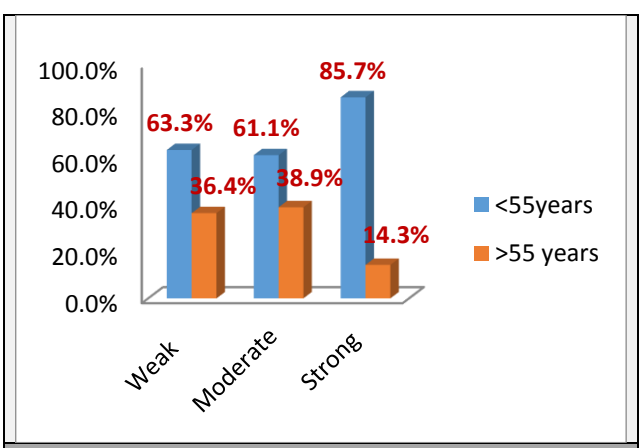

Figure 1. Expression of B-cell lymphoma-2 (Bcl2) among the Two Age Groups of Participants

Extent of *Bcl-2* staining seemed to decrease with increasing tumour grade (Table 1, Figure 2). Majority (81.8%) of the weakly stained tumours were poorly differentiated. Additionally, most of the well differentiated tumours (85.7%) were well differentiated. This trend seen in expression of *Bcl-2* and grades of tumours was statistically significant (P=0.001).

	Characteristic	Weak (n=22)	Moderate (n=18)	Intense (n=7)	P value
Age (in years)	<55 years	14 (63.3%)	11 (61.1%)	6 (85.7%)	0.589
	>55 years	8 (36.4%)	7 (38.9%)	1 (14.3%)	
Histological types	Serous	16 (72.7%)	4 (22.2%)	4 (57.1%)	*<0.001
	Mucinous	1 (4.5%)	13 (72.2%)	2 (28.6%)	
	Endometroid	4 (18.2%)	0 (0%)	1 (14.3%)	
	Brenner	1 (4.5%)	1 (5.6%)	0 (0%)	
Differentiation (grades)	Well	2 (9.1%)	17 (94.4%)	6 (85.7%)	*<0.001
	Moderate	2 (9.1%)	0 (0%)	0 (0%)	
	Poor	18 (81.8%)	1 (5.6%)	1 (14.3%)	

Table 1. B-cell lymphoma-2 (Bcl-2) Expression in Tumours with Respect to Different Characteristics under Study

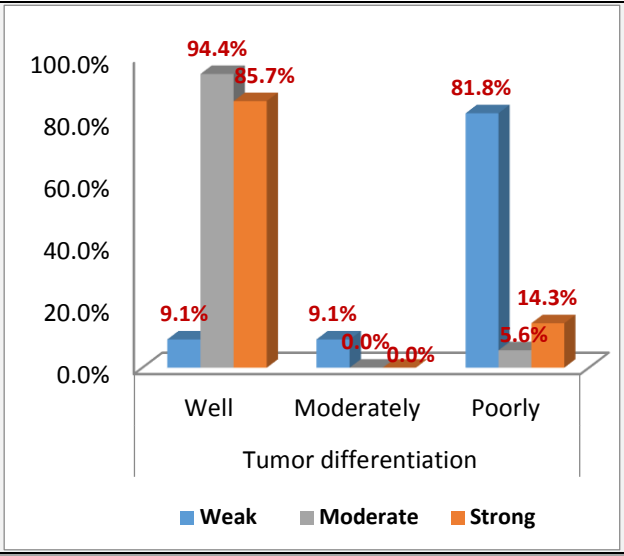

Figure 2. B-cell lymphoma-2 (Bcl-2) Expression in Different Grades of Tumours

Extent of *Bcl-2* Staining in Different Tumours

Number of tumours with <50% staining was slightly more (56.2%) in the <55 years age group when compared to the >55 years group (43.8%). Among the tumours with >50% staining, 71% belonged to the <55 age group. But no statistically significant association was noted between the staining pattern and the age group of the participants (P=0.313; Table 2).

Among the different histological types of ovarian tumours, more than half of the tumours with <50% *Bcl-2* staining were of serous type (62.5%). Rest of the serous type tumours (n=14) and majority of mucinous type tumours (n=15) showed >50% staining of *Bcl-2*. There was a statistically significant relationship between the extent of staining and different histological types of tumours in this study (P=0.004; Table 2).

	Characteristic	<50% (n=16)	>50% (n=31)	P value
Age (in years)	<55 years	9 (56.2%)	22 (71%)	
	>55 years	7 (43.8%)	9 (29%)	0.313
Histological types	Serous	10 (62.5%)	14 (45.2%)	
	Mucinous	1 (6.2%)	15 (48.4%)	
	Endometroid	4 (25%)	1 (3.2%)	
	Brenner	1 (6.2%)	1 (3.2%)	*0.004
Differentiation (grades)	Well	2 (12.5%)	23 (74.2%)	
	Moderate	1 (6.2%)	1 (3.2%)	
	Poorly	13 (81.2%)	7 (22.6%)	*<0.001

Table 2.

There was a clear trend in the staining of *Bcl-2* among the different grades of tumours, with poorly differentiated tumours showing <50% staining and well differentiated tumours having >50% *Bcl-2* staining (Table 2). This

relationship between the different grades of tumours and staining of the protein was statistically significant (P<0.001).

DISCUSSION

With majority of gynaecologic cancer related deaths happening due to ovarian cancer, this is one of the deadliest forms of cancer inflicting females.[11] Majority of patients are diagnosed at later, advanced stages of cancer which considerably decreases the survival rate. Considering the importance of *Bcl-2* in progression and prognosis of various tumours, there are several studies examining the expression and staining of these proteins in epithelial cells. This study focuses on the expression and staining of *Bcl-2* in the ovarian surface epithelial tumours and its correlation with the histopathological as well as grade and differentiation of ovarian cancer. As the most common type of ovarian epithelial tumour, majority of the tumours in this study were ovarian serous adenocarcinoma.

Studies show that more than half of ovarian cancer stain for *Bcl-2*, and the staining is lesser in ovarian cancer specimens when compared to normal and benign ovarian specimens.[12] In the present study on 47 tumours, 46.8% of the epithelial ovarian cancer specimens showed a weak expression of *Bcl-2*. *Bcl-2* proteins play an important role in maintaining the normal physiological function and integrity of ovarian surface epithelium. Decreased expression of this protein stems from dysregulation of *Bcl-2* and leads to tumour progression in ovarian epithelia.[7] A statistically significant relationship was absent between expression of *Bcl*2 and age group in our study, though some studies show a higher rate of expression in menopausal women.[13]

Studies have reported an inversely proportional relationship between *Bcl-2* expression and tumour grade and differentiation. [7,14,15] This is in agreement with our study which shows that 90% of the poorly differentiated ovarian cancers have weak *Bcl-2* expression. A statistically significant association existed between tumour differentiation and *Bcl*2 expression in tumours. Anderson compared the levels of *Bcl-2* expression in cancer cells with that of normal cells and found a similar significant change in expression.[16] Elevated urinary levels of *Bcl-2* was noted in ovarian cancer patients, suggesting a transition from cellular expression to secreted form of *Bcl-2* which is associated with progression of tumour.[16] Moreover, alterations in the stromal cells may also play an active role in the regulation of tumorigenesis. Apoptotic indices were found to be the highest among stage III malignant tumours.[7] Although *Bcl-2* expression plays an important role in ovarian tumorigenesis, there was no correlation between apoptosis and expression of the protein.[7] Thus it is possible that the protein may have roles other than regulation of apoptosis in human ovarian epithelial cancer.

In the present study 66% of the cases showed >50% extent of *Bcl-2* staining, similar to other studies.[16] Studies on expression of *Bcl-2* with respect to clinical outcome are not uniform. Benign and malignant ovarian tumours did not show any statistically significant difference in the expression of *Bcl-2*.[17] Moreover, there was no relationship between *Bcl-2* expression and prognostic criteria like stage and grade of cancer. A study on prognostic significance of *Bcl-2* protein reported improved survival with increased expression of *Bcl-2*.[18] Contrary to this, *Bcl-2* and p53 proteins were associated with poor clinical outcome in ovarian cancer in another study.[13] *Bcl-2* is also noted as an independent predictor of prognosis as well as resistance to chemotherapy, especially in patients with advanced ovarian cancer.[6] *Bcl-2* staining was inversely correlated to initial response to chemotherapy, especially in serous ovarian carcinomas. It is possible that expression of *Bcl-2* and its association with different prognostic factors may depend on various factors including response of the host, expression of other proteins like p53, as well as grade of tumour. Ovarian tumours that are *Bcl*2-positive and p53-negative are found to have the best prognosis in a study.[8] This shows that p53 protein may inhibit the expression of *Bcl-2* in malignant tumours leading to its downregulation in advanced form of cancer.[19]

CONCLUSIONS

There is a significant correlation between *Bcl-2* expression and histological type and tumour differentiation in ovarian cancers. *Bcl-2* expression is decreased in poorly differentiated tumours when compared to well-differentiated tumours. Thus, for primary surface epithelial tumours of ovary, expression of *Bcl-2* may be considered as an indicator of tumour progression. Further studies are warranted since the expression of this protein is an important predictor of prognosis. Moreover, it may also play an important role as a target for treatment in patients with surface epithelial ovarian cancers.

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