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Original Research Paper

Pathology

STUDY OF EXPRESSION OF BCL-2, AN ONCOPROTEIN IN BENIGN AND MALIGNANT OVARIAN NEOPLASMS

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ABSTRACT Ovarian cancer is the fifth most common cancer and it causes more deaths than any other type of female reproductive tract cancer. It is difficult to diagnose ovarian cancer early due to its deep location in pelvis, often there are no warning symptoms and the disease grows relatively slowly. As a consequence, 80% of such cancers are diagnosed only at the late stages when effective treatment could not be achieved. Over the last few years, it has been found that alteration in expression of various oncogenes like bcl-2 are associated with cancer genesis. bcl-2, an oncoprotein which apparently inhibits apoptosis, is expressed in a variety of ovarian tumours and also in normal ovary. The expression of bcl-2 can be detected by immunohistochemistry. The aim of the study is to correlate the expression of bcl-2 in benign and malignant ovarian neoplasm.

KEYWORDS : Benign, Malignant, Bcl-2

INTRODUCTION

The ovaries are paired gonadal structures that lie suspended between the pelvic wall and the uterus by the infudibulopelvic ligament laterally and the uterovarian ligament medially. Ovaries are the source of female fertility, and at the same time the origin of many of the most complex as well as lethal neoplasms. Ovarian lesions include non neoplastic and neoplastic conditions. Non neoplastic conditions include inflammatory lesions, epithelial inclusion cysts, solitary cysts of follicular origin, polycystic ovarian disease and stromal hyperplasia. Neoplastic conditions include benign and malignant neoplasms. Neoplasms are the most serious conditions of the female genital tract(1).

It is the ovarian surface epithelium which is believed to be the origin for majority of epithelial ovarian cancers. The commonest of the ovarian neoplasms are the epithelial tumours forming 80% of all tumours. 80% are benign and 20% are malignant. Of all the malignant tumours, 90% are epithelial in origin(2). Ovarian cancer results from a succession of genetic alterations involving oncogenes and tumour suppressor genes which have a critical role in normal cell growth regulation(3). The deregulation of expression of bcl-2(26 KD membrane associated protein) in neoplastic tissues is of interest for two reasons. First, it may be that inappropriate expression of bcl-2 is involved in neoplastic transformation, and second, expression of bcl-2 by tumours may confer resistance to chemotherapy by enabling cells to avoid apoptosis.bcl-2 gene has been implicated in several diseases including several epithelial tumours, breast carcinoma, prostatic neoplasms, autoimmunity as well as melanoma and cutaneous basal cell carcinoma. Expression of bcl-2 protein can be detected by immunohistochemistry. The influence of bcl-2 overexpression on survival and response to anticancer drugs is highly variable in epithelial malignancies and remains unclear in epithelial ovarian cancer(4).

MATERIAL AND METHOD

The present study was conducted on a total of forty specimens of ovarian neoplasms(twenty benign and twenty malignant) received in the Pathology Department, Government Medical College, Amritsar. Paraffin-embedded tissue sections were stained with conventional Haematoxylin and Eosin followed by Immunohistochemistry staining. IHC was performed using avidin-biotin-peroxidase complex (ABC) technique. New sections of 3 µm-5 µm thickness for each case were then cut from paraffin-embedded blocks and mounted on freshly prepared 0.01% poly-1-lysine-coated slides. Slides were dried overnight at 37°C, dewaxed in xylene, and then gradually rehydrated. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 10 min followed by three washings in phosphate buffered saline (PBS), and then antigen retrieval was achieved in a pressure cooker (4 times, 5 min each; 0.1 M citrate buffer, pH 6.0). The sections were then brought to room temperature and washed in PBS and 2 drops of 3% H_2O_2 and were incubated for 10-30 minutes followed by washing in PBS buffer for 5 minutes twice. Two drops of primary monoclonal mouse antibody, anti-bcl-2 clone 124 procured from the Novacastra were added and incubated for 60 minutes. Following this, sections were incubated with biotin-conjugated secondary antibody for 30 min and then incubated using streptavidin-biotin system for 30 min at room temperature. Each step was followed by PBS wash twice. The reactions became visible after immersion of sections in 3,3 -diaminobenzidine hydrochloride solution and washed with deionised water for 3 minutes. Sections were then counterstained with Mayer's hematoxylin stain for 2 to 5 min, dehydrated, cleared and mounted with mounting media.

Positive control tissue had brown coloured end product at site of target antigen, in the cytoplasm of the cells. Negative control did not have any coloured product. bcl-2 score was then evaluated by taking into account the staining intensity and cytoplasmic positivity of the tumour cell cytoplasm i.e. both quantitatively and qualitatively (5).

Quantitative Scoring:

0 = < 5% tumour cells showing cytoplasmic positivity
1 = 5- 25% tumour cells showing cytoplasmic positivity
2 = 25-50% tumour cells showing cytoplasmic positivity
3 = 50-75% tumour cells showing cytoplasmic positivity
4 > 75% tumour cells showing cytoplasmic positivity

Qualitative Scoring:

0 = No cytoplasmic staining
1 = Weak cytoplasmic staining intensity
2 = Medium cytoplasmic staining intensity
3 = Intense cytoplasmic staining intensity

Then, the final score for each section was obtained by multiplying

the percentage score by the intensity score.

OBSEVATIONS

Of the 20 benign cases, all cases showed bcl-2 staining, with 1 case (5%) showing a score of 2, 10 cases (50%) showing a score of 3 and 9 (45%) cases showed score of 4.

In the 20 malignant cases, 6 cases (30%) showed no positivity, 6 (30%) a score of 2, 4 (20%) a score of 3 and remaining 4(20%) a score of 4. So the number of cases showing a higher bcl-2 score decreased significantly from benign to malignant spectrum with a p value of 0.002.

TABLE I
QUANTITATIVE bcl-2 SCORING

Quantitative scoring of bcl-2	Benign cases		Malignant cases	
	No. of cases	%age	No. of cases	%age
0	0	0	06	30
1	0	0	0	0
2	01	05	06	30
3	10	50	04	20
4	09	45	04	20
Total	20	100	20	100
Mean Rank	26.05	14.95		
Mann Whitney Z value	3.140			
p value	0.002; Significant			

QUALITATIVE bcl-2 SCORING

In the benign cases, majority (55%) showed intense cytoplasmic bcl-2 staining with a score of 3 while just 10% of the malignant cases showed intensity to the same extent. Hence there was a statistically significant difference in the intensity of staining between the two groups.

TABLE II
QUALITATIVE bcl-2 SCORING

Qualitative scoring of bcl-2	Benign cases		Malignant cases	
	No. of cases	%age	No. of cases	%age
0	0	0	6	30
1	01	05	5	25
2	08	40	7	35
3	11	55	2	10
Mean Rank	27.18		13.83	
Mann Whitney Z value	3.792			
p value	<0.001; **Highly Significant**			

TOTAL bcl-2 score

Majority of the benign cases had a higher bcl-2 score while the reverse was true for the malignant cases. None of the benign cases had a score of 0 while 30% of the malignant cases had 0 score which was again found to be statistically significant.

TABLE III
TOTAL bcl-2 SCORE

bcl-2 score	Benign cases		Malignant cases	
	No. of cases	Percentage	No. of cases	Percentage
0	0	0	6	30
1	0	0	0	0
2	0	0	4	20
3	0	0	1	05
4	1	05	2	10
6	6	30	3	15
8	3	15	2	10
9	5	25	0	0
12	5	25	2	10
Mean Rank	27.25		13.75	
Mann Whitney Z value	3.698			
p value	<0.001; **Highly Significant**			

DISCUSSION

bcl-2 score was calculated by observing both the percentage positivity of the tumour cells (quantitative score) as well as the staining intensity (qualitative score). It was seen that the benign neoplasms had a higher score compared to the malignant neoplasms. 100% of the benign cases were positive for bcl-2 while only 70% of the malignant cases showed positivity. This reduced expression of bcl-2 from benign to malignant spectrum was found to be statistically significant. Similarly, Gursan N et al found none of the malignant case positive for bcl-2 while 66.67% of the benign cases were positive for bcl-2 (6).

In a study done by Chan WY et al, 100% of the benign neoplasms showed bcl-2 positivity while only 33% of the malignant cases were positive for bcl-2(7).

This reduced expression of bcl-2 in malignant cases was also seen by Anderson NS et al who found that 100% of the benign cases were positive for bcl-2 while only 79% of the malignant cases showed bcl-2 positivity.(8)

In the present study, 95% of the benign cases showed bcl-2 positivity in more than 50% of the cells while just 40% of the malignant cases showed positivity to the same extent, which is statistically significant. Similarly, In the study done by Anderson NS et al, it was seen that 65% of the benign cases showed bcl-2 positivity in more than 50% of the cells while just 29% of the malignant cases showed positivity to the same extent(8). In the present study, it was seen that only 5% of the benign cases showed weak bcl-2 staining while 95% showed moderate to intense staining intensity. Amongst malignant cases, 55% cases showed nil to weak bcl-2 staining while 45% cases showed moderate to intense staining intensity i.e. the staining intensity statistically decreased from benign to malignant cases with a p value of <0.001.

These findings are supported by the study done by Anderson NS et al who found that 6% of the benign cases showed nil to weak staining while 94% cases showed moderate to intense bcl-2 staining. In the malignant group, 57% cases showed nil to weak staining while 43% cases showed moderate to intense staining intensity(8).

SUMMARY AND CONCLUSIONS

In the present study, it was seen that bcl-2 score was higher in benign cases as compared to malignant which was statistically significant. (p value < 0.001) . It is concluded that bcl-2 expression goes on decreasing both quantitatively as well as qualitatively in malignancy. So, bcl-2 can be used as a valuable marker to differentiate between benign and malignant ovarian neoplasms. As bcl-2 expression decreases in higher grade, it can be used as biological and prognostic marker. Secondly, bcl-2 are only exclusively elevated in patients with ovarian cancer; women without disease and normal healthy women do not seem to have any elevated bcl-2, so there are no false positives, which really would be important for the accuracy. It is very safe, reliable, economic test to detect all ovarian cancers. In that case, we would reduce the mortality of the disease because, while the majority of ovarian cancers are detected in late stages, the mortality is very high.

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