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THE RELATION OF BREAST CANCER TISSUE AND ITS CLINICOPATHOLOGIC FACTORS WITH EXPRESSION OF BCL-2 PROTEIN.

Dr. Naska M. Haseeb
M.B.,Ch.B. M.Sc (path).
naskamansour@gmail.com

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Abstract:

Background: In women worldwide the most common malignant neoplasm is breast cancer, with a lifetime risk of 1 out of every 8 women. The association between Bcl-2 and breast cancer development and progression is still unclear, but a relationship between unfavorable clinical outcome and Bcl-2 overexpression is to be expected. The aim of this study is the detection of Bcl-2 in breast cancer tissues, utilizing the immunohistochemical methods and its association with various clinicopathologic parameters as age, family history, grade and stage.

Materials and methods: Forty eight cases are included in this study and all of them are invasive ductal carcinoma of breast with five control cases, all of them being tonsils. For each case (paraffin block), two sections are taken and each of them is of 5µm thickness. Eosin and hematoxylin stains was used to stain the first section and the other section was stained with immunohistochemical method for Bcl-2. The scoring depended on the percentage of the cells that express Bcl-2 and the intensity of staining.

Results: In using the immunuhistochemical staining to determine the scores of Bcl-2 expression in invasive breast carcinoma tissues and its relation with clinicopathologic factors including age of the patient, stage of the disease, grade, and family history, the highest percentage of Bcl-2 expression was in score 0 and was about 38%, and the lowest percentage was in score 3+ and was about 10%.

Conclusion: Bcl-2 expression is inversely related to the stage of the breast carcinoma, while the age of the patient, family history, and grade of the disease do not have effect on the Bcl-2 expression.

Keywords: Breast cancer, Bcl-2, immunohistochemical method.

INTRODUCTION

Breast cancer is a malignant tumor of breast tissue cells that start in the ducts and lobules.[1] The two keys determinants in the histopathologic examination and study of breast carcinoma are: whether the tumor is in-situ carcinoma or infiltrating carcinoma and whether the cellular detail it is of ductal or lobular type.[2]. The growth of the tumor depends on the balance between two main factors; cell death by apoptosis and cell proliferation a.[3,4] Bcl-2 is a proto-oncogene that act as a gene suppressor and cause death of the cells and regulate the programmed cell death (apoptosis).[5,6] Immunohistochemistry refers to the process that act on tissue cells and detect the proteins in them using the action of antibodies in the solutions binding to antigens in the cells.[3,7]The method used in this research to detect Bcl-2 in breast cancer is immunohistochemical method (DakoCytomation EnVision® +Dual Link System-HRP (DAB+)

Tissue samples

In this study I used a total of forty eight cases (paraffin blocks samples) of malignant breast tissue from patients with malignancy who underwent modified radical mastectomy with axillary clearance. All cases were diagnosed as invasive ductal carcinoma with different stages using TNM staging system and with different grades using Elston modification of the

MATERIALS AND METHODS

Bloom-Richardson grading system, also it included five control cases, all of them being tonsils.

Methods

For each case (paraffin block), two sections of 5µm thickness were taken; the first section was stained with the conventional stain (hematoxylin and eosin) and was reviewed again for diagnostic confirmation. The other section was stained with

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immunohistochemical method for Bcl-2 using DakoCytomation EnVision®+ Dual Link System-HRP (DAB+).

Immunohistochemical Scoring:-

The evaluation of Bcl-2 immunostaining was done by two pathologists. Two parameters used to categoriz the staining as either positive or negative, which are: (1) the immunoreactivity intensity (scale of 0-2 with 0 = negative, 1 = weak or light brown, and 2 = strong or dark brown) and (2)positive staining tumor cells percentage (scale of 0-2 with 0 = < 10% of the cells are positive, 1 = 10-50% of the cells are positive , 2 = > 50% of the cells are positive).

The expression of bcl-2 protein was classified into four-point scale: 0, < 10% of the cells are positive; 1+, 10-50% of the cells are positive; 2+, > 50% of the cells are positive and the staining is weak; 3+, > 50% of the cells are positive and the staining is strong.

Statistical Analysis

In the statistical analysis, to compare the results and determine their significance a Chi-square test was used. Analysis of the variance (ANOVA) was used for evaluating the relation between the expression of Bcl-2 in the breast cancer tissues and the stage, grade, age and family history.

RESULTS

Control group:

Five cases of tonsil tissue were used as a positive control for the study to validate the Bcl-2 stain, as shown in fig (1).

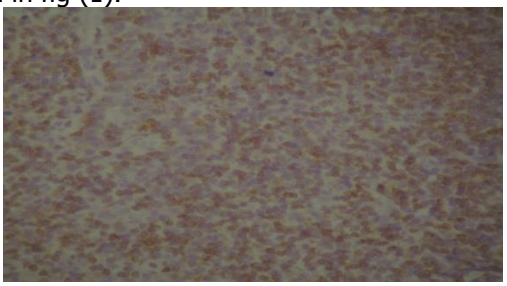

Fig (1): Bcl-2 immunostaining in the control case (Immunostaining X400).

Bcl-2 expression:

Bcl-2 expression was scored as 0, 1+, 2+, 3+. The most frequent score was score 0 with a frequency of 18 (38%), followed by score 1+ with a frequency of 16 (33%). The lowest frequency was for the score 3+ which was 5 (10%), as shown in fig (2).

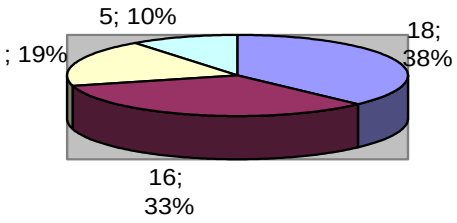

Fig (2): The frequency of Bcl-2 expression scores and their percentages.

Bcl-2 staining of the malignant cells in the breast carcinoma tissue showed brown cytoplasmic granules as shown in fig (3).

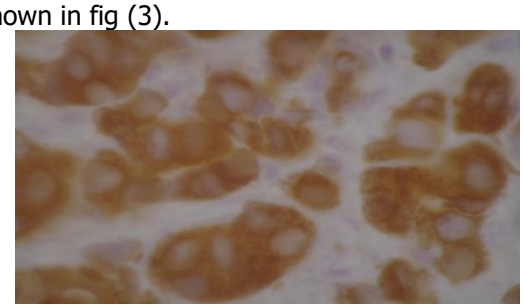

Fig (3): Microscopical appearance of the cytoplasmic Bcl-2 immunostaining of the malignant cells (Immunostaining X 1000).

The presence and staining of Bcl-2 in malignant breast tissue with score 3+, that was >50% population stained with a strong intensity of the stain by immunostaining as shown in fig (4)

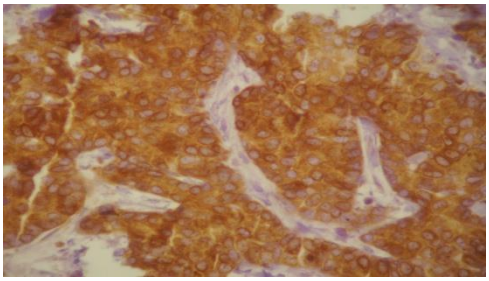

Fig (4): Score 3+ of Bcl-2 expression (immunostaining X400).

The Hematoxylin and eosin staining of the breast cancer tissue, that expressed score 3+ of Bcl-2, as shown in fig (5).

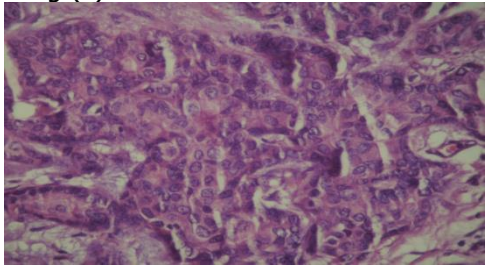

Fig (5): Breast cancer tissue (H&E X400).

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Presence and staining of Bcl-2 in malignant breast tissue with score 2+, that was >50% population stained with a weak intensity of the stain by immunostaining as shown in fig (6).

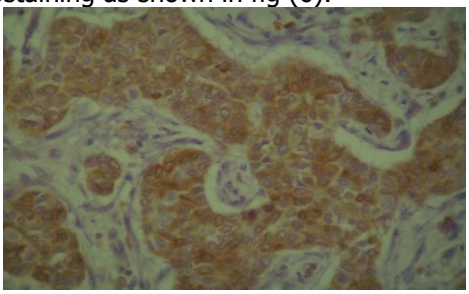

Fig (6): Score 2+ of Bcl-2 expression (immunostaining X400).

Presence and staining of Bcl-2 in malignant breast tissue with score 1+, that was 10-50% population stained by immunostaining as shown in fig (7).

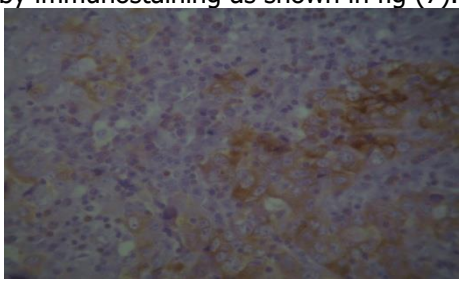

Fig (7): Score 1+ of Bcl-2 expression (immunostaining X400).

Presence and staining of Bcl-2 in malignant breast tissue with score 0, that was <10% population stained by immunostaining as shown in fig (8).

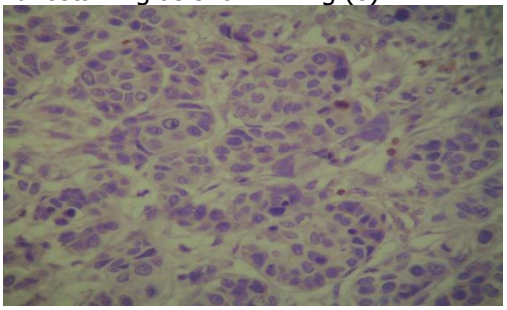

Fig (8): Score 0 of Bcl-2 presence (immunostaining X400)

Bcl-2 and stage:

The score of Bcl-2 presence and detection was different according to the stages of the disease. In stage 1 of the disease there was 2 cases within score 3+ and no cases of the scores 0, 1+, 2+. In stage 2, there was 7 cases was 0 score, 5 cases within1+ score, 3 cases within 2+ score, and 3 cases within 3+ score. In stage 3, there was 9 cases for each of score 0 and score 1+, 6 cases of score 2+, and no cases of score 3+. In stage 4, there was 2 cases for each of score 0 and score 1+, and no cases for each of score 2+ and score 3+, as shown in fig (9).

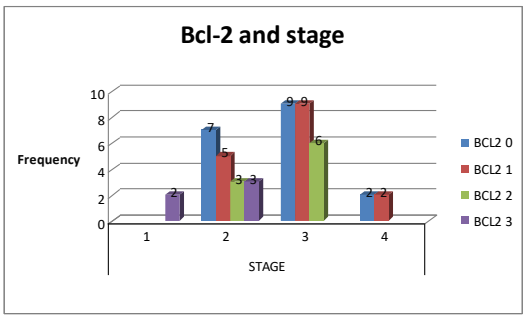

Fig (9): Scores of Bcl-2 expression in various stages of the disease.

Since the P-value is <0.05 (0.0142), statistically there is significant relation between Bcl-2 expression and stage of the disease at the 95% confidence level.

Bcl-2 and grade:

The score of Bcl-2 expression was different in the different grades of the disease. In grade 1, there was 2 cases within 0 score, 1 case within 1+ score, and no cases of both scores 2+ and 3+. In grade 2, there was 4 cases within 0 score, 6 cases within 1+ score, 2 cases within 2+ score, and 4 cases within 3+score. In grade 3, there was 12 cases within 0 score, 9 cases within 1+ score, 7 cases within 2+ score, and 1 case within 3+ score, as shown in fig (10).

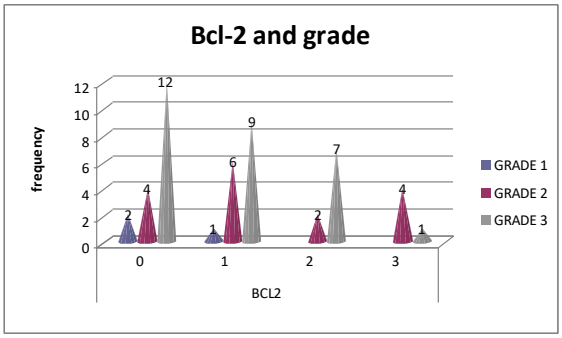

Fig (10): Scores of Bcl-2 expression in various grades of the disease.

Since the P-value is >0.05 (0.7198), statistically there is no significant relation between Bcl-2 expression and grade of the disease at the 95% confidence level.

DISCUSSION

Breast carcinoma is consider as a disease in which its clinical development and prognosis are variable.[8] Although the exact role of Bcl-2 in the

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appearance of growth of malignancy is still not so clear and obvious, the detection of Bcl-2 protein has been reported in many cases of malignancy,[9] and its enhanced expression in human neoplasm, such as breast carcinoma, suggests that this oncoprotein has a role in the pathogenesis of malignancy.[10,11] So Bcl-2 can be consider as one of the most promising members of molecular markers for the evaluation of the behavior of malignancy.[6] By the method of immunohistochemistry we can detect the overexpression of the gene Bcl-2, in the form of accumulated Bcl-2 oncoprotein, using specific monoclonal antibodies.[12] This study assessed the detection of Bcl-2 in tumor cells of the ductal carcinoma of the breast and determined its relation with prognostic factors such as stage and grade of the disease and also with the age and family history of the patient. The detection of Bcl-2 in malignant breast tissue is recognized as brown cytoplasmic granules, and it is variable in both the percentage of population that are stained and in the intensity of the staining.

The study evaluated the detection of Bcl-2 in malignant breast tissues, and found statistically significant inverse relation between the Bcl-2 and the stage of the disease in which the P-value is <0.05 (0.0142) at the 95% confidence level, which is in agreement with that of Seong M.K. et al.[13] and Bhatavdekar J. et al.[14] but disagrees with that of Zhang Y. et al.[10] In the present study, there is no statistically significant association between the Bcl-2 expression and the grade of the disease, the age of the patient, and the family history of the patient.

CONCLUSIONS

1. Bcl-2 expression might be useful in determining the prognosis of invasive breast carcinoma patients.
2. Bcl-2 expression is inversely related to the stage of the breast carcinoma.
3. There is no effect of the age of the patient, family history, and grade of the disease on the Bcl-2 expression.

RECOMMENDATIONS

1-In cases of invasive ductal breast cancer, it is better to be stained by Bcl-2 immunostaining, as it might be helpful in determining the disease prognosis.

2-Further studies on the relation of Bcl-2 expression and other clinicopathologic factors such as ER, PR and HER/2-neu are required to further understand its role in determining the hormonal therapy.

3-Further studies on a wider scale, i.e. more population size, is required to evaluate the actual relationship between Bcl-2 overexpression and the family history.

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