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Immunohistochemical expression of anti-apoptotic protein bcl2 and its correlation with hormone receptors in breast cancers

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Objectives One of the novel benefits of immunohistochemistry in human malignancy (in addition to its major role in confirming the diagnosis) is to discover specific therapeutic targets in cancer cells, so this study was conducted to identify immunohistochemical expression of bcl2 in breast cancers, and to correlate its expression with expression of hormone receptors (estrogen and progesterone) to provide histologic information that may help in clinical practice.
Methods This is a retrospective study that at the histopathology laboratory at Alwasity teaching hospital for orthopedic and plastic surgery in Bagdad, about 52 paraffin-embedded tissue blocks of cases diagnosed as primary mammary carcinoma, which were retrieved from multiple private sectors in Bagdad. From each block, four histological sections were made, one stained with hematoxylin and eosin to examine under light microscope and the other three histological sections were processed for immunohistochemical staining with antibodies to estrogen receptors (ERs), progesterone receptors (PRs) and bcl2. The results of immunohistochemistry were statistically evaluated.
Results Of 52 samples with breast cancer, 42(80.7%) were ductal and 10(19.2%) were lobular. The study showed that 26(50%) of total sample were positive for ERs and 34(65.3%) were positive for PRs. Regarding bcl2, 26(50%) of total samples were immunoreactive for the latter, and there is a highly significant statistical relationship between the expression of bcl2 and (ERs and PRs).
Conclusion Bcl2 immunohistochemical expression can be found in breast cancer and its expression is strongly correlate with ER and PR status and the former can be used as a predictive marker that predicts positive immunohistochemical expression of hormone receptors (thereby hormone receptor therapy) and vice versa and can be used as a quality control marker. This conclusion can be used to standardize immunohistochemistry for this application.
Keywords breast cancer, bcl2, estrogen receptor, progesterone receptor

Introduction

Mammary carcinomas remain one of the most common cancers in the world.[1] Terminal duct lobular units (TDLUs) regarded as the anatomical areas from which breast cancer cells arise.[2,3] Much works were done to classify breast cancers and many classification systems were assigned, but depending on the recent classification of breast cancer, it is divided into four subtypes based on both genetic study and immunohistochemical expressions of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), cytokeratins (CK5 or CK5/6), and epidermal growth factor receptor (EGFR).[4] This classifications having both prognostic and therapeutic benefits;[5] therefore, it's important for every recently diagnosed breast cancers to subject to immunohistochemical analysis[6] because IHC classification correlates with genetic microarray analysis i.e., "ER/PR$^+$, Her2$^+$ with Luminal B; ER/PR$^+$, Her2$^-$ with Luminal A; ER/PR$^-$, Her2$^+$ and ER/PR$^-$, Her2$^-$ with triple negative/basal-like tumors."[7]Breast cancer like other cancers having multiple genetic mutations in the body,[8] and some of them passed on families causing hereditary breast cancer.[9] One of genes frequently mutated in breast cancer cells is bcl2, which encode the anti-apoptotic protein bcl2 that increase cell survival by blocking the programmed cell death (apoptosis) which is a physiological cellular event,[10,11] its name refers to (*B-cell lymphoma 2*) because it is the gene that commonly involved in the famous chromosomal translocation that occur in follicular lymphoma.[12] Antibodies to Bcl-2 can be used immunohistochemically to identify cells that have this antigen. In normal tissue, these antibodies stain mantle zone B cells and some T-cells.[13] There are two isoforms of Bcl-2, Isoform 1(1G5M), and Isoform 2(1G5O/1GJH), both have a similar structure. However, there is a difference in the ability of these isoforms to bind to the BAD and BAK proteins, and in the electrostatic potential of their binding groove, suggest differences in anti-apoptotic property for these two isoforms.[11] The important role of BCL-2 in blocking apoptosis and its expression in breast cancer pay attention to its use as a therapeutic target leading to discovery of therapeutic agents acting as a selective Bcl-2 inhibitors that have the advantage of very low tissue toxicity and destruction when compared with conventional anti-cancer therapy (chemo and radiotherapy), such as ABT-737 and Navitoclax (ABT-263), Genasense, Venetoclax (ABT-199).[13–15]

Materials and Methods

This is a retrospective study done in the laboratory department of histopathology at Alwasity teaching hospital for orthopedic and plastic surgery in Bagdad, 52 paraffin-embedded tissue blocks for cases diagnosed as primary mammary carcinoma were retrieved from multiple private sectors in Bagdad during 1 year period from January 2016 to October 2016. From each block, four histological sections were made, one stained with hematoxylin and eosin to examine under the light microscope to confirm the diagnosis and for histological typing according to WHO classification and grading invasive ductal carcinomas according to Nottingham Modification of the Bloom–Richardson system.[16,17] Informations about patient's age, tumor

Table 1. **Immunohistochemical staining protocols**

Primary antibody	Clone	Dilution	Manufacturer	Localization	Positive control
Monoclonal Mouse anti-human ER* alpha	1D5	1:50–1:100	Dako	Nucleus	Normal breast tissue
Monoclonal mouse anti-human PR**	PgR636	1:50–1:100	Dako	Nucleus	Normal breast tissue
Polyclonal rabbit anti-human Bcl2	124	1:50–1:100	Dako	Cytoplasm	Lymph node

*Estrogen receptor, **Progesterone receptor.

Table 2. **Intensity score of immunohistochemical expression of bcl2**

Intensity score	Histological findings
0	No
1	Weak
2	Intermediate
3	Strong

Table 3. **Proportion score of immunohistochemical expression of bcl2**

Proportion score	Percentage of positive tumor cells
0	0%
1	<10%
2	10–50%
3	51–80%
4	>80%

size, axillary lymph node were missing, which is one of the limitation in each retrospective study. The other three histological sections were processed for immunohistochemical staining with antibodies to ERs, PRss and bcl2 by following the informations found in the manufacturer forms for each antibody and using autostainer (X biogenex i6000), positive and negative control were included in each run as shown in Table 1.

Regarding bcl2 immunohistochemical staining, results obtained by multiply the intensity score with the proportional score to find the total score as shown in Tables 2 and 3 "Total score = PS × IS (= 0–12), 0–4: Negative, 6–12: Positive."[18] While with ER and PR receptor status, positive immunohistochemical results obtained according to Allred scoring system for assessment of ER and PR in breast cancer.[18]

Statistical analysis: Association between Bcl-2 expression and hormone receptors were assessed using Chi-square test and $P < 0.05$ was regarded as statistically significant and that of <0.01 was regarded as highly significant.

Results

Of 52 samples with breast cancer 42(80.7%) were ductal and 10(19.2%) were lobular. Regarding ductal carcinoma, 8(15.38%) were well differentiated, 42(80.7%) were moderately differentiated and only two samples (3.85) were poorly differentiated adenocarcinoma. The study showed that 26(50%) of total sample were positive for ERs and 34(65.3%) were PRs positive. Regarding bcl2, 26(50%) of total samples were immunoreactive.

There is a highly significant statistical relationship between the expression of bcl2 and ERss with *P*- value 0.005

Table 4. **Correlation between immunohistochemical expression of bcl2 and ER**

	ER* positive	ER** negative	Total
Bcl2 positive	18	8	26
Bcl2 negative	8	18	26
Total	26	26	52

*Estrogen receptor, **Progesterone receptor.

Table 5. **Correlation between immunohistochemical expression of bcl2 and PR**

	PR* positive	PR** negative	Total
Bcl2 positive	24	2	26
Bcl2 negative	10	16	26
Total	34	18	52

*Estrogen receptor, **Progesterone receptor.

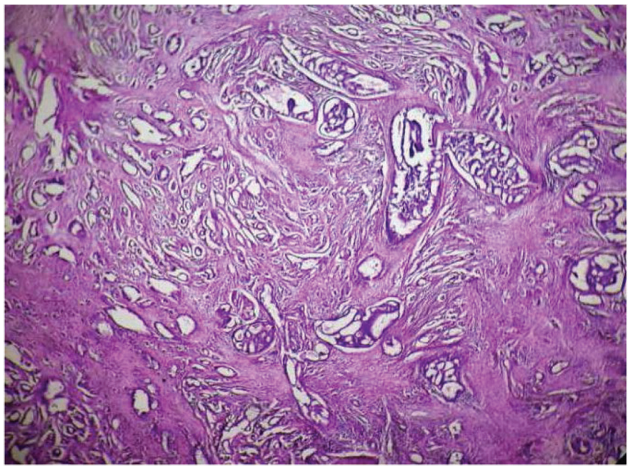

Fig. 1 **(×100) Hematoxylin and eosin stained histological section showed architectural features of invasive ductal carcinoma none other than specified.**

There is a highly significant statistical relationship between the expression of bcl2 and PRs with *P*- value 0.0004.

Discussion

Worldwide, breast cancer is the most common cancer in female.[2] When such diagnosis achieved in a women, she will have a number of treatments options, such as surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy.[19] One of the novel benefit of immunohistochemistry in human malignancy (in addition to its major role in confirming the diagnosis) is to discover specific therapeutic targets in tumor cells, so this study was conducted to identify immunohistochemical

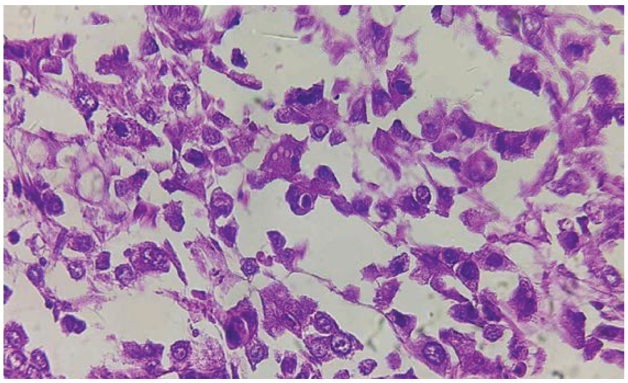

Fig. 2 **(×400) Hematoxylin and eosin stained histological section highlight cytological features of invasive ductal carcinoma none other than specified.**

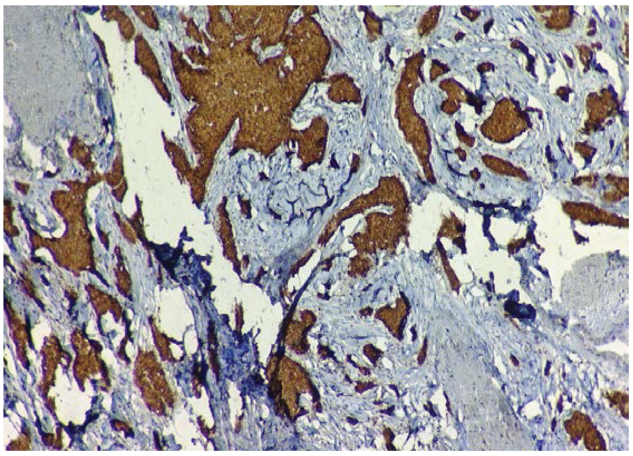

Fig. 3 **(×100) Immunohistochemical stained histological section showed positive immunohistochemical expression of bcl2 in breast cancer cells.**

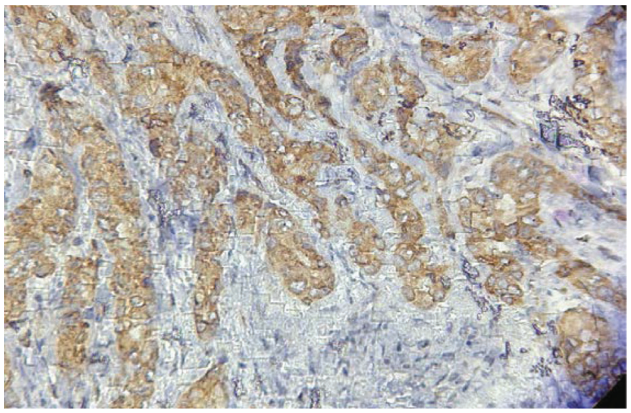

Fig. 4 **(×400) Immunohistochemical stained histological section showed positive immunohistochemical expression of bcl2 in breast cancer cells with a cytoplasmic localization.**

expression of bcl2 in breast cancer in order to provide histological information that may help in clinical practice.

This study showed that (80.7%) of the sample were invasive ductal carcinomas and (19.2%) were lobular type mammary cancer, this results agree with a Iraqi study done by Banan et al.[18] and with the well-known fact supported by the literature which revealed that ductal type mammary carcinomas are more common than lobular type.

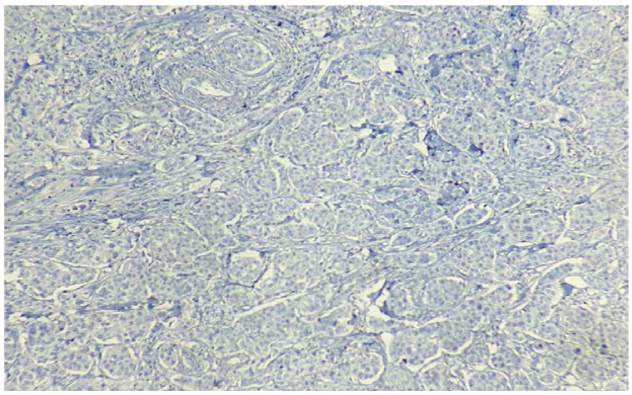

Fig. 5 **(×100) Immunohistochemical stained histological section showed negative immunohistochemical expression of bcl2 in breast cancer cells.**

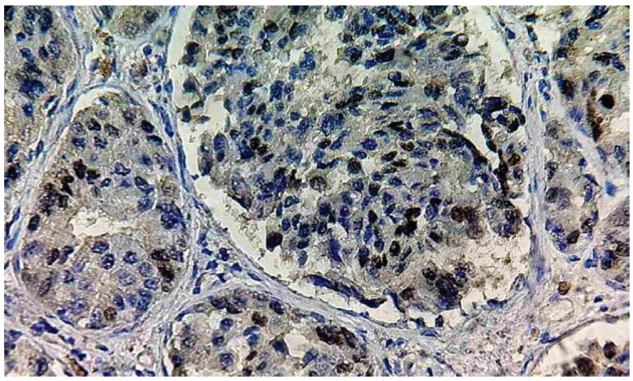

Fig. 6 **(×400): Immunohistochemical stained histological section showed positive immunohistochemical expression of PRs in breast cancer cells.**

This study also showed that bcl2 expression presents in 50% of the samples. Variable results were obtained in studies done by Banan et al,[18] Kitae et al.[20] and Dmerino et al.,[21] which were 46%, 68%, 75%, respectively, which may be due to variation in sample size. In this study, ERss were positive in 50% of samples and 65.3% of samples were PR positive, this result in agreement with the study done by Banan et al.[18]

In this study, there was a significant statistical relationship between immunohistochemical expression of bcl2 and, hormone receptors (ER and PR), which is in agreement with the results of Banan et al.[18] Kitae et al,[20] Dmerino et al.[21] and Dawson et al.[22] This mean that Bcl2 immunohistochemical expression is strongly correlate with positive estrogen and PR status i.e., (positive bcl2 can be of help in predicting hormonal receptor positivity and therefore response to hormonal therapy and vice versa.

Conclusion

1. Bcl2 can have a role in the molecular pathway that involved in the tumerogenesis of breast cancers and can be used as a targeted therapy that has the advantage of low tissue toxicity and destruction of otherwise non-tumoral cells when compared with conventional anti-cancer therapy.
2. Bcl2 immunohistochemical expression is strongly correlate with positive estrogen and PR status.

3. Bcl2 can be used as a predictive marker that can predict positive immunohistochemical expression of hormone receptors and vice versa, and as a quality control marker and this conclusions can be used to standardize immunohistochemistry for this application.

Recommendation

A prospective study using a larger sample size is recommended to evaluate the association between bcl2 expression and various clinical parameters and allow a better follow up of bcl2 positive breast cancers to know the prognostic value of bcl2 expression and to know which cases can predict response to the emerging anti bcl2 therapy.

Conflict of Interest

None.

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