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C-MYC and BCL2 Expression in Normal Tissue Around Proliferative Breast Conditions in Relation to ER, PR in a Sample of Iraqi Women

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Abstract

Breast cancer describes several subtypes of cancer of the breast that differs in clinical presentation, which reveals different gene expression and different molecular characteristics. As new advances in diagnosis and treatment emerge with an already prevalent but still curable disease, more research is required for such advanced diagnostic and prognostic parameters. A Total of 120 tissue samples were included in the current prospective study. Normal breast tissue taken from reduction mammoplasty (40 samples), normal tissue around a breast primary ductal carcinoma (40 samples) and normal tissue around Fibroadenoma (40 samples) were enrolled in the study. Tissue samples were immunohistochemically stained for four markers: BCL2, C-MYC, ER & PR and the score results of the markers were statistically examined and correlated. There was a highly statistically significant expression of BCL2 & C-MYC in normal tissue around breast carcinoma more than other proliferative conditions, with high significant correlation with ER, PR. Though there was overexpression of C-MYC &BCL2 in all three proliferative conditions, it was more pronounced in breast cancer.

1 Introduction

The umbrella term of the breast cancer describes several subtypes of cancer of the breast that differ in clinical presentation, which reveals different gene expression and different molecular characteristics[1,2]. Breast cancer is regarded as the most frequent cancer diagnosed in females where it occurs 1 in 4 of women diagnosed in all cancer. It is the second cause of cancer-related death in women aged 40-59 years[3]. So with multiple subtypes and different molecular characteristics, breast cancer is a heterogeneous disease that requires proper and reliable biomarker to diagnose and treated [4].

MYC is proto-oncogene and nuclear transcription factor that deregulated in different cancer, in breast cancer the MYC is over expressed in 30-50 % of high-grade cancer[5]. MYC show site specific DNA binding activity as a transcription factor with its binding associated factor X (MAX), this MYC-MAX site is rate limiting in cell cycle progression during G1 phase and this process is regulated partly by cyclin dependent kinase. The oncogene C-MYC play important role in cellular pathway that encourage the cancer cells proliferation and anticancer drug resistance[6]

The proto-oncogene BCL2, is a member of BCL-family that involved in apoptosis and first described in translocation (14:18) of human follicular lymphoma[7].The role of proto-oncogene it quickly discovered but its role as anti-apoptotic gene discovered some years later[8]. Due to the influence of BCL2-family in survival and death of the cells, the cells had constricted balance on expression of these proto-oncogenes. This is particularly true for a number of pro-apoptosis molecules like PUMA, Noxa, Bid, and Bad in a p53- dependent manner[9].

Compared to breast cancer, fibroadenomas are the commonest benign tumors in adulthood of the female that arise from TDLU

of the breast[10]. Therefore, a similar and comprehensive study of their nature is as important as studying their malignant counterpart.

The current prospective study was designed to study the immunohistochemical detection and expression of C-MYC and BCL2 in in relation to the concurrent expression of ER & PR in normal breast tissue surround proliferative breast disorders (benifn and malignant) and compare such expression to healthy normal breast without proliferative properties, in an attempt to evaluate the diagnostic and prognostic values of such markers.

2 Material and Methods

2.1 Sample collection and grouping

A Total of 120 tissue samples included in this prospective study. Were obtained from normal tissue of breast either: from totally normal breast tissue that took from reduction mammoplasty (40 samples), or from normal tissue around breast primary ductal carcinoma (40 samples) in addition to (40 sample) from normal tissue around Fibroadenoma lump, from the period of December 2016 until October 2017, which collected from Al Yarmouk teaching hospital and 2 private laboratory.

Breast biopsies with benign results initially were categorized into 1 of the following 3 categories using the criteria outlined by Dupont and Page[11]: (1) nonproliferative (normal breast tissue), (2) proliferative without atypia (Fibroadenoma), or (3) atypical hyperplasia (ductal)

The age of patients was divided into three groups; group1 totally normal tissue that aged <40, group2 normal tissue around proliferative Fibroadenoma that aged <40 years and 40-60 years, and normal tissue around proliferative ductal carcinoma that divided into 3 levels <40—40-60 -- >60 years.

For each sample, 5 serial sections of 4µm were taken, one representative section stained with H&E, the other four sections were stained immunohistochemically with C-MYC, BCL2, ER & PR markers.

2.2 Immunohistochemistry

For maximum staining performance after Deparaffinizing, the slides were heated in the antigen retrieval buffer in a domestic microwave oven at 800W for 20min. Slides were stained using manual method. The standard immuno-peroxidase protocol of Abcam Company was used for detection. Dilutions for the primary antibodies were 1:50 for C-MYC (H00004609-M02 MYC monoclonal antibody), 1:100 for bcl2 (Abcam Clone [Bcl2/100] Code: ab117115). Following counterstain the slide immediately analyze using light microscope (Micros Austria)

All tissues were assessed blindly by two observers. For assessment; six normal lobules were identified for each sample and graded according to the extent and intensity of staining. The positive control for each batch of staining was used as the reference point for assessing intensity. Intensity was scored as; 0 (no staining), 1 (weak), 2 (moderate) and 3 (strong). Extent of staining was categorized by proportion; 0–25% (0.25) of cell stained positively, 26–50% (0.50), 51–75% (0.75), 76–100% (1.00)

An index for each lobule, between 0 and 3, was generated by multiplying these two scores, and the mean of the 6 lobules gave the index for that tissue.

2.3 Statistical analysis

By using analysis of variance (SPSS Software, version 24.0 2017), the results were evaluated statistically, and whenever there was a difference between the correlated groups, student t-test was applied to estimate the degree of significance by comparing the mean of data and standard deviation of each group. Therefore, data are presented as measures of mean ± standard deviation, at 95% confidence interval.

3 Results

3.1 Age distribution and differences

In normal breast tissue 100% of the cases were <40 years. In normal tissue around benign disorders; 60% of the cases were <40 years and 40% of the cases 40-60 years.

In normal tissue around malignant disorders 15.4% of the cases <40 years, 61.5% from 40-60 years and 23.1% >60 years (Table 1 and Fig 1).

Table 1: Frequency distribution of age according to the conditions of breast tissue

Age group	Group		
	Normal	**Benign**	**Malignant**
<40 years	100.0%	60.0%	15.4%
40-60 years	0.0%	40.0%	61.5%
>60 years	0.0%	0.0%	23.1%

The normal control group had more expression of ER when compared to normal tissue around Benign and Malignant conditions, followed by normal tissue around malignant condition. On the other hand, the normal tissue around benign condition had the least expression than other samples with marked statistical significant.

The BCL2 protein was expressed in all conditions of normal breast tissue but it exhibit more expression in benign and malignant conditions than in normal control group. The C-MYC expression didn't differ in control and benign group but it increased in malignant condition especially in age level of 40-60 years.

3.2 Immunohistochemical staining

As shown in figure 2, there was no statistical difference in percentage of staining when comparing different sample from control, benign and malignant for all markers used in this study.

The markers also showed no statistical difference after calculating the final IHS of expression in the three samples of the breast where P value >0.05 (Fig 3).

C-MYC expression showed significant direct correlation with PR not with ER (Fig 4). On the other hand, BCL2 expression correlated directly positively with statistical significance with both ER & PR (Fig 5).

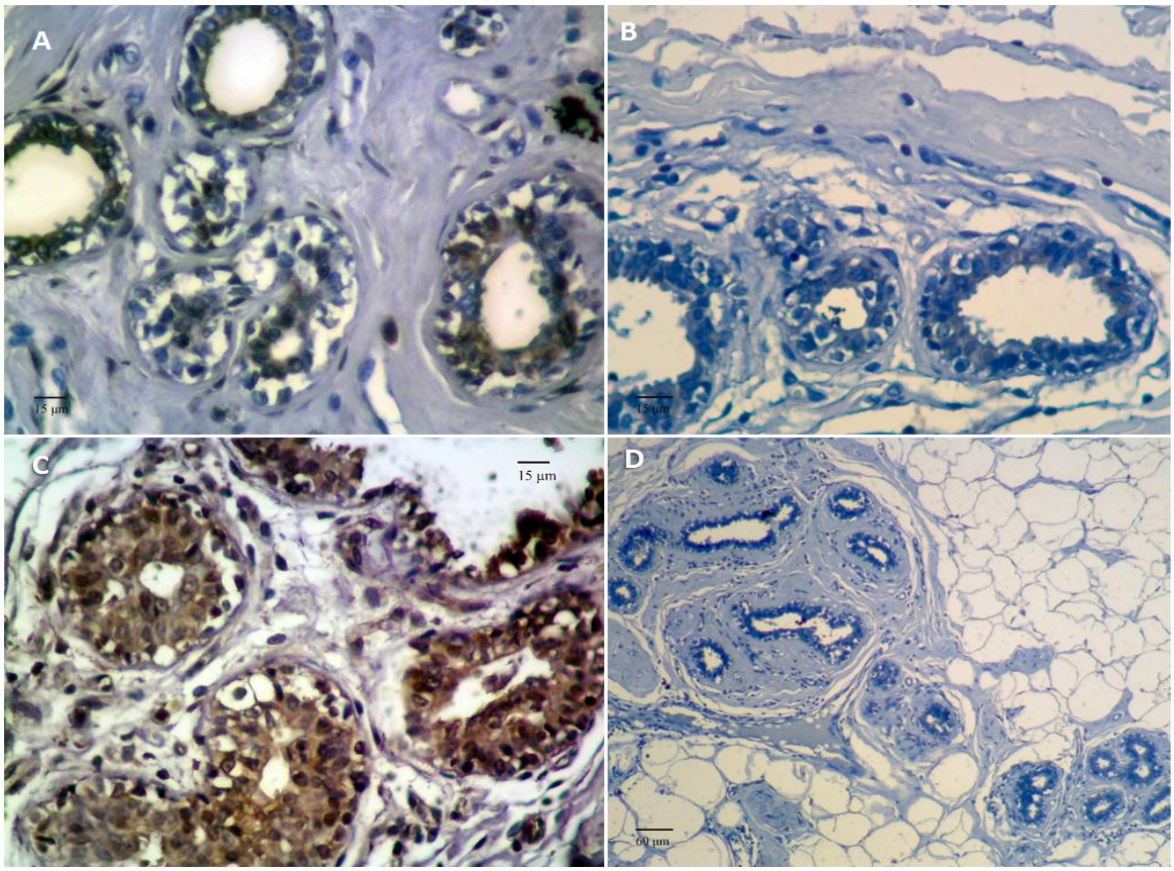

Fig 1: Immunohitochemical expression of different markers in normal breast tissue. (A: positive BCL2 around breast cancer, B: positive ER in normal breast, C: positive C-MYC around cancer, D: Negative ER around cancer)

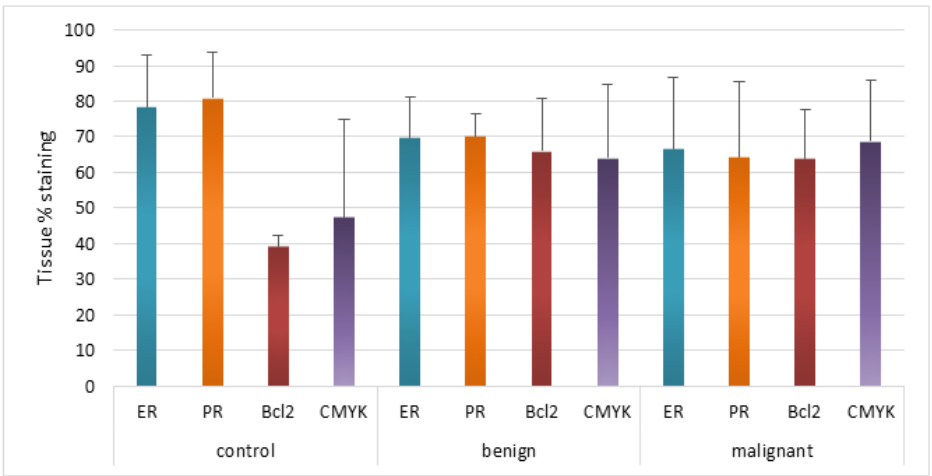

Fig 2: Bar chart showing the percentages of the staining of ER, PR, BCL2 and C-MYC in Control, Benign and malignant groups of breast tissue. (Bars represent mean, error bars represent standard deviation)

4 Discussions

This study was designed to detect presence of ER, PR, BCL2 and C-MYC in normal breast tissue of three proliferative conditions and it revealed that there was presence of ER and PR expression in normal tissue of the breast in any condition that affirm by series of study[12,13] that reported detection of ER, PR in any amount in normal breast tissue.

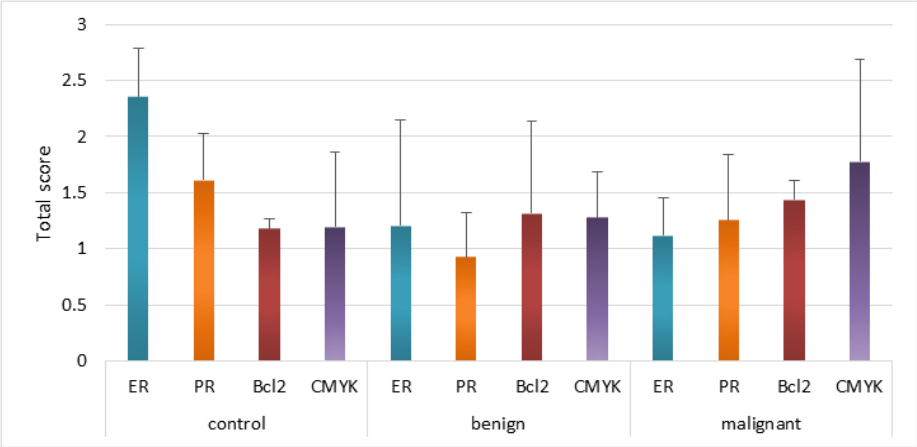

Fig 3: Bar chart showing the immunohistochemical score of ER, PR, BCL2 and C-MYC in Control, Benign and malignant groups of breast tissue. (Bars represent mean, error bars represent standard deviation)

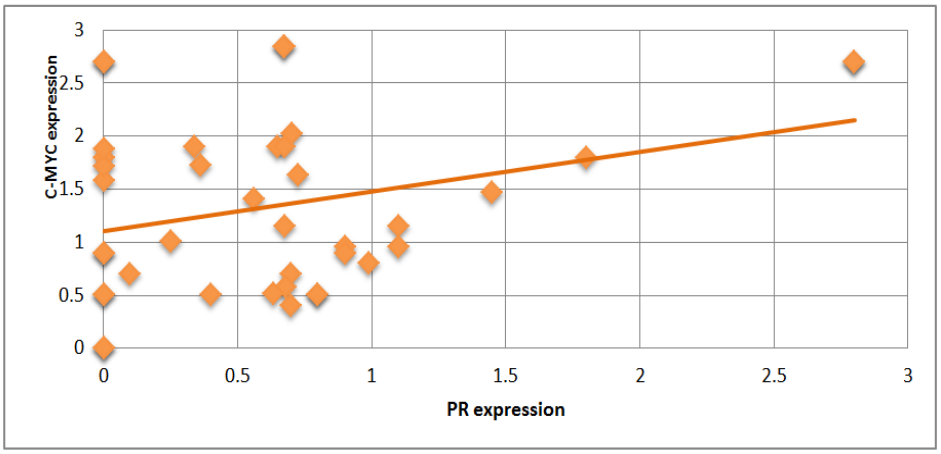

Fig 4: Scatter diagram showing a direct positive correlation of expression between PR & C-MYC markers in breast tissue

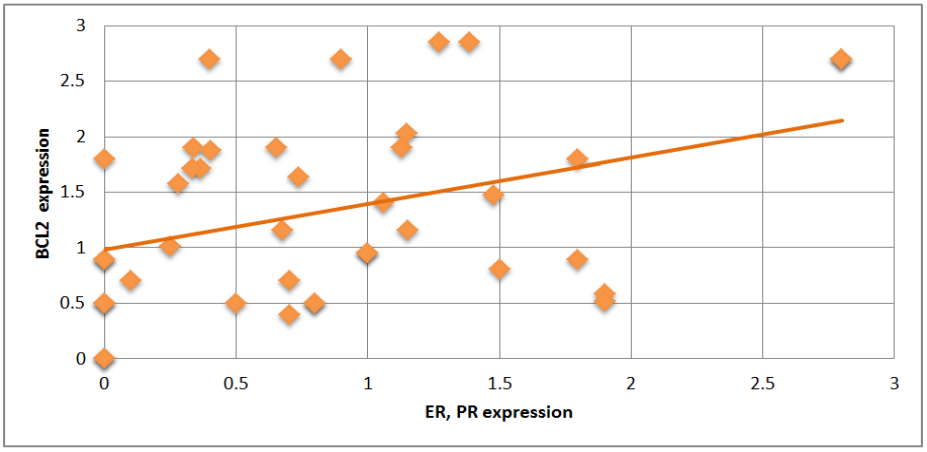

Fig 5: Scatter diagram showing a direct positive correlation of expression between ER & PR with BCL2 markers in breast tissue

Also this study revealed detection of BCL2 in all conditions but it more in normal tissue around malignant that run in same line with other reports[14-16], they all demonstrated there is a significantly higher level of expression of anti-apoptotic protein (BCL2) in the normal tissue around breast carcinoma in comparison to age-matched breast tissue from women without

cancer. In same manner there was an expression of C-MYC in all conditions but it more in malignant condition this agreed with most of studies that mention; C-MYC detected in normal tissue and cancerous from 1-94% of the cases[17-19].

Regarding the correlation, this study found statistically significant direct correlation between BCL2 and ER, PR expression this in concordance of other study[20,21] that reported direct correlation of BCL2 and ER, PR expression in cancer condition than benign. In same line result revealed, there were 60.5% of the cases ER+ had C-MYC overexpression with no significant correlation between them, also there was no effect of ER overexpression that confined with recent study[22] that reported 70.9% of ER+ in C-MYC over expression.

A PR result in this study was; 70% of cases were PR+ with weak direct correlation between them, which confined with other reported of[23] while others, reported negative correlation with PR or didn't find any correlation between C-MYC and PR[24].

5 Conclusion:

C-MYC & BCL2 expressed in all normal breast tissue around proliferative conditions but it more around carcinoma with direct correlation with hormonal receptors.

6 Acknowledgments

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7 Competing Interests

Authors have declared that no competing interests exist.

8 Author's contributions

AFH carried out literature review and histological staining. MMI reviewed the statistical data and finalized the manuscript. BSA gave provided histopathological reviews and helped finalize the manuscript and statistical analysis. All authors read and approved the final manuscript.

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