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Association of Bcl2 Immunohistochemical Expression with Breast Tumors Types

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Abstract*: This is a descriptive case study aimed to study the role of Bcl2 expression in differentiation between malignant and benign breast tumors. Forty paraffin embedded blocks previously diagnosed as breast tumors were collected. Samples include 30(75%) malignant tumors, including invasive ductal carcinoma 27(67.5%) samples, micro papillary carcinoma 1(2.5%) sample, metaplastic squamous cell carcinoma 1(2.5%) sample, and low grade sarcoma 1 (2.5%) sample. And 10(25%) samples were benign tumors, including fibroadenoma 7(17.5%) samples, gynaecomastia 1(2.5%) sample, ductal ectasia 1(2.5%) sample, and granulomatous mastitis 1(2.5%) sample. One section of 3µm thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (modified new indirect method) for detection of Bcl2. Data collected from patients files and results were analyzed using SPSS computer program. The patient's age ranged between 16 and 70 years with mean age of 43 years, most patients were less than 40 years representing 24(60%) and the remaining 16 (40%) patients were more than 40 years. Immunohistochemical expression of Bcl2 was revealed positive result in 14/30 samples and negative result in 16/30 samples in malignant, while all benign tumors gave negative result for Bcl2, with significant statistical association between Bcl2 expression and histopathology diagnosis (P=0.007). 13 breast cancers Bcl2 positive samples, 3(11.1%) samples were grade 1, 3(11.1%) samples were grade11 and 7(25.9%) samples grade111, and negative in 10(37%) samples. With statistical association between Bcl2 expression and grade of cancer (P=0.035). This study concludes that there is association between Bcl2 expression and malignant tumors of breast. There is association between Bcl2 expression and the grade of cancer.*

Keywords— Bcl2; breast tumors; Immunohistochemistry.

1. INTRODUCTION

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008 [(1)]. Breast cancer mortality is high in Sudan and most patients are detected at later stages of the disease due to the lack of awareness and absence of screening programs [(2)].

The diagnosis of breast cancer is accomplished by the biopsy of any suspicious lump or mammographic abnormality that has been identified [(3)].

Bcl-2 (B-cell lymphoma 2), encoded in humans by the Bcl2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis specifically considered an important anti-apoptotic protein and is thus classified as an oncogene [(4)].

Apoptosis is a physiological process following which normal cells die after a given number of replications. Tumor cells tend to interfere with this mechanism by activating genes which inhibit apoptosis, one of the main genes limiting apoptosis is Bcl2. Bcl2 expression has been consistently associated with a better prognosis of breast cancer patients [(5),(6)].

Bcl-2 is frequently expressed in normal breast epithelial cells and breast cancer cells, and is known to be upregulated by estrogen [(7)].

Bcl2 expression in breast cancer has been reported to positively correlate with differentiated markers or favorable prognostic factors [(8)].

Bcl2 protein was found within the tumor epithelial cell cytoplasm of 32/46 breast cancer specimens, inter-patient staining was heterogeneous. Immunostaining for steroid hormone receptors was strongly associated with that for the Bcl2 protein, and it is thus possible that this protein. Bcl2 protein was over expressed in 18 of 42 (43%) invasive breast cancers when compared with adjacent normal breast epithelium [(9)].

Over expression of Bcl2 protein in these tumors was associated with higher tumor grade and increased number of positive nodes. In contrast Bcl2protein was over expressed in 19 of 42 tumors (45%) [(10)].

Grade 1 and 2 tumors were almost three times as likely to be Bcl-2 positive (90%) as grade 3 tumors (33%) (P = 0.0057) [(11)].

2. Materials and methods:

2.1 Materials:

Archived tissue blocks obtained from samples cervical tumors were used in this study.

2.2 Methods:

2. 2.1 Study design:

This is a hospital based descriptive retrospective case study aimed to detect expression of bcl-2 tumor marker in breast tumor using immunohistochemical method.

2.2.2 Sample processing:

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Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60˚C oven for 30 minutes

2.2.2.1 Immunohistochemical staining:

The section of 3µm thickness were obtained from formalin fixed paraffin embedded tissue using a rotary microtome, then immunostained using monoclonal antibodies by new indirect technique as follows:

Sections were dewaxed in hot oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then Ag retrieval by water bath retrieval technique for thirty minutes at 97°c (coplin jar containing citrate buffer pH 6.0), then washed in phosphate buffer saline (pH 7.4) for five minutes, then section use circulated by Dako pen, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffered saline (pH 7.4) for five minutes, then treated with anti Bcl-2 (Bcl-2 alpha Ab-1) primary antibody for thirty minutes, then rinsed in phosphate buffered saline (pH 7.4), then treated with secondary polymer conjugated antibody for thirty minutes, then rinsed in phosphate buffer saline (pH 7.4), then treated with DAB for seven minutes, then washed in phosphate buffer saline (pH 7.4) for five minutes, then counter stained in Mayer's haematoxylin for one minutes, then washed and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant [12].

2.2.3 Result interpretation:

Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining.

2.2.4 Data analysis :

Data analysis was done using SPSS 11.5 computer program. Frequencies mean and chi-square test values were calculated.

2.2.5 Ethical consideration:

Sample collected after taking ethical acceptance from hospital administration .

RESULTS

A total of 40 samples collected from patients with breast tumors were investigated, 30(75%) of them were malignant tumors, including invasive ductal carcinoma 27(67.5%) samples, micro papillary carcinoma 1(2.5%) sample, metaplastic squamous cell carcinoma 1(2.5%) sample, low grade sarcoma 1(2.5%) sample. And the remaining benign tumors 10(25%) samples, include fibroadenoma 7(17.5%) samples, gynaecomastia 1(2.5%) sample, ductal ectasia 1(2.5%) sample, granulomatous mastitis 1(2.5%) sample. As indicated in table (1).

The sex of study population revealed that 2(5%) samples were males and 38(95%) samples were females (Table 2).

The description of cancer grade revealed that 13(43.3) samples were grade 1, 5(16.7%) samples were grade 11, 9(30%) samples were grade 111, 3(10%) samples were not graded (Table 3).

The age study population showed that 40 and less years were 24(60%) patient and more than 40 years were 16(40%) patients, (Table 4).

Malignant breast cancer revealed positive expression of Bcl2 in 14(35%) samples and negative expression in 16(40%) samples, while all benign tumor showed negative expression of Bcl2, this result showed significant association (P.value=0.007) (Table 5).

The comparison between Bcl2 expression and the grade of tumor showed that Bcl2 expression was positive in 3(11.1%) samples were grade1, 3(11.1%) samples were grade 11, 7(25.9%) samples were grade 111. And negative in 10(37%) samples were grade 1, 2(7.4%) samples were grade 11, 2(7.4%) samples were grade 111. With insignificant association (P.value=0.035) (Table 6).

Table (1): Histopathology diagnosis among the study samples

Histopathology diagnosis		Frequency	Percent
Malignant	Invasive ductal carcinoma	27	67.5
	Micro papillary carcinoma	1	2.5
	Met plastic squamous cell carcinoma	1	2.5
	Low grade sarcoma	1	2.5
Benign	Fibroadenoma	7	17.5
	Gynaecomastia	1	2.5
	Ductal ectasia	1	2.5
	Granulomatus mastitis	1	2.5
Total		40	100

Table (2): The distribution of sex among study population

Sex	Frequency	Percent
Female	38	95
Male	2	5
Total	40	100

Table (3): Distribution of cancer grade among malignant breast tumors

Grade	Frequency	Percent
Grade 1	13	43.3
Grade 2	5	16.7
Grade 3	9	30
Not graded	3	10
Total	30	100

Table (4): Distribution of age among study population

Age	Frequency	Percent
40 and less	24	60
More than 40	16	40
Total	40	100

Table (5): Relation between BCL2 expression and histopathology diagnosis

Histopathology diagnosis	Bcl2 expression		P. value
	Positive	Negative	
	N (%)	N (%)	
Malignant	14(35)	16(40)	0.007
Benign	0(00)	10(25)	

Table (6): Relation between BCL2 expression and the grade of breast cancer

Grade	Bcl2 expression		P. value
	Positive	Negative	
	N (%)	N (%)	
Grade 1	3(11.1	10(37)	0.035
Grade 2	3(11.1	2(7.4)	
Grade 3	7(25.9)	2(7.4)	

DISCUSSION

In this study forty samples from patients affected with breast tumor were investigated by immunohistochemical method for detection of Bcl2 expression.

The study revealed that the age of the study population range from 16 to 70 years with mean age of 43 years. Most patients were less than 40 years; this is probably due to production of estrogen hormone in this age. This result was agree with Carey *et al*. [13], who reported that the young women generally face more aggressive cancers and lower survival rates. This result was disagreeing with Howlader *et al*. [14], who reported that the risk of getting breast cancer increases with age.

The study revealed that the majority of the study population sex, this result explain males wear less expected than females with breast cancer, this probably due to reproductive risk factors, this result was agree with Anderson, *et al*. [15], who reported that the gender specific incidence trends differed, most likely reflective of female related changes in surveillance and or reproductive risk factors. Also compatible with result observed by Bagley, *et al*. [16], who reported that male breast cancer is an uncommon disease. Because this disease is rare, compared with female breast cancers. Bcl2 is a mitochondrial protein associated with anti apoptotic function over expression of Bcl2 is found to be in a variety tumor due to degradation of Bcl2. In this study over expression of Bcl2 is observed in malignant breast tumors 14/30, while benign breast tumor showed no expression of Bcl2. This relation showed significant association (P.value =0.007), this finding is compatible with result observed by Samantha, *et al*. [17], who reported that Bcl2 expression is associated with malignant condition. Also compatible with result observed by Leek *et al*. [7], who reported that Bcl2 expression, was then compared with the established indicators of prognosis and biological behavior in malignant breast disease.

The study revealed that the Bcl2 expression and grade of cancer showed increase over expression in high grade tumors, this relation showed significant association (P.value=0.035) indicating that rising of cancer grade is affected by the Bcl2 over expression this finding is compatible with result observed by Lipponen *et al*. [18], who reported that the intensity of Bcl2 expression was inversely related to tumors grade (P<0·0001). Also compatible with result observed by Olopade *et al*. [10], who reported that these findings suggest that expression of Bcl2 protein, is increased in a significant fraction of invasive breast cancers. This result was disagreeing with Hellemans *et al*. [19], who reported that no relationship could be observed between Bcl-2 status and tumor grade.

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