RESEARCH ARTICLE

# Immunohistochemical Expression of B Cell Lymphoma-2 with Clinicopathological Correlation in Triple Negative Breast Cancers in Northern Pakistan

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## Abstract

**Background: Triple negative breast cancers (TNBCs) are high grade aggressive tumors generally with a poor prognosis, not responding to hormonal and anti Her2 Neu therapy. Expression of the antiapoptotic B cell lymphoma 2 gene (Bcl-2) is associated with low grade, slowly proliferating hormone receptor positive tumors with improved survival. Anti Bcl2 agents can be used as alternative targeted therapy in triple negative cancers. Materials and Methods: The objective of this study was to determine the immunohistochemical expression of Bcl2 in triple negative breast cancers and any correlation with clinicopathological variables in Northern Pakistan. Results: All 52 patients were females, aged between 28 and 80 years(average 48.0±12.1). 28 cases (53.8%) were positive for Bcl2, this being associated with low grade invasive ductal carcinomas, lymph node metastasis and lymphovascular invasion. Conclusions: Bcl-2 may be an important prognostic factor and its expression might be used for targeted therapy using Anti Bcl2 drugs.**

**Keywords:** Triple negative breast cancers - B cell lymphoma gene 2 - lymphovascular invasion

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## Introduction

Breast cancer is a heterogenous group of diseases which comprises a spectrum of biological subtypes, natural history, and clinicopathological and molecular features, with different prognostic and therapeutic consequences (Ontilio et al., 2009).

Breast carcinoma is the most common cancer affecting the females worldwide. Each year about 1.4 million new cases of breast cancers are diagnosed worldwide (Formenti et al., 2012). Incidence of breast cancer varies in different region of the world. This difference is attributable to diffence in race, genetic differences, cultural differences and environmental exposures which vary in different parts of the world (Kakarala et al., 2010).

In Pakistan, study from Karachi revealed highest incidence of breast cancer for any Asian country except Israel. In Cancer data 1995-1997 from Karachi, the most common malignancy is women was breast cancer (53.1%). Data set from 1998-2002 showed increased incidence 69.1%, which was highest rate recorded in Asia (Shafqat et al., 2011).

Gene expression profiling (GEP) has resulted in classification of breast cancers into five molecular types, which correlates well with treatment and prognosis (Peppercorn J et al., 2008). Two of these types are estrogen receptor (ER) positive (Luminal A & B), and three types are ER negative [Basal like, ErbB2 (Her2) type and normal breast like] (Reis-Filho et al., 2008). In these types basal like breast cancers lack expression for ER,PR and Her2/Neu (Triple negative), has aggressive behavior (Badve et al., 2011), increased chances of metastasis (Fulford et al., 2007; Hicks et al., 2006) and unfavourable prognosis.

Breast cancers which lack expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (Her2/neu) are called triple negative breast cancers (Pala et al., 2012). Triple negative breast cancer (TNBC) accounts for 10-20% of all breast carcinomas

Although most triple negative cancers are of basal type and most tumors expressing basal markers are triple negative, but both these terms are not completely synonymous. According to a study 71% of Triple negative breast cancers were of basal type on gene expression profiling and 77% of Basal like tumors were triple negative breast trumors (Bertucci et al., 2008).

Immunohistochemistry has proved useful but not perfect surrogate for the molecular subtyping of breast cancers. They are classified into four groups based on IHC profile ER/PR and Her2/neu expression which correlate well with the molecular subtypes. These are Luminal A (ER/or PR +, HER-2 –), Luminal B (ER/or PR +, Her-2 +), Basal cell like (ER/PR-, HER-2 –), Erb-B2 tumors (ER/ PR-, HER-2 +) (Ontilio et al., 2009).

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As these triple negative breast cancers donot respond to current therapies targeting Her-2 (transtuzumab) and hormonal treatment (tamoxifen and aromatase inhibitors), chemotherapy is only choice of treatment. This lack of targeted treatment modalities has led to focus on new treatment strategies for these patients by using the knowledge of molecular characteristics of these tumors. New targeted therapies are being evaluated.

Bcl-2 is an anti-apoptotic protein, but high Bcl-2 expression in breast cancers, was associated with low grade, slowly proliferating, hormone receptor positive breast cancers and has been associated with improved survival. Bcl2 is an important prognostic factor and therapies targeted against bcl2 (like BH3 mimetics ABT-737, ABT-199) (Oakes et al., 2012) are currently in clinical trials. These drugs can be used as targeted therapy against Bcl2 positive triple negative breast cancers.

This study also shows the important prognostic factors (Patient's age, tumor size, histologic subtype, lymph node status and lymphovascular invasion) and their correlation with Bcl-2 expression.

## Materials and Methods

It is a cross-sectional study done at department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan over a period of thirteen months from 1st April 2014 to 31st April 2015. Total 52 cases were included in this study. All patients were females. Sampling technique was Non-probability consecutive sampling. The inclusion criteria was the triple negative breast cancers detected by immunohistochemistry diagnosed on mastectomy specimens. The patient's age, histologic type, histologic grade, lymph node status and lympho vascular invasion was retrieved from the histopathological reports. Those cases with incomplete details were excluded from the study.

Formalin fixed paraffin embedded tissue sections of triple negative breast cancer were selected as per inclusion criteria. Immunohistochemistry (IHC) assay for Bcl2 was performed. IHC results were interpreted using a cut off value. Cytoplasmic or membranous staining of 10% or more tumor cells were taken as positive (Callagy G et al., 2003).

Statistical analysis was done using SPSS version 19.0. Descriptive statistics were calculated for both quantitative and qualitative variables.

## Results

During the study a total of 52 cases of triple negative breast cancer were included. The ages of patients ranged from 28 to 80 years with mean age of 48 years and standard deviation of 12.05. In my study, most of the patients belong to 4th and 5th decade of life followed by 6th and 7th decade as shown in figure1. All patients in my study were females. Out of the total cases, 28 cases of triple negative breast cancer (53.8%) showed positivity for Bcl2 while 24 cases (46.2%) were negative as shown in figure2.

Bcl2 expression was further analyzed and compared with various clinicopathological variables including age, tumor size, histological subtype, histological grade, lymph node metastasis, LVI and their results compared with international studies. These findings are summarized in Table-1.

Bcl2 expression was found to be higher in patients younger than 50 years 62.5% than patients aged more than 50 years 40%. Similarly Bcl2 was frequently expressed

**Table 1. Correlation of Bcl2 Expression with Clinicopathological Features of TNBCs**

| Clinicopathological variable | No of cases | Percentage | Bcl-2 Expression | |
|---|---|---|---|---|
| | | | Positive | Negative |
| Age of Patient (n=52) | | | | |
| < 50 years | 32 | 61.5 | 20 (62.5%) | 12 (37.5%) |
| >50 years | 20 | 38.5 | 8 (40%) | 12 (60%) |
| Tumor size (n=52) | | | | |
| <2 cm | 7 | 13.5 | 4 (57.2%) | 3 (42.8%) |
| 2 – 5 cm | 29 | 55.7 | 17 (58.6%) | 12 (41.4%) |
| >5 cm | 16 | 30.8 | 7 (43.7%) | 9 (56.3%) |
| Histologic Subtype (n=52) | | | | |
| Invasive ductal carcinoma | 46 | 88.5 | 27 (58.7%) | 19 (41.3%) |
| Invasive lobular carcinoma | 4 | 7.7 | 1 (25%) | 3 (75%) |
| Metaplastic carcinoma | 1 | 1.9 | 0 | 1 (100%) |
| Medullary carcinoma | 1 | 1.9 | 0 | 1 (100%) |
| Tumor Grade (n=52) | | | | |
| Grade I | 1 | 1.9 | 1(100%) | 0 |
| Grade II | 36 | 69.3 | 21 (58.3%) | 15 (41.7%) |
| Grade III | 13 | 25 | 5 (38.5%) | 8 (61.5%) |
| Not applicable | 2 | 3.8 | 0 | 2 (100%) |
| Lymph Node Metastasis (n=52) | | | | |
| Seen | 32 | 61.6 | 14 (43.7%) | 18 (56.3%) |
| Not seen | 15 | 28.8 | 10 (66.7%) | 5 (33.3%) |
| Not identified | 5 | 9.6 | 4 (80%) | 1 (20%) |
| Lymphovascular Invasion | | | | |
| Seen | 21 | 40.4 | 9 (42.8%) | 12 (57.2%) |
| Not Seen | 31 | 59.6 | 20 (64.5%) | 11 (35.5%) |

in smaller sized tumors 57.2% and 58.6% for tumors less than 2cm and 2-5 cm respectively as compared to tumor measuring more than 5cm which showed 43.7% expression.

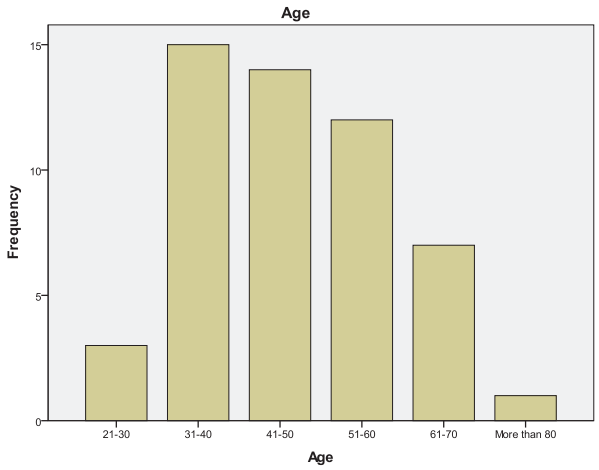


**Figure 1. Bcl2 Expression with Age Distribution in Decades (n = 52)**

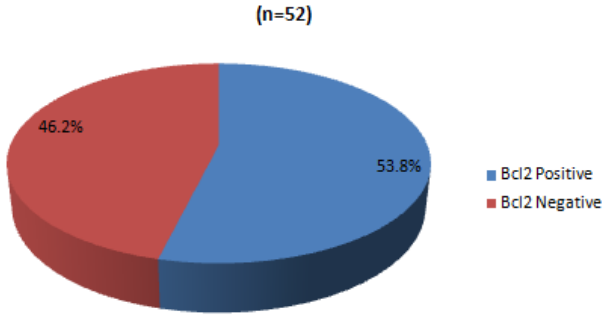


**Figure 2. Bcl2 Expression Results (Total n=52)**

Bcl2 expression was higher in invasive ductal carcinoma (58.7%), as compared to invasive lobular carcinoma (25%), medullary (0%) and metaplastic carcinoma (0%).

There was higher Bcl2 expression in low grade I, II breast cancers 100%, and 58.3% respectively as compared to high grade III cancers (38.5%). Bcl2 was also not expressed in single cases of medullary and metaplastic carcinoma.

Breast cancers with lymph nodes metastasis showed lower Bcl2 expression 43.7% as compared to breast cancers without lymph node metastasis 66.7%. Similarly breast cancer with lymphovascular invasion showed lower Bcl2 expression (42.8%) as compared to those without lymphovascular invasion (64.5%).

## Discussion

As no study in Pakistan is available for comparison. By comparing the findings in my study with few international studies the mean age of presentation was 48±12.05 compared with that of 54 years (Ryu and Lee, 2012), 48.9 years (Alikanoglu et al., 2014) and 52 years (Tawfik et al., 2012), indicating occurrence of disease at relatively younger age in our population. The TNBCs were most common in 4th & 5th decade of life with Bcl2 expression occurring mostly in younger women (<50 years). In my study 62.5% of cases were below 50years out of all Bcl2 positive cases. This is in comparison with other studies 81.3% by Abd El-Hafez et al. (2013).

By comparing the histological subtypes of breast cancers it was found that expression of Bcl2 was higher in

**Table 2. Comparison with International Studies**

| | Abd El-Hafez et al (n=23) | Tawfik et al (n=124) | Ryu et al (n=94) | Alikanoglu et al (n=20) | Zubair et al (n=52) |
|---|---|---|---|---|---|
| Mean Age (yrs) | | 51.5 | 54.2 | 48.9±12.3 | 48±12.05 |
| <50 years | 16 (69.6%) | - | - | - | 32 (61.5%) |
| >50Years | 7 (30.4%) | - | - | - | 20 (38.5%) |
| Histological Subtype | | | | | |
| Invasive ductal carcinoma | 20 (86.9%) | 110 (88.7%) | - | - | 46 (88.5%) |
| Invasive lobular carcinoma | 3 (13.1%) | 2 (1.6%) | - | - | 4 (7.7%) |
| Others | 0 | 12 (9.7%) | - | - | 2 (3.8%) |
| Tumor Grade | | | | | |
| I | | 2 (1.6%) | 6 (8.3%) | 0 | 0 |
| II | 16 (69.6%) | 24 (19.4%) | 20 (27.8%) | 4/15 (26.7%) | 36 (69.3%) |
| III | 7 (30.4%) | 98 (79%) | 46 (63.9%) | 11/15 (73.3%) | 14 (26.9%) |
| Not applicable | | | | | 2 (3.8%) |
| Nodal Status | | | | | |
| Positive | 19 (82.6%) | 40 (32.2%) | 47 (50%) | - | 32 (61.6%) |
| Negative | 4 (17.4%) | 55 (44.4%) | 47 (50%) | - | 15 (28.8%) |
| Not identified | - | 29 (23.4%) | - | - | 5 (9.6%) |
| Tumor Size | | | | | |
| <2cm | 4 (17.4%) | Mean 2.45 cm | 37 (39.4%) | - | 7 (13.5%) |
| 2-5 cm | 19 (82.6%) (>2cm) | - | 44 (46.8%) | - | 29 (55.7%) |
| >5 cm | | - | 13 (13.9%) | - | 16 (30.8%) |
| Bcl2 Expression | | | | | |
| Positive | 17 (73.9%) | 38 (30.6%) | 26 (27.7%) | 18 (90%) | 28 (53.8%) |
| Negative | 6 (26.1%) | 86 (69.6%) | 68 (72.3%) | 2 (10%) | 24 (46.2%) |

Invasive ductal carcinoma (58.7%) followed by invasive lobular carcinoma (25%), which is in comparison with 85% and 30.9% in studies by Abd El-Hafez et al. (2013), and Tawfik et al. (2012), respectively.

High grade breast cancers showed lower expression for Bcl2 (38.5%) in this study, which is in comparsion with international studies 57.1%, and 26.5% by Abd El-Hafez et al. (2013), and Tawfik et al. (2012), respectively. These findings show that expression of Bcl2 decreases with higher grade of breast cancer.

Lymph node metastases were associated with lower expression of Bcl2 (43.7%) than breast cancer without lymph node metastasis (66.7%). Similar findings were seen in study by Abd El-Hafez et al. (2013), to be 68% and 100% expression in breast cancers with and without nodal metastasis. In study by Tawfik K et al. (2012), 24% and 50% expression was seen with and without lymph node metastasis.

Overall expression of Bcl2 in this study was 53.8% in comparison to 74% (Abd El-Hafez et al., 2013), 90% (Alikanoglu et al., 2014), 30.6% (Tawfik et al., 2012), and 27.7% (Ryu and Lee, 2012) in different international studies. These findings are summarized in table-2. The difference in expression of Bcl2 in various studies might be due to the differences in sample size, methodology used and types of cases selected for study.

Triple negative breast cancers are challenging because of unresponsiveness to hormonal therapy. These are high grade tumors with poor prognosis. Bcl2 is an important prognostic factor and its expression in TNBCs is associated with small sized, low grade breast cancers with less chances of lymph node metastasis and lymphovascular invasion. Due to limited options, novel targeted therapies should be developed for treatment of such cases. Such Bcl2 positive triple negative breast cancer patients can be benefitted by targeting this important pathway using various anti Bcl2 drugs.

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