CORRELATION OF PROTEIN EXPRESSION BETWEEN APOPTOTIC REGULATORS BCL-2 AND BAX WITH HER-2/NEU ONCOGENE IN BREAST CARCINOMA

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Abstract: Breast cancer is one of the commonest malignancies effecting women worldwide. It has been estimated that 1 million new cases every year account for about nearly 22.7% of all cancer in women. It is evident from the data that there is an increased rates of breast cancer mostly in urban population worldwide as well as in India. The present study is focusing on the correlation of protein expression between apoptotic regulators Bcl-2 and Bax with Her-2/neu oncogene in breast carcinoma to understanding their interactions in the development of breast cancer so that they can be used them collectively for determining prognosis, therapeutic of breast cancer. The present study was retrospective study in which 165 cases of primary breast tumor site which were diagnosed cases of breast cancer were included. The specimens were processed and paraffin blocks were prepared for histological examination and IHC expression of Bcl-2, Bax and Her-2, Further the Bcl-2 and Bax were correlated with expression of Her-2. The result shows that In positive Her-2 cases 25.97% cases
were positive for Bcl-2 while rest 74.03% cases were negative for Bcl-2. Similarly, Her-2/neu negative cases, 87.50% (77/88) cases were positive for Bcl-2. while 12.50% cases were negative for Bcl-2. Thus, expression of Her-2/neu and Bcl-2 was inversely correlated ($p<0.05$) . Further, Bax expression is correlated with Her -2 expression in which In positive Her-2 case, 55.84% cases were positive for Bax, while rest 44.16% cases were negative for Bax expression. Similarly, amongst 88 Her-2/neu negative cases, 5.68% cases were positive for Bax, while 94.32% cases were negative for Bax expression. Thus, expression of Her-2/neu and Bax was positively correlated ($p=0.09$). It may be concluded that the apoptotic regulators Bcl-2 and Bax and onco-protein Her-2 are correlated with each other and they may be used as candidate for better prognosis and therapeutics of breast cancer.

Keywords: Bcl-2, Bax, Her-2, IHC, breast cancer.

Introduction: Breast cancer is one of the common malignancies effecting women worldwide. It has been estimated that 1 million new cases every year account for about nearly 22.7% of all cancer in women [1]**.** while according to the National cancer registry 2006 is the second most common cause due to cancer of death of females in India. The age standardized incidence rate of breast cancer worldwide is 35.6 per 100 000 females, but rate vary substantially across countries from region to region and even among racial ethnic group within a country. Incidence rate in India is thought to be around 29.1/1, 00,000 [2]. It is evident from the data that there is an increased rates of breast cancer mostly in urban population worldwide as well as in India [3-4]. . This may be due to various reasons which may include lack of early detection or awareness, may contribute to advance presentation, Further, biologically aggressiveness related to poor differentiation. The various receptor and genes involving apoptosis, differentiation, DNA repair mechanism etc. are much more tendencies to affect young population remains to be unexplained. This may interns to alteration in the regulatory proteins and any alteration or deregulation in the genes controlling these activities with the other genetic as well as environment al factor may lead to breast cancer**.** In the present study we are focusing on two apoptotic regulatory proteins (Bcl-2, Bax) and its correlation with an onco- protein Her -2. The Bcl-2 is the main anti-apoptotic regulator and Bax is the pro-apoptotic regulator Earlier studies shows that these genes have been implicated in pathogenesis of breast cancer. Earlier studies have been reported to understand the importance of these alterations in the development, progression of the disease. These genes may be proposed as candidate potential prognostic markers for clinical utility [5-12]. The present study is focusing on the correlation of protein expression between apoptotic regulators Bcl-2 and Bax with Her-2/neu oncogene in breast carcinoma to understanding their interactions in the development of breast cancer so that they can be used them collectively for determining prognosis, therapeutic of breast cancer.

Material and Methods: The present study was a retrospective study which was conducted in the Department of Pathology, Maulana Azad Medical College, New Delhi. In the study a total of 165 diagnosed cases with primary breast cancer (IDC, NOS) were included. The tissue sample required for the presents study was retrieved from paraffin blocks, which were prepared from the primary breast tumor region. The patients with the history of tumor recurrence or prior radiation exposure or on neo

adjuvant therapy were excluded from the present study. The adjacent region of the tumor tissue which was non- malignant was taken as control for the study.

The Hematoxylin & eosin staining was performed to make histological diagnosis as well as the histological grading was done by using Scarf-Bloom-Richardson grading system. Further, the expression of Bcl-2, Bax and Her-2 was done by using Immunohistochemistry method. The primary antibodies used were Bcl 2 & Bax (Purified mouse anti-human Monoclonal antibody, DAKO, USA); Her 2/neu, Purified mouse anti-human Monoclonal antibody, Secondary antibody, Tertiary antibody, DAB (Novacastra, USA).The Immunohistochemical stains were performed using Avidin – Biotin technique. Whole tumor area was observed and overall percentage positivity of tumor cells for Bcl-2, Bax and Her-2/neu was counted under x400 magnification and the expression of Bcl-2 and Bax were correlated with her -2 expression.

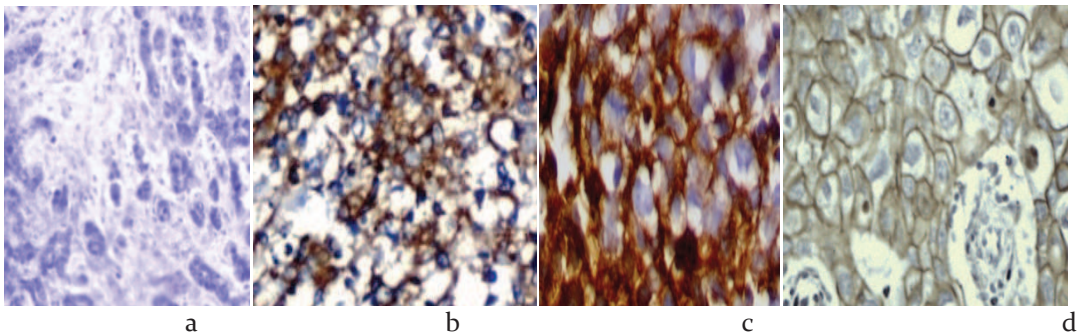
Results: The immunohistochemical expression of two apoptotic regulator genes, anti-apoptotic Bcl-2 & pro-apoptotic Bax was correlated separately with oncogene Her-2/neu.

Correlation between immunohistochemical expressions of anti-apoptotic Bcl-2 with Onco-protein Her-2/neu .The Results Are Summarized In The Table Given Table 1)								
Histopathology & Immunohisto chemistry: Bcl-2 Expression (n=165)	**Grade I (n=35)**		**Grade II (n=91)**		**Grade III (n=39)**		**Total Cases (n=165)**	
	Her-2 + ve (14)	Her-2 –ve (21)	Her-2 +ve (41)	Her-2 –ve (50)	Her-2 +ve (22)	Her-2 –ve (17)	Her-2 +ve (77)	Her-2 –ve (88)
Bcl-2 +ve (n=97)	09	20	09	48	02	09	20	77
Bcl-2 -ve (n=68)	05	01	32	02	20	08	57	11

Table 1: Correlation of protein expression between anti-apoptotic Bcl-2 with Her-2/neu expression (n=165).

The Correlation between immunohistochemical expression of anti-apoptotic Bcl-2 with onco-protein Her-2/neu. The results are summarized in the table given below: (Table 1)

Out of 77 positive cases of Her-2/neu, 25.97% (20/77) cases were positive for Bcl-2 while rest 74.03% (55/77) cases were negative for Bcl-2. Similarly, amongst 88 Her-2/neu negative cases, 87.50% (77/88) cases were positive for Bcl-2. While 12.50% (11/88) cases were negative for Bcl-2. Thus, expression of Her-2/neu and Bcl-2 was inversely correlated ($p<0.05$) (Table 1). Further, Immunostaing of Her-2/neu was again correlated with Bcl-2 expression in different grades. By looking into the (Table 1), we may infer that Her-2/neu and Bcl-2 are significantly inversely correlated in grade II cases ($p<0.05$), while for grade I and grade III cases there was no significant correlation ($p=0.15$).



a b c d

The Correlation between immunohistochemical expression of pro-apoptotic Bax with onco-protein Her-2/neu. The results are summarized in the table2

Table 2: Correlation between immunohistochemical expressions of pro-apoptotic Bax with onco-protein Her-2/neu expression (n=165).

Out of total 77 positive cases of Her-2/neu, 55.84% (43/77) cases were positive for Bax, while rest 44.16% (34/77) cases were negative for Bax expression. Similarly, amongst 88 Her-2/neu negative cases, 5.68% (05/88) cases were positive for Bax, while 94.32% (83/88) cases were negative for Bax

expression. Thus, expression of Her-2/neu and Bax was positively correlated (p=0.09) (Table 2). Further, Immunostaing of Her-2/neu was again correlated with bax expression in different grades. By looking in to the Table 1 we may infer that Her-2/neu and Bax are significantly positively correlated in grade II cases (p<0.05), while from grade I and grade III cases there was no significant correlation. (p=0.23).

Table 1: Correlation between immunohistochemical expressions of anti-apoptotic Bcl-2 with Onco-protein Her-2/neu expression (n=165).

Bax expression (n=165)	**Grade I (n =35)**		**Grade II (n=91)**		**Grade III (n=39)**		**Total Cases (n=165)**	
	Her-2 +ve (14)	Her-2 –ve (21)	Her-2 +ve (41)	Her-2 –ve (50)	Her-2 +ve (22)	Her-2 –ve (17)	Her-2 +ve (77)	Her-2 –ve (88)
Bax +ve (n=48)	06	02	33	02	04	01	43	05
Bax –ve (n=117)	08	19	08	48	18	16	34	83

Fig. IHC expression of (a) Control (b) Bcl-2 (c)Bax (d) Her-2 IHCx 400x.

(b) Correlation of protein expression between pro-apoptotic Bax with Her-2/neu;

Discussion: In the present study along with apoptotic regulators, Bcl 2 and Bax, other gene which we studied was Her-2/neu, an oncogene, which has been found to be most commonly associated with breast cancer pathogenesis as well as prognosis [13]. In the present study, overall expression rate of Her-2 /neu was 46.66%. This was slightly higher than as seen in most of other studies where they have reported its expression from 10 to 40% [13-14]Immunohistochemical expression of the two apoptotic regulators (Bcl-2 and Bax) were correlated with her /*neu* expression. In the present study 79.38 % (77/97) of Bcl-2 positive cases were negative for Her 2/*neu* and 83.82% (57/68) of Bcl 2 negative cases were positive for Her-2/*neu*. Only 20.62% (20/97) Bcl-2 positve cases were also positive for Her-2/*neu* and 16.18% (11/68) of Bcl-2 negative cases were also negative for Her-2/*neu* (Table 1). Thus, there was inverse correlation between expression of Bcl-2 and Her-2/*neu*. In the study of Binder C et al, 1995; 64% of the Her-2/neu negative tumors showed immunostaining for Bcl-2 (57/89), whereas only 45% (20/44) of Her-2/neu positive ones were also Bcl-2-positive (p=0.041), which was similar to our findings. In another studies by Rehman S et al, in 2000 and Al-Moundhri M et al, in 2003, reported no association between Her 2/neu and Bcl-2 expression. In our study, the inverse correlation between Her 2/neu and Bcl-2 expression was also seen in grade II and grade III tumors while grade I tumors did not show such inverse correlation. This lack of inverse correlation in grade I tumors may be due to insignificant association between Her 2/*neu* expression and histological grade. Similarly, bax expression was also correlated with Her-2/neu expression.In the present study 89.58 % (43/48) of Bax positive cases were also positive for Her- 2/*neu* and 70.94% (83/117) of Bax negative cases were also negative for Her- 2/*neu*. Only 10.42 %(5/48) Bax positive cases were negative for Her-2/*neu* and 29.06 (34/117) % of Bax negative cases were positive for Her-2/*neu* (Table 2). Thus, there was positive correlation between expression of Bax and Her 2/*neu*. In a study Binder C et al, (1995), showed positive correlation between Bax and Her-2/*neu*. This result was similar to our finding. Similar correlation was also reported by Joenssu H et al,1994; Doglioni C et al, 1994; Leek RD et al, 1994**.** In another study by Rehman S et al, in 2000 reported no association between Her-2/*neu* and Bax expression. The positive correlation between Bax and Her 2/*neu* expression was also seen in histological grade I and grade II tumors while grade III tumors did not show such positive correlation in our study. The non-significant correlation of Bax as well as Her- 2/*neu* expression with histological grade may be the reason for lack of association between Bax and Her-2/*neu* expression and histological grade. The present study shows significant association of immune histo chemical expression of Bcl-2, Bax and Her-2/*neu* with breast cancer. Bcl-2 and Her-2/neu also showed significant correlation with level of differentiation of breast cancer (i.e. histological grade), Although, Bax showed variable results. These findings are similar to most of the other studies. The apoptotic regulator (Bcl-2 and Bax) also correlates with Her-2/*neu*. Therefore, similar to Her-2/*neu*, Bcl-2 and Bax may also be consider a probable target for breast carcinoma management. However, for this large cohort study is required involving apoptotic regulators in breast cancer.

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