Chapter

Bcl-2 Immunoexpression in Invasive Ductal Carcinoma and Its Evaluative Correlation with Molecular Sub-Types and BR-Grade and TNM Stage

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Abstract

Invasive Ductal carcinoma is the most common histological type of breast cancer. It constitutes about 80 percent of all breast cancer diagnoses. The molecular pathogenesis of breast cancer involves multiple gene types. Bcl-2 is one of them. Bcl-2, is an anti-apoptotic protein which is up regulated by oestrogen in breast cancer patients. The immunoexpression of Bcl-2 detection is being carried out by immunohistochemical methods as described in many published studies. Bcl-2 as is known acts through transcriptional induction in pathogenesis of breast cancer. The present chapter describes the role of Bcl-2 in pathogenesis, significance and its relationship with BR Grade and TNM stage. The present chapter specifically describes its observation of Bcl-2 immunoexpression and relationship with molecular subtypes of breast carcinoma.

Keywords: Bcl-2, breast cancer, BR grade, TNM stage, molecular sub-types

1. Introduction

The Breast cancer has become great concern for global health scenario and health providers [1]. The incidence of it has surpassed the cervical cancers in Indian female [2]. The world over laboratory physicians across the world are engaged in assessing new and novel prognostic and predictive markers that would bring about best possible outcome at breast cancer treatment. The modern day practice of onco-pathology revolves more around predictive prognostic markers that would enable the appropriate adjuvant therapies and management of cancer. The challenges in breast cancer management is to predict its prognostic outcome, benefits of adjuvant therapy, surgical management and immunotherapy.The another challenge in breast cancer treatment is to understand molecular defect and thereby assessment of prognosis, and corrective therapies that would involute the primary tumour as well as metastasis [1, 2].

The conventional pathological prognostic factors in breast cancers which were until relied heavily were lymph node status, tumour size, tumour stage, tumour grade, Nottingham prognostic index and many others [2, 3].

With advent in understanding of pathogenesis of breast cancer many cell surface molecules, cytoplasmic signalling pathways, the nuclear transcriptional activities and many others have come under scanner which relates with breast cancer prognosis and treatment outcomes, especially with chemotherapeutic interventions and monoclonal antibody therapies [4].

Among many such families of the genes, Bcl-2 has been studied extensively for its commonality at participation in the pathogenesis of solid tumours especially the cancers of breast, prostate, lung, colo-rectum and ovaries [5, 6].

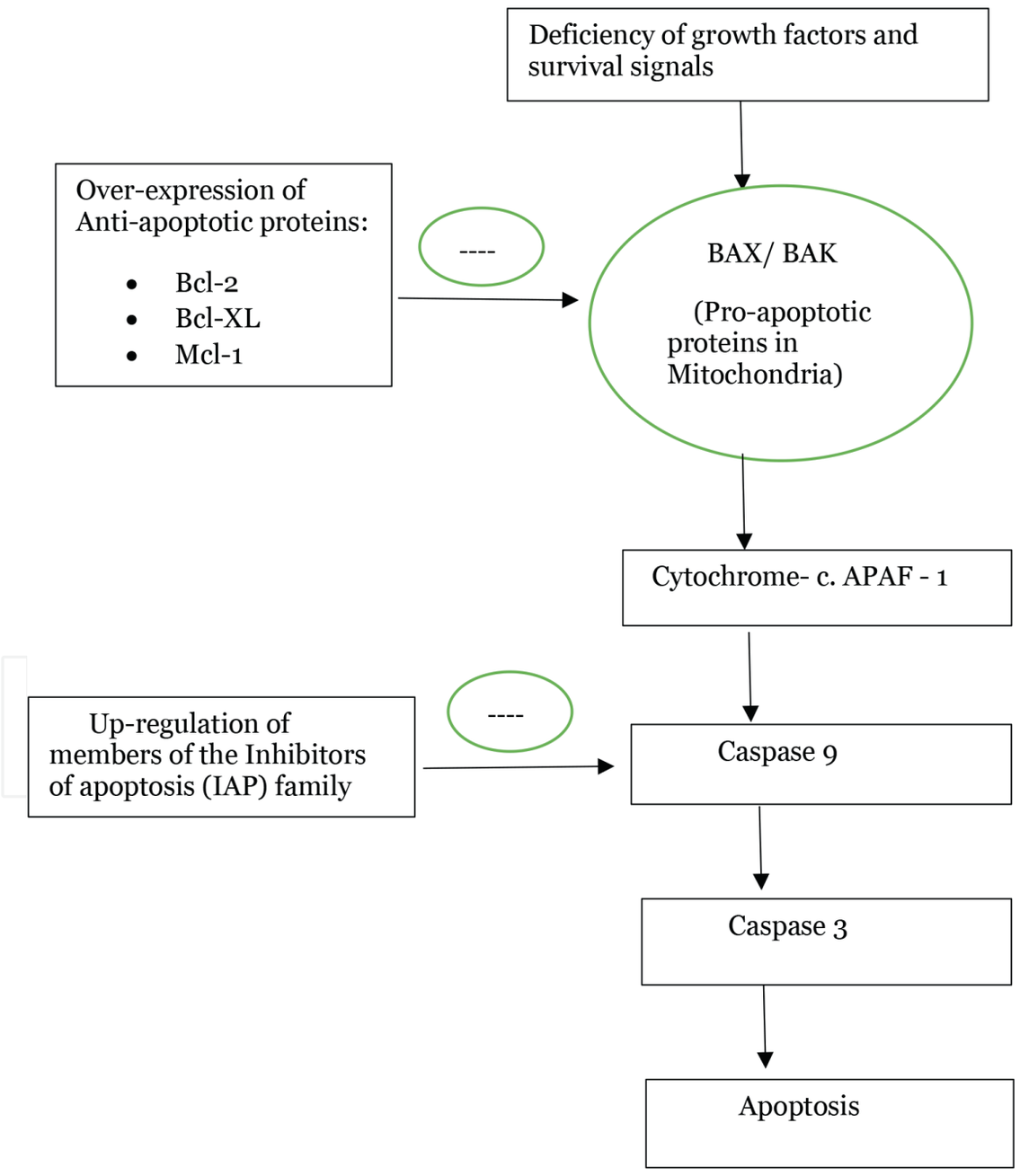

Figure 1.
Flowchart – Evasion of cell death.

Bcl-2 is an anti-apoptotic protein normally expressed in mammary tissue and is up-regulated by oestrogen in breast cancer through direct consequence of transcriptional induction.

Bcl-2 is a principle member of anti-apoptotic proteins along with Bcl-XL and MCL-1. The release of pro-apoptotic proteins in the cells such as cytochrome-c is through the integrity of the mitochondrial outer membrane. This is tightly controlled by Bcl-2 family of proteins. Bcl-2 is overexpressed due to chromosomal translocations and certain mutational changes. Bcl-2 protein also resides in the cytosol and ER membranes. Impermeability by Bcl2 protein prevents the leakage of cytochrome and thereby limits the process of apoptosis. The one way, Bcl-2 genes and their proteins plays an important role in intrinsic pathway of apoptosis [7]. Therefore Bcl-2 genes is one of the genes which is at the centre stage in the pathogenesis of the breast cancer (**Figure 1**).

A few studies in published literature did correlation between Bcl-2 immunoexpression and clinico-pathological variables, disease free survival, prognostic factors, Nottingham prognostic index, TNM stage and treatment outcome [8, 9]. A few studies have proposed that Bcl-2 expression be considered as a molecular subtype of invasive ductal carcinoma because of clinical implications [10, 11].

The search for publications over this topic originating in India was found to be marginal which correlated clinicopathological variables, molecular subtypes of breast cancer and BR-grades [12, 13].

The Bcl-2 expression as published in the western literature have shown its predictive utility and therefore its inclusion in the reporting of histopathology is considered as an essential component [14, 15]. Detecting Bcl-2 immunoexpression in the tumour cell therefore create a frame for appropriate treatment and management of invasive ductal carcinoma.

2. Methodology

The chapter includes the observation on Bcl-2 immunoexpression in invasive ductal carcinoma and its evaluative correlation with BR grade, TNM stage and molecular subtypes as gathered from published literature. Most of the published literature detected Bcl-2 immunoexpression by immunohistochemistry performed on paraffin sections of breast lumps diagnosed as invasive ductal carcinoma.

The authors of the present chapter adopted the methodology for performing the immunohistochemistry in demonstration of Bcl-2, ER, PR and Her 2 on paraffin tissue section as described in the previous studies [6].

The present study included 50 cases whose complete demographic details, clinical examination of the breast lumps, relevant clinical examination, mastectomy details, gross examination finding of specimen, subsequent tissue diagnosis, BR grading, and TNM staging was carried out. The work included only those cases of invasive ductal carcinoma whose complete clinical records and follow up of at least 6 months were available.

The studies whose results are a part of the present chapter performed immunohistochemistry by standard methods meant for it, in detection of ER, PR, Her 2 nu and Bcl-2. The results and interpretation of positivity and score of immunohistochemistry for Bcl-2 and ER, PR, Her 2 were aligned.

The statistical tests used for comparison of the results contained in the present chapter were similar as performed by the authors of other studies.

3. Short review, results and discussion

The chapter contributor's work in this field over the 50 cases of invasive ductal carcinoma is depicted in a tabular forms below.

The Bcl-2 immunoexpression was seen in 33 of 50 cases (66%). There were 30 (60%) women who were below age of 51 and 20 (40%) were 51 and above years. The age versus Bcl-2 immunoexpression is charted in **Table 1**.

Of the 30 women who were below age of 51 showed Bcl-2 immunoexpression on 21 instances (70%) while 12 of 20 (60%) showed Bcl-2 immunoexpression in women more than 50 years of age. The youngest patient of invasive ductal carcinoma was 26 years while oldest one was 84 years. The immunoexpression of Bcl-2 was observed to be 100% in women of invasive ductal carcinoma in between the age of 31 to 40 years.

The distribution of Bcl-2 immunoexpression across BR grade is shown in **Table 2**.

It was observed that Bcl-2 immunoexpression is independent of BR grade.

TNM stage and immunoexpression in 50 cases of invasive ductal carcinoma is shown in **Table 3**.

The TNM stage of invasive ductal carcinoma when plotted against Bcl-2 immuno-expression revealed 15 Bcl-2 immunoexpressions in 20 cases of stage II disease and 7

Age Range	No. of cases/ Percentage	Bcl-2 Immuno-expression				Total No. Cases showing Positive Bcl-2 Expression/ Percentage
		Negative	1(+)	2(++)	3(+++)	
21–30 years	03(06%)	01	01	—	01	02(66.6%)
31–40 years	07(14%)	—	01	03	03	07(100%)
41–50 years	20(40%)	08	04	05	03	012(60%)
51–60 years	11(22%)	03	04	02	02	08(72.7%)
61–70 years	05(10%)	02	01	01	01	03(60%)
71–80 years	03(06%)	02	01	—	—	01(33.3%)
81–90 years	01(02%)	01	—	—	—	00(0%)
Total (21–90) years	50 (100%)	17	12	11	10	33(66%)

Table 1.
Bcl-2 Immuno-expression and age range of invasive ductal carcinoma.

BR-Grade	No. of cases/ Percentage	Bcl-2 Immuno-expression				Total No. Cases showing Positive Bcl-2 Expression/ Percentage
		Negative	1(+)	2(++)	3(+++)	
Grade I	12(24%)	05	—	05	03	08(66.6%)
Grade II	29(58%)	08	07	06	07	20(68.9%)
Grade III	09(18%)	04	05	—	—	05(55.5%)
Total	50(100%)	17	12	11	10	33(66%)

Table 2.
Bcl-2 Immuno-expression and BR-grade.

TNM Stage	No. of cases/ Percentage	Bcl-2 Immuno-expression				Total No. Cases showing Positive Bcl-2 Expression/ Percentage
		Negative	1(+)	2(++)	3(+++)	
Stage I	17(34%)	07	01	04	05	10(58.82%)
Stage II	20(40%)	05	06	06	03	15(75%)
Stage III	09(18%)	02	04	01	02	07(77.7%)
Stage IV	04(08%)	03	01	—	—	01(25%)
Total	50(100%)	17	12	11	10	33(66%)

Table 3.
Bcl-2 Immuno-expression and TNM stage.

Molecular Sub-type	No. of cases/ Percentage	Bcl-2 Immuno-expression				Total No. Cases showing Positive Bcl-2 Expression/Percentage
		Negative	1(+)	2(++)	3(+++)	
Luminal A	18(36%)	05	04	07	02	13(72.2%)
Luminal B	11(22%)	03	03	01	04	08(72.7%)
Triple negative breast cancer (TNBC)	13(26%)	07	02	01	03	06(46.15%)
Her- 2 enriched	08(16%)	02	02	02	01	05(62.5%)
Total	50(100%)	17	12	11	10	33(66%)

(TNBC, Triple negative breast cancer).

Table 4.
Bcl-2 Immuno-expression and molecular sub-type.

of the 9 stage III disease thus the comparisons of Bcl-2 immunoexpression in between TNM stages was found to be non-specific.

The molecular subtype of invasive ductal carcinoma and its relationship with Bcl-2 is shown in **Table 4**.

It was observed that Bcl-2 immuno-expression by percentage was more in Luminal A molecular subtype of invasive ductal carcinoma followed by Luminal B and Her2 enriched (**Figure 2**).

The higher frequency of correlation between the Bcl-2 immunoexpression with molecular subtype Luminal A of breast cancer as observed in present study is attributed to oestrogen up regulating of Bcl-2 imuunoexpression. The higher frequency of immunoexpression of Bcl-2 with molecular subtype of Luminal A of breast cancer too has been observed in the other studies.

The p-value distribution of the various studies for relationship between molecular subtype of invasive ductal carcinoma and Bcl-2 immunoexpression is shown in **Table 5**.

The studies depicted in **Table 5** concluded of evidences of Bcl2 immuno-expression correlates well with molecular subtype of Luminal A of invasive ductal carcinoma to which the contributors of present chapter agree.

The included studies for their observations are cited below paragraphically explaining about Bcl-2 immuno-expression and its evaluative correlation with BR Grade, TNM stage and molecular subtypes of invasive ductal carcinoma including comparisons.

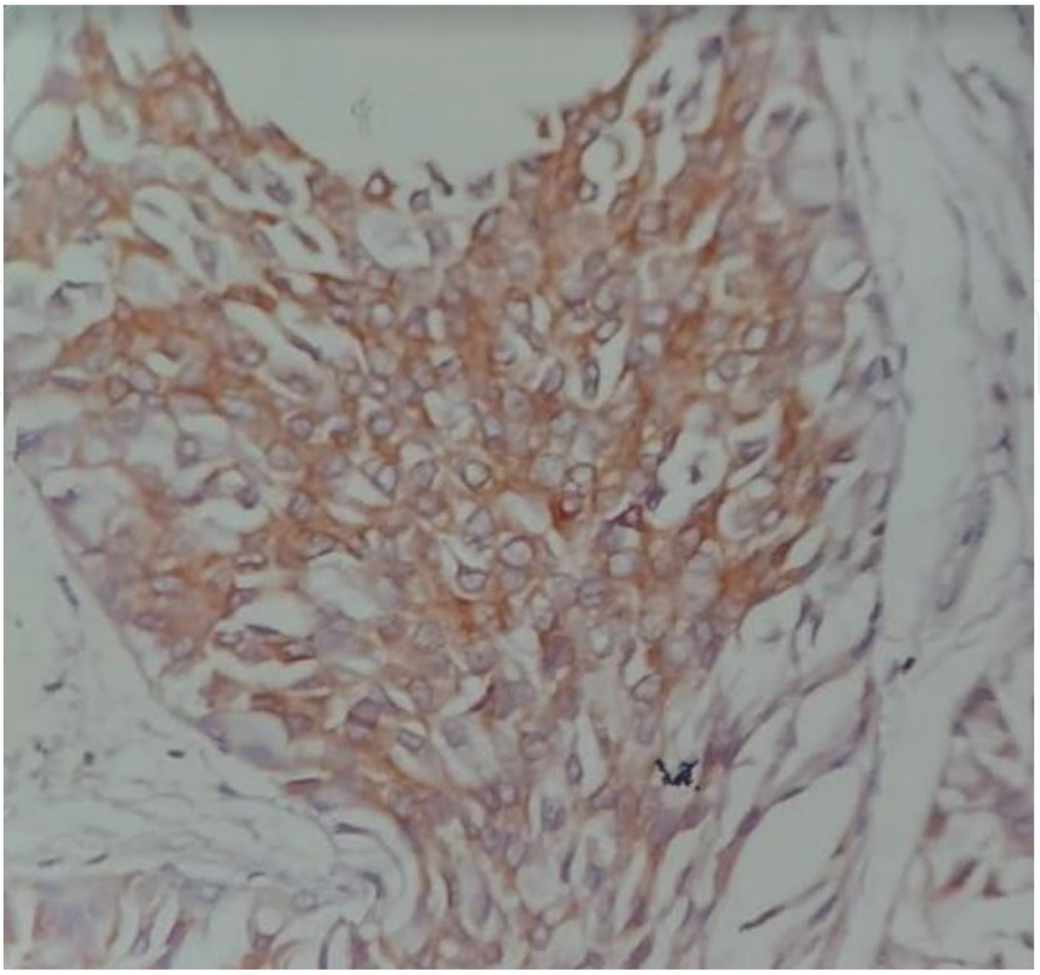

Figure 2.
IHC Bcl-2, invasive ductal carcinoma (luminal a molecular subtype, 40×).

Sharmila, Praba [2] have studied 30 cases of invasive ductal carcinoma for expression of Bcl-2 in immunohistochemistry (IHC). The immunohistochemistry was carried out by standard methods. The objective of study was to analyse Bcl-2 expression and its relationships with ER, PR, HER-2 status, histological grade and Nottingham prognostic index. The study observed that 7 cases of the Invasive Ductal Carcinoma showed intense Bcl-2 staining while 23 cases showed no expression. The Grade I tumour showed 45.5% positive immuno-expression followed by Grade II at 14.3%. The correlation of Bcl-2 expression with ER status showed that 7 out of 12 ER positive cases expressed Bcl-2 with statistically significant values. The study concluded that BCl2 expression in invasive ductal carcinoma, directly related with lower histological grade, small tumour, size, ER and PR positive status. It is inversely related to HER-2 nu status.

Cecka, et al. [3] did study on expression of Bcl-2 in 57 females suffering from primary breast cancer who were treated with neo-adjuvant chemotherapy. The immunohistochemistry for BCl2 were performed either on the surgical specimens or core cut biopsies with Streptavidin and Biotin method with peroxidise detection system. The results of immunohistochemistry when correlated with the findings of Bcl-2 have shown the following p-values with individual variables.

Tumour size (0.56), grading (0.53), ER (0.003), PR (0.36), Ki-67 score (0.07), Her-2 nu (0.24) and p53 (0.88). Hence the study concluded that there exists no significant association of Bcl-2 expression with clinical variable except ER status.

S.No.	Studies	Year	Number of cases in study	Country of Source	Bcl-2 Immuno-expression		p -value	
					Number/ Percentage	Molecular subtype	Total	Molecular subtype
1.	Sharmila,Praba [2]	2020	30	India	44.4% 25.6%	Luminal A and Normal breast like tumours Luminal B, Her2 nu enriched, Triple Negative	$p < 0.05$ $p = 0.236$	Luminal A and Normal breast like tumours Luminal B, Her2 nu enriched, Triple Negative
2.	Cecka et al. [3]	2008	57	Russia	52.8% 23.2%	Luminal A Luminal B, Her2 nu enriched, Triple Negative	$p = 0.003$ $p = 0.561$	Luminal A Luminal B, Her2 nu enriched, Triple Negative
3.	Callagy et al. [4]	2006	930	United Kingdom	57.4%	Luminal A and Normal breast like tumours	$p < 0.001$	Luminal A and Normal breast like tumours
4.	Kamaruzman et al. [5]	2019	53	Malaysia	22.4%	Luminal A and Normal breast like tumours	$p < 0.005$	Luminal A and Normal breast like tumours
5.	Adams, Cory [6]	2007	45	Australia	19.3%	Luminal A and Normal breast like tumours	$p < 0.003$	Luminal A and Normal breast like tumours
6.	Eom et al. [8]	2016	1356	Korea	40.9% 37.1% 9.6% 12.4%	Luminal A Luminal B Her2 enriched Triple Negative	$p < 0.001$ $p < 0.001$ $p < 0.001$ $p < 0.001$	Luminal A Luminal B Her2 enriched Triple Negative
7.	Dawson et al. [9]	2010	11,212	United kingdom	62.3%	Luminal A, Luminal B and Her2 enriched	$p < 0.001$	Luminal A, Luminal B and Her2 enriched
8.	Lehmann et al. [10]	2011	3247	Tennessee(USA)	43.1%	Triple Negative	$p = 0.451$	Triple Negative
9.	Hwang et al. [11]	2012	7230	Seoul (Republic of Korea)	51.2%	Luminal A	$p < 0.001$	Luminal A
10.	Min et al. [12]	2016	203	Seoul (Republic of Korea)	34.2%	Luminal A and Normal breast like tumours	$p < 0.005$	Luminal A and Normal breast like tumours
11.	Wijesinghe et al. [13]	2018	208	Srilanka	33.1%	Her2 nu enriched	$p = 0.001$	Her2 nu enriched

S.No.	Studies	Year	Number of cases in study	Country of Source	Bcl-2 Immuno-expression		p -value	
					Number/ Percentage	Molecular subtype	Total	Molecular subtype
12.	Bayoudh et al. [14]	2010	84	Tunisia (North Africa)	42.1%	Triple Negative	p = 0.002	Triple Negative
13.	Rashid, AL-Sakkal [15]	2015	100	Iraq	61%	Luminal A and Luminal B	p = 0.030	Luminal A and Luminal B
14.	Present Study	2022	50	India	72.2% 72.7% 46.15% 62.5%	Luminal A Luminal B TNBC Her 2 enriched	p = 0.04(S) p = 0.78(NS) p = 0.95(NS) p = 0.68(NS) p = 0.36(NS)	Luminal A Luminal B TNBC Her 2 enriched Overall

Table 5.
Study and results (p-value) distribution.

Callagy et al. [4] did study to evaluate that in first 5 years after diagnosis, Bcl-2 is predictor of breast cancer outcome independently, and serves as a useful tool as prognostic marker besides Nottingham prognostic index. A total of 13 markers expression was evaluated in 930 breast cancer patients on a tissue microarray. Out of all the markers Bcl-2 was the best marker. Through this study it's also evaluated that whether a single marker or a series of markers could improve prognostic potential of Nottingham prognostic index.

Kamaruzman, et al. [5] published study related to nanotherapeutics in breast cancer wherein they observed that the expression of Bcl-2 was observed in 22.4% of molecular subtype of Luminal a of invasive breast cancer.

Adams, Cory [6] did a new study which was innovative as it encouraged to ponder us upon that most of cytotoxic stresses imposed on a cell lead to activation of BH3 only proteins as important signal of stress. These BH3 proteins belong to Bcl-2 family which help us to understand their role in cancer development, and through this search for important class of anticancer drugs can be done.

Eom et al. [8] did study to evaluate the relation between the prognostic outcomes and Bcl-2 expression among the molecular sub-types. A study was conducted taking into account 1356 patients who were newly diagnosed with breast cancer between November 2006 and November 2011. Mainly Immunohistochemistry (IHC) was used to measure status of - ER, progesterone receptor, human epidermal growth factor receptor 2, and Bcl-2 expression. In this study breast cancer was classified into five molecular sub-types namely, luminal A, luminal B with positive status, luminal B with negative status, human epidermal growth factor receptor 2 expression, and triple negative sub-types. The clinico-pathological variables were analysed which assessed the correlation between Bcl-2 expression and clinical outcomes such as relapse free survival and disease- specific survival according to the five molecular sub-types.

Dawson et al. [9] have established the rationale of performing Bcl-2 immunohistochemistry in prognostic stratification of invasive ductal carcinoma. Their work included the conglomeration of 5 studies wherein the relationship between Bcl-2 and molecular subtypes of breast carcinoma was followed. The study observed the significant p-value ($p < 0.01$) in ER positive breast cancer (Luminal A subtype).

Lehmann et al. [10] observed 43.1% of their cases showing Bcl-2 immunoexpression. However, the study observed no relationship between Bcl-2 immuno-expression and molecular subtype of breast cancer. A similar observation of discordant relationship in between molecular subtype of luminal A and Bcl-2 immuno-expression by Wijesinghe et al. [13] and Bayoudh et al. [14]

The study of Hwang et al. [11] observed the Bcl-2 immunoexpression in 51.2% cases and luminal A subtype held its association with significant p-value ($p < 0.01$).

Min et al. [12] observed 34.2% of the breast cancer expressing Bcl-2 and its significant correlation with luminal A subtype of breast cancer ($p < 0.05$).

Rashid, AL-Sakkal [15] studied 61 cases of primary breast cancer in which 71% of ER positive cases and 59% of PR positive cases depicted positive Bcl-2 oncoprotein expression, having p-values ($p = 0.030$) and ($p = 0.001$) respectively.

4. Conclusion

Bcl-2 is an independent prognostic marker for breast cancer although its expression frequency may differ but it plays definite prognostic role in breast cancer. It is observed that Luminal A molecular subtype of invasive ductal carcinoma has a

frequent association with Bcl-2 immuno-expression. There are some limitations to use of immunohistochemistry staining method as the results may be affected by intra-tumoral heterogeneity.

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