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A Study of Bcl-2 Immuno-expression in Invasive Ductal Carcinoma of Breast and it's Correlation with Molecular Sub-types

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Study Protocol

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ABSTRACT

Background: The incidence of Breast cancers has overtaken the cervical cancers amongst Indian females. Invasive Ductal carcinoma is reported to be the most common form of breast cancer. Bcl-2 has been studied extensively as a common factor in the pathogenesis of solid tumours including breast cancer. 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. The present study attempts to look into Bcl-2 immunoexpression as a key parameter for predicting the treatment outcomes and recurrence of Invasive Ductal Carcinoma.
Methodology : This will be an observational study conducted in Department of Pathology, JNMC, Wardha. The study will include clinicopathological detailing of 50 mastectomy specimens of Invasive ductal carcinoma, detailed sectioning of tumour tissues, histopathological BR-grading and molecular subtyping using Bcl-2 immunohistochemistry.
Expected Results: A significant correlation is expected between Bcl-2 expression and treatment outcomes and recurrence of Invasive Ductal Carcinoma.
Conclusion: The conclusion will be drawn based on careful analysis of the results.

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Keywords: Breast cancer; Bcl-2; invasive Ductal Carcinoma; molecular classification; molecular sub-types; prognosis; expression.

1. INTRODUCTION

The Breast cancer by now has become great concern for heath scenario of Indian as its incidence has overtaken the cervical cancers amongst Indian female [1]. The laboratory physicians across the world are engaged in assesing new and novel prognostic and predictive markers that would bring about best possible outcome of breast cancer. The modern day practice of oncopathology revolves more around predictive prognostic markers that would enable the appropriate adjuvant therapies and management of cancer. One of the challenges in breast cancer management is to accurately predict the outcome for each patient, so that the patients will be benefitted from adjuvant therapies or by appropriate surgical interventions [1,2].

The traditional pathological prognostic factors in Breast cancers which were until relied heavily were Lymph node status , tumour size, 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].

Amongst many such families of the genes, Bcl-2 is being studied extensively for its commonality at participation in the pathogenesis of solid tumours especially that includes cancers of breast, prostate, lung, colorectum and ovaries [5,6].

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. The studies though few in published literature, attempted Bcl-2 expression for its correlation with clinicopathological variables, disease free survival, prognostic factors, Nottingham prognostic index, TNM stage and treatment outcome [7,8].

A few studies even have proposed that Bcl-2 expression in tumour be considered as a molecular subtype of Invasive Ductal Carcinoma [9,10].

The search for publications over this topic originating in India was found to be marginal rather, the correlative studies with clinicopathological variable, molecular subtypes of breast cancer and BR-grades are negligible [11].

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 an essentiality [12,13].

1.1 Research Gap

The present study is being carried out to close the gap of understanding about Bcl-2 expression and its prognostic and predictive importance in correlation to clinicopathological variables as there is paucity of such study from India. This study would enable to breach the gap of understanding the relationship of Bcl-2 expression in invasive Ductal carcinoma with grade of the breast cancer and its molecular subtypes. This would create a frame for appropriate treatment and management of breast cancer for favourable outcome.

1.2 Research Question

With understanding of complexities in prognostication and predictive abilities of Bcl-2 in breast cancer, following research question is framed: 'Does Bcl-2 immunoexpression study performed on the tumour tissue of breast cancer, by immunohistochemical method correlates with traditional clinico-pathologic variables of prognosis and molecular sub-types.'

2. METHODOLOGY

Study Design: Observational (retrospective and prospective inclusions)

Place of Study: Department of Pathology, JNMC, Sawangi (Meghe), Wardha, Maharashtra.

Duration: Study will be from 2020 to 2022 (2 years)

Methods: Determination of Bcl-2 immunoexpression in Invasive Ductal Carcinoma, with the help of immunohistochemistry (IHC).

Sample Size: 50 patients (diagnosed Invasive Ductal Carcinoma).

Sample Size formula with desired error of margin-

$$n = (Z^{a/2})^2 \times p \times (1-p)/d^2$$

where, $Z^{a/2}$ is the level of significance at 5% that is 95% confidence interval
p = prevalence
d = desired error of margin
n = sample size

Inclusion criteria

1. The prospective and retrospective specimens (paraffin blocks) of lumpectomy and mastectomy of the breast with histological diagnosis of Invasive ductal carcinoma.
2. The cases with complete details of clinico-surgical workup as indicated for breast cancer.

Exclusion Criteria

1. The cases with incomplete clinico-surgical details.
2. The breast cancers other than Invasive ductal carcinoma
3. The cases of recurrent Invasive ductal carcinoma
4. Male breast in ductal carcinomas

The gross and microscopic examination of surgical specimen of mastectomy: The specimens of the mastectomy for its pathology reporting and histological detailing will be done by standard references.

Immunohistochemistry of the tumour tissue for Bcl-2, ER, PR and Her-2: The following methodology will be adopted for staining and assessment for interpretation results of immunoexpression of Bcl-2, ER, PR and Her-2.

1. Paraffin tissue section of tumour tissue of IDC(Invasive ductal carcinoma)
2. Deparafinisation of section
3. Endogenous peroxidise and serum application
4. Antigen retrieval with treatment of buffer and microwave processing
5. Primary antibody incubation
6. Treatment by wash buffers
7. The secondary antibody in principle to Streptavidin Biotin method
8. The colour development by peroxidise - DAB (3,3'- Diaminobezidine) chromogen.
9. Interpretation – brown granular positivity assessed by percentage of cells
10. The co-run for negative and positive controls

2.1 Statistical Methods

The correlation statistics will be performed by suitable statistical tests along with values of significance compatible with the objectives (p-value), Fischer exact test, uni-variate comparisions.

2.2 Prelimnary Data Recording

i. Name
ii. Age
iii. Gender
iv. Ward
v. OPD
vi. Unit incharge
vii. MRD
viii. Complains
ix. Co-morbid conditions
x. Local examination of breast
xi. Findings of radiological work-up
xii. Details of suspected metastasis
xiii. Clinical stage of disease if assessed

2.3 Expected Results

The study will be conducted for a period of 2 years and all the observations will be depicted in a well tabulated master chart and conclusion will be drawn.

3. DISCUSSION

A short review for the present work is entitled below, citing five studies from the different centres explaining about Bcl-2 immunoexpression in Invasive Ductal Carcinoma and its evaluative correlation with molecular subtypes and BR-grade and TNM stage.

Sharmila and Prabha et al. [1] have studied 30 cases of Invasive Ductal carcinoma (IDC) for expression of BCl2 in immunohistochemistry (IHC). The immunohistochemistry was carried

out by standard methods. The objective of study was to analyse BCl2 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 BCl2 staining while 23 cases showed no expression. The Grade I tumour showed 45.5% positive immunoexpression followed by Grade II at 14.3% . The correlation of BCl2 expression with ER status showed that 7 out of 12 ER positive cases expressed BCl2 with statistically significant values. The study concluded that BCl2 expression in Invasive Ductal carcinoma (IDC), 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. [2] did study on expression of BCl2 in 57 females suffering from primary Breast cancer who were treated with neoadjuvant chemotherapy. The immunohistochemistry (IHC) 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 (IHC) when correlated with the findings of BCl2 have shown the following P-values with individual variables:-

Tumor size (0.56), grading(0.53), ER(0.003), PR (0.36), Ki-67 score(0.07), Her-2/neu(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.

Callagy, et al. [3] did study to evaluate that in first five 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 its also evaluated that whether a single marker or a series of markers could improve prognostic potential of Nottingham prognostic index.

Adams JM, Cory S., et al. [5] 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 YH, Kim HS, et al. [6] 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 1,356 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.

Hence the studies concluded that there are evidences of Bcl2 immunoexpression in Invasive Ductal Carcinoma and it can be correlated with molecular sub-types and BR grade and TNM stage.

Bcl-2 is an independent favourable prognostic marker for breast cancer although its expression frequency may differ but it plays different prognostic roles in breast cancer molecular subtypes. There may be some limitation to this study as in we will be using (IHC) Immunohistochemistry staining method, the results may be affected by intra-tumoral heterogeneity and intra-observer heterogeneity, there may also be presence of selection bias. However our approach to present study would try to overcome such issues by comparing it with other prognostic markers of breast cancer, and thus we will try to establish that indeed there is evidence of Bcl-2 immunoexpression in Invasive ductal carcinoma.

The traditional pathological prognostic factors in Breast cancers which were until relied heavily were Lymph node status, tumour size, tumour grade, Nottingham prognostic index and many others.

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.

The present study attends to an aspect of breast cancer that would enable the clinicians to look into Bcl-2 immunoexpression as a definitive or as an alternative parameter at predicting the outcome of Invasive Ductal Carcinoma, its recurrence and its treatment. 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 being studied extensively for its commonality at participation in pathogenesis of solid tumors that includes cancers of Breast, prostate, lung, colorectum and ovaries. The present study is aimed at knowing the significance of Bcl-2 immunoexpression in Invasive Ductal Carcinoma and its evaluative correlation with molecular sub-types and BR-grade and TNM stage.

The present study is being carried out to close the gap of understanding about Bcl-2 expression and its prognostic and predictive importance in correlation to clinicopathological variables as there is paucity of such study from India. This study would enable to breach the gap of understanding the relationship of Bcl-2 expression in invasive Ductal carcinoma with grade of the breast cancer and its molecular subtypes. This would create a frame for appropriate treatment and management of breast cancer for favourable outcome. Studies on different aspects of breast carcinoma were reported [14-16]. Jain et. al reported a study on correlation of carcinoma antigen (Ca 15-3) serum assay with clinicopathological parameters in women with invasive ductal carcinoma [17]. Mishra et. al. reported on correlation of cytokeratin expression with Bloom Richardson grading of carcinoma breast [18]. Few of the similar studies were reported [19-27].

A few studies even have proposed that Bcl-2 expression in tumour be considered as a molecular subtype of Invasive Ductal Carcinoma. The search for publications over this topic originating in India was found to be marginal rather, the correlative studies with clinico pathological variable, molecular subtypes of breast cancer and BR-grades are negligible.

4. CONCLUSION

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 an essentiality. In this study clinicopathological details of 50 mastectomy specimens of Invasive ductal carcinoma would be taken, detailed sectioning of tumor tissues of IDC would be done and these would be classified according to Histopathological BR-grade , molecular subtypes, using Bcl-2 with help of immunohistochemistry. All the results would be correlated by BR- grade, TNM stage and molecular subtypes.

CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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