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"Study of Galectin -3 Expression in Breast Carcinoma Patients"

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ABSTRACT: -

Background - Breast cancer has always been diverse disease with various phenotypes and distinct biological features. It ishas becomehazardous disease which badly affects health of female. The current overall risk of death is about 20%. Immunohistochemistry is used for cancer diagnosis. Galectin-3 is a 30 kD protein which is part of non-integrin beta galactoside binding lectin family and plays a role in the process of cell adherence, cell cycle regulation, apoptosis, and cell repair**.** Galectin-3 plays an important role in complex gene expression regulation depending on the type of cell. This study aims to compare galectin-3 expression with Nottingham Grading System grading in histopathology in patients diagnosed with carcinoma breast and assess the aggressiveness of the tumour.

Objectives - To acknowledge the association between galectin-3 expression and the Nottingham histological grading system in carcinoma breast.

Methods – The present study is an observational, cross sectional and retrospective study to be conducted for a duration of two years from (October 2020 to October 2022) in the Histopathology and Immunohistochemistry Department of Pathology, Jawaharlal Nehru Medical College, Sawangi (Meghe), in coordination with the Department of General Surgery, Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe). Approval will be obtained from Institutional Ethics Committee and informed consent will be taken from the

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patients participating in this study.In this study we will correlate galectin-3 expression in patients diagnosed with breast cancer on histopathology with grading according to the Nottingham Grading System and determine the aggressiveness of the tumour.

Results- The findings will be tabulated.

Conclusion - Conclusion will be drawn from the results obtained from the study.

INTRODUCTION

The most common dreaded malignancy in females is breast cancer. Women are diagnosed with breast cancer and one in three of those affected die from the disease, leading to an increase in breast cancer.[1]According to Globocan Data, in 2018, the count of new cases registered were, 1,62,468 and the estimated number of deaths was about 87,090 and it makes 14% of female cancer deaths.[1]In India, the rate of incidence starts to increase in the early thirties and peaks at 50-64 years of age.Overall, 1 out of 28,it is inevitable that female will develop breast cancer during their lives..[2]

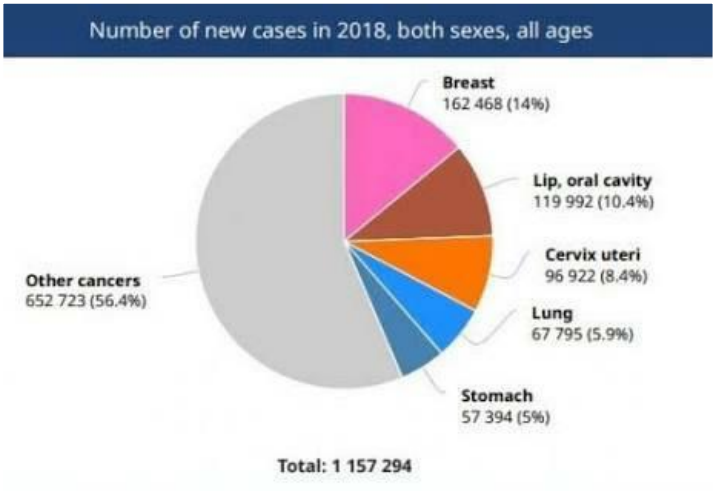

Figure 1: [2]

Breast cancer has been one of the big illnesses affecting the health of women.The reasons behind this pattern are thought to be shifts in social lifestyle, delayed childbearing, less births and decreased breastfeeding, along with lack of access to optimal health care.Oestrogenic arousal and age are the most important risk factors.Gender (99% of those affected are female), genetic inheritance, and environmental and lifestyle variables also play a critical role.[1] In younger and older women, the risk of death in those who develop invasive breast cancer has steadily decreased, most recently by 1% to 2% each year, and the actual overall risk of death is around 20%.This decline is due to both mammographic screening and more successful modalities of treatment.In breast cancer, multiple prognostic indicators play a significant role, including size of tumour, lymph node status of axilla, histological grade, type of tumor, vascular invasion, estrogen receptor status, etc.[3]

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The Nottingham modification of the Bloom Richardson Histologic Score systemconsiders formation of tubule, nuclear pleomorphism and mitotic rate found to determine the grade of carcinoma breast.

Each of these characteristics is graded from 1 to 3, and then the scores are combined to give a final overall score of 3 to 9. To assess the grade, the final total score is used in the following manner:-

- Grade I tumours ,we get total score of 3-5
- Grade II tumours,we gettotal score of 6-7
- Grade III tumours,we get total score of 8-9.[4]

The most prevalent type of carcinoma in women is breast cancer, which is a cause of high mortality and morbidity worldwide.Galectin-3 is a multifunctional protein that is expressed in different types of neoplastic cells at elevated levels and is associated with changes in cell growth, transformation and metastasis. Few studies indicate that the expression of galectin-3 in breast cancer tissue is greater than that of normal breast tissue. We will thus compare Nottingham's breast cancer grading system with the expression of galectin-3 and assess the tumor's activity and aggressiveness for early diagnosis and tumor treatment.

Carcinoma breast is a diversified disease with multiple biological features with several different phenotypes. For many women with breast cancer, if precautionary agents are taken and selective treatment for the estrogen receptor, progesterone receptor, and human epidermal growth factor 2 receptor (HER2/neu) have shown improved clinical outcomes.[5]In recent years, a few studies showthat the inhibition of galectin-3 expression affects tumor cell proliferation and apoptosis.

Galectins are a family of similar amino acid sequences of nonintegrin beta-galactoside binding lectins.Galectin-3 is a lectin family galactose-binding protein that plays a significant role in the interaction of cell and cell matrix, growth of cell, control of cycle of cell, apoptosis, damage to cell and repair, transformation to malignant, and metastasis.

Galectin-3 has a special family structure and consists of—

1) a phosphorylation site for serine is present at a short N-terminal domain of galectin-3
2) A repeated proline, tyrosine and glycine consensus series
3) Recognition of carbohydrates is present at C-terminal domain having one domain .[6]

The only unique galectin found in mammals so far is galectin-3.Through its carbohydrate-binding property, extracellular galectin displays important role such as migration of cell, adherence of celland interactions of cell to cell, where galectin-3 which is expressed in cytoplasm has anti-apoptotic activity and controls multiple signal transduction pathways.In the nucleus, galectin is also found as a nuclear matrix protein.[6]

Immunohistochemistry (IHC) is a diagnostic procedure in histopathology,with the help of antibody it recognize antigen in cells or tissue. The chief use of immunostaining in IHC is the potential to see the desired target without changing the context and tissue structure of tissue sample.In variousneoplasms and diseases of different organs, immunohistochemical expression of galectin-3 is studied, such as endocrine system, GIT, female reproductive

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system, urinary tract, CNS, respiratory system, region of head and neck, breast , gallbladder region.

Immunohistochemistry has become an necessary tool for pathologists in both everyday practice and basic research for better pathophysiology of the diseases.[7]In immunohistochemistry antigen antibody reaction takes place in which galectin-3 acts as a antibody that is present in cytoplasm and nucleus, there is interaction between antigen and antibody giving a reaction ,by which we detect the tumour aggression is high or low by using Nottingham histologic score system.[7]

In the present study,we will correlate the Nottingham histologic grading system with galectin -3 expression.

RESEARCH GAP:

The present study aims to close the gap of understanding between correlation of galectin-3 which significantly expresses in Carcinoma Breast. It will enable to breach the gap of understanding the relationship between galectin-3 and the histological grade i.e Nottingham prognostic index. It will help to create a frame for early diagnosis and effective treatment thus reducing the risk of breast cancer.

RESEARCH QUESTION:

With understanding the effect of galectin-3 expression with the Nottingham prognostic index, the following research question is framed –

'Does galectin-3 expression have a significant association with Nottingham's Prognostic Index in Carcinoma Breast.'

METHODOLOGY:

STUDY DESIGN - Observational, cross-sectional and retrospective study.

PLACE OF STUDY – Department of Pathology, JNMC, Sawangi(Meghe), Wardha, Maharashtra.

DURATION – 2020 to 2022 (2 years)

METHODS –

Haematoxylin and Eosin staining:[8]

- Sections from breast tissue are deparaffinized in xylene : 3 changes of 10 minutes each.
- Dewaxing of sections is done. Sections are rehydrated through descending grades of alcohol.
- Bring sections to water.
- Stain in Harris haematoxylin in a jar for ten minutes.
- Wash well in running tap water for 2-3 minutes.
- Differentiate in 1% acid alcohol (1%HCl in 70%alcohol) for few seconds.

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- Wash in alkaline tap water (bluing) for 5 minutes.
- Stain in 1% aqueous Eosin for 1 minute.
- Dehydrate through 90% alcohol.
- Mount in Dibutyl phthalate polystyrene xylene (DPX).
- Two sections of 3-5 micron thickness from each block at cut at 50 micron intervals on poly l-lysine
coated slides.
- Antigen retrievals performed by heat induced epitope retrieval using pressure cooker and rinsing the slides with Phosphate Buffer Saline three times.
- Peroxidase blocking-Apply 3% hydrogen peroxide for 10 minutes to block the endogenous peroxidase activity.
- Place primary antibody, Galectin -3 (Brand- DAKO™) Glostrup, Denmark ,on top and allow reacting for 30-40 minutes at room temperature.
- Wash with Phosphate Buffer Saline 3 times.
- Allow to react with secondary antibody (streptavidin biotin) for 30 minutes at room temperature and then wash with PBS.
- Flood the slides with DAB (3, 3-diaminobenzidine) solution for 10 to 20 minutes.
- Wash with tap water, then allow to react with haematoxylin for 2 minutes at room temperature.
- Dehydrate, clear and mount the slides

SAMPLE SIZE – 40-50 patients

The sample size was calculated by using Krejcie and Morgan formula with desired error of margin:

Sample size formula with desired error of margin:-

$n = (Z \frac{\alpha}{2})^2 X\ p\ X\ (1-p) / d^2$

where,

$Z\frac{\alpha}{2}$ is the level of significance at 5% i.e. 95% confidence interval = 1.96

p = prevalence of breast carcinoma in women in maharashtra = 1.26% = 0.0126

d = desired error of margin = 3% = 0.03

$n = (1.96)^2\ X\ 0.0126\ X\ (1 - 0.0126) / (0.03)^2$

$= 53.10$

= Approximatey**40 - 50 patients** needed in each study group [9]

Inclusion Criteria:

- All patients diagnosed with Carcinoma Breast on histopathology.
- All female patients.
- Primary cases of Carcinoma Breast without any history of previous treatment.

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- Cases that underwent biopsy, trucut biopsy, mastectomy or modified radical mastectomy.
- Formalin fixed tissues.

Exclusion Criteria:

- All patients diagnosed with lesions other than breast carcinoma on histopathology like myoepithelial lesions, mesenchymal tumours, fibroepithelial tumours, tumours involving nipple, malignant lymphoma, metastatic tumours and adenomas (Tubular adenoma, Lactating adenoma, Pleomorphic adenoma, Apocrine adenoma, Ductal adenoma.
- Male patient with Breast cancer.
- Cases with recurrence or history of neoadjuvant therapy.
- Tissues not fixed in formalin.

Statistical Analysis-

The Chi square test will be used for statistical analysis and multiple linear regression analysis will be used to study the association between galectin-3 protein expressions and the histologic grade of carcinoma breast.

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.

DISCUSSION:

Every year, around 1.7 million females are diagnosed with breast cancer, all of which are diagnosed across the world. The prevalence of breast cancer is increased by risk factors such as estrogen. stimulation, decreased breast feeding, infant birth delay, environmental and lifestyle modifications.[1]

Breast carcinoma's biological activity has a high invasive potential for early recurrence, rapid recurrence andshort survival time[5]The key method for tumour treatment is surgery, chemotherapy and radiotherapy.

Targeted treatments such as the receptor of estrogen(ER), the receptor of progeterone (PR) and humanepidermal growth factor 2 . But in patients with no molecular targets, the prognosis is low. Fresh molecular targets, therefore must be detected in order to boost the prognosis for breast carcinoma.

Galectin-3 is aprotein which has multiple functions and is part of family of lectin . Galectin -3 is identifiedin cytoplasm , cell surface and nucleus , and its biological activity depends on cellular location . It is known that it plays important role in differentiation,maturation ,tumourdevelopment and metastasis [10] . In cancer regression, galectin -3 plays a role and regulates different biological pathways, including formation of cell,variations, adherence,

inflammation,apoptosis.It serves as an immunohistochemical marker that indicates tumour aggressiveness by which the nature of the breast cancer can be defined.[11]

Xiaohong Liu et al, in Hubei University of Medicinea research was conducted to detect expression of galectin-3 levels from tissues of breast cancer and neighbouring tissues from one twenty patients with carcinoma of breast after modified radical mastectomy in hospital. Levels of galectin-3 expression were detected using SP immunohistochemistry procedure . It was found that Higher Galectin-3 expression levels has positive association with the reduced survival rate .The study results showed that in tissues of breast cancer, levels of galetin-3 expression were significantly increased relative to that in normal tissues.The criterion for galectin-3 positive expressions were that the positive signal emerged in the cytoplasm and nucleus as yellow-brown granules.[3]

Hao Zhang et al, conducted a study in the Tumor Hospital of Liaoning Province, China Medical University, and Dalian Medial University. Overall one thousand one hundred and eighty seven patients who were diagnosed with carcinoma breast histologically and those patients in which radical resection was undertaken were included in this study. Immunohistochemical analysis in this study showed that galectin-3 is situated in the cytoplasm and membrane of cells of breast carcinoma and expression was substantial.Of the 1187 cases of breast cancer, 388 displayed elevated galectin-3 expression in particular. A linear association between galectin-3 expression and histological grading was discovered by Spearman correlation regression analysis. Galectin-3 displays optimum expression in grade-3 according to the histological grade.[5]

Andree-Anne Grosset et al, carried out a study entitled "Galectin protein signature in normal and cancerous breast tissues".The research was carried out using tissue micro arraystoanalyse the expression of galectins at protein level by immunohistochemistry using tissue microarrays constructed with clinical data from 213 human breast cancer tumor tissues consisting of each molecular subtype of breast cancer. In grade 3 of the Nottingham Histological Grading System (Elston-Ellis adaptation of the Scarff-Bloom-Richardson grading system), high levels of galectin-3 expression were observed.[11]

A number of other studies related to Breast carcinoma were reported [12-14]. Wankhade et. al. reported on role of Ki 67 in pathological prognostic staging of breast cancer [15]. Aditi et. al studied utility of microvessel density and it's correlation with Nottingham Prognostic Index in carcinoma breast [16]. Few other related studies on breast cancer and immunohistochemical biomarkers of carcinoma were reviewed [17-20].

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