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Study of Expression of KAI-1 in Breast Carcinoma

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ABSTRACT

Background –The KAI1 gene, which is also called as CD82, its a metastasis suppressor gene whichis indicated as development of disease of certain solid tumors, involving breast cancer. In normal breast tissue and non-invasive breast cancer, KAI1 protein levels are elevated and decreased in infiltrating breast tumors by comparison. The level of KAI1 protein is inversely proportionalto the potentialof metastasis of cancer cells of breast. In this studyexpression of KAI1 in breast cancer is done by immunohistochemical technique and to assess the relationship between the KAI1 protein expression with Nottingham Prognostic Index and individual parameters of Nottingham prognostic index.

Objectives - To assess the relationship between the KAI1 protein expression level with Nottingham Prognostic Index and individual parameters of Nottingham prognostic index.

Methods – The present study is an observational, cross sectional and retrospective study to be conducted for a duration of two years in the Histopathology and Immunohistochemistry division of the Pathology department, JawaharlalNehruMedicalCollege, Sawangi (Meghe), in coordination with the Department of General Surgery, AcharyaVinobaBhaveRuralHospital, Sawangi (Meghe). In this study, 40-50 Carcinoma of Breast caseswho have undergone ModifiedRadical Mastectomy will be taken. The Nottingham Prognostic Indexother individual parameters of Nottingham Prognostic Index will be determined in each case and their correlation will be studied through a well-tabulated master chart.

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Results -The observations will be depicted in a well-tabulated master chart.
Conclusion - Conclusion will be drawn from the results obtained from the study.

INTRODUCTION:

Breast carcinoma is the most common cancer which occurs amongfemalesall over the world.[1]Since breast carcinoma is the most common type of cancer in females,itcomprises 14 percentage of cancer in women of India. An Indian woman diagnosed with breast cancer in every four minute. In2018 statistics survey of breast carcinoma,one lakh sixty two thousand four hundred sixty eight new caseswere registered and eighty seven thousand ninety deaths recorded.[2]

The risk of breast cancer is considerable in Indian women between the early thirties and the fifties and the risk of occurrence rises at the age of 50-64.One in twenty eight women in India is likely to experience breast carcinoma during their lifetime. For urban women, this is more, i.e, one in twenty two, than for the rural populationi.e. one in sixty. [2]

The most important risk factors are gender (99 percentage of those affected are female), age, genetic inheritance, early menarche, late menopause, late first pregnancy, nulliparity, absence of breast feeding, exogenous hormone therapy, postmenopausal obesity, physical inactivity, alcohol consumption, environmental and lifestyle factors.[3]

There are many clinical pathological features including the tumour size, involvement of axillary lymph node and extent of metastasis are involved in the prognosis, relapse and survival of breast carcinoma (Soerjomataram et al., 2008). Estrogen Receptor , Progesterone Receptor and Her2-neu are well established predictive and prognostic factors for breast cancer that show the significance of various clinic-pathological parameters in prediction and prognosis in response to number of available therapeutic options in breast cancer (Singh et al).[4]

There are various scoring methodsusedtoassess the grading of breast carcinoma. One of the systems is the **Nottingham Histological Gradingsystem**which is also named as " **TheElston-Ellis modification of theScarff-Bloom-Richardson** grading system". This histological grading system is used to classify breast cancer into low, intermediate and high grade for diagnosis and treatment. There are three factors used to determine breast cancer that is tubule formation, nuclear pleomorphism and mitotic rate.

Breast cancer is categorised as tumour (T)- size of the tumour, (N)-number of lymph nodes, if the cancer has spread to lymph nodes, and metastasis (M) which shows whether the carcinoma has invaded to other part of the body is based on American Joint Committee on Cancer (AJCC) **TNM Classification** .[4] These characteristics are allotted individual scores called the pathological T stage (T0-4), N stage (N1-3) and M stage (M0-1) which are integrated to form final overall pathology stage (stage 0-IV).

Nottingham prognostic index (NPI)is a well-established prognostic tool in the management of breast cancer. It is most commonly used indexfor prognosis and survival of patient of breast cancer. Its value isdetermined with the help of three pathological criteria: tumour size, the no. of lymph nodes involvedand grading of the tumour. Here grading of tumour is done according to Nottingham Modification of Bloom Richardson System and lymph node staging is done according to TNM staging.

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Immunohistochemistry is a procedure for recognising the tissue or cellular constituents (antigens) with the help of antigen-antibody interactions. Thelocation of antibody can be recognised either by direct labelling of the antibody, or by using the secondary labelling method. The selection of immunohistochemistry antibodies is done on the grounds of their specificity of tumour and the probability that they will react with tumour cells under assessment. Traditionally, immunohistochemical studies have focused on markers of specific cell or tumour type as helps in diagnosis of specific tumours.[5] The chromogenic or fluorescent detection can measure protein expression through IHC. Both detectors rely on antigen recognition mediated by a primary antibody.

KAI1 is a metastasis suppressor gene. It is derived from Chinese word Kang ai which means anticancer. First it wasdiscovered ina study of T- cell activation and is a family member of Tetraspanin.[6]In prostate cancer, suppression of metastasis iscaused by this particular gene was studied on somatic cell hybridization of highly metastatic rat prostate cancer cells later.[7] Dong et al, discovered theposition of KAI1 gene on chromosome 11p with 10 exons and 9 introns covering ~80kb.[8]There are two isoforms in KAI-1 protein, with 267 residues in isoform-1 and 242 residues in isoform-2.[9] KAI1 protein found on cell membrane and in cytosol.[1] It participates in an array of cellular mechanism like cell proliferation, cellular motility and cell-cell interaction.(Lazo 2007).[10] KAI1 is also known as CD82, suppressor of tumorigenicity 6(ST6)and leucocyte surface antigen R2 (SAR2). The act of KAI1 in progression of cancer has been limited to prostate cancer andalso has shown to be involved in the progression of pancreatic cancer, non-small cell lung cancer, bladder cancer, breast cancer and gastric cancer.[11]

Breast cancer, the second most common cancer among women in the world and is most frequent cause of death among women. KAI1 protein level is high in normal breast tissue and non- invasive breast cancer and in contrast, the KAI1 expression is reduced in most of the infiltrating breast tumours. KAI1 is an indicator used to correlate along with clinical and pathological parameters in breast cancer progression.

It has been recently proposed that the KAI1 protein expression is frequently downregulated in metastatic cancers and thus KAI1/CD82 could be a favourable biomarker for the diagnosis of patients with malignant disease. It can also helps in predicting the potential of metastasis in various cancers including breast carcinoma. KAI1/CD82 is associated with CD81 and CD9 in tetraspaninfamily and is also associated with integrins , immunoglobin such as EW12 and epidermal growth factor receptors (EFGR) (Helmer , 2005). KAI1/CD82 regulation associated with Integrins and EGFR has been studied very well by different independent groups in context to cancer reduction.[12]

The present study is designed with an aim to correlate KAI1 protein expression in breast cancer with Nottingham histologic grading system and TNM staging using KAI-1/CD82 as an immunohistochemical marker.

RESEARCH GAP:

The present study aims to close the gap of understanding correlation between KAI1 protein expression and Nottingham Prognostic index and individual parameters of Nottingham

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Prognostic Index in breast carcinoma. The measurement of KAI1 protein expression will help to identify the metastatic propensity in breast cancer patients and hence it will guide the doctors to stratify their patients and the necessity for close follow up aggressive treatment plan.

RESEARCH QUESTION:

With understanding the KAI1 protein expression on tumour cells in Carcinoma Breast, the following research question is framed –

'Does KAI1 gene have a significant association with the tumour size, histological grade and lymph node metastasis i.e. Nottingham's Prognostic Index in Carcinoma Breast.'

METHODOLOGY:

STUDY DESIGN - Observational, cross-sectional and retrospective.

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

DURATION – 2020 to 2022 (2 years)

METHODS –

- Biopsy from clinically suspected cases will be taken and sent for histopathological examination to the Pathology department, Jawaharlal Nehru Medical College (J.N.M.C).
- Modified radical mastectomy specimen on histopathological examination will be received in the Department of Pathology, J.N.M.C.
- Gross examination and dissection of the received specimens will be done and appropriate sections will be done and appropriate sections from the margins, tumour mass and axillary lymph nodes will be done.
- Specimens will be subjected to routine tissue processing after which routine Haematoxylin and Eosin (H & E) staining will be carried out.
- The histological grade of the tumour will be determined by the Nottingham modified Bloom-Richardson Grading System.
- The staging of tumour will be determined by the TNM staging based on American Joint Committee on Cancer and Nottingham prognostic index will be calculated.
- Immunostaining for KAI1/CD82 will be carried out to evaluate KAI1 protein expression in each case.
- After which, correlation of KAI1 protein expression of each case with Nottingham prognostic index and its individual parameters will be done.

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,

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

p = prevalence of breast carcinoma in women = 1.266% = 0.0126

d = desired error of margin = 3% = 0.03

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

$= 53.10$

= Approximately **40-50 patients needed in each study group.**

Inclusion Criteria:

1. All cases diagnosed as Carcinoma Breast on histopathology.
2. All Modified Radical Mastectomy Specimens.
3. All female patients presenting with Carcinoma Breast.
4. Primary cases of Carcinoma Breast without any history of previous treatment.

Exclusion Criteria:

1. All cases of mesenchymal tumours, myoepithelial lesions, tumours involving nipple, fibroepithelial tumours, malignant lymphoma, metastatic tumours and adenomas such as (Tubular adenoma, Ductal adenoma, Pleomorphic adenoma, Apocrine adenoma, Lactating adenoma).
2. Cases where Trucut biopsy, wedge biopsy, lobectomy has been done.
3. Male patients with carcinoma breast.
4. Cases with recurrence or history of neoadjuvant therapy.

Staining Protocol: Haematoxylin and Eosin staining:[13]

- Sections from carcinoma of breast 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.
- Wash in alkaline tap water (bluing) for 5 minutes.
- Stain in 1% aqueous Eosin for 1 minute.
- Dehydrate through 90% alcohol.
- Mount in Dibutylphthalate Polystyrene Xylene (DPX).

Immunostaining:[14]

- Four micron thick section of formalin fixed paraffin embedded tissue section were mounted on poly l-lysine coated slides.
- Slides were then deparaffinized and rehydrated conventionally.

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- 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 KAI1/CD82 primary antibody on top and allow reacting for 30-40 minutes at room temperature.
- Wash with Phosphate Buffer Saline three times.
- Allow to react with secondary antibody (streptavidin biotin) for 30 minutes at room temperature and then wash with Phosphate Buffer Saline.
- 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

Statistical Analysis

Statistical analysis will be done by 'chi square test' by analysing the association between KAI1 protein expression and clinicopathological indices. Multiple linear regression analysis is used to clarify the relative factors for metastasis. P value less than0.05 will be considered to indicate significant statistics.

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:

A number of studies on different markers of breast carcinoma were reported [15-18].Kumar et. al reported oneffect of single amino acid mutations on c-terminal domain of breast cancer susceptible protein 1 [19]. Aditi et. al reported about utility of microvessel density and it's correlation with Nottingham Prognostic Index in carcinoma breast [20]. A short review for the present work is entitled below, citing the studies from differentcentres establishing the correlation of KAI1 protein expression with Nottingham Prognostic Index with individual parameters of Nottingham's Prognostic Index.

Zhang et alconducted a study,entitled " The tumour expression of metastasis suppressor gene of KAI1 and matrix metalloproteinase two in breast cancer tissues",here they observed an attenuated expression of KAI1 in patients of breast carcinoma with axillary lymph node metastasis (ALNM) with corresponding to the group of patients of breast cancer withoutaxillary lymph node metastasis(ALNM) and demonstrated that, expression of KAI1 is downregulated in breast cancer and the patient with lymph node metastasis shown decreased expression of KAI1 as compared to patients without lymph node metastasis.[4]

Huang et al conducted a study which is entitled as" Theassociation of reduction in expression of mrp-1/cd9 and kai1/cd82 with the recurrences in patients of breast carcinoma" here theyexplored the association of KAI1 protein expression loss with the recurrences in

breast cancer patients. The studyshowed that the rate of patient's survivalwith disease free with KAI1-negative tumors was notably lower than that of patients with KAI1-positive tumors.[11]

Yang et al conducted a study in 2000 which is entitled "KAI1proteinis downregulatedduring the progressionofhumanbreastcancer" here they demonstrated significantly high expression ofKAI1 protein levels in normal breast tissues than in breast cancer.[4]

The above studies all suggest that there is a association of KAI1 expression with breast cancer progression. Some of the related studies were reported [21-22].

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