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Research Protocol

Pathology Section

Utility of CA15-3 as a Tumour Proliferation Marker in Breast Carcinoma Patients: A Cross-sectional Study Protocol

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ABSTRACT

Introduction: The Carcinoma Antigen (CA) 15-3 is a protein antigen from the mucin family that contains carbohydrates. It has shown potential as a tumour marker in breast cancer for detection, diagnosis, prognosis, therapy monitoring and assessing disease progression. Elevated CA 15-3 levels may correlate with tumour burden, making it a useful tool in preoperative evaluation.

Need of the study: There is a need for reliable, non invasive biomarkers that can support the clinical evaluation of tumour growth in breast cancer. Assessing CA 15-3 levels before surgery may help in predicting tumour behaviour and enhancing the accuracy of preoperative staging.

Aim: To evaluate the usefulness of CA 15-3 as a marker of tumour growth in patients with preoperative breast cancer.

Materials and Methods: This prospective observational study will be conducted over a span of two years (2023-2025) within the haematology and histopathology segment of the Department of Pathology, in collaboration with the General Surgery and Biochemistry Departments at Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, Maharashtra, India. This study will include 50 female patients diagnosed with breast carcinoma based on biopsy. Each case will be staged according to the Tumour, Node, Metastasis (TNM) classification system. CA 15-3 levels will be measured before surgery using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Over two years, CA 15-3 levels will be analysed in biopsy-confirmed breast carcinoma cases, with the expectation of finding higher levels in malignant and progressive tumours. The present study aims to validate CA 15-3 as a prognostic biomarker, supporting early detection, tailored treatment and improved patient outcomes. The study will assess the correlation between CA 15-3 levels, histopathological markers and TNM staging in breast carcinoma. It aims to establish CA 15-3 as a cost-effective diagnostic tool and refine treatment protocols. By integrating CA 15-3 with existing markers, the study seeks to improve early detection and personalise treatment strategies for better patient outcomes.

Keywords: Breast cancer, Carcinoma antigen, Metastasis, Prognosis, Tumour marker

INTRODUCTION

Breast cancer is on the rise among women in India, reflecting an increasing prevalence. Despite this concerning trend, there has been notable progress in improving survival rates. However, it's important to recognise that treatments for breast cancer can profoundly impact the patient's overall quality of life [1]. Moreover, early diagnosis allows women more choices in selecting treatment options. Hence, it is necessary to identify reliable prognostic factors.

Along with the traditional pathological factors such as tumour size, tumour grade, lymph node status and molecular markers-including hormone receptor status and Human Epidermal growth factor Receptor 2 (HER2) expression-serum tumour markers play an essential role in screening, early diagnosis of recurrence and treatment of many malignancies [1].

Breast cancer can be diagnosed through various tests such as breast examinations, mammography, breast ultrasound and tissue diagnosis via biopsy. Among the assessments noted earlier, tissue biopsy is regarded as the definitive standard for diagnosis. Recently, serum concentration of tumour markers has been utilised to detect tumour activity [2]. They provide a less intrusive, less expensive source of data that is critical for monitoring disease progression, establishing prognosis and determining treatment options [2].

In 2022, the average incidence rate for cancer cases was 100.4 per 100,000 individuals, with a particularly high rate of 105.4 per 100,000 for women diagnosed with breast carcinoma. This rate is higher for urban women (1 in 22) compared to the rural group (1 in 60). The likelihood of women succumbing to breast carcinoma is estimated to be approximately 1 in 39, or roughly 2.6% [3].

There has been considerable interest in recent years regarding the predictive value of preoperative CA15-3 levels in breast cancer. Preoperative CA15-3 levels have been shown in studies to be beneficial in detecting and treating breast carcinoma. As a result, the European Group on Tumour Markers has advised that CA15-3 levels be utilised in breast cancer prediction, early identification of disease progression and therapy monitoring [1].

The numerals "15-3" correspond to the antibodies employed in antigen immunoassays. CA15-3 is a mucin-containing carbohydrate antigen [3]. Mucins (MUCs) are large transmembrane glycoproteins with extracellular domains comprising a strongly O-linked glycosylated protein core that contains a variable number of highly conserved 20-amino acid repeat units [2]. According to their genetic and biomolecular characteristics, MUC1 to MUC7 are classified into seven families [3]. CA15-3 belongs to the MUC1 family. The MUC1 gene is found in several tissues and apparently produces an identical core protein [3]. The distinguishing feature between different tissue sources is the variation in the extent of glycosylation (carbohydrate content) [3]. In breast tissue, the carbohydrate content is approximately 50% [4]. MUC1 hinders tumour cell lysis and cell-to-cell contact [3]. However, the precise physiological roles of MUC1 proteins remain incompletely understood. MUC1 is overexpressed in the majority of carcinomas [4]. The MUC1 gene is overexpressed in malignant breast tumours, which allows the use of the gene product CA15-3 as a tumour marker for breast cancer [3].

The CA15-3 levels in the circulatory system can be used to screen for cancers other than breast cancer, such as lung, pancreatic, ovarian, liver and colon cancer. However, it has been observed

to be high in benign liver and benign breast illnesses, leading to misleading positive results [5]. CA15-3 is tested 2 to 3 weeks before surgery to obtain an accurate baseline, thus avoiding surgery-related fluctuations and allowing for a clear comparison of pre- and postsurgery results.

Therefore the aim of the present study is to assess the utility of CA15-3 as a tumour proliferation marker in breast carcinoma patients. The primary objectives of the study are to confirm examination. breast carcinoma by histopathological, to determine the pathological TNM stage of breast carcinoma and to establish the immunophenotypic status of breast carcinoma. The secondary objectives are to assess the utility of CA15-3 as a tumour proliferation marker in breast carcinoma patients, to assess the correlation between the expression of CA15-3, histopathological prognostic markers, pathological TNM staging, and immunophenotypic biomarker status in breast carcinoma patients.

REVIEW OF LITERATURE

Breast carcinoma is the most frequent malignancy in women globally and ranks second only to cervical cancer in India. Breast carcinoma is becoming more prevalent in both developed and developing nations; in developed countries, peak occurrence happens after the age of 50 years, while in India, it occurs after the age of 40 years [6].

Several factors increase the likelihood of breast cancer occurrence, such as being female, advancing age, personal history of breast cancer, familial breast cancer history, early onset of menstruation, later onset of menopause, history of any radiation treatments, nulliparous status, lack of breastfeeding and being overweight. Mutations in certain genes such as BReast CAncer gene (BRCA), Phosphatase and TENsin homolog (PTEN), Tumour Protein 53 (TP53), Serine/Threonine Kinase 11 (STK11), CaDHerin 1 (CDH1) serve as major risk factors for the development of breast cancer [7].

A family history of breast cancer significantly increases an individual's risk, particularly with first-degree relatives (mother or sister), where having such a relative approximately doubles one's risk compared to those without affected family members. About 5% to 10% of women diagnosed with breast cancer have a first-degree relative with the disease, reflecting a clustering effect within families. Second-degree relatives (like aunts or grandmothers) with breast cancer also have an elevated risk, reported in approximately 10% to 20% of cases. This familial risk often correlates with underlying genetic factors, such as mutations in BRCA1 or BRCA2 genes, which further increase susceptibility. Age at diagnosis among relatives and the number of affected family members further modulate risk levels. Consequently, individuals with a family history of breast cancer may benefit from earlier and more frequent screening, genetic counselling and potentially, genetic testing to inform personalised risk management strategies [8].

Mutations in BRCA1 and BRCA2 are responsible for 80% to 90% of single-gene family carcinomas of the breast and 3% to 6% of all breast carcinomas [8].

Apart from that, classic molecular prognostic markers for breast carcinoma include Oestrogen Receptor (ER), Progesterone Receptor (PR) and HER2neu receptor expression, while other non-molecular markers include postmenopausal age, size of the tumour, metastasis and number of lymph nodes [2].

Breast tumours associated with BRCA1 mutations are usually poorly-differentiated, exhibiting distinctive morphological features and are categorised under the TNBC (Triple-Negative Breast Cancer) subgroup. Conversely, BRCA2-related breast carcinomas are also poorly-differentiated but are more commonly ER-positive compared to BRCA1 tumours [9].

Many evolving techniques are used to determine the prognosis of breast cancer, one of which is serum tumour markers. These provide minimally invasive and cost-effective methods for prognosis. They are particularly beneficial in assessing treatment efficacy, as blood concentrations and the percentage of patients with increased levels of this marker tend to rise with disease severity (stage) and/or tumour size [1].

T: Tumour [Table/Fig-1]

The stage of the tumour can be determined using TNM staging, which is accepted by the American Joint Committee on Cancer. Here, T stands for Tumour, N stands for lymph node involvement and M stands for Metastasis to distant organs or structures [10].

Classification	Description
T	Site of tumour
Tx	Tumour cannot be assessed
T0	No evidence of primary tumour
Tis	In-situ cancer (confined to either duct or lobule)
T1, T2, T3, T4	Size and/or extent of tumour

[Table/Fig-1]: Classification of breast carcinoma according to tumour size.

N: Lymph Node Involvement

The lymph nodes that can be involved in breast cancer are primarily the axillary lymph nodes, internal mammary lymph nodes and supraclavicular lymph nodes. Lymph node status is depicted as Nx, N0, N1, N2, or N3.

M: Metastasis to Distant Organs

As per previous research findings, higher presurgery CA15-3 levels were significantly linked to larger tumour sizes, with percentages of 20% for T1, 34.8% for T2 and 48.5% for T3 tumours [Table/Fig-2] [11].

Classification	Description
M	Depicts distant metastasis
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis present

[Table/Fig-2]: Classification of breast carcinoma according to metastasis of the carcinoma.

Four molecular subtypes of breast cancer-Luminal A, Luminal B, HER-2 positive and triple-negative are distinguished based on the levels of ER, PR, HER2 and Ki67 expression [12]. Data indicate that Luminal A shows the highest survival rates among these subtypes, whereas triple-negative exhibits the lowest. Immunophenotypic markers play a critical role in guiding treatment decisions and assessing the risk of carcinoma progression [Table/Fig-3] [12].

Type	Hormone status	Her2neu	Ki67
Luminal A	ER+/PR+	-	<14%
Luminal B	ER+/PR+	+	>14%
Triple negative	ER-/PR-	-	<14%
Her2neu enriched	ER-/PR-	+	>14%

[Table/Fig-3]: Breast carcinoma according to Luminal classification.

Despite no statistical significance, elevated preoperative CA15-3 levels were observed in all breast cancer molecular subtypes. The highest prevalence was found in TNBC (48.0%) and HER2-enriched (47.1%), while the luminal subtypes exhibited the lowest prevalence, with rates of 30.4% for Luminal A, 24.2% for Luminal B (HER2) and 30.4% for Luminal B (HER2+). Notably, presurgical CA15-3 levels were not significantly associated with ER, PR, or HER2 overexpression [13].

The study by Lian M et al., titled "The Association of Five Preoperative Serum Tumour Markers and Pathological Features in Patients with Breast Cancer," indicated that before surgery, CA15-3 levels were

higher in breast cancer patients compared to healthy volunteers and those with benign conditions [14]. CA15-3 levels increased as tumours advanced. Among breast cancer patients, CA15-3 levels were linked to tumour size, with higher levels in stage T3 compared to T2 and T1. Additionally, CA15-3 levels were significantly higher in patients with involved lymph nodes (N1 or N2) compared to those with no lymph node involvement (N0) [14].

Daniele A et al., conducted a research study titled "Clinical Usefulness of Carcinoma Antigen 15-3 in Breast Carcinoma Patients Prior to and Following Surgery," and discovered that patients' mean serum CA15-3 levels were considerably higher before surgery [15]. The study also found that increased preoperative serum CA15-3 levels were significantly associated with the occurrence of metastatic illness [15].

Furthermore, the study by Shao Y et al., titled "Elevated Levels of Serum Tumour Markers CEA and CA15-3 are Prognostic Parameters for Different Molecular Subtypes of Breast Carcinoma," indicates that raised levels of serum CA15-3 have independent prognostic value for breast carcinoma [1]. An elevated preoperative serum marker may be beneficial in evaluating the likelihood of recurrence and metastasis of breast carcinoma following surgery and in future investigations [1].

MATERIALS AND METHODS

This prospective observational study will be conducted over a span of two years (2023-2025) within the haematology and histopathology segment of the Department of Pathology, in collaboration with the General Surgery and Biochemistry Departments at Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, Maharashtra, India. The research protocol for the present study has received ethical approval from the Datta Meghe Institute of Medical Sciences (Deemed to be University) Institutional Ethics Committee on March 31, 2023, with the designated study number DMIHER (DU)/IEC/2023/866. Informed consent will be obtained from the eligible patients who choose to participate.

The present study will enroll women presenting with a breast lump, selected from the Outpatient Department (OPD) of Surgery and Surgical Oncology at Acharya Vinoba Bhave Rural Hospital (AVBRH) in Sawangi, Wardha, Maharashtra. The recruitment process will focus on identifying eligible patients who meet the criteria for participation in the study. Inclusion and exclusion criteria for the selection of participants are mentioned below.

Inclusion criteria:

- All female patients diagnosed with breast cancer on histopathological examination;
- No history of chemotherapy and/or radiotherapy received;
- Serum tumour marker i.e., CA15-3, was detected within two to three weeks prior to surgery.

Exclusion criteria:

- All individuals with lesions other than breast carcinoma, such as benign breast tumours, myoepithelial tumours and mesenchymal tumours;
- History of chemotherapy and/or radiotherapy received;
- Serum tumour marker i.e., CA15-3, detected before two weeks and after three weeks.

Sample size calculation: The formula used:

$$\frac{Z^2 \times P \times (1-P)}{E^2}$$

Z is the level of significance at 5%

i.e., 95% Confidence interval=1.96

P=Incidence of breast cancer=14%=0.14

E=Desired error of margin=7%

$n=(1.96\times1.96)\times0.14\times(1-0.14)/(0.10)^2=46.25$

n=50 patients needed in the study.

Study Procedure

The screening process for potential cases of suspicious breast carcinoma will be carried out in the OPDs of the Department of General Surgery and Surgical Oncology at AVBRH and Shalinitai Meghe College of Medical Sciences (SGMCH).

Upon identification of a suspicious carcinoma case, a biopsy will be performed by the surgeon. The obtained biopsy sample will then be sent to the Pathology Department at Jawaharlal Nehru Medical College (JNMC) for immunohistopathological examination to confirm the diagnosis. The histopathological analysis of the breast carcinoma will adhere to the guidelines set forth by the American Joint Committee on Cancer (AJCC) [10].

Furthermore, blood samples will be collected to test for the serum marker CA15-3. The results of CA15-3 expression will be carefully examined in correlation with histopathological grades, TNM staging and the expression of immunophenotypic markers. This comprehensive approach seeks to deepen the understanding of how CA15-3 levels relate to different clinical and pathological factors in cases of breast carcinoma.

All patient information, both pertaining to and concerning the individual, will be maintained with the utmost confidentiality. Under no circumstances will any information be disclosed. This commitment to confidentiality is unwavering and applies regardless of the situation. The privacy and confidentiality of patient data are of paramount importance and will be rigorously upheld throughout the course of the study.

Methodology of interpretation:

1. **Prospective research study:** A prospective research study will be undertaken for individuals diagnosed with breast carcinoma through histopathological examination of their breast tissue. The tumour stage will be determined using TNM staging (8th AJCC edition) [10].

Interpretation of Overall Stages:

- Stage 0: Tis
- Stage I: T1, N0
- Stage II: T2, N0; T3, N0; T0, N0; T1, N1; T2N1
- Stage III: Invasion in ribs, skin, or matted lymph nodes (T3N1, T0N2, T1N2, T2N2, T3N2 and T N3) indicates locally advanced carcinoma of the breast.
- Stage IV: M1; Advanced breast cancer.

2. **Sample collection:** During the premastectomy phase, a 3 mL blood sample will be systematically collected from each patient. Subsequently, the serum from these samples will be meticulously isolated, placed in a securely sealed container and stored in a refrigerated environment at a temperature of -70°C. These samples will be preserved until they are thawed and processed in separate batches for CA15-3 analysis.

   The Enzyme-Linked Immunosorbent Assay (ELISA) method will be employed for the analysis of CA15-3. This test is characterised by a dual-sided solid-phase enzyme immunoassay. The CA15-3 molecules will be positioned between two monoclonal antibodies in a sandwich-like configuration. One antibody will adhere to the surface of the microtiter plate wells, while the other will be linked to horseradish peroxidase, functioning as the enzyme conjugate. The ensuing enzymatic process will produce a colour directly correlated with the amount of CA15-3 molecules detected in the test. This colour development occurs following a designated period of incubation and subsequent washing. This meticulous analytical process using ELISA ensures accurate and reliable assessment of CA15-3 levels in

the serum samples obtained prior to mastectomy.

3) Correlation of Expression of CA15-3 with the Expression of ER, PR, HER2

Outcome

Primary outcome:

- To evaluate the association between serum CA15-3 levels and tumour progression in breast cancer patients.

Secondary outcomes:

- To assess the role of CA15-3 in detecting metastasis and recurrence.
- To determine the impact of early detection of serum CA15-3 levels on treatment decisions and the selection of appropriate therapeutic modalities.

STATISTICAL ANALYSIS

Statistical analysis will involve the utilisation of the Chi-square test and the software Statistical Package for Social Sciences (SPSS) version 27.0. A significance level of $p<0.05$ will be employed for the analysis.

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