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Research Protocol

Pathology Section

Immunoprofiling of BRAF^{V600E} in Precancerous and Cancerous Lesions of Breast by Immunohistochemistry and its Association with Clinicopathological Features

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ABSTRACT

Introduction: Breast carcinoma in women is one of the few causes of morbidity and mortality which concerns the health providers. The molecular basis of breast carcinoma in recent years has provided the insight for the genesis of carcinoma, the prognostic, and predictive utility for targeted therapies.

Aim: A study has been planned with the aim to know immunoprofiling of BRAF^{V600E} in precancerous and cancerous lesions of the breast by Immunohistochemistry (IHC) and its association with clinicopathological features.

Materials and Methods: The subjects will be divided in two groups of 45 cases each: Precancerous breast lesions; and Invasive ductal carcinoma. The tissue of these cases will undergo IHC of BRAF^{V600E}. The comparison of expression is to be brought out and the clinicopathological association of immunoexpression of BRAF^{V600E} will also be studied.

Keywords: Breast cancer, Molecular subtype, Tumour grade, Tumour stage

INTRODUCTION

Breast cancer in women remains an active area of scientific research for past many decades. The reasons for the comprehensive evaluation of breast cancer will be to understand the progression of precancerous to cancerous lesions. To determine morphological prognostic factors, and molecular genetic aspects for treating breast cancer by antibody therapy and other approaches [1].

The mortality and morbidity of breast cancer in women is considerably high in India and is comparable to global statistics. Therefore, breast carcinoma still remains the major cause of morbidity and mortality in Indian women [2].

Breast carcinoma has multiple facets. Two decades ago, the morphological pathology of breast was considered to be important for decision-making having prognostic implications. However, research over the past two decades has shown a paradigm shift from morphology to molecular [3-5].

Pathogenesis of breast carcinoma is now considered to be genetic alterations impacting cell proliferation and cell cycle parameters. Both sporadic or familial breast carcinoma have been found in association with multiple molecular alterations to BRCA-1 and BRCA-2 genes, genes involving BRAF/MEK/ERK pathways and many others. The previous decade, BRCA-1 and BRCA-2 mutations were studied for their role in the pathogenesis of breast carcinoma. Similarly, mutations in tumour suppressor genes TP53, SKT11, and CDH1 were also studied to establish their link in the pathogenesis of breast carcinoma. Dysregulation of these genes have been associated with breast carcinoma pathology and other clinicopathological aspects [6-9].

Over the last decade, researchers have been interested in immunohistochemical detection of mutant BRAF (BRAF^{V600E}) and its role in pathogenesis, treatment, and prognosis of breast carcinoma, especially intraductal carcinoma. There are studies that have examined BRAF^{V600E} by IHC in precancerous ductal lesions of breast [10-14].

Indian studies on these aspects of BRAF are scarce. Therefore, mutation of BRAF in breast cancer affecting Indian women is not statistically validated [2].

There remains a research gap in understanding the role of mutant BRAF in precancerous and cancerous ductal cell lesions of breast in Indian women. Therefore, the proposed study will carry out immunoprofiling of ductal cells in precancerous and cancerous lesions of breast in Indian women.

The aim of this study will be to study immunoprofiling of BRAF^{V600E} in precancerous and cancerous lesions of breast by IHC and its association with clinicopathological features.

The following are the objectives of the study:

1. To study the immunoexpression of BRAF^{V600E} for its frequency in precancerous and cancerous ductal cell lesions of breast.
2. To compare the frequency of immunoexpression of BRAF^{V600E} between precancerous and cancerous ductal cell lesions of breast.
3. To establish the association of immunoexpression of BRAF^{V600E} with Bloom Richardson (BR) grade, tumour stage, nodal stage, Tumour, Node, Metastasis (TNM) stage, and molecular subtype of invasive ductal carcinoma.

REVIEW OF LITERATURE

Breast cancer is the most common cancer in women, and it is the leading cause of death. Early diagnosis and treatment can stop the disease from progressing and reduce the morbidity and death rate [10]. Therefore, the purpose of this study will be to see the immunoprofiling of BRAF^{V600E} in precancerous and cancerous lesions of breast using IHC to establish its association with clinicopathological features.

A study conducted by Jung YY et al., on BRAF mutation in breast cancer by BRAF^{V600E} mutation-specific antibody revealed that 30 of the 230 breast cancer cases tested positive for the BRAF^{V600E} mutant specific antibody, and 17 (7.4%) had nuclear expression. ER negativity (p=0.003), PR negativity (p=0.031), and TNBC subtype (p=0.009) were all linked with nuclear BRAF^{V600E} positive cases [11]. BRAF^{V600E} mutant specific antibody was positive in 4 (3.0%) of the 132 TNBC cases tested, while 10 (7.6%) of the cases had nuclear expression. Nuclear BRAF^{V600E} positivity was related with a poorer histological grade in TNBC (p=0.012). The presence of BRAF^{V600E}

did not connect with the prognosis of breast tumours or TNBC. Finally, there was an indication of existence of a BRAF mutation due to presence of BRAF^{V600E} mutant specific antibody positivity in a small percentage of breast cancer and TNBC cases. The BRAF mutation was not linked to clinicopathologic variables in breast cancer.

According to a study conducted by Mohamad BJ et al., BRAF mutations were related to various clinicopathological variables and were detected in very few cases of breast cancer [14]. In a study conducted by Perou CM et al., molecular portraits characterised not only the particular tumour 'sample', but also the 'tumour' itself [4]. This was due to the identification of unique expression pattern of a tumour in original independent samples.

According to a study by Tilch E et al., the actionable oncogenic mutations on the OncoCarta Panel^{TM} were usually rare in triple-negative and basal-like breast cancers [3]. The PIK3CA mutation in breast cancer was an exception in the study.

MATERIALS AND METHODS

The patients' name, age and gender as well as other details like registration number, unit, Inpatient Department (IPD), and Outpatient Department (OPD) will be recorded. The observational study will be carried out from 1st January 2019 to 31st May 2022 retrospectively, and 1st June 2022 to 31st December 2023 prospectively at Wardha, Maharashtra.

The sample size will be tentatively calculated as follows:

Precancerous=45 cases

Cancerous=45 cases

Cochran formula for sample size:

$$n=\frac{Z^2_{\alpha/2}\cdot p\cdot(1-p)}{E^2}$$

where,

$Z_{\alpha/2}$ is the level of significance at 5% i.e., 95% Confidence Interval (CI)=1.96

Incidence of breast cancer [15,16]=13.5%=0.135

Error of margin=10%=0.1

$$n=\frac{1.96^2\times0.135\times(1-0.135)}{0.1^2}=44.86$$

n=45 patients will be needed in each group

Study reference: Global Cancer Observatory, WHO [16]

Formula reference: Cochran WG, [17]

This study will stringently follow the ethical guidelines for experimentation on humans as laid down by the Declaration of Helsinki. A clearance certificate has been obtained by the Institutional Ethics Committee for this study with Ref. No. DMIMS(DU)/IEC/2022/1061.

Inclusion criteria: Cases diagnosed for breast cancer on biopsy or surgical specimens of breast cancer.

Exclusion criteria:

1. Breast biopsy with inflammatory histomorphology.
2. Biopsy without representative histomorphology of precancerous and cancerous lesion
3. Deficient clinical data and details

Clinical parameters like symptoms, general and systemic examination, breast examination findings, and provisional diagnosis will be studied.

Laboratory investigations like Complete blood count, basic blood biochemistry, tumor markers will be recorded.

Radiological investigations will be documented from case sheets of the patient, from Ultrasonography (USG), mammography, Computed Tomography (CT) scans, and Magnetic Resonance Imaging (MRI) in appropriate cases. The study material will consist of biopsies of breast lumps and surgically resected specimens of mastectomy in cases suspected of precancerous and cancerous lesions.

The following laboratory methods will be conducted.

1. Grossing of mastectomy specimen [6]:

   Biopsy specimens and mastectomy specimens of breast lump will be grossed by standard techniques.

2. Histopathological processing of tissue [18]:

   Tissue sections will be processed in automated histokinette for preparing paraffin sections.

3. Haematoxylin and eosin staining [6,18]:

   Haematoxylin and eosin staining of paraffin sections will be carried out by standard procedures.

4. Reporting of haematoxylin and eosin sections [18]:

   The histopathological diagnosis of precancerous and cancerous lesions of the ductal cells will be based of standard criteria.

5. IHC of tumour tissue for BRAF^{V600E} [18]

   Paraffin embedded sections or biopsy and surgically resected specimens that represent either precancerous or cancerous ductal cell lesions of breast will be selected for IHC.

IHC will be carried out by biotin avidin peroxidase method using monoclonal antibody GE-BRAF^{V600E} (Abcam, Cambridge, UK) against BRAF^{V600E} in the following steps:

a) Formalin fixed paraffin embedded tissue section will be taken.

b) Slides will be deparaffinised as well as rehydrated.

c) Standard heat epitope retrieval will be done using pressure cooker, and followed by washing the slides three times with Phosphate Buffered Saline (PBS).

d) Peroxidase blocking- Endogenous peroxidase activity will be blocked by applying 3% hydrogen peroxide for 10 minutes.

e) BRAF^{V600E} primary antibody will be added to the section and incubated at room temperature for 30-40 minutes.

f) The slides will be rinsed three times with PBS.

g) Slides will be kept for 30 minutes at room temperature for the secondary antibody (streptavidin biotin) to react, followed by washing with PBS.

Interpretation of immunohistochemical staining [11] was done as follows: Tumour cells staining will be graded as follows:

0: No or weak immunostaining in less than 1% of the tumour (0)

1: Immunostaining in 1-10% of tumour will indicate focused expression (+)

2: Positive in 11-50%of tumours (++)

3: Positive in 51-100% of tumour samples (+++)

The evaluation will cover the complete tumour region. A score of 0 will indicate a negative result and a score of one or more will indicate a positive result. Cases with 20% or more positive tumour cells will be recorded as BRAF^{V600E} positive [11].

STATISTICAL ANALYSIS

The statistics of association and relationship between the immunoexpression of BRAF^{V600E} with clinicopathological characters will be carried out for percentage and Chi-square test with value of significance (Pearson's correlation coefficient p-value). Software to be used will be Statistical Package for the Social Science (SPSS).

The research plan is shown as a flowchart in [Table/Fig-1].

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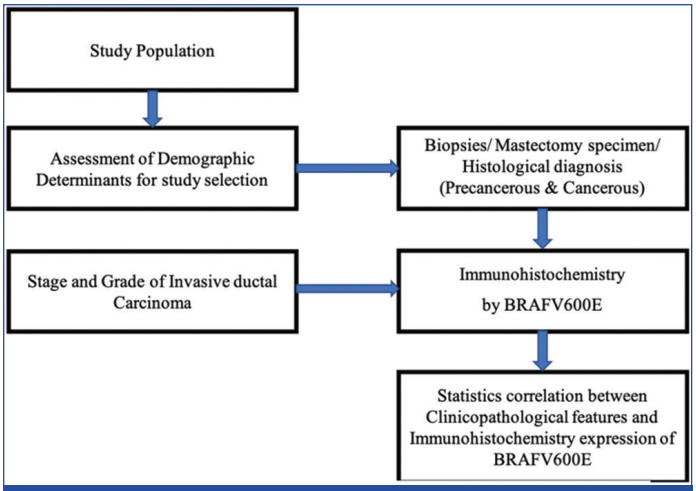

[Table/Fig-1]: Flowchart of research work.

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