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SHORT COMMUNICATIONS

Analysis of Pik3ca Gene Mutation along with Screening of Estrogen, Progesterone and Ki-67 Expression in Breast Cancer Cases

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Abstract

Analysis of Breast cancer exercised and contributed to the screening of the level of estrogen, progesterone and Ki-67 expression with prevalence rate of HER2/neu gene of mainly female population affected by breast cancer in Guwahati and along with detection of PIK3CA mutation in HER2/neu positive cases of other parts of the countries. A preliminary study conducted to analyze the prevalence, prognosis and treatment of breast cancer cases. In this study, we investigate the prevalence and expression patterns of estrogen receptor (ER), progesterone receptor (PgR), Ki-67, and HER2/neu gene, and identifies PIK3CA mutations in breast cancer patients from Guwahati, Northeast India, and other regions. A novel aspect of this study is the comparison of HER2/neu prevalence between Assam and other regions. Thus, the results indicated a lower HER2/neu positivity rate in Assam, with most affected individuals being housewives, that suggests unique regional risk factors. PIK3CA exon 20 mutations were identified in HER2- positive cases, potentially guiding targeted therapy.

Keywords: HER2/neu, breast cancer, PIK3CA mutation, Ki-67 biomarker, risk factors and treatment

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Introduction

Breast Cancer: A Global Health Challenge

Breast cancer (BC) has emerged as a leading cause of morbidity and mortality among women worldwide. It ranks as the second most frequently diagnosed cancer globally, highlighting its significant impact on public health [1]. Despite advancements in early detection and treatment, BC remains a major concern, particularly in low- and middle-income countries (LMICs).

Epidemiology and Mortality

In 2020, the International Agency for Research on Cancer (IARC) reported over 2.26 million new BC cases and approximately 685,000 deaths worldwide. Notably, LMICs accounted for a disproportionately high number of these deaths, with nearly 500,000 women succumbing to the disease in these regions. This represents almost three-quarters of global BC mortality [2].

- Incidence: 2.26 million new cases (2020) [2]
- Mortality: 685,000 deaths (2020) [2]
- LMIC Mortality: 500,000 deaths (2020) [2]
- Male Incidence: Approximately 0.5-1% of all BC cases occur in men [2].

The IARC projects a significant increase in BC incidence and mortality by 2040, with estimates exceeding 3 million new cases and 1 million deaths annually [3]. This anticipated surge is primarily attributed to demographic shifts, such as population aging, and lifestyle changes, particularly in LMICs [2].

Risk Factors

The development of BC is influenced by a complex interplay of genetic, hormonal, and lifestyle factors.

- Genetic Factors:

o Hereditary factors account for 10-30% of BC cases, with 5-10% linked to strong hereditary predispositions [4].

o Mutations in high-penetrance genes, such as BRCA1 and BRCA2, significantly increase BC risk. Approximately 5-10% of breast cancer cases are related to these gene mutations.

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o BRCA1 and BRCA2 mutations, which are inherited in 60% of cases, impair DNA repair, transcription, and cell cycle regulation, leading to increased cancer susceptibility [4].

● Hormonal Factors:

o Prolonged exposure to estrogen, without adequate progesterone counterbalance, is associated with increased BC risk.

o Early menarche and late menopause contribute to increased lifetime estrogen exposure [5, 6].

o Hormone receptor status (ER and PgR) is crucial for determining treatment strategies, with approximately 75-80% of invasive BCs being ER-positive [7].

● Lifestyle Factors:

o Dietary habits, physical inactivity, and exogenous hormone use are modifiable risk factors.

o Obesity, particularly postmenopausal, is associated with increased BC risk [8].

o Alcohol consumption increases breast cancer risk.

o Lack of breastfeeding is also considered a risk factor [9, 10].

● Age:

o The risk of breast cancer increases with age. The lifetime risk of developing breast cancer in the US is 1 in 8. [11].

● Personal History:

o A prior diagnosis of DCIS, or invasive breast cancer, increases the risk of developing future breast cancer [12].

● Family History:

o A family history of breast cancer, especially in a first degree relative, increases breast cancer risk [6].

Molecular Subtypes and Biomarkers

BC is classified into distinct molecular subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2).

● Luminal A: ER-positive and/or PgR-positive, HER2-negative, low Ki-67, favorable prognosis [7].

● Luminal B: ER-positive and/or PgR-positive, HER2-positive or negative, high Ki-67, less favorable prognosis than Luminal A [7].

● HER2-enriched: ER-negative and PgR-negative, HER2-positive, aggressive growth, responsive to HER2-targeted therapies [7].

● Triple-negative (TNBC): ER-negative, PgR-negative, and HER2-negative, aggressive, limited treatment options [7].

Biomarkers such as Ki-67 and HER2 play crucial roles in prognosis and treatment selection.

● Ki-67: A marker of cell proliferation, with higher values indicating more aggressive tumor growth [7].

● HER2: Overexpression is associated with aggressive tumor behavior and responsiveness to HER2-targeted therapies [13].

Regional Considerations: Northeast India

The Northeast region of India, including Assam, has a distinctive sociocultural and dietary profile that influences its disease burden. High prevalence of tobacco and betel nut consumption, often starting at a young age, along with alcohol use and low intake of green vegetables, contributes to systemic inflammation and hormonal imbalances that may increase breast cancer susceptibility [14, 15]. Furthermore, limited access to healthcare facilities, lower rates of regular screening, and socioeconomic barriers often delay diagnosis. Cultural factors also play a role; for instance, housewives often ignore early symptoms due to family obligations. These factors potentially contribute to the observed dominance of hormone receptor-negative and triple-negative breast cancer (TNBC) subtypes in this region, which are known for poor prognosis and limited treatment options [16, 17].

PIK3CA and Targeted Therapies

● PIK3CA mutations are common in BC, particularly in HR-positive subtypes [18].

● Targeting the PI3K pathway offers promising therapeutic strategies, including monoclonal antibodies such as trastuzumab and pertuzumab [19, 20].

● These monoclonal antibodies have shown to increase survival rates for HER2 positive breast cancer. [13].

Methodology

Patient Selection And Ethical Approval

Fifty consecutive, non-duplicative female breast cancer patients diagnosed with HER2 overexpression or amplification, as per ASCO-CAP 2007 guidelines, were enrolled from the State Cancer Institute, Guwahati, between November 2021 and June 2022. Patients were informed verbally and in writing about the potential research use of their tissue samples. At the time of surgery or biopsy informed consent was being obtained from the patients. Patients had the right to decline or withdraw at any stage without affecting their treatment procedures. This study was approved by the Institutional Ethics Committees of Gauhati University and the State Cancer Institute, Guwahati.

Inclusion Criteria

● Female breast cancer patients

● HER2 overexpression or amplification confirmed by IHC scoring of 3+ or via FISH for confirmation

● Availability of formalin-fixed paraffin-embedded (FFPE) tissue blocks.

Exclusion Criteria

1. HER2-equivocal cases (IHC score 2+) were excluded from molecular and statistical analyses. However, they were initially recorded for completeness but were not included in mutation or statistical correlation results.

DNA Extraction And Quantification

Genomic DNA was extracted from 10 µm thick FFPE sections using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. The concentration and purity of extracted DNA were assessed using a Multi Scan GO Thermo Scientific

spectrophotometer.

DNA Extraction

DNA was extracted from samples (bone marrow aspirates or peripheral blood) by using the manufacturer instruction

. The procedure of DNA extraction is given below

Materials Required

Qiagen DNeasy blood and tissue kit (69504), Centrifuge (11000 rpm), 95-100%

ethanol, 56°heat block, Pipette (20-200µL, 200-1000µL), Vortex Mixer.

Procedure

· 3 sections (10 micron thick) of FFPE tissue were taken in 1.5 centrifuge tube and 1mL xylene added.

· Mixed thoroughly by vortexing and centrifuged at 11000 rpm for 5 min.

· Supernatant was discarded and 1mL ethanol added.

· Mixed thoroughly by vortexing, centrifuged at 11000 rpm for 1 minute and the supernatant was discarded.

· The tissue dried at 56°C.

· 200µL buffer ATL + 20 µL Proteinase K added in labelled 1.5 mL lysis tube.

· Mixed thoroughly by vortexing.

· Incubated overnight at 56°C for completely lysis of the tissue.

· Mixed thoroughly by vortexing.

· 200ul AL buffer added to lysed sample and incubated for 15 min. at 56°C.

· 200µL ethanol added.

· Mixed thoroughly by vortexing.

· The mixture transferred into DNeasy mini spin column placed into 2mL collection

tube.

· Centrifuged at 11000 rpm for 1 minute.

· Discarded flow through and collection tube. The column is placed in a 2mL collection tube.

· 500µL buffer AW1 added and centrifuged at 11000 rpm for 1 minute.

· Again, discarded flow through and placed the spin column in new 2mL collection

tube.

· 500µL buffer AW2 added and centrifuged at 11000 rpm for 1 minute.

· Discarded flow through and centrifuged for dry spin and discarded the collection

tube.

· The spin column transferred to the new 1.5mL microcentrifuge tube.

· The DNA eluted by adding 50µL (FFPE) buffer AE to the centre of the column membrane.

· Incubated for 5 minutes at room temperature and centrifuge at 11000 rpm for 1 minute.

· Stored the extracted DNA at -20°C/-80°C till downstream processing.

PIK3CA mutation analysis and Tumor Grading

PIK3CA mutations were detected using the Therascreen® PIK3CA RGQ PCR Kit (Qiagen), which is a qualitative real-time PCR assay targeting known hotspot mutations, including Exon 20.

Tumor Grading Criteria

Tumor were graded histopathologically based on mitotic index, nuclear pleomorphism, and tubule formation, by following the Nottingham grading system:

Grade II: Moderately differentiated tumors with intermediate prognosis.

Grade III: Poorly differentiated tumors with aggressive clinical behavior.

Clinical Significance of Exon 20 Mutation

● PIK3CA Exon 20 mutations are associated with enhanced PI3K pathway activation, promoting cell survival, proliferation, and resistance to anti-HER2 therapies. Their presence may indicate eligibility for targeted therapies such as PI3Kα-specific inhibitors (e.g., alpelisib) in hormone receptor-positive cases.

Detection Of Pik3ca Mutations By therascreen PIK3CA RGQ PCR KIT

PIK3CA mutations were analysed on genomic DNA by PCR amplification.

As per manufacturer instructions of the kit were performed to detect different types of PIK3CA mutations.

Results

Data of patient profiling and expression of Estrogen (ER)- Progesterone (PrG) Hormone, HER2/neu gene and Ki-67 biomarker (Table 1, 2 and 3), (Figure 1 and 2).

Among the total patients

● HER2-positive (3+)cases:11.1%(n=5/45)

● HER2-negative:67%

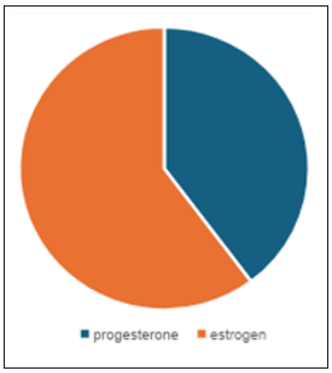

Figure 1. Distribution of ER and PgR expression among breast cancer cases (n = 50). (Note: Data variability may arise due to results pending or missing hormone status in some patients; final percentages are based on interpretable cases.). Expression of ER and PgR (Figure 1). Out of 50 breast cancer patients: ER (Estrogen Receptor) positivity was observed in 50% of cases. PgR (Progesterone Receptor) positivity was seen in 33% of cases.

Table 1. Expression of Estrogen (ER) in Patient Report

Patient Serial Number	Types	Age	Status of ER
1	Invasive breast carcinoma	66/F	Negative
2	Invasive breast carcinoma	37/F	Negative
3	Residual invasive breast carcinoma	48/F	Positive
4	Left breast swelling	27/F	Result Awaited
5	Invasive breast carcinoma	75/F	Positive
6	Invasive breast carcinoma	41/F	Positive
7	Invasive breast carcinoma	33/F	Positive
8	Invasive breast carcinoma	33/F	Negative
9	Invasive breast carcinoma	59/F	Positive
10	Extensive Ductal Carcinoma	42/F	Negative
11	Metastatic CA breast	43/F	Positive
12	Invasive ductal carcinoma	74/F	Positive
13	Metastatic invasive breast carcinoma	60/F	Negative
14	Invasive breast carcinoma	56/F	Negative
15	Invasive breast carcinoma	47/F	Positive
16	Invasive breast carcinoma	55/F	Positive
17	Metastatic breast carcinoma	50/F	Negative
18	Invasive breast carcinoma	52/F	Negative

Table 2. Expression of Progesterone (PrG) Hormone in the Patient Report

Patient Serial Number	Types	Age	Status of PrG
1	Invasive breast carcinoma	66/F	Negative
2	Invasive breast carcinoma	37/F	Negative
3	Residual invasive breast carcinoma	48/F	Positive
4	Left breast swelling	27/F	Result Awaited
5	Invasive breast carcinoma	75/F	Positive
6	Invasive breast carcinoma	41/F	Positive
7	Invasive breast carcinoma	33/F	Positive
8	Invasive breast carcinoma	33/F	Negative
9	Invasive breast carcinoma	59/F	Positive
10	Extensive Ductal Carcinoma	42/F	Negative
11	Metastasis CA breast	43/F	Positive
12	Invasive ductal carcinoma	74/F	Positive
13	Metastatic invasive breast carcinoma	60/F	Negative
14	Invasive breast carcinoma	56/F	Negative
15	Invasive breast carcinoma	47/F	Negative
16	Invasive breast carcinoma	55/F	Negative
17	Metastatic breast carcinoma	50/F	Negative
18	Invasive breast carcinoma	52/F	Negative

HER2-equivocal (2+):17%

Equivocal cases (Patients 7, 16, 17) were excluded from downstream mutation analysis.

HER2/neu status distribution among breast cancer cases.

Triple-Negative Breast Cancer (TNBC)

Of the interpretable cases (excluding equivocal HER2), 30% were triple-negative (ER-/PgR-/HER2-). This aligns with literature reports from similar regions. The discrepancy is reconciled by excluding HER2-equivocal cases from the TNBC analysis denominator (Table 4-7), (Figure 3, 4).

In conclusion, in breast cancer, being the second most affected disease in the population of women worldwide. Larger sample studies are required to identify, analyse and measure the expression prevalence of ER-PR Hormone. It also interferes with result and analyses of the matter in different view by knowing the stages of different types of cancer with the help of Ki-67 biomarker to know the seriousness of the disease. HER2/neu expression and

prevalence may differ with larger sample analyses which can interfere with the results of this study. The HER2/neu positivity was found to be 11.1%, much lower than the numbers reported from Delhi, Mumbai and southern India which have a positive rate between 20% and 25%. There are many reasons why neural connections vary regionally. A challenge in Northeast India is that patients arrive for diagnosis at later stages, often after the time for the most accurate HER2 testing has passed. Furthermore, problems related to HER2 testing equipment such as inconsistent IHC use and too few cases tested with FISH, can influence correct diagnosis. It is possible that choices in sample collection or the amount of genetic and cultural diversity in the Assamese affected the results. Various drugs like for HER2/neu-Herceptin is being used with positive expression of HER2/neu gene. Other than drugs surgery, chemotherapy and hormonal therapy are available for the treatment depending on the different types and level of invasion in the parts of body. Tobacco and betel nut usage, a high salt diet and eating little fruit or vegetables

Table 3. Expression of HER2/neu Gene in the Patient Report

Patient Serial Number	Types	Age	Status of HER2/neu
1	Invasive breast carcinoma	66/F	Negative
2	Invasive breast carcinoma	37/F	Negative
3	Residual invasive breast carcinoma	48/F	Negative
4	Left breast swelling	27/F	Result Awaited
5	Invasive breast carcinoma	75/F	Negative
6	Invasive breast carcinoma	41/F	Negative
7	Invasive breast carcinoma	33/F	Equivocal (2+)
8	Invasive breast carcinoma	33/F	Negative
9	Invasive breast carcinoma	59/F	Negative
10	Extensive Ductal Carcinoma	42/F	Negative
11	Metastasis CA breast	43/F	Positive
12	Invasive ductal carcinoma	74/F	Negative
13	Metastatic invasive breast carcinoma	60/F	Negative
14	Invasive breast carcinoma	56/F	Positive
15	Invasive breast carcinoma	47/F	Negative
16	Invasive breast carcinoma	55/F	Equivocal (SCORE 2+)
17	Metastatic breast carcinoma	50/F	Equivocal (SCORE 2+)
18	Invasive breast carcinoma	52/F	Negative

Table 4. Expression of Ki-67 Biomarker in the Patient Report

Patient Serial Number	Types	Age	Status of Ki-67 (%)
1	Invasive breast carcinoma	66/F	30
2	Invasive breast carcinoma	37/F	60
3	Residual invasive breast carcinoma	48/F	15
4	Left breast swelling	27/F	Result Awaited
5	Invasive breast carcinoma	75/F	90
6	Invasive breast carcinoma	41/F	25
7	Invasive breast carcinoma	33/F	30
8	Invasive breast carcinoma	33/F	80
9	Invasive breast carcinoma	59/F	60
10	Extensive Ductal Carcinoma	42/F	67
11	Metastasis CA breast	43/F	20-25
12	Invasive ductal carcinoma	74/F	0
13	Metastatic invasive breast carcinoma	60/F	60
14	Invasive breast carcinoma	56/F	20-22
15	Invasive breast carcinoma	47/F	40
16	Invasive breast carcinoma	55	25
17	Metastatic breast carcinoma	50	30
18	Invasive breast carcinoma	52	70

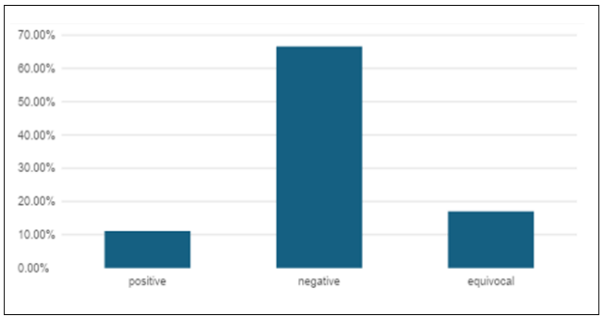

Figure 2. In This Graph, HER2/neu Cases in Breast Cancer were 11.10% Positive, 67% Negative and 17% were Equivocal.

may alter Assam's women's hormone receptors and cancer rates. Also, since many women at the time were mostly housewives dealing with lots of household work, they experienced physical strain and found it hard to go for regular screening. When patients don't know the first signs and come in later, it leads to a later diagnosis. So, with this preliminary study, a lot of knowledge about prevalence, prognosis and treatment planning is being gathered. Furthermore, after got approval by the ethical committee, extension of the work for prognosis outcome and mutational screening gave a better view of the survival rate and observed the chance of relapse of the breast cancer. It gave an insight that in Guwahati the prevalence rate is increasing in a low scale, reason may be due to lack of awareness, food habits, excessive household courses and have witness housewives being affected the most in the patient history. In Guwahati cases of HER2/neu negative cases are highly prevalent in the population, so maximum surgical or chemotherapy treatment is observed, and all are Grade III stage i.e., having later stage of breast cancer having PIK3CA mutation in the Exon number 20 as followed the manufacturer protocol to preformed diagnostic techniques. In the five patients with Exon 20 mutations, these mutations strongly linked to Grade III tumors. When activated, these mutations turn on the PI3K/AKT/mTOR pathway which helps cancer cells multiply and become resistant to treatments designed for HER2. Recently, alpelisib, an inhibitor of PI3Kα, has demonstrated success in treating HR-positive, HER2-negative or HER2-equivocal disease. Conclusively, this work examines the spread of breast cancer subtypes and PIK3CA mutations among people affected in Assam, providing early evidence of the region's special oncology profile. The low HER-2/neu levels, the significant percentage of TNBC and what's more, PIK3CA Exon 20 mutations, suggest that breast tumors in Assam have a different molecular profile to those seen in Delhi and Mumbai.

However, there are some things we must keep in mind when looking at the findings. The analysis is not as powerful and the findings cannot be applied more broadly due to the small number of subjects (n = 50). Excluding HER2-equivocal cases may have made it possible for molecular subtypes between basal and HER2 groups to be overlooked. Because data was taken from just one institution in retrospect, we cannot extrapolate the results across the Northeast Indian population.

Research studies should be carried out at multiple centers with a diversity of patients to make future work more reliable and useful. Introducing NGS to genomic analysis could enhance our understanding of PIK3CA mutations and other clinically important genomic changes. Focusing on public health, by running educational campaigns, implementing screening on a timely basis and

Table 5. For Case of the Expression of Triple Negative Gene in the Carcinoma of Left Breast or Formation Left Breast Lump in the Patient

Patient Serial Number	Types	Age	Status of % Triple negative cases= 30%
1	Invasive breast carcinoma	66/F	Mastectomy
2	Invasive breast carcinoma	37/F	Surgery
3	Residual invasive breast carcinoma		
4	Left breast swelling		
5	Invasive breast carcinoma		
6	Invasive breast carcinoma		
7	Invasive breast carcinoma		
8	Invasive breast carcinoma	33/F	Surgery
9	Invasive breast carcinoma		
10	Extensive Ductal Carcinoma	42/F	Surgery (Carcinoma in Right Breast)
11	Metastasis CA breast		
12	Invasive ductal carcinoma		
13	Metastatic invasive breast carcinoma	60/F	Surgery
14	Invasive ductal carcinoma		
15	Invasive ductal carcinoma		
16	Invasive ductal carcinoma		
17	Metastasis CA breast		
18	Invasive breast carcinoma	52/F	Surgery

Table 6. For the Case of Expression of the Percentage of Estrogen (ER) of the Left or Right Breast of Invasive Carcinoma in the Patient Report

Patient Serial Number	Types	Age	Status of % ER cases= 50%
1	Invasive breast carcinoma		
2	Invasive breast carcinoma		
3	Residual invasive breast carcinoma	48/F	Hormonal treatment (post-menopausal)
4	Left breast swelling		
5	Invasive breast carcinoma	75/F	Hormonal treatment
6	Invasive breast carcinoma	41/F	Hormonal Treatment
7	Invasive breast carcinoma	33/F	Hormonal Treatment
8	Invasive breast carcinoma		
9	Invasive breast carcinoma	59/F	Hormonal Treatment
10	Extensive Ductal Carcinoma		
11	Metastasis CA breast		
12	Invasive ductal carcinoma	74/F	Hormonal treatment (post-menopausal)
13	Metastasis CA breast		
14	Invasive ductal carcinoma	56/F	Hormonal treatment (post-menopausal)
15	Invasive ductal carcinoma	47/F	Hormonal treatment (post-menopausal)
16	Invasive ductal carcinoma	55/F	Hormonal treatment (post-menopausal)
17	Metastasis CA breast		
18	Invasive ductal carcinoma		

Table 7. For the Analysis of PIK3CA Mutation in the Patient Samples

SL. NO.	ER	PgR	PIK3CA Mutation	Grade
1	Negative	Negative		III
2	Negative	Negative		III
3	Negative	Negative		III
4	Negative	Negative		II
5	Negative	Negative		III
6	Negative	Negative		II
7	Positive	Negative		II
8	Positive	Negative		II
9	Positive	Positive		III
10	Positive	Negative		III
11	Negative	Negative		III
12	Positive	Positive		III
13	Negative	Negative		III
14	Positive	Negative	EXON 20	
15	Positive	Positive		II
16	Negative	Negative		III
17	Negative	Negative	EXON 20	
18	Negative	Negative		III
19	Positive	Negative		III
20	Negative	Negative		II
21	Negative	Negative	EXON 20	III
22	Positive	Positive		II
23	Negative	Negative		II
24	Negative	Negative	EXON 20	III
25	Positive	Negative	EXON 20	III

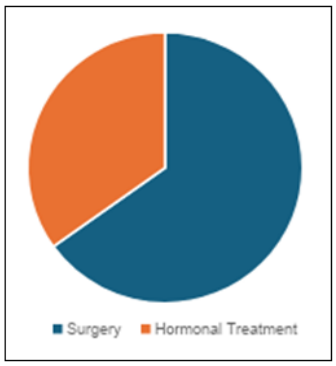

Figure 3. In this Graph, Out of all Patients only 28% Opted for Surgery and 15% Opted for Hormonal Treatment in the Breast Cancer Cases

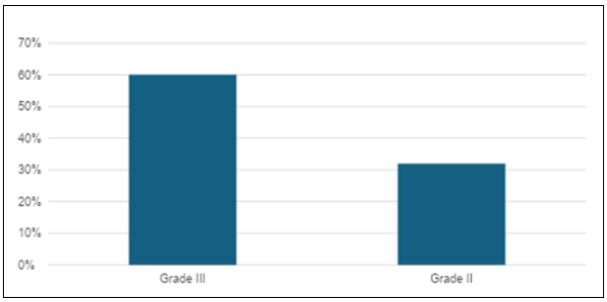

Figure 4. This Graph Shows the Distribution of Tumor Grades and Frequency of Exon 20 mutations (n = 25), with 60% of breast cancer cases are of grade III type and 33% of breast cancer cases are grade II type. Exon 20 mutations were found in 5 cases (20%). All 5 cases with Exon 20 mutations were Grade III, suggesting a strong association with high-grade, aggressive tumors.

increasing access to modern testing, will greatly contribute to better breast cancer care in places like Assam.

Declaration

Author's contribution

Researched, wrote, edited the manuscript: Tandralee Bhuyan, Ria Goswami. Researched, edited and reviewed the manuscript: Tandralee Bhuyan, Dr. Kandarpa Kr. Saikia.Interpretation, analysis and collection in the manuscript: Dr. Dushyant Kumar, Dr. Muktanjalee Deka.

Availability of data and materials

Gauhati university, H-prime lab Pvt. Ltd. and State Cancer Institute/Hospital.

Financial support and sponsorship

None.

Ethical approval and consent to participate

H-prime lab Pvt. Ltd., State Cancer Institute/Hospital, Gauhati University.

Conflict of Interest

Not applicable.

Consent for Publication

Not applicable.

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