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# EXPRESSION OF PCNA AND KI 67 IN BREAST TUMORS AND THEIR CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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**ABSTRACT** Assessment of proliferative changes in breast tumors are used as a tool for recognition of early disease .The objectives of this study were to assess the expression of proliferative markers namely Ki67 and Proliferating Cell Nuclear Antigen in breast tumors and to determine their correlation with various clinicopathological features.

Study was carried out prospectively on 103 patients of carcinoma breast and 46 patients of benign breast disease. Histopathology examination and Immunohistochemistry was carried out to study the PCNA and Ki 67 expression. Ki 67 overexpression was found in 61% of carcinoma breast and 35% of benign breast disease patients. Significant correlation was found in pathologically node negative patients.PCNA overexpression was seen in majority of patients but no significant correlation was found with any clinicopathological parameters.

Conclusion: Ki67 expression could guide in differentiating suspicious proliferative breast lesions from carcinoma breast.PCNA overexpression can be attributed to the commercially available antibodies recongnizing both isoforms of PCNA on normal and malignant epithelium.

**KEYWORDS :**

**Introduction:**

The structure and functioning of a normal breast is a continuous and complex interplay between the different cells which are luminal cells, myoepithelial cells and stromal cells. The same events which occur during puberty and pregnancy, like abrogation of the basement membrane, increased proliferation and escape from growth inhibition, angiogenesis and stromal invasion may go awry and cause uncontrolled proliferation. The earliest such alterations are proliferative changes, which may stem from loss of growth inhibiting signals, aberrant increases in pro growth signals or decreased apoptosis.[1]In the process of development of carcinogenesis, certain populations of cells acquire certain genetic and epigenetic changes that will lead to the conversion of a normal cell to a cancer cell. Proliferative changes are the earliest changes, which are being extensively studied so that they become a tool for recognition of early stage disease which may translate into survival benefit or an increment in disease free survival in carcinoma breast patients.

In most centers , it has become a standard practice to determine the ER, PR,HER2neu status on biopsy specimens prior to therapeutic intervention .[ 2] Tailored therapy based on the presence or absence of receptors for estrogen, progesterone and human epidermal growth factor 2 can be planned. Agents that use novel approaches to target HER 2 as well as targeting different portions of the HER signaling pathway, are in various stages of development.For example, pertuzumab, a humanized monoclonal antibody that binds to a different domain of the extracellular portion of the HER 2 receptor than trastuzumab, was approved for use, as was lapatinib, a small molecule tyrosine kinase inhibitor .[3]Many laboratories have evaluated the usefulness of various proliferation indices using immunohistochemical techniques like p53, HER 2 neu, Proliferating cell nuclear antigen(PCNA), Vascular Endothelial Growth Factor (VEGF) etc.

Proliferating Cell Nuclear Antigen (PCNA) is a 36-KD protein which actively participates in a number of molecular pathways which are responsible for the continuity of life in a mammalian cell. It is actively involved in DNA synthesis as a co-factor for DNA polymerase delta. Expression of PCNA occurs during the S phase and G2 phase of the cell cycle, which makes this protein a good cell proliferation marker. In quiescent cells the level of PCNA is found to be very low but dramatically increase during cell cycle. It is this particular quality of PCNA which can be exploited to garner information regarding cell proliferation .The level of expression of PCNA changes during cell cycles and is associated with cell proliferation or transformation [4]. Deregulation of PCNA expression is a hallmark of many proliferative diseases and PCNA acts as a general proliferative marker, particularly in determining cancer prognosis. [5] PCNA plays crucial roles in DNA replication, DNA repair, the cell cycle and apoptosis and also interact with other proteins to accomplish these roles. PCNA expression relates to cell proliferation and has diagnostic value in many types of cancers. It is also a target for cancer therapy and its inhibitors are currently being developed as potential anticancer drug. [6]

Various other markers are being evaluated to assess the proliferative activity of breast cancer. Ki67 is one of the most studied monoclonal antibody to assess the proliferative activity in breast cancer and has been found to have a significant correlation with histological grade of malignancy and mitotic count. (Brown DC 1990).[7]

The Ki67 antigen, encodes two protein isoforms with molecular weight of 345 and 395 kDa and was originally identified by Scholzer and Gerdes in early 1980s [8]. It has short half-life of 1-1.5 hours and is present in all stages of life cycle except it is absent in resting cells. Its expression is associated with proliferative activity of intrinsic cell populations in malignant tumors, thus can be used as a marker for tumor aggressiveness [9] .The prognostic value of Ki67 has been shown in number of cancers e.g. breast, lung, soft tissue, prostate, CNS. 10]. It can be used as a predictive biomarker which identifies sub population of patients who are most likely to respond to a particular therapy.

p53 (also known as protein 53 or tumor protein 53), is a tumor suppressor protein that in humans is encoded by the TP53 gene. p53 is crucial in multicellular organisms, where it regulates the cell cycle and thus, functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. The name p53 is in reference to its apparent molecular mass: it runs as a 53-kilodalton (kDa) protein on SDS-PAGE. It has anticancer function, and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis through several mechanisms like activating DNA repair gene after DNA insult, cell cycle arrest at G1/S regulation point, promoting apoptosis of irreparable gene. Mutation in TP53 gene destroys ability of protein to bind to its target DNA sequence and preventing transcriptional activation thus reducing tumor suppression [11]

The objectives of this study were to assess the expression of proliferative indices namely Ki67 and Proliferating Cell Nuclear Antigen (PCNA) by Immunohistochemistry in breast carcinoma and benign breast disease and to determine their relationship with various

clinico- pathologic variables.

## MATERIALS AND METHODS

The study was carried out prospectively in a single surgical unit from January 2013 to December 2015. The study was approved by the Institute's Ethics committee. 103 patients of histologically proven carcinoma breast and 46 patients of benign breast disease were included in the study. Biomarker analysis was done on paraffin fixed tissue samples obtained after modified radical mastectomy or lumpectomy specimens and trucut biopsy specimens in case of benign breast disease patients.

A detailed history and meticulous clinical examination was carried out in each patient. A proforma was completed which included various clinic-pathological features like age, parity, menstrual status, size of lump, lymph node status , grade of tumor , TNM staging , chemotherapy status .

Fresh tissue samples were collected and fixed in 10% formalin and paraffin embedded to be used for histopathological examination (HPE) and Immunohistochemistry (IHC).Histological grade according to modified Bloom and Richardson Classification was noted for each patient.[ 12 ] IHC kits (Biogenex Company, The Netherlands) ,Ready to use, containing all reagents along with primary and secondary antibodies were used to assess Estrogen Receptor(ER), Progesterone receptor(PR), HER-2neu, PCNA and Ki 67 expression.

### Immunohistochemistry (IHC)

IHC was performed on paraffin embedded tissue which was cut into 4µm thin section, deparaffinized with xylene and rehydrated through graded ethanol washes. The sections were autoclaved in antigen retriever in citrate buffer (pH 6.0) at $95^0$ C for 10min for 1st cycle and at $97^0$ C for 10 minutes in second cycle, then cooled to $26^0$ C , treated with 3% $H_2O_2$ for 20 minutes to block endogenous peroxidase activity, followed by washing with Tris Buffer and (pH 7.6) for 3 minutes thrice. The sections were then incubated at $4^0$ C for overnight with anti-PCNA (Ready to use Antibody Monoclonal, clone PC 10, Biogenex).

On the next day, sections are washed with Tris buffer thrice and incubated with secondary antibody for 30 minutes and then again washed with Tris Buffer. Horseradish peroxidase polymer conjugate is then applied to section at $37^0$ C for 30 minutes followed by Tris Buffer washes. Finally sections were incubated with 3-3' Diaminobenzidine (DAB) for 5-10 minutes. A negative control was run simultaneously by omitting the primary antibody. The slides were then assessed by a dedicated pathologist.

The following scoring system is used for ER and PR status-

| Proportion Score (PS) | Observation | Intensity Score (IS) | Observation |
|---|---|---|---|
| 0 | None | 0 | None |
| 1 | 1% | 1 | Weak |
| 2 | 1-10% | 2 | Intermediate |
| 3 | 10-33% | 3 | Strong |
| 4 | 33-66% | | |
| 5 | 66-100% | | |
| Sum of proportion score and intensity score (PS + IS) | | | |
| Total score | | | Interpretation |
| 0-2 | | | Negative |
| 3-8 | | | Positive |

**Hercep Test Guidelines for scoring HER 2neu expression**

| Score | HER 2 protein Overexpression Assessment | Staining Pattern |
|---|---|---|
| 0 | Negative | No staining observed or membrane staining observed in < 10% of tumor cells |
| 1+ | Negative | A faint /barely perceptible membrane staining detected in >10% of tumor cells.The cells exhibit incomplete membrane staining |
| 2+ | Weakly positive | A weak to moderate complete membrane staining observed in >10% of tumor cells |
| 3+ | strongly positive | A strong complete membrane staining is observed in >10% of tumor cells |

*Assessment for PCNA:*

Nuclear staining of 500 nuclei in the designated histological category was counted. If >10% cells showed when trabeculated, intensely red or cloudy red stain, it was assigned overexpressed as coded as 3. If <10% nuclei were stained it was considered as normal and coded as 0. The final score was calculated by adding the score of number of cells stained along with intensity. [12]

*Assessment for Ki67:*

The results of the tissue sections staining were estimated according to the following: positive, nuclear staining; negative, no nuclear staining. The intensity of staining was categorized according to the following criteria: negative, 0; weak, 1; moderate, 2; and strong, 3. Staining was semi quantitatively scored according to proportion of stained cells by the following scale: 0, no cells stained; 1, <10%; 2, 10-50%; and 3, >50% of cells stained. The staining intensity scores and percentage of the stained cells were added; the cutoff value for positive expression of Ki67 was defined as moderate staining with >10% of cells stained..[13]

Statistical Analysis: Statistical analysis was performed using SPSS software version 16.0.Chi Square test was applied to assess the association between the parameters. For all tests, p value of <0.05 was considered significant.

### Result:

*Expression of Ki67 in breast cancer tissues and benign breast diseases:*

The expression of Ki67 in breast cancer tissues is shown in Fig. 1. The Ki67-positive cells exhibited brown Stain in the nucleus of the cell. While negative expression of Ki67 in fibro adenoma patients shows no brown stained cells. Total, 63 of the 103 cases of breast cancer tissues were showing Ki67-positive, accounting for 61%. In contrast, tissues from the 46 cases of Fibro adenoma, 30 cases of Ki67-negative expression were observed (65%), indicating a statistically significant difference compared with breast cancer tissue. (P=0.003; Table I).

**Table I. Expression of Ki67 in breast cancer and benign breast disease tissues.**

| Groups | Patients (n) | Expression of ki67: Over-expression | no over-expression | p- value |
|---|---|---|---|---|
| Ca breast | 103 | 63 (61%) | 40 (39%) | 0.003 |
| Benign breast disease | 46 | 16 (35%) | 30 (65%) | |

*Correlation of Ki67 expression with clinicopathological data of breast cancer and benign breast disease patients.*

In breast cancer patients, <50 years of age, 61% of the patient showed ki67 positive expression and 67% ki67 positive patients were premenopausal females. 78% patients of ca breast were associated with breast pain that were showing ki67 positive expression. The expression of Ki67 in breast cancer tissues was significantly associated with the breast pain. (P<0.01; table II); however, no correlation was found with the age, menstrual status and laterality of the patient (P>0.05; Table II). In Fibro adenoma, majority of patients exhibited negative expression of ki67 and was found no correlation associated with age, menstrual status, laterality and breast pain.

**Table II. Correlation between the expression of Ki67 in breast cancer tissues and benign breast tissues with clinicopathological data.**

| Variables | Ca Breast | | | BBD | | |
|---|---|---|---|---|---|---|
| | Over expression | no over expression | p- value | Over expression | no over expression | p- value |
| Age, years | | | | | | |
| ≤50 (70) | 43 (61%) | 27 (39%) | 0.93 | 16 (36%) | 29 (64%) | 0.46 |
| >50 (33) | 20 (61%) | 13 (39%) | | 0 (0%) | 1 (100%) | |
| Menstrual status | | | | | | |
| Pre menopausal (36) | 24 (67%) | 12 (33%) | 0.4 | 16 (36%) | 28 (64%) | 0.29 |
| Post menopausal (67) | 39 (58%) | 28 (42%) | | 0 (0%) | 2 (100%) | |
| Laterality | | | | | | |
| Left (64) | 39 (61%) | 25 (39%) | 0.95 | 9 (39%) | 14 (61%) | 0.53 |

| Right (39) | 24 (62%) | 15 (38%) | | 7 (30%) | 16 (70%) | |
|---|---|---|---|---|---|---|
| Breast Pain | | | | | | |
| No (67) | 35 (52%) | 32 (48%) | 0.01 | 2 (33%) | 4 (67%) | 0.93 |
| Yes (36) | 28 (78%) | 8 (22%) | | 14 (35%) | 26 (65%) | |

*Expression of PCNA in breast cancer and benign breast diseases:*

The expression of PCNA in breast cancer tissues is shown in Fig. 1. The PCNA-positive cells exhibited intense brown Stain in the nucleus of the cell. 98 out of 103 patients of breast cancer showed over expression of PCNA, accounts for 95% while all the patients out of 46 of fibro adenoma were showing positive expression of PCNA accounting for 100%. PCNA were highly expressed in both, Ca breast and benign breast disease group. Although, expression of PCNA were not associated significantly with both the groups. (Table III).

**Table III. Expression of PCNA in breast cancer and benign breast disease.**

| Groups | Patients (n) | Expression of PCNA | | |
|---|---|---|---|---|
| | | Over-expression | no over-expression | p- value |
| Ca breast | 103 | 98 (95%) | 5 (5%) | 0.12 |
| Benign breast disease | 46 | 46 (100%) | 0 (0%) | |

Correlation of PCNA expression with clinicopathological data of breast cancer and benign breast disease patients.

In both, breast cancer patients and benign breast patients, PCNA was highly expressed. However, there was no correlation found between the expression of PCNA and clinicopathological parameters of Ca breast and benign breast disease summarized in Table IV.

**Table IV. Correlation between the expression of PCNA in breast cancer tissues and benign breast tissue with clinicopathological data.**

| Variables (n) | Ca Breast | | | BBD | | |
|---|---|---|---|---|---|---|
| | Over expression | no over expression | p- value | Over expression | no over expression | p- value |
| Age, years | | | | | | |
| ≤50 | 67 (96%) | 3 (4%) | 0.69 | 45 (100%) | 0 | - |
| >50 | 31 (94%) | 2 (6%) | | 1 (100%) | 0 | |
| Menstrual status | | | | | | |
| Pre menopausal | 34 (94%) | 2 (6%) | 0.8 | 44 (100%) | 0 | - |
| Post menopausal | 64 (96%) | 3 (4%) | | 2 (100%) | 0 | |
| Laterality | | | | | | |
| Left | 60 (94%) | 4 (6%) | 0.39 | 23 (100%) | 0 | - |
| Right | 38 (97%) | 1 (3%) | | 23 (100%) | 0 | |
| Breast Pain | | | | | | |
| No | 63 (94%) | 4 (6%) | 0.47 | 6 (100%) | 0 | - |
| Yes | 35 (97%) | 1 (3%) | | 40 (100%) | 0 | |

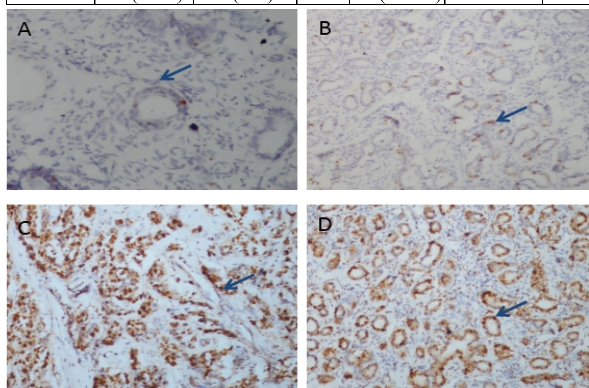


Figure 1. (A) Negative expression of Ki67 in fibro adenoma tissues; (B) Positive expression of Ki67 in breast cancer tissues; (C) Positive expression of PCNA in breast cancer tissues; (D) Positive expression of PCNA in fibro adenoma tissues. (A-D, magnification, x200).

**Table V. Correlation between the expressions of Ki67 in breast cancer tissues with the clinicopathological data.**

| Variables (N) | Ca Breast | | |
|---|---|---|---|
| | Over expression | no over expression | p- value |
| **Parity** | | | |
| > 2 (74) | 46 (62%) | 28 (38%) | 0.74 |
| ≤ 2 (29) | 17 (59%) | 12 (41%) | |
| **Addiction** | | | |
| No (99) | 61 (62%) | 38 (38%) | 0.64 |
| Yes (4) | 2 (50%) | 2 (50%) | |
| **Chemotherapy** | | | |
| Neo ad. (51) | 32 (63%) | 19 (37%) | 0.74 |
| Adjuvant (52) | 31 (60%) | 21 (35%) | |
| **Tumor size (cm)** | | | |
| 0.1-2 (8) | 5 (63%) | 3 (38%) | 0.16 |
| 2.1-5 (58) | 31 (53%) | 27 (47%) | |
| > 5 (37) | 27 (73%) | 10 (27%) | |
| **Stage** | | | |
| Early (42) | 23 (55%) | 19 (45%) | 0.26 |
| Late (61) | 40 (66%) | 21 (34%) | |
| **Histological grade** | | | |
| I (5) | 5 (100%) | 0 | 0.18 |
| II (21) | 12 (57%) | 9 (43%) | |
| III (77) | 46 (60%) | 31 (40%) | |
| **LN status (pN)** | | | |
| Positive (62) | 32 (52%) | 30 (48%) | 0.01 |
| Negative (41) | 31 (76%) | 10 (24%) | |

**Table VI. Correlation between the expressions of PCNA in breast cancer tissues with the clinicopathological data.**

| Variables (N) | Ca Breast | | |
|---|---|---|---|
| | Over expression | no over expression | p- value |
| Parity | | | |
| > 2 (67) | 63 (94%) | 4 (6%) | 0.67 |
| ≤ 2 (36) | 35 (97%) | 1 (3%) | |
| Addiction | | | |
| No (99) | 94 (95%) | 5 (5%) | 0.64 |
| Yes (4) | 4 (100%) | 0 | |
| Chemotherapy | | | |
| Neo ad. (51) | 48 (94%) | 3 (6%) | 0.63 |
| Adjuvant (52) | 50 (96%) | 2 (4%) | |
| Tumor size (cm) | | | |
| 0.1-2 (8) | 8 (100%) | 0 | 0.47 |
| 2.1-5 (58) | 56 (97%) | 2 (3%) | |
| > 5 (37) | 34 (92%) | 3 (8%) | |
| Stage | | | |
| Early (42) | 40 (95%) | 2 (5%) | 0.97 |
| Late (61) | 58 (95%) | 3 (5%) | |
| Histological grade | | | |
| I (5) | 5 (100%) | 0 | 0.49 |
| II (21) | 19 (90%) | 2 (10%) | |
| III (77) | 74 (96%) | 3 (4%) | |
| LN status (pN) | | | |
| Positive (62) | 58 (94%) | 4 (6%) | 0.35 |

| Negative (41) | 40(98%) | 1 (2%) | |
|---|---|---|---|

**Table VII. Correlation between the expressions of Ki67 in breast cancer tissues with the ER, PR and HER2 neu.**

| Receptors Status | Ki67 in Ca breast | | |
|---|---|---|---|
| | Over expressio (%) | no over expression (%) | p- value |
| ER | | | |
| Positive (36) | 26 (72.2) | 10 (27.8) | 0.09 |
| Negative (67) | 37 (55.2) | 30 (44.8) | |
| PR | | | |
| Positive (32) | 21 (65.6) | 11 (34.4) | 0.53 |
| Negative (71) | 42 (59.2) | 29 (40.8) | |
| HER2 neu | | | |
| Positive (34) | 21 (61.8) | 13 (38.2) | 0.93 |
| Negative (69) | 42 (60.9) | 27 (39.1) | |

**Table VIII. Correlation between the expressions of PCNA in breast cancer tissues with the ER, PR and HER2 neu.**

| Receptors Status | PCNA in Ca breast | | |
|---|---|---|---|
| | Over expression(%) | No over expression(%) | p-value |
| ER | | | |
| Positive (36) | 35 (97.2) | 1 (2.8) | 0.47 |
| Negative (67) | 63 (94.0) | 4 (6.0) | |
| PR | | | |
| Positive (32) | 31(96.9) | 1 (3.1) | 0.58 |
| Negative (71) | 67 (94.4) | 4 (5.6) | |
| HER2 neu | | | |
| Positive (34) | 32 (94.1) | 2 (5.9) | 0.73 |
| Negative (69) | 66 (95.7) | 3 (4.3) | |

**Discussion:**

The molecular signatures which may help to detect early disease or residual disease after treatment can be of aid in the treatment of breast cancer. Assessment of proliferation of tumor cells may suggest the aggressiveness of the tumor. Various techniques like thymidine labelling and flow cytometry accurately measure the proliferative capacity of a tumor. But, these techniques are expensive, difficult and laborious. Immunohistochemistry is a good surrogate, which allows the proliferative cells to be demonstrated in situ. We have thus used commercially available antibodies against Ki67 and PCNA to assess its immunoreactivity in formalin fixed and paraffin-embedded tissue sections.These procedures are easily replicable and can be carried out in any pathology laboratory with modest facilities.

Clinical utility of Ki67 has been studied extensively by various study groups and clinical trials like IMPACT (Immediate Preoperative Anastozole Tamoxifen or combined with Tamoxifen) study, ATAC (Armiden, Tamoxifen Alone or combined) trial and Breast International Group (BIG) 1-98 trials. The assessment of Ki67 in breast cancer is not uniform among different laboratories and an effort has been made by the International Ki67 in Breast Cancer Working Groups [14]. And certain recommendations have been put forward, based on current evidence which would allow harmonization of methodology.

Ki67 score is defined as the percentage of positively stained cells among the total number of malignant cells scored. As Ki67 is a nuclear protein, only nuclear staining and mitotic figures stained by Ki67 should be incorporated in the Ki67 score. The recommendation is to count at least 3 randomly selected high-power (x40) fields. To achieve adequate precision at least 1000 cells should be scored by the pathologist and 500 cells being the absolute minimum. [14].T Haerslev et al [15] have concluded that the immunoreactivity for Ki 67 was independent of the length of formalin fixation if the sections were microwave processed before incubation with the primary antibody.

In our study, Ki67 positivity was seen in 61% of patients (63 out of 103 patients) in CA breast and only 39% in benign breast disease patients. The p-value being 0.003, this finding was statistically significant. In a study by A. Nieto et al 2000, on canine mammary tumors, ER-α value were compared with proliferative activity [16]. PCNA index was closely correlated with ER- α and correlation with Ki67 index was close to significant, indicating that well differentiated tumors can maintain same hormonal regulatory mechanisms and have a low proliferation index. For IHC of Ki67, many cutoffs have been used, although staining levels of 10%-20% have been most commonly used by most investigations [17] we have used >10% as positive.

Keshing Li [14] et al found 90.56% Ki67 positivity in age <59 years and 80.64% in patients above 60 years of age. The relation of Ki67 positivity with stage of disease was not found to be statistically significant. In our study also, correlation of Ki67 expression with stage of disease was not found to be statistically significant, the p value being 0.26. Studies by Isolla JJ et al (1990)[18] and Railo M et al (1993)[19] have demonstrated the association between a high Ki-67 labeling index, histological grade and a large tumor size. In our study, high Ki67 index (73%) correlated with larger size (>5cm) of tumor. As regarding histological grade of tumor, Ki67 overexpression was found to be 100%, 57% and 60% respectively in Grade I, Grade II and Grade III tumors. no statistical significance was found in our study.

Lymph node status being an independent prognostic marker, also found statistical correlation with Ki67 labeling index in our study. In 52% patients, overexpression was found in LN positive patients and 76% patients had Ki67 over expression among lymph node negative population, p-value was 0.01.In studies by Keshing Li et al [14] Ki 67 closely correlated with lymph node positivity (97.82%) were showing over expression, p value being <0.01 and Kristina Joshua et al 2013 study demonstrated a p value of 0.027.[20]

Correlation was seen between Ki67 expression and receptor status. Estrogen Receptor positive population showed Ki67 overexpression in 72% of patients and 55% overexpression in Estrogen Receptor negative patients. Progesterone receptor positivity did not have a bearing on Ki67 expression. As Progesterone receptor positive patients showed 65% overexpression while PR negative patients also have almost similar expression of about 59%. Similarly HER-2 neu positivity did not have a statistically significant bearing of Ki67 labelling index, the p-value being 0.93. As the receptor status was not evaluated in BBD patients, the above correlation could not be done in these patients.

PCNA expression was seen in both carcinoma and BBD patients and correlated with clinico-pathological data like age, menstrual status, laterality and mastalgia. No significant correlation was found with the above parameter, the expression of PCNA being between 90-100% in all groups. This marked overexpression could be attributed to the fact that non-malignant breast epithelium cells contain a single isoform of PCNA protein that has a basic isoelectric point (nm PCNA). Malignant breast epithelial cell cultures and breast epithelial cell cultures in tissues, on the other hand, were found to harbor the basic form of the protein as well as an acidic isoform (caPCNA). It was observed in other studies by Malkas LH et al, that the commercially available antibodies readily recognized the PCNA present in either the normal or malignant breast tissue extracts..[21]

In our study, the positivity rate of PCNA was found to be 95%, while in study by G. Terry et al 2006, 69% were found to be positive for PCNA.[12] Their positivity rate was comparable to studies by Steck K, El- Naggar 1994[22], Fabian et al 2002[23] and Honrado et al 2005[24]. The high percentage of PCNA positivity could be attributed to the longer half life of PCNA compared with Ki 67 and it being expressed not only during cell proliferation but also during DNA repair, (Bravo R, 1986)ref 22[25]

The synthesis of PCNA is closely associated with the normal G1/S transition of the cell cycle and the protein has a comparatively long half-life. Therefore PCNA is a good proliferative marker denoting proliferative activity in breast lesions where cells are at different phases of growth and accounts for its expression in 94-100% of in situ and invasive CA and 61% of histologically non- proliferative BBD.[12]

Lack of correlation between PCNA and clinocopathological variables could also be attributable to several factors like fixation time.[26] Study by Torben Haerslev et al[15] showed 52% PCNA positivity.Amit Kumar K et al 2017[27] report a 90% positivity in carcinoma breast patients. Likewise different researchers have

reported highly variable and diverse positivity rates for PCNA expression.Schonborn et al in a study on 471 breast cancer patients that on a median follow up of 5 years, survival rates could be significantly associated with PCNA expression in node negative patients and concluded that PCNA could prove to be a valuable prognostic marker. On the other hand, Masakuni Noguchi[28] in a study on 91 patients showed that PCNA expression did not correlate with clinicopathologic parameters as is observed in our study.

## CONCLUSION

According to the findings of our study, Ki67 expression would guide differentiating suspicious proliferative breast lesions from carcinoma breast. Statistical significance was found only between Ki67 and lymph node status ,thus emphasizing it's role as a prognostic marker. PCNA over expression was seen in majority of patients of both benign and malignant disease of breast, thus not of much significance as a prognostic marker. Majority of our patients lacked expression of steroid receptors, suggesting towards a more aggressive course of disease in Indian patients. The findings of our study suggests that only estimating proliferative indices would not suffice to prognosticate the patient regarding the course of the disease and further studies are needed to assess their role in determining disease free interval and overall survival.

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