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# Immunohistochemical Analysis and Correlation of *p*53, Ki-67 and PCNA in Core Needle Biopsy Specimens of Carcinoma Breast

**Kalaivani Amitkumar*[1], S Srigayathri[1], AR Praburaj[2] and S Jayanthi[3]**

[1]Department of Pathology, SRM Medical College Hospital and Research Centre, Kancheepuram, India
[2]Department of Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India.
[3]Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India.

## ABSTRACT

In India, the incidence of carcinoma breast is rapidly increasing with an estimated 80,000 new cases diagnosed annually. The chances of cure who develop the disease related to early diagnosis. Immunohistochemistry (IHC) technique allows identification of cell types expressing antigens and core needle biopsy is appropriate for analysis. Prognostic factors that are considered as independent variables include lymph node status, grade, tumor size, hormone receptors, *p*53 and proliferation markers like Ki-67 and proliferating cell nuclear antigen (PCNA). Sixty carcinoma breast patients diagnosed by fine needle cytology and for whom core needle biopsy tissue was available were included in the present study. Histopathological grading and clinical staging were assessed and immunohistochemical expressions of markers p53, Ki-67, PCNA were done. Finally grade, clinical stage and immunohistochemical expression were correlated with each other. According to our study results only histological grade correlated significantly with other parameters. Among the proliferation markers Ki-67 correlated significantly with other parameters but not PCNA. *p53* positively correlated with histological grade, clinical stage and Ki-67 index indicating the aggressiveness of the tumor. Clinical stage correlated only with histological grade and *p53* expression. Menopausal status had no correlation with any parameter.

**Keywords:** Breast cancer, immunohistochemistry, *p*53, Ki-67, PCNA

***Corresponding author***

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## INTRODUCTION

In India, the incidence of carcinoma breast is rapidly increasing with an estimated 80,000 new cases diagnosed annually [1]. If detected early the breast can be conserved by lumpectomy alone without mastectomy. Prognostic factors may select patients most likely to recur without adjuvant therapy and therefore potentially benefit from therapy. Prognostic factors that are considered as independent variables include lymph node status, grade, tumor size, hormone receptors, *p*53 and proliferation markers like Ki-67 and proliferating cell nuclear antigen (PCNA). Immunohistochemistry (IHC) technique allows identification of cell types expressing antigens and core needle biopsy is appropriate for analysis [2]. It has been shown that diagnostic accuracy is higher in core needle biopsy than fine needle aspiration cytology (FNAC) [3].

The product of *p*53 tumor suppressor gene is one potential marker currently under investigation; mutation and deletion are relatively early in carcinogenesis. This is associated with aggressive behavior and a significant prognostic marker for survival in early breast cancer [4]. Proliferation fraction is the single most independent prognostic marker for node negative breast cancer. Ki-67 is a proliferation marker which is a good objective substitute for mitotic count when used in a grading system. High level is associated with increased risk of recurrence [5]. PCNA is a nuclear auxiliary protein directly involves in DNA synthesis and replication. It is useful to detect prognosis in certain tumors including breast cancer. But in few studies it failed to show any prognostic relevance [6]. Hence, it's necessary to correlate these markers with grade and stage to find out the prognostic reliability in carcinoma breast patients.

Sixty carcinoma breast patients diagnosed by fine needle cytology and for whom core needle biopsy tissue was available were included in the present study. Histopathological grading and clinical staging were assessed and immunohistochemical expression of markers *p53*, Ki-67, proliferating cell nuclear antigen (PCNA) were done. Finally grade, clinical stage and immunohistochemical expression were correlated with each other.

## MATERIALS AND METHODS

This study was performed in the Department of Pathology in collaboration with the Department of Surgery, JIPMER. Patients diagnosed by FNAC as carcinoma breast were included in the study. The patients who had received neoadjuvant therapy and patients with recurrent breast carcinoma were excluded from the study group. Patients were staged according to TNM staging system from the AJCC Cancer staging manual, determined by clinical and radiological findings [7].

10% buffered formalin fixed core needle biopsy cores were processed in an automated tissue processor (Histokinette). Paraffin embedded blocks were cut by HM 320 micro rotary microtome into four to six microns sections. The slides were stained for Haematoxyllin and eosin (H&E). Slides were independently examined by two authors and the findings were compared. There was >88% agreement between these two authors. Nottingham's modification of Bloom Richardson system was used to grade the tumours [8].

Only infiltrating ductal carcinomas, not otherwise specified (NOS) were included in the study, other types were excluded.

The monoclonal antibodies used in the present study for IHC are Ki-67, PCNA and *p53.* Novocastra kits were used. Streptavidin biotin method using di-amino-benzidine (DAB) as chromogen was applied. Sections were made from paraffin blocks on silane coated slides. Antigen retrieval was attempted with microwave. 30 minutes heat retrieval time was used for Ki-67, 20 minutes for *p53* and PCNA. Section from the Tonsil from our routine files was used as a positive control for Ki-67 and *p53*. Skin biopsy was used for PCNA.

**Evaluation of immunoreactive scores for *p53* [9]:**

Only unequivocal nuclear staining was accepted as a positive reaction for *p53*. The degree of expression of *p53* was expressed semi quantitatively by calculating immunopositive score method. With this method the intensity of the immunohistochemical reaction was recorded as 0 to 3. The proportion of tumor nuclei showing positive staining was also scored as 0 to 4. The score for intensity was multiplied by the score for proportion, giving the final score, with the range of 0 to 12 for each individual tumour.

**Evaluation of immunoreactive scores for Ki-67 and PCNA [10]:**

Only unequivocal nuclear staining was accepted as a positive reaction for Ki-67 and PCNA. These scores were determined by counting a minimum of 1000 tumour cells. The labeling index was expressed as the percentage of positive cells.

**Statistical Analysis**

For statistical analysis, SPSS software version 13.0 was used. The clinical stage, histological grade and immunohistochemical markers were correlated by using chi square test, Spearman's rank correlation test and Kendall's tau-b test. For all statistics, significance was accepted for $p < 0.05$.

## RESULTS

All patients were females, age ranged from 24 to 78 years, majority of patients were in the age group of 40 - 50 years. 39% of patients were premenopausal and 61% were postmenopausal. Patients were categorized into clinical stage I, II, III or IV. Patients in stage II and III were further subdivided into sub stages. As can be observed, 4.9%cases presented with stage I. 37.7%cases with stage II and 57.6 % with stage III. No patient was with stage IV disease. The maximum number of cases (20) presented with stage IIIB disease. 15 biopsies studied were grade 1 tumours, 33 cases were found to be complying with grade 2 and 12 were grade 3.

***p53* status**

*p53* overexpression was detected by means of calculating immunoreactive score. Score 0 and 1 were considered negative for *p53* expression. Only cells exhibiting nuclear staining were considered positive. *p53* positivity was detected in 41 cases (66.2%) of breast carcinomas and **(Picture 1,2,3)** immunoreactive score ranged from 0 to 12 points.

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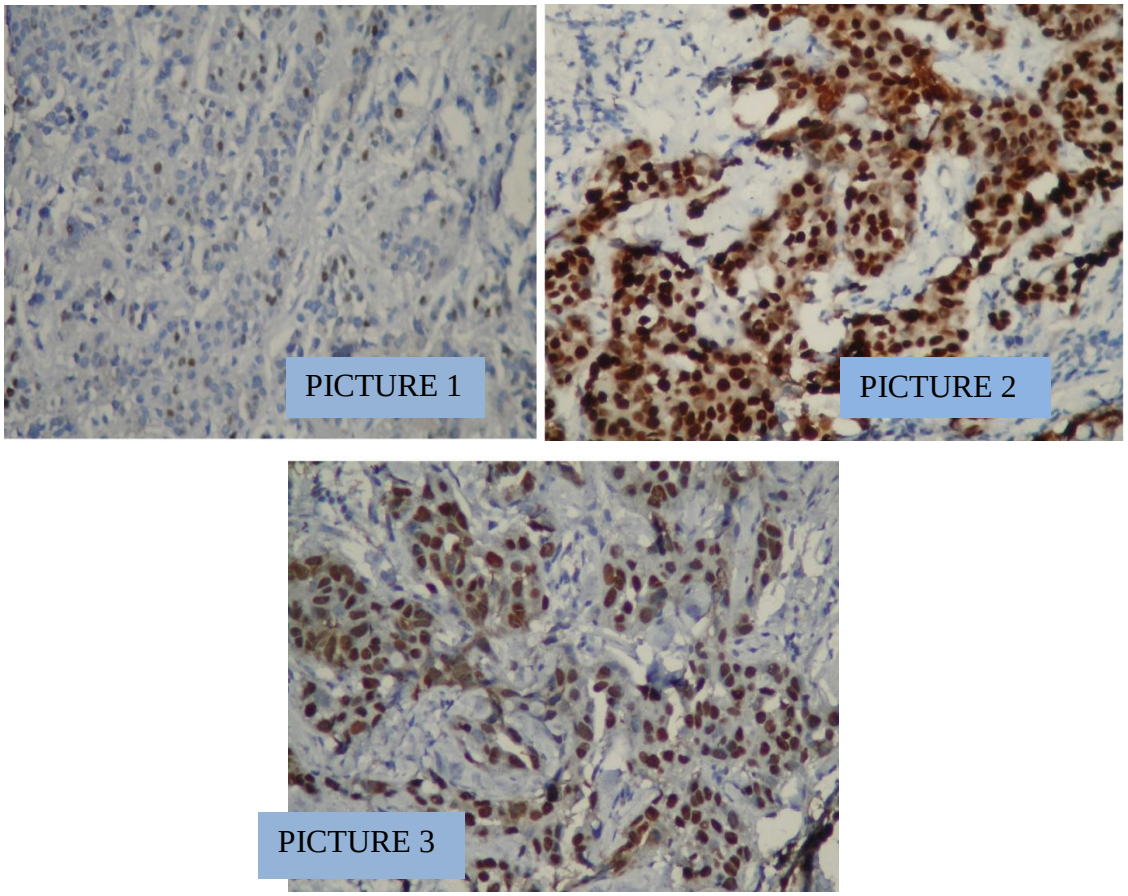
PHOTOMICROGRAPH SHOWING IHC STAINING OF *p*53




Picture 1 – grade 1, score 6 (IHC, 200X)
Picture 2 - grade 2, score 12 (IHC, 400X)
Picture 3 – grade 3, score 12 (IHC, 400X)

**Correlation of altered *p53* expression with histological grade, clinical stage and other IHC markers: (Table 1)**

*p53* expression showed positive correlation with Ki-67 LI (P=0.01). It was observed that 25(60.9%) tumours with *p53* positivity also showed high Ki-67 LI, similarly 15(75%) tumours with *p53* negativity showed low Ki-67 LI. Significant p values were obtained between *p53*+/high Ki-67 LI group and *p53*-/low Ki-67 LI group. *p53* overexpression positively correlated with high tumour grade (p=0.03). Only 8(19.5%) *p53* positive cases were observed in grade 1 category whereas 33(80.4%) *p53* positive cases were found to be in higher grade (grade 2 and grade3). There was a positive correlation between *p53* positivity and clinical stage (p=0.01). 29(80.6%) cases with *p53* positivity were seen in higher stage (stage III), whereas only 1(2.4%) case with *p53* positivity was seen in stage I disease. There was no significant correlation found with PCNA index (p=0.18). *p53* expression did not reveal any significant association with patient's menopausal status (p=0.32).

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A detailed study of correlation of *p53* with other clinicopathological factors revealed a positive correlation with Ki-67 LI, histological grade and clinical stage but did not show any correlation with PCNA index and menopausal status.

**TABLE 1: CORRELATION OF ALTERED *p53* STATUS WITH OTHER PARAMETERS**

| Parameters | *p53+* | *p53-* | p value |
|---|---|---|---|
| **Ki-67 Low LI** | 16 (39.0) | 15 (75.0) | **P = 0.016** |
| **Ki-67 High LI** | 25 (60.9) | 5 (25.0) | |
| **PCNA Low value** | 18 (43.9) | 13 (61.9) | P = 0.18 |
| **High value** | 23 (56.0) | 8 (38.0) | |
| **Menopausal status Premenopausal** | 19 (46.3) | 7 (33.3) | P = 0.32 |
| **Postmenopausal** | 22 (53.61) | 14 (66.6) | |
| **Grade G1** | 8 (19.5) | 8 (38.0) | **P = 0.03** |
| **G2** | 20 (48.7) | 12 (57.1) | |
| **G3** | 13 (31.7) | 1 (4.7) | |
| **Stage I** | 1 (2.4) | 2 (9.5) | **P = 0.01** |
| **II A** | 2 (4.8) | 7 (33.3) | |
| **II B** | 9 (21.9) | 6 (28.5) | |
| **III A** | 10 (24.3) | 2 (9.5) | |
| **III B** | 18 (43.9) | 4 (19.0) | |
| **III C** | 1 (12.4) | 0 | |
| **IV** | 0 | 0 | |

***p <0.05 is considered significant, parenthesis shows percentage***

**Ki-67 expression:**

Positive Ki-67 labeling Index was detected in 51 out of 60 (85.5%) breast carcinomas. Ki-67 immunostaining was clearly evident as nuclear and nucleolar granular staining. Some degree of intratumoural heterogeneity of staining was found. Every stained nucleus was considered positive independent of intensity **(Picture 4, 5, 6)**.

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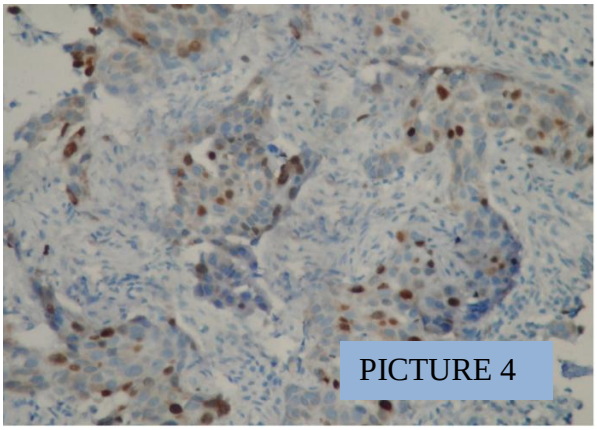
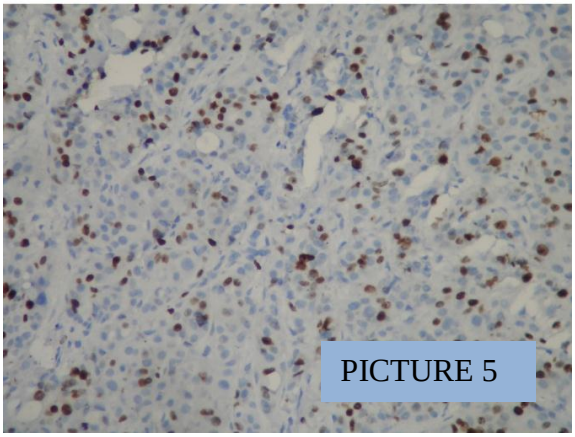
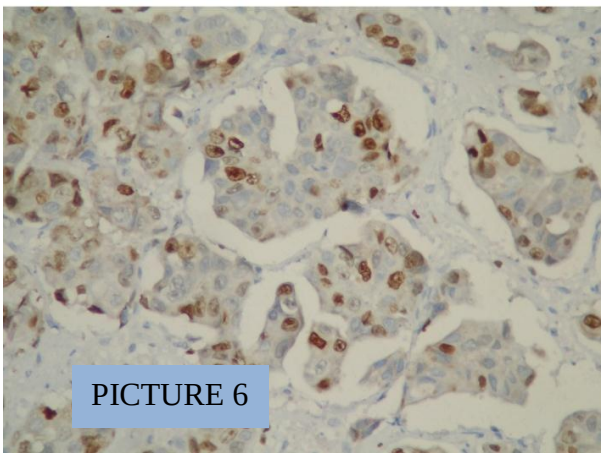
PHOTOMICROGRAPH SHOWING IHC STAINING OF Ki-67




Picture 4 – grade1, low LI – 21% (IHC, 400X)

Picture 5 – grade2, High LI – 30% (IHC, 200X)
Picture 6 – grade3, high LI – 37% (IHC, 400X)

The analysis of Ki-67 value in 60 breast tumours showed their exponential distribution with a median and mean ± standard deviation of 24.50, 29.71±24.56 respectively. Median value was used to classify lesions as high and low Ki-67 LI. **(Figure 1).** Ki-67 LI was correlated with histological grade, stage and all IHC parameters.

**Correlation of Ki-67 with clinical and IHC parameters: (Table 2)**

Ki-67 LI positively correlated with histological grade (p=0.00). Only 1(0.3%) tumour with high Ki-67 LI expression was seen in grade 1 tumours while 11(35.4%) tumours with high Ki-67 expression were found in grade 3 tumours. Conversely 3(0.9%) tumours in grade 3 showed a low Ki-67 expression. and 15(48.3%) grade 1 tumours showed low Ki67 LI. Ki-67 LI was also positively associated with clinical stage (p=0.01). 24 (74.7%) tumours with high Ki-67 LI were seen in stage III (IIIA, IIIB and IIIC) and no case with high Ki-67 LI was seen in stage I. There was a significant increase in the mean ± SD of Ki-67 LI values from grade 1 to grade 3 categories **(Table 3).** Mean ± SD of Ki67 LI values of all stages are shown in **Table 4** to depict the strong positive association of Ki-67 with clinical stage. There was no statistically significant association found with *p53* status (p=0.16). Ki-67 LI showed a positive correlation with PCNA index. 20(64.5%) tumours with high Ki-67 LI also showed high PCNA

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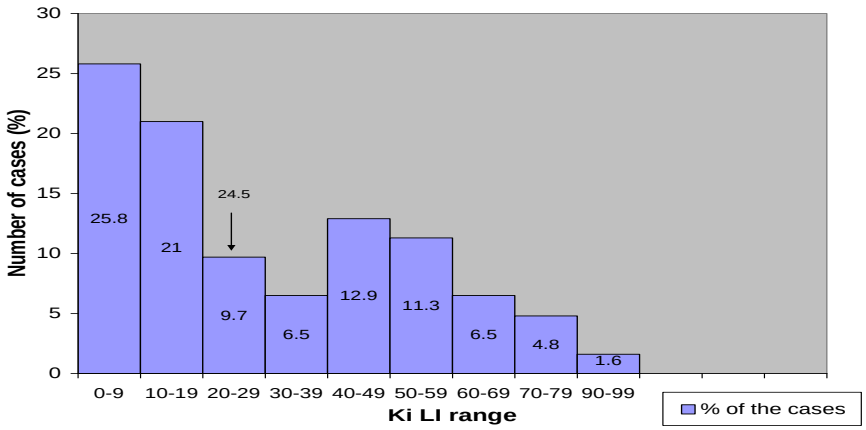
index. Similarly 20(64.5%) tumours with low Ki-67 LI showed low PCNA index. (p=0.02). Though there was no association found with menopausal status of the patients (p=0.12) when mean ± SD of Ki-67 LI values of pre and postmenopausal women were compared, increased expression of Ki-67 LI value was found in the premenopausal women.

Thus Ki-67 status of the tumours positively correlated with histological grade, PCNA index and clinical stage. No association was found with *p53* expression and menopausal status.

**FIGURE 1: FREQUENCY DISTRIBUTION OF Ki-67 LABELING INDEX ACCORDING TO THE NUMBER OF THE CASES (%)**




The upper arrow represents the 24.5% median value

**TABLE 2: CORRELATION OF Ki-67 CASES WITH OTHER PARAMETERS**

| Parameters | Low Ki-67 LI | High Ki-67 LI | p value |
|---|---|---|---|
| ***p53* +** | 16 (51.6) | 25 (80.6) | P = 0.16 |
| ***p53*-** | 15 (48.3) | 6 (19.3) | |
| **PCNA Low value** | 20 (64.5) | 11 (35.4) | **P = 0.02** |
| **High value** | 11 (35.4) | 20 (64.5) | |
| **Menopausal status Premenopausal** | 10 (32.2) | 16 (51.6) | P = 0.12 |
| **Postmenopausal** | 21 (67.7) | 15 (46.3) | |
| **Grade G1** | 15 (48.3) | 1 (0.3) | **P = 0.00** |
| **G2** | 13 (41.9) | 19 (61.2) | |
| **G3** | 3 (0.9) | 11 (35.4) | |
| **Stage I** | 3 (0.9) | 0 | **P = 0.01** |
| **II A** | 5 (16.1) | 4 (12.9) | |
| **II B** | 12 (38.7) | 3 (0.9) | |
| **III A** | 4 (12.9) | 8 (25.8) | |
| **III B** | 7 (22.5) | 15 (48.3) | |
| **III C** | 0 | 1 (0.3) | |
| **IV** | 0 | 0 | |

**p <0.05 is considered significant, parenthesis shows percentage**

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TABLE 3: MEAN ± SD VALUES OF Ki-67 ACCORDING TO TUMOUR DIFFERENTIATION

| Grade | Number of cases | Ki-67 Labeling index Mean ± SD | Range |
|---|---|---|---|
| I | 16 | 11.50 ± 14.0 | 0-58 |
| II | 32 | 31.19 ± 23.0 | 0-75 |
| III | 14 | 47.14 ± 23.8 | 17-96 |

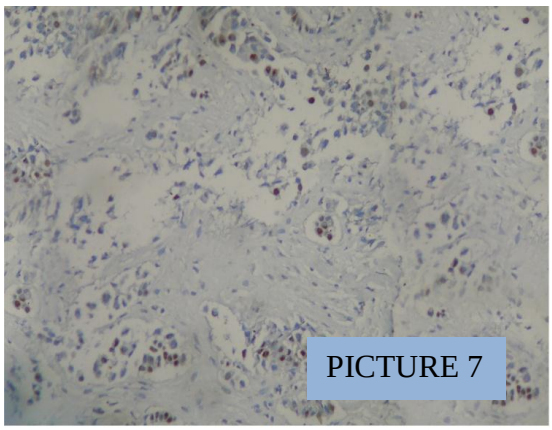
TABLE 4: MEAN AND STANDARD DEVIATION VALUES OF Ki-67 LABELING INDEX BASED ON TNM STAGING

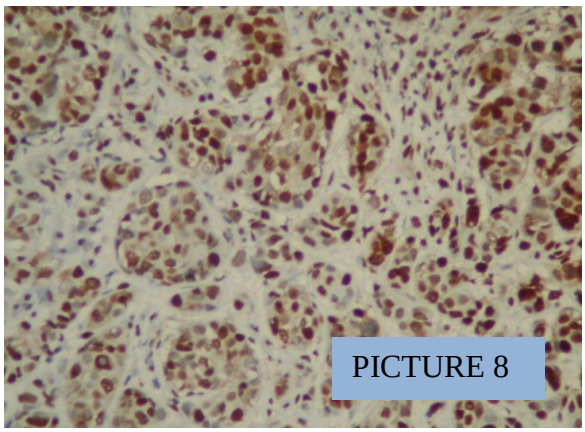
| Stage | Number of cases | Ki-67 Labeling index Mean ± SD | Range |
|---|---|---|---|
| I | 3 | 10 ± 4.3 | 5-13 |
| II | 24 | 19.52 ± 21.6 | 0-58 |
| III | 35 | 38.46 ± 24.0 | 0-96 |

**PCNA INDEX:**

PCNA staining was confined to nuclei and showed diffuse, granular or a mixture of both types of staining. Some cells stained more strongly than others, but all identifiable staining was regarded as positive. 55 (88.5%) tumours were positive for PCNA index **(Picture 8, 9, 10)**. PCNA positivity showed pronounced variation among tumours and the index ranged from 0 to 100% with the median value of 47.50. To evaluate the correlation between PCNA index and clinicopathological factors, tumours were separated on the basis of a median value (47.5) as cases with high PCNA Index (>47.50) and cases with low PCNA Index (≤ 47.50). **(Figure 2)**

PHOTOMICROGRAPH SHOWING IHC STAINING OF PCNA


PICTURE 7


PICTURE 8

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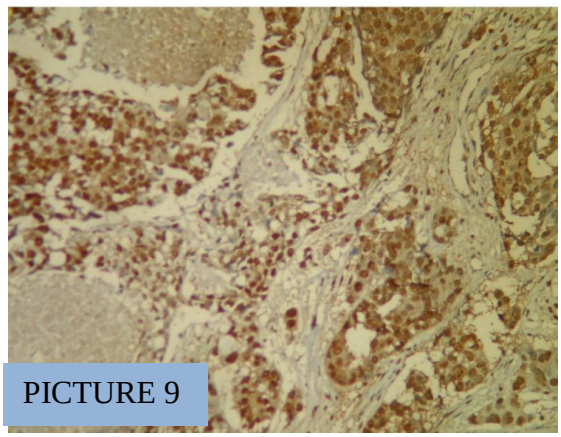
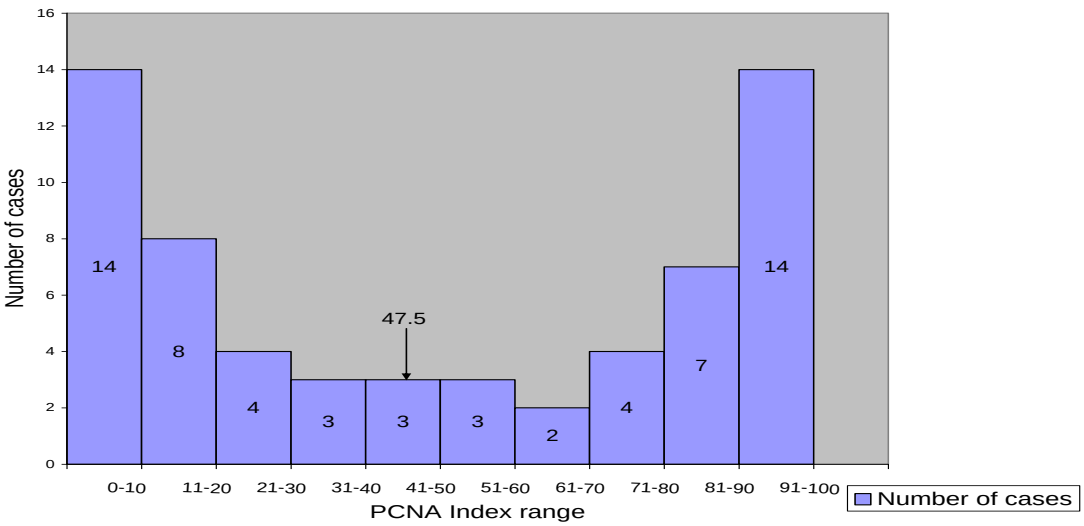



Picture 7 – grade 1, low index – 26% (IHC, 200X)
Picture 8 – grade 2, high index – 88% (IHC, 200X)
Picture 9 – grade 1, high index – 97% (IHC, 200X)

**FIGURE 2: BREAK-UP OF THE CASES BASED ON PCNA INDEX RANGE (%)**




**The arrow represents the 47.5% median value**

## Correlation of PCNA with other factors: (Table5)

PCNA index positively correlated with Ki-67 LI (p=0.02). It was observed that 20(64.5%) cases with low PCNA index also showed low Ki67 LI, similarly 20(64.5%) of high PCNA index cases were found to have high Ki67 LI. p value was not significant when PCNA was correlated *p53*. PCNA index was found to be not significantly associated with histological grade and clinical stage. Similarly menopausal status also did not correlate with PCNA LI. p value for all these markers was >0.05.

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**TABLE 5: CORRELATION OF PCNA INDEX WITH OTHER PARAMETERS**

| PARAMETERS | PCNA low | PCNA High | p value |
|---|---|---|---|
| ***p53* +** | 18 (58.06) | 23 (74.19) | P = 0.18 |
| ***p53*-** | 13 (41.93) | 8 (25.80) | |
| **Ki-67 Low value** | 20 (64.51) | 11 (35-48) | **P = 0.02** |
| **High value** | 11 (35.48) | 20 (64.51) | |
| **Menopausal status Premenopausal** | 13 (41.93) | 13 (41.93) | P = 1.0 |
| **Postmenopausal** | 18 (58.06) | 18 (58.06) | |
| **Grade G1** | 11 (35.48) | 5 (16.12) | P = 0.17 |
| **G2** | 15 (48.38) | 17 (54.83) | |
| **G3** | 5 (16.12) | 9 (29.03) | |
| **Stage I** | 2 (6.95) | 1 (3.22) | P = 0.12 |
| **II A** | 6 (19.35) | 3 (9.61) | |
| **II B** | 11 (35.42) | 4 (12.90) | |
| **III A** | 4 (12.90) | 8 (25.8) | |
| **III B** | 8 (25.80) | 14 (45.16) | |
| **III C** | 0 | 1 (3.22) | |
| **IV** | 0 | 0 | |

**p <0.05 is considered significant, parenthesis shows percentage**

## DISCUSSION

The basis of this present study was to correlate clinical stage and histological grade of 60 breast carcinoma cases on core needle biopsies with IHC score of p53, KI-67 and PCNA. Tumour size and axillary lymph node status are independent prognostic factors used in adjuvant therapy decision making; the presentation was similar to previous studies [11]. In the present study clinical stage showed positive correlation with *p53* overexpression. 80.6% of *p53* positive cases were observed in higher stage. Josma Isola et al[12] studied 289 breast cancer patients and showed that clinical stage positively correlated with *p53* expression.

In the present study clinical stage was not significantly associated with PCNA index or menopausal status. These results were comparable to previous studies[13]. In the present study grade 1 tumors showed the lowest Ki-67 labeling index whereas grade 3 carcinomas showed the highest Ki-67 LI. This is comparable to the study done by Surowiak et al [14] who observed a positive correlation between tumour grade and the Ki-67 labeling index, indicating that higher grade tumours have high proliferating capacity.

In the present study a positive correlation with histological grade and stage was found. This was comparable with the study done by Gurjeet et al[15]. No correlation was found between the histological grade and PCNA index in the present study. Surowiak et al[14] studied 60 ductal breast carcinomas and showed that intensity of PCNA index did not show any differences among the different grades. No correlation between the histological grade and menopausal status was found in this study similar to previous studies [16].

**Ki-67 Labeling Index (LI):**

Ki-67 IHC staining is a simple method of assessing tumor proliferation index and can be widely employed in many laboratories. Proliferative rate has a strong correlation with clinical outcome[17]. In breast carcinomas the Ki-67 reactivity observed by several groups was more divergent, the median value of positive cells ranging from 7.2% to 32.4%. Caly M[18] et al observed the highest median value (32.4%) of Ki-67 LI. In the present study a median value of 24.5% was obtained. These differences can be explained by different assessment methods applied. In the present study 9 (14.5%) cases showed no nuclear Ki-67 staining. It is attributed to processing instability of Ki-67 antigen. Wintzer et al[19] showed that Ki-67 LI was positively associated with tumour grade, altered *p53* overexpression and clinical stage. Similar results were obtained in the present study. A high Ki-67 proliferating activity was seen in stage 3, grade3 tumours while a low Ki-67 proliferating activity was seen in stage 1, grade1 tumours.

Thomas et al[13] studied 144 cases of primary breast carcinomas for the expression of PCNA and correlated with other clinicopathological parameters, and found no association between Ki-67 LI and PCNA index . Surowiak et al[14] also showed similar results. But in the present study results were comparable with Leonardi et al who found a positive correlation between PCNA index and Ki-67 LI in carcinoma breast patients [20]. When menopausal status was considered premenopausal patients had higher Ki-67 LI (34 ± 24.4 %) than postmenopausal patients, similar to the study done by Veronese et al [20].

**Proliferating cell nuclear antigen (PCNA) index:**

Immunohistochemical labeling of PCNA was considered as a marker for proliferating activity. PCNA Immunostaining results on breast cancer in the literature are partly contradictory [6]. PCNA staining detects not only cells in S phase but also those in late G1, G2, M phases. Horiguchi et al[22] evaluated PCNA index in 167 carcinoma breast specimens. The PCNA index ranged from 1% to 76% (mean, 23.9%). Our results showed 0 to 100% variation which is comparable with this study. In the present study PCNA index correlated positively with Ki-67 LI. Not all studies have confirmed the correlation of PCNA with Ki-67 labeling index. Since PCNA involves in DNA repair, it is up regulated in non proliferating cells also. In some tumours 100% cells show positive staining. Therefore PCNA was not considered to be a very reliable marker [10]. Other studies have shown that PCNA index did not correlate with Ki-67[6]. There was no association found with clinical stage, histological grade, *p53* expression and menopausal status which is similar to previous studies [13].

***p53* altered expression:**

Mutations of the *p53* tumour suppressor gene often result in over expression of the *p53* protein which can be detected by immunohistochemistry on paraffin embedded tumours. The percentage of the positive tumours which showed *p53* immunopositivity was 66.2%. The percentage of *p53* immunoreactive breast cancers has been reported to be 54% and 45.5% by Oskowsky et al [23], Cattoretti and associates [24] respectively. The present study showed a high positive tumor for *p53*. This is attributed to the fact that most of these studies had considered 20% positive cells as a cut off value to divide positive and negative

cases, whereas in the present study 10% cells with positive staining was set as a cut off value.

In the present study *p53* overexpression positively correlated with high histological grade and stage. It is not associated with menopausal status. The results from the present study correlated with the data of Cattoretti and associates and partly similar to Josma et al[12] study. Cattoretti and associates [24] showed that altered *p53* expression was positively associated with high histological grade, higher stage, and Ki-67 proliferative activity.

Over expression of the *p53* protein in breast cancers reveal a high malignant potential. The correlation between over expression of *p53* and high Ki-67 labeling index suggested that they together give a proliferative advantage to cancer cells, because the mutant *p53* protein lack the property to inhibit the cell growth and also forms complex with *p*53 and inactivates it [12]. High *p*53 staining is associated with high Ki-67, higher stage and higher grade tumors which imply that it is associated with aggressive behavior [16]. A high degree of variability between the tumours in the immunoreactive score for *p53* was found in the present study [0 to 12 scores] which may result due to the true intratumour heterogeneity in *p53* expression [24].

**Menopausal Status:**

In the present study menstrual status of the patient did not correlate with all other parameters studied. Josma Isola et al[12] studied 289 breast carcinoma cases and found that there was no significant association of menopausal status with *p53* expression, histological grade and clinical stage. Barbereshchie et al[16] studied 97 unselected breast carcinomas for the expression of *p53* and Ki-67 and found that all the markers were not significantly associated with menopausal status. Results were similar in the present study also.

## CONCLUSION

According to our study results only **histological grade** correlated significantly with other parameters. Among the proliferation markers **Ki-67** correlated significantly with other parameters but not PCNA. *p53* positively correlated with histological grade, clinical stage and Ki-67 index indicates the aggressiveness of the tumor. Clinical stage correlated only with histological grade and *p53* expression. Menopausal status had no correlation with any parameter. It seems that a combination of parameters is needed in the treatment protocol of carcinoma breast patients. To identify the prognostic significance of these markers, minimum of five years follow up study is suggested.

## REFERENCES

1. Murthy NS, Chaudhry K, Nadayil D, Agarwal UK, Saxena S. Indian J Cancer 2009; 46:73-4.
2. Michael F Press. Advances in Pathology and Laboratory Medicine, 1993;6:12-13.
3. Parker SH, Stavros AT, Dennis MA. Radiol Clin North Am 1995; 33:1171-1186.

4. Baccouche S, Daoud J, Frika M,Gargouri RM, Gargouri A, Jlidi R, Ann NY Acad Sci 2003; 1010: 752-763.
5. Trihia H, Murray S, Price K. Cancer 2003; 97: 1321-1331.
6. Biesterfeld S, Kluppel D, Koch R. Schneider S, Steinhagen G, Michalcer A. et.al. J Pathol 1998; 185: 25-31.
7. Greene FL, Page DL, Fleming ID, et al. AJCC Cancer Staging Manual, 6th Edition. New York, NY, Springer-Verlag, 2002; pp.171-180.
8. Simpson, JF, Gray R, Dressler LG, Cobau CD, Falkson CI, Gilchrist KW. et al. J Clin Oncol 2000; 18: 2059-69.
9. Rudolph R, Olsson H, Bonatz G, Ratjen V, Bolte H, Baldetorp B et al. J Pathol 1999;187: 206-216.
10. Van Diest, Paul J, Brugal, Gerard, Baak, Jan PA. J Clin Pathol 1998; 51(10): 716-724.
11. Linjawi A, Kontogiannea M, Halwani F, Edwardes M, Meterissian S. Jl of the Am Coll of Surg 2004; 198: 83-90.
12. Isola J, Visakorpi T, Holli K, Olli P, Kallioniemi. J Natl Cancer Inst 1992; 84: 1109-1114.
13. Thomas M, Noguchi M, Kitagawa H, Kinoshita K, Miyazaki I. J Clin Pathol 1993; 46: 525-528
14. Surowiak P, Pudelko M, Maciejczyk A. Ginekol Pol 2005; 76: 9-14.
15. G. Kaur, R. Ismail, L. Suk Kam,, S. Sabaratnam, N. Ahmad. The Internet J Pathol 2007; 5( 2): 10.5580/193b.
16. Barbareschi M, Leonardi E, Mauri FA, Serio G. Am J Clin Pathol 1992; 98: 408-418.
17. Brown DC, Gatter KC. Histopathol 1990; 17: 489-503.
18. Caly M, Genin P, Ghuzlan AA. Anticancer Res 2004; 24: 3283-88.
19. Wintzer HO, Zipfel I, Monting JS. Cancer 1991; 67: 421-428.
20. Leonardi E, Girlando S, Serio G. J Clin Patrhol 1992; 45: 416-419.
21. Vernose SM, Gambacorta M. Am J Clin Pathol 1991; 95: 30-34.
22. Horiguchi J, Iino Y, Takei H, Maemura M, Takeyoshi I, Yokoe T. Oncol Rep 1998; 5(3): 641-644.
23. Ostrowsky JL, Sawan A, Henry L. J Pathol 1991; 164: 75-81
24. Cattoretti G, Andreola S. Br J Cancer 1988; 57: 353-357.