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Original Research Article

Correlative Study of Ki67 with Other Molecular Markers in Breast Cancer in Tamil Nadu Population: Retrospective Study

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Conflict of interest: Nil

Abstract:

Background: Although Ki-67 protein antigen has a half-life of only 1–1.5 hours, it is an important prognostic marker in breast cancer and used to differentiate sub-types of breast cancer by a cut-off value to be considered in distinguishing sub-types of cancer.

Method: 190 cases of breast carcinoma were analyzed. Haematoxylin and Eosin (H and E)-stained sections from the paraffin blocks representing the tumour tissue were performed, along with IHC staining for ER, PR, HER2, and Ki-67. The study was performed under the guidance of two pathological experts, and their findings were compared and calculated with ER and PR scores recommended by St. Gallens. Moreover, an automated method was also observed.

Results: The mean value of both experts, average and automated, had a significant p value ($p<0.001$). The median percentage value of both experts' average values and automated values had a significant p value ($p<0.001$). The highest mean of the automated Ki-67 value was in subgroup B (48%), and the least was in subgroup A (29%).

Conclusion: It is concluded that all the subgroups of ER and PR had positive findings as per the molecular subtypes recommended by Gallen's, which helps the pathologist predict the prognosis of breast cancer.

Keywords: St. Gallens, Ki-67, PR, ER, Her-2, immunohistochemistry, H and E staining.

Introduction

The Ki-67 antigen, which encodes two protein isoforms with molecular weights of 345 and 395 KDa, it was originally identified by Scholzer and Gerdes in the early 1980s [1]. The Ki-67 protein has a half-life of only 1-1.5 hour. It is present during all the active phases of cell cycle but absent in the resting cells. In later phases of mitosis, a sharp decrease in Ki-67 levels occurs [2].

Expression of the Ki-67 protein is associated with the proliferative activity of intrinsic cell populations in malignant tumours, allowing it to be used as a marker of tumour aggressiveness. The prognostic value of Ki-67 has been studied, with its potential as a reliable marker having been shown in breast, soft tissue, lung, prostate, cervix, and central nervous system cancers [3].

The quantity of Ki-67 present at any time during the cell cycle is regulated by a precise balance between synthesis and degradation, as indicated by its short half-life of 1–1.5 hours. Ki-67 protein expression coincides with the transit of cells through mitosis and undergoes phosphorylation and dephosphorylation during mitosis in vivo, rendering it susceptible to protease degradation [4]. Hence, an attempt was made to study the Ki-67 values in various subgroups to justify the prognostic value of breast cancer as it is an indicator of cell proliferation.

Materials and Methods

190 (one hundred and ninety) breast cancer cases from the pathology department of Dr. A L Mudaliar Postgraduate Institute of Basic Medical Sciences, Taramani, Chennai, Tamil Nadu (600113) were studied.

Inclusive Criteria: Study included all lumpectomy, simple mastectomy, and modified radical mastectomy specimens of carcinoma breast with known ER, PR, and HER2 status.

Exclusion Criteria: Benign and unpreserved specimens were excluded from the study.

Method: The specimens received were grossed after proper fixation, and adequate sections were taken. Then, for all the cases H and E, IHC staining for ER, PR, HER2, and Ki-67 was performed. (Fig 1-5)

Around 4-5 µm sections were cut from a paraffin block of tumour tissue and taken on glass slides. Coated glass slides with adhesive were taken for immunohistochemistry.

Clone used for ER was ventana anti ER Rabbit monoclonal primary antibody SP1, PR was ventana anti PR. Rabbit monoclonal primary antibody 1E2 and for HER2/neu was ventana anti-Her2. Ki-67 was the Dako monoclonal mouse anti-human antibody MIB-1. All of these were ready-to-use kits. No dilutions were performed. Ventana Benchmark XT-automated IHC slide staining was performed. All the cases were then divided into the following four subgroups based on ER, PR, and Her2 status [5].

Two pathological experts (professors) were selected as observers/ guides to participate in the present study.

Immunohistochemistry (IHC) stained Ki-67 slides were submitted to two experts (observers) independently. Both experts were blinded regarding the patient outcome, and the observers scoring of ki-67 was done independently, irrespective of fields of study and number of cells (preferably 1000, but a minimum of 500 cells to be counted) [6]. After scoring, a category of high or low is designated based on a cut-off of 50% [7]. Then, the same slides were used with the automated image analysis software IMMUNORATIO (automated determinator of the Ki-67 Index) [8]. The categories of high or low were analyzed for the interpretation and scoring of Ki-67 [9]. Mouse anti-human Ki-67 monoclonal antibody MIB-1 was used in IHC staining of Ki-67.

The invasive edge of the tumour was selected to score Ki-67. At least three fields were scored at the periphery of the tumour.

Only nuclear staining (plus mitotic figures, which are stained by Ki-67) of any intensity was considered positive. Internal positive controls of mitotic figures, normal ducts, lymphocytes, or endothelial and stromal cells were taken.

The duration of the study was August 2010–2013 (retrospective).

Statistical analysis: The Ki-67 index value was calculated in all 190 cases of breast cancer. The distribution of the range was observed, and the median value was obtained. The mean Ki-67 index values in each group were calculated separately. To compare manual scoring with automated scoring, a cutoff 15% was considered to categorize high and low Ki-67. Therefore, cases with Ki-67 levels, ≤15% were taken as low Ki-67. The average values of the two experts were taken for each case. An ANOVA test was used to calculate different parameters, and significant values were noted. The statistical analysis was carried out in SPSS software.

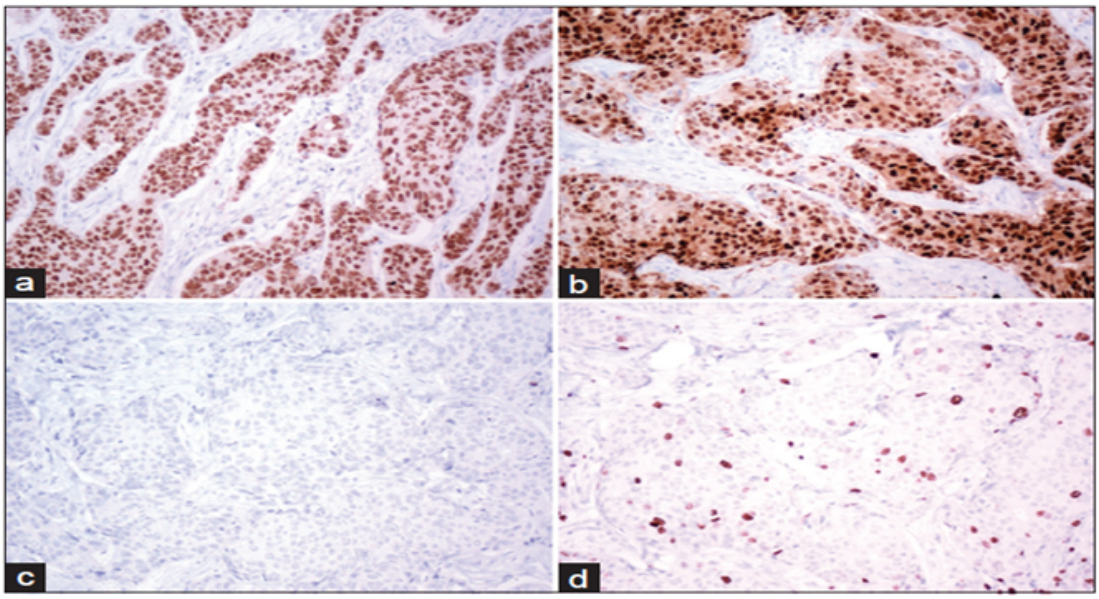

Figure 1: Luminal A molecular subtype; (a) ER positive, (b) PR positive, (c) HER2 negative, (d) Low MIB-1

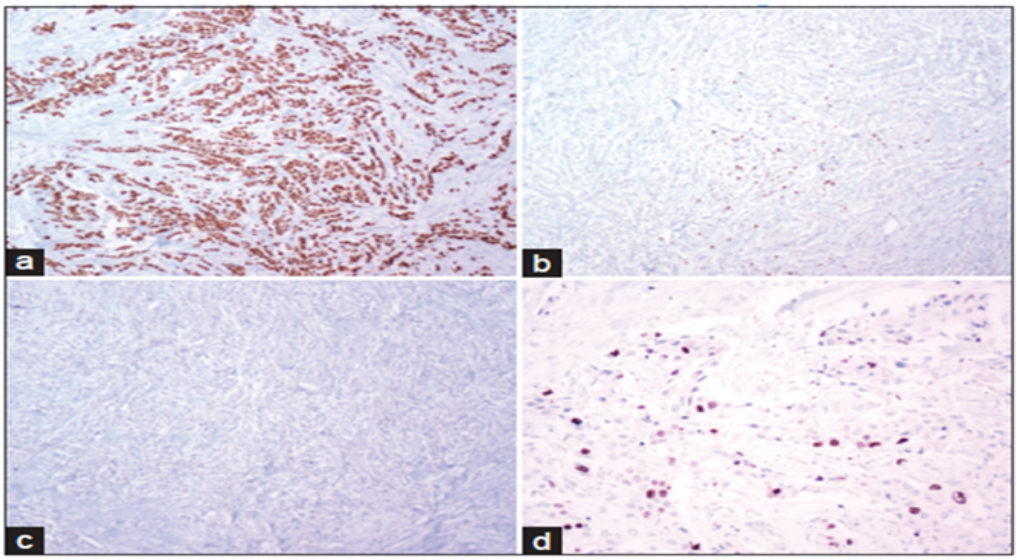

Figure 2: Luminal B Her2 negative molecular subtype; (a) ER positive (b) PR low positive, (c) HER2 negative, (d) MIB-1

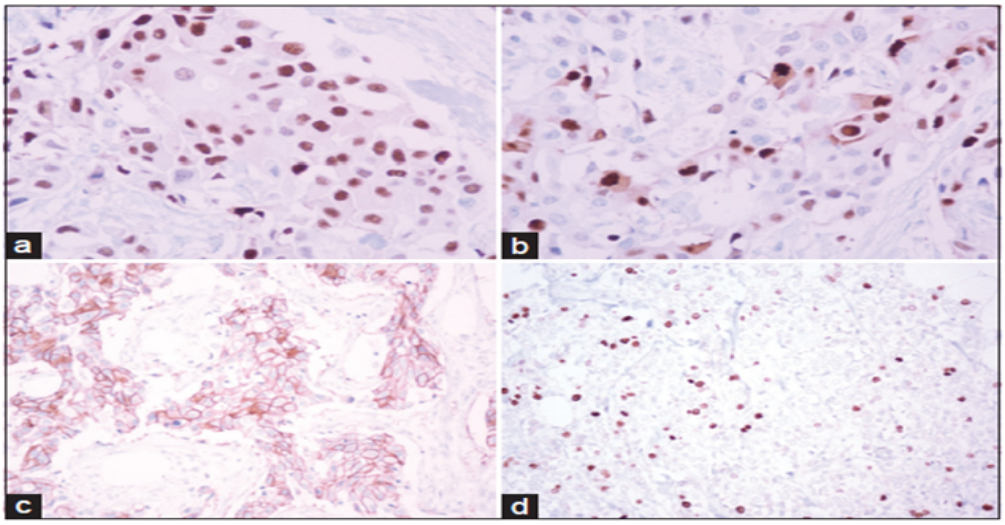

Figure 3: Luminal B HER 2 positive molecular subtype; (a) ER positive, (b) PR positive, (c) HER2 positive, (d) MIB-1

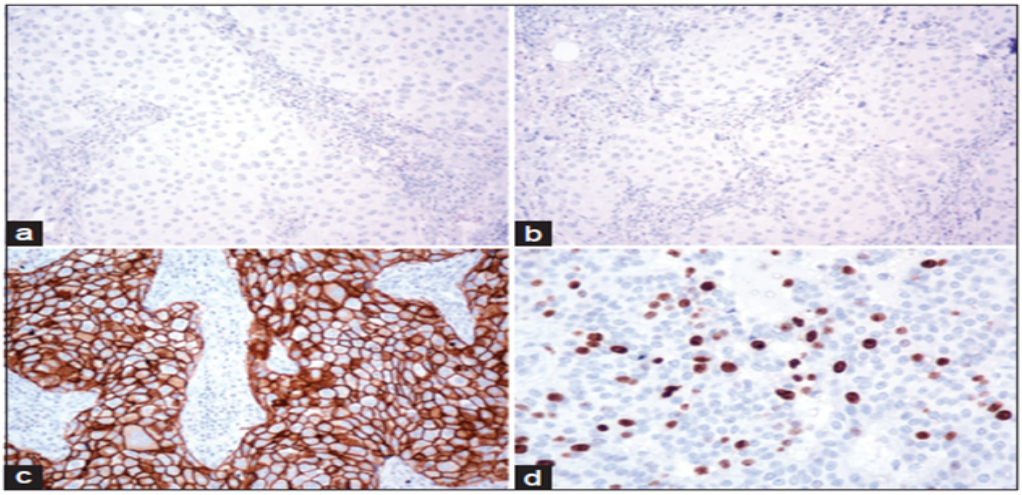

Figure 4: HER2/neu enriched molecular subtype; (a)ER negative, (b) PR negative, (c) Her2 positive (d) MIB-1

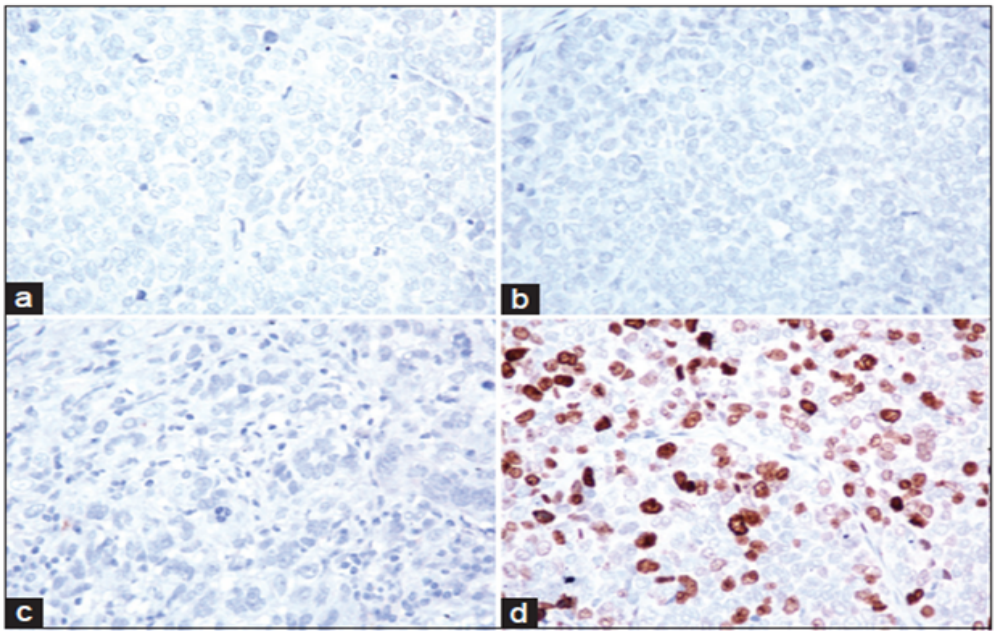

Figure 5: Triple negative; (a)ER negative, (b) PR negative, (c) Her2 negative (d) MIB-1

Observation and Results

Table 2: Comparison of both expert studies, average, automated values of Ki-67 values were done.

In the mean, 30.11 (expert I), 28.45 (expert II), and 29.78 were the average values. 38.25 was the automated value, and p<0.001 (the p value was highly significant).

The % study had a value of 28.60 (expert I study value). 27.88 (expert II value) 28.30 was average, 35.40 was automated value, and p<0.001 (p value was highly significant).

Table 3: Mean Ki-67 value in subgroups. In subgroup A, 21.05 was the mean value % and 29 were the automated. In subgroup B, 37.17 was the mean value % and 48 was the automated value. In subgroup C, 33.70 was the mean value % and 40 was the automated value. In the subgroup of D, 27.25 was the mean value % and 30 was the automated value.

Table 1: The molecular sub types were divided according to the St. Gallen's recommendations

Group	ER	PR	HER2/neu
A	Positive	Positive (>20%)	Negative
B	Positive	Negative or low positive (<20%)	Negative
C	Negative	Negative	Positive
D	Negative	Negative	Negative

(ER=Estrogens, PR=Progesterone, HER2=Human epidermal growth factor receptor 2)

Table 2: Comparison both parts, Average and automated mean and Median Ki-67 values of 190 cases

Group	Observed (expert-1)	Observed (expert-2)	Average	Automated	O/f	p value
N	190	190	190	190		
Mean	30.11	28.45	29.78	38.25	3	P<0.001
Median	28.60	27.88	28.30	35.40	3	P<0.001
SD±	0.193	0.178	0.180	0.253		

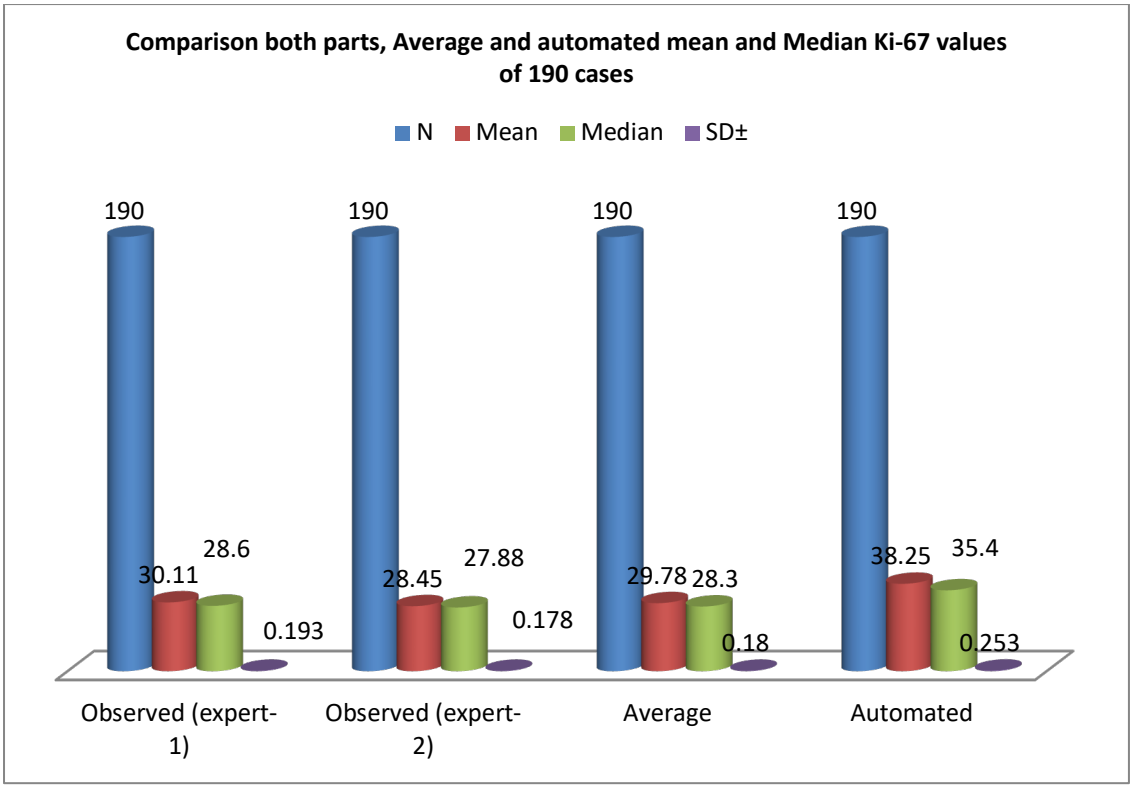

Figure 6: Comparison both parts, Average and automated mean and Median Ki-67 values of 190 cases

Table 3: Mean Ki-67 value in sub group

Group	Mean Ki-67 (%)	Mean Ki-67 of automated values (%)
Subgroup A	21.05	29
Subgroup B	37.17	48
Subgroup C	33.70	40
Subgroup D	27.25	30

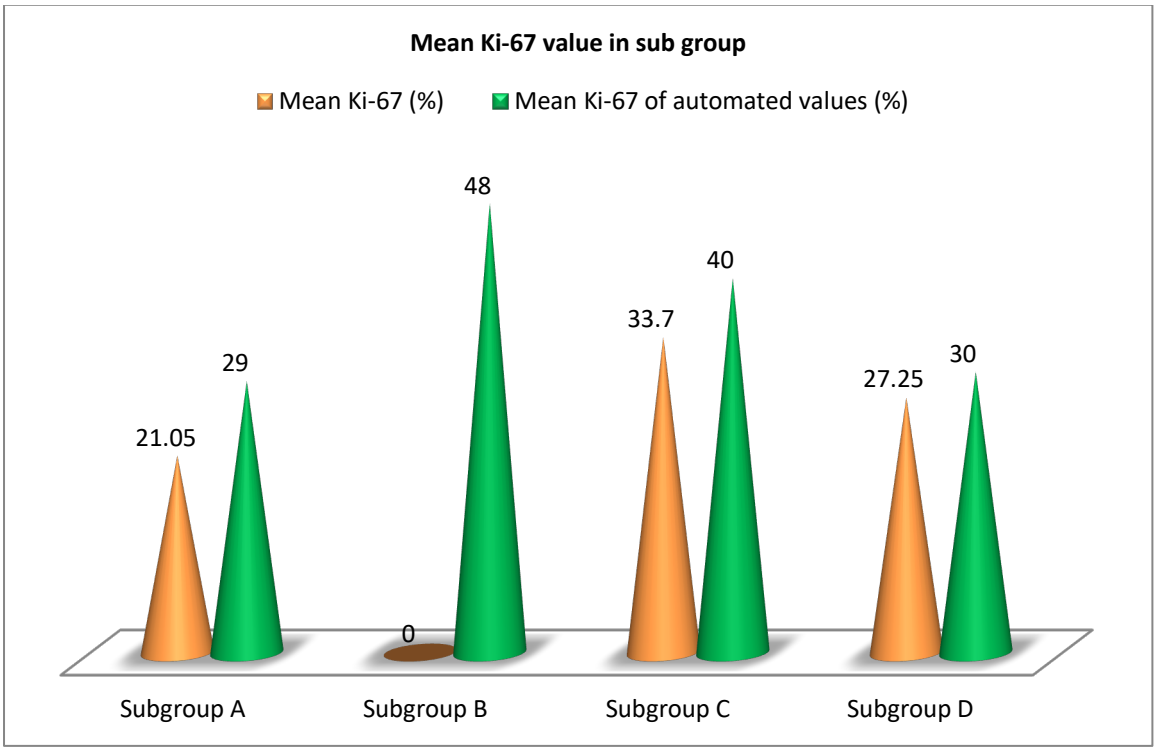

Figure 7: Mean Ki-67 value in sub group

Discussion

Correlation of the Ki-67 marker with other molecular markers in the breast cancer studies of the Tamil Nadu population. As per the recommendation of St. Gallen's, progesterone receptor, PR was in position (>20%) in group A and PR was in position (<20%) in group B along with positive ER.

In the negative of both ER and PR, HER2 neu is positive in group C. Group D is triple negative (Table 1). The mean percentage value of expert I, expert II average, and automated has a significant p value ($p<0.001$). In the median percentage study, expert I, expert II average, and automated values had significant p values ($p<0.001$) (Table 2). The study of mean values of Ki-67 subgroup B had the highest mean value percentage (48%) and the least observed in subgroup A (29%) (Table 3) (Figures 1 to 5). These findings are more or less in agreement with previous studies [10,11]. Luminal A subgroups have the best prognosis among all subgroups, and subgroup D has a (30%) mean value. India has the second-best prognostic value. On the other hand, subgroup B has a better prognosis due to an increased mean value (48%); hence, Ki-67 plays an important role in determining the prognosis and also for the treatment modality.

Hence, since Ki-67 is an independent prognostic factor for disease-free survival, standardization of the staining techniques and scoring methods is necessary in order to incorporate this biomarker into routine practice because Ki-67 is a sensitive protein associated with cell proliferation [12]. Owing to high cell proliferation, frequently associated with the Ki-67 protein labeling index Ki-67 may be a promising factor for targeted molecular therapies, as targeting pathways [13] and molecular markers implicated in cancer cell growth is a promising avenue for the development of effective therapies.

Summary and Conclusion

As a proliferation marker to measure the growth fraction of cells in human tumours, the expression of Ki-67 is strongly associated with cell proliferation and is widely used in routine pathology.

The present study demands the standardization of techniques. Although Ki-67 is a key marker associated with proliferating cancer cells and has a poor prognosis.

Limitation of Study: The limitation of the present study was the lack of follow-up with our patients and the correlation of the molecular subtypes with clinical stage.

The present research study was approved by ethical committee of the Dr. A L Mudaliar Postgraduate Institute Basic of Medical Sciences, Taramani, Chennai, Tamil Nadu-600113

References

1. Scholzen T and Gerdes J: The Ki-67 protein from the known and the unknown J. cell physiology 2000, 182; 311-322.
2. Modlin IM, Moss SF: Priorities for improving the management of neuroendocrine tumours J. Natl. Cancer Inst. 2008, 100; 1282–89.
3. Ishihara M, Mukai H: Retrospective analysis of risk factors for central nervous system metastasis in breast cancer effects of biological subtypes and Ki-67 overexpression on survival oncology 2013, 84; 135–140.
4. Castro LA, Elias LS: Long-term effects of nifidepine on human gingival epithelium: a histo-pathological and immunohistochemical study J. oral science 2010, 52; 55–62.
5. Goldhirsch A, Winer EP: Personalizing the treatment of women with early breast cancer: high lights of the St. Gallen international expert consensus on the primary therapy of early breast cancer 2013, Ann. Oncol. 2013, 29; 2206–23.
6. Dowsett M., Nielsen TO: Assessment of Ki-67 in breast cancer Recommendations from this international Ki-67 breast cancer working group J. Natl. Cancer Inst. 2011, 103; 1654-64.
7. Mohammed ZMA, McMillan DC: Comparison of visual and automated assessments of Ki-67 proliferative activity and their impact on outcomes in primary operable invasive breast cancer Br. J. Cancer 2012, 106; 383–8.
8. Yoe M, Kim HE, and Kim SH: Clinical usefulness of a free web-based image analysis application for immune ratio assessment for the Ki-67 labelling index of breast cancer J. Clin. Potho-2017, 10; 1–5.
9. Polley M-Y C, Leung Scu: An international Ki-67 reproducibility study JVCI J. Natl. Cancer Inst. 2013, 105; 1897-906.
10. PenaultCiorca F: Ki-67 assessment in breast cancer. An update on pathology, 2017, 49; 166-7
11. Sun J, Chen C: Associations and indications of Ki-67 expressions with clinic-pathological parameters and molecular subtypes in invasive breast cancer. A population-based study. Oncol. Lett. 2015, 10; 1741–8.
12. Tashima R, Nishimura R, and Osako T: Evaluation of an optimal cut-off point for the Ki-67 index as a prognosis factor in primary breast cancer, PL OS One 2015, 10; 1–10.
13. Kausch L, Lingnau A: Antisense treatment against Ki-67 mRNA inhibits proliferation and tumour growth in vitro, Int. J. Cancer 2003, 105; 710–76.