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Assessment of Response of Neoadjuvant Chemotheraphy in Locally Advanced Breast Carcinoma Using Ki-67 As A Proliferative Marker

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Abstract
BACKGROUND *:This study is aimed at assessing the response of neoadjuvant chemotheraphy in locally advanced breast carcinomas using Ki-67 as a proliferative marker.*
METHODOLOGY *:This is a PROSPECTIVE RANDOMISED CASE CONTROL STUDY done for the period of one year from May 2018 to May 2019. Patients from surgical OPD or casualty presenting with breast lump proven for malignancy in GRH Madurai are recruited. The patients were diagnosed on the basis of history, clinical examination and investigations like core needle biopsy, USG breasts with axilla. ER , PR , HER2 neu status were assessed along with Ki-67 index. 60 patients were recruited for this study. Patient were categorized into 2 groups based on Ki-67 index (low - < 20% ; High- > 20%). Patients were subjected to neoadjuvant chemotheraphy and reassessed following its completion.*
RESULTS: *In low Ki-67 index group 53.30% were in Stage IIIc and 46.70% were in Stage IIIB. Following Neoadjuvant chemotheraphy 53.30% downstaged to Stage IIIA , 30% to Stage IIB.In High Ki-67 index group 66.70% were in Stage IIIC and 33.30% were in Stage IIIB. Following Neoadjuvant chemotheraphy 66.70 % downsatged to Stage IA and 20% to Stage IB and 13.30% to Stage IIA.*
CONCLUSION: *From our study we concluded that KI-67 a proliferative marker can be used to assess the response of neoadjuvant chemotheraphy. Tumors with high index of Ki-67 respond significantly well to chemotheraphy and it can be used to assess the achievement of a pathological complete response. Neoadjuvant chemotherapy reduces tumor size, which enables patients who were initially inoperable to undergo mastectomy and makes breast-conserving surgery possible in patients who otherwise would have required mastectomy.*
Keyword: *ca breast, ki67,chemotherapy*

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I. Introduction

While an enormous amount of information still remains intricately hidden with the human genome, research today is slowly but steadily decoding it. Every piece of data thus garnered alters to some extent our current understanding of malignancies and opens up new avenues for their management.

Carcinoma [CA] of the breast is one of the most common malignancies encountered in the General Surgery Out Patient Department & operated on at Government Rajaji Hospital, Madurai. Patients, mainly from lower socioeconomic strata, often present with either palpable lump or in a more advanced stage.
They are treated as per standard protocols with:

- Modified Radical Mastectomy followedby adjuvant Chemotherapy, Radiation or both in early breastcarcinomas,
- Neoadjuvant Chemotherapy followed by Surgery, Adjuvant Chemotherapy and Radiation in locally advanced breast cancer and
- Palliative Chemotherapy for advanced metastatic disease.

The follow up in our hospital is excellent as this is a tertiary care center for the people of this region. For a condition such as cancer of the breast, all the patients are treated here and it is possible to follow the patient from initial presentation, through diagnosis, treatment and during adjuvant therapy as well, except in rare extenuating circumstances.

Neoadjuvant chemotheraphy has been established as a standard treatment strategy for patients with not

only locally advanced but also operable breast cancer. This strategy allows patients to benefit a reduction in the extent of surgery and provide information on the efficacy of chemotheraphy. Recently, it has been demonstrated that patients who achieved pathological complete response (pCR) to NAC were likely to also have a favourable long term outcome in certain subtypes of breast cancer patients. As such, clinical and molecular biomarkers capable of predicting pCR have been assessed following neoadjuvant treatment in breast cancer patients.

Conventional variables such as tumor size, nodal status and histological grade do not correlate well with sensitivity to specific types of chemotheraphy drugs. Several retrospective breast cancer studies have suggested that tumor expression of ER, PR, epidermal growth factor receptor (EGFR), HER2, Ki-67 and p53 may be associated with chemotherapy sensitivity. Compared with other biomarkers, Ki-67 expression has been reported to correlate with tumor cell proliferation rate, which is a nuclear protein that is expressed during all phases of cell cycle, except the G0 phase and many studies have investigated the IHC expression of Ki-67 as a prognostic and predictive marker for breast cancer.

II. Materials And Methods

Design Of Study: PROSPECTIVE RANDOMISED STUDY
Study Location: The study was done in a tertiary care teaching centre – Govt. Rajaji Hospital, Madurai, Tamilnadu in Department of General Surgery
Duration Of Study: 1 year
Collaborating Department: Nil
Selection of study subjects: All surgical in-patients of Govt. Rajaji Hospital during the study period satisfying the inclusion criteria were recruited for the study after obtaining valid consent.
Sample size: 60 patients were enrolled for the study after obtaining proper informed consent. The patients were randomly allotted to case and control groups.
Total sample size (n) = 60
No. Of Cases = 30
No. Of controls = 30
No. Of dropouts = NIL

– **Data collection:** Patients from surgical OPD or casualty presenting with breast lump proven for malignancy in GRH Madurai are recruited.
– The patients were diagnosed on the basis of history, clinical examination and investigations like trucut biopsy, USG breasts with axilla
– The patients presenting with locally advanced breast carcinoma in GRH Madurai will be included in this study.

Ethical Clearance: Obtained
Conflict Of Interest: None
Financial Support: NIL FROM THE INSTITUTION
Participants: from surgical OPD or casualty presenting with breast lump proven for malignancy in GRH Madurai
Follow Up: Upto 1 year
PRIMARY OBJECTIVE
To assess the response of neoadjuvant chemotheraphy in locally advanced breast carcinomas with ki-67 as a proliferative marker.

SELECTION CRITERIA

- **INCLUSION CRITERIA**

– Patients presenting with breast lump proven for malignancy, in GRH Madurai.
– Locally advanced breast carcinoma.
– Patients consented for inclusion in the study according to designated proforma.

- **EXCLUSION CRITERIA**

– Breast carcinoma with metastasis
– Recurrent breast tumors

PROCEDURE

– Patients from surgical OPD or casualty presenting with breast lump proven for malignancy in GRH Madurai are recruited.

– The patients were diagnosed on the basis of history, clinical examination and investigations like trucut biopsy, USG breasts with axilla

- The patients presenting with locally advanced breast carcinoma in GRH Madurai will be included in this study.
- Following consent, a questionnaire will be filled to record the patient's demographic data, duration of disease, symptoms, treatment history.

Trucut biopsy

Histological subtype estrogen receptor status progesterone receptor status HER2 status	Grading and proliferation status as assessed by Ki-67 staining(by using monoclonal anibody against ki-67antigen)

- 60 patients who are presenting with locally advanced breast carcinoma will be included in the study.
- They will be divided into 2 groups of 30 each.

Group A	Group B
Patients with low ki-67 index (< 20%) i.e, less than 20% of cells staining positive for ki-67.	**Patients with high ki-67 index (> 20%)** **i.e, more than 20% of cells staining positive for ki-67**

These 60 patients were subjected to neoadjuvant chemotheraphy. Following neoadjuvant chemo the parameters were reassessed and were documented.
Comparison between the parameters before and after neoadjuvant chemotheraphy was made.

III. Results

The present study of 60 cases of locally advanced carcinoma breast was studied during a period of May 2018 to May 2019. Both outpatient and inpatient basis patients diagnosed as locally advanced breast carcinoma were selected and patients were investigated with measurement of Ki-67 levels from the core needle biopsies taken. Patients were categorized into low And high depending on the values of Ki-67 and were subjected to Neoadjuvant chemotheraphy. Results were estimated.

This study mainly focuses on assessing the response of neoadjuvant chemotheraphy in locally advanced breast cancers with Ki-67 as a proliferative index.

The mean age of 60 patients who were included in the study is 54.4 with the mean of 53.3 in Low Ki-67 index group and 55.5 in High Ki-67 index group.

Fig 1: chart showing age distribution in the two groups

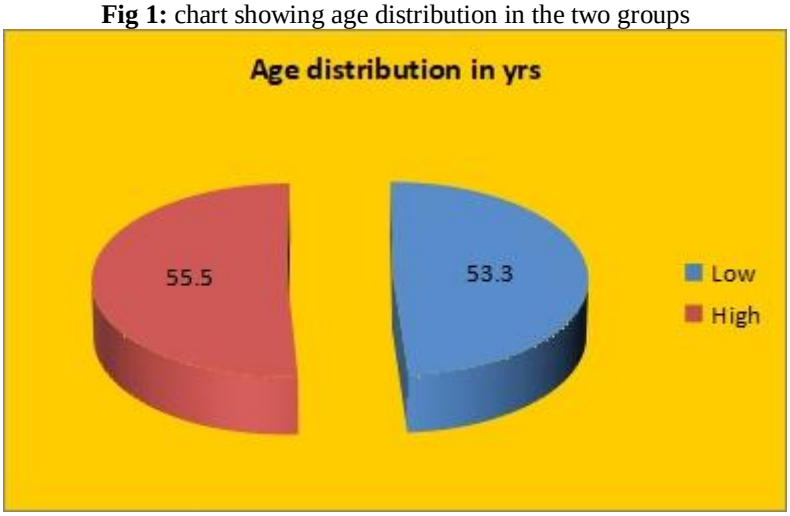

Fig 2

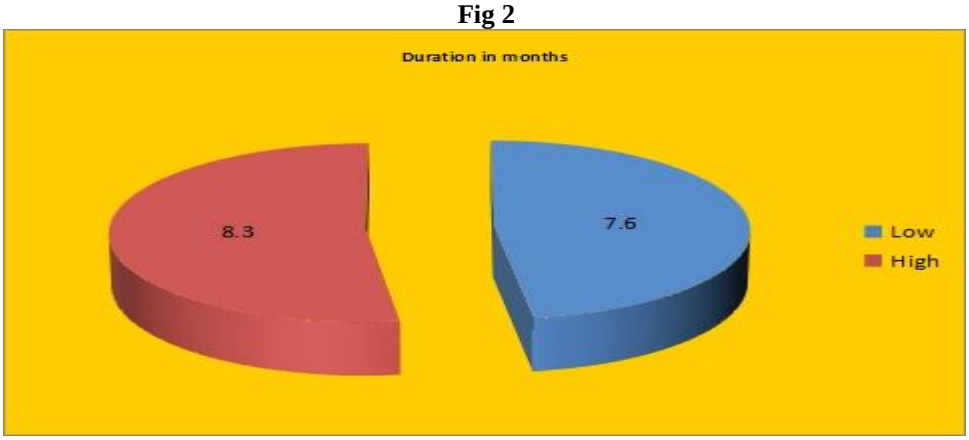

Fig 2 : Pie chart depicting the duration of symptoms in both group of patients.

Fig 3

Indicates the characteristic distribution of patients selected for the study.

The P value is not significant indicating that these factors do not decide the outcome of neoadjuvant chemotheraphy in locally advanced breast carcinomas.

	Ki67Index	N	Mean	Std. Deviation	Std. Error Mean	P value
Age	Low	30	53.57	7.118	1.300	0.640
	High	30	55.57	7.074	1.292	
Duration	Low	30	7.67	5.598	1.022	0.340
	High	30	8.33	6.692	1.222	

Fig 3 Patient characteristics distribution

Fig 4

Mann whitney U test; Not significant

Of the patients in Low Ki-67 group, carcinoma had involved Left breast in36.70 % and carcinoma had involved Right breast in 63.30%

Of the patients in High Ki-67 group, carcinoma had involved Left breast in33.30 % and carcinoma had involved Right breast in 66.70%

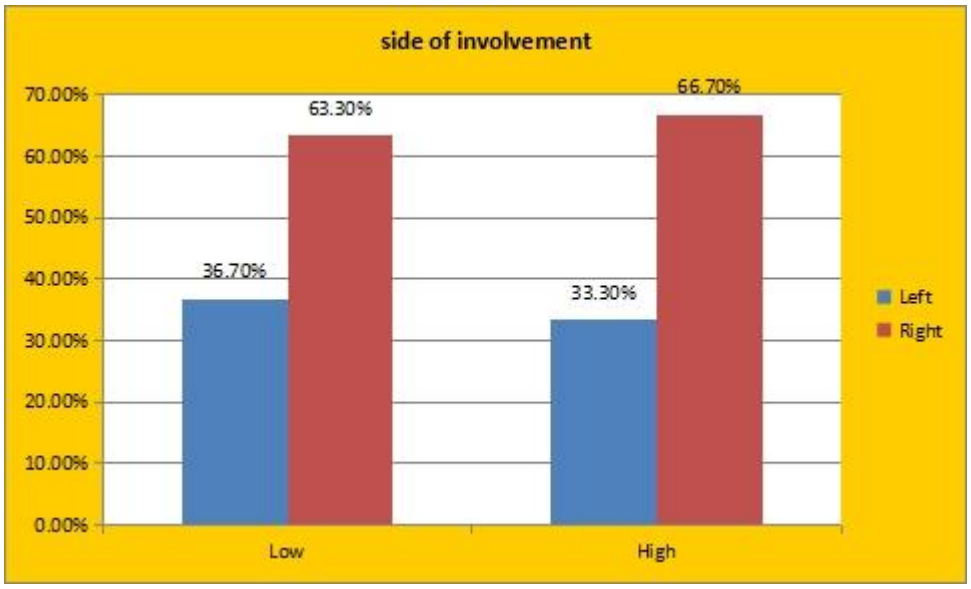

Fig 4 Indicates the side of involvement in both groups.

Fig 5

The mean size of tumor before chemotheraphy in Low Ki-67 index group was 6.5 x 5.8 on clinical examination and by USG estimation was 6.77 x 6.5 .Post chemotherapeutic mean size was 4.5 x 4 both clinically and USG wise.This was indicative of reduction in tumor size following neoadjuvant chemotheraphy.

Fig 5
Responsive Change in tumor size with chemotheraphy for low Ki 67 index

Low Ki 67	Before chemotheraphy				After chemotheraphy				
Tumor size	**Mean**	**Std.Dev**	**median**	**IQR**	**Mean**	**Std.Dev**	**median**	**IQR**	**P value**
Cl. length	6.5	1.656	6	2.25	4.73	1.363	4.5	2	0.001**
Cl .breadth	5.8	1.297	6	2	4.53	1.332	4	2.25	0.001**
Us. length	6.77	1.455	6.5	2	4.73	1.363	4.5	2	0.001**
Us. breadth	6.5	1.196	7	2	4.53	1.332	4	2.25	0.001**

Wilcoxon's statistical test ; shows * ($p<0.001$**)

Fig 6

This chart compares the tumor size before neoadjuvant chemotheraphy and after neoadjuvant chemotheraphy in patients with Low ki-67 index group.

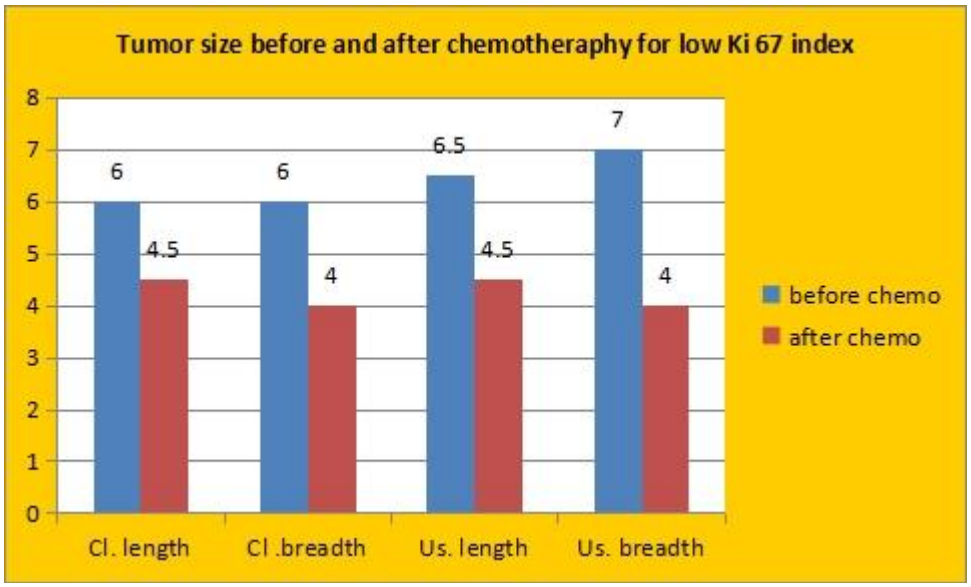

Fig 7

The mean size of tumor before chemotheraphy in High Ki-67 index group was 6.47 x 5.87 cm on clinical examination and by USG estimation was 6.63 x 6.33.Post chemotherapeutic mean size was 1.1 x 1.04 cm clinically and 1.02 x 0.97 USG wise.This was indicative of significant reduction in tumor size following neoadjuvant chemotheraphy.

Fig 7
Responsive Change in tumor size with chemotheraphy for high Ki 67 index

High Ki 67	Before chemotheraphy				After chemotheraphy				
Tumor size	**Mean**	**Std.Dev**	**median**	**IQR**	**Mean**	**Std.Dev**	**median**	**IQR**	**P value**
Cl. length	6.47	1.383	6	2	1.1	0.317	1	2	0.001**
Cl .breadth	5.87	1.224	6	2	1.04	0.344	1	2	0.001**

Us. length	6.63	1.326	6	1.2	1.02	0.359	1	2	0.001**
Us. breadth	6.33	1.155	6	2.2	0.97	0.37	1	2	0.001**

Wilcoxon's statistical test ; shows * (p<0.001**)

Fig 8

This chart compares the tumor size before neoadjuvant chemotheraphy and after neoadjuvant chemotheraphy in patients with High ki-67 index group

Fig 8

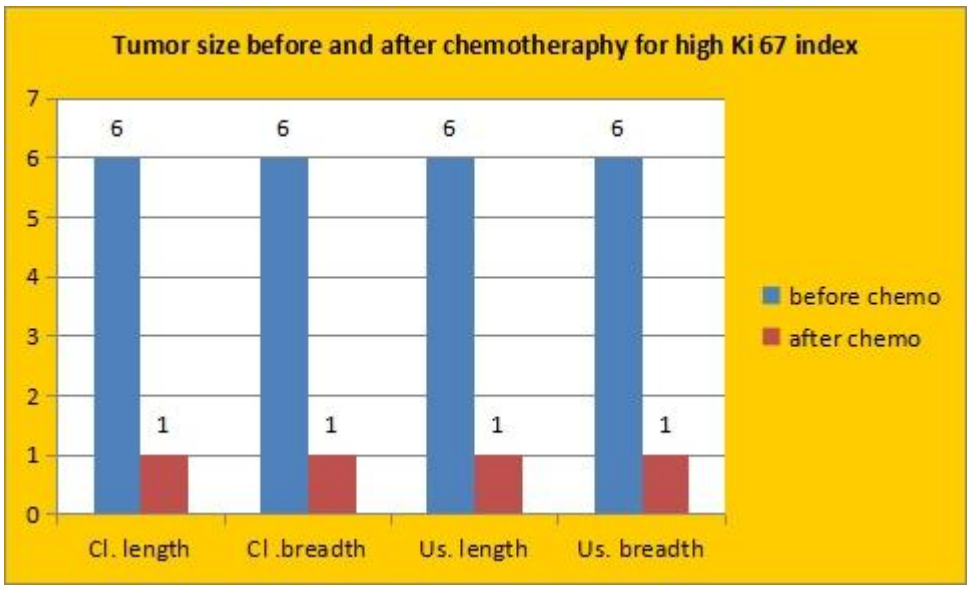

Fig 9

Indicates the responsive change in tumor size for low Ki-67 and High Ki-67 index groups following neoadjuvant chemotheraphy.

From this itis evident that tumors having high proliferation index have more cells in their proliferating phase where the chemotherapeutic drugs act producing significant change in reduction of tumor size.

Fig 9

Association of Responsive Change in tumor size for low and high Ki 67 indexwith chemotheraphy

	LOW Ki67 index			High Ki67 index			
Tumor size (Cms)	**Mean**	**Std. Deviation**	**Std. error**	**Mean**	**Std. Deviation**	**Std.error**	**P value**
Before chemotheraphy							
Cl. length	6.5	1.656	0.302	6.47	1.383	0.252	0.921
Cl.breadth	5.8	1.297	0.237	5.87	1.224	0.224	0.885
Us. length	6.77	1.455	0.266	6.63	1.326	0.242	0.76
Us. breadth	6.5	1.196	0.218	6.33	1.155	0.211	0.532
After chemotheraphy							
Cl. length	4.73	1.363	0.249	1.1	0.317	0.062	0.001**
Cl .breadth	4.53	1.332	0.243	1.04	0.344	0.067	0.001**
Us. length	4.73	1.363	0.249	1.02	0.359	0.066	0.001**
Us. breadth	4.53	1.332	0.243	0.97	0.37	0.068	0.001**

Mann whitney U test; shows ** (p<0.001**)

Fig 10

Indicates the nodal status of patient before starting neoadjuvant chemotheraphy and after completing chemotheraphy.

Following neoadjuvant chemotheraphy in high Ki-67 index group most of the patients I.e., 83.30% of them became N0.

From this it is evident that high KI-67 index group ahs a better response to neoadjuvant chemotheraphy compared to low ki-67 index group

Fig 10

Change in nodal involvement grading based on Ki 67 index

		Low		High	p value
Before chemotheraphy	N	(%)	N	(%)	
	N2a	6	20.00%	6	20.00%	
	N2b	8	26.70%	4	13.30%	
	N3a	7	23.30%	6	20.00%	0.586
	N3b	5	16.70%	6	20.00%	
	N3c	4	13.30%	8	26.70%	
Response after chemotheraphy					
	N0	2	6.70%	25	83.30%	
	N1	18	60.00%	5	16.70%	0.001**
	N2a	6	20.00%	0		
	N2b	2	6.70%	0		
	N3a	2	6.70%	0		

Fisher's exact test; shows ** ($p<0.001$**)

Fig 11

Indicates the distribution of nodal involvement in both groups before chemotheraphy

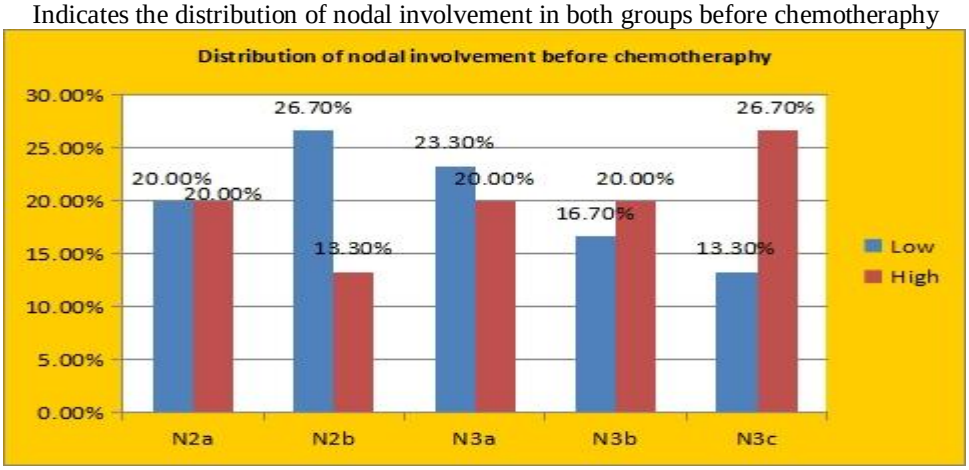

Fig 11

Fig 12 Indicates the distribution ofnodal involvement in both groups afterchemotheraphy

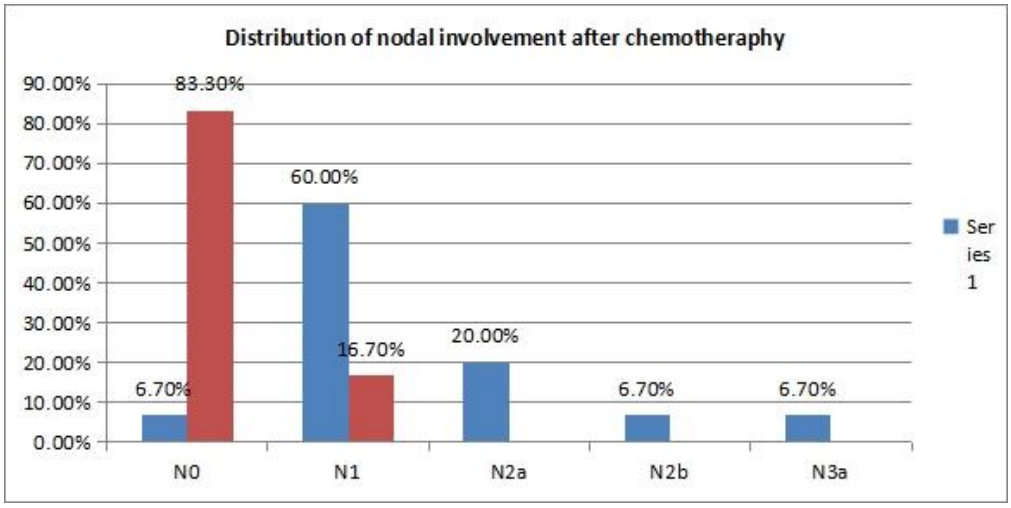

Fig 13

Summarises the change in nodal stages based on ki-67 index.

Following neoadjuvant chemotheraphy in high Ki-67 index group most of the patients I.e., 83.30% of them became N0. From this it is evident that high KI-67 index group ahs a better response to neoadjuvant chemotheraphy compared to low ki-67 index group.

Fig 13

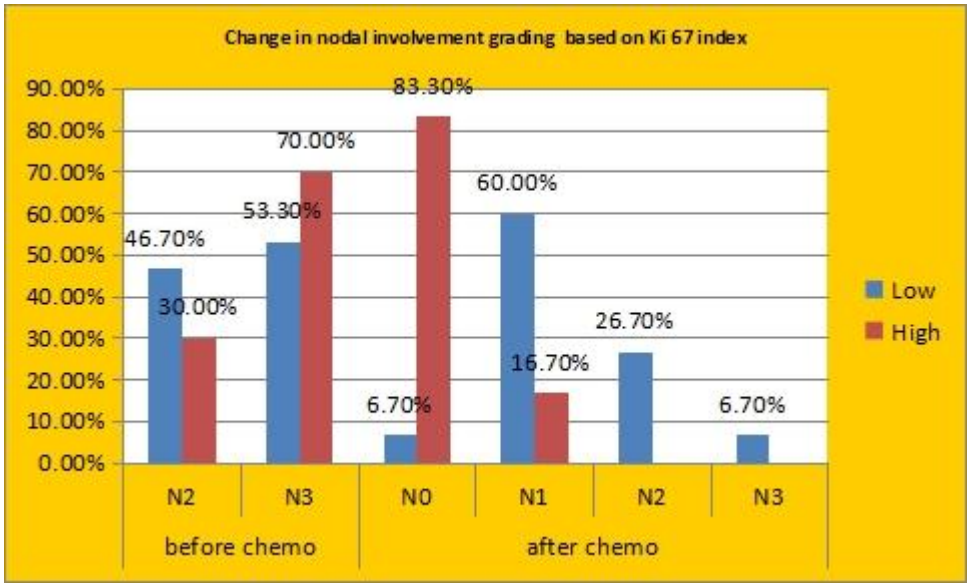

Fig 14

Demonstrates the responsive chsnge in Staging of breast cancer following Neoadjuvant chemotheraphy based on KI-67 index

In low Ki-67 index group 53.30% were in Stage IIIc and 46.70% were in Stage IIIB. Following Neoadjuvant chemotheraphy 53.30% downstaged to Stage IIIA , 30% to Stage IIB.

In High Ki-67 index group 66.70% were in Stage IIIC and 33.30% were in Stage IIIB. Following Neoadjuvant chemotheraphy66.70 % downsatged to Stage IA and 20% to Stage IB and 13.30% to Stage IIA

Fig 14

TNM staging		Low		High		p value
Before chemotheraphy	Stage IIIB	14	46.70%	10	33.30%	0.252
	Stage IIIC	16	53.30%	20	66.70%	
Response after chemotheraphy	Stage IA	0		20	66.70%	0.001**
	Stage IB	1	3.30%	6	20.00%	
	Stage IIA	0		4	13.30%	
	Stage IIB	9	30.00%	0		
	Stage IIIA	16	53.30%	0		
	Stage IIIB	2	6.70%	0		
	Stage IIIC	2	6.70%	0		

Fisher's exact test: shows ** ($p<0.001$**)

Summarises a change in staging of breast cancer following Neoadjuvant chemotheraphy in High and Low Ki-67 index groups

Fig 15
Responsive Change in TNM grading based on Ki 67 index

Fig 15

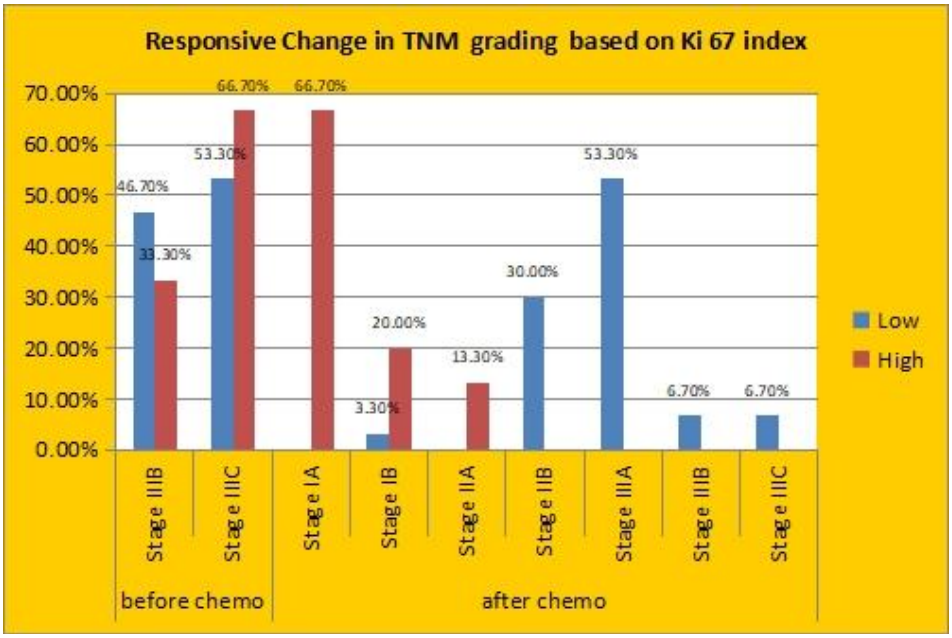

Fig 16
Responsive Change in Nodal involvement grading and TNM grading with chemotheraphy for low and High Ki 67 index

	Before chemotheraphy	Response After chemotheraphy	
	Median	**Median**	P value
Low Ki 67 index			
Nodal involvement	N3	N1	0.001**
TNM staging	Stage IIIC	Stage IIIA	0.001**
High Ki 67 index			
Nodal involvement	N3	NO	0.001**
TNM staging	Stage IIIC	Stage IA	0.001**

Wilcoxon's statistical test ; shows * ($p<0.001$**)

IV. Discussion

Uncontrolled proliferation is a hallmark of malignancy and may be assessed by a variety of methods, including counting mitotic figures in stained tissue sections, incorporation of labeled nucleotides into DNA, and flow cytometric evaluation of the fraction of the cells in S phase (1–3). The most widely practiced measurement involves the immunohistochemical (IHC) assessment of Ki67 antigen (also known as antigen identified by monoclonal antibody Ki-67 [MKI67].

Ki-67 is a nuclear antigen that is expressed in the growth and synthesis phases of the cell cycle, but not in the resting phase. Since its discovery in the early 1980s, Ki-67 has received a lot of attention as a proliferation marker in almost all types of cancers. The expression of Ki67 is strongly associated with tumor cell proliferation and growth, and is widely used in routine pathological investigation as a proliferation marker.

The nuclear protein Ki67 (pKi67) is an established prognostic and predictive indicator for the assessment of biopsies from patients with cancer. Clinically, pKi67 has been shown to correlate with metastasis and the clinical stage of tumors. In addition, it has been shown that Ki67 expression is significantly higher malignant tissues with poorly differentiated tumor cells, as compared with normal tissue. According to its predictive role, pKi67 expression identifies subpopulations of patients who are more likely to respond to a given therapy. The Ki67 protein is well characterized at the molecular level and is extensively used as a prognostic and predictive marker for cancer diagnosis and treatment.

In many cases, neoadjuvant chemotherapy reduces tumor size, which enables patients who were initially inoperable to undergo mastectomy and makes breast-conserving surgery possible in patients who otherwise would have required mastectomy. The outcome of neoadjuvant chemotherapy can be determined in a relatively short time, which makes this approach useful for deciding which drugs or regimens are effective for specific pathologic conditions. Moreover, this is a useful modality for investigating the efficacy of specific biologic markers as predictive and prognostic factors.

Neoadjuvant chemotherapy does not provide a survival advantage compared to postoperative adjuvant therapy. However, patients who achieve a pathologic complete response (pCR) have significantly improved disease-free survival and overall survival compared to those with residual cancer. The objectives of this study were to assess the potential value of Ki-67 in predicting the therapeutic response to neoadjuvant chemotherapy in locally advanced breast cancer patients.

V. Conclusion

From our study we concluded that

- KI-67 a proliferative marker can be used to assess the response of neoadjuvant chemotheraphy.

- Tumors with high index of Ki-67 respond significantly well to chemotheraphy and it can be used to assess the achievement of a pathological complete response.

Neoadjuvant chemotherapy reduces tumor size, which enables patients who were initially inoperable to undergo mastectomy and makes breast-conserving surgery possible in patients who otherwise would have required mastectomy.

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