ISSN No:-2456-2165

Ki-67 Index in Patients with Invasive Ductal Carcinoma (IDC) of Breast and an its Correlation with Other Prognostic Markers

Dr. Yashodha D.
DNB Resident,Department of Pathology,Bangalore Institute of Oncology,Bangalore,Karnataka, India
Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India

Prof. Dr. Diganta Hazarika
MD, Head of Department of Pathology, Bangalore Institute of Oncology, Bangalore, Karnataka, India
Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India

Abstract:- Surprising progress has been made in the biology of breast cancer in recent years. Breast cancer is the commonest cancer in women worldwide. In India it is the second most common female malignancy , accounting 28% of all malignancies in women. The age standardized rate is 54.9 per 1,00,000 per year. Biomarkers expression in breast cancer is used as a prognostic indicator and predictor of response to hormonal and chemotherapy. To date, the leading parameters that guide therapy in breast cancer are estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER2neu). In recent years, gene expression analysis studies have demonstrated the vitality of proliferation signatures not only in the prognosis of breast cancer but also as a predictive response to subsequent therapy ,(3,4,5). The aim of the current study is to analyse the correlation between various pathological prognostic markers of Invasive ductal carcinoma (IDC) of the breast with Ki67 index and provide a rough estimate of the cut-off values for the latter. The methodology used is formalin fixed paraffin embedded tissue blocks and slides (H&E histology and Immunohistochemistry) were retrieved and reviewed. Re-cutting and re-staining of faded material were carried out when needed. This is a two-year study from February 2015 to February 2017. Data were analysed using "SPSS Version 23." The outcomes of biomarker analysis showed that immunohistochemical expression of Ki67 appears to be associated with absence of ER, PR expression and Her2neu positivity. These findings underlined the relationship between Ki67, a relatively new biological marker which is a valuable predictive factor in invasive ductal carcinomas of breast. index" and the (p =0.04), PR and Ki 67 (p=0.026). A slightly higher incidence of Her-2/neu positivity was seen, and there was a significant connection between "HER2-Neu" and "Ki-67" index and the (p =0.029). The molecular groups and Ki67 have no discernible relationship.

Keywords: *Ki-67, Prognostic Markers, Invasive Ductal Carcinoma, Breast Cancer*

I. INTRODUCTION

Normally, cells grow in a controlled and coordinated way. However, occasionally, due to external causes, cells grow uncontrollably. Additionally, this unchecked expansion results in tumour formation (Aroef et al., 2020). Both benign and malignant tumours are possible. While malignant ones not only create cancer but also metastasis to other areas of the body, which results in death, benign ones do not do so. Cell cycle and regulatory pathway disruptions are to blame for all of this. The cause may be due to a genetic mutation, a functional aberration in the cell cycle, the impact of environmental factors, or ageing (Ercisli et al., 2021). There are many distinct varieties of cancer that affect various body areas, and the majority of them are classified based on where they first appeared.

The second-most common kind of female cancer in India is cancer of the breast, which accounts for 28% of all malignancies in women (Johnson et al., 2018). As per the data from the Indian "Population-Based Cancer Registry (PBCR)," cancer of the breast accounts for 22.1% of all cancer instances in women and represents the most common cancer form among them (Harris et al., 2007). The tumour has the following characteristics: it is grey-white, firm, poorly defined, contracts from the surrounding tissue, has a hard cartilaginous substance, makes a grating sound when scraped, and calcified.

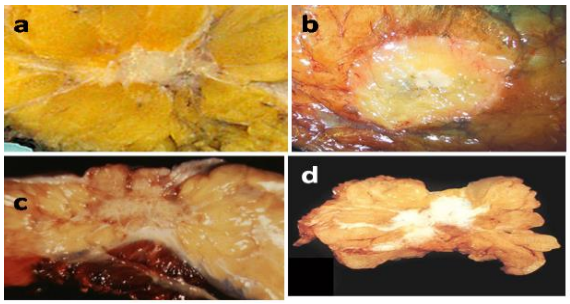

Fig 1 (a,b,c,d): A typical infiltrating ductal carcinoma with uneven boundaries and a radiating infiltration pattern into the surrounding fibro adipose tissue and pectoral fascia. The desmoplastic stromal response is represented by yellow striations within the tumor's core. The tumour has spread to the skin's surface, forming an ulcer (Pictures taken from the case studied).

ISSN No:-2456-2165

Ki-67 is a proliferation marker that is found in all proliferating cells and is of significant importance (Albarracin and Dhamne, 2014). Moreover, "Ki-67" is one of the 21 genes selected prospectively to anticipate the probability of recurrence and treatment intensity advantages for women with "node-negative, ER-positive" breast cancer (Siow et al., 2018). It is crucial to figure out how to administer chemotherapy to hormone-sensitive patients with "ER-positive" and "HER2-negative" tumours since they represent the vast majority of people with primary breast cancer are women. One alternative is to contemplate the "Ki67 index" while choosing a method of treatment. The study aims to investigate the connection between the "Ki-67 index" and other prognostic indicators in patients with cancer of the breast with "Invasive Ductal Carcinoma (IDC)."

II. METHODS AND MATERIALS

- *Site and Design of Study*

This study was conducted at the Triesta Reference Laboratory of Bangalore Institute of Oncology, Bangalore. This is a two-year study from February 2015 to February 2017 (prospective and retrospective study design).

For the prospective study, the mastectomies, lumpectomies specimens, and "formalin-fixed paraffin-embedded blocks" of patients with IDC of breast received at Triesta laboratory from February 2015 to February 2017 were integrated. On "formalin-fixed, paraffin-embedded tissue blocks," histopathological and immunohistochemical analyses were conducted.

- *Sample Size and Study Population*

The sample size is calculated using the correlation coefficient value r=0.23, with an alpha of 0.05 and 80% power, using the following formula for calculating sample size from the correlation coefficient. The correlation coefficient of tumour size with Ki67 expression was 0.23.

- *Methods*

In this investigation, all patients diagnosed with IDC of the breast who had been tested for Ki67 at this institute were included. Histopathological information was extracted from "Haematoxylin and Eosin (H&E) stained slides/formalin-fixed paraffin-embedded tissue blocks" of the study's participants. Case files or archived reports in the laboratory were accessed for other clinical information.

- *Interpretation of Ki67 Scoring and Staining:*

With the MIB1 antibody, cytoplasmic and occasionally membrane Ki67 staining might occur, particularly in breast cancers with squamous metaplastic alterations. In an otherwise homogeneously stained sample, hot spots, defined as regions where Ki67 staining is especially frequent, may exist.

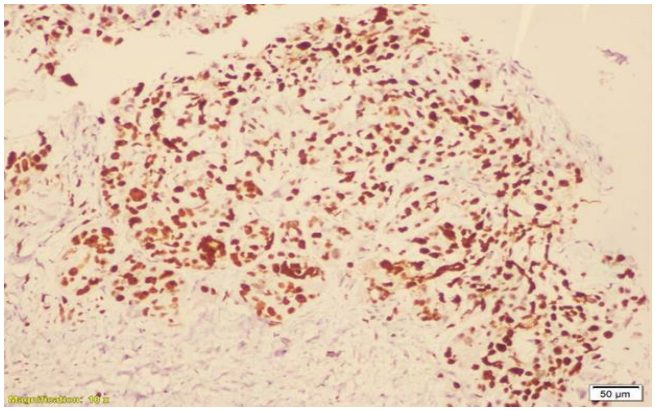

Fig 2 Invasive Edge Showing the Hot Spots

- *Statistical Analysis:*

SPSS (statistical software for social science) VERSION 23 for Windows was used to analyse the data.

III. RESULT & DISCUSSION

In this investigation, the "Ki-67 index" was used as a predictive and prognostic marker in patients with cancer of the breast in order to assess the clinical importance of proliferative activity. This study investigates the relevance, clinical, of the "Ki-67 index" as a forecaster of IDC of the breast in 147 patients from our institution. Additionally, the associations between the clinicopathological variables that represent prognosis and the "Ki-67 index" were assessed.

The 147 patients with IDC of the breast who fulfilled the requirements for inclusion were considered for the study. This includes 46 cases of lumpectomy specimens and 101 cases of mastectomy specimens. The connection between the "Ki 67 index" and several pathological prognostic markers was examined, such as pathological stage and biomarker expressions like ER, PR, and Her2/neu.

In this study, Indian women's IDC findings using Ki 67 were immunohistochemically stained. The full set of slides was inspected under a light microscope to assess the immunostaining.

Whereas the other predictive pathological indicators were associated with the Ki 67 index of patients with IDC. Additionally, individuals IDC in this hospital were estimated to have an ideal Ki-67 cut-off index.

Table 1 Categorization of Ki 67

Group	Ki67 percentage%
Low	0 – 10% (< 10%)
Intermediate	11-20%
High	< 20%

A. Clinicopathological Features of Patients with IDC of Breast and its Correlation with Ki67 Expression:

- *Ki67 Expression:*

Ki-67 index value and its cut-off value: Of 147 cases studied, Ki67 values were categorised as high, intermediate, and low. Comparable studies include those by Munzone et

ISSN No:-2456-2165

al. (2012) and Haroon et al. (2013). Moreover, these tumours were categorised as "Luminal A," "Luminal B," and "Her2/neu" rich & "triple negative (basal-like)."

The study found the majority of 101 cases (68.71%) of these patients had a Ki 67 value > 20%, followed by 27 cases (18.37 %) having Ki 67 value of 11%-20% and 19 cases (12.93%) having a Ki 67 value < 10% (see table 2).

Table 2 Frequency and Percentage-Wise Distribution of Cases According to their Ki-67 Positivity:

Ki-67 positivity	Frequency	Percent %
Low grade (<10%)	19	12.93
Intermediate (11-20%)	27	18.37
High grade (>20%)	101	68.71
Total	147	100.0

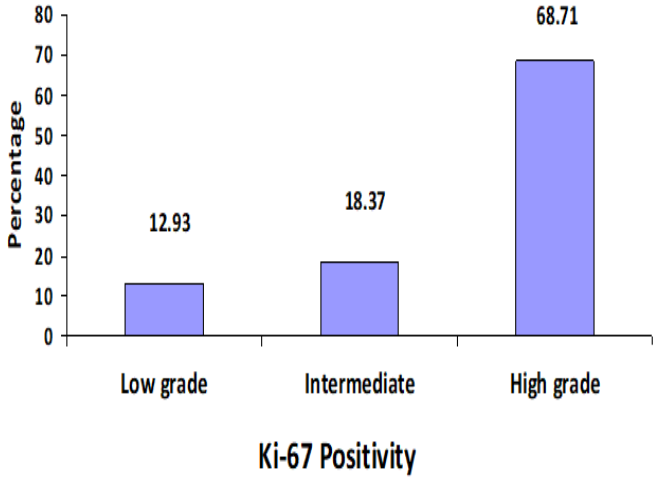

Fig 3 Frequency and Percentage-Wise Distribution of Cases According to their Ki-67 Positivity

➢ *ER:*

Of 147 cases, 82 cases were ER Negative. ER, +ve tumors had significantly lesser positivity of high-grade Ki67 (Ki 67> 20%) compared to ER-ve tumors (58%vs76%). The correlation between "ER" and "Ki-67 index" was substantial (p = 0.04).

Table 3 Association between ER and Ki-67

ER	Ki-67 positivity						X^2-value p-value
	Low grade (<10%)		Intermediate (11-20%)		High grade (>20%)		
	F	%	f	%	f	%	
Positive (n=65)	10	10.98	17	26.15	38	58.46	6.17 0.04*
Negative (n=82)	9	10.98	10	12.20	63	76.83	
Total	19	12.93	27	18.37	101	68.71	

*-highly significant

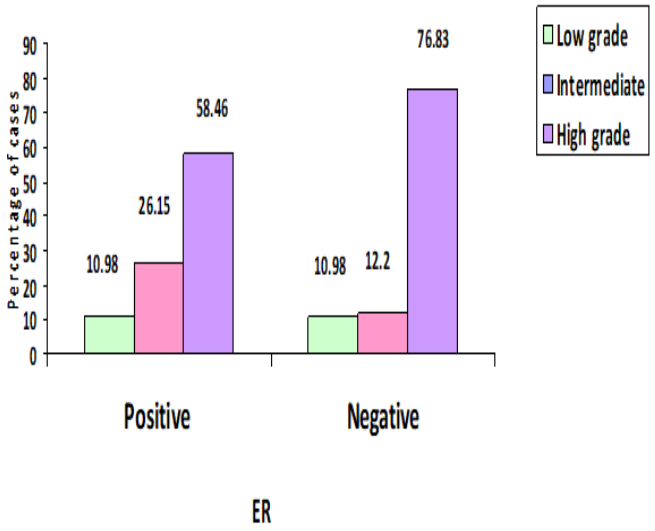

Fig 4 Association between ER and Ki-67

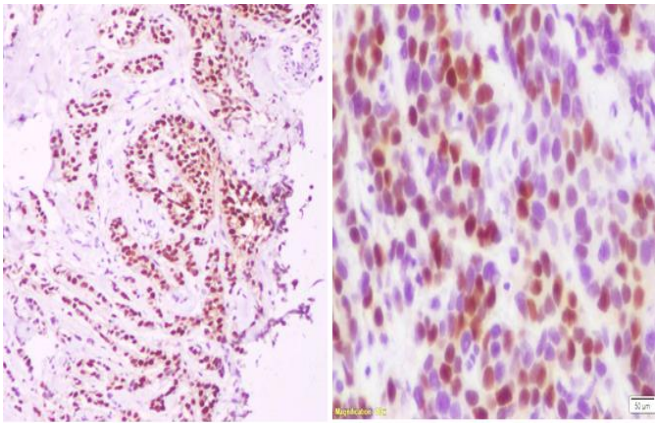

Fig 5 ER Positivity

➢ *PR*

Of 147 cases, 83 cases were PR negative. PR +ve tumors had significantly higher positivity of high-grade Ki67 (Ki 67> 20%) compared to PR-ve tumors (79.68%vs60.24%). There was a significant association between PR and Ki-67 index "(p =0.026)" (Table 4).

Table 4 Relationship between Ki-67 and PR

PR	Ki-67 positivity						X^2-value p-value
	Low grade (<10%)		Intermediate (11-20%)		High grade (>20%)		
	F	%	f	%	f	%	
Positive (n= 64)	7	11.29	6	9.68	51	79.68	7.33 0.026
Negative (n= 83)	12	14.46	21	25.30	50	60.24	
Total	19	12.93	27	18.37	101	68.71	

*-Highly Significant

ISSN No:-2456-2165

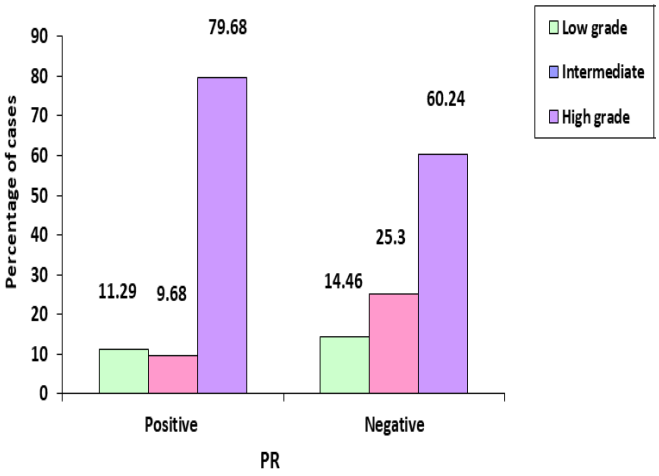

Fig 6 Association between PR and Ki-67

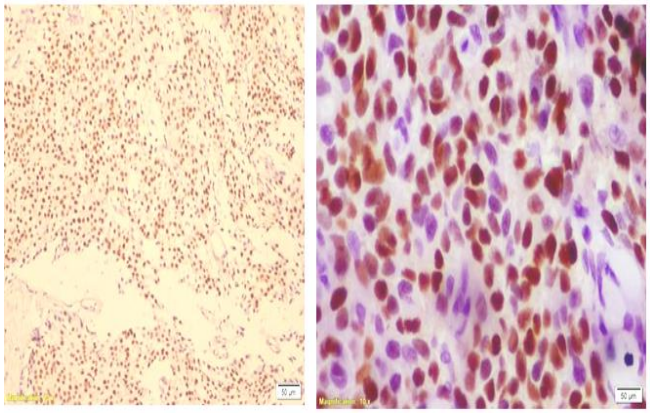
Fig 7 PR Positivity

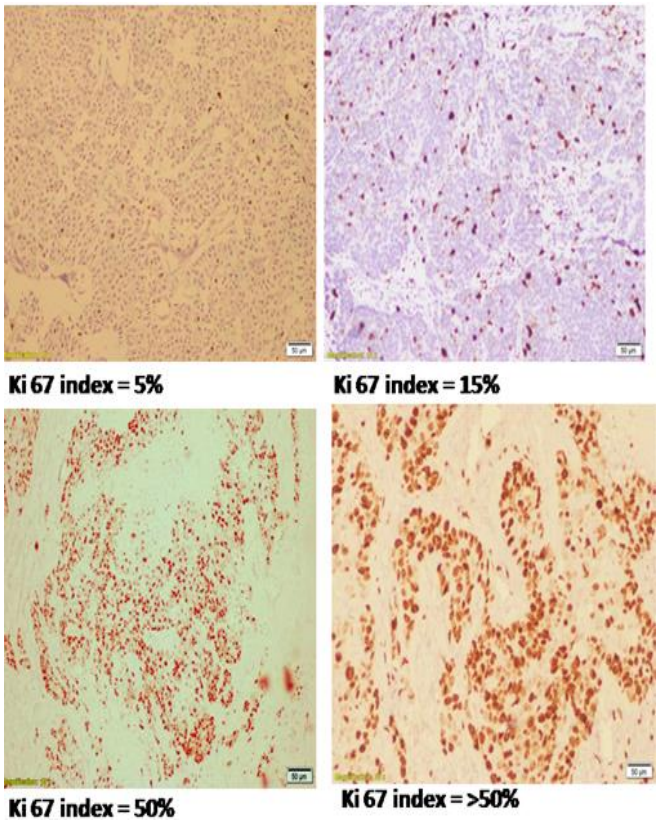

Fig 8 Ki 67 Index in Different Percentage

➢ *Her2 / Neu:*

Of 147 cases, 85 cases were Her2/Neu negative. Her2/Neu +ve tumors had significantly lesser positivity of high-grade Ki67 (Ki 67> 20%) compared to Her2/Neu -ve tumors (72.58%vs 65.88%). There was a significant association between ER and Ki-67 index and the "(p =0.029)" (Table 5).

Table 5 Relationship between HER2 & Ki-67

HER2	Ki-67 positivity						X^2-value p-value
	Low grade (<10%)		Intermediate (11-20%)		High grade (>20%)		
	f	%	f	%	f	%	
Positive (n=62)	3	4.84	14	22.58	45	72.58	6.650.029*
Negative (n=85)	16	18.82	13	15.29	56	65.88	
Total	19	12.93	27	18.37	101	68.71	

*-Significant

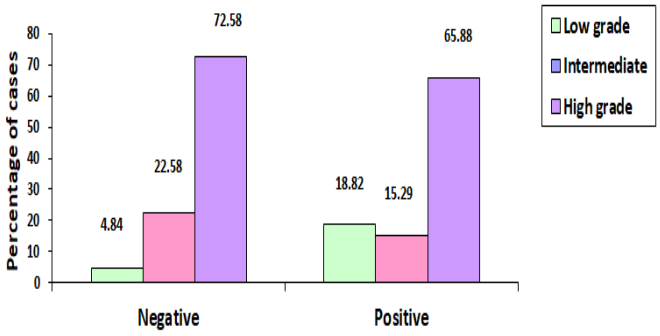

Fig 9 Association between HER2 and Ki-67

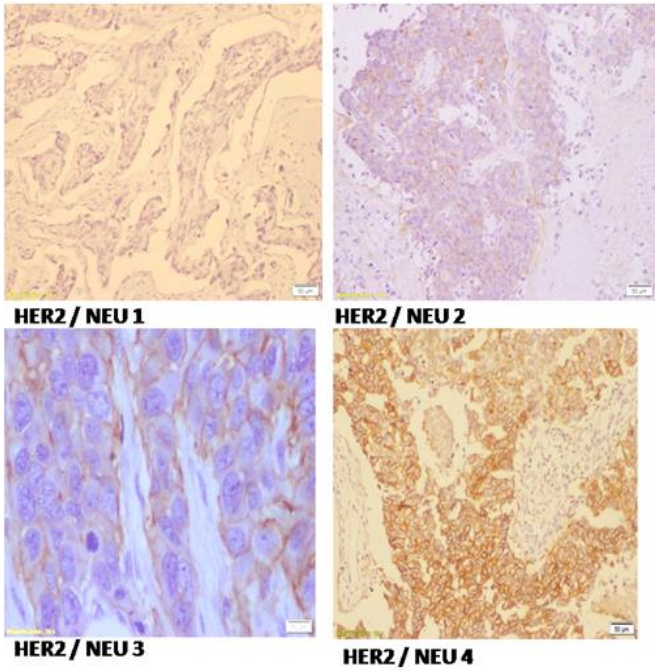

Fig 10 HER2/NEU Stages

ISSN No:-2456-2165

➢ *Molecular Classification:*

The 2013 "St. Gallen Consensus" established a fresh threshold of 20%. (17). Randomized prospective studies verify that a Ki-67 cut-off value of 20% is optimal. This study explored the role of Ki 67 value with the other immunohistochemical markers PR /ER/Her-2/neu positivity and categorised into groups (Table 5).

Table 5 Molecular Groups

Group 1:	**ER/PR+,Her2- = ER+/PR+,Her2-; ER-/PR+,Her2-; ER+/PR-,Her2-**
G 2:	ER/PR+,Her2+ = ER+/PR+,Her2+; ER-/PR+,Her2+; ER+/PR-,Her2+
G 3:	ER/PR-,Her2+ = ER-/PR-,Her2+
G 4:	ER/PR-,Her2- = ER-/PR-,Her2-

Of 147 cases studied, the majority was group 1 with 44 cases, followed by group 2 cases with 38 cases. There was no significant association between the molecular groups and Ki67 (Table 6).

Table 6 Association between Molecular Groups and Ki-67.

Molecular Groups	**Ki-67 positivity**						**X^2-value p-value**
	Low grade (<10%)		**Intermediate (11-20%)**		**High grade (>20%)**		
	f	%	f	%	f	%	
Group-1 (n=68)	13	19.12	11	16.18	44	64.71	7.52
Group-2 (n=52)	3	5.77	11	21.15	38	73.08	0.271
Group-3 (n=10)	0	0	3	30.0	7	70	
Group-4 (n=17)	3	17.65	2	11.76	12	70.59	
Total	19	12.93	27	18.37	101	68.71	

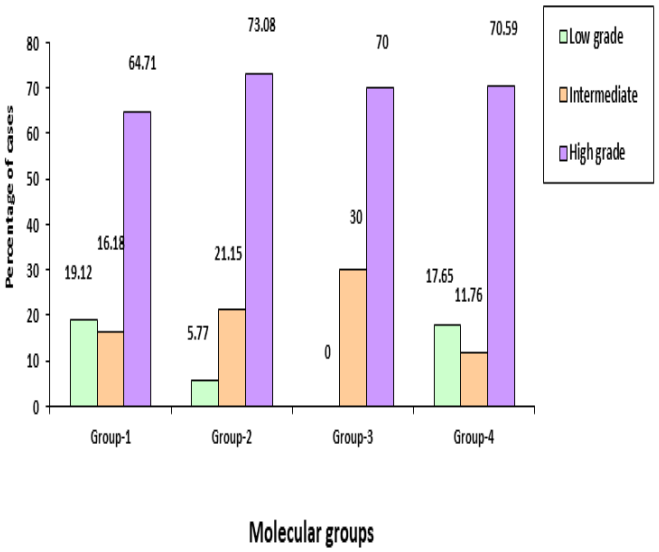

Fig 11 Association between Molecular Groups and Ki-67

The recognised 2011 "St. Gallen Ki 67" cut-off value of 14% to distinguish "luminal subtypes" (58).

Table 7 Molecular Classification

Molecular Groups	**"ER/PR/HER2/Ne"**	**"Ki 67 index"**	**Molecular subtypes**
Group-1	ER+ and/or PR+, HER2-,	Low Ki67 (<14%)	Luminal A(n=19)
G-2	ER+ and/or PR+, HER2+	HighKi 67 (=/>14 %)	Luminal B(n=128)
G-3	ER-,PR-,HER2+		HER2 rich type
G-4	ER-,PR-,HER2-		Basal-like(Triple negative).

The fraction of "Ki67-positive" cancer cells seems to be closely connected to the aggressiveness of breast cancer. Consequently, the same reality is reflected in the results. We noticed that immunohistochemistry Ki67 expression appeared to be related to the omission of "ER," "PR," and "Her2neu" expression. These results demonstrated that Ki67, a relatively new biological marker, is a valuable prognostic factor for IDC of the breast (Albarracin and Dhamne, 2014).

A substantially stronger relationship between the "ER" and "Ki-67 index" was discovered in the analyses. and the (p =0.006). ER, +ve tumors had significantly lesser positivity of high-grade Ki67 (Ki 67> 20%) compared to ER-ve tumors.

The same was seen with PR, and the Ki-67 index in 83 cases was PR negative, and PR +ve tumors had significantly higher positivity of high-grade Ki67 (Ki 67> 20%) compared to PR-ve tumors.

"HER2/neu" was significantly associated with the "Ki-67 index" and the (p =0.036).Her2/Neu +ve tumors had significantly lesser positivity of high-grade Ki67 (Ki 67> 20%) compared to Her2/Neu -ve tumors. This is in accordance with other studies which have demonstrated the same (Haroon et al., 2013 and Alco et al., 2015).

Molecular subtypes: This study reaffirmed that breast cancer is a complex illness with various biological subtypes and varied natural histories that display a wide range of pathologic, clinical, and molecular characteristics with varying prognostic and therapeutic consequences.[194] The findings show that pathologic, clinical, and outcome characteristics varied significantly between subtypes. Of 147 cases studied, 44 cases were of group 1 type, followed by the group 4 subtype, 38 cases. There was no significant association between these four groups and the Ki67 index.

ISSN No:-2456-2165

IV. CONCLUSION

- The conclusions were drawn from a two-year study (prospective and retrospective) on a sample size of 147 cases diagnosed with invasive ductal carcinoma who underwent Ki 67 index analysis and its correlation with other pathological prognostic markers Biomarker analysis showed the following results:
- A significantly higher proportion of ER & PR negativity was seen and there was a significant associationbetween ER and Ki-67 index and the (p =0.04), PR and Ki 67 (p=0.026).
- A slightly higher incidence of Her-2/neu positivity was seen, and there was a significant associationbetween HER2-Neu and Ki-67 index and the (p =0.029).
- There was no significant association between the molecular groups and Ki67. Of 147 cases studied, the majority was the group 1 with 44 cases followed by the group 2 cases with 38 cases.
- In this study we also categorised invasive ductal carcinoma patients depending on with Ki 67cut off value of 14% in to Luminal A and Luminal B and the majority 128 cases were of Luminal B subtype.
- The association between the poor prognostic factors and the high Ki-67 index was revealed in the study .Ki 67 index value is the prognostic pathological marker in patients with Inavasive ductal carcinoma.

REFERENCES

[1]. Albarracin, C., & Dhamne, S. (2014). Evolving role of Ki67 as a predictive and prognostic marker in breast cancer. *J Clin Exp Pathol*, *4*, e117.

[2]. Alco, G. U. L., Bozdogan, A., Selamoglu, D., Pilancı, K. N., Tuzlalı, S., Ordu, C., ... & Ozmen, V. (2015). Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. *Oncology letters*, *9*(3), 1046-1054.

[3]. Aroef, C., Rivan, Y., & Rustam, Z. (2020). Comparing random forest and support vector machines for breast cancer classification. *TELKOMNIKA (Telecommunication Computing Electronics and Control)*, *18*(2), 815-821.

[4]. Ercisli, M. F., Kahrizi, D., & Aziziaram, Z. (2021). Environmental factors affecting the risk of breast cancer and the modulating role of vitamin D on this malignancy. *Central Asian Journal of Environmental Science and Technology Innovation*, *2*(4), 175-183.

[5]. Haroon, S., Hashmi, A. A., Khurshid, A., Kanpurwala, M. A., Mujtuba, S., Malik, B., & Faridi, N. (2013). Ki67 index in breast cancer: correlation with other prognostic markers and potential in pakistani patients. *Asian pacific journal of cancer prevention*, *14*(7), 4353-4358.

[6]. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007 Nov 20;25(33):5287–312.

[7]. Johnson, R. H., Anders, C. K., Litton, J. K., Ruddy, K. J., & Bleyer, A. (2018). Breast cancer in adolescents and young adults. *Pediatric blood & cancer*, *65*(12), e27397.

[8]. Munzone, E., Botteri, E., Sciandivasci, A., Curigliano, G., Nole, F., Mastropasqua, M., ... & Viale, G. (2012). Prognostic value of Ki-67 labeling index in patients with node-negative, triple-negative breast cancer. *Breast cancer research and treatment*, *134*, 277-282.

[9]. Siow, Z. R., De Boer, R. H., Lindeman, G. J., & Mann, G. B. (2018). Spotlight on the utility of the Oncotype DX® breast cancer assay. *International journal of women's health*, 89-100.