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ASSESSMENT OF TUMOUR PROLIFERATION BY USE OF THE MITOTIC ACTIVITY INDEX, Ki67, AND PHOSPHOHISTONE H3 EXPRESSION IN INFILTRATING DUCTAL CARCINOMA OF BREAST

Oncopathology

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ABSTRACT

Introduction: Infiltrating ductal carcinoma (IDC) of the breast is the most common subtype of breast cancer, necessitating precise prognostic markers. Phosphohistone H3 (PHH3) is a novel marker of mitotic activity that may offer superior prognostic information compared to traditional markers like Ki67 and the mitotic activity index (MAI). Despite the widespread use of proliferation markers like Ki67 and the Mitotic Activity Index (MAI) in breast cancer prognosis, their low reproducibility and subjective interpretation limit their usefullness. Phosphohistone H3 (PHH3) has shown promise as a more reliable marker due to its specificity for mitotic cells, yet its relationship with traditional markers like Ki67 in breast cancer remains underexplored. This study is needed to clarify the potential of PHH3 in improving prognostic accuracy, particularly in infiltrating ductal carcinoma, and to establish its role in enhancing breast cancer grading and guiding personalized treatment decisions. **Aims:** To assess PHH3 and Ki67 expression in infiltrating ductal carcinoma of the breast and correlate it with mitotic activity index. Additionally, to investigate their association with histological grade and TNM stage of the carcinoma. **Objectives:** The study aims to evaluate PHH3 expression in IDC and compare its effectiveness with Ki67 and MAI in correlating with histological grade and tumor staging. Specifically, it seeks to:
1. To assess PHH3 expression levels in IDC.
2. To correlate PHH3, Ki67, and MAI with histological grade and TNM stage of IDC.
Results: PHH3 expression was observed in 40.3% of cases and showed a stronger correlation with MAI compared to Ki67. High PHH3 expression was more consistently associated with higher histological grades and advanced tumor stages, demonstrating its superior prognostic value over Ki67 and MAI. **Conclusions:** PHH3 is a superior biomarker for assessing proliferation in IDC compared to Ki67 and MAI. Its stronger correlation with histological grade and tumor staging underscores its potential to enhance prognostic accuracy and guide treatment decisions, leading to improved patient outcomes.

KEYWORDS

INTRODUCTION

The heterogeneity of breast cancer poses a challenge in predicting its malignant behavior, distinguishing it from other types of human malignancies.[1] Therefore, the evaluation of proliferation is essential for categorizing and forecasting the basic characteristics of breast tumors. The two most commonly accepted techniques for quantifying cell proliferation are Ki67 and the mitotic activity index (MAI). MAI, which is included in standard breast cancer pathology reports as part of histologic grading, is regarded as the most critical factor for predicting prognosis.[2] Ki67 is frequently used in laboratories to distinguish tumors that have a high likelihood of recurrence, necessitating further chemotherapy, and is a factor in the classification of breast cancer subtypes.[3,4] Nevertheless, despite their crucial functions, both Ki67 and MAI demonstrate significant limitations in terms of reproducibility, which present diagnostic difficulties.

Traditionally, MC can be expressed as either Mitotic Index (MI) or Mitotic Activity Index (MAI). MI is commonly described as the proportion of cells undergoing mitosis compared to cells that are not undergoing mitosis, regardless of the phase of the cell cycle, in a specific location of a tumor. It is typically expressed as a percentage or as the number of mitotic events per 1000 neoplastic cells.[5] MAI, or Mitotic Activity Index, is a measurement of the number of mitotic figures inside a specific region of a tumor. It is expressed as an index, calculated by dividing the number of mitotic figures by the area that was enumerated.[5-7]

Nevertheless, even with the use of a uniform counting procedure, there continues to be a prevalence of low concordance rates. Interpretative schemes show that there are low rates of agreement for MC, especially in tumors with high scores, highlighting the difficulties in accurately assessing them.[8] In ordinary practice, there are often substantial discordance rates when it comes to characterizing mitotic forms on hematoxylin and eosin (H&E)-stained slides, as this process is subjective.[6,9-12] Standardization efforts have been made, but challenges persist, including defining the optimal area size for MC assessment and adapting criteria for digitized whole slide images(WSI).[13-16] Modifications in criteria and incorporation of defined mitotic figure counts into histological grading are needed to address these challenges effectively.

Ki67, which is especially challenging in diverse tumors such as breast cancer, causes discrepancies in the choice of field for evaluation. In addition, the interpretation of Ki67 as positive can vary widely based on the subjective criteria of individual raters, leading to a lack of consistency in the results.[17] Another significant drawback of Ki67 is its limited ability to accurately depict proliferation, as it is present in all active stages of the cell cycle (G1, S, and G2 phases).

Several studies have shown the predictive significance of Ki67. Nevertheless, the practical usefulness of this marker has been questioned due to its limited ability to be replicated accurately. The flaws in Ki67 assessment can be related to the absence of a consensus among specialists regarding the score and the lack of a clearly defined cut-off point for making clinical decisions.[18]

Histone H3 is a crucial protein found in the nucleus that forms the core of DNA chromatin. It plays a significant role in the condensation of chromosomes and the advancement of the cell cycle during mitosis and meiosis. This occurs after the phosphorylation of specific serine residues, namely serine-10 and serine-28.[19] Phosphorylation takes place from the late G2 phase to the early prophase, whereas dephosphorylation proceeds gradually from late anaphase to early telophase. FourDuring metaphase, histone H3 is consistently phosphorylated and shows a positive result for PHH3. In contrast, interphase cells either do not produce PHH3 or express it to a lesser extent. This feature enables PHH3 to specifically stain cells that are actively undergoing mitosis, making it a marker for cell proliferation.[19]

The utilization of PHH3 IHC stain analysis greatly improves consensus among observers when calculating the mitotic rate and enables a quick and unbiased determination of the number of mitotic events.[20] It helps differentiate between apoptotic cell debris and mitoses and improves the chances of identifying mitotic cells with abnormal morphologies, such as metaphase or anaphase, which may not be detected by H&E staining alone. Nevertheless, studies have demonstrated that PHH3 analysis can elevate the mean mitotic rate by 86-200%.[21-25] The immunohistochemical (IHC) staining technique for PHH3 has been suggested as a possible substitute indicator for mitotic count. It has been examined in several types of tumors such as melanoma, meningioma, pulmonary carcinoid, and well-differentiated neuroendocrine tumors of the pancreas.[26,27] Furthermore, the examination of PHH3 requires less time and is more

straightforward to interpret compared to the standard method of mitotic counting using H&E stain.

However, the connection between MAI, PHH3 and KI67 in infiltrating ductal carcinoma of the breast is not well documented in India. Hence, this study aims to investigate the relationship between the Mitotic activity index, PHH3, and Ki67 in infiltrating ductal carcinoma of the breast.

AIMS

To assess PHH3 and Ki67 expression in infiltrating ductal carcinoma of the breast and correlate it with mitotic activity index. Additionally, to investigate their association with histological grade and staging of the carcinoma.

OBJECTIVES

- To determine the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast.
- To correlate mitotic activity index with the expression of PHH3 and Ki67 in infiltrating ductal carcinoma breast.
- To study the association between mitotic activity index, Ki67 and phosphohistone H3 expression with Histological grade and staging of ductal carcinoma breast.

MATERIAL AND METHODS

This study is a cross-sectional observational analysis conducted in the Department of Pathology from July 2019 to June 2023. The study population consists of all histologically confirmed cases of invasive ductal carcinoma from the medical college's pathology department. Patients eligible for inclusion were those diagnosed with infiltrating ductal carcinoma of the breast. Excluded from the study were individuals who had undergone neoadjuvant radiotherapy or chemotherapy before surgical excision of the tumor, as well as those who had received chemotherapy for other cancers within the past five years.

The primary tools utilized in the study are immunohistochemical staining methods for PHH3 and Ki67, applied to evaluate the tumor characteristics.

SAMPLE SIZE

The sample size for this cross-sectional study is **102 cases of IDC Breast**

METHODOLOGY:

GRADING OF IHC:[28]

Evaluation of slides was done by two pathologist. This dual review process was implemented to ensure reliable assessment of the histopathological features and findings.

Grading of IHC was done by calculating the **filed diameter as per the WHO criteria to calculate mitotic figures**

FOR MAI: MAI was categorized on the basis of the total number of mitotic figures in an area of 0.196 mm^2, as follows: 0– 7 mitotic count = Score 1, 8–14 mitotic count = Score 2, ≥15 mitotic count = Score 3.

FOR PHH3: Similar to MAI

FOR KI67:

1- A cut-off value of 14% of nuclei positively stained for Ki67 was used for Molecular classification

2- According to the International Ki67 Working Group (IKWG), a Ki67 score of less than 5% or greater than 30% is more robust for estimating prognosis in patients with stage I or II breast cancer that is ER+HER2−. Patients are categorized into low (≤ 5%), intermediate (6-29%), and high (≥30%) based on IKWG recommendations[29-31]

TILS[32,33]

TILS scoring was done according to International TILS Working Group as <10%=Low TILS, 10-40%=Intermediate TILS and >40% High TILS

T:S RATIO[34]

Tumor stromal ratio was divided into in two categories as stroma-low tumor having <50% stroma and >50%stroma is considered as stroma-high

The method that was folllowed for T:S Ratio was that initially using a 4x objective the most invasive tumor area of the whole slide was selected, subsequently, using 10x objective, only fields were scored where both stroma and tumor were present and, most importantly tumor cells were seen on all sides of the microscopic filed.

Statistical Analysis:

The data was analyzed using SPSS version 22 and Microsoft Excel. Categorical data was summarized with frequencies and proportions, and statistical significance was assessed using the Chi-square test or Fischer's exact test for 2x2 tables. Pearson correlation was used for correlation analysis. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

The patient cohort primarily comprised women aged 50-59 years (32.35%), with a mean age of 51.46 years. Tumor laterality showed a slight predominance on the left side (53.92%), and 62.7% of tumors were less than 5 cm in size. Tumor staging revealed that 52% of tumors were classified as T2 (2-5 cm), with T3 tumors (=< 5 cm) in 29.4%, T1 tumors (=< 2 cm) in 10.8%, and T4 tumors (extending to chest wall or skin) in 8.8%. Regarding nodal involvement, 46.1% of cases were N0 (no regional lymph node involvement), while 53.9% had nodal involvement (N1-N3), with N1 (1-3 axillary lymph nodes) being the most common. The study found 53.92% of cases had positive lymph node metastases. Tumor-infiltrating lymphocytes (TILs) were low (<10%) in 66.7% of cases, moderate (10-40%) in 25.5%, and high (>40%) in 7.8%. The tumor-to-stroma ratio was greater than 50 in 72.5% of cases. Mitotic activity, assessed using PHH3 staining, showed that 82.4% of cases had a high mitotic score (score 3), compared to 50% with H&E staining. This increase in high-grade cases with PHH3 reflects its sensitivity in detecting mitotic activity. In the modified Bloom-Richardson (mBR) grading, PHH3 identified 14.7% of cases as Grade 1, 67.6% as Grade 2, and 17.6% as Grade 3, compared to H&E's 31.4% Grade 1, 60.8% Grade 2, and 7.8% Grade 3. Lymphovascular invasion (LVI) was present in 34.3% of cases, indicating a higher likelihood of metastasis. The Nottingham Prognostic Index (NPI) categorized 42.2% of cases with moderate prognosis, 25.5% with good prognosis, 19.6% with poor prognosis, and 12.7% with excellent prognosis. The Ki67 proliferation index revealed intermediate levels (6-29%) in 71.6% of cases, with high levels (>30%) found in 13.7%, correlating with more aggressive tumor behavior and significant associations with lymph node involvement and stage.

Table 1 : Distribution Of Cases Based On MAI(mitotic count – H&E) And PHH3

MITOTIC COUNT Score	MITOTIC COUNT score - H&E	MITOTIC COUNT score – PHH3
1	25	7
2	51	11
3	26	84

Comparison of Mitotic Count Scores (H&E) with Mitotic Count Scores (PHH3)

Table 2: Distribution Of Mitotic Count Scores (H&E) by Mitotic Count Scores (PHH3):

MITOTIC COUNT score-H&E	MITOTIC COUNT score- PHH3					
	1		2		3	
	N	%	N	%	N	%
1	7	28.0%	10	40.0%	8	32.0%
2	0	.0%	1	2.0%	50	98.0%
3	0	.0%	0	.0%	26	100.0%

Comparison of mBR GRADE (PHH3) with mBR GRADE (MAI)

Table 3: Comparison Of mBR GRADE (PHH3) With mBR GRADE (MAI)

mBR Grade H&E/ MAI	mBR Grade- PHH3					
	1		2		3	
	N	%	N	%	N	%
1	13	40.6%	18	56.3%	1	3.1%
2	2	3.2%	51	82.3%	9	14.5%
3	0	.0%	0	.0%	8	100.0%

The comparison of Mitotic Count Scores (H&E) with Mitotic Count Scores (PHH3) reveals significant upgrading trends, with 10 cases moving from score 1 to 2, 50 cases from score 2 to 3, and 8 cases from score 1 to 3, with no downgrades observed. Similarly, the comparison of mBR Grade (PHH3) with mBR Grade (MAI) shows considerable

upgrading, particularly from Grade 1 to 2 in 18 cases and Grade 2 to 3 in 9 cases, while downgrades was seen only in 2 cases.

Comparison of MAI with Ki67 Scores

Table 4: Comparison Of MAI With Ki67 Score

MAI	Ki67 SCORE					
	1		2		3	
	N	%	N	%	N	%
1	5	20.0%	7	28.0%	13	52.0%
2	8	15.7%	11	21.6%	32	62.7%
3	2	7.7%	3	11.5%	21	80.8%

Upgrade	N	%
score 1 to score 2	7	28
score 2 to score 3	32	62.7
score 1 to score 3	13	52
Downgrade		
score 2 to score 1	8	15.7
score 3 to score 2	2	7.7
score 3 to score 1	3	11.5

Comparison of Mitotic Count Scores (PHH3) with Ki67 Scores

Analysis of MAI with Ki67 Scores also indicates a notable upgrade from score 2 to 3 seen in 32 cases and score 1 to 3 seen in 13 cases, though some downgrades were present, particularly from score 2 to 1 (15.7%).

Table 5: Mitotic Count Scores (PHH3) with Ki67 Scores:

Mitotic count score-PHH3	Ki67 SCORE					
	1		2		3	
	N	%	N	%	N	%
1	2	28.6%	1	14.3%	4	57.1%
2	1	9.1%	7	63.6%	3	27.3%
3	12	14.3%	13	15.5%	59	70.2%

Table 6 : Upgrade And Downgrade Rates Between Ki67 Scores And PHH3

Upgrade	N	%
Score 1 to Score 2	1	14.3
Score 2 to Score e 3	3	27.3
Score 1 to Score 3	4	57.1
Downgrade		
Score 2 to Score 1	1	9.1
Score 3 to Score 2	13	15.5
Score 3 to Score 1	12	14.3

Finally, comparing Mitotic Count Scores (PHH3) with Ki67 Scores shows that most cases tend to upgrade, with 4 cases moving from score 1 to 3 and 3 cases from score 2 to 3, although there were downgrades, especially from score 3 to 2 seen in 13 cases and score 3 to 1 seen in 12 cases. (Table 2-6)

DISCUSSION

Breast cancer is the leading cause of cancer in women around the world, accounting for one-quarter of all female cancers. Breast cancer deaths in the Southeast Asia region are expected to increase to 61.7% by 2040.[35] Breast cancer is the most common cancer in India, accounting for 28.2% of all female cancers, with an estimated 216,108 cases by 2022.[36]

Early diagnosis of breast cancer is an important factor for the reduction of the mortality rate because its treatment plan is advised on the basis of the grade and prognosis of the cancer. To determine the grade of breast cancer, the Modified Bloom Richardson grading system has been widely used. According to this system, there are three biomarkers for the grading of breast cancer in histopathology images. These biomarkers are nuclear atypia, tubule formation, and the mitotic cell count. Among these biomarkers, the mitotic cell count is the most important biomarker as the mitosis cell division process is directly related to the prognosis of tumor. [37,38]

The mitotic score is a key component of breast cancer (BC) grading and is a strong predictor of survival, reflecting the underlying biological behavior of the disease, but because of the poor reproducibility of mitotic count is mainly attributed to the presence of mitotic mimickers such as hyperchromatic nuclei, karyorrhectic or apoptotic cells, even cells in prophase are usually not considered during routine scoring of mitotic figures. Additionally, the heterogeneity of mitotic activity in different regions, and cell density variations, might all be aggravating factors. Such factors can be avoided by use of IHC marker[19]

This study was done to assess the expression and association of PHH3, Ki67, and MAI in infiltrating ductal carcinoma breast with various parameters.

The present study shows notable differences in mBR grades when compared to previous studies. A significantly higher percentage of mBR Grade I cases (31.4%) is reported, in contrast to Ghosh et al. (6.3%), Steenhoven et al. (16%), and Gogai et al. (16.96%). This suggests that the current cohort may consist of tumors with lower histological grades or that there are differences in grading methodologies or population characteristics. The percentage of mBR Grade 2 cases (60.8%) is consistent with Steenhoven et al. (67%) and Gogai et al. (59.82%), implying similar grading standards or patient demographics across these studies. However, the present study reports a significantly lower proportion of mBR 3 cases (7.8%) compared to Ghosh et al. (67.5%), Steenhoven et al. (27%), and Gogai et al. (23.21%). These differences may be due to population characteristics or subjective variation in the mitotic figure.

Table 7- Comparison Of The Different Studies With Respect To mBR Grade

STUDIES	YEAR	TOTAL CASES	mBR 1	mBR 2	mBR 3
Ghosh et al[39]	2014	320	6.3%	26.3%	67.5%
Steenhoven et al[40]	2020	159	16%	67%	27%
Gogai et al[41]	2015	112	16.96%	59.82%	23.21%
Present study- H & E	**2024**	**102**	31.4%	60.8%	7.8%

Comparison Of mBR Grade Changes Using PHH3 vs. MAI

In comparing the mBR Grade (PHH3) with the mBR Grade (MAI), the present study observed significant changes in grading when compared to Woo et al.'s findings. Specifically, in the current study, the majority of grade upgrades occurred from Grade 2 to Grade 3, with 50 cases (compared to Woo et al.'s 41 cases), and from Grade 1 to Grade 2, with 10 cases (compared to Woo et al.'s 53 cases). There were also upgrades from Grade 1 to Grade 3 in 8 cases (versus 7 cases in Woo et al.'s study). Conversely, downgrades were rare in the present study, with no cases observed for downgrades from Grade 2 to Grade 1 or from Grade 3 to Grade 2, while Woo et al. reported 2 and 4 cases, respectively, in these categories. This indicates a trend toward higher grading with PHH3 in the current study.

These findings indicate that PHH3 staining could be superior to traditional H&E-based mitotic activity index (MAI) in identifying higher-grade tumors with greater proliferative activity, potentially influencing clinical decision-making and prognostic assessments in breast cancer management.

Table 8: Comparison Of Ki67 With Various Studies According To IKWG

STUDIES	YEAR	CASES	<5%	6-29%	>30%
Shim et al.[42]	2024	307	8.8 - 16.0	61.5 - 79.8	11.4 - 22.5
Arora et al.[43]	2023	73	15.0%	28.8%	56.2%
Present study	**2024**	**102**	12.7	74.5	12.7

Ki67 is a well-known proliferation marker used to assess the growth fraction of tumors. In 2021, the Ki67 International Working Group (IKWG) highlighted the importance of Ki67 as a critical biomarker in breast cancer, issuing updated guidelines to standardize its visual assessment. These guidelines align with the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) standards for HER2 and hormone receptor testing, emphasizing the use of core needle biopsies to minimize fixation issues that can compromise test validity. The IKWG specifically identified Ki67 immunohistochemistry (IHC) cut points of ≤5% and ≥30% as clinically meaningful for patients with ER+HER2− stage I/II breast cancer. These cut points guide treatment decisions, with ≤5% suggesting that chemotherapy might be avoidable, and ≥30% indicating the need for chemotherapy. By providing these standardized protocols, the IKWG aims to reduce variability in Ki67 assessment, enhancing its reliability and clinical utility, and ultimately improving personalized treatment strategies for breast cancer patients.

The variation in Ki67 expression levels across these studies could be due to differences in patient populations, tumor characteristics, or methodological approaches to Ki67 staining and scoring. Despite

these differences, the findings underscore the importance of standardizing Ki67 assessment to improve its prognostic utility in breast cancer.

Table : Pearson Correlation Showing Correlation Between MAI, PHH3, and Ki67

		Mitotic count- PHH3	Ki67
Mitotic count- H&E	Pearson Correlation	.761**	.184
	p value	.000	.064

		Ki67
Mitotic count- H&E	Pearson Correlation	.184
	p value	.064
MITOTIC COUNT- PHH3	Pearson Correlation	.107
	p value	.283

Pearson correlation analysis was used to examine the relationships between mitotic counts (H&E), PHH3 expression, and Ki67 expression in the present study.

Mitotic Count (H&E) vs. PHH3: A strong positive correlation was found between the mitotic count (H&E) and PHH3 expression ($r = 0.761$, $p < 0.001$). This statistically significant correlation suggests that higher mitotic counts are closely associated with higher PHH3 expression, supporting the idea that PHH3 is a reliable marker for tumor proliferation.

Mitotic Count (H&E) vs. Ki67: The correlation between the mitotic count (H&E) and Ki67 expression was weak and not statistically significant ($r = 0.184$, $p = 0.064$). This indicates that there is no strong linear relationship between these two markers in this sample, which may reflect the broader range of cell cycle phases that Ki67 stains compared to the more specific mitotic focus of H&E and PHH3.

PHH3 vs. Ki67: The correlation between PHH3 and Ki67 expression was very weak and also not statistically significant ($r = 0.107$, $p = 0.283$). This further suggests that Ki67 and PHH3 may provide different insights into tumor proliferation, with PHH3 potentially being more specific for identifying active mitosis

CONCLUSION

The comparative study emphasizes the superiority of PHH3 staining over traditional H&E staining(MAI) in accurately assessing mitotic activity and MBR grades in IDC breast cancer cases. The high upgrade rates from H&E to PHH3 scores suggest that PHH3 is more sensitive in detecting higher mitotic counts, potentially leading to more precise grading and prognosis. Additionally, Ki67 expression showed a significant association with lymph node involvement, reinforcing its role as a prognostic marker. These findings advocate for the integration of PHH3 staining in routine diagnostic practices to enhance the accuracy of breast cancer grading and prognosis.

MICROSCOPIC

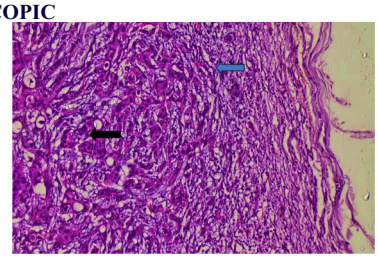

Figure 1: Microphotograph showing lymph node metastasis in IDC breast

Blue arrow- Shows the lymph node capsule and black arrow shows the tumor metastsis into the lymph node

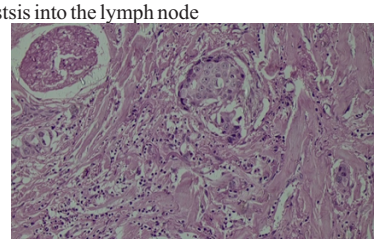

Figure 2: Microphotograph showing IDC breast with low TIILs (<10%), stain used is H&E (20x magnification)

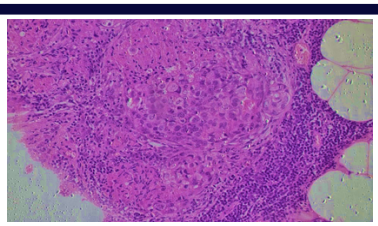

Figure 3: Microphotograph showing IDC breast with intermediate TIILs (10%-40%) (20x magnification)

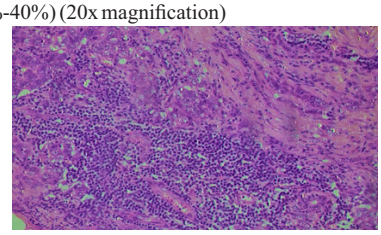

Figure 4: Microphotograph showing IDC breast with high TIILs (>40%) (20x magnification)

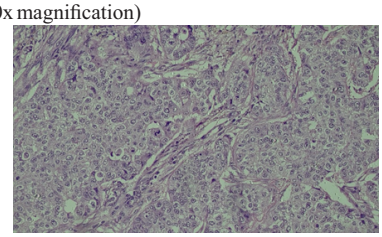

Figure 5: Microphotograph Showing IDC breast with Low T:S Ratio (<50%) under 20x magnification

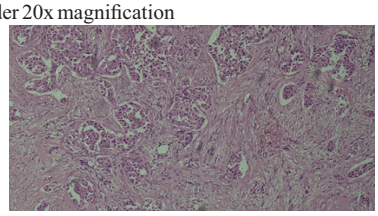

Figure 6: Microphotograph Showing IDC breast with Low T:S Ratio (>50%) under 10x magnification

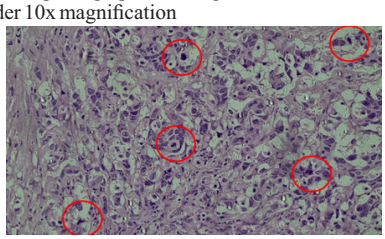

Figure 7: Microphotograph showing Mitotic figures stained with H and E stain (40x magnifiction)

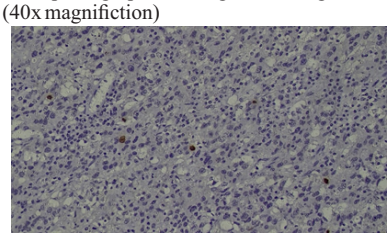

Figure 8: Microphotograph of IDC breast showingmitotic figures stained by PHH3 - Score 1 (<7 mitotic figures) under 40x magnification

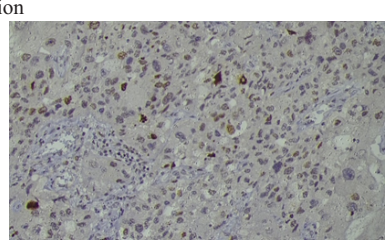

Figure 9: Microphotograph of IDC breast showing mitotic figures stained by PHH3 - Score 2 (8-14 mitotic figures) under 40x magnification

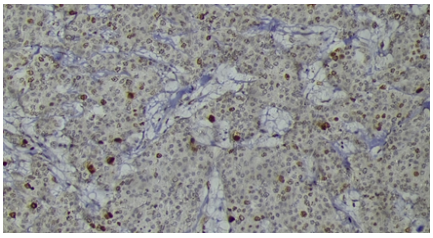

Figure 10: Microphotograph of IDC breast showing mitotic figures stained by PHH3 - Score 3 (>15 mitotic figures) under 40x

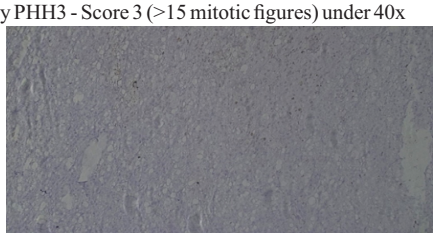

Figure 11: Microphotograph showing Ki67 expression of <5% in IDC breast (4x magnification)

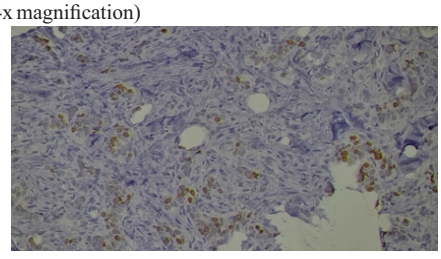

Figure 12: Microphotograph showing Ki67 expression of <6-29% in IDC breast (20x magnification)

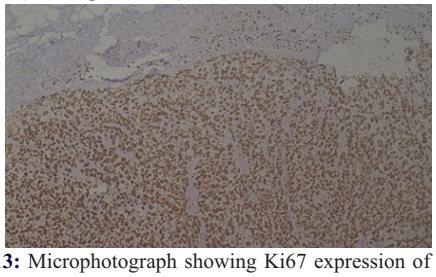

Figure 13: Microphotograph showing Ki67 expression of >30% in IDC breast (20x magnification)

REFERENCES:

1. Rosai and Ackerman's Surgical Pathology, 10e - 1st Edition | Elsevier 2011
2. Abdel-Fatah TM, Powe DG, Ball G, Lopez-Garcia MA, Habashy HO, Green AR, et al. Proposal for a modified grading system based on mitotic index and Bcl2 provides objective determination of clinical outcome for patients with breast cancer. J Pathol. 2010;222(4):388–99.
3. Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer. J Natl Cancer Inst. 2009;101(10):736.
4. Curigliano G, Criscitiello C, Andrè F, Colleoni M, Di Leo A. Highlights from the 13th St Gallen International Breast Cancer Conference 2013. Access to innovation for patients with breast cancer: how to speed it up? Ecancermedicalscience. 2013;7(1).
5. Sarli G, Benazzi C, Preziosi R, Della Salda L, Bettini G, Marcato PS. Evaluating Mitotic Activity in Canine and Feline Solid Tumors: Standardizing the Parameter. Biotech Histochem. 1999;74(2):64–76
6. van Diest PJ, Baak JPA, Matze-Cok P, Wisse-Brekelmans ECM, van Galen CM, Kurver PHJ, et al. Reproducibility of mitosis counting in 2,469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project. Hum Pathol. 1992;23(6):603–7.
7. Van Diest PJ, Brugal G, Baak JPA. Proliferation markers in tumours: interpretation and clinical value. J Clin Pathol. 1998;51(10):716–24.
8. Tan PH, Ellis I, Allison K, Brogi E, Fox SB, Lakhani S, et al. The 2019 World Health Organization classification of tumours of the breast. Histopathology [Internet]. 2020;77(2):181–5.
9. Rakha EA, Bennett RL, Coleman D, Pinder SE, Ellis IO. Review of the national external quality assessment (EQA) scheme for breast pathology in the UK. J Clin Pathol. 2017;70(1):51–7.
10. Baak JPA, van Diest PJ, Ariens AT, van Beek MWPM, Bellot SM, Fijnheer J, et al. The Multicenter Morphometric Mammary Carcinoma Project (MMMCP). A nationwide prospective study on reproducibility and prognostic power of routine quantitative assessments in The Netherlands. Pathol Res Pract. 1989;185(5):664–70.
11. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000;124(7):966–78.
12. Rakha EA, Aleskandarani M, Toss MS, Green AR, Ball G, Ellis IO, et al. Breast cancer histologic grading using digital microscopy: concordance and outcome association. J Clin Pathol. 2018;71(8):680–6.
13. Bonert M, Tate AJ. Mitotic counts in breast cancer should be standardized with a uniform sample area. Biomed Eng Online. 2017;16(1):1–8.
14. Aubreville M, Bertram CA, Donovan TA, Marzahl C, Maier A, Klopfleisch R. A completely annotated whole slide image dataset of canine breast cancer to aid human breast cancer research. Sci Data. 2020;7(1).
15. Bertram CA, Aubreville M, Gurtner C, Bartel A, Corner SM, Dettwiler M, et al. Computerized Calculation of Mitotic Count Distribution in Canine Cutaneous Mast Cell Tumor Sections: Mitotic Count Is Area Dependent. Vet Pathol. 2020;57(2):214–26.
16. Williams BJ, Hanby A, Millican-Slater R, Nijhawan A, Verghese E, Treanor D. Digital pathology for the primary diagnosis of breast histopathological specimens: an innovative validation and concordance study on digital pathology validation and training. Histopathology. 2018;72(4):662–71.
17. Pathmanathan N, Balleine RL. Ki67 and proliferation in breast cancer. J Clin Pathol [Internet]. 2013 Jun;66(6):512–6.
18. Cuylen S, Blaukopf C, Politi AZ, Muller-Reichert T, Neumann B, Poser I, et al. Ki-67 acts as a biological surfactant to disperse mitotic chromosomes. Nature. 2016;535(7611):308.
19. Kim JY, Jeong HS, Chung T, Kim M, Lee JH, Jung WH, et al. The value of phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers: A comparative study with Ki67. Oncotarget. 2017 May 10;8(39):65064–76.
20. Schimming TT, Grabellus F, Roßner M, Pechlivanis S, Sucker A, Bielefeld N, et al. pHH3 immunostaining improves interobserver agreement of mitotic index in thin melanomas. Am J Dermatopathol. 2012;34(3):266–9.
21. Angi M, Damato B, Kalirai H, Dodson A, Taktak A, Coupland SE. Immunohistochemical assessment of mitotic count in uveal melanoma. Acta Ophthalmol. 2011;89(2):e155–60.
22. Casper DJ, Ross KI, Messina JL, Sondak VK, Bodden CN, McCardle TW, et al. Useof anti-phosphohistone H3 immunohistochemistry to determine mitotic rate in thin melanoma. Am J Dermatopathol. 2010;32(7):650–4.
23. Ladstein RG, Bachmann IM, Straume O, Akslen LA. Ki-67 expression is superior to mitotic count and novel proliferation markers PHH3, MCM4 and mitosin as a prognostic factor in thick cutaneous melanoma. BMC Cancer. 2010;10(1):1–15.
24. Glatz K, Hartmann C, Antic M, Kutzner H. Frequent mitotic activity in banal melanocytic nevi uncovered by immunohistochemical analysis. Am J Dermatopathol. 2010;32(7):643–9.
25. Nasr MR, El-Zammar O. Comparison of pHH3, Ki-67, and survivin immunoreactivity in benign and malignant melanocytic lesions. Am J Dermatopat