ORIGINAL RESEARCH

Analysis of Proliferative Indices (Ki-67 & Agnors) With Grades of Breast Carcinoma

[1]Dr. Neha Nautiyal, [2]Dr. Kanika Munish, [3]Dr. Ankita Varma

[1,2]Assistant Professor, [3]Senior Resident, KKBM, Subharti Hospital, Dehradun, Uttarakhand, India

Corresponding Author
Dr. Ankita Varma
Senior Resident, KKBM, Subharti Hospital, Dehradun, Uttarakhand, India

Received: 19 March, 2023 Accepted: 23 April, 2023

ABSTRACT

Aim: To analyse proliferative indices (Ki-67 & Agnors) with grades of breast carcinoma. **Material and Methods:** The present observational cross-sectional study was conducted among 42 Breast biopsies reported histopathologically as Breast carcinoma. Demographic details of the patient were documented. Presenting complaints of the patient along with detailed history was recorded. Relevant cytological and radiological findings were documented. We prepared two sections, first one was stained with special stain i.e. AgNOR and second with immunohistochemical marker i.e. Ki-67. Histopathological grading of all breast malignant biopsies were done according to Notthingham grading system. Expression of AgNORs and Ki-67 was documented and analysed using SPSS version 24. **Results:** Notthingham Grade I was revealed maximum in subjects with Ki67 <1%, grade II in subjects with Ki67 1-10% while grade III was found maximum in subjects with Ki67 >10%. Hence higher Notthingham Grade was related more with higher Ki67 score. Mean AgNOR score was found maximum in Notthingham Grade III while least in Notthingham Grade I, though no statistically significant difference was found as p>0.05. **Conclusion:** This research demonstrated that Ki-67 index can be used to classify tumours into distinct prognostically meaningful clinical outcomes.

Keywords: Breast Carcinoma, Ki-67, Agnors

INTRODUCTION

Cancer of the breast is a heterogeneous illness, meaning that it consists of a number of separate entities that each have their own unique biological characteristics and behaviour in the clinic.[1-2] The publishing of research based on microarrays, which found several genetic subgroups, brought to light the extensive heterogeneity of cancer of the breast.[3-4]

Cancers of the breast are clonal proliferations that originate from cells that have various genetic aberrations. These genetic aberrations are caused by hormonal exposures, and inherited susceptibility genes also play a role.[5] In 2018, about 2.1 million women had the diagnosis of cancer of the breast; this equates to around one new case being diagnosed every 18 seconds on average. When compared to the numbers from earlier years, this figure shows a substantial rise.[6,7] Taking into consideration the incidence which is adjusted to age, which is 25.8 cases per lakh women each year, this form of cancer that affects Indian women at a rate that is greater than any other type. In fact, the rate is higher than any other type.[8]

There is a rising interest in the use of immunohistochemistry markers for the classification of the tumours into different sub-types. This is a direct result of the previous point.[9,10] A significant amount of new understanding about cancer of the breast has been obtained over the last two decades. This new molecular categorization is a very essential one for the overall framework of the breast cancers research field. Consideration should be given to the possibility that cancer of the breast is no longer a single illness characterized by varying levels of oestrogen receptor (ER) and Her-2 expression. It is important to note that the cancer of the breast may possibly originate from a variety of different progenitor cells, and there are atleast three illnesses that may be distinguished from one another both molecularly and clinically. Understanding the molecular profile of cancer is now possible because to the advancement of improved technology, in particular the microarray.[11]

In Luminal A-like and Luminal B-like subtypes, which are both HER2 negative, positive findings for hormone receptors are reported whereas negative results for HER2 are shown. These classifications are: the 1st classification is Ki67 more than 14%, the second classification is Ki67 less than 14% and PR less than 20% or Ki67 more than 14%, the third classification is Ki67 less than 20% and PR less than 20% or Ki67 more than 20%, and the most recent classification is Ki67 more than 20% or Ki67 between

14 and 19% and PR (Progesterone Receptor) less than 20%.[12]

Estimating a patient's prognosis may be accomplished in a number of different methods, each of which makes use of an important element known as the cancer of the breast's potential for proliferation. One example of such a method is the mitosis counts per 10x microscope field (HPF), which measures the number of mitoses in a given area.[13]

One kind of molecular cancer marker is referred to as AgNOR's, which stands for silver stained nucleolar organiser regions (NORs). NORs are the loops of DNA that may be found in the nucleus of a cell, namely on the acrocentric chromosomes 13,14,15,21 and 22. There is a relationship that can be established between NORs and the proteins in question. By using the silver staining method,[14] it is possible to complete the identification of these argyrophilic related proteins in a manner that is rather uncomplicated.

Cancer of the breast is characterised by rapid cell division, a characteristic of the disease. For the purpose of diagnosing cancer of the breast, many distinct proliferative index signals may be used.[14] However the histopathological examination is always considered the gold standard for the diagnosis of breast carcinoma. The current study was conducted to provide an additional tool for precise and accurate grading of tumours, which could be used as an adjunct to routine histopathological findings in order to obtain more accurate prognostic information. This was accomplished by determining the proliferative activity of tumour cells using the ki-67 and AgNOR stains. The aim of the present study was to associate proliferative indices (Ki-67 & Agnors) with various grades of breast carcinoma.

MATERIALS AND METHODS

After taking into consideration the criteria for inclusion and exclusion, this cross-sectional research was carried out with a total of 42 instances that were reported as CA breast in the department of pathology.

Inclusion Criteria: All the Breast biopsies reported histopathologically as Breast carcinoma.

Exclusion Criteria

1. Autolysed specimen
2. Inadequate Breast biopsies
3. Other malignancies which are metastasizing to the breast
4. Patients not giving consent for study

For this particular investigation, each specimen was preserved in formalin, and tissue blocks were prepared by embedding them in paraffin. Every patient that was reported as having a breast tumour was subjected to a comprehensive history pertaining to breast tumours as well as a full clinical physical examination, both of which were recorded. Either the patient or the attendant provided their written informed permission so that further research work could be done on cases. The information pertaining to each patient was kept personal at all times and was never divulged to any third parties under any circumstances.

We prepared two sections, one was stained with special stain i.e. AgNOR and second with immunohistochemical marker i.e. Ki-67.

Assessment of IHC staining:

Ki-67: Depending on nuclear staining of the tumor cells that were positively stained Ki-67 was calculated as % expression by tumor cells. The scoring was done on the basis of criteria which was given by Yamashita et al.[15]

Total 100-500 tumor cells were counted and out of 500 tumor cells positive cells for Ki-67 were counted & multiplied by 100.

0. = None
1. = <1%
2. = 1–10%
3. = 10–50%
4. = > 50%

Tumours with score of 2 or greater were considered to be positive for the Ki-67 expression.

Assessment of AgNOR count:

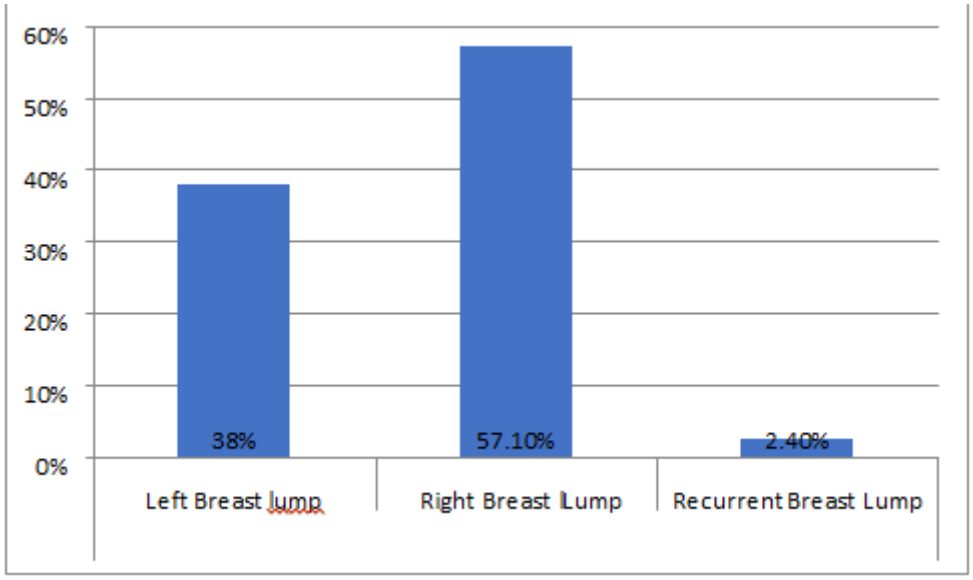
Enumerating AgNOR: AgNOR are visualised as blackish or brown dots in a pale yellow background, both in the nucleolus and within the nucleoplasm.

Mean AgNOR count: A 100X objective was used in order to determine the number of AgNORs present inside the nucleus of one hundred cancer cells. After that, the mean numbers of NORs per nucleus were computed, and the findings were reported as the mean plus or minus the standard deviation.

Result was correlated with histological grading of tumors and appropriate statistical analysis was performed using SPSS software version 24.

RESULTS

The mean age of the individuals was 50.88±14.59 years. Left, right and recurrent breast lump was reported among 38%, 57.1% and 2.4% of the subjects respectively (graph 1).



Graph 1: Chief complaint among the study subjects

Invasive ductal carcinoma (NST), carcinoma with medullary features, invasive lobular carcinoma and low grade ductal carcinoma among the study subjects was found in 90.5%, 4.76%, 2.38% and 2.38% of the subjects respectively. Notthingham Grade I, II and III was found in 16.7%, 23.8% and 59.5% of the subjects respectively (table 1).

Table 1: Histological diagnosis and Notthingham Grade among the study subjects

Diagnosis	**N**	**%**
Invasive ductal carcinoma (NST)	38	90.5
Carcinoma with medullary features	2	4.76
Invasive Lobular Carcinoma	1	2.38
Low Grade Ductal Carcinoma	1	2.38
Grade		
Grade I	7	16.7
Grade II	10	23.8
Grade III	25	59.5
Total	42	100

Mean AgNOR score among the study subjects was 4.59±1.46. AgNOR score viz. 2-4, 4-6 and 6-8 was found among 45.2%, 42.9% and 11.9% of the study subjects respectively. Mean Ki-67 score among the study subjects was 26.69±26.51. Ki-67% score viz. <1, 1-10, 1050 and >50 was reported in 21.4%, 28.6%, 33.3% and 16.7% of the study subjects respectively (table 2).

Table 2: AgNOR score and Ki-67% positivity cells among the study subjects

AgNOR Score	**N**	**%**
2-4	19	45.2
4-6	18	42.9
6-8	5	11.9
Mean±SD	4.59 ±1.46	
Ki-67%		
<1	9	21.4
1-10	12	28.6
10-50	14	33.3
>50	7	16.7
Mean±SD	26.69±26.51	

Notthingham Grade I was revealed maximum in subjects with Ki67<1% while grade III was found maximum in subjects with Ki67>10%. Hence higher Notthingham Grade was related more with higher Ki67 score. When Notthingham Grade distribution was compared according to Ki67 (table 3). Mean Ki67 score was found

maximum in Notthingham Grade III while least in Notthingham Grade I with statistically significant difference as $p<0.05$.

Table 3: Notthingham Grade distribution according to Ki67

Notthingham Grade		Ki 67 score			
		<1%	**1-10%**	**10-50%**	**>50%**
Grade I	N	5	1	1	0
	%	55.6%	8.3%	7.1%	0.0%
Grade II	N	2	4	2	2
	%	22.2%	33.3%	14.3%	28.6%
Grade III	N	2	7	11	5
	%	22.2%	58.3%	78.6%	71.4%
Total	N	9	12	14	7
	%	100.0%	100.0%	100.0%	100.0%
Chi Square		14.79			
p value		0.022*			

*: statistically significant

Notthingham Grade I was revealed maximum in subjects with AgNOR score of 4-6, grade II in subjects with AgNOR score of 2-4 while grade III was found maximum in subjects with AgNOR score of 4-6. When Notthingham Grade distribution was compared according to AgNOR score, statistically insignificant difference was found as $p>0.05$ (table 4). Mean AgNOR score was found maximum in Notthingham Grade III while least in Notthingham Grade I, even though there was no discernible change was found as $p>0.05$.

Table 4: Notthingham Grade distribution according to AgNOR score

Notthingham Grade		AgNOR Score		
		2-4	**4-6**	**6-8**
Grade I	N	3	4	0
	%	15.8%	22.2%	0.0%
Grade II	N	6	1	3
	%	31.6%	5.6%	60.0%
Grade III	N	10	13	2
	%	52.6%	72.2%	40.0%
Total	N	19	18	5
	%	100.0%	100.0%	100.0%
Chi Square		7.89		
p value		0.09		

DISCUSSION

AgNORs have been recognised as the loops of DNA that transcribe to the ribosomal RNA and therefore reflect the cell kinetics of the tumour. The mitotic figure counts and the count of the nucleolar organiser regions (AgNORs) can be found in the mitotic figure counts. The immunohistochemical (IHC) assessment is the most promising method for detecting the nuclear proteins that are related to DNA replication. These proteins are produced by cells that are in the proliferative phase of the cell cycle, such as Ki-67, which is a labile non-histone nuclear protein that is expressed in the G1 phase through the M phase of the cell cycle and is not detected in the resting phase of the cells, the G0 phase. Because of this, Ki-67 is an extremely useful marker.[16]

The mean age of the study subjects was 50.88±14.59 years with minimum and maximum of 22 and 80 years respectively in this study. It was in concordance with studies conducted by Ansari et al[17] in which the mean age was 48.2 years, Setyawati et al[18] revealed mean age as 52 years and in study by Cheng et al[19] the mean age was found to be 48.5 years. Hence breast cancer is related to old age.

In this study; invasive ductal carcinoma (NST), carcinoma with medullary features, invasive lobular carcinoma and low grade ductal carcinoma among the study subjects was found in 90.5%, 4.76%, 2.38% and 2.38% of the subjects respectively. In the study conducted by **Karangdan et al**[1], 54 cases (90%) were of IDC, NST subtype and similar findings were observed by the study done by **Mittal et al**[20] too. In the study conducted by Ansari et al[17] out of 516, majority (496) were of IDC, NST followed by 12 cases lobular carcinoma, 3 cases of mucinous carcinoma, 2 cases of medullary carcinoma and 1 case each of secretory carcinoma, papillary carcinoma and metaplastic carcinoma.

Mean Ki-67 score among the study subjects was 26.69±26.51. Ki-67% score viz. <1, 110, 10-50 and

>50 was reported in 21.4%, 28.6%, 33.3% and 16.7% of the study subjects respectively. Notthingham Grade I was revealed maximum in subjects with Ki67 <1%, grade II in subjects with Ki67 1-10% while grade III was found maximum in subjects with Ki67 >10%. Hence higher Notthingham Grade was related more with higher Ki67 score. When Notthingham Grade distribution was compared according to Ki67 sing chi square test, statistically significant difference was found as p<0.05. Mean Ki67 score was found maximum in Notthingham Grade III while least in Notthingham Grade I with statistically significant difference as p<0.05 in this study. In a study conducted by Manisha Sharma et al[21], Ki-67 has a direct relationship with the grade of the tumour, which is consistent with the findings of the current investigation. This is in agreement with the findings of the research that was conducted by other scientists (Wojnar A et al[22], Azambuja ED et al[23]), who discovered that the grade III tumours had a significantly higher mean number of Ki-67 positive cells when compared to the grade II and grade I tumours, with a p value of less than <0.05.

In the current investigation, fifty percent of the study individuals had a Ki-67% value more than 10. It was observed by Manisha Sharma et al[21] that the proportion of Ki-67 positive was 30%, although other research have shown it to be anything from 49% to 53.6%. The percentage of Ki-67 immunostained nuclei ranged anywhere from 3 to 70 percent, and this conclusion was consistent with the ranges (1 to 64 percent) that were reported by other investigations.[24]

Mean AgNOR score among the study subjects was 4.59±1.46. Agnor score viz. 2-4, 4-6 and 6-8 was found among 45.2%, 42.9% and 11.9% of the study subjects respectively. Notthingham Grade I was revealed maximum in subjects with AgNOR score of 4-6, grade II in subjects with AgNOR score of 2-4 while grade III was found maximum in subjects with AgNOR score of 4-6. When Notthingham Grade distribution was compared according to AgNOR score, statistically insignificant difference was found as p>0.05. Although there was no statistically significant difference identified (p>0.05), the mean AgNOR score was found to be highest in Notthingham Grade III and lowest in Notthingham Grade I. However, this difference was not found to be statistically significant. According to the findings of Manisha Sharma and colleagues[21], the average number of AgNORs found in their investigation ranged anywhere from 2.42 to 6.68. With a p value of =0.0137, the mean AgNOR count was significantly higher in the grade III tumours (4.28+/1.07) than it was in the grade II tumours (3.39+/-0.79). Dube MK et al[25] also found that the grade III population had considerably higher mean AgNOR counts than the grade II population.

It is possible to molecularly classify breast cancer by using IHC surrogate markers, and this classification is able to encompass a variety of pathologic characteristics, each of which indicates a distinct pattern of biological behavior. In addition to this, it offers valuable information from a clinical perspective and may be utilised in everyday practise.

In our study, there was no statistically significant correlation between Ki67 and AgNOR score with respect to grade I and grade II of CA Breast whereas, statistically significant correlation was found between Ki-67 and AgNOR with respect to grade III of CA Breast. Similarly, Manisha Sharma et al[21] reported that in their study, when they attempted to find a correlation between Ki-67 and the mean AgNOR counts, they did not find a significant correlation (p = 0.606), despite the fact that both the parameters (score and count) rose with an increase in the grade of the tumors.

The limitation of this study is small sample size. There is a difference of up to 39% between the molecular categorization provided by IHC and that provided by gene expression, according to the research that has been done so far. In addition to this, there is need to investigate the association between molecular subtypes and risk factors in a sizable population spread out throughout the nation and in more than one location.

CONCLUSION

This research endeavoured to demonstrate that Ki-67 index can be used to classify tumours into distinct prognostically meaningful clinical outcomes. These features of tumours may be used in clinical practise to direct patient care, improve the way patients are treated, and increase the likelihood that patients will survive their cancer. The limited expression of the proliferative markers and the other prognostic markers of the breast, the non-correlation of Ki-67 and the mean AgNOR counts, sometimes has to be correlated with other such parameters for further evaluation. This is because the breast has limited proliferative markers and other prognostic markers.

REFERENCES

1. Simpson PT, Reis-Filho JS, Gale T, Lakhani SR. Molecular evolution of breast cancer. J Pathol. 2005; 205(2): 248-54.
2. Vargo-Gogola T, Rosen JM. Modelling breast cancer: one size does not fit all. Nat Rev Cancer. 2007; 7(9): 659-72.
3. Perou CM, Sørlie T, Eisen MB, Van De Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge Ø. Molecular portraits of human breast tumours. Nature. 2000; 406(6797): 747-52.
4. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, Van De Rijn M, Jeffrey SS, Thorsen T. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci.USA. 2001; 98(19): 10869-74.
5. Susan C. Lester. The Breast. In: Vinay Kumar, Abul K. Abbas, Jon C. Aster, Editor Robbins and Cotran Pathologic Basis of Disease:South Asia Edition. 9th Edition; p.1051-1066

6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jernal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2018; 68(6): 394-424
7. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019; 144(8): 1941-53.
8. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11; 2013.
9. Callagy G, Cattaneo E, Daigo Y, Happerfield L, Bobrow LG, et al. Molecular classification of breast carcinomas using tissue microarrays. Diagn Mol Pathol. 2003; 12: 27–34.
10. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, et al. Subtyping of Breast Cancer by Immunohistochemistry to Investigate a Relationship between Subtype and Short and Long Term Survival: A Collaborative Analysis of Data for 10,159 Cases from 12 Studies. PLoS Med.2010; 7(5): e1000279.
11. George P. Stathopoulosa, Nikolaos A. Malamosb, Christos Markopoulosc, et al . The role of Ki-67 in the proliferation and prognosis of breast cancer molecular classification subtypes. Anti-Cancer Drugs 2014, 25:950–957.
12. Gándara-Cortes M, Vázquez-Boquete Á, Fernández-Rodríguez B, et al. Breast cancer subtype discrimination using standardized 4-IHC and digital image analysis. Virchows Archiv. 2018; 472(2): 195-203.
13. Park D, Karesen R, Noren T, Sauer T. Ki-67 expression in primary breast carcinomas and their axillary lymph node metastases: clinical implications. Virchows Archiv: an international journal of pathology. 2007; 451(1): 11-8.
14. Uma S, Ritu S, Srivastava AN, et al. AgNOR count and its diagnostic significance in cervical intraepithelial neoplasia. Journal Obstet Gynecol India. 2006; 56(3): 244-6.
15. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, Iwase H. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. Breast Cancer Research. 2003; 6(1): 1-7.
16. Kunc M, Biernat W, Senkus-Konefka E. Estrogen receptor-negative progesterone receptor-positive breast cancer–"Nobody's land "or just an artifact?. Cancer Treatment Rev. 2018; 67: 78-87.
17. El Ansari R, Craze ML, Althobiti M, Alfarsi L, Ellis IO, Rakha EA, Green AR. Enhanced glutamine uptake influences composition of immune cell infiltrates in breast cancer. Br J Cancer. 2020; 122(1): 94-101.
18. Setyawati Y, Rahmawati Y, Widodo I, Ghozali A, Purnomosari D. The Association between Molecular Subtypes of Breast Cancer with Histological Grade and Lymph Node Metastases in Indonesian Woman. Asian Pac J Cancer Prev. 2018; 19(5): 1263
19. Cheang MC, Martin M, Nielsen TO, Prat A, Voduc D, Rodriguez-Lescure A, Ruiz A, Chia S, Shepherd L, Ruiz-Borrego M, Calvo L. Defining breast cancer intrinsic subtypes by quantitative receptor expression. Oncologist. 2015; 20(5): 474.
20. Mittal A, Mani NS. Molecular classification of breast cancer. Indian J Pathol Oncol 2021; 8(2): 241-247.
21. Sharma M, Manjari M, Kahlon SK. Proliferative indices, ki-67 immunostaining and nucleolar organizer region associated protein and their association with various grades of breast carcinomas. J Clin Diagn Res. 2011; 5(7): 1371-4.
22. Wojnar A, Kobierzycki C, Krolica A, Pula B, Podharska OM, Dziegeil P. Correlation of the Ki-67 and the MCM-2 proliferative markers with the grade of the histological malignancy(G) in ductal breast cancers. Folia Histochem Cytobiol 2010; 48(3): 442-46.
23. Azambuja ED, Cardasa F, Castro G, Mano MS, Durbecq V, Sotiriou C. Ki-67 as a prognostic marker in early breast cancer : meta-analysis of published studies involving 12155 patients. British Journal of Cancer 2007; 96: 1504-13.
24. Domenica DS, Pietro LM, Lvgi S, Mssimo D, Vittoria M. A comparative study of the histopathology, the hormone receptors, peanut lectin binding, Ki-67 immunostaining and the nuclear organizer region associated proteins in human breast cancer. Cancer 1991; 67: 463-71.
25. Dube MK, Govil A. Evaluation of the significance of the AgNOR counts in differentiating a benign from a malignant lesion in the breast. Indian J Pathol Microbiol 1995; 38: 5-10.