Original Article

Pathology

Proliferative Indices, Ki-67 Immunostaining and Nucleolar Organizer Region Associated Protein and their Association with Various Grades of Breast Carcinomas

MANISHA SHARMA, MRIDU MANJARI, SK KAHLON

ABSTRACT

Introduction: Clinical outcome of the tumour in many cases is related with cell proliferation which can be detected by mitotic figure counts, AgNOR counts and most promising approach of immunohistochemical (IHC) assessment of Ki-67.

Material and Methods: The present study was conducted on 40 specimens of breast carcinoma out of which 12 cases were of Grade II and 28 cases were of Grade III. Percentage positivity and scoring of Ki-67 and mean AgNOR count was done with relation to grades.

Observations: Ki-67 positivity was seen in 12 cases with the percentage positivity of 2.33±5.516 in grade II and 9.67±7.671 in grade III tumours . Similarly Mean AgNOR count in grade II was 3.39±0.79 and 4.28±1.07 in grade III cases. Mean Ki-67 positivity was 7.2% in cases showing metastatic deposits and it was 6.3% in cases with reactive pathology in lymph nodes.

Conclusion: It is concluded that Ki-67 positivity and AgNOR count increases with the increase in the grade of carcinoma breast and metastasis in lymph node. Therefore, Ki-67 expression and high mean AgNOR count can be associated with poor prognosis in carcinoma breast.

Key Words: Ki-67, AgNOR count, Breast cancer, Prognosis

INTRODUCTION

Breast cancer is one of the most common malignancies all over the world in women and it accounts for 13.7% of all the cancer deaths in women [1]. In India, among females, breast cancers account for 19% to 34% of all the malignancies [2]. The clinical outcome of the tumour in many cases, is related to cell proliferation, which can be detected by the mitotic figure counts and the count of the nucleolar organizer regions (AgNORs) which have been recognized as the loops of DNA which transcribe to the ribosomal RNA and therefore reflect the cell kinetics of the tumour. The most promising approach is immunohistochemical (IHC) assessment, for detecting the nuclear proteins which are related to DNA replication, which are produced by the cells which 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 G_1 phase through the M phase of the cell cycle and is not detected in the resting phase of the cells, the G_0 phase and this makes it a very useful marker for distinguishing the benign lesions from the malignant ones. Various studies have positively correlated the Ki-67 expression and the AgNOR counts with higher histological grading. The present study was undertaken to study the relationship of Ki-67 and the AgNOR counts with the histological grading, individually and between each other.

AIMS OF STUDY

- To find out the Ki-67 protein expression in forty cases of breast cancer cases.
- To find the AgNOR count in the same cases.
- To grade the lesions histopathologically.
- To study the relationship between the three.

MATERIALS AND METHODS

In the present study, forty histologically proven cases of breast cancer (irrespective of the grade and the lymph node status) were taken up for Ki-67 expression and the AgNOR counts. Individual grading was done according to the Nottingham grading system [3]. All the cases were then subjected to Ki-67 and AgNOR staining.

Ki-67 Expression

A primary antibody-Mouse anti Ki-67 antibody clone MB67 (Diagnostic BiosystemsCode: E762) was employed in the present study. The Ki-67 expression status was assessed according to the estimated proportion of the nuclear staining of the tumour cells that were positively stained. The scoring was done on the basis of the criteria which were laid down by Yamashita et al [4].

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

Tumours with score of 2 or greater were considered to be positive for the Ki-67 expression. The intensity of the staining, whether it was strong, moderate or weak, was also noted.

AgNOR Counts

The stain, which was a mixture of gelatin-formic acid and 50% aqueous silver nitrate, was employed under dark room conditions. For each sample, the number of AgNORs within the nuclei of 100 tumour cells was calculated and the results were expressed as mean +/– standard deviation.

OBSERVATIONS (PART I)

Out of the forty cases, 12(30%) were of the histological grade II, while 28(70%) were of grade III. The IHC staining for Ki-67 of all the malignant cases which were included, showed positivity in 12/40 cases (30%) only. 28/40(70%) cases which showed < 1% of Ki-67 positivity, were categorized as Ki-67 negative, despite the grade 2 and 3 lesions.

Of the cases which exhibited Ki-67 postivity: the maximum number of cases (75%) demonstrated a weak to moderate staining intensity. This was followed by cases (25%) with a heavy staining intensity.

No definite correlation was seen between Ki-67 expression and the parameters like age and size of the lesion. A definite correlation was seen between the grade of the tumour and lymph node involvement. As the grade increased, the Ki-67 expression also increased significantly [Table/Fig-3) and a significantly higher Ki-67 percentage positivity was noted in the cases which showed metastatic deposits in the lymph nodes [Table/Fig-4].

PART II

Similarly, AgNOR staining was done in all the 40 cases of the malignant lesions. The maximum number of cases (22/40-55%) showed AgNOR counts which ranged from 2-4, 16/40-40% cases showed a mean count of 4-6 and 2/40-5% cases had a mean AgNOR count of 6-8.

No definite correlation was seen between AgNOR and the parameters like the age and size of the lesion. Although the comparison of the mean AgNOR count with histological grade showed a definite correlation as the grade increased, the mean AgNOR count was also found to be increased. The cases with metastatic deposits had a higher mean AgNOR count than the others. Out of the 12 grade II breast carcinomas, 10 cases (83.3%) had counts which ranged from 2-4, but none had a mean count of more than 6, whereas in the grade III tumours, most of the cases (16/28) had counts above 4 and in 5 cases, the mean count was above 6.

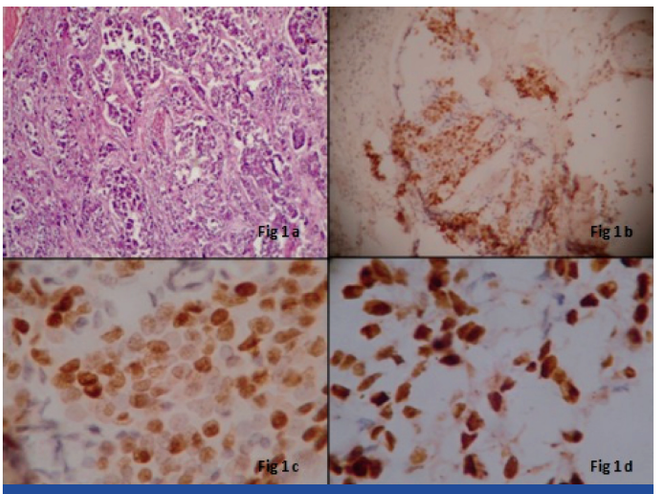
Thus, by deriving a correlation between the histological grade, the mean AgNOR count and the Ki-67 percentage positivity, it was found that the mean AgNOR counts for the grade II and III tumours was 3.39+/–0.79 and 4.28+/–1.07 respectively. The mean Ki-67 percentage positivity for tumours of grades II and III were 2.33+/–5.51 and 9.67+/–7.67 respectively. The unpaired t-test was applied and the p-value was calculated to check for a significant correlation between the histological grade, the Ki-67 expression and the AgNOR count respectively. A statistically significant correlation ($p<0.05$) was found between the Ki-67 positivity and the histological grade, as the mean Ki-67 positivity was higher in the grade III tumours as compared to the grade II cases. A significant difference was also observed statistically ($p=0.0137$) between the AgNOR count and the histological grades of breast carcinoma.

DISCUSSION

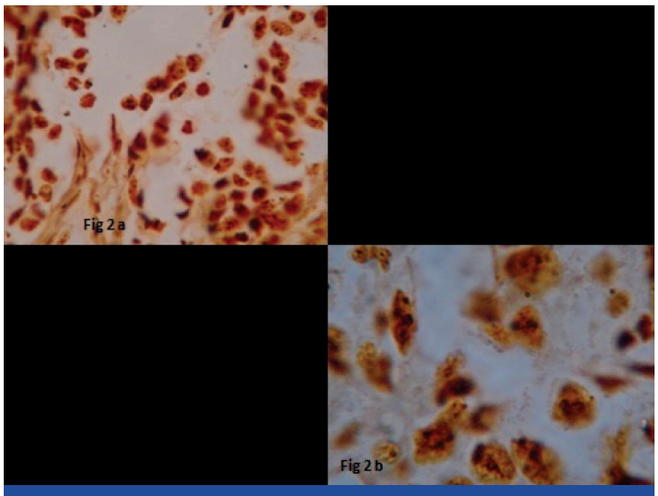
Ki-67 Immunostaining

Ki-67 is a proliferative marker which is produced by the cells which are in the proliferative phase of the cell cycle (the G1 through the M phase) [5,6]. Hence, strictly speaking, the Ki-67 positive cell fraction is the growth fraction which is the proportion of the actively cycling cells within a defined population of cells [7].

The authors of the present study and other researchers also have found Ki-67 to be directly correlated to the grade of the tumour and to the other prognostic variables like mitotic index and lymph node metastasis.



[Table/Fig-1]: (a) Sections showing infiltrating ductal carcinoma breast (H& E × 100); (b) Low power magnification of nuclear positivity of Ki-67 in tumor cells (IHC × 100); (c) Moderate intensity of Ki-67 nuclear positivity in tumor cells (IHC × 400); (d) Strong intensity of Ki-67 nuclear positivity in tumor cells (IHC × 400).



[Table/Fig-2]: (a) Nucleolar organizer regions (NOR's) seen in tumor cells (AgNOR × 100); (b) Same focus as above but in higher magnification demonstrating NOR's in the tumor cells (AgNOR × 400)

In the present study, the Ki-67 percentage positivity was found to be 30%, while other studies have reported it to be in the range of 49% to 53.6% [4,8]. The proportion of the Ki-67 immunostained nuclei varied from 3%–70% and this finding concurred with the ranges (1%-64%) which were reported by other studies [9].

Also the % positivity of Ki-67 was significantly higher in the grade III tumours (9.67+/–7.67) than that of the grade II tumours (2.33+/–5.51) in our study, with a p value of <0.05. This is in concordance with the findings of the work which was done by other researchers, where a higher mean of the Ki-67 positive cells was found in the grade III tumours as compared to that in the grade II and grade I tumours, with a p value of < 0.001 [10,11].

Among the other prognostic factors which were revealed to be significantly associated with the Ki-67 positivity, one was lymph node metastasis. The mean Ki-67 positivity was 7.2% in the breast cancer cases with lymph node metastasis, which was significantly higher than the percentage positivity which was seen in the cases with reactive lymph nodes (6.3%). This fact was also corroborated by Stump et al, who concluded that the cases with a distant metastasis was associated with a higher mean Ki-67 percentage positivity (21.3%) than the other cases (13.9%) [12].

Histological Grade	Number of cases	Ki 67				AgNOR			
		Percentage positivity of cells				Mean counts			
		<1%	1-10%	10-50%	>50%	0–2	2–4	4–6	6–8
I	0	0	0	0	0	0	0	0	0
II	12	10	0	2	0	0	10	2	0
III	28	18	1	7	2	0	12	11	5
Total	40	28	1	9	2	0	22	13	5

[Table/Fig-3]: Comparative analysis of Ki-67 and AgNOR with histological grade of the tumour

Lymph node involvement	Number of cases	Ki 67 Percentage positivity of cells				AgNOR Mean counts			
		<1%	1–10%	10–50%	>50%	0–2	2–4	4–6	6–8
Metastatic	22	18	0	2	2	0	11	10	1
Reactive	9	6	0	3	0	0	5	3	1
Absent	9	4	1	4	0	0	6	3	0
Total	40	28	1	9	2	0	22	16	2

[Table/Fig-4]: Comparative analysis of Ki-67 and AgNOR with Lymph Node involvement

In a study which was done in Malaysian women also, this percentage was found to be higher, with a p value of = 0.033 [13].

Of the other prognostic factors such as age and size of the tumour, no significant correlation was found. The two parameters which were found to be directly correlated with the Ki-67 expression were the grade of the tumour and lymph node metastasis.

AGNOR STAINING

The nucleolar organiser regions (NORs) have been recognized as loops of DNA which transcribe to the ribosomal RNA. In human beings, the five acrocentric chromosomes [13,14,15,21,22] bear NORs [14]. The number of AgNORs have been related to the cellular activation and so the mean AgNOR counts have found to be more in the malignant lesions as compared to their benign counterparts. This corollary also holds true when a higher grade lesion is compared with a lower grade one [15]. An increased mean AgNOR count is associated with a poor prognosis and it has been found to be directly correlated with other prognostic variables like grade of the tumour, mitotic count and lymph node metastasis [16,17].

The mean AgNOR count in our study varied from 2.42-6.68 and other researchers reported it to range from 2.7–9.9 [18].

In the present study, the mean AgNOR count was significantly higher in the grade III (4.28+/-1.07) than that in the grade II tumours (3.39+/-0.79), with a p value of =0.0137. Similar findings were observed by other researchers, with significant p values [18,19].

In our study, 50% of the breast cancer cases with metastatic deposits showed a mean AgNOR count of >4, while only 44% of the cases with reactive lymph nodes had a mean AgNOR count of >4. This fact corresponded to the findings which were observed by Giri et al, where the cases with metastatic deposits had higher AgNOR counts [19].

Similarly, as in Ki-67, no significant correlation was found between the mean AgNOR count and the age of the patient and the size of the tumour. The two parameters which were found to be directly correlated with the AgNOR count were grade and lymph node metastasis, as was also seen with Ki-67.

On attempting to correlate between Ki-67 and the mean AgNOR counts, no significant correlation was observed (p = 0.606), although both the parameters (score and count) increased with an increase in the grade of the tumours. However, other researchers have found a statistically significant correlation between these two parameters [20]. This could be explained on the basis of the lesser number of Ki-67 positive (percentage positivity); 12/40 cases in the present study.

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, at times has to be correlated with other such parameters for further evaluation.

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AUTHOR(S):
1. Dr. Manisha Sharma
2. Dr. Mridu Manjari
3. Dr. SK Kahlon

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, SGRDIMSR, Amritsar (Punjab) India.
2. Professor, Department of Pathology, SGRDIMSR, Amritsar (Punjab) India.
3. Professor, Department of Pathology, GMC Amritsar, Amritsar (Punjab) India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Manisha Sharma, C/O Mr S. L Sharma
B-241, Ranjit Avenue, Amritsar-143001
Punjab, India.
Phone: +919876842942
E-mail: manisha_salwan@yahoo.com

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