ORIGINAL RESEARCH PAPER

Pathology

"STUDY OF PROLIFERATIVE INDICES USING MITOTIC INDEX AND AGNOR AND THEIR CORRELATION WITH VARIOUS GRADES OF BREAST CARCINOMA"

KEY WORDS:

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ABSTRACT

Background: Breast cancer is a leading cause of death in women. The objectives of this study were to compare the argyrophilic nucleolar organizer region (AgNOR) count and mitotic index with various histological grades of infiltrating ductal carcinoma of breast.

Aim: To study the relationship of the proliferative indices and various grades of breast carcinoma.

Objective: "Study of proliferative indices using mitotic index and Agnor and their correlation with various grades of breast carcinoma"

Material and Method: In this prospective study all specimens of infiltrating ductal carcinoma of female breast belonging to all age groups received in the department of histopathology of Gajra Raja Medical College, Gwalior during the period of three years were graded using Bloom and Richardson score and correlated with proliferative indices mitotic index and AgNOR counts.

Result: The mean Age (Years) was 46.16 ± 8.68. The mean B&R Score was 6.52 ± 1.20. The mean Mitotic Index was 8.51 ± 2.61. The mean AgNOR Count was 4.24 ± 2.34. There was significant correlation between AgNOR count, mitotic index and histological grades.

Conclusion: AgNOR count and mitotic index can be used as reliable proliferative indices which correlate well with the histological grades and are cost effective too.

1. INTRODUCTION

Breast cancer is one of the most common malignancies and leading cause of death in middle aged women. It accounts for 13.7% of all cancer deaths in women. Altered proliferative activity as a prognostic factor is important as it points out to the biological aggressiveness and behaviour of malignancy. This altered proliferative activity can be detected by mitotic figure counts, the count of nucleolar organizer region (AgNORs) and also by Ki-67.

The ductal carcinoma of the breast can be graded using Nottingham modification of Bloom and Richardson grading system.

This system uses 1).Tubule formation 2)Nuclear pleomorphism 3).Mitotic count.

The nucleolar organizer regions are chromosomal loops of DNA involved in ribosomal synthesis. These NORs are located on each of short arms of the chromosomes 13,14,15,21 and 22. These proteins are identified by a silver colloid staining technique and visualised as dark intrancuclear dots under the microscope.

Silver staining is an easy and very quick technique that can be performed on formalin fixed paraffin embedded sections. These dots are fine and dispersed singly in benign tumors, the dots are coarse and present in clusters in malignant tumors. Hence AgNOR is a useful tool to differentiate various grades of malignancy. There are other techniques to estimate the proliferative indices that use immunohistochemistry these techniques are expensive and need lot of expertise. Therefore this study is proposed to study the proliferative indices using AgNOR and to understand its correlation with various grades of Breast carcinoma.

In this study all specimens of infiltrating ductal carcinoma of female breast belonging to all age groups received in the department of histopathology of Gajra Raja Medical College, Gwalior during the period of one and half year were assessed and scored using Nottingham modification of Bloom and Richardson scoring system.

MATERIAL AND METHOD

In this prospective study All specimens of infiltrating ductal carcinoma of female breast belonging to all age groups received in the department of histopathology of Gajra Raja Medical College, Gwalior during the period from 1st January 2020 to 31st June 2021.

Sample size: 50 cases
- AgNOR count and Mitotic index were calculated and correlated with the histological grade.
- Correlation of these proliferative indices and the various histological grades were determined.

Staining procedure
1. Sections were dewaxed in xylene, hydrated through alcohols to water.
2. Then the sections were rinsed with deionized water 3 times.
3. Sections were incubated with working staining solution (freshly prepared) for 45 minutes under dark room temperature conditions.
4. Sections were washed with deionized water for 10-15 minutes.
5. Sections were dried cleared in xylene and mounted in DPX mountant.
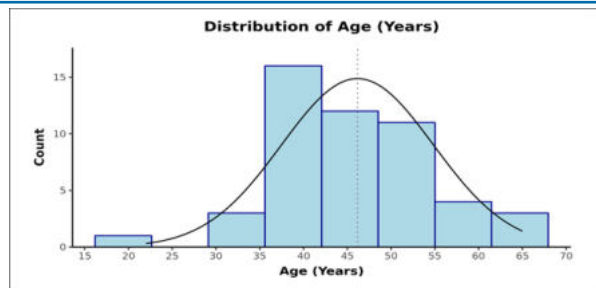
AgNOR counting procedure
The stained slides were viewed under oil immersion and after counting intranuclear silver dots in atleast 100 cells, AgNOR score was calculated i.e., mean number of AgNOR dots per nucleus.

RESULT:

AGE:

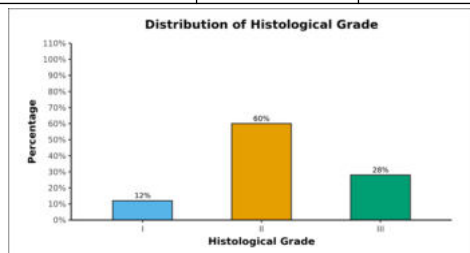**Table 1 : Distribution of the Participants in Terms of Age (Years) (n = 50)**

Age (Years)	
Mean (SD)	46.16 (8.68)
Median (IQR)	46.5 (40-50)
Range	22 – 65

Graph 1 Distribution Of Age

Table 2 :Distribution of the Participants in Terms of Histological Grade (n = 50)

| Histological Grade	Frequency	Percentage
I	6	12.0%
II	30	60.0%
III	14	28.0%

Graph 2. Percentage distribution of Histological Grade

**Table 3: Comparison of the 3 Subgroups of the Variable Histological Grade in Terms of Mitotic Index (n = 50)**

Mitotic Index	Histological Grade			
	I	II	III	p value
Mean (SD)	4.42 (1.36)	7.59 (0.40)	12.22 (0.31)	<0.001
Median (IQR)	4.1 (3.85-4.42)	7.6 (7.4-7.8)	12.25 (12.12-12.4)	
Range	3 – 7	6.8 - 8.4	11.5 - 12.8	

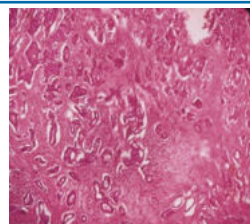**Chart : The means of Mitotic Index in the 3 different groups:**
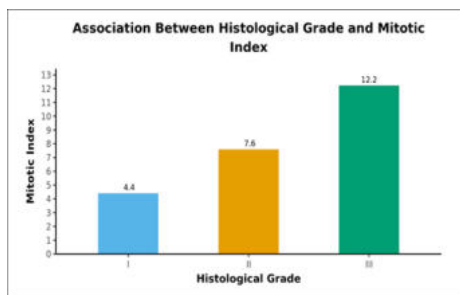
Comparison of the 2 Subgroups of the Higher Histological Grade in Terms of AgNOR Count (n = 50)
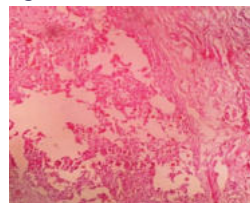
AgNOR Count			
	II	III	p value
Mean (SD)	4.20 (1.73)	6.14 (1.35)	<0.001
Median (IQR)	4 (3-5)	6 (5.25-7)	
Range	0 – 7	3 – 8	
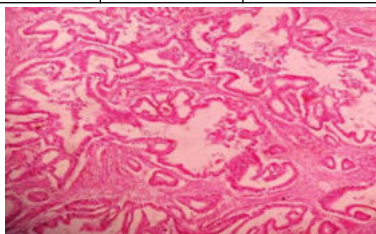
Grade I Infiltrating ductal Carcinoma Breast

Grade II Infiltrating ductal Carcinoma Breast

Grade III Infiltrating ductal Carcinoma Breast

DISCUSSION:

Age:
In our study the the age of patients ranged from 22-63 years. The mean Age (Years) was 46.16 ± 8.68.

In the study of Ahtesham et al the ages of the patients ranged from 24 – 85 years with a mean age of 46.84 years ± 11.8 SD. This is in concordance with our study.

Histological grade:
In our study 12.0% of the participants had Histological Grade: I, 60.0% of the participants had Histological Grade: II, 28.0% of the participants had Histological Grade: III which is concordance with the study conducted by Blamey et al in 2009 having grade I- 29%, grade II- 41%, Grade III- 30%.

• **AgNOR count :**
In the present study **mean AgNOR Count** was 4.24 ± 2.34.In studies done by Nepal N et al, Ansari et al and Simha et al the mean AgNORs were 4.50,4.0 and 3.5.These studies are in concordance with the present study. In our study the mean (SD) of AgNOR Count in the Histological Grade: II group was 4.20 (1.73).The mean (SD) of AgNOR Count in the Histological Grade: III group was 6.14 (1.35). In the study by Kazuhiko H et al the mean AgNOR count in grade-II it was 4.38 and in grade-III it was 5.4.

AgNOR counts were correlated with tumor grade in malignant breast lesions. These studies are in concordance with our study.

Research conducted by Sharma et al in 2011 revealed that mean AgNOR counts increase with an increase in grade of breast cancer which is in concordance with our study. It could therefore be concluded from the results that, there is a significant positive correlation between AgNOR count and the histological grade of breast cancer.

• **Mitotic index:**
In our study The mean mitotic index of histological grade I, II and III was 4.42, 7.59 and 12.22. In the study conducted by Lik Hang Lee et al Mitotic count/10 hpf grade I,II,III was 5.5 , 9.52, 53.25.This is in concordance with our study.

CONCLUSION
High AgNOR count in higher grades of breast carcinoma showed that higher grade the cancer peaks in its, proliferation and aggressiveness and has high number of cells which are mitotically active when compared with the lower grade of the malignant cases.

AgNOR staining technique is a simple procedure for measuring the cellular proliferative activity and uses light

microscopy for visualizing.

Immunohistochemical techniques are known to be an expensive technique compared with AgNOR which is a cheaper technique.

The use of AgNOR and mitotic index has the advantage that it can be used with the material that we obtained for routine process in a histological laboratory and we did not need special fixation, which Ki-67 needs.

AgNOR staining and mitotic indexing are much cheaper, and can be useful tools to discriminate between low and high grade tumors, and can guide in management of patients.

AgNOR and mitotic index showed a good positive correlation with the Bloom and Richardson grading system and the AgNOR values increased as the histological grade of the neoplasm progressed higher.

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