COMPARISON OF AGNORS SIZE AND DISPERSION WITH ER STAINING IN INVASIVE DUCTAL CARCINOMA OF BREAST

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ABSTRACT

Introduction: Breast cancer is the most common malignancy and a leading cause of death in women throughout the world. It is the most frequently reported malignancy and a major cause of cancer morbidity and mortality in Pakistan as well.

Objective: To compare the Argyrophilic nucleolar organizer regions size and dispersion in estrogen receptor positive and ER-ve tumors of invasive ductal carcinoma of breast.

Materials and Methods: Fifty cases of invasive ductal carcinoma of breast diagnosed on trucut, core biopsy/incision/excision were collected from Lahore General Hospital, Lahore. The slides prepared were stained with H&E, AgNOR, and ER immunostains.

Results: The mean age of the patients was 46.84 years ± 11.8 SD. AgNOR size and dispersion were significantly of higher grade ($p = 0.001$ and $p < 0.05$) in ER-ve tumors than ER+ve tumors.

Conclusion: It is concluded that AgNOR size and dispersion correlateinversely with ER status in invasive ductal carcinoma of breast and the results are statistically significant.

Key words: Argyrophilic Nucleolar Organizer Regions, Estrogen receptor, Breast carcinoma.

INTRODUCTION

Breast cancer is the most common malignancy and a major cause of death in women throughout the world.[1] Improved methods of detection and treatment have had a significant influence on disease outcome.[2]

Assessment of ER status is an essential component of the evaluation of breast cancer patients.[3] In 96.8% cases the ER status of the primary and metastatic tumor was the same, while in 3.2% cases with ER-positive primary tumor, the metastasis was ER-negative. There were no ER-negative primary tumors with ER-positive metastasis. Moreover, the time for distant metastasis to appear was significantly longer in ER-positive tumors.[4]

Prognostic factors for breast cancer are age, tumor size, grade and metastasis. Molecular factors associated with prognosis are hormone receptor status, HER 2 status, Ki-67 and Argyrophilic Nucleolar Organizer Regions (AgNORs) silver stains.[5,6]

Study of proliferation markers may help in the proper diagnosis of differentbreast lesions which lie in the gray zone on routine histopathology.[2]

The nucleolar organizer regions are choromosomal loops of DNA involved in ribosomal synthesis. The NORs were first described by Heitz in 1931 and by McClintock in 1934.[7] These NORs are located on each of the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22. These proteins are identified by a silver colloid staining technique and visualized as dark intranuclear dots under themicroscope.[8]

AgNOR technique has been applied in many areas of tumor pathology.[9] Statistically significant difference in the mean number of AgNORs has been found between normal, ordinary hyperplasia and neoplastic breast lesions.[10] The amount of AgNORs is related to the estrogenreceptor status, and the S-phase fraction on flow cytometry, withKi 67 staining and with Proliferating cell nuclear antigen (PCNA) and therefore has been proposed asan alternative measure of tumor proliferation.[11,12] The AgNOR parameters in human breast cancer have been suggested to be affected by the status of the oncosuppressor proteins p53 and Rb.[13]

Aims and Objectives were to compare the AgNORs size and dispersion with ER status in invasive ductal carcinoma of breast.

SUBJECTS AND METHODS

Fifty samples were collected from Lahore General Hospital, Lahore. H&E staining, AgNOR staining, and ER immunostaining were done. Histological diagnosis and

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grading was done on H&E stained slides using Nottingham Modification of Bloom and Richardson grading system. A specimen was considered ER-positive if more than 10% of the counted nuclei were positive.[14] For AgNOR staining the method used by Khalid et al (1996) was followed.[9] The nuclei were stained light yellow and AgNORs were visualized as brown black discrete dots of variable size within the nuclei. Variation in AgNOR size and dispersion were graded according to Khan et al (2006),[15] as follows: **Size Variation** 0 = More or less uniform, 1+ = Two different sizes, 2+ = More than two different sizes but not those of 3+, 3+ = All grades and sizes including too minute to be counted.

Dispersion
0 = Limited to nucleoli, 1+ = Occasional dispersion outside nucleoli, 2 + = Moderate dispersion outside nucleoli, 3+ = Widely dispersed throughout the nucleus.

Statistical Analysis
The data was analyzed using SPSS 21.0. The variation in AgNOR size and dispersion inER +ve and ER -ve tumors was compared using chi square test. $P \leq 0.05$ was considered significant for statistical analysis.

RESULTS
This study included 50 cases of invasive ductal carcinoma of the breast. Histological diagnosis and grading of the tumor was done on H&E stained slides (Fig. 1). Estrogen receptor staining and AgNOR staining was carried out on all cases, and variation in AgNOR size and dispersion were determined (Fig 2, 3).

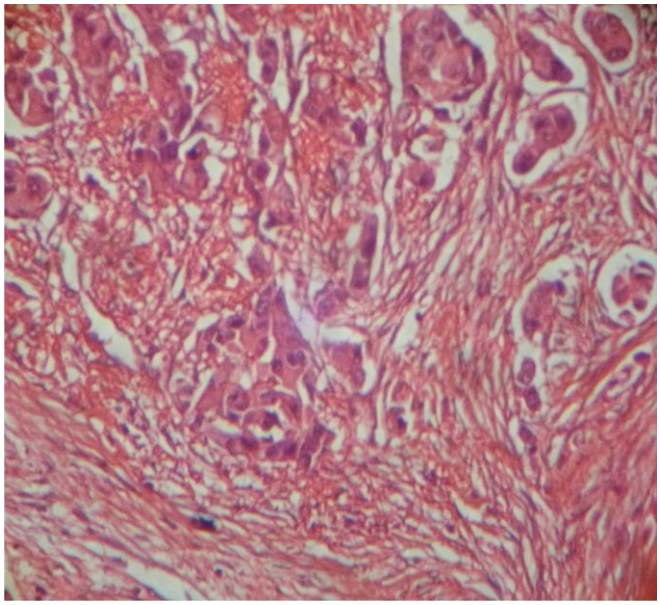

Fig. 1: *Photomicrograph of a section of invasive ductal carcinoma breast-grade II (H&E x400).*

The ages of the patients ranged from 24 – 85 years with a mean of 46.84 years ± 11.8 SD. Maximum number of cases were in 40 – 49 years age group.

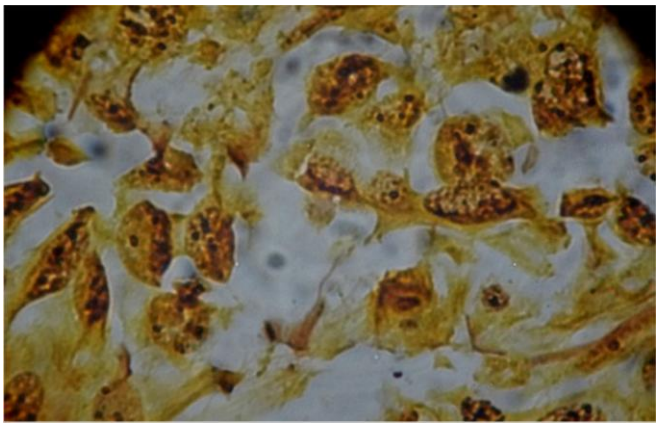

Fig 2: *Photomicrograph of a section of invasive ductal carcinoma of breast grade II (AgNOR staining x1000).*

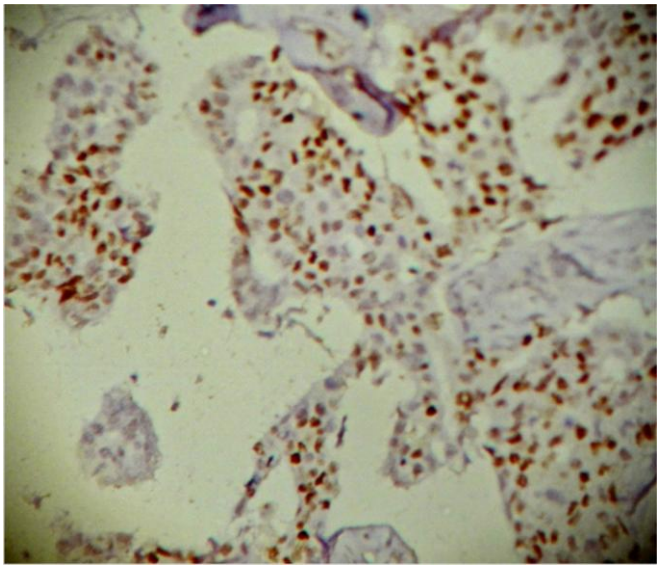

Fig 3: *Photomicrograph of a section of invasive ductal carcinoma of breast grade II (ER stainingx400).*

AgNOR size in estrogen receptor +ve tumors was predominantly 2+ whereas AgNOR size of ER-ve tumors was predominantly 3+. The difference was statistically significant ($p = 0.001$, Table 1).

AgNOR dispersion in ER+ve tumors was predominantly 2+ and 3+, whereas AgNOR dispersion was 3+ in 25 out of 27 ER-ve tumors. The difference was statistically significant ($p < 0.05$, Table 2).

DISCUSSION
This study included 50 cases of invasive ductal carcinoma of breast which is the most common form of breast cancer. The ages of the patients ranged from 24 –

85 years with a mean age of 46.84 years ± 11.8 SD. Maximum number of cases were in 40-49 year age group. These findings are in accordance with the findings of El-Dosoky and Shahba (2011).[16]

Table 1: *Table showing comparison of ER status with variation in AgNOR size.*

ER Status	*No. of Cases with AgNOR Size*		*Total*
	2+	*3+*	
+ve	16	7	23
-ve	5	22	27
Total	21	29	50

P = 0.001

Table 2: *Table showing comparison of ER status with AgNOR dispersion.*

ER status	*No. of Cases with AgNOR Dispersion*		*Total*
	2+	*3+*	
+ve	8	15	23
-ve	2	25	27
Total	10	40	50

P < 0.05

AgNOR size and dispersion in ER +ve tumors was predominantly 2+ whereas AgNOR size and dispersion of ER-ve tumors was predominantly 3+. The difference was statistically significant (p = 0.001, and p < 0.05, Table 1, 2). This is in accordance with the study conducted by Ruschoff J in 1990 and Masiuk in 2007.[17,11]

AgNOR parameters can be used for the diagnosis as well as for assessing the prognosis of cancer.[10]ER positivity, PR positivity and HER2/neu status are useful in identifying patients who would benefit from systemic adjuvant therapy.[18] In this study 23 cases were ER +ve and 27 were ER –ve. The study conducted by Mudduwa (2009) also showed that prevalence of hormone receptor positive breast cancer in Asian countries is lower than Western world where more than 50% tumors express hormone receptors.[19]

Chemotherapy is expensive and has toxic effects. Moreover, patients show a varying response to treatment. Patients with advanced breast cancer when treated with neoadjuvant chemotherapy, may show complete recovery, stable disease or progression of disease. So, there is a need to identify biological markers to predict response and resistance to chemotherapy in our population.[20] Markers of biological aggressiveness are required in breast cancer to select those patients who are suitable for adjuvant radiotherapy and chemotherapy.[21] Hormone receptor status is a useful predictor of overall survival and response to adjuvant hormone therapy.[22] The association of AgNOR will be particularly useful in patients with same estrogen receptor status.

In this study it was ***concluded*** that AgNOR size and dispersion correlate with ER status. Therefore, AgNOR staining may be employed to assess tumor aggressiveness and possible response to therapy.

Contribution of Authors

KA: Did the research work and wrote the article. RJ: Helped in providing material for research and in doing research. AAI: Helped in research work and writing the article. SMS: Helped in checking the research work. MTH: Helped in checking the references.

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