CORRELATION OF MEAN ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS AND AgNOR PROLIFERATION INDEX WITH ESTROGEN RECEPTOR STATUS IN CARCINOMA OF BREAST

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ABSTRACT

Background and Objectives: Breast cancer is the most common malignancy and a leading cause of death in women throughout the world. Improved methods of detection and treatment have had a significant influence on disease outcome. Studies conducted at various places in Pakistan show that breast cancer is the most frequently recorded malignancy and a leading cause of cancer morbidity and mortality in our population. Moreover, in the majority of our population the tumors are already of large size when women first seek medical attention. Carcinoma of breast is one of the hormone dependent tumors.The objectives of this study were to compare the argyrophilic nucleolar organizer regions pattern in ER+ve and ER-ve tumors of invasive ductal carcinoma of breast.

Methods: Fifty cases of invasive ductal carcinoma of breast diagnosed on trucut biopsy, core biopsy, incision biopsy or excisional biopsy were collected from Lahore General Hospital, Lahore. Haematoxylin and Eosin staining, estrogen receptor staining and Argyrophilic nucleolar organizer regions staining were done.

Results: The mean age of the patients was 46.84 years. Maximum number of cases was in 40-49-year age group.The mean duration of symptoms was 4.62 months. Mean AgNOR in ER-ve tumors was not significantly higher than in ER+ve tumors. AgNOR proliferation index was significantly higher (p < 0.01) in ER-ve tumors than ER+ve tumors.

Conclusion: In this study of 50 cases of invasive ductal carcinoma of breast, ER+ve cases had low AgNOR parameters. Whereas the ER-ve cases had high AgNOR parameters. It is concluded that AgNOR parameters correlate with ER status. So, AgNOR staining can be done in place of ER staining as it is economical and more feasible in poor countries.

Key words: Argyrophilic Nucleolar organizer regions, estrogen receptor, breast carcinoma, Bloom and Richardson grade.

INTRODUCTION

Breast cancer is the most common malignancy and a leading cause of death in women throughout the world.[1] Improved methods of detection and treatment have shown a significant influence on disease outcome.[2] Studies conducted at various places in Pakistan also show that breast cancer is the most frequently recorded malignancy and a leading cause of cancer morbidity and mortality.[3]

Conditions which expose to prolonged effect of reproductive hormones e.g early menarche, late menopause, postmenopausal hormone therapy, and obesity increase the risk of breast cancer.[4-6] Genetic factors like BRCA1 and BRCA2 mutations, PTEN mutation and Peutz Jeghars syndrome also predispose to increased risk of breast cancer.[7]

Carcinoma of the breast is one of the hormone dependent tumors.[8] Estrogen receptor positive tumors are well differentiated.[9,10] Prognostic indicators of carcinoma of the breast other than histological classification and lymph node metastasis are proliferation activity analysis using oncogenes expression, such as c-erb B2, epidermal growth factor receptor, proteolytic enzyme cathepsin and Argyrophilic Nucleolar Organizer Regions (AgNORs) silver stains.[11] Proliferation markers also help in the categorization of different breast lesions which lie in the gray zone on routine histopathology.[12]

The nucleolar organizer regions are chromosomal loops of DNA involved in ribosomal synthesis. These are located on each of short arm of the acrocentric chromosomes.[13-15,21,22] The two argyrophilic proteins

associated with rRNA transcription and processing are nucleolin and nucleophosmin.[13] These proteins are identified by a silver colloidal staining technique and visualized as dark intranuclear dots under the microscope.[14] AgNOR technique has been applied in many areas of tumor pathology.[15] Statistically significant difference in the mean number of AgNORs has been found between normal, hyperplastic breast lesions and neoplastic breast lesions.[16]

OBJECTIVES
To compare the AgNORs pattern in patients of estrogen receptor positive and estrogen receptor negative breast carcinoma.

PATIENTS AND METHODS
Fifty samples of invasive ductal carcinoma of breast were collected in 10% formalin solution from Lahore General Hospital, Lahore. The tissues were processed in an automatic processor. The sections were taken on albuminized slides. H&E staining,[17] estrogen receptor staining[18] and AgNOR staining[15] were done. Histological diagnosis and grading of the tumor was done on H&E stained slides using Nottingham modification of Bloom and Richardson grading system.[12]

On ER staining, all the reactive nuclei were considered positive regardless of the intensity of the staining and the fraction of the positive cells were determined. A specimen was considered positive if ≥ 10% of the counted nuclei were positive.[18]

AgNOR staining showed the nuclei were stained light yellow and AgNORs were visualized as brown black discrete dots of variable size within the nuclei. Mean number of AgNOR per nucleus was counted on hundred random nuclei. The pAgNOR was the percentage of cells with ≥ 5 AgNORs per nucleus.

The data was analyzed using SPSS 19. The quantitative variables were expressed as mean ±SD. Mean AgNOR count and mean pAgNOR count in ER +ve and ER-ve carcinoma of breast was found. The collected data was analyzed by applying t-test. For statistical analysis a p value ≤ 0.05 was considered significant.

RESULTS
The ages of the patients ranged from 24-85 years with a mean of 46.84 years. Maximum number of cases was in 40 − 49 year age group (Fig. 1).

The duration of symptoms ranged from 2 − 12 months with a mean of 4.62 months (Fig. 2).

The size of the tumor ranged from 2-12cm. Mean tumor size was 3.7 ± 1.8 cm.

ER+ve tumors had a mean AgNOR count of 3.83 ± 0.87, whereas ER-ve tumors had a count of 4.4 ± 1.38 (Table 1, Fig. 4, 5).

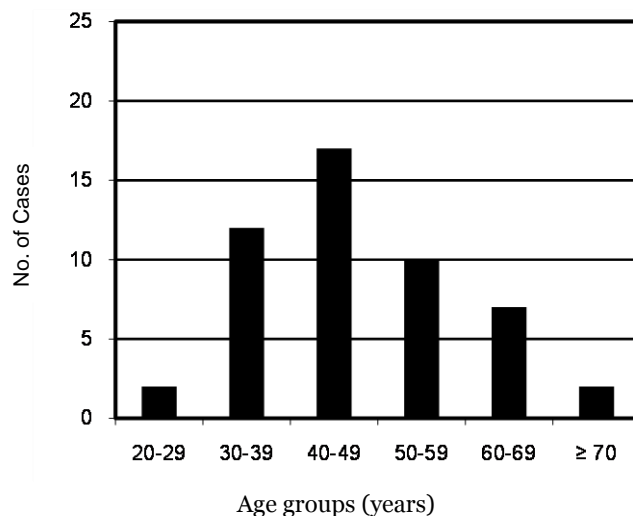ER-ve tumors had a higher (p < 0.01) AgNOR proliferative index of 37.6 ± 23.01 as compared to ER+ve

Age groups (years)
Fig. 1: *Age distribution of patients included in the study.*

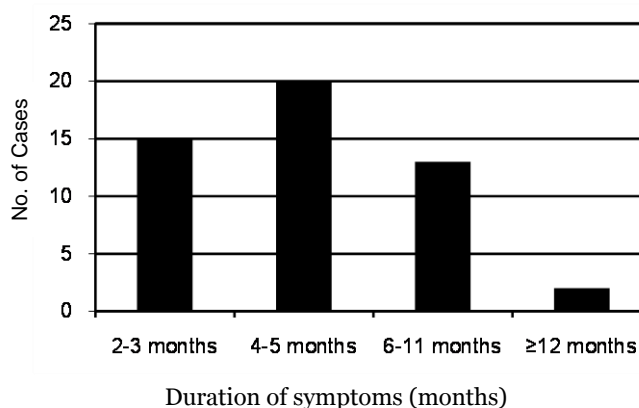Duration of symptoms (months)
Fig. 2: *Duration of symptoms at the time of diagnosis.*

Table 1: *Comparison of mean AgNOR count with ER status.*

ER status	Number of Cases	MAgNOR
		Mean ± SD
+ve	23	3.83 ± 0.87
-ve	27	4.4 ± 1.38

P = NS

Table 2: *Comparison of AgNOR proliferation index with ER status.*

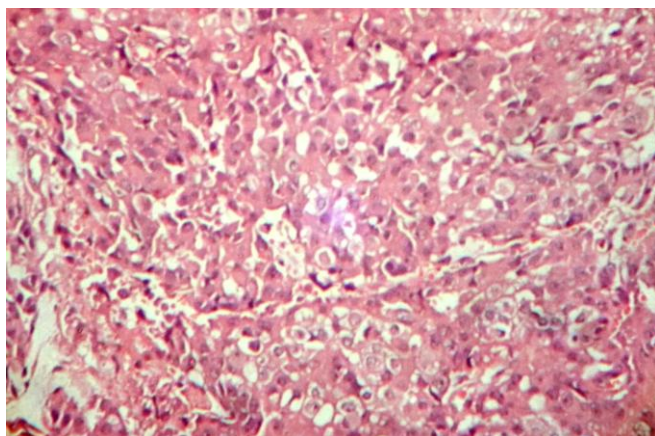| ER status	Number of Cases	PAgNOR
		Mean ± SD
+ve	23	20.82 ± 16.269
-ve	27	37.6 ± 23.015

P < 0.01

Fig. 3: *Photomicrograph of a section of invasive ductal carcinoma breast Grade III (H&E Staining × 400).*
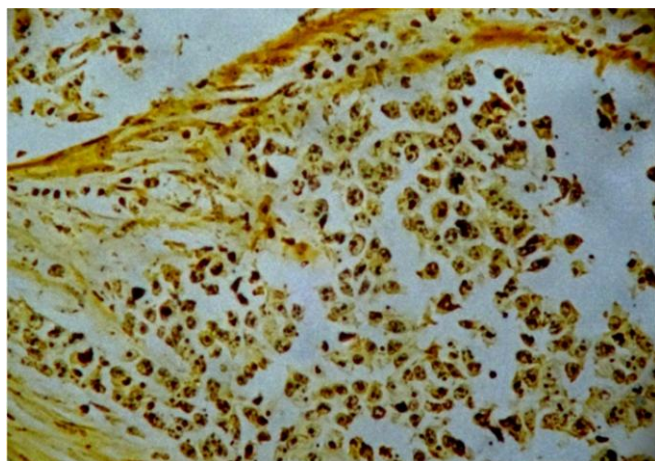

Fig. 4: *Photomicrograph of a section of invasive ductal carcinoma breast Grade III. (AgNOR Staining × 400).*
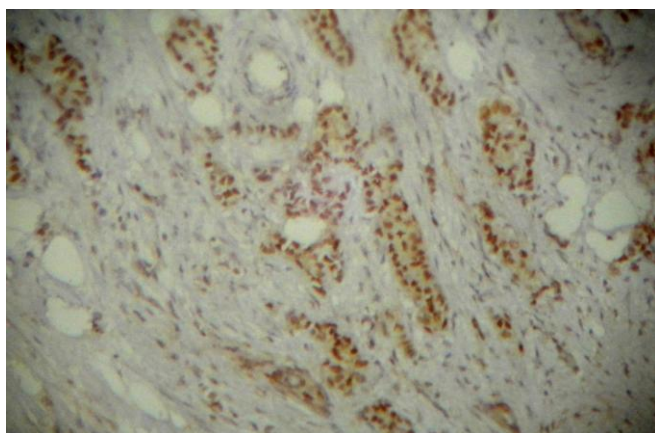

Fig. 5: *Photomicrograph of a section of invasive ductal carcinoma breast Grade II (ER Staining × 400).*

tumors which had pAgNOR 20.82 ± 16.26. The difference was statistically significant (Table 2, Fig. 4, 5).

DISCUSSION

Breast cancer is the major cause of mortality.[1,12] In Pakistan the incidence of breast cancer is increasing in the reproductive age.[6]

In our study the ages of the patients ranged from 24 - 85 years with a mean age of 46.84 years. Maximum number of cases was in 40-49 year age group. This is in accordance with the studies conducted at AFIP Rawalpindi, Pakistan and at Institute of Nuclear Medicine and Oncology, Lahore, Pakistan.[19,20]

In the present study the duration of symptoms ranged from 2 - 12 months. The mean duration of symptoms was 4.62 months and maximum number of cases had duration of symptoms of 4 to 5 months. Similar results were shown by Ahmed et al., in 2009.[3]

ER positivity, PR positivity and HER2/neu status are useful in identifying patients who benefit from systemic adjuvant therapy.[8] In this study 23 cases (46%) were ER +ve and 27 (54%) were ER -ve. The study conducted by Mudduwa[21] 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.

ER+ve tumors had a mean AgNOR count of 3.83 ± 0.87, whereas ER-ve tumors had a count of 4.4 ± 1.38. The difference was not statistically significant. This is in accordance with the study conducted by Ohri et al.[22] They found no significant relationship between AgNOR score and ER status. On the other hand ER-ve tumors had a significantly higher ($p<0.01$) proliferation index of 37.6 ± 23.01 as compared to ER+ve tumors which had pAgNOR value 20.82 ± 16.26 (Table 2, Fig 5). Therefore, this study showed that pAgNOR is a more useful parameter than AgNOR.

Chemotherapy is expensive and has toxic effects. Moreover, patients show a varying response to treatment. So, there is a need to identify biological markers to predict response and resistance to chemotherapy in our population.[20] Estrogen receptor status is a useful predictor of response to adjuvant hormone therapy.[23]

This study showed that different AgNOR parameters correlate with ER status. Therefore, AgNOR staining is a useful parameter to differentiate ER+ve and ER-ve tumors, as tumors having higher AgNOR or pAgNOR are more likely to be ER-ve.

It is ***concluded*** that in the light of our findings it may be suggested that AgNOR staining, which is far more economical than hormone receptor staining may be employed to assess tumor aggressiveness and possible response to adjuvant therapy.

CONTRIBUTION OF AUTHORS
K. A. did the research work and wrote the article. R. J. helped in providing material for research and in doing research. A. A. I. helped in research and writing the article. M. H. helped in modifying the article in final shape. S. M. S. helped in research.

REFERENCES
1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer Statistics. CA: a Cancer J Clin. 2008, March 1; 58 (2): 71-96.
2. Kraeina J, Siesling S, Vliegen IMH, Klaase JM, Ijzerman MJ. Individual risk profiling for breast cancer: towards tailored follow-up schemes. Brit J Cancer, 2013; 109: 866-871.
3. Ahmed Z, *Khurshid* A, Qureshi A, Idrees R, Asghar N, Kayani N. Breast carcinoma grading, estimation of tumor size, axillary lymph node status, staging and Nottingham Prognostic Index scoring on mastectomy specimens. Indian J Pathol & Microbiol. 2009; 52 (4): 477-481.
4. Rosai, J.Rosai and Ackerman's Surgical Pathology. 9th Ed. Missouri: Mosby; 2004: pp 1763-1876.
5. Bhurgri Y, Bhurgri A, Nishter S, Ahmed A, Usman A, Parvez S et al. Pakistan – country profile of cancer and cancer control 1995 – 2004. J Pak Med Assoc, 2006; 56 (3): 124-130.
6. Bhurgri Y, Pervez S, Kayani N, Bhurgri A, Usman A, Bashir I, et al. Cancer profile of Larkana – Pakistan (2000 – 2002). Asian Pacific J Cancer Prev. 2006; 7: 518-521.
7. Lester SC. The Breast. In: Kumar, V., Abbas, A.K., Fausto, N. Pathologic basis of disease. 8th Ed. Robins and Cotran. Philadelphia. Elsevier. 2010: pp 1065-1095.
8. Elzagheid A, Kuopio T, Pyrhonen S, Collan Y. Lymph node status as a guide to selection of available prognostic markers in breast cancer: the clinical practice of the future? Diagnostic Pathol, Online (cited 2006, November 8).
9. MdPaiman N, Md Ali SA, MdZin R, Moer KMZ, Md Amin WA, Nallusammy M et al. Estrogen Receptor Negative Breast Ductal Carcinoma: Clinicopathological Features and MIB-1 (Ki-67) Proliferation Index Association. PLOS ONE On line (cited in 2014).
10. Putti TC, Abd El-Rehim DM, Rakha EA, Paish CE, Lee AHS, Pinder SE et al. Estrogen receptor – negative breast carcinomas: a review of morphology and immunophenotypical analysis. Mod Pathol. 2005; 18: 26-35.
11. Mourad WA, Devloo S, Setrakian S. Predictors of invasion in ductal carcinoma in situ of the breast: the value of a scoring system. Annals Saudi Med. 1997; 17 (4): 427-431.
12. Manna AK, Pathak S, Sarkar DK. Role of proliferative markers in Breast lesions. Indian J Surg. 2010; 72: 57-61.
13. Derenzini M. The AgNORs. Micron. 2000; 31: 117-120.
14. Alarcon Romero L C, Illades Aguiar B, Flores Al-faro E, Teran Porcayo MA, Antonio Vejar, V, Reyes Maldonado E. AgNOR polymorphism association with squamous intra epithelial lesions and invasive carcinoma with HPV infection. Salud Publica Mex. 2009; 51: 134-140.
15. Bukhari MH, Niazi S, Khan SA, Hashmi I, Parveen S, Shafqat S et al. Modified methods of AgNOR staining for tissues and interpretation in histopathology. Int J Exp Pathol. 2007; 88 (1): 47-53.
16. Mijovic Z, Stefanovic N, Mihailovic D, Kostov M. Quantification of Argyrophylic Nucleolar Organiser Regions in estrogen receptor positive and estrogen receptor negative ductal breast carcinomas. *Facta Universitatis,* 2006; 13: 65-69.
17. Gamble M, Wilson I. The Haematoxilins and Eosins. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 5th Ed. London: Churchil Livingston; 2005: pp 125-38.
18. Ahmed HGK, Al-Adhraei MA, Ashankyty IM. Association between AgNORs and Immunohistochemical Expression of ER, PR, HER2/neu, and p53 in Breast carcinoma. PatholRes International, On line (cited in 2011).
19. Jamal S, Moghal S, Mamoon N, Mushtaq S, Luqman M, Anwar M. The patterns of malignant tumors: Tumor registry data analysis, AFIP, Rawalpindi Pakistan (1992-2001). J Pak Med Assoc. 2006; 56: 359-62.
20. Khokher, S., Mahmood, S., Khan, S.A. Response to neoadjuvant chemotherapy in patients with advanced breast cancer: A local hospital experience. Asian Pacific J Cancer Prev. 2010; 11: 303-308.
21. Mudduwa LKB. Quick score of hormone receptor status of breast carcinoma: Correlation with the other clinico-pathological prognostic parameters. Indian J Pathol Microbiol. 2009; 52 (2): 159-163.
22. Ohri AK, Ohri SK, Herbert A, Royle G, Taylor I. The relationship between clinical staging, oestrogen receptor status and silver – binding nucleolar organizer regions (AgNOR) in breast carcinoma. Eur J Surg Oncol. 1992; 18: 103-107.
23. Andre F and Pusztai L. Molecular classification of breast cancer: implication for selection of adjuvant chemotherapy. Oncology, 2006; 3 (11): 621-632.