# Role of AgNOR in Benign and Malignant Breast Lesions

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## Abstract

**Objectives:** To assess usefulness of argyrophilic nucleolar organizer regions (AgNORs) in distinguishing between the benign and the malignant breast lesions.

**Study design:** The studied samples included 48 formalin-fixed, paraffin-embedded breast tissues which consist of following diagnostic categories: Adenosis (n=8), ductal hyperplasia (n=6), infiltrative ductal carcinomas (n=14), infiltrative lobular carcinomas (n=5), and ductal carcinomas in situ (n=5). Standardized AgNORs analysis was performed on the above mentioned samples according to the guide lines of the committee on AgNORs. Quantification of AgNOR content was done by the usual counting method.

**Results:** The mean AgNORs counts of these studied cases were as follow : Adenosis 2.59+/-0.54, ductal hyperplasia 3.15+/-0.54, benign cysts 1.7+/-0.42, phylloides tumor 4.2+/-1.18, infiltrative ductal carcinomas 8.92+/-1.68, infiltrative lobular carcinomas 8.79+/-1.11and ductal carcinomas in situ 8.84+/-2.54. The differences in mean AgNORs counts between benign (2.58) and that of malignant lesions (8.84) were statistically significant (p-value 0.001).

**Conclusions:** The use of AgNORs analysis is found to be a helpful tool in addition to the conventional hematoxylin and eosin staining technique for the distinction between benign and malignant breast lesions.


## دور حساب المواقع المنظمة للنوية في التمييز بين الاورام الحميدة والخبيثة للثدي


### الخلاصة

ان التمييز بين الاورام الحميدة والخبيثة للثدي في بعض الاحيان يعاني من بعض الصعوبات. كما ان بعض الاورام الحميدة قد تتحول الى اخرى سرطانية. لذلك ولتشخيص اكثر دقة يمكن استعمال طرق تحديد المواقع المنظمة للنوية والتي تعطي انطباع عن قابلية تكاثر الخلايا باستعمال نترات الفضة.

**الاهداف:** لتقييم فائدة المواقع المنظمة للنوية المصبوغة في التمييز بين الاورام الحميدة والسرطانية للثدي.

**خطة البحث:** تضمنت الدراسة ٤٨ نموذجا لمرضى مثبتة بالفورمالين ومطمورة بالبارافين والتي تشمل ٨ حالات تكاثر غدي، ٦ فرط التنسج القنوي، ٨ اكياس حميدة، ٢ ورم ورقاني، ١٤ سرطان قنوي مرتشح، ٥ سرطان فصيصي مرتشح، و٥ حالات سرطان قنوي موضعي. وقد اجري التحليل القياسي للمواقع المنضمة للنوية المصبوغة وباستعمال طريقة التعداد المعروفة.

**النتائج:** كانت معدلات المواقع المنضمة للنوية المصبوغة كما ياتي: تكاثر غدي ٢,٥٩، فرط التنسج القنوي ٣،١٥، اكياس حميدة١،٧، ورم ورقاني٤،٢، سرطان قنوي مرتشح٨،٩٢، سرطان فصيصي مرتشح٨،٧٩، و حالات سرطان قنوي موضعي٨،٨٤. وكانت الفروقات بين المعدل الكلي للحالات الحميدة والمعدل الكلي للحالات السرطانية ذو اهمية من الناحية الاحصائية.

**الاستنتاج:** ان استعمال التحليل القياسي للمنظمة للنوية المصبوغة بالفضة يمكن ان يعتبر وسيلة مساعدة اضافية للتمييز بين الاورام الحميدة والسرطانية للثدي.


## Introduction

In the United States, each year approximately 100,000 new cases are diagnosed and around 30,000 patients die from the disease. The incidence is high in North America and northern Europe (91.4 new cases / 100,000 women/year), intermediate in southern Europe and Latin American countries, and low in most Asian and African countries [1]. In North America, North-Europe and Australia,

breast cancer is the commonest type of malignancy in women [2]. In the United Kingdom, it accounts for 20% of all cancers and is the commonest cause of death among women in the 35-55 age groups. Recent reports show there to be 39,500 new cases each year in U.K.[2] Breast carcinoma show increase incidence in the last years among Iraqi women in the last few years, it form about 31% of females cancers and about 31% of female cancers in Iraq, the highest number of patients were in $5^{th}$ decade of their life.[3] In one study performed in Saudi Arabia, here a retrospective analysis of malignant breast tumors seen at King Fahad University Hospital between 1982 to 1987. 1,658 Saudi nationals were admitted with different malignant conditions, out of this 69 (4.1%) females suffered from breast cancer [4].

**The argyrophilic staining of nuclear organizer region associated proteins (Ag-NOR):** Cells proliferation under normal physiological condition is accompanied by increased ribosomal biogenesis. The level of transcriptional activity is related to the amount of rDNA transcription machinery present in nuclear organizer region.[8] Nuclear organizer region (NORs) are segments of chromosomes where ribosomal genes are located and which correspond to secondary constrictions [9]. During interphase, NORs are located in both fibrillar centers and the closely associated dense fibrillar components of the nucleolus [10, 11, 12]. These structures contain all the necessary components of rRNA synthesis and are the site where the transcription of ribosomal genes occurs [13]. A peculiar group of highly argyrophilic acidic proteins is present in NORs, thus allowing NORs to be clearly and selectively visualized at the light microscopic level by specific silver nitrate staining procedure [14]. The silver stained NORs are called AgNORs. AgNORs were introduced in to tumor pathology in 1986 by Ploton et al[15], who noted that AgNORs were more abundant in malignant than in the benign neoplastic cells. Initially used as a parameter for the diagnosis of malignancy, the AgNORs parameter was found to be more useful for assessing the prognosis of cancer disease [16]. The relation between AgNORs values and cancer prognosis was explained on the basis of biological function of the NORs. In vitro and in vivo studies have shows that the quality of AgNORs is directly related to the ribosome biogenesis rates, which in proliferating cell is tightly related to the length of the cell cycle. The shorter the cell cycle, the greater the synthesis of rRNA for each time unit and, therefore, the quality of AgNORs present in the nucleolus. Thus, the AgNORs value was thought to be a measure of the rate of cell proliferation [17,18]. Because the tumor mass growth rate is one of the most important factors influencing the clinical outcome of cancer, faster growth was suggested to explain the worse prognosis of cancers with high AgNOR values compared with cancers with low AgNOR values [16].

## Material and Method

This is a retrospective study using 48 cases of different breast lesions which are selected randomly from a laboratory in Najaf all are formalin fixed paraffin-embedded tissue blocks, two sections were taken from each block each 4 micron thick, one section was stained with AgNOR method and the other was stained with hematoxylin and eosin stain for comparative morphological assessent. The nucleolar organizer regions are stained black/dark brown.

**Quantitative analysis of AgNOR proteins; The counting method**: After staining, representative areas in each section were selected and NORs were counted by single observer. In each case, the number of NORs were counted in 100 glandular epithelial cells using an oil-immersion lens at a magnification of x1000. with in large aggregates of AgNORs. Smaller dots were resolved by careful focusing and were counted individually. The results were expressed for each case as the mean AgNOR count per cell, and the results for each diagnostic category were expressed as the total of mean counts for each case divided by the number of cases.

**Statistical analysis:** The mean number of AgNOR, and S.D for each diagnostic group was calculated and the mean AgNOR for benign and malignant cases was also calculated separately. "t-test" was used to compare between the mean AgNORs of the different groups. P-value of less than 0.05 was considered statistically significant. Analysis of data (ANOVA) was used for evaluation of the differences in the mean AgNORs between benign and malignant conditions. All data were analyzed with SPSS software (statistical package for the social Sciences, version 11.0 for Window XP; SPSS, Inc. Chicago, I11).

## Results

**Table 1** showing mean AgNOR values, S.D., and range of the different studied groups.

| Groups | No.of cases | Mean AgNORs value | S.D | Ranges |
|---|---|---|---|---|
| **IDS** | 14 | 8.93 | 1.68 | 6.60-11.88 |
| **ILC** | 5 | 8.79 | 1.11 | 7.24-10.12 |
| **DCIS** | 5 | 8.84 | 1.56 | 5.96-10.24 |
| **Adenosis** | 8 | 2.54 | 0.54 | 2.12-3.84 |
| **Ductal hyperplasia** | 6 | 3.15 | 0.54 | |
| **Benign cysts** | 8 | 1.7 | 0.42 | 1.23-2.24 |
| **Phylloides tumor** | 2 | 4.2 | 1.18 | 3.36-5.04 |

**Table 2** comparison of mean AgNOR counts of malignant lesions with mean mean AgNOR counts of benign lesions

| Comparison | No. of cases | Mean AgNOR | P values* |
|---|---|---|---|
| **Benign** | 24 | 2.58 | |
| VS | | | 0.001 |
| **malignant lesion** | 28 | 8.84 | |

**P- value considered statistically highly significant if it is 0.001*

**Table 3** comparison of mean AgNOR of IDC with mean AgNOR counts of other benign and malignant cases.

| Comparison | No. of cases | Mean AgNOR | P-value* |
|---|---|---|---|
| **IDC** | 14 | 8.92 | |
| VS | | | 0.001 |
| **Adenosis** | 9 | 2.59 | |
| **IDC** | 14 | 8.92 | |
| VS | | | 0.001 |
| **Ductal hyperplasia** | 9 | 3.15 | |
| **IDC** | IDC | 8.92 | |
| VS | VS | | 0.001 |
| **Benign cysts** | Benign cysts | 1.70 | |
| **IDC** | 14 | 8.92 | |
| VS | | | 0.002 |
| **Phlloides tumor** | 2 | 4.20 | |
| **IDC** | 14 | 8.92 | |
| VS | | | 0.868 |
| **ILC** | 5 | 8.72 | |
| **IDC** | 14 | 8.92 | |
| VS | | | 0.733 |
| **DCIS** | 4 | 8.59 | |

**Table 4** comparison between mean AgNOR count of ILC with the mean AgNOR counts of other benign and malignant lesions in the studied groups.

| Comparison | No. of cases | Mean AgNOR | P-value* |
|---|---|---|---|
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.001 |
| **Adenosis** | 9 | 2.59 | |
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.001 |
| **Ductal hyperplasia** | 8 | 3.15 | |
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.001 |
| **Benign cysts** | 9 | 1.70 | |
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.005 |
| **Phylloides tumor** | 2 | 4.02 | |
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.868 |
| **IDC** | 14 | 8.92 | |
| **ILC** | 5 | 8.79 | |
| **VS** | | | 0.851 |
| **DCIS** | 4 | 8.59 | |

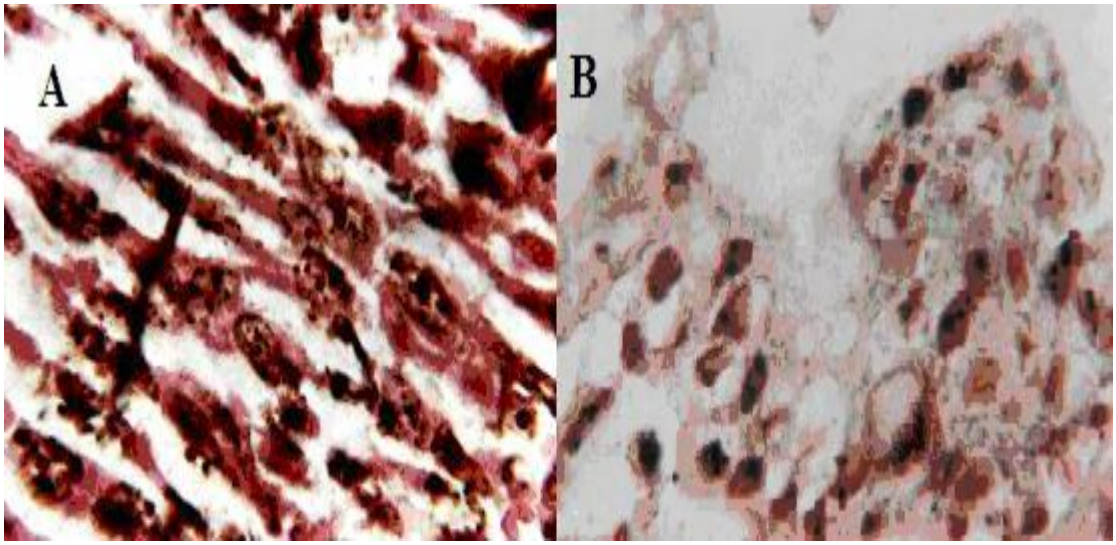


**Figure 1 A:** Adenosis of the breast showing uniform regular blak dots of AgNOR. Silver stain X100. **B:** Invasive ductal carcinoma showing more numerous and more irregular black dots for AgNOR. Silver stain X100.

## Discussion

In our study we found that the mean AgNOR count of adenoses was 2.59+/-0.54, for ductal hyperplasia 3.15+/- 0.15, for benign cysts 1.70+/-0.42 and for combined benign lesions was 2.58+/-0.42. In study by Giri DD et al they found that mean AgNOR count of adenoses was 1.96+/-0.24 and for ductal hyperplasia 2.21+/-0.30 [19]. These were near to our results. In study by Kumar et al , found that the mean AgNOR count of benign breast lesions was 1.88+/-0.19 and this is near to the AgNOR count of benign breast lesions in our study (2.21+/-0.30) [20]. In study by Dasgupta et al, found that the mean AgNOR count in fibrocystic disease was 1.61, this compare to 2.21+/-0.30 which was the mean AgNOR count of benign lesions in our study. [21] In our study, the mean AgNOR count of infiltrative ductal carcinomas was 8.92+/-1.68, for infiltrative lobular carcinomas 8.79+/-1.11, for ductal carcinoma in situ 8.59+/-1.94 and for combined malignant lesions the mean AgNOR count was 8.84+/-1.56. In study by Dasgupta et al[21] ,found that the mean AgNOR count for breast carcinomas was 12.10 and this was near to our results of mean AgNOR count of breast carcinomas 8.84+/-1.56 (mean of all malignant lesions in the study). Kumar A et al found that the mean AgNOR count for breast carcinomas was 6.61+/-1.75 and this lower than that of our study (8.84+/-1.56). [20]. Giri DD et al found that mean AgNOR count of ductal carcinoma in situ was 3.75+/-1.83 and that for invasive carcinoma was (4.22+/-1.18) [20]. These results were lower than those in our study. Rzyrmowska J found in their study that the mean AgNOR count in lobular carcinomas was 3.55+/-0.56 and in intraductal carcinoma was 4.83+/-1.20. These were lower than the the mean AgNOR count in lobular carcinomas which was8.79+/-1.11 and that of ductal carcinoma in situ (8.59+/-1.94) [22]. In study by Gupta JR et al found that the mean AgNOR count of breast carcinomas may reach up to 16.90 dots per tumor cell nucleus and the mean count was 6.26 dots [23]. These agreed with the mean AgNOR count of breast carcinomas which was (8.84+/-1.56). In study by siviridis E and Sims B found that the mean AgNOR count of

breast carcinomas was 8.57+/-2.63 especially in breast carcinoma that had metastases in the axillary lymph nodes. This near to the results in our study that the mean AgNOR count of breast carcinomas was(8.84+/-1.56) [24]. In our study we found that the differences in mean AgNOR count between carcinoma 8.84+/-1.56 and that of benign breast lesions (2.58+/-0.90) is highly significant (p-value 0.001). In study by Aubele M found that their was significant differences in AgNOR count between breast carcinoma non carcinomatous ductal epithelium (p-value<or=0.001) [25], and this goes with our results. In our study we found that the differences in mean AgNOR count between malignant groups were not significant (p-value 0.785). Aubele M et al found that considering metastases free interval of patients the same AgNOR features showed an independent prognostic validity)[25]. In our study we found that the differences in mean AgNOR count between infiltrative ductal carcinomas and ductal hyperplasia , infiltrative lobular carcinomas and ductal hyperplasia , and between ductal carcinoma in situ and ductal hyperplasia is significant (p-value 0.001, p-value 0.001, value 0.001) respectively. This was agreed with study by Guski et al [26] who found that differences in mean AgNOR count between ductal hyperplasia and ductal carcinoma in situ or infiltrative ductal carcinomas was statistically significant. Dasgupta et al found that the differences in mean AgNOR count between benign and malignant breast disease was significant[21] and this agreed with our results. Bankfalvi et al found significant differences in mean AgNOR count between benign and malignant breast diseases[27]. This agreed with our results. Also they found that AgNOR may give insight into the biological background of breast carcinogenesis, differentiation and tumor progression and may also underlie the independent prognostic value of AgNORs in breast cancer [27]. In study by Simha et al, they found that AgNOR value could sharply distinguish benign from malignant lesions. Among the malignant lesions, an attempt to determine the value of AgNOR counts in prognostication was made. AgNOR counts correlated with tumor size, mitosis and desmoplasia. ER/PR negative tumors did not show a tendency for high NOR did count, but lymph nodes metastasis, which is considered one of the most reliable prognostic indicators, not concur with AgNOR count in the study. These results indicate that AgNOR counts cannot be used as a sole independent marker in breast cancer prognostication[28]. This agreed with our results that show significant differences in AgNOR count between benign and malignant breast diseases. Cecarelli D et al in their study demonstrate a highly significant association between AgNOR proteins quantity and tumor prognosis, AgNOR value showed independent prognostic value together with ki67-labelling index (L1), nodes state and tumor size[29]. Subramanian S et al found that AgNOR count was significantly related to the cell size , histological grade and presence of metastasis. They observed that the number of AgNOR count significantly related to the cell size , histological grade and presence of metastases[30]. Derenzini M et al in their study found that the prognostic value of AgNOR parameter depend on the status of the tumor expression proteins pRb and p53[31]. Agarwal PK et al found that the malignant breast lesions have higher number of mean AgNOR counts than benign breast lesions. Also their results

showed correlation between the AgNOR counts and size of the tumor, axillary lymph nodes status and age of the patients [32]. This agreed with the results in our study which showed significant differences in AgNOR counts between benign and malignant breast diseases (p-value <or=0.001). Dr. Faiza Al-Rawi found in her study that the mean AgNOR of benign breast lesions was 2.58 , while that of malignant breast lesions was 8.95 and this significantly exceed the mean AgNORs of benign breast lesions (p-value <0.001). [33].

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