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Original Research Paper

Pathology

AGNORS IN BENIGN AND MALIGNANT BREAST LESIONS

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ABSTRACT Objective of our study was to assess usefulness of argyrophilic nucleolar organizer regions (AgNORs) in distinguishing between the benign and the malignant breast lesions. 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.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).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.

KEYWORDS : AgNORs,Benign and Malignant breast lesions.

INTRODUCTION

In India,one of the commonest cause of death among women in the 35-55 age groups is the breast lesions.. 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 . The silver stained NORs are called AgNORs. AgNORs were introduced in to tumor pathology in 1986 by Ploton et al, 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.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 . 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 .

MATERIAL AND METHOD

In our study,we used 48 cases of different breast lesions which are selected randomly from laboratory, 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. 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. 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.

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).

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. 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.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 ,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).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). 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. 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). 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),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). 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, 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 and this agreed with our results. Bankfalvi et al found significant differences in mean AgNOR count between benign and malignant breast diseases.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. 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.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.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.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. 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.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).

CONCLUSION

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.

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