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AgNOR, Ki-67 and PCNA expression in fibroepithelial tumours of the breast in correlation with morphological features

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The authors retrospectively reviewed the cytological slides of 44 histopathologically confirmed fibroepithelial lesions of the breast, of which 11 were fibroadenoma (FA), 19 benign phyllodes tumours (PTLGM), 8 borderline (PTBM) and 6 malignant (PTHGM). The 2 FA misdiagnosed as PTLGM in cytological smears were both of cellular type. NORS were quantified in a series of the above cases using the silver-colloid method. Expression of Ki-67 and PCNA were evaluated by immunohistochemistry on sections from the corresponding paraffin blocks. The results were compared with morphological parameters.

In phyllodes tumours (PT), the AgNOR scores showed a tendency to increase with degrees of malignancy. There was significant correlation between AgNOR counts and proliferation rates as determined by Ki-67 and PCNA immunostaining. Ki-67 and PCNA expression correlated with mitotic count, stromal overgrowth, cellularity and atypia in PT. Determination of the AgNOR number per cell revealed an overlap between FA and PTLGM. The proliferating activity determined by immunohistochemistry with Ki-67 and PCNA antibodies did not reveal any significant difference between FA and PTLGM.

In summary, Ki-67 and PCNA expression is suggested as a marker of stromal element proliferation. The results obtained confirm the diagnostic difficulties in distinguishing PTLGM from FA of the cellular type using fine needle aspiration cytology.

Key words: AgNORs , Ki-67, PCNA, fibroepithelial tumours of the breast

INTRODUCTION

Fibroepithelial tumours of the breast include fibroadenoma and phyllodes tumours. These are biphasic lesions, whose biology is mainly determined by the stromal element. FA is benign neoplasm. Phyllodes tumours are well known for their unpredictable behaviour [4, 7]. Previous studies have emphasised that several characteristics of the stromal component in PT must be considered together in assessing their malignant potential [7]. In addition to conventional pathological parameters, several methods, including the determination of proliferative activity, provide information concerning the biological behaviour and clinical outcome of these neoplasms [8]. Proliferative activity can be determined by different immunohistochemical markers, such as Ki-67 and PCNA or by quantitative analysis of AgNORs [2]. Some investigators have tried to prove the usefulness of

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such determination in differential diagnoses of benign and malignant lesions of the breast [2]. With regard to fibroepithelial tumours it is extremely important to establish the correct diagnosis from the therapeutic point of view [6].

The aim of our study was the evaluation of AgNOR, KI-67 and PCNA expression in fibroepithelial tumours of the breast in correlation with morphological parameters.

MATERIAL AND METHODS

Cytological slides and formalin-fixed, paraffin-embedded tissues from 44 fibroepithelial tumours of the breast diagnosed in the Centre for Oncology in Białystok were retrospectively reviewed. These included 11 fibroadenomas (FA), 19 benign phyllodes tumours (TLGM), 8 borderline phyllodes tumours (PTBM) and 6 high-grade malignant phyllodes tumours (PTHGM). These were histologically classified, taking into account mitotic activity, atypia, stromal overgrowth and cellularity, using a scale of 1–3. The cytological material, obtained by FNAB, was routinely stained with HE, destained and then stained again using the silver-colloid method to visualise NORs. Mean AgNOR count was evaluated in each case in 100 randomly chosen nuclei. Expressions of Ki-67 and PCNA were evaluated by immunohistochemistry on sections from the corresponding paraffin blocks. Immunostaining was performed with monoclonal antibodies PCNA (PC-10, Dako) and Ki-67 (Ki-67, Dako) using a LSAB KIT with DAB as a chromogen. The percentages of cells expressing Ki-67 and PCNA were determined by counting 500 stromal cells per slide. The percentage of positive nuclei was expressed as the labelling index (LI). The significance of the differences in scores between all the groups was assessed using the chi-squared and Mann-Whitney test. The correlation between the scores and counts and pathological variables was evaluated using Pearson and Spermans correlation analysis. Values of $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Fine needle aspiration cytology, now an integral part of the pre-operative investigation of breast lesions, does not always precisely define the character of a fibroephitelial tumour. In our series the initial cytological diagnosis was confirmed histopathologically, although there were some difficulties in correct qualification of the lesion. The 2 FA misdiagnosed as PTLGM in cytological smears were FA of the cellular type. Determination of the AgNOR number per cell revealed an overlap between FA and PTLGM. Proliferating activity determined by immunohistochemistry with Ki-67 and PCNA antibodies did not reveal any significant difference between the lesions. Krishnamurthy et al. [5] distinguish both neoplasms in H-E slides based on a proportion of long, short, round and oval nuclei. The presence of long nuclei above 30% qualifies the lesion to the PT group. The lesions with 10–30% long nuclei should be categorised as indeterminate. The stromal cellularity of some FA may overlap with that of benign PT. Immunohistochemical evaluation of proliferating activity using Ki-67 antibodies performed by Kaya et al. [3] revealed no significant difference between FA of the cellular type and PTLGM. Hasebe et al. [1] noted a significant relationship between proliferating activity and stromal cellularity regulated by fibroblast growth factor and fibroblast growth factor receptor. Expression of the factors examined was higher in FA of the cellular type in comparison with FA of the conventional type. The above data may explain the diagnostic difficulties in distinguishing PTLGM from FA of the cellular type using FNA. In the series of PT examined the mean number of AgNOR dots per nucleus was 1.38; 1.94; 3.18; 4.98 in FA, PTLGM, PTBM and PTHGM respectively. Mean Ki-67 — LI was 1.98; 2.91; 2.13; 7.63 in FA, PTLGM, PTBM and PTHGM respectively. Mean PCNA LI was 3.93; 5.94; 31.78; 83.87 in FA, PTLGM, PTBM and PTHGM respectively. The AgNOR values showed a tendency to increase with higher degrees of malignancy. There was a significant correlation between AgNOR counts and proliferation rates as determined by Ki-67 and PCNA immunostaining. Ki-67 and PCNA expression correlated significantly with mitotic counts, stromal overgrowth, cellularity and atypia. Our results are in agreement with others [8] and prove Ki-67 and PCNA antigens to be useful in the analysis of stromal proliferation in phyllodes tumours.

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