Turk J Med Sci
31 (2001) 523-528

Sema HÜCÜMENOĞLU[1]
Handan KAYA[2]
Esin KOTİLOĞLU[2]
Gülben ERDEM[3]
Evrim DEMİRAY[1]
Gülsün EKİCİOĞLU[2]

Hormonal Profile and Agnor Values in Pituitary Adenomas

Received: May 09, 2001

[1]Department of Pathology, SSK Ankara Training and Research Hospital, [2]Department of Pathology, Faculty of Medicine, Marmara University, [3]Department of Pathology, SSK Istanbul Training and Research Hospital, İstanbul - TURKEY

Abstract: Nucleolar organizer regions (NORs) are chromosomal segments in which ribosomal RNA is encoded. The AgNOR technique, which reveals these regions, has rarely been used in diagnosis and preceding the prognosis of pituitary adenoma. The purpose of this study was to evaluate the correlation of hormonal profile with the AgNOR counts, and the correlation of recurrence with the AgNOR counts and to determine the concordance between the evaluations of two different observers. This study included 33 patients with pituitary adenoma. The slides were stained with hematoxylin and eosin, silver colloid method for NORs and immunohistochemistry for the hormone expressions. Consistency was strong among the two pathologists (for hormonal profiling, the kappa test was used and $p<0.001$; for AgNOR count, the intraclass correlation coefficient was 0.83). There was no significant relationship between mean AgNOR counts and hormone expression ($p>0.05$). In addition, we found no difference between recurrent patients and non-recurrent ones in terms of mean AgNOR numbers ($p>0.05$). However, in recurrent patients, nucleolar silver staining was more frequently detected as clusters of AgNORs.

Key Words: Pituitary adenoma, AgNOR, immunohistochemistry, recurrence.

Introduction

Pituitary adenomas constitute 10% of all intracranial tumors. Although these are benign lesions, their growth rate is variable, and the recurrence risk is about 10-35%. They may be hormonally inactive or synthesize one or more hormones, i.e., FSH, LH, TSH, GH, ACTH and PRL (1).

NOR related proteins are acidic non-histone proteins stained by silver (2-4). Although their real biological functions are disputed, the AgNOR result for each cell is considered as a marker of cellular activity (2, 5). Today, AgNOR staining has diagnostic and prognostic value in hyperplastic events and malignancies such as lymphomas, breast carcinomas and hepatomas, although there are some opposing opinions (2, 3, 6, 7). Some researchers also consider it to be a marker showing the differentiation grade of malignant neoplasms (3).

Three main configurations of AgNOR are identified (4):

1. Round argyrophylic structures indicating the small nucleolus of stable cells

2. AgNOR groups in a matrix indicating the nucleolus

3. Small point-like structures in nucleoplasm

In this study, we investigated the correlation between hormone profile and AgNOR values in addition to the correlation between recurrence and AgNOR values, considering the conformity among the observers.

Materials and Methods

Thirty-three pituitary adenoma patients, diagnosed in the pathology department of SSK Ankara Education Hospital between 1984 and 1998, were examined. Five cases were recurrent and the average follow–up period was 3.4 years (41 months). In 30 patients, adenomas were intracapsular and were almost totally excised, but in three patients, partial excision was carried out. Two cases recurred because of the residual tumor. Only five cases recurred in the totally excised group.

AgNOR Staining

Sections of 3-4 micron thickness were cut from the routinely processed paraffin blocks. These were dewaxed in xylene and then rehydrated through ethanols to distilled, deionized water. The AgNOR solution was

prepared by dissolving gelatine in 1g/dl aqueous formic acid at a concentration of 2g/dl. This solution was mixed (1: 2 volumes) with 50 g/dl aqueous silver nitrate solution. The sections were washed off with deionized water after keeping them in darkness for 30 min at room temperature. They were closed after dehydration.

Counting procedure

AgNOR stained sections were examined by two pathologists using a x1000 oil immersion objective. For each case, all dark points indicating the AgNOR proteins in the nucleus were counted in 100 tumor cells. Stromal cells and inflammatory cells were excluded. Dark points were counted one by one when it was possible to see the inside of the nucleolus, but counted as one when the interior was not clear. Then the mean AgNOR value for each case was calculated.

In addition, for hormone profiling the sections taken from all patients were stained with FSH, TSH, PRL, LH, GH and ACTH immunohistochemically by using the streptavidin-biotin technique. Each case was stained for all hormones. These slides were also examined by two pathologists, under a light microscope. In order to determine the differences between the two observers, the kappa test was used for hormone profiling and intraclass correlation coefficient test for mean AgNOR values. The Mann-Whitney test was used to determine the correlation between hormone profiles and AgNOR values. T test was used to compare the recurrent cases with the others.

Results

The ages of the patients ranged from 16 to 69. Eighteen cases were women and 15 were men. Among the recurrent cases, two were in women and three in men, with ages ranging from 30 to 62. In both groups, there were complaints of headache, decreased vision and enlargements of the hands and feet. In female patients, galactorrhea and amenorrhea were additional complaints. Immunohistochemically detected hormone profiles in comparison with the clinical findings are shown in Table 1. In 27 cases, one hormone was synthesized, whereas in six cases, the number of hormones synthesized was more than one. It was observed that the immunohistochemically detected hormone profile had a correlation with clinical findings.

Table 1. Hormonal profiles of the cases immunohistochemically (IHC) and clinically. Recurrent cases are marked with an asterisk.

Case	1st observer(IHC)	2nd observer(IHC)	Clinically
1	Hormone --	Hormone --	Hormone --
2	Hormone --	Hormone --	Hormone --
3	FSH, GH	Hormone --	FSH, GH
*4	Hormone --	Hormone --	Hormone --
5	Hormone --	Hormone --	Hormone --
6	PRL	PRL, GH	PRL, GH
7	Hormone --	Hormone --	Hormone --
8	PRL	PRL	PRL
9	GH	GH	GH
10	PRL	PRL	Hormone --
11	PRL	PRL	PRL
12	PRL	PRL	PRL
*13	PRL, GH	PRL, GH	PRL
14	PRL	PRL	PRL
15	GH	GH	GH
16	FSH	FSH	FSH
*17	Hormone --	Hormone --	Hormone --
*18	Hormone --	Hormone --	GH
19	GH	Hormone --	GH
20	Hormone --	Hormone --	PRL
21	FSH, GH	FSH, GH	Hormone --
22	ACTH	ACTH	ACTH
23	PRL, GH	PRL, GH	PRL
24	Hormone --	Hormone --	Hormone --
25	Hormone --	Hormone --	Hormone --
26	Hormone--	Hormone --	PRL
27	Hormone --	Hormone --	Hormone --
28	PRL	Hormone --	PRL
29	GH	GH	GH
30	PRL, LH, GH	GH, PRL	PRL, LH
31	Hormone --	Hormone--	Hormone --
32	GH	GH	GH
*33	PRL	PRL	PRL

AgNORs were observed as dark points of variable size in the nucleus and in some cases in the nucleolus (Figures 1, 2).

The mean AgNOR values are shown in Table 2. The mean AgNOR values according to the hormones were as follows: hormone (-): 2. 06, GH: 1. 85, FSH: 1. 79, PRL: 1. 96, ACTH: 2. 63, PRL and GH mixed: 1. 63, FSH and GH mixed: 1. 15, LH, GH and PRL mixed: 1. 83.

AgNOR values according to the hormones are shown in Figure 3. There was no correlation between hormones and the AgNOR values ($p>0.05$). For the five recurrent cases, the mean AgNOR value was 1. 66, and it was 2. 02 for the others. No significant difference between these

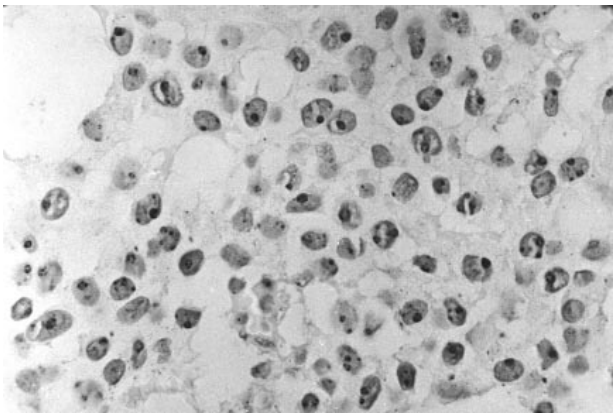

Figure 1. AgNOR spots in nuclei in a case of pituitary adenoma (AgNOR x1000).

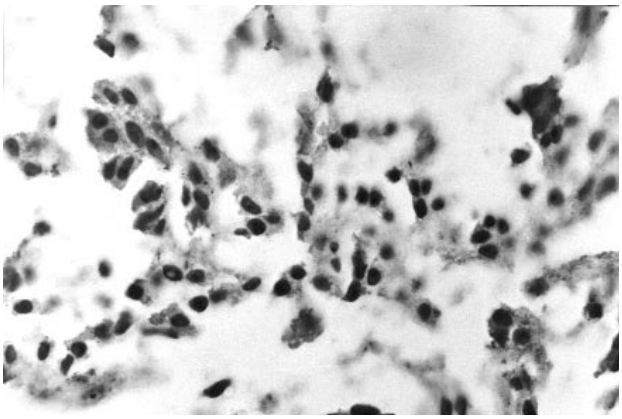

Figure 2. A recurrent pituitary adenoma. AgNOR clusters in nuclei are shown in black (AgNOR x1000).

two groups was observed. Mean AgNOR values for recurrent and nonrecurrent cases are shown in Figure 4. Interior of the nucleolus was counted as one in five recurrent (100%) and 12 non-recurrent (50%) cases. There is strong conformity between the two observers in evaluating the immunohistochemical staining and the AgNOR values ($p<0.01$). Immunohistochemical GH positivity in the tumor cells is seen in Figure 5.

Discussion

NOR proteins are the r DNA areas. They are located in the nucleus and contain the gene for ribozomal RNA. Five chromosomes contain NOR. NOR-related argyrophilic molecules (RNA polymerase 1, C23pr, a molecule like C23 but smaller, another molecule with a molecular mass of 80 kDa) can be demonstrated immunologically (8).

Table 2. Mean AgNOR values. Recurrent cases are marked with an asterisk

Case	1st observer	2nd observer
1	3. 11	3. 46
2	2. 58	2. 57
3	1. 32	1. 30
*4	1. 69	1. 69
5	1. 06	1. 00
6	1. 44	1. 42
7	3. 12	3. 10
8	2. 18	1. 88
9	2. 06	2. 01
10	2. 87	2. 65
11	1. 82	1. 80
12	3. 75	1. 62
*13	1. 62	1. 51
14	1. 00	1. 00
15	1. 28	1. 23
16	1. 80	1. 79
*17	1. 57	1. 37
*18	1. 50	1. 49
19	4. 17	4. 15
20	2. 18	2. 17
21	1. 00	1. 00
22	2. 81	2. 46
23	2. 02	1. 79
24	1. 90	1. 15
25	2. 56	1. 64
26	1. 91	1. 82
27	2. 60	2. 32
28	1. 35	1. 35
29	1. 85	1. 75
30	1. 83	1. 83
31	1. 99	1. 76
32	1. 87	1. 87
*33	2. 10	2. 10

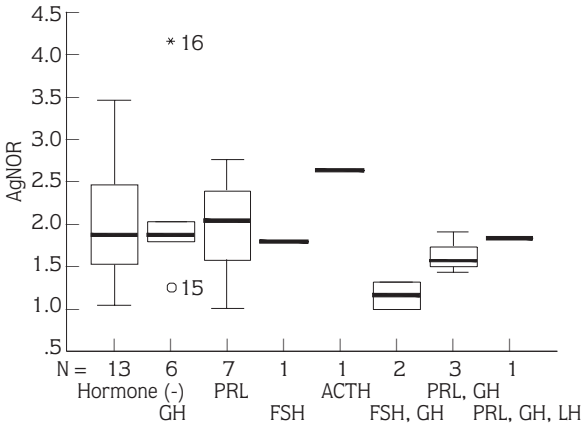

Figure 3. Graph of the mean AgNOR values for hormones.

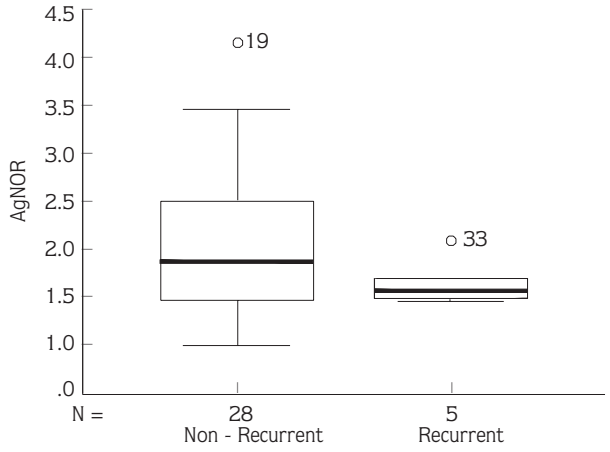

Figure 4. Graph of the mean AgNOR values of recurrent and non-recurrent cases.

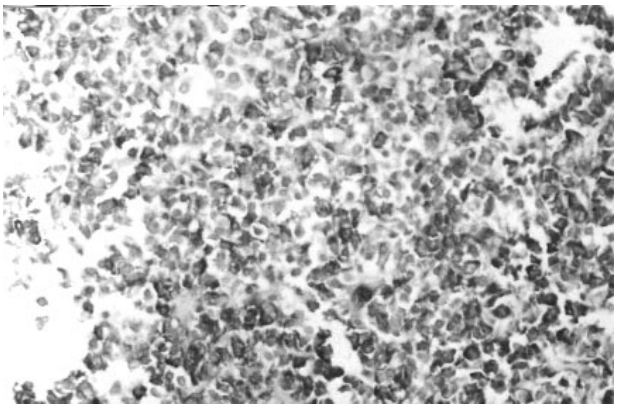

Figure 5. Pituitary adenoma. (+) immunoreactivity for growth hormone in tumor cells (immunoperoxidase x 400).

The AgNOR technique is a practical and simple technique. It is used as a marker for tumor cell proliferation by pathologists. AgNOR values are correlated with Ki-67 immunoreaction and DNA flow cytometry (4, 9, 10).

There are two different methods for counting AgNORs. In the first method, the AgNOR clusters are considered to be a single structure (2). In the second, if it is possible to see inside, it is counted one by one, and if it is not possible it is again counted as a single structure. Orrell et al. proposed that it was necessary to count intranucleolar spots as well as the extranucleolar spots (11). However, Howatt et al. Stated that counting the intranucleolar spots led to intraobserver variability (12). Other scientists did not find either technique to be superior (13). As a result of these differences in the counting techniques, it is expressed that the morphology and the distribution pattern of AgNOR, in addition to the AgNOR values, were meaningful (13, 14).

In the present study, the AgNOR counting was done by evaluating the spots in the nucleus and nucleolus (2). The spots in the nucleolus were counted as one since the intranucleolar area was not clear. No significant difference was detected between the AgNOR values of recurrent and nonrecurrent cases. NOR regions in the nucleolus were not clear in all recurrent and 50% of nonrecurrent cases. For this reason, we think that cases with nucleolar AgNOR staining as clusters have a higher probability of recurrence. The importance of clusters and spots in counting varies (4, 15, 16). Clusters indicate nucleolar structures and they are formed when ribosomal genes are active and the interphase is long enough (17, 18). Grotto et al. observed widespread multiple points in rapidly proliferating cells. Clusters are more common in undifferentiated cells and NORs not only show the activation level but also the differentiation degree. As the cells become differentiated, AgNOR staining becomes point-like.

The number of nuclei to be counted for obtaining AgNOR values is also a subject for discussion. While Orrell et al. preferred 30 nuclei, Aoki et al. preferred 200 (2). In the present study, we counted 100 nuclei.

Higher AgNOR values are observed in malignant cells than in benign and non-neoplastic cells (7). Therefore they have been studied in many different tumor types (2, 3, 6). Crocker and Nar used the AgNOR technique to differentiate high and low grade non-Hodgkin lymphomas (19). Crocker and Skilbeck applied this method to nevus and malignant melanoma cases, whereas Egan et al. used it in small round cell carcinomas of childhood (8, 20-22). In a study by Elagöz et al., the value of the AgNOR method alone in the diagnosis of melanotic lesions is discussed. It was concluded that the value of the AgNOR method alone is limited and other techniques such as visual analysis are required (13).

In our study, mean AgNOR values for pituitary adenomas were between 1 and 4.17. The highest values were observed in hormonally inactive cases and no significant relation between AgNOR values and hormones was demonstrated. In recurrent cases, the AgNOR values were observed at low levels. Although hormone (-) cases have the highest AgNOR values, in three recurrent cases no hormone expression was observed.

The differences between the values obtained by each observer is about 10-12% in the literature (13, 23, 24). The reasons for these differences are the nonstandardized method, fixation conditions, thickness of sections, time for staining, heterogeneity of tumoral tissues, evaluation of different areas by each observer, and including the lymphocytes, histiocytes and endothelial cells while counting (13, 15, 25, 26). These problems are less significant in cytological preparations, since all cells are smeared on slides. When the counting technique is standardized, the conformity between observers increases. In our study this conformity was strong (intraclass correlation coefficient test: 0. 83).

Ten to thirty-five percent of pitutary adenomas recur in 4-20 years (1). Of our cases, 15. 1% are recurrent. Mindermann et al. found 6. 4% recurrent cases in a study composed of 65 pituitary adenoma cases (27). In a study by Hsu et al. the ages of patients with recurrent pituitary adenomas were between 23 and 74. Our recurrent cases occurred in patients between 30 and 62 years old.

In two cases in our study, no hormone or related clinical findings were detected, while there was immunohistochemical positivity. It is known that 30-40% of pituitary adenomas are nonsecretory, although most of them stain positive for hormones immunohistochemically (28). In another two cases, although there was a hormonal increase in the blood and corresponding clinical findings, no immunohistochemical positivity was detected in the tissue. This can be explained by the mechanism of stalk inhibition in the pituitary gland (29). Two of the five recurrent cases were hormone (-) both clinically and immunohistochemically. One of the remaining cases was PRL (+) both clinically and immunohistochemically. Another one was hormone (-) immunohistochemically and GH (+) clinically. While the last case was PRL and GH (+) immunohistochemically, it was only PRL (+) clinically. Carboni et al. found no prominent relation between hormone expression in pituitary adenomas and the recurrence and the proliferation index (30).

In conclusion, in our opinion, although AgNOR values, morphology and distribution properties are used for many organs, their value is limited in pituitary adenomas and they should be used with other proliferation markers.

Correspondence author:
Sema HÜCÜMENOĞLU
Hoşdere C. No. 164/3
06550 Y. Ayrancı, Ankara - TURKEY

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