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SILVER STAINING OF NUCLEOLAR ORGANIZER REGIONS IN GIANT-CELL TUMORS OF BONE AND CLINICAL CORRELATIONS

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ABSTRACT: A silver colloid staining technique was applied to 30 cases of giant-cell tumor of bone (GCT) and 14 cases of aneurysmal bone cysts (ABC) for identifying nucleolar organizer region-associated protiens. (Ag-NORS). It has been suggested that the number of NOR's may reflect nuclear and cellular activity. The mean number of Ag-NOR's per cell were found to be twice as much for GCT(3,30 ± 0,0308) as the mean numbers of Ag-NORs per cell for ABC (1,603 ± 0,634) ($p<0,01$). GCT's of radiologic grade III revealed higher Ag-NOR counts per cell as compred to Grade I+II tumors ($p<0.01$). Tumors consisting of mostly rounded stromal cells showed higher Ag-NOR counts than tumors with mostly spindle shaped stromal cells ($p<0,01$). Patients older than 30 years and younger than 20 years of age have higher Ag-NOR counts than 20-30 years age range ($p<0,01$). Tumors with soft tissue invasion and/or lung metastasis have higher Ag-NOR contents per cell ($p<0,01$). Tumors which are located at weight-bearing locations have lower mean Ag-NOR countings than tumors at non-weight bearing locations ($p<0,01$). A count of more than 2,0 Ag-NORs per cell would favor a diagnosis of GCT rather than ABC.A count of more than 4,0 Ag-NORs per cell would favor a proliferating course.

INTRODUCTION: The nucleolar organizer regions (NORs) are loops of DNA which possess ribosomal ribonucleic acid (rRNA) genes. They are located in the nucleoli. The argyrophil technique stains NOR-associated proteins whose nature is not fully understood. It has been suggested that the numbers of NORs may reflect nuclear and cellular activity (1,4,5,10,13,14,18,19,22,29). Most specialists agree that the giant cell tumor (GCT) of the bone is a distinct clinicopathologic entity. Not all agree how to classify this tumor biological (2,20,21). In this study, we applied the argyrophil stain to giant cell tumors of bone and to aneurysmal bone cysts (ABC) to see if it would facilitate the distinction between these two and to predicit any prognostic significance in giant cell tumors.

MATERİALS AND METHODS

30 GCT and 14 ABC were examined. Clinical findings for each GCT case were obtained from relevant hospital records. Percent distributions of age and sex of GCT are shown in (Fig. 1).

Radiologic grading was made according to grading system used by Campanacci et. al. (3).

3 milimicron thick slices belonging to both GCT and ABC were routinely rehydrated. NOR staining was performed according to method developed by Ploton and colleagues (9,23,24). Briefly, the silver colloid solution for staining of Ag-NORs was prepared by dissolving gelatin in 1% aqueous formic acid at a concentration of 2%; this solution was mixed 1/2 volumes, with 50% aqueous silver nitrate to obtain the final working solution. This was dropped onto the sections and left for 30 minutes at room temperature under darkroom conditions. The sections were taken to xylene and were mounted in a synthetic medium.

NORs were visualised as distinct silver positive intranuclear dots. In each tumor, 100 stromal mononuclear cells were examined using a x 100 oil-immersion lens and the mean number of Ag-NORs per cell was calculated. Giant-cells were not included in counting, because of its non-tumoral reactive nature. In order to test the reproducibility of data, same determinations were made by two observers for each case. Previous studies had shown that 100 cell samples were representative and this number was examined from randomly selected fields (10). Student's t-test was used to compare mean Ag-NOR countings. Interobserver variation was tested using the sample correlation coefficient test.

RESULTS

Nucleoli stained as a single ring structure by the colloidal silver nitrate were conted as one. A single nucleolus may contain many well defined dots. Smaller, distinct, brownish-black dots may also be present in the nuclei, lying away from the main nucleolar structures. Ag-NOR scores recorded by two observers are shown as a "scattergram" in Fig. 2. The Ag-NOR counts for ABC were statistically significantly different from those of GCT ($p<0,01$). The interobserver variation was moderate for ABC ($r=0,72$, slope=0,83) and minimal for GCT ($r=0,84$, slope=0,998). The number of Ag-NORs per cell in ABC was usualy 1,2 or occasionally 3 with little variation in morphology (Fig. 3).

The number of Ag-NORs per GCT's stromal mononuclear cells was variable with marked Ag-NOR pleomorphism in many lesions (Fig. 4).

When we grouped the cases according to their clinical or radiological features, it was noted that the Ag-NOR score was higher in tumors which showed soft tissue invasion

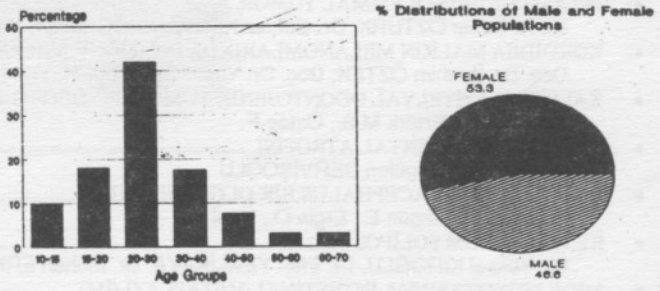

Fig 1: Percent distribution of age and sex in giant cell tumors of bone.

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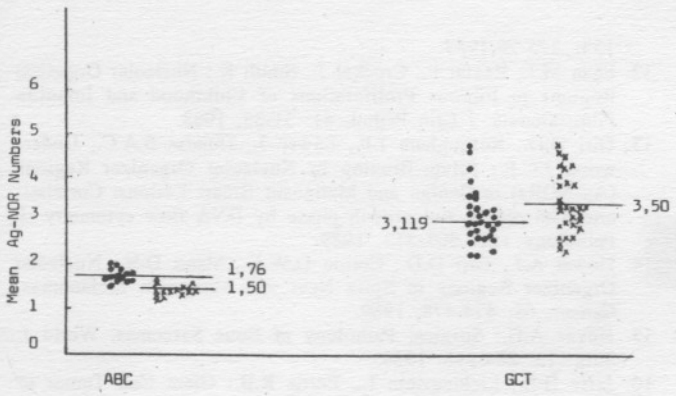

Fig 2: Scattergram of mean Ag-NOR numbers belonging to aneurysmal bone cysts and giant-cell tumors of bone.

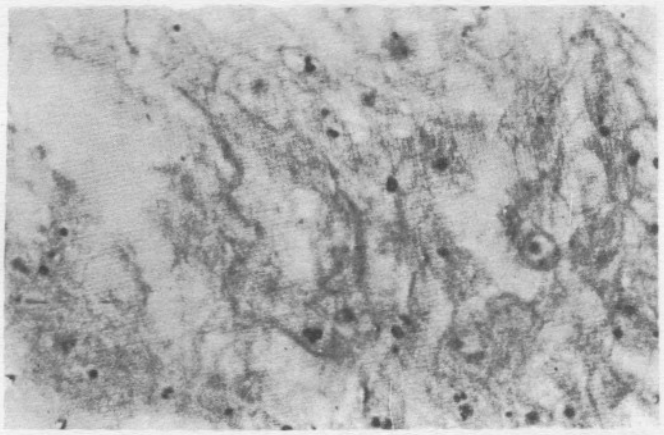

Fig 3: Ag-NOR stained paraffin block section of aneurysmal bone cyst. Almost all nuclei contain 1 or 2 Ag-NORs in the form of small black dots (x 1250).

and/or lung metastasis (p<0,01). It was also noted that tumors with radiologic Grade III, showed higher Ag-NOR counts than Grade I and II (p<0,01).

Patients who were not included in usual age range (20-30 years) of GCT revealed higher Ag-NOR numbers as compared to this group (p<0,01).

Tumors with rounded stromal cells have higher Ag-NOR content than tumors with spindled stromal cells (p<0,01).

Tumors which are at weight-bearing locations (such as knee) have lower mean Ag-NOR countings than tumors at non-weight bearing locations (p<0,01).

DİSCUSSİON

NOR's are loops of DNA that transcribe ribosomal RNA (rRNA). Although activated NORs are found in the nucleolus, inactive NORs may also be detected in the extranucleolar nuclear compartment (11,12,13).

They are easily demonstrated by the simple, specific, one-step staining technique used in this study. Since they are reported to be markers of rRNA transcriptional activity, an analysis of their numbers and forms is of great interest to tumor pathologists (4,6,7,10,23,24,27).

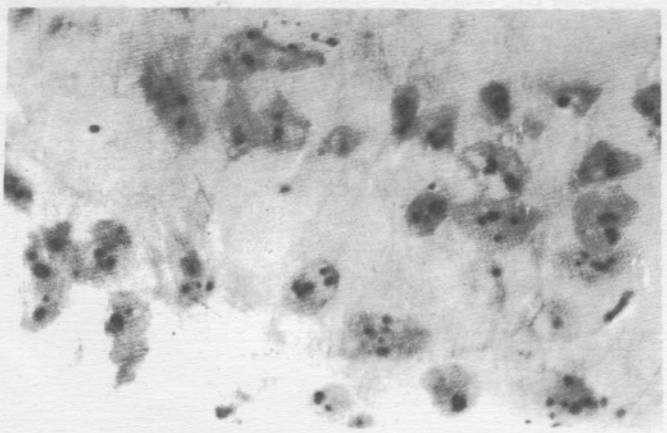

Fig 4: Ag-NOR stained paraffin block section of giant cell tumor of bone. Almost all nuclei contain 3 to 7 Ag-NORs in different sizes (x 1250).

The giant cell tumor of the bone is a distinct clinicopathologic entity. Not all agree how to classify this tumor biologically. Of all bone lesions its behaviour is the most difficult to predict in any single case (20).

As many as 50% of all GCTs recur following curettage. Recurrent lesions may seed the joint and local soft and subcutaneous tissue. Rare conventional cases result in metastasis to the lungs. Most of these features fit with the definition of a low-grade malignancy (2,8,20,28).

Jaffe originally proposed a histologic grading system. Eventually, he discontiuned grading, because he found that cases could recur or metastasize independent of prospective grading (16,17,20,21). On the other hand, some authors indicate that the presence or absence of cytogenetic abnormalities did not correlate with histologic grading systems of GCT and systems that have proved to be of limited value (2).

In this study, the silver staining technique for Ag-NORs was applied to GCT of bone to determine whether the method was effective in analyzing their grade of biological malignancy.

We also included aneurysmal bone cysts for comperative purposes because of its some what similar morphology to GCT ant its locally aggressive behaviour. On the other hand, one can see some secondary ABC formation in a GCT case (20,21). There was significant difference in the mean number of Ag-NORs per cell between GCT and ABC (Fig. 3 and 4). The Ag-NOR countings may facilitate to differentiate between these two entities.

Radiological agressivity was directly related to mean Ag-NOR numbers for GCT. Due this agressiveness soft tissue invasion and even lung metastasis can be seen in GCT. Consequently, we observed higher Ag-NOR countings in such cases.

Some authors indicate that GCT later than 40 years of age usually shows a malignant course clinically (15,21). We found relatively higher Ag-NOR numbers in these kind of cases. We also had similar observations in younger age groups which were below 20-30 years of usual age range.

We did not use histological grading because of the existing controversy and its somewhat subjective interpretation (25). On the other hand, none of our cases was recorded as histological Grade III. However, we found that tumors with

rounded stromal cells revealed much higher Ag-NOR contents as compared to spindled stromal cells. This may be due to a kind of spontaneous regression of tumor by maturation of the stromal cells (17,26).

The lower Ag-NOR counts of tumors located at weight-bearing locations can be explained by similar reasoning. Some microtraumatic changes in such locations may cause a few secondary reactive responses, such as reparative fibroblastic tissues or hemorrhage organization. These reparative fibroblastic tissues with more mature non-proliferating, non-tumoral, fusiform appearance may lower Ag-NOR countings per cell (17,20,26).

This study provided some interesting information regarding the prognosis of patients with GCT of bone. Patients harboring tumors with less than 3 per cell could have a better prognosis than those with 4 Ag-NORs or more. It may be an important tool in determining the clinical behaviour of giant-cell tumors of bone. It may be helpful in cases where the biopsy material is insufficient to differentiate between GCT and other giant cell rich benign conditions.

This silver colloid staining method is effective for analyzing long-term prognosis becuase it allows retrospective study using formalin- or -alcohol fixed, paraffin embedded specimens.

We believe that more reliable results may be achieved by increasing the number of cases wich should be evaluated along with the results of clinical observations.

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