Evaluation of AgNOR staining in human bladder transitional cell carcinoma

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Abstract

The aim of this study was to evaluate the diagnostic value of AgNOR (Argyrophilic Nucleolar Organizer Proteins Region) staining in differentiation between malignant and non-malignant lesion, and histological grading of bladder transitional cell carcinoma. We have also showed the rate of AgNOR distribution score in the nuclear of malignant and non-neoplastic cells of bladder epithelium as an important diagnostic marker at light microscopic level.

Forty nine paraffin block samples selected from the Pathology Department of Ghaem Hospital including 6 chronic cystitis, 5 transitional cell carcinoma (Grade I), 15 transitional cell carcinoma (Grade II) and 23 transitional cell carcinoma (Grade III). Nucleolar AgNOR dots were studied in epithelial cells of chronic cystitis and transitional cell carcinoma of bladder Grades I, II, III by a light microscope to distinguish the average number of AgNOR dots and their distribution rate in nuclei of 100 cells which had three or more AgNOR dots.

There was a significant stepwise increase in the percentage of cells exhibiting nucleoli with at least three or more distinct silver dots in chronic cystitis and transitional cell carcinoma of bladder Grades I, II, III.The counting of AgNOR dots and determining their distribution score are simple, suitable and cheap method to distinguish malignant neoplastic and non-neoplastic lesion of bladder. This method has also a high diagnostic value for grading of bladder transitional cell carcinoma.

Keywords: Bladder, AgNOR, Transitional cell carcinoma.

Introduction

Bladder transitional cell carcinoma is a tumor with unpredictable clinical behavior and prognosis (1). In most cases histological grading and clinical staging are the main criteria for treatment and follow-up of patients with transitional cell carcinoma (TCC). On the basis of research, which has been done, there are no careful or correct criteria for prognosis estimation of TCC (6,19). Furthermore, in some cases even experienced pathologists are unable to make a crucial decision in grading bladder transitional cell tumors by use of numerous criteria such as mitosis, atypia, necrosis and morphological studies. Many studies have been done to discover new methods and techniques for useful and influential steps relating to more crucial decisions concerning bladder transitional neoplasm.

According to the recent studies, cell grade and proliferation could be determined by use of various methods including flowcytometry (S-phase fraction), morphometry, immunohistochemistry (Ki- 67) and AgNORs (3). Chromosomic changing such as activation of oncogen (H-Ras) and inactivation of genes controlling tumors such as P53 and Cer-bB2 has been seen in high-grade bladder tumors. On the other hand, immunological researches show that the existence and increase of epidermal factor (EGF) in bladder tumoral cells are a good criterion to determine prognosis and bladder wall muscular invasion (9). During the last decade, numerous studies have been done concerning DNA flowcytometry and nucleolar diagnosis as an important criterion for malignant neoplasm. The number and size of nucleoli of each cell and its attachment to the membrane have been noticed. DNA flowcytometry in bladder tumors showed DNA anoploidy with a range of 37% to 66% (8). As the grading increase, the anoploidy increases too, and in most articles there is an important relationship between relapse of disease in patients with

TCC and DNA anoploidy (10,20,21). The argyrophilic nucleolar organizer region (AgNOR) parameter has been introduced as an important marker in diagnosis of malignant neoplasm during the last decade (9,10). AgNORs are chromatin areas within the nucleus that are formed around the nucleoli at the end of telophase when it disappears and indeed, it carries coding genes of rRNA, which are taken to be ribosomic nucleoli DNA. These acidic proteins, along with a series of non-histonic proteins, are selectively stained by silver in the argyrophilic technique (9,10). The argyrophilic dots such as RNA polymer become specifically obvious as B_{23}, C_{23} protein (26). These nucleolar parts are called interphase NORs and non-histonic argyrophilic proteins, which are stained by AgNOR and called AgNOR protein (9,10). The number and size of AgNOR dots have a direct relation to cell division and proliferation (11,12,14,20) indicating chromosomic activity, ploidy and state of cell division. Therefore, AgNORs is an important marker in distinguishing malignant and benign neoplasm and even in grading of various neoplasm's (11,13). Regarding this promising data we decided to differentiate between benign and malignant lesions and different grades of bladder transitional cell carcinoma existing in the Pathology Department of Ghaem Hospital by the AgNORs method.

Material and methods

Specimens: 49 paraffin block samples selected from the Pathology Department of Ghaem Hospital including: 6 cases of chronic cystitis, 5 cases of TCC (grade I), 15 cases of TCC (grade II), 23 cases of TCC (grade III). Slides were graded by two expert pathologists on the basis of criteria presented by WHO, and then two cuts (4-micron thick) of paraffin blocks were prepared. One of the sections was stained with hematoxylin - Eosin method, and the other was stained by the argyrophilic technique.

Staining methods: After deparaffinising the slides and hydrating them, fresh AgNOR solution was poured on the slides and then they were put inside an incubator (37 ^{o}C) for 45 minutes. After staining the background with neutral red, in order to count the nucleolar AgNOR dots, specific areas on each slide were selected for staining and histological quality. In other words, areas of concentrated diagnostic criteria were selected on each slide. Then they were magnified up to x 1000 to be studied and count by a light microscope (10,15,16).

Evaluation methods: Epithelial cells (100 cells) of all samples of chronic cystitis and transitional cell carcinoma (Grades I, II, III) were randomly selected and the AgNOR dots were counted. In order to determine the distribution score, one hundred cells in contagious method of the mentioned samples counted in which the cell nuclei had three or more AgNOR dots.

Statistical analyses: The number of AgNOR dots in chronic cystitis, transitional cell carcinoma grades I, II, III, are presented as the mean ± S.D. Analysis of variance (ANOVA) followed by Turkey-Kramer test was used to determine any statistical differences. $P < 0.05$ was regarded as statistically significant.

Results

The number of nucleolar AgNOR dots increased as the histological grading increased so that the average number of AgNOR dots of cystitis was 2.93 and the values for bladder transitional cell carcinoma grades I, II and III were 3.56, 4.45 and 5.82 respectively (Table 1, Figures 1). The percentage of epithelial cells exhibiting three or more nucleolar AgNORs were as follow: chronic cystitis (0.8%), transitional cell carcinoma (grade I) (3.88%), transitional cell carcinoma (grade II) (6.9%), transitional cell carcinoma, grade III (32.43%). There was a

Table 1 : The values of mean AgNORs dots ± SD in chronic cystitis and TCC (GI, GII, GIII).

Diagnostic group	No of cases	Mean AgNOR dots	SD	Variance	PV
Chronic cystitis	6	2.93	0.57	0.33	<0.05
TCC I	5	3.56	0.23	0.05	<0.05
TCC II	15	4.45	0.34	0.12	<0.05
TCC III	23	5.83	0.77	0.6	<0.05

Table 2 : The mean Percentage of cells exhibiting at least 3 or more AgNORs dots in chronic cystitis and TCC (GI, GII, GIII).

Diagnostic Group	No of cases	Mean Percentage of cells with 3≥AgNOR dots	SD	Variance	P.V
Chronic cystitis	6	0.8	0.25	0.06	<0.05
TCC I	5	3.88	0.52	0.27	<0.05
TCC II	15	6.9	1.5	2.25	<0.05
TCC III	23	32.43	11.78	138.7	<0.05

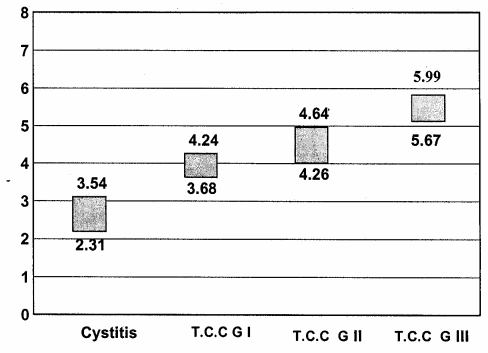

Figure 1: Range of mean with 95% confidence limit of AgNOR a Dots per cell in chronic cystitis and TCC (GI, CII, GIII).

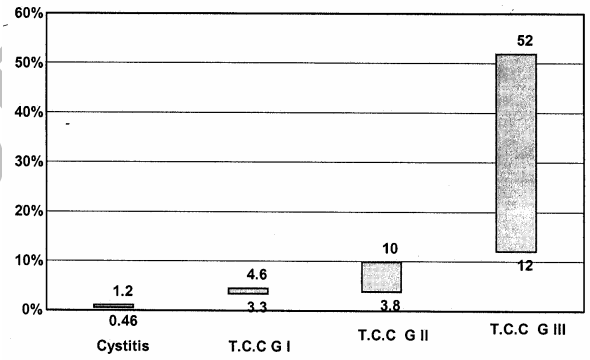

Figure 2: Range of mean with 95% confidence limit of percentage of cells with at least three or more AgNORs dots in TCC (GI, GII, and GIII).

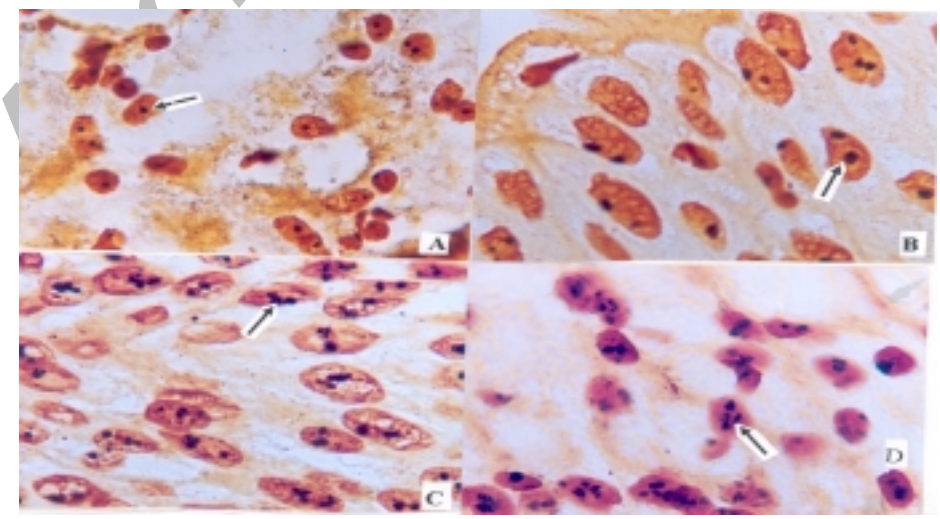

Figure. 3: Intranuclei AgNOR dots in A: chronic cystitis, B: TCC (GI), C: TCC (GII), D: TCC (GIII) (x.1000).

significant increase in AgNOR dots as the grading increased (Table 2, Figure 2,3). In other words, in TCC (grade III) the AgNOR dots are 4.63 times in comparison to TCC (grade II), TCC (grade II) is 1.77 times of TCC (grade I), and TCC (grade I) is 4.85 times of chronic cystitis. The other finding was that in chronic cystitis, AgNOR dots within the nucleus are often placed central whereas in malignant lesion they were settled marginally and irregular.

Discussion

Bladder transitional cell carcinoma forms 90% of bladder malignancies (1) in which 70% to 80% of cases are superficial type and relapse almost in 70% of patients (4,5).

One of the new methods innovated for diagnosis of carcinoma is AgNOR nucleolar staining of the cell nucleus (2,5,9) by use of silver staining to estimate as far as possible an accurate grading and prewarning of bladder transitional cell carcinoma.

In this study, the results of AgNOR staining showed that the grading of transitional cell carcinoma could be performed more carefully by this method. It is clear that during the last decade, by studying the various tumors with the AgNOR technique, the nucleolar AgNOR has a direct relation with cell division and proliferation (11,12,14) and it is an important marker in distinguishing relapse and prewarning of various organ neoplasm (5,9,13,20,21) such as thyroid (24, 23), lung (22) and prostate (18, 21). Many studies have shown that there is a parallel and significant increase in grading of transitional cell carcinoma as the number of nucleolar AgNOR dots is increased and our data confirms the previous results. In conclusion we believe that by use of nucleolar AgNOR staining we can distinguish different grades of bladder transitional cell carcinoma with accuracy and determination of the distribution score (percentage of cells exhibiting three or more AgNOR dots in their cell nuclei) is the most valuable and valid criteria for diagnosis of non-neoplastic and neoplastic of bladder and also for differentiation between different grades of transitional cell carcinoma.

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