Evaluation of AgNOR Count as Diagnostic Marker of Prognosis and Proliferative Activity in Thyroid Neoplasm

Rand Mohammed Abdul-Hussain Al-Hussaini, Asad A. Al-Janabi

Abstract — Thyroid cancer is the most common endocrine malignancy. The aim of this study was to assess the diagnostic and prognostic significance of argyrophilic nuclear organizer region (AgNOR) counts in the cells of malignant and benign thyroid cancer. We analyzed paraffin-embedded tissue sections, from one hundred and two tissue samples were: 20 cases of normal thyroid tissue as control, 34 cases of thyroid adenoma, and 48 cases of thyroid carcinoma. Counting AgNORs helps with the differential diagnosis of thyroid cancers. AgNOR counts in control group ranged from 1.2 to 1.6 with a mean of 1.4±0.01. In all the 34 cases of thyroid adenoma the AgNOR counts ranged from 3.2 to 4.1 with a mean of 3.65±0.58, which was significantly higher than that in the control group (P<0.05). In 48 patients with thyroid carcinoma, the AgNOR value ranged from 5.2 to 8.45 (mean 6.82 ± 0.36), which were significantly (P <0.05) higher than the values obtained from the control group and from the patients with thyroid adenoma. AgNOR counts were also compared in the different grades of thyroid cell carcinoma. A progressive significant increase in the counts was observed with increasing grades of carcinoma and noted a significant difference (P <0.05) in AgNORs between the three grades. These findings strongly support the view that the use of AgNOR number, as cell proliferative activity marker, can be recorded as an inexpensive and reliable diagnostic method and prognostic tool in assessment of thyroid neoplasm. And by use of nucleolar AgNOR staining we could distinguish different grades of thyroid carcinoma.

Index Terms —: AgNOR s, Thyroid cancer, Prognosis, Cell proliferative activity, Cancer diagnostics, Tumors.

1 INTRODUCTION

The thyroid is the largest endocrine gland and by far the most common site of all primary endocrine cancers, therefore thyroid cancer is the most common malignancy of the endocrine system, its incidence had been increasing over the past 20 years and it was the sixth most common cancer among women [1], [2].

Assessment of the proliferative activity markers have been applied to evaluation of malignancy of many neoplastic lesions, such as PCNA, Ki-67 (they are nuclear protein involved in DNA synthesis), and AgNORs (loops of DNA)[3], [4].

Of the various techniques used for assessing proliferating cells of tumor tissue based on nuclear studies, staining of the nucleolar organising regions by silver compound (AgNOR) has become popular for its simplicity, low cost, and its good correlation with other proliferative markers [5].

Nucleolar organizer regions (NORs) are DNA sequences on the short arms of acrocentric chromosomes (13, 14, 15, 21 & 22), which involved in ribosomal synthesis [6]. Associated with NORs there are some nucleolar proteins, which have a strong affinity with silver (argyrophilic proteins). Since NORs are closely related to the ribosomal protein synthesis, they are claimed to reflect cellular proliferative activity [7].

Nucleolar organizer regions may be visualized by number of ways. The simplest and most widely used method is silver colloid impregnation. This technique has been shown to be specific for carboxyl and sulphydryl-rich proteins associated with NOR (Nuclear organizer region). These groups have been proposed as reaction sites and reduced the silver solution forming deposits (black dots) of silver visible at low microscopic magnification [8].

In normal cells, the AgNORs are tightly packed in the nucleoli and are indiscernible. In rapidly proliferating cells such as neoplastic cells, nucleolar disaggregation may take place resulting in dispersion of individual AgNOR [9]. Pich et al. [10], in other study, provided that, the expression of AgNOR proteins is associated with several
biological properties of neoplastic cells: metabolic activity, DNA content, histological grade of differentiation and, especially, the rapidity of cellular proliferation.

Many studies [11], [12], [13], [14],[15] showed that AgNORs were significantly higher in malignant cells as compared to benign cells.AgNOR number and distribution in the nucleus (configuration) were useful in the detection of the proliferative activity and prognosis of some neoplasias, such as urinary bladder [16], breast [17], colorectal carcinoma [18], bone tumors [19], and lung cancers[20].

In thyroid cancer, many studies evaluated AgNOR in patients with benign and malignant tumors. Kawasaki et al.[21] detected AgNORs, as a well-known indicator for proliferative potential of the cancer cells, in 89 cases of DTC and found that AgNOR score might be clinically applicable as a useful indicator for disease recurrence in DTC. Mehrotra et al. [22] proved that AgNOR counting is more sensitive, simple and cost effective as compared to Ki-67 for differentiating between benign and malignant thyroid follicular neoplasms.

Słowińska-Klencka et al. [23] results recorded the usefulness of silver staining of nucleolar organizer regions (AgNORs) in the preoperative diagnosis of follicular lesions in the thyroid. Camargo et al. [24] correlated the subjective AgNOR counting method with histologic diagnoses of thyroid cancer and invasion. Finally, Eroz et al.[25] recorded AgNOR staining as an easy and reliable method for evaluating proliferation activity of cells in malignant and benign thyroid lesions.

2 METHODOLOGY

This study was carried out in the laboratory of Histopathology in Alsader Teaching Hospital in Al-Najaf province.

2.1 Sampling of Cases

a) Study group: Paraffin blocks of eighty two (70 females and 12 males) cases with the thyroid tumors; 34 cases with thyroid adenoma, and 48 cases with thyroid carcinoma were included in this study. These samples were collected from laboratory of histopathology in Alsader Teaching Hospital and from some private laboratories in this governorate.

b) Control group: Twenty samples with normal thyroid tissues were considered as control group for this study.

2.2 Histopathological Examination

Five μm-thick sections were obtained from formalin fixed –paraffin embedded tissue blocks. Sections were stained with the routine haematoxylin and eosin (H&E) staining method [26]. Then examinations of these histological sections were done under the supervision of pathologist, who determines the grades of cancer according to the American Joint Commission on Cancer Staging Manual for grading thyroid tumors [27].

2.3 Standardized AgNOR Staining Technique

According to the recommendations of Trerre[28], the “International Committee on AgNOR Quantitation” guidelines for AgNOR protein evaluation must be considered as standard method. This “standardized” silver-staining protocol, proposed by the Committee, consisted of staining the 5 micron paraffin sections in the dark at a constant temperature of 37ºC using pre-warmed solutions. A very important point in these guidelines was the introduction of a “retrieval” method for formalin-fixed samples based on the exposure of sections to high temperatures using a wet autoclave, microwave oven or pressure cooker. The result of staining is observed under the light microscope as black dots scattered all over the nucleus and the nucleolus, dots of silver (AgNOR dots or AgNORs).

2.4 Quantitative Analysis of AgNOR Proteins (The Counting Method):

AgNORs were counted as brown-black dots in the nuclei of cells (within the yellowish background of the nucleus) using an oil-immersion lens.100 cells (chosen randomly) were studied in each case; the mean AgNOR per cell was calculated.AgNORs were counted in the normal tissue, benign and malignant tumors.

2.5 Statistical Analysis

Statistical analyses of all results were carried out by the help of SPSS version 17 software statistical package using chi square (P value at level of significance less than 0.05). Data were recorded as mean± SD (standard deviation), range or percentage.
3 EXPERIMENTAL RESULTS

3.1 Histopathological variants of thyroid tumors

During this study, out of 82 cases with thyroid tumors, benign tumors have been found in 41.4% of cases, whereas thyroid carcinomas in 58.5% of cases. The tumors with highest frequency of occurrence were papillary carcinomas (51.22 %) followed by follicular adenoma (40.24%) (results are shown in Fig. 1).

Fig. 1. Histopathological variants of thyroid tumors.

Grading of the presented malignant cases was assessed according to the American Joint Commission on Cancer grading system of carcinoma which shown in Fig 2; revealing that grade I was reported in 34 (70.83%) cases, grade II 10 (20.83%), while those of grade III were 4 (8.34%) cases.

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Fig. 2. The percentage of grades of thyroid carcinoma patients.

3.2 AgNOR Counts as Diagnostic Marker

The values of the mean AgNOR (mAgNOR) counts in nuclei of normal thyroid cells, adenoma cells, and thyroid carcinoma cells are presented in Table 1.

AgNOR counts in control group ranged from 1.2 to 1.6 with a mean of 1.4± 0.01. In all the 34 cases of thyroid adenoma the AgNOR counts ranged from 3.2 to 4.1 with a mean of 3.65±0.58, which was significantly higher than that in the control group (P<0.05) (Table 1).

In 48 patients with thyroid carcinoma, the AgNOR value ranged from 5.2 to 8.45 (mean 6.82 ± 0. 36) (Table1), which were significantly (P <0.05) higher than the values obtained from the control group and from the patients with thyroid adenoma.

<table>
<thead>
<tr>
<th>Thyroid Cells</th>
<th>No. of Cases</th>
<th>AgNOR Count/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cells</td>
<td>20</td>
<td>1.2-1.6</td>
</tr>
<tr>
<td>Adenoma cells</td>
<td>34</td>
<td>3.2-4.1</td>
</tr>
<tr>
<td>Carcinoma cells</td>
<td>48</td>
<td>5.2-8.45</td>
</tr>
</tbody>
</table>

*P< 0.05 significant

Table 1

Distribution of AgNORs in Normal and Neoplastic Thyroid Tissue (Benign and Malignant)

3.2 AgNOR Counts as Prognostic Marker

Mean AgNOR values were calculated for the three grades of thyroid cell carcinomas, well differentiated (G1), moderately differentiated (GII), and poorly differentiated (GIII).

AgNOR counts were also compared in the different grades of thyroid cell carcinoma(Table 2). A progressive significant increase in the counts was observed with increasing grades of carcinoma. Counts of 5.65± 0.62 were recorded in well differentiated carcinoma while moderately and poorly differentiated carcinomas yielded counts of 6.87 ± 0.18 and 7.67± 0.02 respectively. The difference was statistically significant (P<0.05) between the three groups.

Table 2
Distribution of AgNORs According to Grade of Thyroid Carcinoma Patients.

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of Cases</th>
<th>AgNOR Count/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Grade I</td>
<td>34</td>
<td>5.2-6.1</td>
</tr>
<tr>
<td>Grade II</td>
<td>10</td>
<td>5.9-7.85</td>
</tr>
<tr>
<td>Grade III</td>
<td>4</td>
<td>6.9-8.45</td>
</tr>
</tbody>
</table>

*P< 0.05 significant

5 Conclusions

It has been found that the means of AgNOR counts were the highest in malignant tumor cells and the lowest in non-neoplastic thyroid cells.

Our results were agreed with many studies, that AgNOR count are useful in discriminating between benign and malignant conditions being significantly higher in malignant cells than in normal cells.

The same results were found by Bukaeva et al. [29], their data indicated that AgNORs counts can be used as differential diagnosis between thyroid carcinoma and adenoma. Also Mehrotra et al. [22] had used AgNOR to differentiate hyperplastic nodules (HPN), follicular adenoma (FA) and follicular carcinoma (FCA). Their result proved that AgNOR counting is more sensitive, simple and cost effective for differentiating between benign and malignant thyroid follicular neoplasms.

Lewy-Trenda et al. [30] found that only AgNOR numbers (in comparing with other proliferations markers) were helped in differentiation between thyroid nodules and follicular carcinoma.

Eroz et al. [25] recorded that Patients with PTC had significantly (p<0.001) higher AgNOR count (4.6 ± 1.2%) than in the patients with benign lesions (2.0 ± 0.5%), also they mentioned that AgNOR staining was an easy and reliable method for evaluating proliferation activity of cells in malignant and benign thyroid lesions.

The results of this study were in agreement with previous data that had been recorded by Slowinska-Klencka et al. [31]; they demonstrated that the mean values with at least five AgNORs allowed for differentiation between malignant and benign follicular lesions.

Cornianu et al. [32] study had indicated that the AgNOR methods were commonly used as the markers of proliferative activity, and mentioned that there were a good correlation between the mean number of NORs nucleus and the rate of cell proliferation appreciated by mitotic index.

Since their number corresponds to the proliferation rate, they can be used for tumor grading, i.e. the number of nucleolar AgNOR dots increased as the histological grading increased [33].

Kawasaki et al. [21] study had shown that in tumors with hematological metastasis, AgNORs score was significantly higher than in tumors without metastasis, as well as, higher risk for disease recurrence was demonstrated in those cases with high AgNOR score than cases with lower score.

In this study, the Mean AgNOR values had calculated for the three grades of thyroid cell carcinomas and noted a significant difference (P<0.05) in AgNORs between these three grades (Table 4.28).

In conclusion we believed that by use of nucleolar AgNOR staining we could distinguish different grades of thyroid carcinoma.

These findings strongly support the view that proliferative activity and malignant potential of neoplastic lesions of the thyroid increase progressively as the grade of the lesion becomes higher.

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REFERENCES


