Limited value or argyrophil nucleolar organizer regions in the assessment of esophageal lesions

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ABSTRACT
Objective: To determine the usefulness of the argyrophil nucleolar organizer scores in predicting malignant or aggressive behavior in neoplastic and non-neoplastic lesions of the esophagus. Methods: The silver staining technique for argyrophil nucleolar organizer regions was performed on 66 specimens of formalin fixed paraffin wax embedded esophageal biopsies: Twenty six (26) normal biopsy specimens, 4 cases of squamous papilloma, 10 cases of well differentiated squamous cell carcinoma, 16 cases of moderately differentiated squamous cell carcinoma and 10 cases of adenocarcinoma were studied. Results: The mean AgNOR scores and range of values were as follows: Normal esophageal squamous epithelium, mean 6.3 (range 4.8 to 7.4); squamous papilloma, mean 8.6 (range 6.9 to 10.2) well differentiated squamous cell carcinoma, mean 6.0 (range 4.5 to 7.7); moderately differentiated squamous cell carcinoma mean 7.2 (range 5.5 to 8.7); adenocarcinoma mean 5.8 (range 4.5 to 6.5). The results of the different diagnostic categories were compared using the Kruskal-Wallis test but did not achieve statistical significance. Conclusion: The AgNOR scores of different esophageal lesions did not predict aggressive behavior or malignancy.

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The nucleolar organizer regions (NOR) are loops of DNA that encode for ribosomal DNA located in the short arms of the five acrocentric chromosomes 13,14,15,21 and 22 in humans. The NOR are demonstrated as silver stained nuclear dots in the interphase nuclei (AgNOR). The silver binds to NOR associated proteins including RNA polymerase I, C23 protein (nucleolin) and B23 protein. Quantitation of the AgNOR has been applied to the study of malignant and non malignant conditions of various organs. It has been suggested that AgNOR numbers correlate with malignant grades of lymphoma and carcinoma. Few studies are available on the application of AgNOR technique in the study of esophageal lesions and its role in the diagnosis of esophageal lesions remains controversial. In one study AgNOR counts were shown to correlate with prognosis in esophageal carcinoma, while in another study, AgNOR counts of esophageal lesions did not predict malignant or aggressive behavior. In this study, we attempted to determine whether AgNOR plays a role in predicting malignancy or biologically aggressive behavior by studying 66 formalin fixed, paraffin wax embedded specimens of non-neoplastic and neoplastic esophageal epithelium.

Material and methods. Sixty-six esophageal specimens were studied. These included twenty-six normal (non-malignant) stratified squamous epithelium which served as controls, four cases of squamous papilloma, ten cases of well differentiated squamous cell carcinoma, sixteen cases of moderately and poorly differentiated squamous cell carcinoma and ten cases of adenocarcinoma. The cases were retrieved from the archival files of the pathology department of the King Khalid University Hospital, Riyadh. The specimens had been fixed in 10% buffered formalin and embedded in paraffin wax. Sections (5μm thick) were dewaxed in xylene and hydrated through graded ethanol to deionized water. Adjacent sections from each block were stained with hematoxylin and eosin, and silver stain for NOR associated proteins, AgNOR staining was carried out as described by Crocker et al. Briefly, the AgNOR staining solution was prepared by dissolving 2% solution of gelatin in 1% formic acid.

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<table>
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<th>Normal (n=26)</th>
<th>Well-differentiated D-cell squamous carcinoma (n=10)</th>
<th>Moderately poorly differentiated squamous carcinoma (n=16)</th>
<th>Adeno-carcinoma (n=10)</th>
<th>Squamous papilloma (n=4)</th>
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<tbody>
<tr>
<td><strong>Mean AgNOR scores</strong></td>
<td>6.3</td>
<td>6</td>
<td>7.2</td>
<td>5.8</td>
<td>8.6</td>
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This solution was then mixed in a ratio of 1:2 with 50% aqueous silver nitrate. The tissue sections were immersed in the final working solution and left for 30 minutes at room temperature in a dark room. The slides were then fixed, washed in distilled water, dehydrated in ascending grades of ethanol and mounted. The presence of NOR was indicated by the appearance of black silver granules in the nucleus. AgNORs present in the nuclei of at least 100 consecutive normal or lesion-related esophageal epithelial cells were examined with regard to the number and morphological pattern and were counted at a magnification of X1000 using an oil immersion lens by a single observer. The number of separate black dots in each nucleus were counted, overlapping dots were counted as one. The mean number of AgNOR count per nucleus was calculated using the cumulative means technique. In each case adjacent H and E sections were reviewed to make sure that AgNOR counts were carried out at the appropriate areas. The AgNOR scores for the different categories were compared with Kruskal-Wallis test using program 3S from the BMDP statistical package.

**Results.** In all specimens, AgNORs were clearly visible as black dots of varying sizes in the nuclei. These were arranged into one or more clusters or as individual satellites extranucleolar. The mean AgNOR counts for each specimen in all the five categories shown in Table I. The mean AgNOR counts and the range for each diagnostic category are as follows: normal esophageal squamous epithelium, mean 6.3 (range 4.8 to 7.4); squamous papilloma, mean 8.6 (range 6.9 to 10.2); well differentiated squamous cell carcinoma mean 6.0 (range 4.5 to 7.7); moderately differentiated squamous cell carcinoma, mean 7.2 (range 5.5 to 8.7); adenocarcinoma mean 5.8 (range 4.5 to 6.5). There was a trend towards increased mean AgNOR counts with moderately differentiated squamous cell carcinoma as compared to normal squamous epithelium but this did not achieve statistical significance. Also statistical analysis of the AgNOR scores between the different groups using the Kruskal-Wallis test did not achieve significance.

The highest mean AgNOR count was observed in a squamous papilloma (Fig. 1). Squamous cell carcinoma tended to have small AgNOR dots that tended to be extranucleolar (Fig 2). The mean AgNOR count for well differentiated squamous cell carcinoma and adenocarcinoma were actually lower that the mean AgNOR count for normal squamous epithelium.

**Discussion.** AgNOR technique has been applied to the study of malignant, premalignant, and non neoplastic conditions in various organs and tissue. Numerous studies have shown the usefulness of AgNOR counts in discriminating between benign and malignant condition while other studies have shown

![Figure 1 - Squamous papilloma. AgNOR are predominantly nucleolar in distribution](image1)

![Figure 2 - Moderately differentiated squamous cell carcinoma. AgNOR appear as dark dots in the nucleus. (magnification x 1000)](image2)
prognostic value of AgNOR counts in various malignancies. Furthermore, the AgNOR technique was found to be of limited use in the diagnostic assessment, of thyroid neoplasms and certain neuroendocrine neoplasms.

In the lesions of the esophagus, the application of the AgNOR technique has yielded conflicting results. Morita et al. have found AgNOR counts of esophageal squamous cell carcinoma to be an independent prognostic factor with a direct relationship between the AgNOR count and patient survival. However, these workers did not achieve statistical significance when they compared the AgNOR count between well differentiated moderately differentiated and poorly differentiated, squamous cell carcinoma of the esophagus.

In a recent study Miyazaki S. et al. did not observe statistically significant differences between the mean AgNOR counts per nucleus in the non pathological parabasal layer of the esophagus, displastic esophageal epithelium and carcinoma of the esophagus. The results of the present study showed no statistical significance and considerable overlap of the mean AgNOR counts per cell in the non-neoplastic esophageal squamous epithelium, squamous cell carcinoma and adenocarcinoma of the esophagus. Our findings confirm that AgNOR count is of limited value in the diagnosis of the different benign and malignant lesions of the esophagus. The AgNOR numbers have been shown to correlate, in general, with the level of cell proliferation. Ploidy as assessed by DNA flow cytometry or karyotypic analysis has little or no effect on interphase AgNOR counts. Thus, the AgNOR count has been shown to correlate with Ki67 antibody scores and the proliferating cell nuclear antigen labelling index.

References


