QUANTITATIVE STUDY OF GASTRIC EPITHELIAL LESIONS BY NUCLEOLAR ORGANIZER REGION STAINING

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Abstract- Nucleolar organizer regions (NOR) are defined as nucleolar components containing a set of argyrophilic proteins which are selectively stained by colloidal silver nitrate staining. Although studies have shown that the number of NOR dots or particles is directly related to the rapidity of cell proliferation in cancer cells, prognostic or diagnostic value of NOR remains controversial. The aim of the present study was to asses the proliferative activity of the NOR in different gastric epithelial lesions. For these purposes 60 biopsy and surgical specimens of stomach from pathology files of Khatamalanbia and Imam Hospitals were chosen. For each patient, 3-5 paraffin sections were prepared and stained by one step colloidal silver nitrate solution. In each section intranuclear dots in 100 cell nuclei were counted by two of authors in randomly selected fields and data were analyzed by ANOVA. Statistical analysis showed significant difference for NOR number between gastritis, different grades of dysplasia and carcinoma. The shape and number of NOR showed a grater variability in carcinoma compared to other lesions. It seems that NOR could reflect the proliferative activity of cells.

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Key words: Nucleolus organizer region, Stomach neoplasms, Silver staining

INTRODUCTION

The silver staining technique for nucleolar organizer regions (NOR) in formalin fixed, paraffin embedded tissue is a simple method for demonstrating intranucleolar-stained structures, which have also been termed AgNORs (1). NORs are loops of DNA, which in human are present on each of the short arms of chromosomes 13, 14, 15, 21 and 22 and transcripts to rRNA (2, 3).

Argyrophilia of NOR is attributed mainly to C23 (Nucleolin) and B23 (Numartin) proteins. NOR numbers reflect nuclear activity, in particular rDNA transcriptase activity and ribosome biogenesis and hence are indicators of cellular proliferation rate (4,5). Histopathologists have recently shown much interest in the AgNORs staining method, because numerous studies have already shown differences in the number of silver dots per nucleolus between normal, benign or cancerous lesions in various organs and between different tumors with similar morphologic appearance (6). Although studies have shown that the number of NOR dots or particles is directly related to the rapidity of cell proliferation in cancer cells (4, 7), prognostic or diagnostic value of NOR particles remains controversial (6).

Although gastric carcinoma is declining in some regions, it has remained a significant cause of mortality (8, 9). Furthermore, studies have shown that chronic gastritis and dysplasia are possible precursors of gastric carcinoma especially the intestinal type (9, 10). The aim of the present study was to compare the proliferative activity of AgNORs in patients with chronic gastritis, low and high grade dysplasia and carcinoma.

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MATERIALS AND METHODS

Sixty biopsy and surgical specimens of stomach from patients with diagnosis of chronic gastritis (30 case) and adenocarcinoma (30 case) with mean ages of 49 were chosen from pathology files of Imam and Khatamalanbia Hospitals. To study dysplasia, samples were taken from periphery of cancer lesion. For each patient, 3-5 sections with thickness of 4-5 micrometer were stained by Hematoxylin Eosin and freshly prepared solution of colloidal silver nitrate solution for 1 hour in darkness and room temperature.

AgNOR solution was prepared as follows: two volumes of a 50 percent aqueous of $AgNO_3$ were mixed immediately before use with one volume of 2 percent aqueous porcine gelatin in one percent formic acid. The sections were then treated for 5 minutes in 5 percent solution of thiosulphate and then washed, dehydrated and mounted (1, 8).

In each microscopic slide random fields were chosen and NOR dots were counted independently and blinded in 100 cell nuclei by two of the authors using × 100 objective and oil immersion. Data were analyzed with ANOVA by SPSS (version 9); further analysis were done by Mann Whitney and Pearson correlation tests and $P<0.05$ was defined as the conventional level of significance.

RESULTS

Loss of cellular polarity, hyperchromasia and cell crowding were more obvious in high-grade dysplasia than low grade dysplasia. We found areas of low and high-grade dysplasia in periphery of cancer lesion in 11 (37%) and 23 (77%) cases of carcinoma patients, respectively

NOR particles appeared as black round dots or particles in cell nuclei. The number and shape of these particles in patients with carcinoma was more variable than in subjects with gastritis, low and high grade of dysplasia. In each microscopic field of gastritis, majority of cells had only one NOR particle in their nuclei, while it was 2 and 3 for dysplasia and carcinoma, respectively. In dysplasia and gastritis NOR particles typically had a more regular shape than carcinoma (Figures 1-3, Table 1).

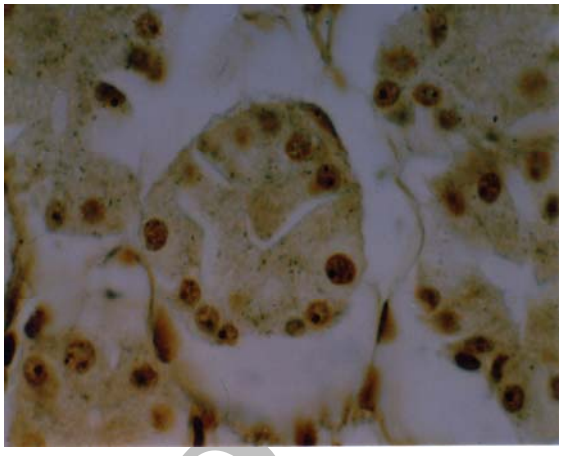

Fig. 1. NOR particles appear as black round dots in cell nuclei in cases of gastritis. Colloidal silver staining × 1000

Statistical analysis with ANOVA showed significant difference between NOR number in all of studied groups ($P<0.001$) (Fig. 4).

Further analysis with Mann Whitney test showed significant difference for NOR number between gastritis and low-grade dysplasia ($P<0.01$), gastritis and high-grade dysplasia ($P<0.01$), gastritis and carcinoma $P<0.01$) and grades of dysplasia with carcinoma ($P<0.01$). Pearson's correlation test showed minimal interobserver variation for NOR counting between two of observers (r=0.97, $P<0.001$).

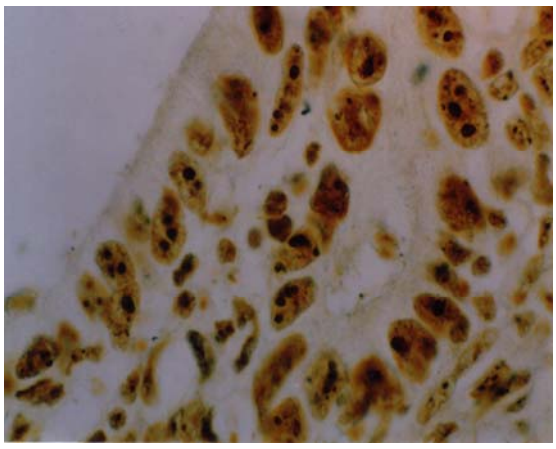

Fig. 2. NOR particles appear as heterogeneous dots in neoplastic cells. Note different sizes of NOR particles compared with Fig. 1. Colloidal silver nitrate staining × 1000

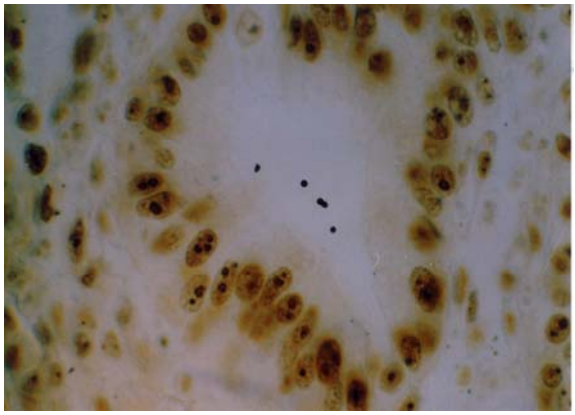

Fig. 3. NOR particle appears as black round dots in cases of severe dysplasia × 1000.

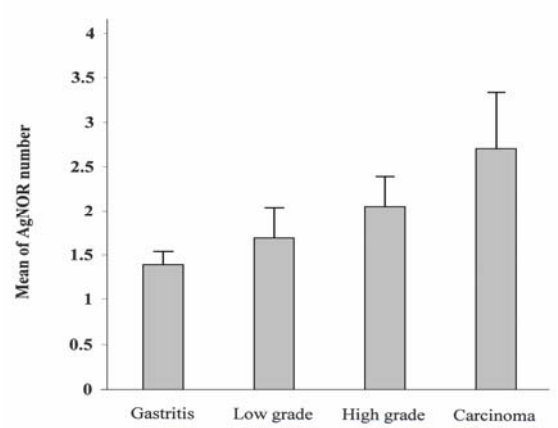

Fig. 4. Comparison of AgNOR number between different gastric epithelial lesions

DISCUSSION

NORs have been shown to be the site of rDNA which are transcribed to rRNA. They can be routinely demonstrated by virtue of argyrophilia of their associated proteins (7). It has been reported that potentially human somatic cells could contain 10 demonstrable NORs in nuclei, but many resting cells contain only one NOR particle (11). Due to increased proliferative activity of neoplastic cells, higher number of NOR particles in cancerous cells might be expected (12). The results of the present study showed that number of NORs increases from gastritis to carcinoma and the number and shape of NOR particle shows greater variability in carcinoma than low and high grade dysplasia and gastritis. Although there was some overlapping of NOR count between gastritis and low grade dysplasia and between severe dysplasia and carcinoma, Mann Whitney test for NOR number showed significant difference between them. Furthermore Pearson correlation tests showed minimal interobserver variation between counting of NOR by two of the authors (r=0.97 P<0.001). The amount of NOR proteins increase in proliferating cells; this phenomenon has been observed in non-Hodgkin's lymphoma (11), carcinoma of the cervix (6), squamous cell carcinoma of the oral cavity (5), carcinoma of the stomach (13,14) and carcinoma of the breast (2). Derenzini *et al.* showed that the amount of NOR proteins increases in neoplastic cells and it is independent of cancer type (2). The current finding clearly indicates that this technique could be a useful method to help separate neoplastic and non-neoplastic cases from each other.

Piffko *et al.* introduced the coefficient of variation as a useful parameter for the analysis of AgNOR measurement (5). The results of the present study showed that the number of NOR particles showed greater variability in carcinoma patients than in those suffering from gastritis. The increased variability of NOR number and shape in carcinoma specimens indicates the heterogeneous morphology of NORs within neoplastic in comparison with non-neoplastic cells.

Table 1. Statistical data of NOR numbers for epithelial lesions of the stomach

Lesions	Mean	SD	Max NOR number	Min NOR number	Coefficient of variance	Range of variable	Median	Mode
Gastritis	1.39	0.15	4	1	0.1	3	1	1
Low grade dysplasia	1.7	0.35	2	1	0.2	1	2	2
High grade dysplasia	2.07	0.34	4	1	0.16	3	2	2
Carcinoma	2.73	0.63	7	1	0.23	6	3	3

Abbreviations: NOR, nucleolar organizer regions; SD, standard deviation; Max, maximum; Min, minimum.

The number and size of NOR particles are thought to reflect the degree of differentiation and the synthetic activity of the cell (6). The results of the present study for NOR indicates that there was little variability in patients with gastritis, where usually the cells show only one small round NOR in comparison to dysplastic and cancerous cells which have more than one NOR particle.

Our results are in agreement with those of Kakeji *et al.* who studied the number of NOR particles in endoscopically obtained material and found an increase of AgNOR number from normal to carcinoma subjects and showed a significant difference between early and advanced gastric carcinoma (13).

Our results are also in accordance with studies of Derenzini *et al.* who showed that high number of NOR particles in neoplastic cells is a reflection of high proliferative index of cancer cells (2). The studies of Krecicki *et al.* showed value of NOR staining in laryngeal cancer. They reported a significant difference in NOR count between benign and laryngeal squamous cell carcinoma. They also found that there was no significant correlation between NOR count and patient age and sex, but there was a strong correlation between stage and lymph node metastasis with AgNOR and histologic grade of tumor (15).

Misra *et al.* showed that NOR number increased in chronic gastritis of the stomach and proposed that increase proliferative index of cells in gastritis make the cells more susceptible to carcinogens (16). Antonangelo *et al.* showed the prognostic value of NOR particles in squamous cell carcinoma of the lung (17).

In conclusion we can propose that NOR staining is an economic and simple technique that could help pathologist differentiating reactive lesions from low-grade dysplasia and also carcinoma in situ from severe dysplasia.

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