

Original Research Article

Evaluation of AgNORs in gallbladder lesions

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ABSTRACT

Background: Quantification of argyrophillic nucleolar organizer regions (AgNORs) is a good indicator of cellular proliferation activity and is useful diagnostic tool to estimate the malignant potential of lesions in gallbladder. The aim of the present study was to study the AgNORs and assess their correlation with various lesions of the gallbladder.

Methods: This study was conducted in the department of pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. One hundred specimens of gall bladder lesions (50 cases chronic cholecystitis, 30 cases epithelial hyperplasia and 20 cases of carcinoma) were study subjects. AgNOR staining was done on three micron thin sections of paraffin embedded tissue, as per the method of Crocker and Smith with safranin counterstain. The number of AgNORs, stained as black dots was counted in one hundred adjacent cells in different lesions.

Results: Mean AgNOR count in chronic cholecystitis, epithelial hyperplasia and carcinoma was 2.44 ± 0.31 , 3.88 ± 0.39 and 7.90 ± 0.76 respectively. AgNOR counts in various lesions gradually increased from chronic cholecystitis to carcinoma and the increase was statistically significant ($p < 0.05$).

Conclusions: Despite inter-laboratory variations and lack of standardization of counts for a particular lesion, AgNOR technique is easy to perform, economical and reliable indicator of malignant potential of the gall bladder lesions, hence can be used in resource poor set up as an adjunct to histopathology.

Keywords: AgNOR technique, Carcinoma, Cholelithiasis, Gallbladder lesions

INTRODUCTION

The silver stained nucleolar organiser region (AgNOR) associated proteins are in the nucleolar components and represent the interphase counterpart of metaphase nucleolar organizer regions.¹ NORs are loops of deoxyribonucleic acid in the nucleolus which transcribe to rRNA. These are seen in the acrocentric chromosomes (13, 14, 15, 21 & 22) in the metaphase and in the form of fibrillar centres in the nuclei of cells in the interphase.²

There has been a linear correlation between cell proliferation activity and the amount of AgNOR staining, independent of the type of cancer cells. Therefore,

AgNORs are useful indicators of tumour proliferation, particularly having, diagnostic value in distinguishing between benign and malignant lesions.¹ Cholelithiasis is known to produce changes in the gallbladder mucosa ranging from acute cholecystitis, chronic cholecystitis, polyp, granulomatous cholecystitis, empyema, eosinophilic cholecystitis, metaplasia, hyperplasia and dysplasia to carcinoma.³ The incidental detection of gallbladder cancer in routine cholecystectomy specimens is 0.3%-2%. Early clinical and radiological detection of carcinoma is difficult as only 40% cases present as solid intraluminal masses. Hence, in countries with high incidence of gallbladder cancer, it is a routine practice to subject all specimens of cholecystectomy for

histopathological examination, so that the early preneoplastic lesions, are detected.⁴

In addition to histomorphology, various proliferative indices such as DNA content, S phase fraction, oncogenes, Ki67, monoclonal antibodies, proliferating cell nuclear antigen (PCNA), fibroblastic growth factor receptor (FGFR), and argyophilic nucleolar organizer regions (AgNORs) can be used to differentiate between benign, dysplastic and malignant lesions. NORs have been used to differentiate between benign, dysplastic and malignant lesions of various organs such as prostate, uterus, cervix, breast, oral cavity, lymphoma and gallbladder.⁵ The AgNOR is established economical, simple, quick and reliable adjunct to routine histopathological examination. Hence, the present study was done with the aim to evaluate the role of AgNORs in differentiating various lesions of the gallbladder.

METHODS

This prospective study was conducted in the department of pathology, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India. One hundred specimens of gall bladder lesions comprising (50 case chronic cholecystitis, 30 cases epithelial hyperplasia and 20 cases of carcinoma) were selected and subjected to silver colloidal staining for AgNORs as per the method of Crocker and Smith⁶ and number of AgNORs in the different lesions was counted.

Staining technique

Special silver colloidal staining was used in this study, as described by Smith et al.⁶ Paraffin sections were dewaxed and hydrated through descending concentrations of

alcohol, washed for 15 minutes in running tap water and then distilled water. These sections were placed in the dark in working AgNOR solution for 15 minutes, washed for 3 minutes in 3 changes of distilled water. Counter staining was done with 0.01% saffranin solution. Then the sections were dehydrated, cleared and mounted in DPX. AgNORs stain as black dots within the nucleus and their number was counted in 100 adjacent cells in the lesion.

Statistical analysis

Mathematical and statistical tests were applied to get the average AgNOR counts for each histological lesion by using statistical software Epi-info version 7 (7.1.1.0). Results were summarized in tables and percentages. Quantitative data was summarized using means & standard deviation. A p-value of less than 0.05 was considered statistically significant.

RESULTS

AgNOR count was performed on 100 cases which included 50 cases of chronic cholecystitis, 30 cases of chronic cholecystitis with hyperplasia and 20 cases of carcinoma. AgNORs in the stained sections were identified as black dots within the nucleus and contrasted with light pink background. Their number was counted in 100 adjacent glandular cells of various gall bladder lesions. The mean AgNOR counts in relation to gallbladder lesions is shown in Table 1. Average AgNOR counts gradually increased from chronic cholecystitis to dysplasia and carcinoma. The increase in AgNOR counts from cholecystitis to hyperplasia and hyperplasia to carcinoma was found to be statistically significant ($p < 0.05$) Table 1.

Table 1: Gall bladder lesions and AgNOR counts.

Lesions	No of cases	AgNOR count			p value
		Min	Max	Mean±SD	
Chronic cholecystitis	50	2.10	3.22	2.44±0.31	
Epithelial hyperplasia	30	3.44	4.86	3.88±0.39	<0.000001
Carcinoma	20	6.25	8.98	7.90±0.76	<0.000001

DISCUSSION

The epithelial changes associated with chronic cholecystitis and cholelithiasis range from flat regenerative epithelium to marked villous proliferation which can be difficult to differentiate from hyperplasia, dysplasia and carcinoma. The proliferative activity can be measured by the AgNOR count which varies according to the activity of proliferation and the degree of differentiation.² The number of AgNORs per nucleus are a marker of proliferative activity.⁷ AgNOR counts are

useful indicators of the tumour proliferation and their quantification can be used to differentiate between the benign and malignant lesions. In the present study, AgNOR counts observed in chronic cholecystitis (2.44±0.31) were higher in comparison to Gupta et al and Gupta et al (1.89±0.96 and 2.08±0.37 and Misra et al (1.970.28).^{2,8,9} In cases of hyperplasia, the average AgNOR count in the present study was lower than that found in studies by Gupta et al and Misra et al.^{7,8} However the mean AgNOR counts in carcinoma were higher in our study than that observed by Gupta et al and

Misra et al but much less than those of Gupta et al though they had counted AgNORs separately in different grades of carcinoma.^{2,7,8} Suzuki et al had found that mean AgNOR scores in carcinoma were low in comparison to other studies (3.28 ± 1.38).¹⁰ The increase in AgNOR counts from chronic cholecystitis to hyperplasia and hyperplasia to carcinoma were found to be statistically significant ($p < 0.05$).

CONCLUSION

AgNOR technique is simple, quick and effective technique for rapid and reliable differentiation of benign and malignant lesions in gallbladder. It is economical and hence can be used in resource poor set up as an adjunct to histopathology or genetic analysis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Takano J, Ayabe H, Hatano K, Hatano K, Yamaguchi H, Tagawa Y. Gall bladder Cancer. A comparative study among clinicopathologic features, AgNORs and DNA content analysis. *Digestive Dis and Sci.* 1996;41(5):840-7.
2. Gupta N, Kaur A, Pal S, Kahlon S. Study of nucleolar regions (NORs) in lesions of gallbladder. *Indian J Pathol Microbiol.* 2003;46(3):371-4.
3. Zaki M, Al-Refeidi A. Histological changes in the human gall bladder epithelium associated with gallstones. *Oman Med J.* 2009;24(4):269-73.
4. Kalita D, Pant L, Singh S, Gaurav J, Kudesia M, Gupta K, et al. Impact of routine histopathological examination of gall bladder specimens on early detection of malignancy-A Study of 4115 cholecystectomy specimens. *Asian Pac J Cancer Prev.* 2013;14:3315-8.
5. Khanna A, Yadav S, Dixit V, Mohan K. AgNOR Count and Subjective AgNOR Pattern Assessment (SAPA) Score in Carcinoma of the Pancreatic Head Including Periapillary Tumors. *J Pancreas.* 2005;6(6):575-80.
6. Smith R, Crocker J. Evaluation of nucleolar organiser region-associated proteins in breast malignancy. *Histopathol.* 1988;12:113-25.
7. Kaur A, Dubey V, Mehta K. Gallbladder mucosal changes associated with chronic cholecystitis and their relationship with carcinoma gallbladder. *JK Sci J Med Edu Res.* 2012;14(2):89-92.
8. Gupta S, Misra S, Goyal M, Kumar V, Chattervedi A, Arya NK, et al. Prognostic significance of AgNOR Proliferative index in gallbladder carcinoma-A potential alternative to frozen section analysis. *J liver.* 2014;3:143.
9. Misra V, Misra S, Dwivedi M, Gupta S. Role of AgNORs in Diagnosis of Early Malignant Lesions of Gall Bladder. *Indian J Pathol Microbiol.* 1995;38(4):383-8.
10. Suzuki T, Kida Y, Takano Y. Silver binding argyophilic nucleolar organizer regions in gall bladder cancer. Correlation with malignant grading and prognosis. *Acta Pathologica Japonica.* 1993;43(1-2):36-43.

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