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AgNOR STAIN IN NORMAL, CIRRHOTIC AND CARCINOMATOUS LIVER

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In this era of tumour marker AgNOR stain has still retained the diagnostic yield in tumour pathology. This study was conducted to evaluate the role of AgNOR number and morphological features in cirrhotic and carcinomatous liver. A total of one hundred liver biopsy specimens were included, twenty cases were of hepatocellular carcinoma, sixty were those of cirrhosis of the liver and twenty cases with normal histology as control. The mean AgNOR count, size and distribution were significantly of higher grade in hepatocellular carcinoma as compared to cirrhosis of liver.

NORs (nucleolar organizer regions) are the structures present in the short arms of acrocentric chromosomes in human and were first detected on giemsa banding as" achromatic gaps" with much reduced staining. The areas are of course not geunine gaps but are, areas of specialized chromosome configuration number 13, 14, 15, 21&22 .It was shown that the achromatic gap areas on the achrocentric chromosomes were argyrophil and the silver method became a standard technique[1]. NOR represent loops of DNA that possess the genes of ribosomal RNA and are associated with specific proteins including RNA polymerase 1 B_{23} protien, and C_{23} protien[2-3].

These proteins can be easily demonstrated by means of argyrophilic techniques for NORs[4]. Application of AgNOR staining to conventionally fixed and processed, paraffin sections has made this technique a useful tool in diagnosis of human malignancies[5]. Derezini et al in their study showed that higher number of interface AgNOR are associated with increased risk of hepatocellular carcinoma in patients with chronic liver disease[6].

MATERIALS AND METHODS

One hundred liver biopsy specimens were collected from various hospitals of Lahore. They were fixed in formaline, processed and embedded in paraffine wax. Each block was cut into multiple sections of 4µm thickness and stained with H&E. Twenty cases were diagnosed as hepatocellular carcinoma (HCC), 60 as cirrhosis of the liver and 20 had normal histology.

AgNOR STAINING

Sections 4µm thick were cut for each case and stained by the modified AgNOR staining technique introduced by Ploton et al[7].and Crocker et al[8]. The optimum incubation period with the staining solution in our experience was 37 minutes at room temperature.

AgNOR ENUMERATION

The AgNOR count in 100 randomly selected hepatocytes were counted using an x100 oil immersion objective and x10 eye piece (total magnification: x1000). To minimize the inter-observer counting error, a second count of the AgNOR dots was made without knowing the previous count.

The interval between the first and the second count was at least 2 weeks. The mean number of AgNORs per cell was calculated taking both these counts into consideration. Bile ductules, vascular, inflammatory and Kupffer cells were not included in the enumeration procedure.

The grading of the size variation and distribution of AgNORs was preformed by the following criteria used by Ahsan et al[9].

RESULTS

The AgNOR staining was performed on all the 100 liver biopsies, and detailed results are tabulated in table 1-3. The mean AgNOR count in hepatocellular carcinoma (14.96) was significantly higher ($p<0.001$) than the mean AgNOR count (4.70) in cirrhosis as well as in normal liver (1.57). Similarly mean AgNOR count in cirrhosis was significantly higher (0.001) than normal liver (Table 1).

AgNOR size and distribution was of significantly higher grade in hepatocellular carcinoma ($p< 0.001$) than in cirrhosis and in normal liver (Table 2 and Figure 1, 2). Mean AgNOR count in well differentiated hepatocellular carcinoma

(13.20) was significantly low or ($p<0.001$) than the mean count (14.96) of moderately differentiated hepatocellular carcinoma. This in turn was lower than that of poorly differentiated hepatocellular carcinoma (17.88) fig 3.

Table 1: *Comparison of mean AgNOR count in hepatocellular carcinoma, cirrhosis of the liver and normal liver*

Groups	No. of cases	AgNOR count Mean±S.D
Hepatocellular carcinoma	20	*14.96 ± 1.62
Cirrhosis of the liver	60	4.70 ± 0.66
Normal liver	20	1.57 ± 0.13

*$p<0.001$ as compared with other groups.

Table 2: *Comparison of AgNOR size in hepatocellular carcinoma, cirrhosis of the liver and normal liver.*

Groups	AgNOR Size		Total
	0 and 1+	2+ and3+	
Hepatocellular carcinoma	0	*20	20
Cirrhosis of the liver	32	28	60
Normal liver	20	0	20

*$p< 0.001$ significantly higher when disease group was compared with other and normal liver.

SIZE VARIATION
0 = More or less uniform
1+ = Two different sizes
2+ = More then two different sizes(but not those of 3+).
3+ = All grades and sizes including too minute to be counted.

DISCUSSION

The number and morphological features of AgNOR are thought to reflect the cellular proliferative activity and grade of malignancy. In liver diseases, it has been reported that AgNOR scores for hepatocellular carcinoma were significantly higher than those for benign and borderline lesion; the score increased with histologic tumour grade. In the present study mean AgNOR count was 14.966±1.62 for hepatocellular carcinoma 4.70± 0.66 for cirrhosis of the liver and1.57±0.13 for normal liver. It is in accordance with other studies. Siddiqui et al[10] in their study reported a gradual increase in mean AgNOR count from normal liver through cirrhosis to hepatocellular carcinoma. The difference in AgNOR count was also significant in these three groups. A similar significant difference

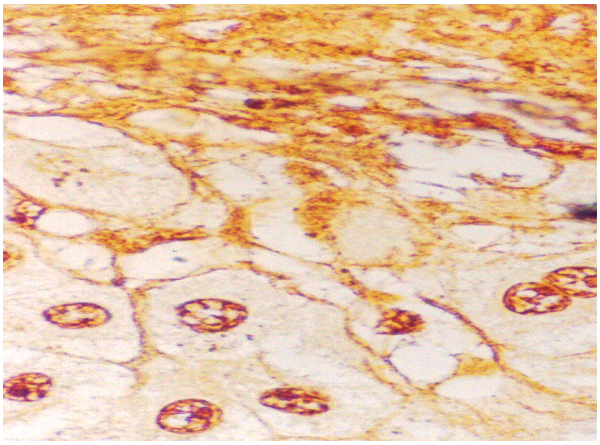

Figure 1: Photomicrograph of a section of liver with cirrhosis of the liver. Hepatocytes show 4 to 5 intranuclear AgNOR dots. (AgNOR stain x 4000).

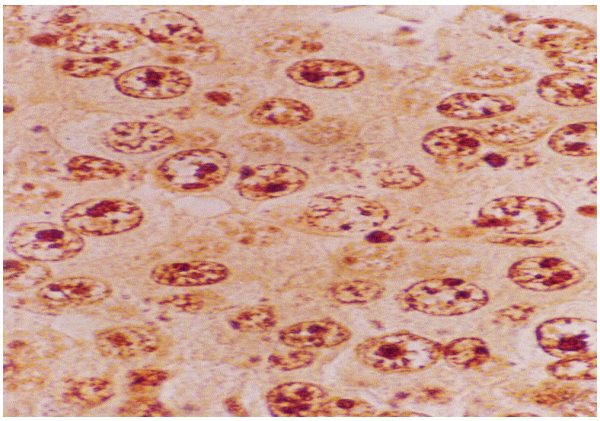

Figure 2: Photomicrograph of a section of liver showing hepatocellular carcinoma. Most of the hepatocytes contain large number of small dispersed AgNOR dots. (AgNOR stain x 4000).

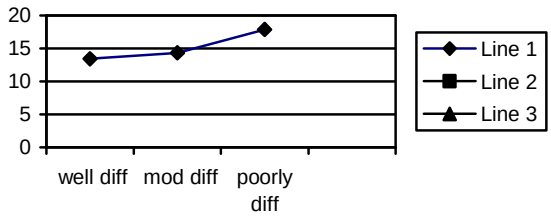

Figure 3: Comparison of AgNOR count according to the grade of hepatocellular carcinoma.

($p<0.001$) was found between the mean AgNOR score of the normal and the pathological biopsies and between the non-neoplastic and the carcinomatous lesions in a study conducted by Anselmi[8].

REFERENCES

1. Underwood JCE. AgNOR measurements as indices of proliferation, ploidy and prognosis. J Clin pathol: Mol pathol 1995; 48: M239-M240.
2. Nikiciez EP, Norback DH, Argyrophilic nucleolar organizer region(AgNOR)staining in normal bone marrow cells. J Clin pathol 1990; 43: 723-727.
3. Yang P, Huang GS, Zhu XS. Role of nucleolar organizer regions in differentiating malignant from benign tumors of the colon. J Clin pathol 1990; 43: 235238.
4. Terasaki S, Terada T, Nakanuma Y, Nonmura A, Unoura M, Kobayashi K. Argyrophilic nucleolar organizer regions and alpha fetoprotein in adenomatous hyperplasia in human cirrhotic liver. Am J Clin pathol 1991; 95: 850-857.
5. Aubele M, Auer G, Jutting U, Falkmer U, Gias P. Prognostic value of quantitatively measured AgNOR in ductal mammary carcinoma. Cyto Histol 1994; 16: 211-218.
6. Ploton D, Menager M, Jeannnesson P, Himber G, Pigeon F, Improvement in the staining and visualization of the argyrophilic proteins of the nucleolar organizer regions at the optical level. Histochem J 1986; 18: 5-14.
7. Croker J, Ayers J, McGovern J. Nucleolar organiser regions in small cell carcinoma of the bronchus. Thorax 1987; 42: 972-75.
8. Anselmi L, Sementa AR, Borgiani L,Banderali A, Rovida S. Nucleolar organiser regions (NOR) in normal and pathological liver; a quantitative analysis. Pathol 1990; 82 (1082): 653-54.
9. Ahsan S, Tayyab M,, Chaudrhy NA, Khan SA. Silver staining nucleolar organizer regions (AgNOR) typing in nodular hyperplasia and carcinoma of the prostate PPM J 1991-92; 2-3 (1-4, 1-2): 67-72.
10. Siddiqui MS, Soomro IN, Kayani N, Muzzafar S, Hasan SH. Assessment of nucleolar organiser regions (NORs) in proliferative conditions. Pathol Res Pract1999; 194 (6): 421-426.