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AgNORs Scoring in Benign Intermediate and Malignant Soft Tissue Tumor

Monika Gupta, A.N.Shrivastava, Nirupma Lal, Asha Agarwal, M.S.D Jaiswa*

Abstract

One of the most recent methods to determine the proliferative activity is silver stained nucleolar organizer region(AgNORs).The purpose of present study is by using mean AgNOR count per nucleus was found to be higher in benign soft tissue tumour as compared to apparently normal soft tissue and found to be statistically significant. An increase AgNOR score in benign, intermediate, malignant soft tissue tumour as compared to apparently normal soft tissue tumour. After histological confirmation, one more section was prepared which was subjected to AgNOR staining. Study was done in taking 32 benign, 05 intermediate and 23 malignant soft tissue. Benign soft tissue neoplasm which comprised 32 cases showed a range of mean AgNORs count 2.2-3.20 and intermediate group showed 3.10-4.61 and malignant group 4.90-6.70, so on comparing benign, intermediate and malignant group p value is found to be <0.0001. Hence results are statistically significant.

Key Words

AgNORs, Soft tissue tumor

Introduction

AgNORs are loops of DNA which contains ribosomal rRNA genes and are stained by silver colloidal technique they are transcribed by RNA polymeraseI.They are located on the short arm of chromosome 13,14,15,21,22 & are of vital importance for ultimate synthesis of protein, thus the quantification of these portion has been a useful maker in differentiating benign, pre malignant and malignant lesion. NORs are seen to be useful in diagnosing pre malignant and malignant lesions of gallbladder, prostate, stomach, colon, cervix endometrium, ovaries, skin, mesothelium, breast, CNS, NHL. NORs appear as black dots of metallic silver about 0.5-1 μm diameter localized within secondary constrictions of metaphase chromosome or in nuclei (1).

Material and Methods

Present study comprised of 60 cases of soft tissue tumor the diagnosis of tumors selected by based upon routine histopathological examinations of H& E stained sections & silver staining was further applied on them for demonstration of AgNORs in an attempt to determine the AgNORs dots in soft issue tumor.

AgNORs Staining Procedure: - Histological sections were stained with haematoxylin & eosin. Also, AgNORs by method of Crocker *et al* (1) by taking 4μ thick sections were made for each case, deparaffinise in Xylol, hydrate through graded alcohol to water, incubate the sections in freshly prepared working solution i.e. silver colloid developer (containing one part by volume of 2% gelatin in 1% Formic acid and two parts by volume of 50% aqueous silver nitrate solution) in a closed coplin jar for 30 to 45 min in dark room at room temperature. This silver colloidal solution was washed with three changes of de-ionized water for 2 minutes each & blot the section and dehydrate through ascending grade of alcohol cleared in xylene mounted.

AgNORs count -For counting, most cellular representative areas were elected. In each case approx 200 nuclei were examined under X 100 oil immersion lens. Multiple regions were examined in each section by selecting microscopic fields at random & by taking 200 cells into account, the mean no of AgNORs per cell is then calculated simultaneously. We also observe the morphology of NOR dots to avoid biased results. The counts were done by two different observers.AgNORs dots are intra nuclear black to brownish black against pale yellow to golden yellow nuclear background.

From the Department of Pathology & Surgery*, Era's Lucknow Medical College and Hospital - Lucknow- India
Correspondence to : Dr. Monika Gupta, 191/62, Mashakganj, Bagh Sher Jung, Lucknow

Results

Among the benign neoplasm(n=32) maximum (65.62%) cases were present in age group of 21 to 35 yrs, among the intermediate group(n=5) maximum cases (60%) were again observed in 21 to 35 yrs and among the malignant neoplasm(n=23) maximum cases (30.43%) were observed in the age group of 51-65Yrs of age (*Table1*).Among the benign neoplasm, Neurofibroma(n=14) constituted the highest (43.75%), among the Intermediate neoplasm, Dermatofibrosarcoma protuberans(n=5) constitute 60% and in malignant Neoplasm(n=23) maximum cases were of malignant fibrous histiocytoma (34.78%) (*Table 2*). Among the above mentioned benign neoplasm mean AgNORs dot / nucleus were seen in case of neurofibroma 3.4 and low grade fibrous tumor were 3.4 followed by leiomyoma having mean AgNORs per nucleus were 2.4 & pelvic fibromatosis by 2.95 & among the intermediate grade of neoplasm mean AgNORs dot / nuclei were seen in case of dermatofibrosarcoma protuberans 3.2 intermediate grade of malignant fibrous histiocytoma by 4.4. Among the malignant neoplasm highest mean AgNORs per nuclei in case of malignant fibrous histiocytoma 7.3 while the liprosarcoma show 7.2 & rest synovial sarcoma, Rhabdomyosarcoma, fibrosarcoma, leiomyosarcoma shows 6.9, 6.4, 6.3, 5.9 respectively.(*Table 3*)

We have also made observation regarding morphology of AgNORs count per nucleus. In benign lesions, the NORs were slightly small but were still conspicuous uniformly staining, round dots with well defined margins.

Table 1. Age Distribution of Cases In Soft Tissue Tumor

Sl. No.	Age Group (yrs)	Benign		Intermediate		Malignant	
		Nos	%	Nos	%	Nos	%
1	5-20	-	-	-	-	2	8.69
2	21-35	21	65.62	3	60	6	26.08
3	36-50	10	31.25	-	-	6	26.08
4	51-65	1	31.25	2	40	7	30.43
5	66-80	-	-	-	-	2	8.69

Table 2. Distribution of Case in Various Grades of Neoplasm

Sl. No.	Histological Type of Tissues	No of cases	Percentage
Benign			
1.	Neurofibroma	14	43.75
2.	Low Grade fibrous tumour	10	31.25
3.	Pelvic fibromatosis	06	18.75
4.	Leiomyoma (Retroperitoneum)	02	6.25
	Total	**32**	**100**
Intermediate			
1.	Dermatofibrosarcoma protuberans	03	60
2.	Intermediate grade of malignant fibrous histiocytoma	02	40
	Total	**05**	**100**
Malignant			
1.	Malignant fibrous histiocytoma	08	34.78
2.	Liposarcoma	05	21.73
3.	Synovial sarcoma	05	21.73
4.	Rhabdomyosarcoma	02	8.69
5.	Fibrosarcoma	02	8.69
6.	Leiomyosarcoma	01	4.3
	Total	**23**	**100**

Table 3. AgNORs Score in Various Grade of Tumor

Sl. No.	Type of Tumor	No of cases	Mean AgNORs/ nucleus	Range of mean AgNORs/ nucleus	Mean ± SD
BENIGN					
1.	Neurofibroma	14	3.4	2.90-3.40	3.12 ±0.1761
2.	Leiomyoma	02	2.4	2.20-2.81	2.46 ±0.2547
3.	*Low grade fibrous tumor*	*10*	*3.4*	*2.90-3.40*	*3.18 ±0.1813*
4.	Pelvic fibromatosis	06	2.95	2.50-3.31	2.95± 0.288
INTERMEDIATE					
5.	Dermato fibro sarcomaprotuberans	03	3.2	3.10-3.30	3.30.±100
6.	Intermediate Grade of malignant fibrous histiocytoma	02	4.40	4.21-4.60	4.40±0.28
MALIGNANT					
7.	Malignant fibrous histiocytoma	08	7.3	7.10-7.60	7.28±0.193
8.	Liposarcoma	05	7.2	6.80-7.60	7.15±0.24
9.	Synovial sarcoma	05	6.9	6.80-7.00	6.88± 0.83
10.	Rhabdomyosarcoma	02	6.4	6.10-6.70	6.40 ±0.4242
11.	Fibrosarcoma	02	6.3	6.20-6.40	6.30± 0.14
12.	Leiomyosarcoma	01	5.9	5.9	5.9±0.00

Table 4. P Value of Difference In Agnors Score In Various Soft Tissue Tumor

Sl. No.	Type of tumor	p value	t value
1.	Benign Vs Intermediate	4.25	<0.001
2.	Intermediate Vs Malignant	40.63	<0.001
3.	Benign Vs Malignant	14.45	<0.001

By contrast the NORs dots observed in malignant lesions were usually finer, small, irregular in shape and size and irregularly scattered throughout and nucleus.*Fig.1&2*

Benign soft issue neoplasm which comprised 32 cases showed a mean AgNORs count of 3.067 ± 2.80 and a range of mean 2.20-3.20 as a whole. Intermediate group of neoplasm showed a mean AgNORs count of 3.74 ± 0.62 and range of 3.10 - 4.61. Similarly malignant group showed a mean AgNORs count of 6.92 ± 0.43 and range of mean 4.90 - 6.70 (*Table 4*). Hence it is seen that among these lesion malignant group showed highest mean AgNORs per nucleus. On comparing benign with the malignant group p value is found to be <0.0001, hence the results are statistically significant our finding co-related with the studies that the AgNORs count ranging from 1.4 - 16.1(mean 7.5) a good relation with cellularity they also shown that the count was higher in malignancy. For the purpose of statistical analysis in this benign intermediate & malignant are grouped together & only histological grading is consider an relation to AgNORs count. On comparing benign Intermediate & malignant p value is found to be statistically significant.

Discussion

AgNORs that stain for NOR associated proteins is known to increase with increase in cell ploidy, increased transcriptional activity and in states of active cell proliferation. It has been observed that mean numbers of AgNORs count per nucleus is higher in malignant cells as compared to benign or normal precursor. Kuratsu *et al* (2) in 1991 observed AgNORs count ranging from 1.4 -16.1 (mean7.5) showed a good correlation with cellulaity & histological grade but less with mitotic count . Kuratsu *et al* (3) in 1993 observed usefulness of argyrophilic nucleolar staining for histological grading of soft issue sarcomas, histological grading is essential for making proper therapy decision in soft tissue sarcoma ' each category divided into high and low count group, the low count group showed a significantly better prognosis than high count group. Kuratsu *et al* (4) 1994 also observed increased proliferative activity in recurrent tumour as compared to primary tumour using AgNORs & it implication for predicting prognosis is assessed. Arora HL *et al* (5) also compared mean AgNORs count in benign & malignant soft tissue tumour & observed that AgNOR count was found to be higher in benign and malignant soft tissue tumor as compared to normal soft

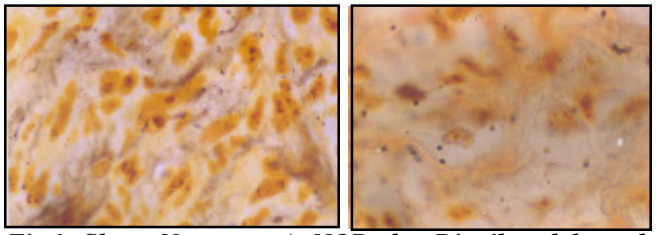

Fig 1. Shows Numerous AgNORs dots Distributed through out Nuclei in a Case of Fibrosarcoma(AgNOR x 1000)
Fig 2. Shows single AgNOR in the Region of Nucleoli in a Case of Low Grade Fibrous Tumour (AgNOR x1000)

tissue tumour. Manera *et al* (6) studied fibromyxoma cell proliferation by silver staining nueleolar organizer region to evaluate cell proliferation by using different areas like myxoid, collagenous & mixed area, a significant ($p<0.01$) difference in cell proliferation activity between different area was found by using AgNORs and confirmed by mitotic index. Johnson *et al* (7) showed to comparison of AgNORs & mitotic index in distinguishing Benign from malignant smooth muscle tumour & It was found AgNOR in 100 nuclei was about as efficient as mitotic index. Matheu RS *et al* (8) evaluation of tumour growth by using AgNORs because proliferative activity of tumour can influence the growth rate of primary carcinoma & account for their aggressiveness. Steininger *et al* (9) also observed a continuous increase in AgNOR from normal to carcinoma & the size of individual AgNOR dots decrease. Our findings correlated which also demonstrate inverse relationship between AgNORs number and their size. Small size, large number and scattered distribution were characteristic of malignant lesion, whereas large size, small number and clustered distribution were characteristic of benign tumor cells.Extensive work has been done on AgNORs as a marker of proliferative potential in a no of lesion Layfield et al (10) in smooth muscle neoplasm, Nagao *et al* (11),Okhi *et al* (12).

Recent Litrature also indicated AgNORs Score to be higger in smear from malignant tumor than benign tumors and thus an important and usefull tool for dignosis (13,14)

Conclusion

On the basis of the present study that AgNORs counts showed a good correlation with cellularity and histological grade. The mean AgNORs score was found to increase with high grade of the tumor, their quantity in terms of number of AgNORs per nucleus is strictly a marker of proliferative activity of the cell. Our study also show inverse relationship between AgNORs number and their size and continuous increase in AgNORs from normal to carcinoma along with decrease in size of individual AgNORs.

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