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AgNOR Scoring in Benign, Intermediate and Malignant Soft Tissue Tumors

Dr. Ramanpreet kaur, Dr. R.K Kundal , Dr. Harpal Singh, Dr. Rohan Bains, Dr. Shivanshu Kundal, Dr. Amitoj Sandhu
Corresponding Author – Dr. R.K Kundal

Abstract: ***Background :*** *Soft tissues are defined as the complex of nonepithelial extraskeletal structures of the body exclusive of the supportive tissue of the various organs and the haematopoietic/lymphoid tissue. It is composed of fibrous tissue, adipose tissue, skeletal muscle, blood and lymph vessels and the peripheral nervous system. Many times histopathological assessment correlates poorly with clinical outcome. Thus some limitations have motivated development of new technique so as to improve accuracy and reproducibility of prognostication. The newer technique is called "Silver stained nucleolar organizer regions (AgNORs)" which is cheap,and simple to use to assess its role in histopathological diagnosis and prognosis of disease.*
Objectives : *(1) To evaluate the various benign, intermediate and malignant soft tissue tumors. (2) To study the AgNOR counts and their correlation with soft tissue tumors.*
Material and Methods *All the specimens of soft tissue tumors submitted for histopathological examination were included in the material to study. The relevant data of patient was recorded in pre designed performa. Total of 80 cases were included in the studies. Paraffin sections were dewaxed and hydrated through series of graded alcohol, washed for 15 min in running tap water and then distilled water. These sections were placed in working AgNOR solution for 15 min, and then washed for 3 min in 3 changes of distilled water. Counter staining with saffranin solution 0.01% was done. Sections were dehydrated cleared and mounted in DPX. AgNOR stain as black dots within nucleus and their number was counted in 100 cells. Mathematical and statistical tests were applied for results.*

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I. Introduction

Nucleolar organiser regions (NORs) are defined as nucleolar components containing a set of argyrophilic proteins, which are selectively stained by silver methods. After silver-staining, the NORs can be easily identified as black dots exclusively localised throughout the nucleolar area, and are called "AgNORs". The NORs' argyrophilia is due to a group of nucleolar proteins, which have a high affinity for silver (AgNOR proteins). A number of studies carried out in different tumour types demonstrated that malignant cells frequently present a greater AgNOR protein amount than corresponding non-malignant cells. Moreover, in cancer tissues AgNOR protein expression was found to be strictly related to the cell duplication rate. Over the past 12 years, the "AgNOR method" has been applied in tumour pathology for both diagnostic and prognostic purposes. However, the lack of a standardised silver-staining protocol has led to much misinterpretation of actual structures evaluated in individual studies. Indeed, the absolute AgNOR scores reported by different authors for the same types of tumour are scarcely comparable and the results produced by these investigations sometimes seem to be conflicting. In order to achieve definitive standardisation of the AgNOR method and produce comparable data in all laboratories, the "International Committee on AgNOR Quantitation" was founded, and during the first Workshop "AgNORs in Oncology" held in Berlin in 1993 guidelines for AgNOR protein evaluation were first defined.[1]

II. Material And Methods

All the specimens of soft tissue tumors submitted for histopathological examination were included in the material to study. The relevant data of patient was recorded in pre designed performa. Total of 80 cases were included in the studies.

The specimens received from surgery department were fixed in 10% buffered formalin. After fixation, the specimen were subjected to gross examination for size external appearance.The tumor was divided into slices with a large shape knife. Several pieces from different areas in formalin, were fixed for several hours and overnight and trimmed to be placed in cassettes.

AgNOR Staining procedure
Staining was done by Crocker and Smith 1988.[2]

Requirement
1. Colloidal developer solution –It was prepared by dissolving 2gm of gelatin powder in 100ml of distilled water, to which 1 ml of formic acid was added
2. Aqueous silver nitrate – it was prepared by dissolving 5gm of silver nitrate in 10 ml of distilled water.
3. Final working solution – Colloidal solution and aqueous solution were mixed in ratio 1:2 to obtain working solution.
4. Safranin-It was prepared by dissolving 1gm of safranin in distilled water. To get 1ml stock solution was mixed with 10 ml of distilled water.

Method
1. Paraffin sections were dewaxed and hydrated through series of graded alcohol, washed for 15 min in running tap water and then distilled water.
2. These sections were placed in working AgNOR solution for 15 min, and then washed for 3 min in 3 changes of disilled water.
3. Counter staining with saffranin solution 0.01% was done.
4. Sections were dehydrated cleared and mounted in DPX.
5. AgNOR stain as black dots within nucleus and their number was counted in 100 cells.
6. Mathematical and statistical tests were applied for results.

III. Results

All the specimens of soft tissue tumors submitted for histopathological examination were included in the material to study. The relevant data of patient was recorded in pre designed performa. Total of 80 cases were included in the studies.

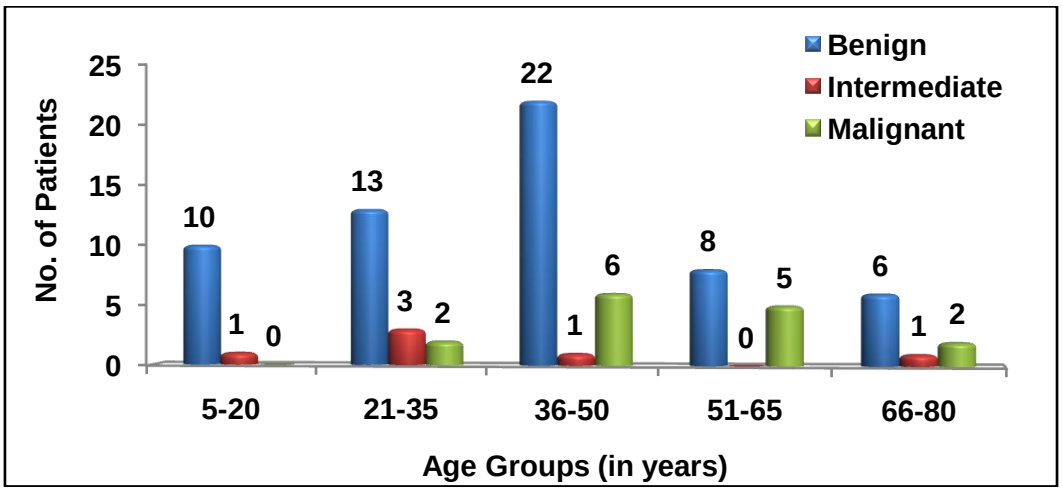


Figure 1 : Age Wise Distribution Of Soft Tissue Tumor

In the present study patients were divided into 5 groups, according to age. In the benign category and malignant category the maximum number of patients were in age group 36-50 years i.e 22 (37.29%) and 6 (40%) cases respectively. In the intermediate category the maximum number of patients were in age group 21-35 years i.e 3 (50%). The mean age of patients in benign category was 39.56 years with the range of 7-80 years. The mean age of patients in intermediate category was 38.50 with the range of of 17-70 years. The mean age of patients in malignant category was 41.55 with the range of 23-75 years.

Table-1
The Agnor Scoring In Benign Soft Tissue Tumors [Group A]

Histological Type of Tumor	No. of Cases	Mean AgNOR/ Nucleus	Range of mean AgNOR/ Nucleus
Lipoma	23	1.65	1.30-2.10
Hemangioma	13	2.40	1.90-2.90
Endothelial hemangioma	1	1.9	1.9
Neurofibroma	6	2.65	2.20-2.90
Schwannoma	2	2.65	2.50-2.80
Nodular Fasciitis	2	1.95	1.90-2.00
Angiofibroma	1	2.50	2.50-2.50
Benign Fibrohistiocytic	4	2.40	2.20-2.40
Tenosynovial Giant cell	4	2.37	2.10-2.60
Glomus Tumor	1	2.10	2.10-2.10
Fibromyxoma	1	2.50	2.50-2.50
Angiomyxoma	1	1.50	1.50-1.50

Statisical Analysis Of Group A

Group	Mean	SD	Std. Error Mean
A	2.08	0.45	0.059

In the present study the group A included the mean AgNOR/nucleus and range of mean AgNOR/nucleus of benign soft tissue tumors. These were analysed and further the mean AgNOR/ nucleus in benign soft tissue tumors was calculated which came out to be 2.08.

Table-2
The Agnor Scoring In Intermediate Soft Tissue Tumors [Group B]

Histological Type of Tumor	No. of Cases	Mean AgNOR /Nucleus	Range of mean AgNOR /Nucleus
DFSP	2	3.75	3.60-3.90
Fibromatosis	4	3.15	3.00-3.20

Statistical Analysis Of Group B

Group	Mean	SD	Std. Error Mean
B	3.33	0.34	0.141

In the present study group B included the mean AgNOR/nucleus and range of mean AgNOR of intermediate soft tissue tumors. These were analysed and further the mean AgNOR/ nucleus of intermediate soft tissue tumors were calculated which came out to be 3.33.

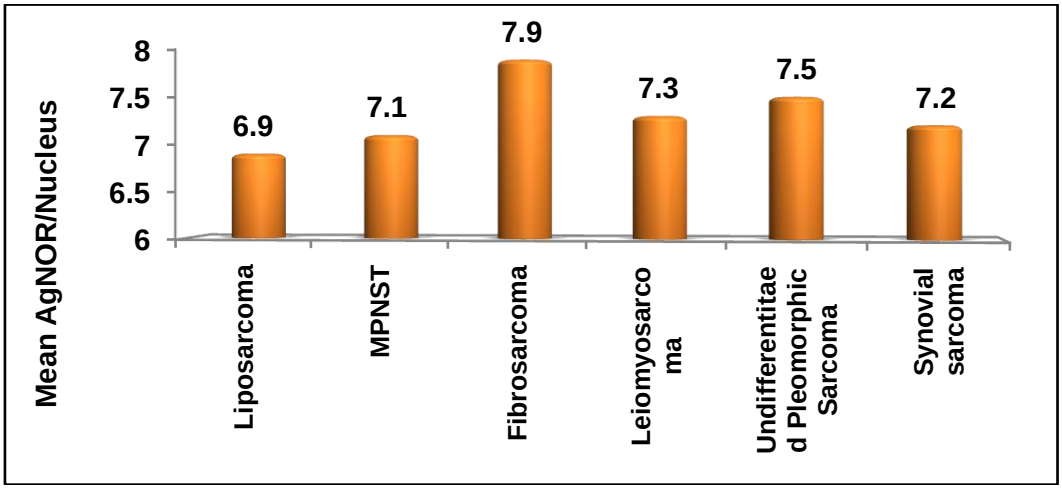


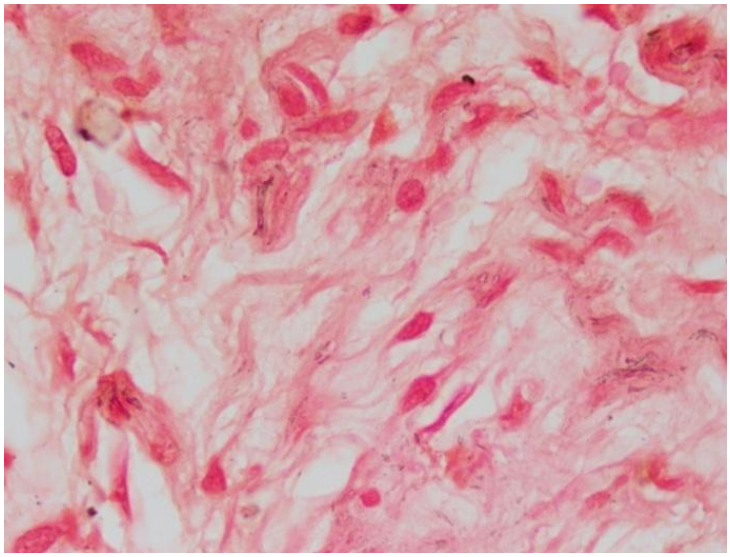
FIGURE 2 : THE Agnor SCORING IN MALIGNANT SOFT TISSUE TUMORS IN GROUP C

In the present study the group C, included the mean AgNOR/nucleus and range mean of AgNOR of malignant soft tissue tumors. These were analysed and further the mean AgNOR/ nucleus of malignant soft tissue tumors was calculated which was 7.33.

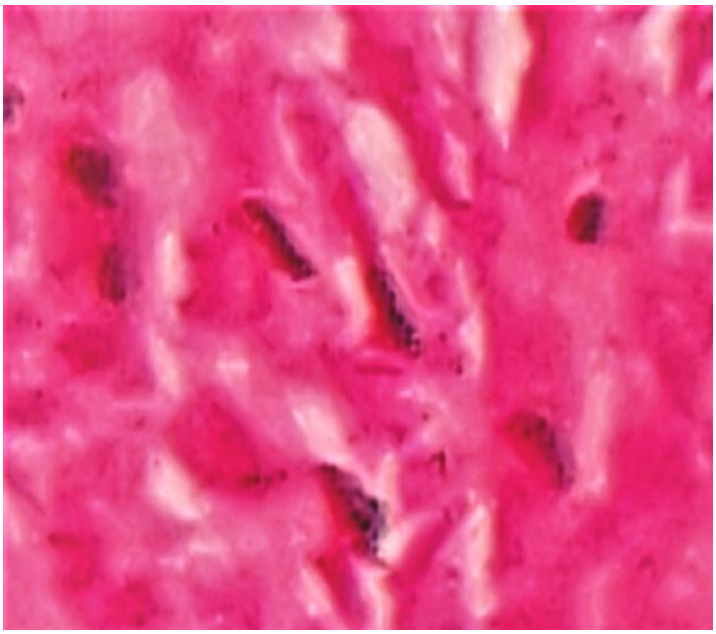
Table-20: Correlation Of Agnor Scoring In All The Three Groups

Group		χ^2	p value	Sign.
I	A-B	8.192	0.001	HS
II	A-C	42.090	0.001	HS
III	B-C	24.222	0.001	HS

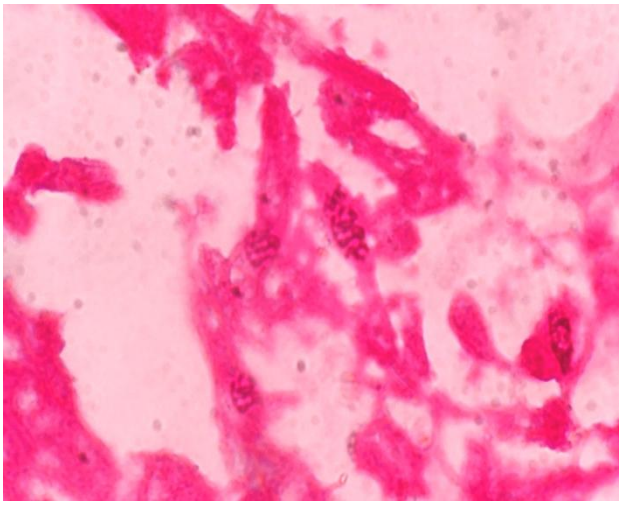
In the present study the comparsion was made between all the three groups. GROUP I - benign vs intermediate, GROUP II - intermediate vs malignant and GROUP III - benign vs malignant
The results of all the groups had p value of 0.001, which was highly significant.



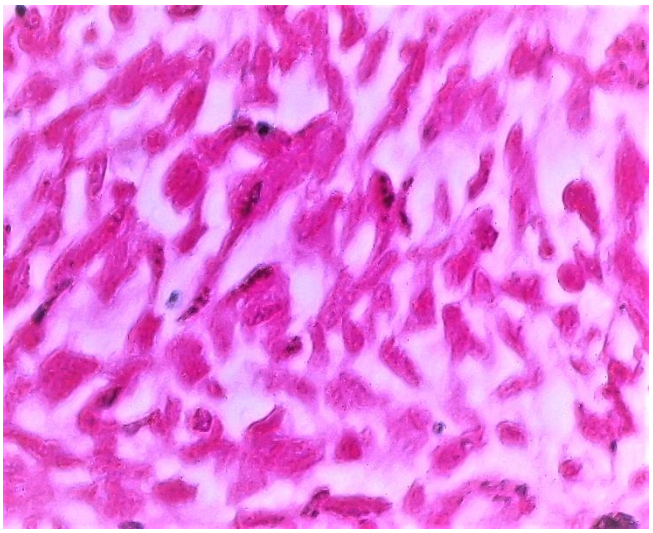
Photomicrograph 1 : Section showing lesser number of AgNOR dots in a case of neurofibroma compared to malignant tumors (AgNOR 1000X)



Photomicrograph 2 : Section showing multiple dots in case of DFSP (intermediate grade) ,these are increased as compared to benign tumors but less than malignant tumors at higher magnification (AgNOR 1000 X)



Photomicrograph 3 : Section showing AgNOR dots in a case of leiomyosarcoma, which are increased as compared to benign tumors (AgNOR 1000X)



Photomicrograph 4 : Section showing AgNOR dots in malignant cells in a case of MPNST (AgNOR 1000X)

IV. Discussion

All the specimens of soft tissue tumors submitted for histopathological examination were included in the material to study. The relevant data of patient was recorded in pre designed performa. Total of 80 cases were included in the studies. In the present study, the mean AgNOR count in benign tumors was 2.08, which is in accordance with the study conducted by **Khan et al[3] (2006)** in which the benign group had AgNOR score in the range of 1.1 to 2.1, and **Gupta et al[4] (2012)** in which the range was 2.20 to 3.20.

The mean AgNOR score in intermediate group was 3.33 in the present study which is in accordance with the study conducted by **Gupta et al[4] (2012)** in which the range of mean AgNOR score was 3.10 to 4.61.

The mean AgNOR score was 7.33 in the present study which is in accordance with the study conducted by **Kurastu et al[5] (1991)** where the mean AgNOR score in malignant tumors was 7.5, **Johnson et al[6] (1995)** where mean AgNOR count was 7.4, **Khan et al[3] (2006)** where range of mean AgNOR score was 2.57-7.21 and **Gupta et al[4] (2012)** where mean AgNOR score was 4.90-6.70.

In the present study, the mean AgNOR count was found to be higher in malignant than in benign tumors, which is in accordance with the study conducted by **Wrba et al[7] (1991),** in which the mean AgNOR was higher higher in case of malignant soft tissue tumors than intermediate and benign soft tissue tumors.

The mean AgNOR count in the case lipoma was 1.60, which is in accordance with the study conducted by **Arora et al[8] (1996)** where the mean AgNOR count was more than 0.94.

In the present study, the benign peripheral nerve sheath tumor i.e neurofibroma had mean AgNOR score of 2.65 which was in close accordance with the study conducted by **Gupta et al[4] (2012)** in which mean AgNOR score was 3.4.

In the present study,mean AgNOR score for intermediate tumor i.e for DFSP was 3.75 which is in accordance with the study conducted by **Arora et al[8] (1996)** in which the mean AgNOR score was more than 1.02 and **Gupta et al[4] (2012)** in which mean AgNOR score was 3.2.
In the present study, the AgNOR score in case of fibromatosis was 3.15 which is in accordance with study conducted by **Gupta et al[4] (2012)** where the mean AgNOR score was 3.4.
In the present study , the mean AgNOR score in the case of liposarcoma was 6.9 which is in accordance with the study conducted by **Gupta et al[4] (2012)** where mean AgNOR score was 7.2.
In the present study , the mean AgNOR count in case of leiomyosarcoma was 7.30 which is in accordance with study conducted by **Johnson et al[6] (1995)** where mean AgNOR score was found 7.4, **Arora et al[8] (1996),** where mean AgNOR score was more than 4 and **Gupta et al[4] (2012)** where mean AgNOR score was 5.9.
In the present study, the mean AgNOR score in case of synovial sarcoma was found 7.2 which is in accordance with the study conducted by **Gupta et al[4] (2012)** where the mean AgNOR count was 6.9
In the present study, the mean AgNOR count in case of fibrosarcoma was found to be 7.9 which is in accordance with the study conducted by **Arora et al[8] (1996)** where mean AgNOR count was more than 4 and in study by **Gupta et al[4] (2012)** where mean AgNOR score was 6.3.
In the present study, the mean AgNOR count of undifferentiated pleomorphic sarcoma was 7.50 which is in accordance with the study conducted by **Gupta et al[4] (2012)** where mean AgNOR score was 7.3.
In the present study , the p value of AgNOR scoring was significant in all the three groups made which included benign vs intermediate GROUP I, intermediate vs malignant GROUP II, benign vs malignant GROUP III which are in accordance with the study by **Kanamouri et al[9] (1999)** and **Gupta et al[4] (2012),** where all the three groups had highly significant p values.

V. Conclusion

The benign soft tissue tumors are most common compared to intermediate and malignant tumors. The adipocytic tumors had higher incidence followed by vascular tumors and PNS tumors. Various techniques can be used to diagnose soft tissue tumors such as FNAC, core needle biopsy, histopathological examination along with radiological findings. The AgNOR score was found higher in malignant tumors followed by intermediate tumors and the lowest counts were found in benign tumors. The study of proliferation with Nucleolar organiser regions using simple silver stained techniques is an important milestone. Many benign proliferation can be differentiated from malignant. It also helps in grading and staging of soft tissue tumors.

Bibliography

[1]. Berlin O, Stener B, Kindblom LG, Angervall L. Leiomyosarcomas of venous origin in the extremities. A correlated clinical, roentgenologic, and morphologic study with diagnostic and surgical implications. Cancer 1984;54(10):2147-59.
[2]. Crocker J, Boldy DAR, Egan MJ. How should we count AgNORs proposals for a standardized approach. J Pathol. 1989;158(3):185-88.
[3]. Khan N, Sood P, Vasenwala SM, Afroz N, Verma AK. Significance of AgNOR score in benign and malignant soft tissue tumours. Indian J Pathol Microbiol. 2006;49(1):17-20.
[4]. Gupta M, Shrivastava AN, Lal N, Agarwal A, Jaisava MSD. AgNORs scoring in benign intermediate and malignant soft tissue tumour. JK Science 2012;14(1):18-21.
[5]. Kuratsu S, Aozasa K, Myoui A, Tsujimoto M, Ueda T, Uchida A et al. Prognostic significance of agyrophilic nucleolar organizer staining in soft tissue Sarcoma. Int J Cancer. 1991;48(2):211-4.
[6]. Johnson GC, Miller MA, Ramos-Vara JA. Comparison of argyrophilic nucleolar organizer regions (AgNORs) and mitotic index in distinguishing benign from malignant canine smooth muscle tumors and in separating inflammatory hyperplasia from neoplastic lesions of the urinary bladder mucosa. J Vet Diagn Invest. 1995;7(1):127-36.
[7]. Wrba F, Augustin I, Fertl H. Nucleolar organizer regions in soft tissue sarcomas. Oncology. 1991;48(2):166-70.
[8]. Arora HL, Arora N, Solanki RL.Argyrophilic nucleolar organiser region in soft tissue sarcoma. Indian J pathomicrob 1996;39(4):251-63.
[9]. Kanamori M, Matsui H, Ohmori K, Maeda Y, Tatezaki S. Significance of proliferating cell nuclear antigen and argyrophilic nucleolar organizer regions in bone and soft-tissue spindle-cell tumors–PCNA-AgNORs index as a new parameter. International Journal of Clinical Oncology. 1999;4(2):101-6.

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