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Research Article

AgNOR Staining in Prostatic Carcinoma - Comparative Study with p53 and Gleason Score

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Abstract
Introduction: *AgNOR, a synonym for silver stained nucleolar organizing regions (NORs) of DNA located on the short ends of acrocentric chromosomes are emphasized in actively proliferating cells. When compared with immunohistochemistry, AgNOR can give information related to the proliferation status of the tumours as inferred by ki67 other proliferation and apoptotic markers. In this study we compare the AgNOR staining patterns in prostatic carcinoma with the Gleason score and immunohistochemical markers p53.*
Methods: *The present study was done on 62 cases of Prostatic carcinoma. In this study we compare the AgNOR staining patterns in prostatic carcinoma with Gleason score and immunohistochemical markers p53.This study will be able to ascertain the efficacy of AgNOR staining as a substitute p53. Statistical analysis of mAgNOR count with Gleason score and p53 was done by SPSS software statistics By using Kendalls tau b test.*
Results: *mAgNOR shows statistically significant correlation with Gleason score and p53.*
Conclusions: *Silver staining of Nucleolar organizer regions is a useful method in identifying and grading of prostatic carcinoma. mAgNOR count show positive correlation with Gleason score and p53 IHC so, AgNOR staining may substitute the p53 IHC in lab where IHC facility is not available.*
Key words: *AgNOR- Silver stained nucleolar organizing regions (NORs) mAgNOR – Mean AgNOR, Gleason score, carcinoma prostate.*

Introduction

Prostate cancer is predominantly a disease of the elderly men, with more than 75% of new prostate cancers being diagnosed in men older than 65 years. AgNOR, a synonym for silver stained nucleolar organizing regions (NORs) of DNA

located on the short ends of acrocentric chromosomes are emphasized in actively proliferating cells[1]. NORs can be selectively visualised by silver staining in routinely processed histological samples. Extensive evidence shows that the quantity of AgNOR protein reflects the state of cell proliferation. So, NORs can serve as an independent indicator of differentiation in malignant tumours, and/or as prognostic factor. AgNOR staining can be performed on routine paraffin sections and is inexpensive. When compared with immunohisto-chemistry, AgNOR can give information related to the proliferation status of the tumours inferred by ki67, p53, Bcl-2, and other proliferation and apoptotic markers. Prostatic carcinoma is a common and growing public health problem. There are several criteria available for the diagnosis and prognostigation of prostate cancer; such as histological grade, clinical stage, IHC markers like ki67, PCNA. But these features are seldom looked at routinely, as IHC is expensive. In general, increase in mean AgNORs could result if any of the following has occurred in,

In active cell proliferation, nucleolar dissociation occurs so that AgNORs are dispersed throughout the nucleus.

- Defective nucleolar association.
- Increased cell ploidy, resulting in real increase of AgNOR bearing chromosomes.
- Increased transcriptional activity.

The number mean AgNOR correlate with the level of DNA transcription the degree of cell proliferation as evidenced by the percentage of cells in S phase and the growth fraction determined by Ki-67 immunostaining and bromodeoxyuridine labelling index. The argyrophil, AgNOR technique is remarkably specific and simple as a means for detection of NORs which it demonstrates by virtue of silver binding to a wide array of NOR- associated proteins (NORAPs). In this study we compare the AgNOR staining patterns in prostatic carcinoma with the Gleason score and immunohistochemical p53. This study will be able to ascertain the efficacy of AgNOR staining as a substitute for p53.

Materials and Methods

The Study design was Hospital based Cross sectional study with Univariate analysis using mean, standard deviation and proportion and Bivariate analysis by using Chi square test, student t test. Duration of study was 18 months and on sample received in Histopathology. Intra observer bias is prevented by first observing all AgNOR then observing the routine sections separately. Evaluation by a second pathologist prevented inter observer error. Inclusion criteria included all biopsy of prostate where adequate tissue available(Adequate tissue is atleast one bit 0.75cm of prostate tissue having epithelial and stromal elements).

Morphometry

For quantitative analysis of AgNOR staining, The AgNOR count will be done counting nuclei of 100 cells under oil immersion lens .Clusters of black dots within nucleoli will be counted as one AgNOR and dispersed dots throughout the nucleus will be counted as discrete AgNORs Atleast 100 tumour cells were counted per patient. For quantitative analysis of AgNOR staining, 100 nuclei per patient specimen were counted and expressed as the mean number of positive-stained grains per nucleus per patient specimen.

The extent of p53 nuclear staining was expressed using a four-point scale of:

0 No nuclear staining

1 (1 – 33%) positive nuclear staining

2 (34 – 67%) positive nuclear staining

3 (68 – 100%) positive nuclear staining

Gleason score

Tumour Pattern	Glandular-Architectural Differentiation	Tumour-stromal relation	
		Boundary of tumour mass	Stromal Infiltration
1	Distinct glands, uniform size and shape,closely packed.	Sharply defined rounded	Negligible
2	Distinct glands, irregularities in size and shape, varyig interglandular spacing.	Defined but less sharp	Along major stomal planes
3	Distinct glands, accentuated irregularities in size, shape interglandular spacing Or Abortive minute glands and cell clusters. Or Rounded masses, cribriform or papillary.	ill defined	Along major and smaller fiber planes or Expansile
4	Apparently fused glandular tumour. Or conglomerates pale cells with hypernephroid appearance.	iII defined ragged	Severe across smaller fiber plane
5	Solid tumour masses Or Diffusely infiltrating anaplastic carcinoma	Sharply defined Or Poorly defined ragged	Expansile severe across stromal fiber

Statistical analysis of mAgNOR count with Gleason score and mAgNOR count with p53 was done by PSS software statistics By using Kendalls tau b test

Observations

During the study period 18 month , Sixty two cases of Prostatic carcinoma biopsies were analysed. These included trucut biopsies and prostatectomy specimens

Table 01 Age distribution

Age range	Frequency	Percentage
50-60	9	14.51
61-70	27	43.54
71-80	16	25.8
81-90	10	16.12
Total	62	100

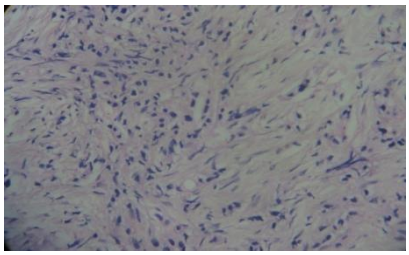
Maximum cases seen in the age range of 61-70yrs 27/62cases (43.54%)

In each case the architecture of acini, nuclear features, and stroma were studied and prostatic carcinoma cases were grouped into 5 grades according to Gleason scoring system.

Table 02 Distribution of prostatic carcinoma cases according to Gleason score

Gleason score	Frequency	Percent
6	1	1.61
7	11	17.74
8	21	33.87
9	24	38.7
10	5	8

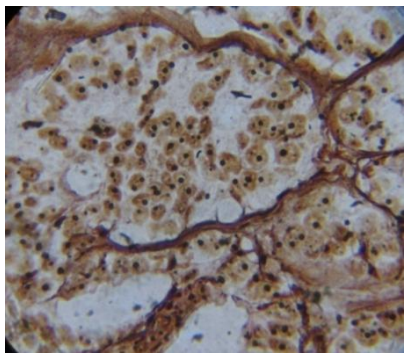
Maximum cases are seen in the Gleason score 9 24/62 (38.7%) with high grade tumour



Gleason score 10

Table 03 Distribution of prostatic carcinoma cases according to mAgNOR Number

mAgNOR Number	Frequency	
1.01-1.50	15	
1.51-2	14	22.58
2.01-2.5	18	29
2.51-3	5	8
3.01-3.5	6	9.7
3.51-4	3	4.83
4.01-4.5	1	1.61

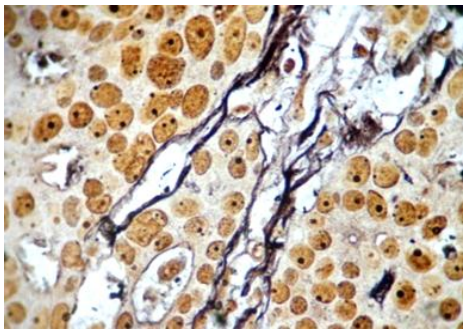


mAgNOR Number 1.32

Table 04 Comparison of mAgNOR number with Gleason score

mAgNOR Number	Frequency	Gleason score				
		6	7	8	9	10
1.01-1.50	15	1	8	3	3	0
1.51-2	14	0	1	12	1	0
2.01-2.5	18	0	2	5	11	0
2.51-3	5	0	0	1	4	0
3.01-3.5	6	0	0	0	4	2
3.51-4	3	0	0	0	1	2
4.01-4.5	1	0	0	0	0	1

As mAgNOR count increase the grading and gleasons score also increases.



mAgNOR Number 2.39

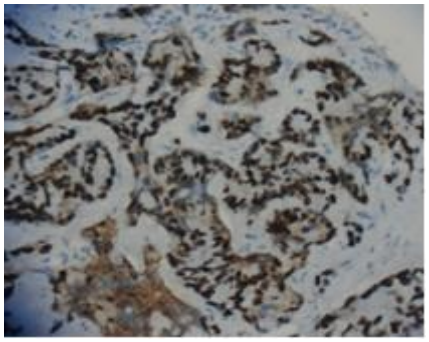
Table 05 p53 Status

p53	Frequency	Percent
p53 Positive	36	58%
p53 Negative	26	42%

36/62 cases 58% are positive for p53 staining

Table 06 Comparision of p53 nuclear staining score and frequency of the cases

P53 Nuclear staining Score	Frequency	Percent
0	26	42%
1	16	25.8
2	9	14.51
3	11	17.74



p53 staining score 3

Table 07a Comparison of p53 positive case with Gleason score

Gleason score (No. of cases)	P53 Positive (Frequency)	P53 Negative
6 (1)	0	1
7 (11)	3 (27.27%)	8
8 (21)	11 (52.4%)	10
9 (24)	18 (75%)	6
10 (5)	4 (80%)	1
Total	36	26

Table 07b Comparison of p53 positive case with Gleason score

Gleason score	P53 Positive (Frequency)	P53 Score 0 (Negative)	P53 Score 1	P53 Score 2	p53 Score 3
6	0	1	0	0	0
7	3	8	1	1	1
8	11	10	2	3	6
9	18	6	11	4	3
	4	1	2	1	1
Total	36	26			

It shows that maximum percentage positivity in high Gleason score so, it shows p53 positivity indicates possibility of high grade of disease

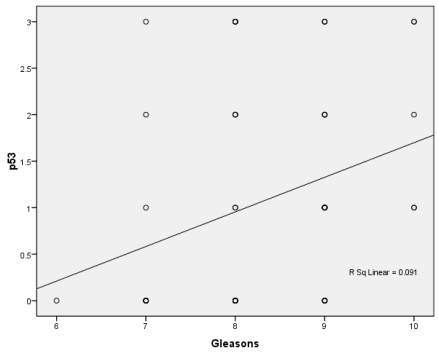
Table 08 Comparision of mAgNOR and p53 positive and negative cases

mAgNOR Number	P53 Positive	P53 Negative
1.01-1.50	4 (26.66%)	11
1.51-2	7 (50%)	7
2.01-2.5	13(72.22%)	5
2.51-3	4 (80%)	1
3.01-3.5	5 (83.33%)	1
3.51-4	2(66.67%)	1
4.01-4.5	1(100%)	0
Total	36	26

Shows a maximum percentage of positive cases in high mAgNOR number

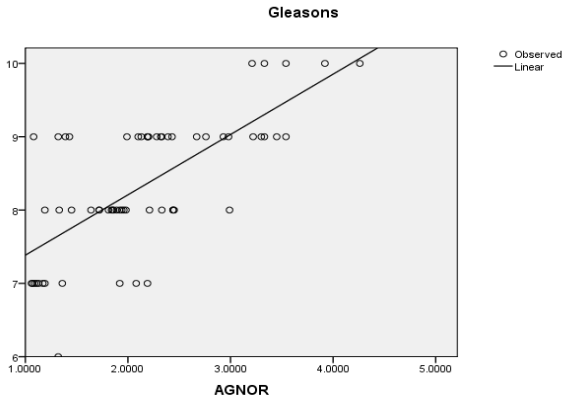
Statistical analysis

Figure 01 Scatter diagram showing relation between mAgNOR and Gleason score



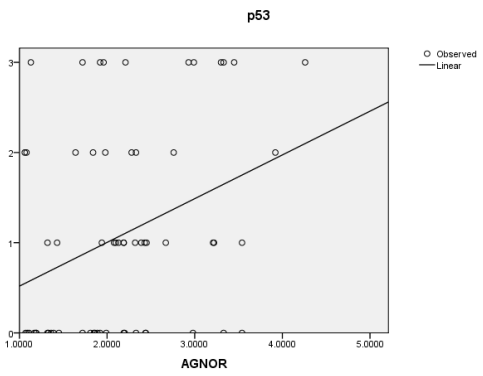
Statistical analysis of mAgNOR count and Gleason score was done by SPSS software statistics By using Kendalls tau b test Shows positive correlation with P value 0.001 (Significant)

Figure 02: Scatter diagram showing relation between p53 and Gleason score



Statistical analysis of Gleason score and p53 was done by SPSS software statistics, By using Kendalls tau b test, shows Positive correlation between gleasons score and p53 with p value 0.012 which is significant. It shows that p53 and Gleason score positively correlate

Figure 03 Scatter diagram showing relation between mAgNOR and p53



Statistical analysis of mAgNOR count and p53 was done by SPSS software statistics By using Kendalls tau b test, shows Positive correlation between mAgNOR and p53 with p value 0.012 which is significant

Discussion

The study group included 52 to 87 years. Maximum case seen in the age range of 61-70yrs i.e 27 out of 62 cases (43.54%). Maximum cases are seen in the Gleason score 9 i.e 24 out of 62 cases(38.7%) with high grade tumour. This study analyses the AgNOR count in carcinoma of the prostate[2,3]. The histopathological grouping of prostate lesions was done based on architecture, nuclear features and the stroma[5]

Hansen and ostergard[9] had observed that satellite AgNORs predominates in hyperplasia and granular AgNORs were seen in prostatic Intraepithelial Neoplasia (PIN) and carcinoma of prostate. Orrel JM et al[8] had observed that it was essential to count Intranucleolar AgNORs separately in addition to those lying outside the nucleolus to obtain clear separation of naevi from melanoma. Deschenes and Weidner [17] observed a mean AgNOR count 1.35 in Prostatic Intraepithelial Neoplasia. The low AgNOR count in PIN, in the present study could be due to occurrence of only PIN grade 1 lesions. In carcinoma the AgNOR counts is consistent and increased progressively from Gleason score 6 to 10.

mAgNOR count 1.01-1.50,1.51-2.0 shows maximum cases in Gleason score 7 & 8 respectively

mAgNOR count 2.51-3.50 shows maximum cases in Gleason score 9 mAgNOR count 3.51-5.0 shows maximum cases in Gleason score 10 .It shows that as mAgNOR count increase the grading and gleasons score also increase that is positive correlation.

P53 over expression has been investigated independently in a large number of different malignancies for their potential value as a prognosticmarker. Mutation of the p53 tumor suppressor gene is a common genetic alteration in malignant human tumors and can be immunohistochemical detected. Importance of p53 in the pathogenesis of prostatic adenocarcinoma was first postulated by Rubin SJ *et al.*[10]and Isaacs WB et al [11]demonstrated

mutations of p53 gene in prostate cell lines and in a primary human prostatic adenocarcinoma. While a number of groups demonstrated a high p53 mutation and/or protein accumulation rate in prostate cancer[12] others reported rare mutation. In this study shows that 36 out of 62 cases i.e 58% are positive for p53 staining. It shows that maximum percentage of p53 positivity in high Gleason score so, it shows p53 positivity indicates possibility of high grade of disease. Bookstein R *et al.*[13] reported that 23% of stage III or IV tumors and 4% of stage 0–II tumors had abnormal nuclear p53 accumulation and that 20–25% of advanced cancers, but none of early Prostatic carcinoma had mutations of the p53. Kubota Y *et al.*[14] screened Prostatic carcinoma specimens for p53 gene mutations in axons 1–11 and found that 9% of well and moderately differentiated and 30% of poorly differentiated Prostatic carcinoma had p53 mutations. This result also supports that p53 mutation is a late event in the development of Prostatic carcinoma. In this study By using Kendalls tau b test, shows Positive correlation between gleasons score and p53 with p value 0.012 which is significant.

In this study we have done a Comparision of mAgNOR and p53 positive and negative cases**.** It Shows a maximum percentage of positive cases in high mAgNOR number as 2.5-3,3.01-3.50,3.51-4,4.01-4.5 shows 80%,83.33%,66.67%,100% positive cases respectively**.** By using Kendalls tau b test, shows Positive correlation between mAgNOR and p53 with p value 0.012 which is significant.

Conclusion

Silver staining of Nucleolar organizer regions is a useful method in identifying and grading of carcinoma prostate. The differences in AgNOR count in benign and malignant lesions can be observed on casual examination of slides. Hence the study of AgNOR can be used as a method routinely, when diagnosis of malignancy is equivocal on routine Haematoxylin-Eosin stained section.

mAgNOR count show positive correlation with Gleason score and p53 IHC so, AgNOR staining may substitute the p53 IHC in lab where IHC facility is not available.

Significant association between p53 protein over expression and Gleason score were found so, p53 IHCcan help to asses grading and prognosis of prostatic carcinoma

Acknowledgment

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