AgNOR Staining in Prostatic Carcinoma - Comparative study with Bcl-2 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 Bcl-2.*
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 Bcl-2.This study will be able to ascertain the efficacy of AgNOR staining as a substitute Bcl-2. Statistical analysis of mAgNOR count with Gleason score and Bcl-2 was done by SPSS software statistics By using Kendalls tau b test.*
Results: *mAgNOR shows statistically significant correlation with Gleason score and insignificant correlation with Bcl-2.*
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 so, AgNOR staining may substitute the proliferative marker IHC in lab where IHC facility is not available.*
Keywords: *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 immunohistochemistry, 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 Bcl-2. This study will be able to ascertain the efficacy of AgNOR staining as a substitute for Bcl-2.

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 Bcl-2 cytoplasmic staining was expressed using a four-point scale of:

0 No cytoplasmic staining

1 Weak diffuse ctyoplasmic staining

2 Moderate cytoplasmic staining

3 Strong cytoplasmic 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 Bcl-2 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

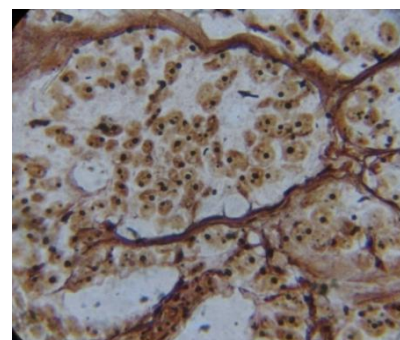| Gleason score	Frequency	Percent
6	1	1.61
7	11	17.74
8	21	33.87
9	24	38.7
10	5	8

Maximum cases are seen in the Gleason score 9 24/62 (38.7%) with high grade tumour
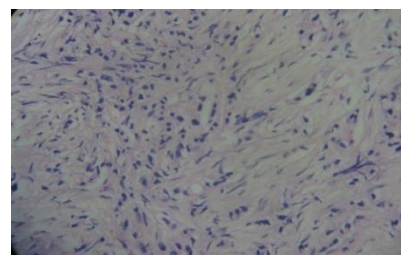
Gleason score 10

Table 03 Distribution of prostatic carcinoma cases according to mAgNOR Number

mAgNOR Number	Frequency	Percent
1.01-1.50	15	24.19
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.
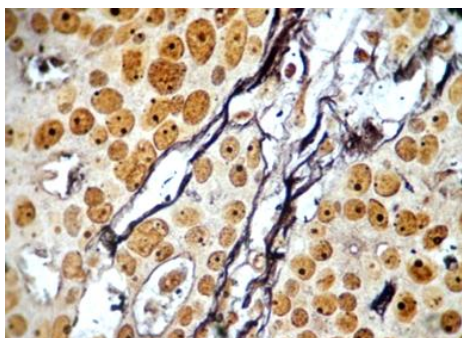
mAgNOR Number 2.39

Table 05 Bcl-2 Status

Bcl-2	Frequency	Percent
Bcl-2 Positive	36	58%
Bcl-2 Negative	26	42%

36/62 cases 58% are positive for Bcl-2 staining

Table 06 Comparision of Bcl-2 cytoplasmic staining score and frequency of the cases

Bcl-2 cytoplasmic staining Score	Frequency	Percent
0	26	42%
1	9	14.5
2	16	25.8
3	11	17.7
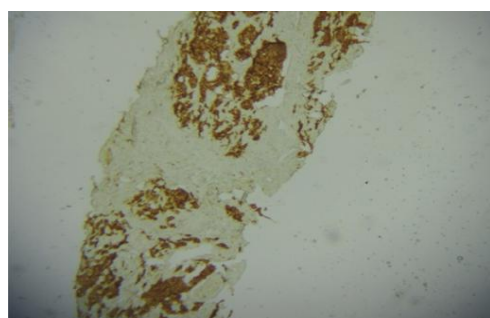
Bcl-2 staining score 3

Table 07a Comparison of Bcl-2 positive case with Gleason score

Gleason score (No. of cases)	Bcl 2 Positive (Frequency)	Bcl-2 Negative
6 (1)	1(100%)	0
7 (11)	5(45.4%)	6
8 (21)	13(62%)	8
9 (24)	14(58.3%)	10
10 (5)	3(60%)	2
Total	36	26

Table 07b Comparison of Bcl-2 positive case with Gleason score

| Gleason score	Bcl-2 Positive (Frequency)	Bcl-2 Score 0 (Negative)	Bcl-2 Score 1	Bcl-2 Score 2	Bcl-2 Score 3
6	1	0	0	1	0
7	5	6	0	1	4
8	13	8	5	6	2
9	14	10	3	6	5
	3	2	1	0	2
Total	36	26			

It shows that maximum percentage positivity in low Gleason score so, it shows Bcl-2 positivity may indicates possibility of low grade of disease

Table 08 Comparision of mAgNOR and Bcl-2 positive and negative cases

| mAgNOR Number	Bcl-2 Positive	Bcl-2 Negative
1.01-1.50	10(66.7%)	5
1.51-2	10(71.4%)	4
2.01-2.5	8(44.3%)	10
2.51-3	4 (80%)	1
3.01-3.5	3(50%)	3
3.51-4	1(33.3%)	2
4.01-4.5	0(0%)	1
Total	36	26

Shows a maximum percentage of Bcl-2 positive cases in low mAgNOR number

Statistical analysis

Figure 01 Scatter diagram showing relation between mAgNOR and Gleason score
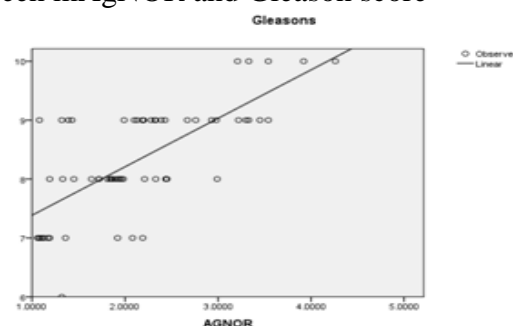
Statistical analysis of mAgNOR count and Gleason score was done by SPSS software statistics By using Kendalls tau b test Shows positive correlation with P value 0.001 (Significant)

Figure02 Scatter diagram showing relation between mAgNOR and Bcl-2
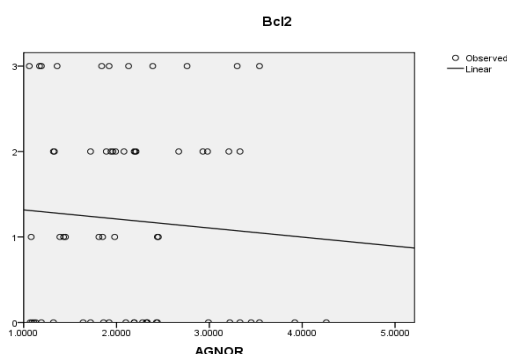
Statistical analysis of mAgNOR count and Bcl-2 was done by SPSS software statistics. By using Kendalls tau b test, shows inverse correlation between mAgNOR and Bcl-2 with p value 0.635 which is insignificant.

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[3] 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 gleason score also increase that is positive correlation.

Bcl-2 proto-oncogene has been shown previously to be expressed in tissue from normal prostate. McDonnell et al[10] showed a strong correlation between bcl-2 overexpression and progression of androgen dependence to hormone- resistance in prostate cancer. Recently Apakama et al.[11] showed this effect to be more pronounced when bcl-2 overexpression was combined with p53 nuclear protein accumulation. However, in tumours from other tissues, an increased expression of bcl-2 has been shown to be associated with better differentiation and favourable outcome. In this study shows that 36 out of 62 cases i.e 58% are positive for Bcl-2 staining Out of 36 positive staining maximum cases are staining score 2 and 3.It also shows that maximum percentage Bcl-2 positivity in low Gleason score so, it shows Bcl-2 positivity indicates possibility of low grade of disease

In this study Comparision of mAgNOR and Bcl-2 positive and negative cases Shows a maximum percentage of positive cases in low mAgNOR count as 1.01-1.50,1.51-2, 2.5-3 show 66.67%,71.4%,80% positive cases respectively. When the mAgNOR count were high the percentage positivity of Bcl-2 very low. 3.51-4, 4.01-4.50 mAgNOR count showed 3.33% and 0% respectively. So, there is inverse correlation between mAgNOR count and Bcl-2 positivity. By using Kendalls tau b test, shows inverse correlation between mAgNOR and Bcl-2 with p value 0.635 which is insignificant.

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 so, useful in grading of disease and AgNOR staining may substitute the proliferative marker IHC, in lab where IHC facility is not available.

Bcl-2 shows inverse correlation with mAgNOR so, Bcl-2 role in grading of disease is less significant.

Acknowledgment

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