



Original Research Article

Extend of neuroendocrine differentiation in adenocarcinoma prostate needle biopsies

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ABSTRACT

Neuroendocrine differentiation in prostatic adenocarcinoma is now one of the emerging prognostic factor that determine treatment outcome. This study aims to identify the extent of neuroendocrine differentiation amidst the tumour cells in adenocarcinoma prostate with the help of immunohistochemical marker, Chromogranin A (CgA) and correlate with its Gleason score. A semiquantitative scoring system was utilised to estimate and quantify cells having neuroendocrine differentiation.

Results: Out of 40 cases of prostatic adenocarcinomas in needle core biopsies, 93% of patients with a Gleason score >6 showed CgA positivity of more than 5% and 2 cases (16.6%) having Gleason score 9 showed >50% of CgA positive cells in the tumour area.

Conclusion: Hence higher Gleason score correlated with higher degree of neuroendocrine differentiation in our study.

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1. Introduction

In men worldwide, after lung cancers, the second most frequently diagnosed malignancy is Prostate cancer. As per the GLOBOCAN 2018 estimates 1,276,106 new cases are reported worldwide and 3,58,989 deaths (3.8% of all deaths caused by cancer in men) is due to prostate malignancy. Indian statistics, GLOBOCAN 2018, prostate cancers are among the top 10 leading sites of cancer in India with 25,696 new cases and 17,184 deaths, with a 5 year prevalence of 47,558.^{1,2} The average age of diagnosis of prostate cancers is about 66 years which may be correlated to the increasing mortality. In African men, the incidence and mortality rates are higher when compared to the White men, about 158.3 new cases are diagnosed per 100,000 men.³

Individual prostatic cancers show substantial variation in its outcome and so it is important to stage the disease because of its variable biological potential. The

various prognostic indicators include clinical staging, serum PSA levels, the percentage of biopsy core involved and histological grade. Of all these the most relevant is the histological grade of tumour which correlates both with local invasiveness as well as with the metastatic potential of the tumour. In a subset of prostatic carcinomas, both localized and locally advanced cancers, the existing markers are often unable to differentiate poor from good outcome cancers.⁴

1.1. Neuroendocrine differentiation in prostate cancer

Neuroendocrine differentiation appears to be more common in prostatic carcinoma than in carcinomas arising in any other organs of the male (or female) urogenital tract.⁵ This can be explained by the fact that the prostate gland has the largest population of neuroendocrine [NE] cells as compared to any other organ in the male or female urogenital tract.⁶ The incidence of NE cells in prostatic carcinoma has been reported in 50-100%, with a steady increase in the detection rate of these cells due to the development of more sensitive

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staining techniques. Focal NE differentiation, particularly when extensive, reflects poor prognosis with refractoriness to endocrine therapy.⁷ There is discrepancy in the prevalence between different studies which may be attributable to factors such as sample type (e.g., biopsy or prostatectomy specimen), the type and extent of fixation, and the antibody used. Neuroendocrine differentiation is usually determined as a positive immunoreactivity for neuroendocrine markers such as Non Specific Esterase (NSE) and/or Chromogranin A (CgA) or bioactive hormones (e.g., serotonin, somatostatin).⁸

Neuropeptides which the neuroendocrine cells release may pave way for the development of androgen independence in the prostatic neoplasms. They also act as autocrine and paracrine growth factors for the malignant cells. In prostate carcinoma cells, these neuropeptides have been shown to accelerate and promote cell growth, migration and protease expression.⁹ Neuroendocrine (NE) differentiation in prostate cancer has received increasing attention in the recent years due to prognostic and therapeutic implications.

The term NE differentiation in prostatic carcinoma includes tumors composed exclusively of NE cells (the rare and aggressive small cell carcinoma and carcinoid/carcinoid like tumor) or prostatic adenocarcinoma with focal neuroendocrine differentiation.¹⁰ The prognostic importance of focal neuroendocrine differentiation in prostate cancer is controversial, but current evidence suggests that it has an influence on prognosis related to hormone resistant tumors or a role in the conversion to a hormone resistant phenotype. Various neuroendocrine markers like Chromogranin A, Synaptophysin, Neuron specific enolase, HCG have been studied and ChromograninA(CgA) appears to be the best among all. No study on NE differentiation in prostate cancer has been done in this part of the world so far. This study intends to look at the neuroendocrine differentiation of prostate cancers and its correlation, if any, with the Gleason score which is the histological grading system followed in prostatic adenocarcinomas.

2. Materials and Methods

A total of 49 prostatic needle core biopsies with a histological diagnosis of adenocarcinoma prostate was studied for a duration of 2years. 9 cases out of these was excluded from the study because of unavailability of tissue sections for IHC studies. 40 cases were thus included, with patients having a mean age of 70 years(57-93). 55% of these patients were in the age group of 61-70 years and 75% of cases were above the age of 65years. Serum PSA values were available in 29 cases out of 40 of which 79% had a PSA level more than 10ng/ml.

Needle core prostatic biopsy specimens were fixed in 10% buffered formalin for 24 hours followed by processing

and paraffin embedding to make tissue blocks. Routine hematoxylin and eosin stained slides were prepared. Those cases with a diagnosis of adenocarcinoma were only considered in this study. These cases were further graded and score was given based on ISUP revised Gleason's scoring system. All the H&E sections were examined independently and patients were categorised as Group A, B and C with Gleason score ≤ 6 , 7 and ≥ 8 respectively.

2.1. Immunohistochemistry

Representative 3 μ m sections were taken from each paraffin block and immunohistochemical staining by Dako technique using Chromogranin A(CgA) was performed for each case. The sections were dewaxed, rehydrated, then placed in Tris EDTA Borate buffer. Antigen retrieval was done by heat method in pressure cooker. The sections were submerged in quenching solution, washed and dried. Primary antibody was added (mouse monoclonal anti-human Chromogranin A) followed by secondary antibody, chromogen Diamino Benzidine (DAB) and washed. Nuclear counterstain was done with Harris Hematoxylin and slides were dehydrated, cleared and mounted.

The percentage of CgA positive cells in the tumor area was evaluated by a semiquantitative method, originally described by Deliu et al¹¹ by counting at least 1000 tumor cells in representative fields of immunostaining using a 40x power of Labomed microscope. The percentage of cytoplasmic Chromogranin A (CgA) positive cells was evaluated semi quantitatively and was given a score from 0 to 3. Neuroendocrine differentiation(NED) amidst the tumour cells were estimated and categorised as, NED score 0 - with no staining or <5% CgA positive neoplastic cells, NED score 1- with 5-25% CgA positive cells, NED score 2 - with 26-50% CgA positive cells and NED score 3 - with >51% CgA positive cells in tumour area.

This percentage positivity in the tumor area was evaluated and compared with the serum PSA levels and histological Gleason's score of the patient using statistical programme Epiinfo.

3. Results

The histological grading of all the 40 cases of adenocarcinoma was done using the Gleason scoring system. The mean Gleason score of our patients were 7.15 with a range of 5 to 9. 14 patients (35%) had a score of ≥ 8 while 11 patients had a score of ≤ 6 . Most of the patients (37.5%) had a Gleason score of 7 as shown in Table 1.

Table 1: Number of patients based on Gleason scores.

Gleason score	No. of cases	Percentage
≤ 6	11	27.5%
7	17	42.5%
≥ 8	12	30%

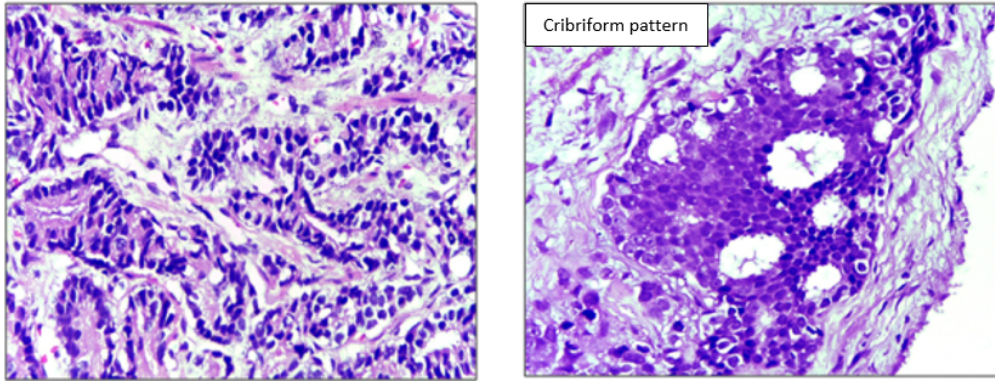


Fig. 1: H&E Gleason score 4

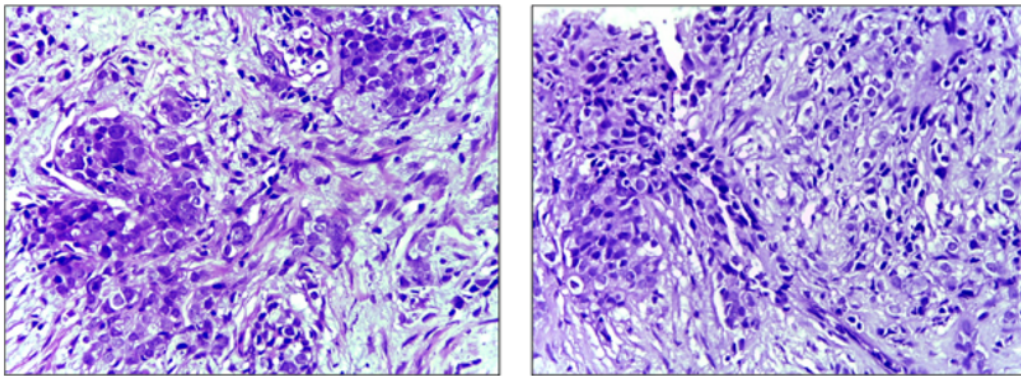


Fig. 2: H&E Gleason score 5

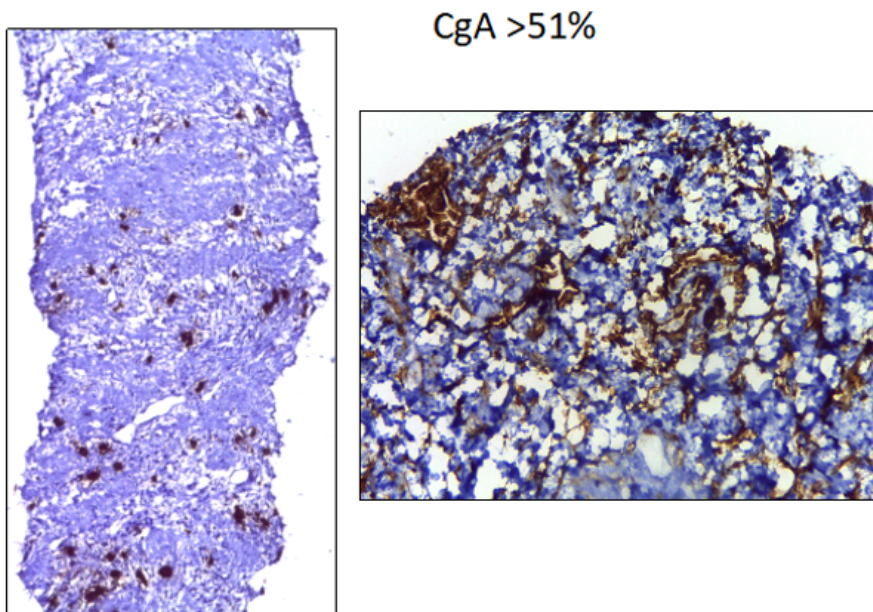


Fig. 3: IHC score 3 (CgA>51%)

Table 2: Comparison of serum PSA and Gleason score.

Serum PSA	Gleason score ≤ 6	Gleason score 7	Gleason score ≥ 8	P value
≤ 10 ng/ml	7	0	0	<0.00001
>10ng/ml	4	17	1	

Table 3: Comparison of %CgA positivity with Gleason score.

%CgA positivity	Gleason score ≤ 6	Gleason score 7	Gleason score ≥ 8	P value
<5% (score 0)	5	1	0	<0.00001
5-25% (score 1)	6	13	9	<0.00001
25-50% (score 2)	0	0	0	NA
>50% (score 3)	0	0	2	<0.00001

Linear regression analysis of age with Gleason score showed a correlation coefficient (r^2) of 0.02. The age was compared with the Gleason score of the patients and it was found that higher the age, greater the Gleason score. 36 out of 40 patients were in the age group of more than 60 years and they had higher Gleason score as compared to patients with age less than 60 years ($P < 0.0001$).

Serum PSA values were obtained in 29 of our 40 cases and 79% of the cases had a level of >10ng/ml. A comparison of serum PSA value and the Gleason score was done and the patients with a serum PSA of >10ng/ml was found to have a higher grade of tumour compared to those with serum PSA of ≤ 10 ng/ml. This difference was statistically significant ($P < 0.00001$) and is given in Table 2.

Neuroendocrine differentiation was estimated by the percentage of cytoplasmic positivity of Chromogranin A (CgA) in the tumour area. 6 cases (15%) had a %CgA score of 0. 30 cases (75%) had a %CgA score of 1. 2 cases (5%) had a %CgA score of 2 and 2 out of 40 cases with %CgA score 3 which accounted for 5% of all cases. The percentage positivity of CgA in individual cases was compared with their corresponding Gleason scores and it was found that the higher Chromogranin A positivity (CgA) score correlated with a higher grade of carcinoma prostate depicted by a high Gleason score as given in Table 3.

Linear regression analysis of percentage Chromogranin A positivity with the Gleason score showed a P value of 0.00012 and correlation coefficient (r^2) of 0.40. Both cases with a %CgA score of 3 had a Gleason score of 8 or more. None of the cases with %CgA score of 0 had a Gleason score $> \text{or} = 8$. Both were statistically significant with a P value of <0.00001.

5 out of 40 cases were associated with Prostatic intraepithelial neoplasia (PIN) of which 4 cases were PIN 2 and 1 case of PIN 3. Perineural invasion was detected in one of our case in the needle core biopsies.

4. Conclusions

The aim of our study was to quantify the neuroendocrine differentiation in prostatic adenocarcinoma using a

immunohistochemical marker Chromogranin A and correlate it with its Gleason score. The results of our study showed that tumors having a higher Gleason score had a higher percentage of neuroendocrine cells. A Neuroendocrine differentiation also had a positive correlation with the serum PSA. In our study, we concluded that serum PSA, Gleason score and neuroendocrine differentiation can be the most relevant prognostic predictors in higher grade tumors. Neuroendocrine differentiation and hormone refractory disease is an associated phenomenon: extensive NED of a tumor renders it androgen-independent. Prostatic carcinoma in advanced stage is the main indication for hormone therapy. Such tumors having higher grade may have a significant neuroendocrine component. This can in turn render the tumor hormone refractory. As shown in our study, it would be desirable to establish the extend of neuroendocrine differentiation before planning the treatment of hormone refractory cases with newer modalities like immunotherapy (antibodies to growth hormone peptides), endocrine therapy or chemotherapy.

5. Source of Funding

None.

6. Conflict of Interest

None.

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