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Original Article

Correlation of expression of Cyclin D1 and E-cadherin with gleason grade, serum prostate specific antigen levels and prostatic volume in prostatic carcinoma

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ABSTRACT

Background: Prostate cancer is increasingly being diagnosed early due to easy access and availability of better diagnostic modalities. Gleason scoring is important for prognosis and is used to determine the management protocol. As the behavior of these tumors is unpredictable, many markers are being investigated. Cyclin D1 and E-Cadherin have been linked to the development, progression, and aggressiveness in some studies. We studied the expression of E cadherin and cyclin D1 in various grades to understand their relationship with clinicopathologic features, PSA, and prostatic volume.

Materials & methods: 52 patients diagnosed with prostatic adenocarcinoma on core biopsy after ethical clearance in our tertiary care center were included in the study and their records were retrieved. Hematoxylin and Eosin stained sections were used for Gleason scoring as well as the Gleason grade group as per existing protocols. Immunohistochemistry using antibodies against Cyclin D1 and E-Cadherin along with appropriate controls was performed. Studied parameters were correlated with E-Cadherin and cyclin D1. We used a statistical software package (version 22, IBM, US).

Results: Cyclin D1 showed increasing immunoexpression with an increase in Gleason score and Gleason grade group emphasizing a significant correlation between these parameters (p= 0.001). There was a significant negative association between the E-cadherin score, Gleason score, and Gleason grade group (p=0.001). No correlation was obtained with serum PSA or prostatic volume.

Conclusions: Cyclin D1 and E-cadherin immunohistochemical markers hold promise in assessing tumor aggressiveness and providing insights into cancer severity

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INTRODUCTION

Prostate malignancy (PCa) is the second most common cancer in males and the sixth leading cause of cancer death worldwide. Prostate cancer was formerly assumed to be far less common in India than in Western countries, but as knowledge has risen, lifestyles have changed, and medical facilities have improved, more and more instances have been identified and more cases are being diagnosed due to easy access and availability of better diagnostic modalities.[1,2]

Several diagnostic and pathological characteristics at the time of surgery for prostate cancer have been studied for their

ability to predict survival. Indicators of tumor aggressiveness include a high PSA at diagnosis, an increased tumor stage (clinical and pathological), and a higher Gleason score (grade). Clinically, the Gleason score is the best predictor of how aggressively prostate cancer will spread. Standard risk stratification factors include PSA at diagnosis, and it continues to be a part of most clinical decisions. There is ongoing research to pinpoint the molecular determinants of prostate cancer so that they may be combined with clinical indicators to better predict prognosis. There is an urgent need to find clinically meaningful indicators of prognosis in prostate cancer due to the disease's high frequency and the variability in clinical outcomes across patients.

Cyclin D1 may be used as a marker to predict a patient's prognosis. In addition, cell adhesion markers such as E-cadherin have predictive importance after surgery in prostate cancer.[3,4] The cyclin D1 proto-oncogene is an important regulator of G1 to S phase progression in many different cell types. Together with its binding partners cyclin-dependent kinase 4 and 6 (CDK4 and CDK6), cyclin D1 forms active complexes that promote cell cycle progression by phosphorylating and inactivating the retinoblastoma protein (RB). The overexpression of cyclin D1 has been linked to the development and progression of cancer. [5] Few reports have explored cyclin D1 expression or distribution in primary prostatic adenocarcinomas, and the criteria for determining positive cyclin D1 staining have varied. Few studies have reported a correlation between PSA and Gleason scoring.[6,7]

E-cadherin is a 120-kDa calcium-dependent transmembrane glycoprotein encoded by the CDH1 tumor suppressor gene located on chromosome 16q21. It is a cell adhesion molecule that connects epithelial cells via homotypic interaction. Disruption of this interaction promotes the detachment of cancer cells from their primary sites, the first step in the tumor invasion process. Reduced or aberrant E-cadherin expression appears to be associated with various parameters such as the aggressiveness of the tumor, enhanced invasion, and metastatic potential in several malignancies.[8]

The role of Cyclins & E-cadherin in the pathogenesis of malignancy is well known, however not many studies have been carried out to assess the immunoexpression of these molecules in PCa and explore their potential as reliable prognostic markers, especially in the Indian population. We conducted a study to evaluate the immunoexpression of Cyclin D1 and E-cadherin in PCa of various grades and correlate their expression with histopathological grading, serum PSA, and prostatic volume to ascertain their role as markers in assessing tumor aggressiveness. We compared pre-operative blood PSA levels and prostate size to levels of cyclin D1 and E-cadherin, and to determine whether or not there was a correlation between the two. We analyzed cyclin D1 expression with immunohistochemistry in 52 patients who had a radical prostatectomy for prostate cancer.

MATERIALS AND METHODS

The study was a retrospective study conducted in a tertiary care hospital and was approved by the Institutional Ethics Committee. A total of 52 patients diagnosed with prostatic adenocarcinoma on core biopsy, were included in the study whose clinical data was available for review. The required laboratory and radiological parameters of these patients were gathered from patient registries and records were extracted. Transurethral resection of the prostate (TURP) chips and holmium laser enucleation of the prostate (HOLEP) procedure was done on these patients in our tertiary care center between Jan 2018 and Aug 2020. Cases of prostatic intra-epithelial neoplasia, recurrent prostatic malignancies, and who underwent neo-adjuvant therapies were excluded from the study. The H & E-stained sections were reviewed by two pathologists for confirmation of diagnosis and graded according to the existing guidelines for the Gleason score and Gleason grade group.

After selecting appropriate blocks, sections of 3-4 μm thickness were cut onto coated slides for IHC using rabbit monoclonal antibody against Cyclin D1 and E-cadherin. IHC was performed using the heat-induced epitope retrieval using the pressure cooker method according to the manufacturer's instructions. A section of normal breast and tonsil was used as a control for E-cadherin and Cyclin D1 respectively. 15 normal prostatic tissue obtained from the autopsy of men with less than or equal to 40 years of age with no signs of malignancy tissues were used as controls.

Immunoscoring for Cyclin D1 was performed based on the intensity of nuclear staining as well as the proportion of positive tumor cells. Cytoplasmic staining was disregarded. 200 tumor cells were counted in this regard. For proportion, <5 % nuclear positive tumor cells were taken as negative (score of 0) and >5% was taken as positive (score of 1). For intensity, the scores were provided as follows 0: Negative, 1: Weak nuclear positivity 2: Strong nuclear positivity. The final scores were calculated by adding the proportion and intensity scores and scores ranged from 0 to 3.[9]

The intensity of membranous staining for E-cadherin was evaluated blinded by the grade or stage. All the cases were allotted into 3 categories according to the pre-decided scoring system. Nuclear and cytoplasmic staining were disregarded. The intensity was assessed in the predominant Gleason pattern and was scored as 0: No expression, 1: Weak membranous positivity2: Strong membranous positivity.[10]

Serum PSA levels were obtained from the patients from records. Estimation was done using an enzyme immunoassay. Ultrasonography reports of these patients were obtained from the patients and records from the radiology department. The prostatic size measured in volume (cc) was obtained for analysis.

The data was compiled in an Excel sheet. Continuous and

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categorical variables were presented as mean ± standard deviation or median as deemed appropriate. The statistical software package IBM®, SPSS® (version 22. Maryland, New York, US) was used for analysis. Categorical variables were expressed as the number of patients and percentage of patients and compared across the groups using Pearson's Chi Square test for Independence of Attributes/ Fisher's Exact Test. Continuous variables are expressed as Mean, Median, and Standard Deviation and compared across the groups using Kruskal-Wallis Test. The association between continuous variables was captured by Spearman's Rank Correlation Coefficient. An alpha level of 5% has been taken, i.e. if any p-value is less than 0.05 was considered significant.

RESULTS

In the present study, the mean age of patients was 67.9 years with an age range from 42 years to 90 years. Maximum patients belonged to the age group 61-70 yrs(30.8%) and 71-80 yrs(30.8%). The mean PSA levels were 38.77ng/dl with an SD of 31.14. Mean prostatic volume was found to be 37.92cc with an SD of 13.97. No significant correlation was obtained between age and Gleason score or Gleason grade group.

The Gleason patterns ascertained according to the existing guidelines in the present study showed that Gleason pattern 5+4 was the most common pattern constituting about 23.1% of the cases while the least common patterns were 5+3 and 3+5. The most common grade group was 5(51.9%). Figure 1 shows the Gleason scoring and patterns on H&E. There was no significant correlation between the Gleason score with serum PSA and prostatic volume.

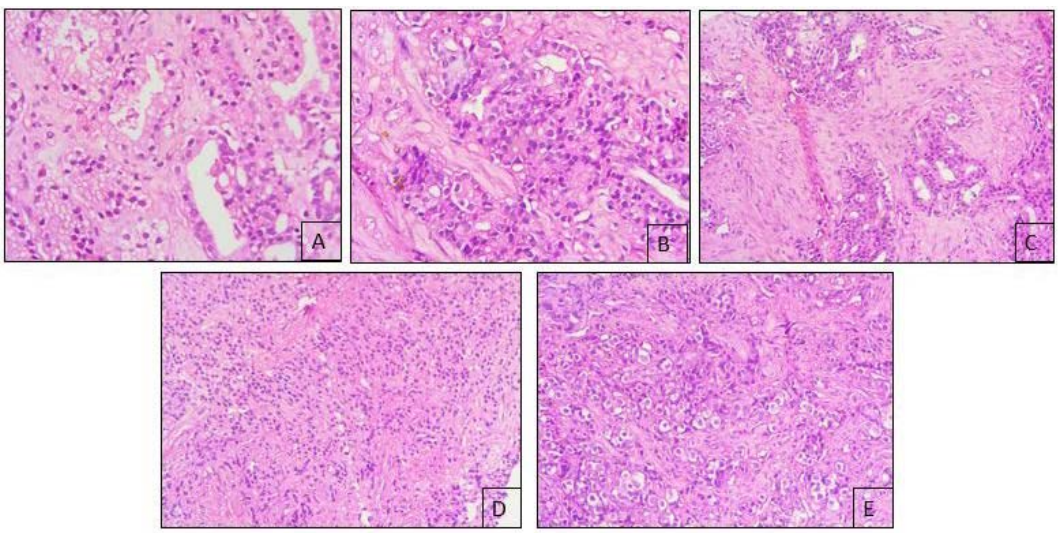

Figure 1: *Gleason pattern 3 shows discrete glands (A), Gleason pattern 4 showing fused glands (B) (400x, H&E), showing cribriform architecture (C) (200x, H&E), Gleason pattern 5 shows singly infiltrating cells (D) (100x, H&E), showing diffuse infiltration in sheets(E) (HE stain, X200)*

Expression of E-cadherin and Cyclin D1 were analyzed at the tumor site showing the most predominant Gleason pattern. Immunoexpression of E-cadherin was retained in areas showing a low Gleason pattern while Cyclin D1 showed overexpression in the form of nuclear positivity in most of the cases with a higher Gleason pattern. Normal prostatic tissue did not show any staining for Cyclin D1.24(46.2%) cases showed a score of 3, 18(34.6%) cases showed a score of 2, 6(11.5%) cases showed a score of 1 and 4(7.7%) cases showed a score of 0. Maximum cases showed a score of 3. Figure 2 shows the cyclin D1 expression and scoring.

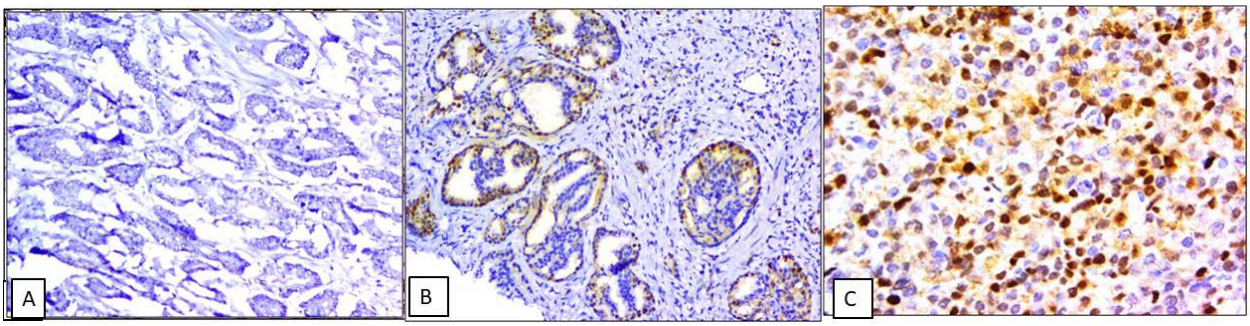

Figure 2: *Immunoexpression of Cyclin D1 in Gleason pattern 3 with a score of 0 (A), in Gleason pattern 4 with a score of 2 (B) in Gleason pattern 5 with a score of 3 (C)*

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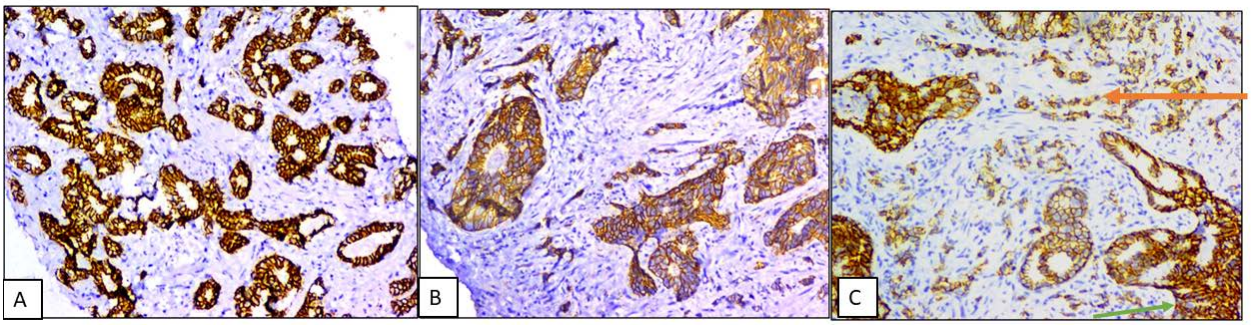

Figure 3: *Immunoexpression of E-cadherin in Gleason pattern 3 (2+) (A), Gleason pattern 4 (1+) (B) Gleason pattern 5 (1+, red arrow) with foci showing areas of Gleason pattern 4 (2+, green arrow)(C)*

Figure 3 shows the expression of E cadherin in various patterns of prostatic carcinoma. 11(21.2%) showed a score of 21.2%, 39(75%) showed a score of 2 and 2(3.8%) showed a score of 3. Maximum cases showed a score of 2.

Cyclin D1 and E-cadherin showed a significant positive association with the Gleason score and Gleason Grade group (p= 0.001). No significant correlation was obtained between the immunoexpression of Cyclin D1 and E-cadherin with pre-operative PSA levels and prostatic volume. (Table 1 and 2)

Table 1: Correlation between immunoexpression of Cyclin D1 with Gleason score, serum PSA levels, and prostatic volume

			Gleason score	Serum PSA	Prostatic volume
Cyclin D1 score	0	Mean	6.75	21.81	34.25
		Median	6.00	22.64	35.00
		Std. Deviation	2.36	11.54	7.85
	1	Mean	5.83	36.60	36.83
		Median	6.00	38.25	36.50
		Std. Deviation	0.98	21.04	7.44
	2	Mean	8.28	32.77	35.78
		Median	9.00	30.00	35.50
		Std. Deviation	1.07	28.89	7.73
	3	Mean	8.63	46.64	40.42
		Median	9.00	27.66	36.00
		Std. Deviation	1.01	35.72	18.87
	p Value		0.001	0.595	0.968

Table 2: Correlation between immunoexpression of E-cadherin with Gleason score, serum PSA levels, and prostatic volume

			Gleason score	Serum PSA	Prostatic volume
E-cadherin score		Mean	9.27	36.69	45.09
		Median	9.00	28.00	42.00
		Std. Deviation	0.47	33.02	12.88
	2	Mean	7.77	38.85	36.18
		Median	8.00	27.00	34.00
		Std. Deviation	1.48	31.53	14.02
	3	Mean	6.50	48.70	32.50
		Median	6.50	48.70	32.50
		Std. Deviation	0.71	23.05	9.19
		p Value	0.001	0.659	0.077

The study observed that most of the tumor cells expressed strong membranous positivity for E-cadherin especially with a lower Gleason pattern and lower Gleason grade group. The immunoexpression was reduced in cases with higher Gleason scores and Grade groups. There was a significant negative association between E-cadherin score and Gleason score and Gleason grade group (p=0.001). Table 3 shows the Correlation between the immunoexpression of E-cadherin with the Gleason grade group. (p-value <0.05)

Perineural invasion was seen in 22 cases (42.3%) and was absent in 30 cases (57.7%). Cribriforming was noted in 16(30.8%) and was absent 36(69.2%) cases. We also studied the correlation of perineural invasion and cribriform with Cyclin D1 expression and E cadherin expression. There was no correlation between perineural invasion and cribriform with either Cyclin D1 expression or E cadherin expression.

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Table 3: Correlation between immunoexpression of Cyclin D1 and E-cadherin with Gleason grade group

		Cyclin D1 score				E-cadherin score		
		0	1	2	3	1	2	3
Gleason grade group	1	2(50)	5(83.33)	1(5.56)	0(0)	0(0)	7(17.95)	1(50)
	2	1(25)	1(16.67)	4(22.22)	2(8.33)	0(0)	7(17.95)	1(50)
	3	0(0)	0(0)	0(0)	3(12.5)	0(0)	3(7.69)	0(0)
	4	0(0)	0(0)	3(16.67)	3(12.5)	0(0)	6(15.38)	0(0)
	5	1(25)	0(0)	10(55.56)	16(66.67)	11(100)	16(41.03)	0(0)
Total		**4(100)**	**6(100)**	**18(100)**	**24(100)**	**11(100)**	**39(100)**	**2(100)**
p Value		0.000				0.017		

DISCUSSION

This study was carried out to study the expression of Cyclin D1 and E Cadherin in 52 cases of prostatic carcinomas and explore their potential role as prognostic markers. The mean age of patients in this study was 67.9 years with an age range from 42 years to 90 years. As per the American cancer society's publication,[11] Cancer Facts and Figures 2020, the average age of diagnosis of prostatic carcinoma is 66 years and around 60% of the cases were in men above 65 years of age. The disease is rarely diagnosed in men below 40 years of age, as in our study where all the cases were above 40 years of age. The incidence of prostate cancer in India has been showing an increasing trend with peak incidence observed in the age group of >65 years indicating that prostate cancer is a disease of advancing age.[12]

The Gleason patterns ascertained according to the existing guidelines showed that Gleason patterns 5+4 and 4+5 were the most common patterns each constituting about 23.15% and 17.3% respectively of the cases while the least common patterns were 5+3, 3+5. Gleason 3 pattern was the most prevalent pattern with 38.4% of the cases showing this pattern as either the most predominant pattern or the second most predominant pattern which agrees with many series that designate Gleason pattern 3 as the most predominant pattern. [13]

In our study, we observed that Cyclin D1 showed increasing immunoexpression with an increase in Gleason score and Gleason grade group emphasizing a significant correlation between these parameters (p= 0.001). This is similar to the observations made by Pereira et al[9] in a study conducted on patients with prostatic carcinoma. In this study, among patients with a high-grade Gleason score (≥7), 86% of the patients demonstrated Cyclin D1 immunostaining of >5% ($p<0.05$). In another study conducted by Comstock et al, they observed that nuclear Cyclin D1 expression was associated with higher grade and increased Ki-67 labeling. These results are in agreement with the present study.[6]

In an Indian study by Gupta et al[14], they concluded that Cyclin D1 immunoexpression was higher in carcinomas and may help distinguish between BPH and carcinoma of the prostate but may not be used as a reliable indicator of grading prostatic adenocarcinoma because of overlapping of values in various grades. However, in contradiction to this study, we found a significant correlation between the immunoexpression of Cyclin D1 and the histological Gleason score and grade. In a study by Ahmed et al[7], Cyclin D1 staining was associated with high-grade Gleason score and perineural invasion. We did not find any correlation between perineural invasion and cyclin D1 expression.

There was no correlation between the serum PSA levels and immunoexpression of E-cadherin or Cyclin D1. This contrasts with the study conducted by Liu et al[15] in which clinical data of cases of prostate cancer was analyzed for correlation between immunoexpression of E-cadherin and clinicopathological parameters including serum PSA levels. It was found that E-cadherin expression was higher in patients with PSA levels < 20microg/L than those with serum PSA >20microg/L.

The correlation between Cyclin D1 immunoexpression and Serum PSA levels was similar to the findings observed by Pereira et al[9] in a study that analyzed the correlation between Cyclin D1 expression, serum PSA levels, and other clinic-morphological parameters. No significant correlation was obtained between the immunoexpression of these markers and the prostate size measured on ultrasonography.

In our present study, it was observed that tumor cells showed strong membranous positivity for E-cadherin with a lower Gleason pattern and weak immunostaining with a higher Gleason pattern. This correlation was observed between the immunoexpression of E-cadherin and the Gleason grade group as well. This is consistent with the observations made by Jaggi et al[16] in a study performed on 16 radical prostatectomy specimens to evaluate the immunoexpression of E-cadherin. In this study, cases with a Gleason score

≥7 showed significantly lower expression of E-cadherin compared to Gleason <7 PCa. In another study performed by J S Ross et al[17] which compared tumor grade and DNA content with the expression of E-cadherin, it was observed that E-cadherin expression was decreased in high-grade (44%) versus low-grade lesions (54%). Cheng et al[10] observed the expression of E-cadherin in 53 primary prostate carcinomas and 14 lymph node metastasis and found that the expression of E-cadherin was reduced in poorly differentiated prostate cancers correlating with the Gleason grading (p=0.03).

The limitations of the study are the small sample size. Studies with a larger sample size may be required for further confirmation.

CONCLUSIONS

Given the high incidence of prostate cancer and the wide variation in patient outcomes, the identification of clinically meaningful indicators of prognosis is urgently needed. We explored the expression of Cyclin D1 and E-Cadherin in prostatic carcinoma and their correlation with clinicopathologic prognostic indicators. There was a significant positive association of Gleason score and grade with Cyclin D1 expression and a significant negative association with E cadherin expression. We did not find any association of either Cyclin D1 or E cadherin with Serum PSA and prostatic volume. Based on the findings of the study, immunohistochemical markers Cyclin D1 and E-cadherin exhibit potential in assessing tumor aggressiveness, providing valuable insights into cancer severity.

Conflict of interest: None

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