



Original Research Article

Study Of The Relationship Between Immunohistochemical Erg Expression And Histopathological Parameters In Prostatic Adenocarcinoma: A Cross-Sectional Study

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Abstract: Background: Prostatic adenocarcinoma is a biologically heterogeneous malignancy in which molecular alterations play a crucial role in tumor behavior and prognosis. Among these, ERG overexpression, most commonly due to TMPRSS2-ERG gene fusion, has emerged as a potential diagnostic and prognostic biomarker. However, the relationship between ERG expression and histopathological parameters remains variable across populations.

Aim: To study the relationship between immunohistochemical ERG expression and histopathological parameters in prostatic adenocarcinoma. **Materials and Methods:** This cross-sectional study was conducted in the Department of Pathology, Yenepoya Medical College, Mangaluru, from August 2023 to February 2025. A total of 40 cases of prostatic adenocarcinoma diagnosed on needle biopsy, TURP, or radical prostatectomy specimens were included. Histopathological evaluation was performed using hematoxylin and eosin-stained sections, with Gleason scoring and Gleason Grade Group assignment as per ISUP guidelines. Immunohistochemistry for ERG was performed using the EnVision-labelled polymer method. ERG expression was evaluated based on nuclear staining intensity and categorized as positive or negative. Statistical analysis was carried out using SPSS version 25.0, employing non-parametric tests and Spearman's correlation analysis. **Results:** ERG expression was positive in 60% of cases. A significant negative correlation was observed between ERG expression and preprocedural serum PSA levels ($\rho = -0.360$, $p = 0.023$), Gleason score ($\rho = -0.318$, $p = 0.045$), and Gleason Grade Group ($\rho = -0.320$, $p = 0.044$). ERG-positive tumors were more frequently associated with lower Gleason scores and lower grade groups, while ERG-negative tumors predominated in high-grade disease. No significant association was found between ERG expression and age, perineural invasion, lymphovascular invasion, or prostatic intraepithelial neoplasia. **Conclusion:**

ERG expression in prostatic adenocarcinoma is significantly associated with lower PSA levels and less aggressive histopathological features. ERG immunohistochemistry may serve as a useful adjunct marker in the histopathological evaluation and risk stratification of prostatic adenocarcinoma.

Keywords: Prostatic adenocarcinoma; ERG expression; Immunohistochemistry; Gleason score; Gleason Grade Group; Prostate cancer biomarkers

INTRODUCTION

Prostatic adenocarcinoma is one of the most frequently diagnosed malignancies among men worldwide and represents a significant public health

concern due to its rising incidence and associated morbidity. According to

GLOBOCAN 2020, prostate cancer was the **second most commonly diagnosed cancer in men globally**,

accounting for approximately **1.41 million new cases**, and was responsible for over **375,000 deaths** worldwide [1]. The incidence of prostate cancer shows marked geographic variation, with higher rates reported in developed regions such as North America, Europe, and Australia, while traditionally lower rates have been observed in Asian countries. However, recent epidemiological trends indicate a steady increase in incidence in developing nations, attributed to population aging, lifestyle changes, and improved diagnostic practices [2].

In India, prostate cancer has emerged as a **leading cancer among elderly men**, with a consistent upward trend over the past two decades. Data from the **National Cancer Registry Programme (NCRP) 2020–2022** indicate that prostate cancer ranks among the **top ten cancers in men**, particularly in urban registries such as Delhi, Mumbai, Bengaluru, and Chennai [3]. The **age-adjusted incidence rate** in India ranges from **5 to 9 per 100,000 men**, with higher incidence observed in metropolitan areas, reflecting increased awareness, screening, and longer life expectancy [4]. Mortality rates remain relatively high, largely due to late-stage presentation and limited access to early diagnostic facilities in many regions [5]. These observations highlight the growing clinical and pathological burden of prostate cancer in the Indian population.

Histopathological evaluation remains the cornerstone of prostate cancer diagnosis and prognostication. The **Gleason grading system**, refined by the International Society of Urological Pathology (ISUP), remains the most powerful predictor of disease aggressiveness, recurrence, and survival [6,7]. Despite its robustness, tumors with similar Gleason scores often demonstrate heterogeneous biological behavior, underscoring the need for **molecular and immunohistochemical markers** that complement conventional morphology and improve risk stratification [8].

Among molecular alterations implicated in prostate carcinogenesis, rearrangements involving ETS family transcription factors are particularly significant. The **TMPRSS2-ERG gene fusion**, first described in 2005, leads to androgen-driven overexpression of ERG and is detected in approximately **40–60% of prostate cancers** in Western populations [9]. Although the prevalence appears lower in Asian and Indian cohorts, ERG rearrangements have been consistently documented and remain biologically relevant [10]. Immunohistochemistry for ERG has emerged as a reliable surrogate for detecting ERG gene rearrangements, demonstrating high specificity for malignant prostatic epithelium and absence of staining in benign glands [11,12].

The association between ERG expression and clinicopathological parameters such as **Gleason score, Grade Group, serum PSA levels, perineural invasion, and lymphovascular invasion** has been widely studied, yet results remain conflicting. Several studies have reported higher ERG positivity in low-to intermediate-grade tumors, suggesting a possible association with less aggressive disease, while others have found no significant correlation or variable associations depending on cohort characteristics and specimen type [13–15]. Indian studies evaluating ERG expression remain limited, and regional data correlating ERG expression with histopathological parameters are sparse, emphasizing the need for institution-based studies to better understand its prognostic significance in the Indian setting [10,16]. The aim of this cross-sectional study is to evaluate the relationship between **immunohistochemical ERG expression** and **histopathological parameters** in prostatic adenocarcinoma. The primary objectives are to determine the Gleason score and Gleason Grade Group based on detailed morphological assessment, to evaluate ERG expression using immunohistochemistry across different Gleason grades, and to correlate ERG expression with key histopathological parameters including serum PSA levels, Gleason score, Gleason Grade Group, perineural invasion, lymphovascular invasion, and presence of prostatic intraepithelial neoplasia. Through this comprehensive analysis, the study seeks to assess whether ERG expression is associated with tumor aggressiveness and pathological risk stratification. The findings of this study are expected to contribute to a better understanding of the biological behavior of prostatic adenocarcinoma in the regional population and may support the incorporation of ERG immunohistochemistry as an adjunct prognostic marker in routine histopathological reporting. Future outcomes include the potential use of ERG expression in refining risk stratification models, guiding personalized management strategies, and serving as a foundation for larger multicentric and prospective studies integrating molecular markers with clinical outcomes.

Materials and Methods

This cross-sectional study was conducted in the **Department of Pathology, Yenepoya Medical College, Mangaluru**, over a period of **one year and seven months, from August 2023 to February 2025**. The study included a total of **40 cases of prostatic adenocarcinoma**, diagnosed on histopathological examination. All **needle biopsy specimens, transurethral resection of prostate (TURP) chips, and radical prostatectomy specimens** received during the study period and confirmed as prostatic adenocarcinoma were included, irrespective of patient age and histological subtype. Specimens

received after treatment with radiotherapy, chemotherapy, or hormonal therapy, as well as inadequate or poorly preserved samples, were excluded from the study.

All specimens were fixed in **10% neutral buffered formalin** and examined grossly according to standard pathological guidelines. Following routine tissue processing, paraffin-embedded blocks were prepared, and **5-micron-thick sections** were cut using a rotary microtome. These sections were stained with **Hematoxylin and Eosin (H&E)** for histopathological evaluation. Detailed microscopic examination was performed to assess tumor morphology, and **Gleason scoring** was assigned based on the primary and secondary architectural patterns in accordance with the **International Society of Urological Pathology (ISUP)** recommendations. Gleason scores were further grouped into **Gleason Grade Groups (1–5)** for standardized prognostic stratification. Additional histopathological parameters, including the presence of **prostatic intraepithelial neoplasia (PIN)**, **perineural invasion**, and **lymphovascular invasion**, were systematically evaluated and recorded.

Representative tumor-containing paraffin blocks were selected for **immunohistochemical (IHC) analysis**. ERG immunostaining was performed on formalin-fixed, paraffin-embedded tissue sections using the **primary antibody ERG [EP111]** (Biocare Medicals, USA). Immunostaining was carried out using the **PolyExcel HRP non-biotin, micro-polymer-based detection system** with diaminobenzidine (DAB) as the chromogen, following the manufacturer's protocol. Appropriate positive and negative controls were included with each staining batch to ensure quality assurance. Nuclear brown staining of tumor cells was

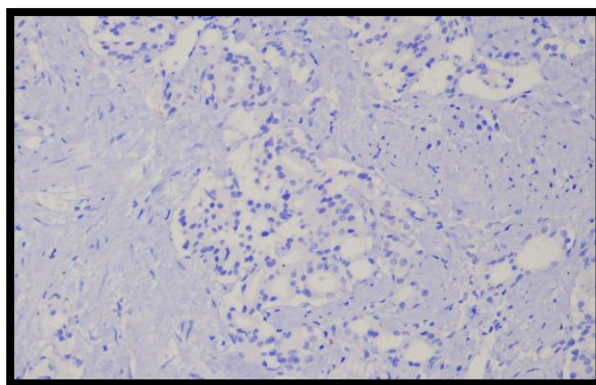
considered positive for ERG expression.

ERG immunoreactivity was evaluated using a **semiquantitative scoring system**, adapted from Suh et al., based on staining intensity. The staining intensity was graded as **score 0 (absent)**, **score 1+ (weak, visible only at high magnification)**, **score 2+ (moderate, visible at low magnification)**, and **score 3+ (strong, striking at low magnification)**. For analysis, ERG expression was categorized as **negative (score 0)** or **positive (scores 1+, 2+, and 3+)**. All immunostained slides were independently evaluated by **two pathologists**, and any discrepancies were resolved by mutual consensus to minimize observer bias.

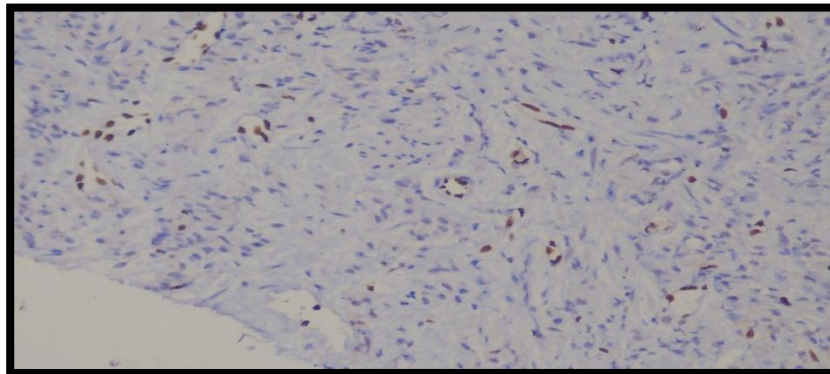
Clinical and pathological data, including patient age, preprocedural serum PSA levels, specimen type, Gleason score, Grade Group, and other histopathological parameters, were compiled and entered into **Microsoft Excel 2019**. Statistical analysis was performed using **Statistical Package for the Social Sciences (SPSS) version 25.0**. Continuous variables were expressed as **mean $\pm$ standard deviation** or median with interquartile range, while categorical variables were expressed as **frequency and percentage**. Since the data showed a skewed distribution, **non-parametric statistical tests** were applied. The **Mann–Whitney U test** was used to compare continuous variables between two independent groups, while the **Chi-square test** or **Fisher's exact test** was applied for categorical variables, as appropriate. **Spearman's correlation coefficient (ρ)** was used to assess the correlation between ERG expression and various clinicopathological parameters. A **p-value < 0.05** was considered statistically significant.

RESULTS

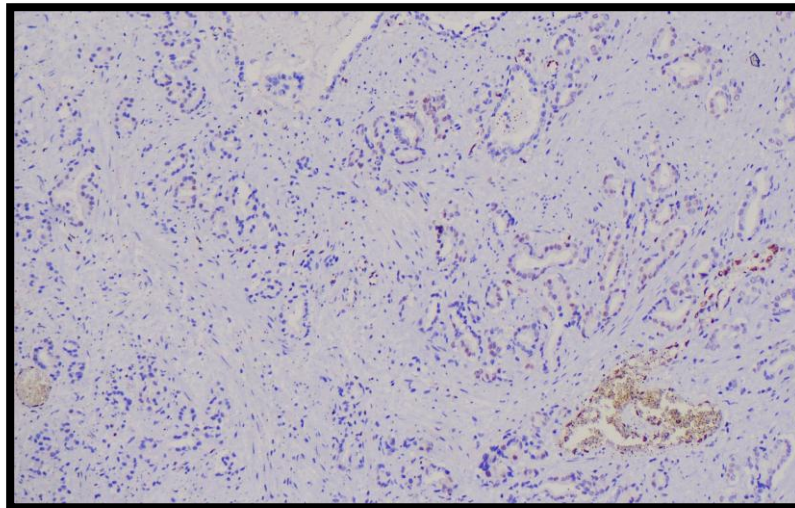
Photomicrograph: 1- IHC ERG nuclear staining- Score 0: Negative in tumor cells (40x)



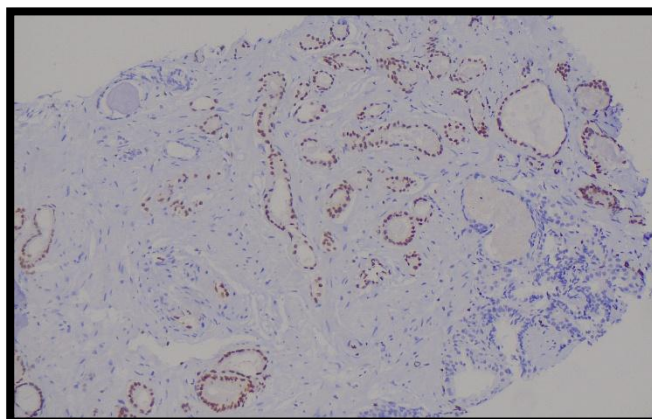
Photomicrograph: 2- IHC ERG nuclear staining- Score 1: Positive in tumor cells with vessels serving as an internal control (40x)



Photomicrograph: 3 - IHC ERG nuclear staining- Score 2: Positive in tumor cells (10x)



Photomicrograph: 4 IHC ERG nuclear staining- Score 3: Positive in tumor cells (10x)



RESULT

A total of **40 cases of prostatic adenocarcinoma** were included in the final analysis. The study population predominantly consisted of elderly patients, with a **mean age of 70.93 ± 9.52 years** (range: 52–89 years). Most cases were observed in the **71–80 years age group (35%)**, followed by the **61–70 years group (32.5%)**. Preprocedural serum PSA levels showed a wide range, with a **mean PSA of 62.70 ± 35.39 ng/ml**. Notably, **45% of cases had PSA levels >75 ng/ml**, indicating advanced disease in a substantial proportion of patients. The majority of specimens were obtained through **needle (Trucut) biopsy (67.5%)**, followed by **radical prostatectomy**

specimens (30%), with TURP contributing minimally (2.5%).

Morphological assessment and **Gleason scoring** revealed a predominance of high-grade tumors. The most common primary Gleason pattern was **Pattern 4 (57.5%)**, while **Pattern 5** was frequently observed as the secondary pattern (40%). The most prevalent Gleason score was **4+5 = 9**, accounting for **35% of cases**, followed by scores of **7 (35%)**. Overall, **55% of cases had Gleason scores ≥ 8** , reflecting a high burden of aggressive disease. Correspondingly, analysis of **Gleason Grade Groups** showed that nearly half of the cases (**47.5%**) belonged to **Grade Group 5**, while Grade Groups 1 and 2 together accounted for only 25% of cases. These findings fulfilled the first objective of detailed Gleason scoring and grading based on histopathological evaluation.

Evaluation of **ERG immunohistochemical expression** demonstrated **positive nuclear ERG staining in 60% (24/40) of cases**, while **40% (16/40)** were ERG-negative. Among ERG-positive cases, strong ERG expression (score 3+) was observed in **35%**, indicating robust nuclear positivity in a significant subset. Comparison of ERG expression with patient age showed **no statistically significant association** with either age categories or mean age ($p > 0.05$). However, ERG-positive tumors were associated with **significantly lower mean serum PSA levels** compared to ERG-negative tumors (**51.4 ± 36.1 ng/ml vs. 79.6 ± 27.5 ng/ml, $p = 0.012$**), suggesting a relationship between ERG expression and biochemical disease burden.

Correlation of ERG expression with **histopathological parameters** revealed that ERG-positive cases tended to have **lower Gleason scores and lower Grade Groups**, although categorical comparisons did not reach statistical significance. Specifically, **75% of ERG-negative cases had Gleason scores ≥ 8** , compared to **41.7% of ERG-positive cases**. Spearman's correlation analysis demonstrated a **significant negative correlation** between ERG expression and **serum PSA levels** ($\rho = -0.360$, $p = 0.023$), **Gleason score** ($\rho = -0.318$, $p = 0.045$), and **Gleason Grade Group** ($\rho = -0.320$, $p = 0.044$), indicating that ERG positivity was associated with less aggressive tumor features. No significant correlation was observed between ERG expression and **perineural invasion, lymphovascular invasion, prostatic intraepithelial neoplasia, or patient age** ($p > 0.05$). These findings address the remaining objectives by demonstrating a meaningful association between ERG expression and key histopathological parameters in prostatic adenocarcinoma.

Table 1. Clinicodemographic and Baseline Characteristics of Study Cases (N = 40)

Parameter	Category	Frequency (n)	Percentage (%)
Age group (years)	≤ 60	7	17.5
	61–70	13	32.5
	71–80	14	35.0
	> 80	6	15.0
Mean age $\pm$ SD (years)	—	70.93 ± 9.52	—
Preprocedural PSA (ng/ml)	≤ 25	11	27.5
	> 25 –50	3	7.5
	> 50 –75	6	15.0
	> 75 –100	9	22.5
	> 100	11	27.5
Mean PSA $\pm$ SD (ng/ml)	—	62.70 ± 35.39	—
Specimen type	Needle biopsy	27	67.5
	Radical prostatectomy	12	30.0
	TURP	1	2.5

Table 2. Distribution of Gleason Score and Gleason Grade Group (N = 40)

Parameter	Category	Frequency (n)	Percentage (%)
Gleason score	6	4	10.0
	7	14	35.0
	8	3	7.5
	9	17	42.5
	10	2	5.0
Gleason Grade Group	Group 1	4	10.0
	Group 2	6	15.0
	Group 3	8	20.0
	Group 4	3	7.5
	Group 5	19	47.5

Key highlight: 55% of cases had **high-grade disease (Gleason ≥ 8)**, with **Grade Group 5** being the most common.

Table 3. ERG Immunohistochemical Expression Profile (N = 40)

Parameter	Category	Frequency (n)	Percentage (%)
ERG score	Score 0	16	40.0
	Score 1+	6	15.0
	Score 2+	4	10.0
	Score 3+	14	35.0
ERG expression	Negative	16	40.0
	Positive	24	60.0

Key highlight: ERG positivity was observed in 60% of prostatic adenocarcinoma cases.

Table 4. Correlation of ERG Expression with Histopathological Parameters (N = 40)

Parameter	Spearman's ρ	p-value
Age	-0.246	0.126
Preprocedural PSA level	-0.360	0.023*
Gleason score	-0.318	0.045*
Gleason Grade Group	-0.320	0.044*
Perineural invasion	-0.026	0.876
Lymphovascular invasion	0.057	0.726

* $p < 0.05$ statistically significant

Key highlight: ERG expression showed a **significant negative correlation** with PSA level, Gleason score, and Grade Group, indicating association with **less aggressive tumor features**.

Figure 1: Distribution of ERG Expression Across Gleason Grade Group (%)

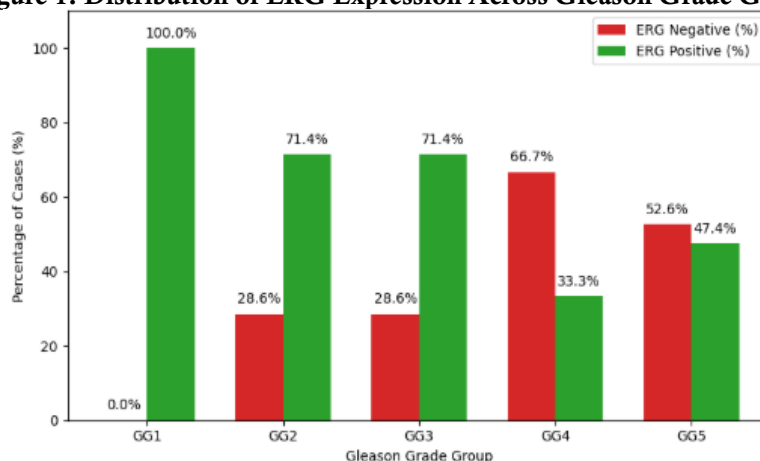
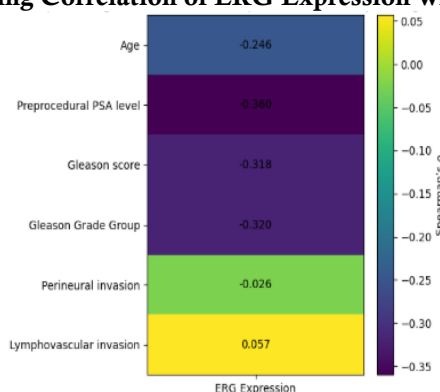


Figure 2: Heat Map Showing Correlation of ERG Expression with Histopathological Parameters



DISCUSSION

Prostatic adenocarcinoma remains a biologically heterogeneous malignancy, with molecular alterations increasingly recognized as key determinants of tumor behavior and prognosis. Among these, ERG overexpression, most commonly resulting from TMPRSS2-ERG gene fusion, has

been extensively studied for its potential clinicopathological significance. In the present study, ERG expression was observed in 60% of cases, which is comparable to the prevalence reported in Western literature, where ERG positivity ranges between 40% and 70% [17,18]. Indian studies have shown relatively variable ERG expression, often

lower than Western cohorts, likely reflecting ethnic, genetic, and environmental differences [19]. The ERG positivity rate observed in this study thus lies within the higher spectrum of reported values and underscores the relevance of ERG as a common molecular alteration in prostatic adenocarcinoma.

A key observation in this study was the significant negative correlation between ERG expression and serum PSA levels, with ERG-positive tumors demonstrating substantially lower mean PSA values compared to ERG-negative tumors. Similar findings have been reported by Pettersson et al. and Minner et al., who observed that ERG-positive prostate cancers tend to present with lower PSA secretion relative to tumor burden, possibly due to altered androgen receptor signaling pathways [17,20]. In contrast, some Indian studies have failed to demonstrate a significant association between ERG expression and PSA levels [19,21]. The statistically significant inverse relationship observed in this study strengthens the hypothesis that ERG-positive tumors may exhibit distinct biological behavior, with PSA being a less reliable surrogate marker of disease aggressiveness in this subgroup.

With respect to histopathological grading, this study demonstrated that ERG expression was more frequently observed in tumors with lower Gleason scores and lower Gleason Grade Groups, while ERG-negative tumors predominated in high-grade disease. Spearman's correlation analysis further confirmed a significant negative correlation between ERG expression and both Gleason score and Grade Group. These findings are in concordance with studies by Park et al. and Hoogland et al., who reported that ERG positivity was more common in low- to intermediate-grade tumors, whereas ERG-negative cancers were associated with more aggressive histological features and poorer differentiation [18,22]. However, conflicting evidence exists, with some authors suggesting that ERG expression may be associated with aggressive behavior and early disease progression [21,23]. The predominance of high-grade tumors in the present cohort may explain the relatively lower proportion of ERG positivity among Grade Group 5 cases, supporting the concept that ERG-negative pathways may drive tumor aggressiveness in advanced disease. The association of ERG expression with other histopathological parameters such as perineural invasion, lymphovascular invasion, and prostatic intraepithelial neoplasia (PIN) was also evaluated in this study. No statistically significant associations were observed, which aligns with findings from several prior studies indicating that ERG expression alone may not independently predict local invasive features [20,22]. While some investigators have suggested a possible link between ERG positivity and precursor lesions such as PIN, the low prevalence of

PIN in the present study limits definitive conclusions. The lack of association with perineural and lymphovascular invasion further suggests that ERG expression may reflect tumor differentiation rather than invasive potential, reinforcing its role as a biological marker rather than a standalone prognostic indicator.

Overall, the findings of this study contribute to the growing body of evidence suggesting that ERG-positive prostatic adenocarcinoma represents a biologically distinct subgroup, characterized by lower PSA levels and relatively less aggressive histomorphology, whereas ERG-negative tumors appear to follow alternative molecular pathways associated with higher grade disease. When interpreted alongside existing literature [17–23], the results support the utility of ERG immunohistochemistry as an adjunctive tool in the histopathological assessment of prostatic adenocarcinoma, particularly in risk stratification and understanding tumor heterogeneity.

CONCLUSION

This cross-sectional study demonstrates a significant relationship between immunohistochemical ERG expression and key histopathological parameters in prostatic adenocarcinoma. ERG positivity was observed in a substantial proportion of cases and showed a significant negative correlation with serum PSA levels, Gleason score, and Gleason Grade Group, indicating an association with relatively less aggressive tumor morphology. In contrast, ERG-negative tumors were more frequently associated with higher Gleason grades and advanced pathological features. No significant association was identified between ERG expression and patient age, perineural invasion, lymphovascular invasion, or prostatic intraepithelial neoplasia. These findings suggest that ERG expression reflects a distinct biological subset of prostatic adenocarcinoma and may serve as a valuable adjunct marker in histopathological evaluation and risk stratification.

Limitations

The present study has certain limitations. The sample size was relatively small and derived from a single tertiary care center, which may limit the generalizability of the findings. The cross-sectional study design precluded assessment of long-term clinical outcomes such as biochemical recurrence, disease progression, or patient survival. Additionally, molecular confirmation of TMPRSS2-ERG gene fusion by techniques such as fluorescence in situ hybridization (FISH) or PCR was not performed, and correlation with treatment response was beyond the scope of this study. Inter-observer variability in histopathological assessment, although minimized by consensus evaluation, cannot be completely excluded.

Recommendations

Based on the findings of this study, ERG immunohistochemistry may be considered as an adjunctive marker in the routine histopathological evaluation of prostatic adenocarcinoma, particularly for refining prognostic assessment alongside Gleason grading. Future studies with larger sample sizes, multicentric design, and prospective follow-up are recommended to validate the prognostic significance of ERG expression and its impact on patient outcomes. Incorporation of molecular techniques to confirm ERG gene rearrangements and integration of ERG status with emerging biomarkers may further enhance personalized risk stratification and therapeutic decision-making in prostate cancer.

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