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Original Research Article

IMMUNOHISTOCHEMICAL EXPRESSION OF NKX3.1 IN PROSTATE CANCER AND ITS ASSOCIATION WITH TUMOR GRADE AND INVASION

Unnikrishnan S[1], Jayalakshmy P. S.[2], Sebina Asmi A. T.[3]

[1]*Post Graduate student, Department of Pathology, MES Medical College, Perinthalmanna, Kerala, India.*
[2]*Professor, Department of Pathology, MES Medical College, Perinthalmanna, Kerala, India.*
[3]*Assistant Professor, Department of Pathology, MES Medical College, Perinthalmanna, Kerala, India.*

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Corresponding Author:
Dr. Sebina Asmi A. T.,
Assistant Professor, Department of Pathology, MES Medical College, Perinthalmanna, Kerala, India.
Email: sebinaasmi@gmail.com

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ABSTRACT

Background: Prostate cancer is one of the most common malignancies among men worldwide, with increasing incidence in India. Accurate diagnosis and grading are critical for management. NKX3.1, a prostate-specific homeobox gene, has emerged as a potential immunomarker with diagnostic and prognostic value. Aim of the study was to determine the expression pattern of NKX3.1 in prostatic adenocarcinoma and correlate it with clinicopathological features including Gleason score, WHO grade, serum PSA levels, and invasive characteristics.

Materials and Methods: A cross-sectional study was conducted in 48 patients with histologically confirmed prostatic adenocarcinoma. NKX3.1 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections. Expression was categorized as strong, moderate, or weak. Correlation with Gleason patterns, WHO grades, PSA levels, perineural invasion, and lymphovascular invasion was analyzed using chi-square tests.

Results: Among the 48 cases, 56.25% were WHO grade 5 tumours. Strong NKX3.1 expression was observed in 100% of Gleason pattern 3 segments, but declined in higher grade; only 9.09% of pattern 5 segments showed strong expression, while 69.70% showed weak expression. Moderate expression was most prevalent overall (41.67%). Perineural invasion was present in 52.08% of cases, and lymphovascular invasion in 10.41%. Strong NKX3.1 expression was associated with lower grade, while moderate expression correlated with higher lymphovascular invasion (75%).

Conclusion: NKX3.1 expression shows a significant inverse relationship with tumor grade and aggressiveness in prostatic adenocarcinoma. Its consistent expression in lower-grade tumours and decline in poorly differentiated cases supports its utility as a reliable prognostic and adjunct diagnostic marker.

Keywords: Prostatic adenocarcinoma, NKX3.1, Gleason score, Tumour Grade, Perineural invasion, Lymphovascular invasion

INTRODUCTION

Prostate cancer is the second most frequently diagnosed malignancy among men globally and remains a leading cause of cancer-related morbidity and mortality.[1] In India, the average incidence is estimated to be around 9–10 per 100,000 men.[2] Histopathological evaluation using the Gleason grading system remains essential for diagnosis, prognostication, and treatment planning. However, with the growing emphasis on precision medicine, molecular markers such as NKX3.1 have gained prominence. NKX3.1 is a prostate-specific homeobox gene located on chromosome 8p21; a region frequently associated with loss of heterozygosity in prostate carcinoma. Notably, its expression tends to decrease with tumor dedifferentiation, making it a potential indicator of disease progression.[3]

While prostate-specific antigen (PSA) and prostatic-specific acid phosphatase (PSAP) are widely used diagnostic markers, their expression may be reduced

or absent in poorly differentiated and metastatic lesions, limiting their utility in such contexts. Immunohistochemical panels combining AMACR (alpha-methylacyl-CoA racemase) and p63 are commonly used to distinguish benign from malignant lesions; however, these can sometimes yield ambiguous or misleading results, especially in morphologically variant tumours.[4] NKX3.1, with its consistent nuclear expression in prostatic tissue may offer improved specificity in low grade tumours. As such, evaluating NKX3.1 expression may enhance our ability to stratify tumor grade and predict biological behavior.[3,4]

Given the potential diagnostic and prognostic utility of NKX3.1, it is important to further explore its expression patterns in prostate adenocarcinoma and established clinicopathological features. This includes its association with Gleason pattern, grade group, serum PSA levels, perineural and lymphovascular invasion.[4] This study aimed to determine the expression pattern of NKX3.1 in prostatic adenocarcinoma and correlate it with clinicopathological parameters.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, MES Medical College, Perinthalmanna, Kerala. The study period was from 1st September 2022 to 31st July 2023. The study population consisted of all histologically proven cases of prostatic adenocarcinoma. In cases where morphology was equivocal, confirmation was done using immunohistochemical markers P63 and AMACR. Cases with insufficient tumour for immunohistochemical analysis and malignancies other than acinar adenocarcinoma were excluded from the study.

Following ethical clearance approval, clinical and histopathological data were retrieved from hospital records using a structured proforma. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were retrieved, and new sections were prepared for immunohistochemistry (IHC) along with review of hematoxylin and eosin (H&E) stained sections.

The IHC panel included NKX3.1 (Clone: EP356, Rabbit Monoclonal Antibody) and in equivocal cases the diagnosis was confirmed with a cocktail of P63(Clone: 4A4, Rabbit Monoclonal Antibody) , and AMACR(Clone: 13H4, Rabbit Monoclonal Antibody). Sections of 4 µm thickness were cut and mounted on gelatin-coated slides, followed by deparaffinization and rehydration. Antigen retrieval was carried out using citrate buffer (pH 6.0) at 150°C in a microwave oven. After blocking endogenous peroxidase activity with hydrogen peroxide, slides were incubated with primary antibodies for 30 minutes. Visualisation was achieved using the Polyexcel HRP-DAB system, followed by counterstaining with hematoxylin. Normal prostatic glands served as the positive control, and non-stainable tissue as negative control.

NKX3.1 immunoreactivity was evaluated and scored as negative or positive. Positive cases were further classified as weak, moderate, or strong based on staining intensity. The staining pattern was then correlated with clinicopathological parameters including Gleason pattern, Gleason score, WHO/ISUP grade group, serum PSA levels, perineural invasion, and lymphovascular invasion. All data were tabulated in Microsoft Excel and analyzed using IBM SPSS Statistics version 26. Descriptive statistics were reported as frequencies and percentages, and associations were assessed using the chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study cohort consisted of 48 patients with prostatic adenocarcinoma, stratified across four age groups. Among them, eight patients (16.67%) were below 60 years of age, while 17 patients (35.42%) fell within the 60–70 years category. The largest group included patients aged between 70 and 80 years, accounting for 20 individuals (41.67%), indicating that prostate carcinoma predominantly affects this age group in the study population. Only three patients (6.25%) were above 80 years.

Serum PSA data was available for 38 out of 48 patients and was grouped into five categories: <4.0 ng/ml (2.63%), 4.0–9.9 ng/ml (15.79%), 10.0–19.9 ng/ml (23.68%), 20.0–100.0 ng/ml (55.26%), and >100.0 ng/ml (2.63%). The majority of patients presented with PSA levels between 20.0 and 100.0 ng/ml, emphasizing a trend toward elevated PSA values in most cases.

Histopathological analysis revealed that 25 patients (52.08%) had evidence of perineural invasion, suggesting an increased risk of local spread. Lymphovascular invasion was detected in only five patients (10.41%), indicating a smaller subset at risk for systemic dissemination. According to WHO grading, the majority of cases (54.16%) belonged to grade 5, denoting poorly differentiated, high-grade carcinoma. Grade 4 constituted 31.25% of cases, while grades 1, 2, and 3 were rare, comprising 8.33%, 4.1%, and 2.08%, respectively. Evaluation of NKX3.1 expression across tumor segments showed moderate expression in 41.67% of cases, strong expression in 34.38%, and weak expression in the remaining cases. Expression was notably linked to tumour grade: all segments with Gleason score 3 showed strong NKX3.1 expression, while Gleason score four segments demonstrated moderate (62.26%) and strong (37.74%) expression, with no weak expression observed. In contrast, segments with Gleason score 5 exhibited predominantly weak expression (69.70%), with moderate (21.21%) and strong (9.09%) staining being less common.

Serum PSA levels also correlated with NKX3.1 expression intensity. Among patients with strong

NKX3.1 expression, the majority (56.25%) had PSA levels between 20.0–100.0 ng/ml, while smaller proportions were seen in the 10.0–19.9 (18.75%) and 4.0–9.9 (18.75%) categories. Only one case (6.25%) was found with PSA <4.0 ng/ml. Similarly, moderate NKX3.1 expression was most frequent (52.38%) in the 20.0–100.0 ng/ml group, followed by 38.10% in the 10.0–19.9 range. Weak NKX3.1 expression also followed this trend, with 63.64% occurring in patients with PSA between 20.0–100.0 ng/ml. Perineural invasion was most common in WHO grade 5 (72%) and significantly lower in grade 4 (28%), with no cases in grades 1 to 3. Lymphovascular invasion was exclusively seen in WHO grade 5 (100%). Interestingly, perineural invasion was present in only 16% of patients with strong NKX3.1 expression but was more frequent among those with moderate (60%) and weak (24%) expression. Lymphovascular invasion, conversely, was absent in cases with strong NKX3.1 expression, but notably higher in those with moderate (75%) and weak (25%) expression, suggesting an inverse relationship between NKX3.1 expression strength and invasive potential.

Table 1: Basic characteristics of the study population

Age group	No. of patients	Percentage (%)
<60 years	8	16.67
60 – 70 years	17	35.42
70 - 80 years	20	41.67
>80 years	3	6.25
Serum PSA levels (ng/ml)		
<4.0	1	2.63
4.0-9.9	6	15.79
10.0—19.9	9	23.68
20.0-100.0	21	55.26
>100.0	1	2.63
Perineural Invasion		
Absent	23	47.91
Present	25	52.08
Lympho vascular Invasion		
Absent	43	89.58
Present	5	10.41
WHO grade		
1	4	8.33
2	2	4.1
3	1	2.08
4	15	31.25
5	26	54.16

Table 2: Pattern of NKX 3.1 Expression among study population

Pattern of NKX 3.1 Expression among (N= 96) segments in the total study population	No of segments	Percentage (%)
NKX 3.1 Expression		
Strong	33	34.38
Moderate	40	41.67
Weak	23	23.96
Expression of NKX3.1 among the segment categorized by Gleason pattern 3 (N=10 segments)		
NKX 3.1 Expression		
Strong	10	100
Moderate	-	-
Weak	-	-
Expression of NKX3.1 among the segment categorized by Gleason pattern 4 (N=53 segments)		
NKX 3.1 Expression		
Strong	20	37.74
Moderate	33	62.26
Weak	-	-
Expression of NKX3.1 among the segment categorized by Gleason pattern 5 (n=33 segments)		
NKX 3.1 Expression	No of segments	Percentage (%)
Strong	3	9.09
Moderate	7	21.21
Weak	23	69.7

Table 3: Relationship between NKX 3.1 expression levels and serum PSA (Prostate-Specific Antigen) levels

Serum PSA levels	NKX 3.1 Expression		
	Strong N (%)	Moderate N (%)	Weak N (%)
<4.0	1(6.25%)	0	0
4.0-9.9	3(18.75%)	2(9.52%)	1(9.09%)

10.0—19.9	3(18.75%)	8(38.10%)	2(18.18%)
20.0-100.0	9(56.25%)	11(52.38%)	7(63.64%)
>100.0	0	0	1(9.09%)

Table 4: Proportion of Perineural invasion, Lympho vascular invasion with different WHO grades of prostate cancer and NKX 3.1 Expression

Variable	Perineural Invasion	Lympho vascular Invasion
WHO grade		
	No. of patients n (%)	No. of patients n (%)
1	0	0
2	0	0
3	0	0
4	7(28.0%)	0
5	18(72.0%)	5(100.0%)
NKX 3.1 Expression		
	No. of patients n (%)	No. of patients n (%)
Strong	4(16.0%)	0
Moderate	15(60.0%)	3(75.0%)
Weak	6(24.0%)	1(25.0%)

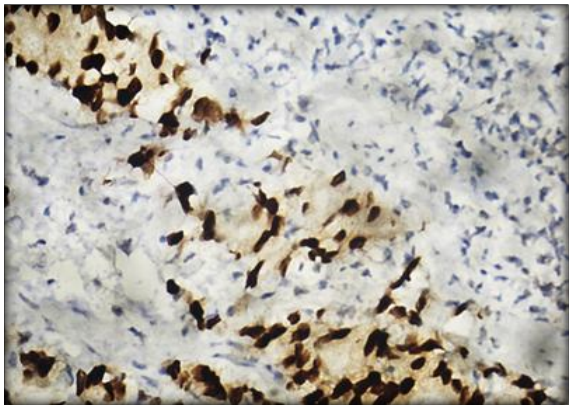

Figure 1: Strong expression of NKX3.1 in Gleason score 3+3 prostatic adenocarcinoma

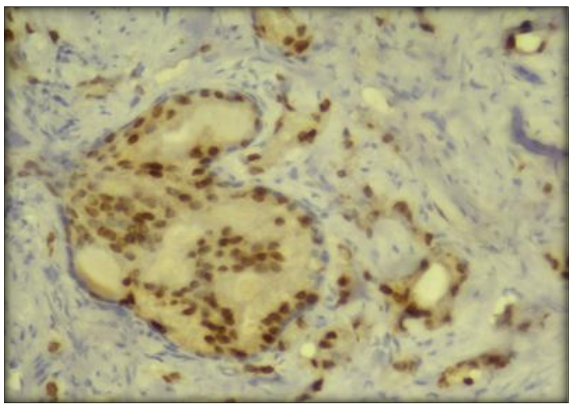

Figure 2: Moderate expression of NKX3.1 in Gleason score 4+4 prostaticadenocarcinoma

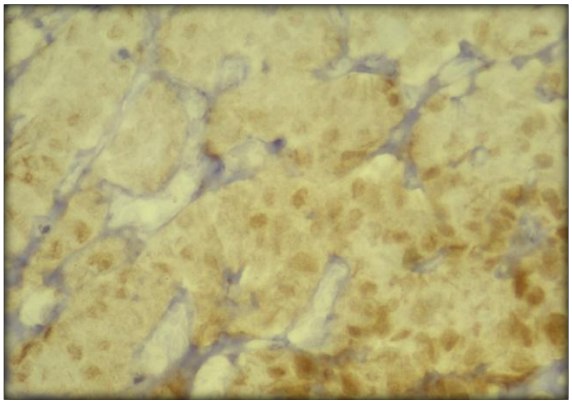

Figure 3: Weak expression of NKX3.1 in Gleason score 5+5 prostaticadenocarcinoma

DISCUSSION

This study evaluated the clinicopathological features of prostatic adenocarcinoma about NKX3.1 expression, with a focus on age distribution, PSA levels, histological grade, and patterns of invasion. The majority of patients fell within the 70–80 years age group (41.67%), consistent with age-related trends in prostate cancer reported in Indian and global populations.[5,6,7] PSA levels were markedly elevated in most patients, with over half (55.26%) having values between 20.0 and 100.0 ng/ml, similar to findings by Gerstenbluth et al. , which reinforce the diagnostic utility of PSA in advanced disease.[8,9,10] Perineural invasion was present in 52.08% of patients, aligning with reports by Ramos and Lee et al. , highlighting its frequent occurrence and prognostic importance.[11,12] In contrast, lymphovascular invasion was observed in only 10.41% of cases, reflecting the variable incidence reported in literature and underscoring the heterogeneous nature of prostate cancer spread.[13]

The WHO grade distribution in this cohort skewed heavily toward higher grades, with 54.16% of patients in grade 5 and 31.25% in grade 4, indicating a predominance of high-grade tumours. NKX3.1 expression showed a clear inverse correlation with Gleason pattern and tumour differentiation. Strong

expression was seen in Gleason pattern 3 segments (100%) declined progressively with moderate expression in patterns 4 (33 cases/62.26%) and there was no weak expression. In pattern 5 weak expression dominated (69.70%). These findings support earlier observations by Bethel et al. and Bowen et al, reinforcing NKX3.1's utility as a surrogate marker for tumour differentiation.[14,15] However in some of the cases of pattern 4 and 5 strong expression was also found (37.74% and 9.09% respectively). In the study by GuvenAslan et al. no correlation was seen between NKX3.1 expression and tumor progression, emphasizing the complexity of NKX3.1 regulation in prostate cancer biology and the need for further studies that consider molecular subtypes, androgen sensitivity, and gene-environment interactions.[16]

The study also explored the relationship between NKX3.1 expression and PSA levels. Strong and moderate NKX3.1 expression were both most commonly observed in patients with PSA values between 20.0 and 100.0 ng/ml, suggesting a potential association between elevated PSA and preserved NKX3.1 expression in biologically active tumors. This finding contrasts with Aslan et al, who reported no significant association between NKX3.1 and PSA levels.[16] The discrepancy underscores ongoing debate regarding NKX3.1 's prognostic reliability and its behaviour in androgen-dependent versus independent tumour pathways. Taken together, the results of this study reaffirm NKX3.1 as a valuable diagnostic and differentiation marker, while also raising new questions about its prognostic role and dynamic expression across tumour grades and stages.

CONCLUSION

This study demonstrates that NKX3.1 expression is inversely correlated with tumour grade and aggressiveness in prostatic adenocarcinoma. Strong nuclear expression of NKX3.1 was predominantly observed in well-differentiated tumors (Gleason pattern 3), while weak expression was more frequent in high-grade, poorly differentiated tumors (Gleason pattern 5). Furthermore, its expression pattern showed notable associations with serum PSA levels, perineural invasion, and WHO grade, suggesting its relevance as a reliable prognostic and adjunct diagnostic marker. Further studies are advised in this field to explore the utility of NKX3.1 in diagnostic and prognostic arena of prostatic cancer including other subtypes.

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