IMMUNOHISTOCHEMICAL EXPRESSION OF NKX3.1 IN PROSTATIC ADENOCARCINOMA AND BENIGN PROSTATIC HYPERPLASIA AT A TERTIARY CARE HOSPITAL IN KARACHI

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ABSTRACT

OBJECTIVES

To determine NKX3.1 expression in prostatic adenocarcinoma and benign prostate hyperplasia on Immunohistochemistry at a tertiary care hospital in Karachi.

METHODOLOGY

74 prostatic specimens were recruited in this comparative cross-sectional study at the Department of Pathology, Pakistan Navy Station Shifa Hospital, Karachi, from January 2018 to February 2019. Of these, 37 specimens were of prostatic adenocarcinoma, and 37 were benign prostate hyperplasia. All specimens were subjected to immunohistochemical staining with NKX3.1. Statistical analysis was done by using SPSS version 23.0. The association of the extent of NKX3.1 staining between the adenocarcinoma and hyperplasia group was assessed using the Chi-square test x2.

RESULTS

Of the 37 cases of benign prostatic hyperplasia subjected to NKX3.1 staining, 32 showed positive staining with strong to moderate intensity. No staining was observed in 5 cases. 37 cases of adenocarcinoma prostate stained for NKX3.1 revealed positive staining in 30 cases with strong to moderate intensity. Negative staining was seen in 7 cases. The prostatic adenocarcinoma showed a statistically significant association of NKX3.1 positivity compared to benign prostatic hyperplasia cases. The p-value was found to be 0.03.

CONCLUSION

NKX3.1 staining was highly specific for prostate epithelium, as it was positive in most cases. This immune marker was useful for distinguishing prostatic origin in the context of metastatic lesions. Adding NKX3.1 protein staining to a panel of features may add value to the diagnosis if applied in the appropriate clinicopathologic context.

KEYWORDS: *Adenocarcinoma prostate, NKX3.1, Benign prostate hyperplasia, Immunohistochemistry.*

INTRODUCTION

Prostate cancer is the second most diagnosed malignancy in males and the fifth major cause of mortality around the globeA total of eight studies were analyzed to evaluate prostate cancer's prevalence. The overall prevalence of prostate cancer in these studies was approximately in the range of 2 to 8 %, and the overall majority was found to be 5% Various risk factors might be implicated in the pathogenesis of this malignancy; these include older men, the role of androgens, certain genetic factors, geographical influence, hereditary predisposition, consumption of a high-fat diet, drinking alcohol, and epigenetic alterations.[1,2,3,4,5] Benign prostatic hyperplasia (BPH) is a nonmalignant prostate enlargement due to cellular hyperplasia. It is one of the most frequently diagnosed pathologies of elderly males. By 50 years, about 50% of men are recognized as BPH, and 70% of men aged 70 years or over generally suffer from this lesion.[6] Even though certain distinctive histological features on biopsy are decisive for diagnosing prostate cancer, including perineural invasion, the diagnosis is based on an array of morphological features, including gross and histological, cytological, and ancillary findings. H and E staining alone cannot be used to establish, and therefore, the advent of Immunohistochemistry has revolutionized the field of histopathology and helped histopathologists make appropriate diagnoses[7]Various immunohistochemical markers have been utilized to diagnose prostate cancer. These include: AMACR, p63, NKX3.1, PTEN, Interleukin-6, transforming growth factor- 1, Glutathione S-transferase P1and chromogranin A.[8] NKX3.1 gene is located on chromosome 8p21 and plays a vital role in normal prostate development, maintaining the proliferation of

the glandular epithelium. It also helps in the formation of ducts. It prevents the recombination of the TMPRSS2 and ERG gene loci by binding to androgen receptors at the ERG gene breakpoint.[9] NKX3.1 expression can be immunohistochemically detected in most primary prostate cancers. It is pathogenic in the human prostate and may lead to early malignant change NKX3.1 maintains the differentiation of the normal prostate gland; on the contrary, its loss represents a predisposing event for prostate carcinogenesis.[10,11] NKX3.1 expression is also seen in Sertoli cell tumours of the testes and ovaries.[12] Various immune markers have been identified and are being used to diagnose prostate cancer. Moreover, work on NKX3.1 has not been documented in Pakistan; this study will provide baseline findings regarding this immune marker for further studies.

METHODOLOGY

This is a comparative cross-sectional study based on the analysis of prostatic samples, comprising of both prostatectomies and transurethral resected prostatic biopsies received at the Department of Pathology, PNS Shifa Karachi during one year from January 2017 to February 2018. The samples were collected by using a non-probability convenience sampling technique. Histologically, confirmed cases of adenocarcinoma prostate and benign prostatic hyperplasia were subjected to immunohistochemistry evaluation for NKX3.1. Ethical approval was taken from the ERC (Ethical Review Committee) of the Bahria University of Medical & Dental College (BUMDC). The sample size was calculated using the method of single proportion on www.openepi.com software with a prevalence of 4.71%with 5%margin of error and a 95% confidence interval. Male patients who were willing to participate between 50 years to 85 years were included in the study. Poorly fixed tissue samples and samples having inadequate material having atrophy were excluded. H&E stained slides were reviewed to confirm the diagnosis. The most representative section was used for immunohistochemical analysis. To perform Immunohistochemistry, 3 to 5 µm thickness sections were taken from Formalin Fixed Paraffin Embedded blocks and picked up on poly-L- lysine coated slides. Antigen retrieval was done by using a retrieval solution (pH 6.0 citrate buffer 10X) in a water bath at 98-99ºC for 40 minutes. The container was removed from the water bath and cooled at room temperature (15 to 20 minutes). The retrieval solution was discarded, and the section was rinsed two to three times. Endogenous peroxide was blocked using a hydrogen peroxide-blocking solution. Primary antibodies were applied to cover the section. NKX3.1 dilution was done per the company-specified protocol and was incubated for 1 hour at room temperature. Slides were then incubated with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes, and all the slides were counterstained with Hematoxylin, dehydrated, and mounted. The slides were washed with phosphate buffer solution (PBS) between each step.NKX3.1 (clone EP356) ready-to-use monoclonal antibody procured from Cell Marque. Normal prostate tissue was used as a positive control for NKX3.1. NKX3.1 showed continuous diffuse nuclear staining of glandular epithelium.The percentage of stained tumour cells (brown colour) was expressed as 3(+) when >80% of nuclei were degraded, 2(+) when 26-50% of cells were degraded, (1+) when 1-25% cells were stained and (0) when negative staining was observed. The intensity of staining was graded as strong (3+), Moderate (2+), Weak (1+), and no staining (0). Relevant data were collected on self-designed proformas. Statistical analysis was performed using SPSS version 23. Mean, and standard deviation were calculated for quantitative variables, while percentages and frequencies were calculated for qualitative variables. Chi-square was applied to calculate the p-value. A p-value of less than 0.05 was considered significant at a 95% confidence interval.

RESULTS

Among 74 patients studied, the mean age of patients with benign prostate hyperplasia was 64.11±8.75 years, while those of adenocarcinoma prostate was70.0±7.4 years. Out of the 37 cases of adenocarcinoma prostate subjected to NKX3.1 staining, 16 patients showed reactivity in 26-50% of cells, 8 points showed reactivity in >80%of cells, 6 cases showed reactivity in 51-80% of cells, 7 patients showed no reactivity. Nineteen cases showed moderate (+2) staining intensity, whereas 11 points showed strong (+3) staining intensity. Out of the 37 cases of benign prostate hyperplasia, subjected to NKX3.1 staining, 3 cases showed reactivity in 26-50% of cells, 15 cases showed reactivity in >80%of cells, 14 cases showed reactivity in 51-80% of cells, 5 cases showed no reactivity. Eighteen cases showed moderate (+2), whereas 14 showed strong (+3) staining intensity. The prostatic adenocarcinoma showed a statistically significant association of NKX3.1 positivity compared to benign prostatic hyperplasia cases. The p-value was found to be 0.03.

Table 1: Extensiveness of Nkx3.1 Immunohistochemical Staining in Benign Prostate Hyperplasia and Adenocarcinoma Prostate

% of stained cells	NKX3.1 (Benign prostate hyperplasia)		NKX3.1 (Adenocarcinoma prostate)	
	No.	%	No.	%
>80%	15	40.5	08	21.6
51 -80%	14	37.9	06	16.2
26-50%	03	8.1	16	43.2
1-25%	03	8.1	01	2.7
0	02	5.4	06	16.3

Table 2: Immunohistochemical Staining Intensity of Nkx3.1 in Benign Prostate Hyperplasia and Adenocarcinoma Prostate

	NKX3.1(Benign prostate hyperplasia)		NKX3.1(Adenocarcinoma prostate)	
Intensity of staining	No.	%	No.	%
Negative(0)	02	5.4	06	18.9
Weak(+1)	03	8.1	01	10.9
Moderate(+2)	18	48.6	19	48.6
Strong(+3)	14	37.9	11	21.6

DISCUSSION

The diagnosis of prostate cancer can be a very challenging task for histopathologists. Morphologically, prostate cancer is difficult to diagnose in that the clues leading to the diagnosis of malignancy may be profound, leading to underdiagnosis. There are also many benign pathologies mimicking malignancy that can lead the histopathologist to an inaccurate diagnosis.[13] Immunohistochemistry plays a pivotal role in diagnosing various malignancies Although studies have been conducted in Pakistan on the immunohistochemical profile of prostate cancer. No study has not been conducted on NKX3.[14,15,16] Our research findings coincide with research carried out in Sudan. Out of the 40 cases, Immunohistochemistry revealed loss of NKX3.1 expression in 26 cases of prostatic adenocarcinoma, whereas 14 cases showed positive expression. The study, therefore, concluded that NKX3.immune expression is strongly associated with tumours of higher Gleason grade, proving that NKX3.1 plays a part in tumour progression.[17]Bowen et al. exhibited that loss of NKX3.1 protein expression, as assessed by Immunohistochemistry (IHC), correlated with prostate cancer progression with a significant p-value of ($p< 0.0001$).[18] Gurel et al. reported positivity for NXK3.1in 40 cases; the mean per cent of tumour cells staining positive in their nuclei was 84.7%. Reactivity in more than 25% of nuclei with moderate to strong intensity of staining was taken as positive in this study.[19] Irer et al. revealed NKX3.1 expression is raised in BPH tissues when compared with normal tissues, which may be important in the development of BPH Ihab et al. performed Immunohistochemistry on 60 samples of BPH and prostate adenocarcinoma respectively, NKX3.1 revealed positivity in 86.6% of cases of BPH and 70% in carcinoma cases.[20,21] However, there is some disagreement. The most widely accepted theory about the expression of the NKX3.1 protein in human prostatic adenocarcinoma states that levels are decreased in initial prostate malignancies and are further decreased and frequently lost in metastatic lesions.[22] When NKX3.1 expression is lost, defective protein secretion and abnormal duct formation lead to carcinogenesis. Chuang et al. proclaimed that NKX3.1 could be used as one of the markers in an immunohistochemical panel to differentiate between adenocarcinoma prostate and high-grade urothelial carcinomas. Loss of NKX3.1 expression was seen in 5% of cases of benign prostate hyperplasia, which contradicts our study since all cases of BPH stained positively for NKX3.1.[23] This is the first study carried out on the immunohistochemical expression of NKX3.1 expression and provides baseline findings for further studies to be carried out on this marker. It is recommended that further studies should be carried out on a larger scale with a greater sample size to validate our findings. Studies should be carried out on a molecular level to further assess this marker's role in diagnosis in our part of the world.

LIMITATIONS

It is a single-centre study with a small sample size.

CONCLUSIONS

In this study, we found NKX3.1 staining highly specific for prostate epithelium as it was positive in most cases. If applied in the appropriate clinicopathologic context, adding NKX3.1 protein staining to a panel of markers may add diagnostic value to the diagnosis.

CONFLICT OF INTEREST: None

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