SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: 10.36346/sarjpm.2023.v04i02.002

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Immunohistochemical Expression of NKX3.1 in Prostatic Adenocarcinoma Correlates with Tumors Grade among Sudanese

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Article History: | Received: 11.03.2023 | Accepted: 27.04.2023 | Published: 30.04.2023 |

Abstract: ***Background***: The second most frequent cancer in males and the fourth most common cancer overall for both sexes is prostate cancer. On chromosome 8p, there is a prostatic tumor suppressor gene called NKX3.1. Despite the fact that the majority of primary prostatic adenocarcinomas exhibit positive NKX3.1 protein staining. ***Objective:*** The purpose of this study was to evaluate the immunohistochemical expression of NKX3.1in prostate adenocarcinoma and correlate it with tumor grades among Sudanese patients. ***Materials and Methods:*** This study is a retrospective descriptive cross- sectional study, was conducted in Khartoum state, (Radiation& Istopes Center- Khartoum (RICK)). Tissue sections were stained by immunohistochemical staining against NKX3.1 (indirect dextral polymers (Dako-EnVision TM Flex kit)) was carried out on forty (40) archival formalin fixed paraffin embedded tissue blocks from patients diagnosed as prostate carcinoma. Data were collected from hospital records and the immunohistochemical results and then analyzed using SPSS 25.0 frequency, and Chi-Square and mean were calculated. ***Results:*** The study found that the most frequent age group is between (71 – 80) years old, twenty (20) cases have prostate adenocarcinoma grade III, twelve (12) cases were grade II, and eight (8) cases were grade I. Any nuclear NKX3.1 staining was regarded as positive. Thirty-five (35%) have NKX3 positive immunostain while (65%) have a negative result. There is a statistically significant correlation between the NKX3.1 expressions and tumor grade as the *P. value* was (0.000), while in a statistically significant correlation between the NKX3 expressions and patients' age as the *P. value* was (0.957). ***Conclusions:*** NKX3 immuno expression is strongly associated with higher tumor grade and may prove the role of this protein in the progression of prostate cancer.

Keywords: NKX3, prostate adenocarcinoma, immunohistochemical staining**.**

INTRODUCTION

The second most frequent cancer in males and the fourth most common cancer overall for both sexes is prostate cancer [1]. Northern America, Australia/New Zealand, and Western and Northern Europe have the highest prostate cancer incidence rates. The incidence rates are still low among Asian populations but are significantly higher in less developed regions including the Caribbean, Southern Africa, and South America. In the Caribbean, the incidence rate is 29 per 100,000, while in sub-Saharan Africa, it ranges from 19 to 24 per 100,000 [2]. The most frequent disease in Sudanese men is prostate cancer, which affects the country's tribes equally. At a mean age of 72.2 9.25 years, 85.4% of these men have stage III or IV cancer [3]. Although it is occasional among Sudanese men under the age of 50, after that the rates increase significantly. The main risk factors for prostate cancer in Sudan include age, education level, and good family history. Additional characteristics include occupation, body mass index

Citation: Ibrahim Bakhit Yousif Elemam, Duaa Sharaf Eldin Safi Eldin Ahmed, Tibyan Abd Almajed Altaher, Ghanem Mohammed Mahjaf, Mosab Nouraldein Mohammed Hamad (2023). Immunohistochemical Expression of NKX3.1 in Prostatic Adenocarcinoma Correlates with Tumors Grade among Sudanese. *SAR J Pathol Microbiol*, 4(2), 20-23.

(BMI), and a history of smoking or drinking [4]. Because of allelic loss, promoter methylation, and posttranscriptional silencing, NKX3.1 expression is typically reported to be reduced in human prostate carcinomas and prostatic intraepithelial neoplasia (PIN). Prostate cancer and other metastatic lesions coming from the prostate can both be diagnosed with NKX3.1. On chromosome 8p21, which experiences loss of heterozygosity (LOH) more frequently as prostate cancer progresses, is the human NKX3.1 gene [5]. An outstanding illustration of a gene that is essential for both embryogenesis and oncogenesis is the Nkx3.1 homeobox gene. The first marker of prostate development is Nkx3.1, which is still expressed in maturity and at all phases of prostate differentiation. Due to abnormalities in prostatic protein secretions and ductal morphogenesis caused by its loss of activity, Nkx3.1 plays a crucial role in the normal development of the prostate. As a result of Nkx3.1's cooperation with other tumor suppressor genes in the development of cancer, its loss of function also leads to prostate carcinogenesis. Additionally, NKX3.1 expression is decreased in non-invasive and early-stage human prostate cancer by several pathways, indicating that this decreased expression is one of the first stages in the majority of cases of human prostate cancer [6]. This is the first research project in Sudan to look for NKX3.1 expression in prostate cancer patients. We speculate that NKX3.1 can be employed as a useful prognostic and diagnostic marker for the detection of prostate cancer since it will be significantly expressed in the late stages of the disease.

MATERIALS AND METHODS

Study Design: This was a Retrospective descriptive cross-sectional hospital-based study.

Study Area: The study was conducted in Khartoum state, an oncology hospital.

Study Population: The study involved archival tissue blocks of patients diagnosed with prostate adenocarcinoma.

Sample Size & Technique

Non-probability Purposive sampling techniques were used in this study (40) samples from Paraffin-embedded tissue blocks were enrolled according to this equation: n =z2*pq/d2

n = sample size
Z = power (1.096)
P = prevalence of disease d = stander deviation (0.05) q = 1-p

Data Collection: A formalin-fixed tissue sample from patients diagnosed with prostatic carcinoma and master sheet.

Samples Processing

By using a Rotary microtome (Leica RM2125RTS, semi-automated) all blocks were trimmed with the hot knife before section cutting (15µm thickness). After trimming the blocks were placed on melting ice for 5 minutes. Then ribbons of sections were cut from blocks (3µm thickness) after adjusting the Clearance and a certain angle. The slide was translated to the lab of work inside the box. Ribbons were then flooded in a water bath with a temperature adjusted to be less than the wax melting point (40˚c) then sections were picked up into a dry slide to allow complete drying, then carried to a metal rack into a hot oven adjusted previously with a melting point of the wax. One section was cut from each block. Sections were stained using the Immunohistochemistry protocol for formalin fixed-paraffin embedded sections.

Data Analysis

Data were analyzed using SPSS 25.0, descriptive statistics in terms of frequency, percentages, means and standard deviations, and Chi-square tests were calculated. A *p value* ≤0.05 is considered statistically significant.

Ethical Considerations

Ethical approval for the study was obtained from the Board of the Faculty of medical laboratories sciences, at University of Al-Neelain. The written informed consent form was obtained from each guardian of the participant as well as from the subject himself before recruitment into the study. All protocols in this study were done according to the Declaration of Helsinki (1964).

RESULTS

Table 1: Correlation between the immune expression of NKX3 and age group of the study population

Variables		***NKX3***		***Total***	***P value***
		Positive	***Negative***		
Age group	51-60	1	1	2	0.957
	61-70	4	9	13	
	71-80	8	14	22	
	81-90	1	2	3	
Total		**14**	**26**	**40**	

Table 2: Correlation between the immune expression of NKX3 and tumor grade

Variables		***NKX3***		***Total***	***P value***
		Positive	***Negative***		
Grade	Grade I	0	8	8	0.000
	Grade II	0	12	12	
	Grade III	14	6	20	
Total		**14**	**26**	**40**	

DISCUSSION

An outstanding illustration of a gene that is essential for both embryogenesis and oncogenesis is the Nkx3.1 homeobox gene. The first marker of prostate development is Nkx3.1, which is still expressed in maturity and at all phases of prostate differentiation. Due to abnormalities in prostatic protein secretions and ductal morphogenesis caused by its dysfunction, Nkx3.1 plays a crucial role in the normal development of the prostate [6]. There are some differences between the results of studies on the expression of NKX3.1 in human prostate cancer and prostatic intraepithelial neoplasia (PIN). Studies of NKX3.1 mRNA were over-expressed in 31% of prostatic adenocarcinomas, reduced in 21% of cases, and resembled normal epithelium in 48% of cases [7]. Moreover, more tumor samples from non-organ confined malignancies (40%) than from organ-confined disease (22%) displayed NKX3.1 mRNA overexpression. Contrarily, in their investigation of early-stage prostate malignancies, Ornstein did not discover a change in NKX3.1 mRNA levels by quantitative in situ hybridization in prostatic adenocarcinomas compared with normal prostate [8]. These researchers hypothesized that NKX3.1 would be a helpful marker of malignant prostate epithelium because it was expressed almost exclusively in the prostate in adult tissues. Particularly, they found that NKX3.1 staining was completely lost in 20% of high-grade PIN, 6% of stage T1a/b samples, 22% of stage T3/4 samples, 34% of hormone-refractory prostate tumors, and 78% of metastases. Bowen reported that loss of NKX3.1 protein expression, as assessed by immunohistochemistry (IHC), correlated with prostate cancer progression [9]. Nevertheless, Korkmaz On adjacent TMA slides, in situ hybridization for mRNA expression and IHC for protein staining were performed [10]. According to Gelmann, the results showed that the majority of prostatic adenocarcinoma patients tested positive for both mRNA and protein and that neither level was correlated with tumor grade or clinical stage. In prostatic adenocarcinomas, NKX3.1 protein was detected by IHC in 66% of initial untreated tumors, 44% of metastatic tumors, and 27.3% of castrate-resistant/hormone- refractory tumors [11]. According to Asatiani *et al.*, only 4.6% of primary tumor samples had completely lost all of their NKX3.1 protein, despite there being a decreased staining intensity for the protein as measured by image analysis [12]. Although almost all cases still had moderate to strong levels of NKX3.1 staining Bethel *et al.*, found that PIN and primary prostatic adenocarcinomas had lower levels of NKX3.1 protein [13]. Chuang and his colleagues recently showed that NKX3.1 protein staining was a highly specific and somewhat sensitive sign when utilized as a diagnostic tool as part of a panel of IHC markers in aiding to identify high-grade bladder urothelial carcinoma from high-grade prostate carcinoma [14]. They did this by using the same rabbit polyclonal antibody as Bethelet [13]. The work by Chuang *et al.*, did not, however, comprehensively investigate the expression of NKX3.1 in metastatic prostatic adenocarcinomas or establish the specificity for prostate carcinoma beyond a review of malignancies of the urinary bladder [14]. 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 further decreased and frequently lost in metastatic lesions [15]. In contrast to this assumption, the current research revealed that NKX3 outcomes were 35% positive and 65% negative. Results and patient age group had no statistically significant association. The grade of the disease, however, significantly correlates with the NKX3 results. Its significance suggests that as the stage advances, it becomes more likely that the outcomes will be favorable. The effectiveness of the various antibodies that have been used is likely a factor in some of the discrepancies that have been identified in the literature so far regarding the existence of residual NKX3.1 protein in prostatic adenocarcinomas. More metastasis was detected in the work of Bora G than in the present investigation [16]. According to the Cai B study NKX3.1 expression was completely lost in 5% of cases with benign prostatic hyperplasia [17].

CONCLUSION

NKX3immuno expression is strongly associated with higher tumor grade and may prove the role of this protein in the progression of prostate cancer.

SOURCES OF FUNDING

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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