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The Contrary Role of Nkx3-1 in Malignant and Benign Prostatic Tissues

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Abstract: ***Nkx3-1 the homeobox gene (located on chromosome 8p21.2) is an androgen-regulated and mainly prostate-specific gene, which was observed primarily in secretory prostatic benign and neoplastic epithelia, Nkx3.1down-regulation is considered one of the earliest events in prostate cancer initiation. Our study was designed to investigation the role and expression of Nkx3-1 proteins in initiation and development of prostate cancer (PCa) as well as in benign prostate hyperplasia (BPH).This study included sixty tissue were collected from samples of prostate tumors patients, twenty nine of them were diagnosed as PCa and thirty one were recorded as a benign patients. Malignancy patients were divided according to aging and grading. Those patients' samples then compared with twelve non-tumors prostate tissues as control group. This study was carried out in Laboratories of the College of Science/ Department of Biology, Wasit University, during period between October 2017 and May 2018. The study of Nkx3-1 expression was done by immunohistochemistry (IHC) staining technique. The results showed highly significant (p≤0.01) increasing in expression of Nkx3-1 in prostate cancer patients when compared with benign and control group. Also the result showed significant (p≤0.05) correlation between Nkx3-1 expression with overlapping effects of grading. From the results presented, it was concluded that the present study confirms that Nkx3-1 play necessary roles in carcinogenesis and development of prostatic cancer. This study suggests using Nkx3- as additional biomarker in diagnosis and following up the treatment of prostatic cancer.***

Keywords: prostate, cancer, benign, Nkx3-1, expression, IHC

1. Introduction

Prostate gland considered as the largest accessory sex gland of males. It is a musculo-glandular, exocrine gland that excretes alkaline fluid which constitutes about 20–30 % volume of the seminal fluid, also this gland is often associated with disorders of elderly, benign prostatic hyperplasia and carcinoma [1].Prostate cancer is the second most common cancer in men globally, as approximately 1.3 million men were diagnosed with the disease in 2018, accounting for 13.5 % of the cancers diagnosed in men during that year with 6.7% mortality rate [2]. This malignancy is also the 5th leading cause of death from cancer in men throughout the world [3]. Almost all prostate cancers are adenocarcinomas that develop when glandular prostate cells become malignant, these cells grow into localized tumors that can either be contained in the prostate, or spread to surrounding tissues such as seminal vesicles, bladder, or the rectum [4]. The aim of this study was to investigation the role and expression of Nkx3-1 proteins in initiation and development of prostate cancer as well as in benign prostate hyperplasia.The exact cause of developing prostate cancer are not known though ageing, ethnicity and heredity are important factors involved in the initiation and development of this cancer, aging is the most significant risk factor, the mean age at diagnosis is between 72 and 74 years and 85% of the patients are diagnosed after the age of 65, Before the age of 50 PCa is still rarely diagnosed though an increased incidence in younger men has been observed lately[3]. The most commonly used system for grading adenocarcinoma of the prostate is the Gleason score [5]. BPH refers to the increase in size of the prostate in middle-aged and elderly men, BPH can be seen in the vast majority of men around the world as they age, particularly in men over the age of 70 years [6]. It occurs almost exclusively in the transitional and periurethral zones. It is characterized by hyperplasia of prostatic stromal and epithelial cells [7]. A variety of molecular and cytogenetic abnormalities are associated with PCa[8]. Nkx3-1 the homeobox gene (chromosome 8p21.2) is an androgen-regulated and mainly prostate-specific gene, which was observed primarily in secretory prostatic benign and neoplastic epithelia [9]. Moreover, Nkx3-1 is essential for prostatic epithelial specification and proper differentiation, and is required for maintenance of luminal stem cells [10]. A hallmark of prostate cancer initiation is loss of the Nkx3-1 homeoboxgene, whose functions have been associated with promotion of lineage plasticity, cellular differentiation, and response to inflammation [11]. Nkx3-1 protein has been found to be positive in the vast majority of primary prostatic adenocarcinomas, also a recent study showed that NKX3.1 staining was highly sensitive and specific for high-grade prostatic adenocarcinomas [12].Some studies reported that Nkx3.1down-regulation is considered one of the earliest events in prostate cancer initiation [13]. Mechanistically, Nkx3.1 has been shown to play a critical role in protecting prostate cells from DNA damage [14]. There was 3–15% deletion of Nkx3.1 in PC patients, therefore considered a tumor suppressor gene and loss of this gene might correlate with disease initiation and progression [15].

2. Materials and Methods

This study was carried out in the laboratory of the Faculty of Science / Wasit University in the period from October 2017 to May 2018. Sixty formalin fixed paraffin embedded sections were collected from samples of prostate tumor patients, twenty nine of them were diagnosed as PCa from patients with age ranged between 50 and 88 years, while the rest (thirty one) were recorded as a benign tumor from patients with age range between 50 and 83 years were included in this study and twelve non tumors prostate tissues

that embedded in paraffin were taken as a control group. Anti-Nkx3.1 antibody [EPR16653], and ABC immune staining system, were supplied from Abcam Biotechnology. Procedure of IHC for NKx3-1 was done as described in manufactured sheet. Slides were backing at 60- 65^{0}C for overnight. Statistical analyses of results were carried out by the help of Minitab using version SPSS statistical package. Spearman test and person factor was used to investigate comparison between the expression of each marker in patients group and control group. The level of significance was 0.05 (or less) in all statistical testing, (p value less than 0.05).

3. Results and Discussion

Nkx3.1 expression and intensity in prostatic cancer and control group

Table (1) referred to expression of Nkx3-1 in prostate cancer patients in comparison with control group. Expression of Nkx3-1 was reported positive in 27 (93.1%) of prostate cancer patients out of 29 cases, while in control group 5 (45.6%) out of 12 cases were showed positive staining for Nkx3-1. There was highly significant (p≤ 0.01) difference between patients of prostate cancer and control group in relation to Nkx3-1 expression. Intensity assessment of Nkx3-1 expression inprostate cancer patients showed that highest percentage in 15 (55.5%) cases with score +1, 7(25.9%) cases with score +2 and 5 (18.5%) cases with score +3. While in control group, one (20%) case with score +1 and +2, highest percentage in 3 (60%) cases with score+3.There was no significant (p≥0.05) difference between patients of prostate cancer and control in relation to staining intensity of Nkx3-1 expression (Table 1) and (Fig 1).

Table 1: Nkx3-1 expression and intensity in prostatic cancer patients and control group

Case	Expression + - NO% NO%		Total	P Value	Intensity 1 2 3 No% No% No%			Total	P Value
Prostatic cancer patients	27 93.1%	2 6.9%	29	P ≤ 0.01	15 55.5%	7 25.9%	5 18.5%	27	P≥0.05
Control group	5 41.6%	7 58.4%	12		1 20 %	1 20%	3 60%	5	
Total	32	9	41		16	8	8	32	

Our results showed strong correlation between Nkx3-1 expression and prostate cancer tissues, although most of the previous results indicated significantly decrease in Nkx3.1 in prostate cancer tissues, but our amazing results reversed the opposite as Nkx3.1 was significantly increases. Studies of Bowen, (2000), Qunying, (2006), Bethel, (2006), Aslan, (2006) and others were showed decreases of Nkx3.1 , due to Nkx3.1 has growth-suppressor effects on cancer cells and promotes oxidative damage in prostate tissues [16-19].Our findings may be provide a molecular principle for additional understanding of prostate cancer initiation. According to intensity of Nkx3-1 in PCa, in previous study [20], the author showed weak intensity in 16.7% cases, moderated staining in 38.3% cases and strong staining in 13.3% cases noted the same result [12].

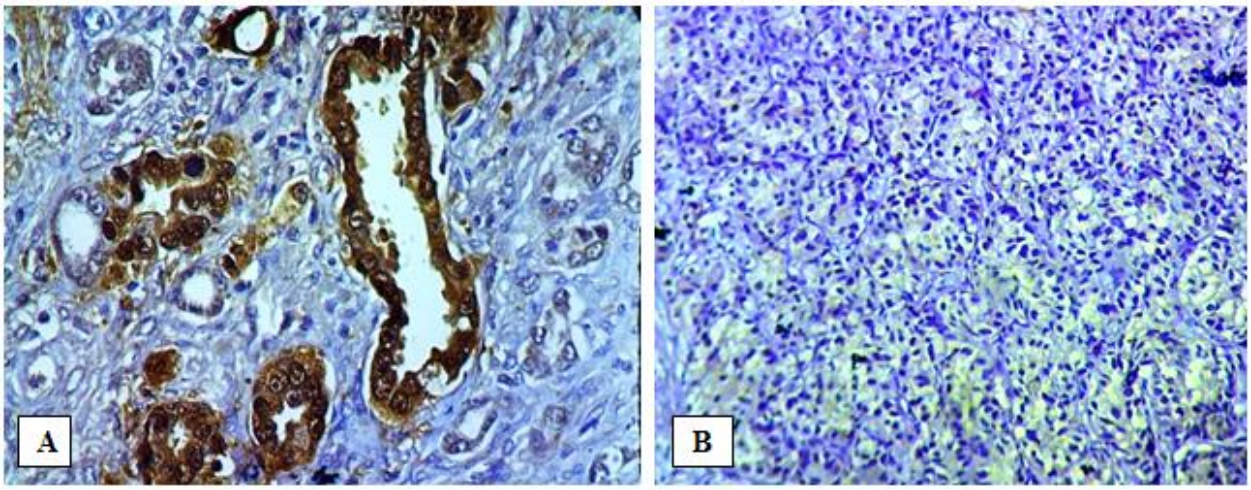

Figure 1: Nkx3-1 IHC staining in prostate patients.A: Cancer (positive) B: Cancer (negative) (40X)

Bowen et al., (2000) reported that loss of Nkx3-1 protein expression, as assessed by IHC, correlated with prostate cancer progression [16]. Gelmann et al. (2003) reported that Nkx3-1 protein was present in prostatic adenocarcinomas in 66% of primary untreated tumors, 44% of untreated metastatic tumors, and 27.3% of castrate resistant/hormone refractory tumors[21]. Xu et al., (2000)reported comparison of Nkx3-1 expression between normal and tumor tissues

revealed over expression in 31% tumor specimens (16 of 52), decreased expression in 21% tumor specimens (11of 52) and there was no change in 48% specimens (25 of 52)[22]. Also Asatiani et al. (2005) showed that although there was reduced intensity of staining for Nkx3-1 protein [23]. Chuang *et al*, (2007) reported that NKX3.1 protein was a highly specific marker with high-grade prostate carcinoma [24].Our result demonstrated that most cases with score I (55.5%), this result is not coming in agreement with results of Curel *et al.*, (2010), who showed the intensity of Nkx3-1 staining I strong in normal prostatic than in prostatic adenocarcinoma [12].Gurel *et al.*, (2010) showed a highly sensitive and specific of Nkx3.1 with metastatic prostatic adenocarcinoma [12]. In conclusion the Nkx3-1 is knows as an inhibitor of prostate epithelial cells growth, and act as cancer suppressor gene for prostate cancer, so, most studies have observed down expression of this gene in prostate cancer cells [25]. Conversely in our study, we observed overexpression of this protein in prostate cancer, this is possibly because of ability of this gene to induce mutation in the adjacent epithelial cells [26], and this mutation may be responsible for prostate cancer initiation. Our result suggests using nkx3.1 protein expression in prostate cancer as additional marker for prostate cancer prognosis.

Nkx3-1 gene expression and intensity in prostatic cancer and benign patients

Expression of Nkx3-1was reported positive in 27 (93.1%) cases of prostatic cancer patients out of 29 cases, while inbenign patients 18 (58%) out of 31 cases was showed positive staining for Nkx3-1.There was highly significant (P≤0.01) difference between prostatic cancer patients and benign group in relation to Nkx3-1 expression. Staining intensity assessment of Nkx3-1 expression in prostatic cancer patients showed that 2 (6.9%) cases with negative staining, 15(55.5%) cases with score +1, 7(25.9%) cases with score +2, 5(18.5%) cases were scored+3.While in benign patients, 13(32%)cases with negative staining, 4(22.2%) cases with score +1 , 6(33.3) with score +2, 8(44.4%) cases with score +3. There were no significant (P≥0.05) difference between patients of prostatic cancer and benign in relation to staining intensity of Nkx3-1 expression (Table2). Ornstein et al. (2001) reported that Nkx3-1 gene expression is restricted to benign and malignant secretory epithelium within the prostate but Nkx3-1 does not appear to be a classic tumor suppressor gene responsible for prostate cancer initiation [27]. While Aslan *et al.* (2006) showed that Nkx3-1 expression is significantly decreased in prostate cancer patients when compared to BPH. Atta (2017), showed 70% of benign prostate patients were positive for Nkx3-1, while Bowen et al., (2000) showed complete loss of Nkx3-1 expression in benign prostate patients [19], [16]. Nodouzi *et al.*, (2015), recorded that there is a significant correlation between prostate cancer and BPH in relation to Nkx3-1 expression [28]. Regarding to staining intensity in PBH, the same study (Bowen et al., 2000), reported that Nkx3-1 intensity showed in 5% with negative score, 12% with score I and 84% with score II [16]. We think these differences may be due to difference in IHC procedures or due to genetic diversity.

Table 2: Nkx3-1 expression and intensity in prostatic cancer patients and benign patients

Case	Expression - No %	Expression + No %	Total	P Value	Intensity 1 No%	Intensity 2 N0%	Intensity 3 No%	Total	P Value
Prostatic cancer patients	27 93.1%	2 6.9%	29	P≤0.01	15 55.5%	7 25.9%	5 18.5%	27	P≥0.05
Benign Patients	18 58%	13 32%	31		4 22.2%	6 33.3%	8 44.4%	18	
Total	45	15	60		19	13	13	45	

Nkx3-1 expression and intensity in benign patients and control group

Expression of Nkx3-1 was reported positive in18 (58.1%) of benign patients out of 31 cases, while in control group 5 (41.6%) out of 12 cases were showed positive staining for Nkx3-1. There was no significant (p>0.05) difference between benign patients and control group in relation to Nkx3-1 expression (Table 3and Fig 2). Staining intensity assessment of Nkx3-1 expression in benign patients showed, 4(22.2%) cases with score +1, 6 (33.3%) cases with score +2 and 8(44.4%) cases with score +3. While in control group, 1 (20%) case with score +1, and score +2 , 3 (60%) cases with score +3. There was no significant (p>0.05) difference between BPH and control group in the intensity of Nkx3-1expression (Table3). The result of Irer *et al.*(2009) showed that Nkx3-1 gene expression in BPH patient tissues were higher compared with normal prostate tissues by using Western blot analyses [29]. In the study of Bowen et al., (2013), the result found no malignant prostate cancer appears Nkx3-1 expression, while only two specimens of BPH displayed negative staining for Nkx3-1 [30].

Table 3: Nkx3.1 expression and intensity in benign patients and control group

Case	Expression + No %	Expression - No %	Total	P Value	Intensity 3 No%	Intensity 2 No%	Intensity 1 No%	Total	P Value
Benign patients	18 58.1%	13 41.9%	31	P≥0.05 *	4 22.2%	6 33.3%	8 44.4%	18	P≥0.05 *
Control group	5 41.6%	7 58.4%	12		1 20%	1 20%	3 60%	5	
Total	23	20	43		5	7	11	23	

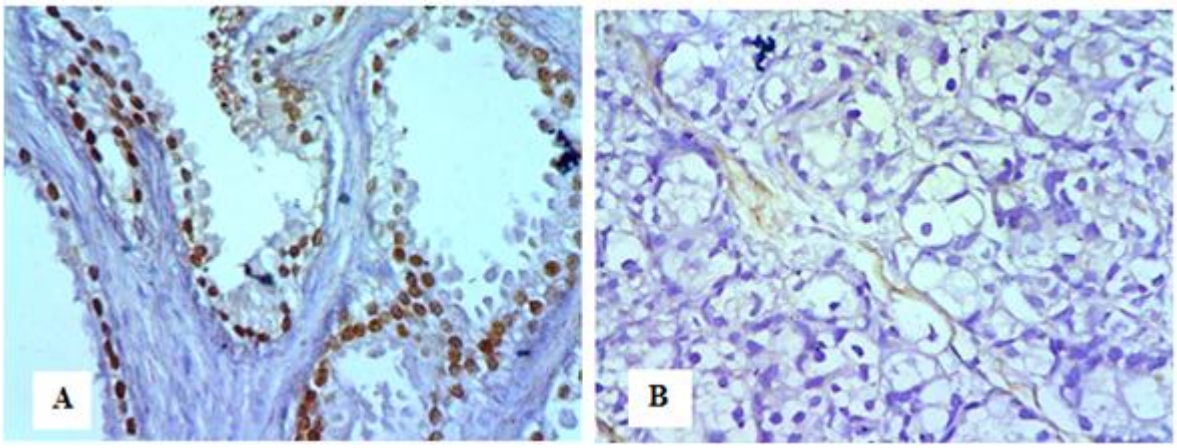

Figure 2: Nkx3-1 IHC staining in prostate patients. A: Benign (positive)B: Benign (negative) (40 X)

Correlation between Nkx3-1 expression and overlapping effects of clinicopathological variables in prostatic cancer patients

The statistical analysis between Nkx3-1 expression with overlapping age and grade of prostatic cancer using correlation coefficient (Person and Spearman's rho), showed no-significant correlation coefficient in Nkx3-1 with age groups (P≥0.05) (P≥0.05), while highly significant in tumor grade (P≤0.01) (P≤0.01). The statistical analysis of Asatiani et al., (2005) result for Nkx3-1 expression and age or Gleason score showed no-significant trends toward lower expression of Nkx3-1 in tumors from younger patients (P = 0.39) and higher Gleason score (P = 0.37) [23]. Also Bethel et al., (2006) provided that Nkx3-1 expression association with Gleason score with highly significant (P≤0.0001) [18]. Yu, (2012) reported thatNkx3-1 was observed a slightly higher sensitivity in prostate cancer with grade IV [31].Padmanabhan et al., (2016) concluded in their study the post-translationally modifications of Nkx3.1 play an important role in Nkx3-1 stability [32]. Atta, (2017) was showed that the Nkx3-1 express more in low Gleason score, while low or lose of Nkx3-1 was showing in tumor progression and poorly prognosis [20].

4. Conclusion

In conclusion we think most events, such as mutation or overlapping interaction, may be effect on Nkx3-1 transcription process lead to modifying in its role causing prostate cancer initiation and progression, or modulated by androgen hormone.

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