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Immunohistochemical Detection of Androgen Receptors in Prostate Tumors

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Abstract:

This study aimed to detect the expression of androgen receptors (AR) in prostate tumors. Forty formalin fixed paraffin blocks (FFPB) previously diagnosed as prostate tumors were selected for this study (27 (67.5%) of them were prostate adenocarcinoma and 13 (32.5%) of them were benign prostatic hyperplasia). The ages of the study subjects ranged between 50 to 88 years with mean age of 66 years. FFPB were cut by rotary microtome then stained by immunohistochemical method (new indirect technique) for detection of androgen receptors. The data obtained was analyzed using SPSS computer program.

Concerning the grade of adenocarcinoma the study showed that 10 (37%) samples were well differentiated tumors, 13(48%) samples were moderate differentiated tumors and 4 (15%) samples were poorly differentiated tumors. The expressions of AR were found as 2/13 benign hyperplasia and 14/27 in adenocarcinoma lesions, with significant relation between AR expression and type of lesions. The relation between cancer grade and AR expression showed that 6/10 positive result in well differentiated tumors, 4/13 positive in moderate

differentiated tumors and 0/4 in poorly differentiated tumors with insignificant relation between AR expression and cancer stages.

Conclusion: *The study concludes AR expression differentiate between benign hyperplasia and adenocarcinoma, with no association with cancer grade.*

Key words: androgen receptor, prostate adenocrcinoma, prostatic hyperplasia.

INTRODUCTION:

Cancer is a disease of DNA, it is a silent killer that creeps upon us without warning, and there are over 10 million of people living with cancer in the world today [(1)].

Prostate cancer is one of most serious cancers in men in many countries, in United Kingdom (UK), it accounts for 33% of all newly diagnosed malignancies among men in the United States [(2)]. According to the American Cancer Society, an estimated 220,900 men will be diagnosed with prostate cancer in 2003, and 28,900 men will die of it, making it the second most common cause of cancer death in men. The incidence of prostate cancer varies worldwide, with the highest rates found in the United States, Canada, and Scandinavia, and the lowest rates found in China and other parts of Asia [(3)(4)]. In United States of America (USA) it was found to be the second diagnosed cancer in men and the second most cause of cancer related death in men older than 50 years old [(5)].

In India the people who have the prostate cancer die within ten years of having the disease [(6)]. Prostate cancer is not common in Asians. The adjusted incidence per 100.000 among Japanese is in the range of 3 – 4 hence in the Chinese is only one [(7)].

The number of men of 65 years is expected to increase 4-fold worldwide between the years 2000 and 2050, representing

an increase from 12.4% of the population in 2000 to 19.6% in 2030 [8].

Cancer diagnosis is the first step to cancer management, the most common tests for localized asymptomatic prostate cancer is digital rectal examination or more recently magnetic resonance imaging can be considered. Cytological examination for prostate secretions is not recommended because of large number of false positive results [9]. A core needle biopsy of the prostate under transrectal ultrasound guidance is the main method used to diagnose prostate cancer [10].

Histological examinations are considered as an essential tool in the diagnosis of prostate cancer, the histological grade of the tumor influences the therapy and correlate well with prognosis [11].

Immunohistochemistry has rapidly become an integral part of most diagnostic tool, which confirms histological results, this reflects its considerable ability to facilitate histological diagnosis. The identification of specific or highly selective cellular epitopes in routinely formalin fixed paraffin wax embedded tissues with an antibody and appropriate labeling system has a significant impact on histological diagnosis [12]. The normal development and maintenance of the prostate is dependent on androgen acting through the androgen receptor (AR). AR remains important in the development and progression of prostate cancer. AR expression is maintained throughout prostate cancer progression, and the majority of androgen-independent or hormone refractory prostate cancers express AR. Mutation of AR, especially mutations that result in a relaxation of AR ligand specificity, may contribute to the progression of prostate cancer and the failure of endocrine therapy by allowing AR transcriptional activation in response to anti androgens or other endogenous hormones [13].

Materials and methods:

Sample collection:

Paraffin embedded tissue blocks previously diagnosed as prostate tumors were selected from different centers for this study.

Slides preparation:

Sections of 4µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for immunohistochemistry which is then taken in thermal coated slides and dried in hot plate oven at 80ºC for one hour.

Immunohistochemical staining:

Sections were brought to water and retrieved using water bath retrieval technique at 97ºC, then treated with hydrogen peroxide solution for 15 minutes, then washed in phosphate buffer saline (pH 7.4) for 5 minutes, then treated with anti AR primary antibodies for 30 minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for 30 minutes, then rinsed in phosphate buffer saline, then treated with DAB for 7 minutes, then washed in phosphate buffer saline for 5 minutes, then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) 2 minutes for each then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media[14].

Result interpretation:

Results obtained were detected by researcher and confirmed by experienced histopathologist. Negative and positive controls were used for evaluation of the test sections.

Statistical analysis:

All information about the study population was entered a computer as well as obtained results. The data was analyzed using SPSS computer program. Frequencies, means, chi-square tests were calculated.

RESULTS:

Forty paraffin blocks previously diagnosed as prostate lesions were collected in this study, 13 (32.5%) of them with benign prostatic hyperplasia whilst 27 (67.5%) prostatic adenocarcinoma (table 1). The age of the patients ranged between 50-88 years with mean age (66) year. Most of them 21 (52.5%) between 61-70, 10 (25%) ranged between 60 and less while the age group 71 and above count about 9 (22.5%) (Table 2).

Positive and negative expression of AR is common among malignant lesions with frequencies 14/27, 13/27 respectively with significant correlation between AR expression and lesions type (P = 0.02) (Table 3).

Cancer grade and AR expression showed that 6/10 positive result in well differentiated tumors, 4/13 positive in moderate differentiated tumors and 0/4 in poorly differentiated tumors with insignificant relation between AR expression and cancer grade (P=0.303) (Table 4).

Table (1) Distribution of histopathology results among study population

Histopathology diagnosis	Frequency	Percent %
Benign	13	32.5
Malignant	27	67.5
Total	40	100%

Table (2) Distribution of age group among the study population

Age group(year)	Frequency	Percent %
60 and less	10	25
61-70	21	52.5
71 and above	9	22.5
Total	40	100%

Table (3) Relation between AR expression and histopathological diagnosis

Expression of AR	Histopathology results				Total	P.value
	Benign		Malignant			
	N	%	N	%		
Positive	2	15.4	14	51.9	16	
Negative	11	84.6	13	48.1	24	
Total	13	100	27	100	40	0.02

Table (4) Relation between AR expression and cancer grade

Cancer grade	AR expression		Total	P.value
	Positive	Negative		
	N	N		
Well differentiated tumor	6	4	10	0.303
Moderate differentiated tumor	4	9	13	
Poor differentiated tumor	0	4	4	
Total	10	17	27	

DISCUSSION:

Prostate cancer is one of the most serious problems worldwide. In this study about forty patients previously diagnosed with prostatic lesions by histopathology. The age of patients above 50 years, which explain that the risk of prostate cancer increase with age with a peak around 65 - 70 years Bardan *et al.,* [(15)]. Similar finding described by Zatzkin, [(16)] , who reported that the risk increases significantly after the age of 50 and about two-thirds of all prostate cancers are diagnosed in men age 65 and older. Also the study results were consistent with those of Bostwick *et al.,* [(17)]. They reported that the risk of developing prostate cancer increases quickly over the age of 50 in white men and over the age of 40 in black men. Prostate cancer is

almost never seen in men under 40, but it is still possible. In fact, over two-thirds of all prostate cancer patients are over the age of 65.

There is a significant relation between AR expression and histopathological diagnosis, the high expression of AR was seem in 88% of adenocarcinoma, while the expression has 12% in benign lesions. Because of AR immunoreactivity was observed in tumor cells, non-neoplastic glandular epithelial cells and AR-positive epithelial cells was significantly higher in cancer tissues than that in normal prostate tissues Qiu *et al*., [18]. Similar result described by Loda *et al.,* [19]. They reported that AR expressed in hyperplastic and normal prostatic glands with strong positive in prostate cancer. Also the study results were consistent with those of Hobisch *et al*., [20]. They reported that prostatic carcinoma cell lines, distant prostatic carcinoma metastases do express the AR. These findings indicate that the AR may be involved in the progression of prostate cancer.

In this study, the immunohistochemical results show insignificant correlation between AR expression and cancer grades. This result was consolidated by the finding of Linja *et al*., [21], they reported that prostate tumors expressed significantly more AR with uniform staining. Similar result described by Qui *et al*., [18]. They reported that histological stages were not correlated with AR expression. Also our result was consistent with those of **Ruizeveld de Winter** *et al*., [22]. they reported that almost all human prostatic cancers revealed of AR expression, regardless of tumor differentiation and progression.

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