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In Vitro Validity of Some Tumor Markers in the Identification and Differential Diagnosis of Prostate Tumors

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ABSTRACT: The objective of this study was to evaluate the validity of prostate specific antigen (PSA), carcino embryonic antigen (CEA), human epidermal growth factor 2 (Her2), B cell 2 (BcL2), protein 53 (P53) and high molecular wight cytokeratin (HMW) tumor markers in the identification and differential diagnosis of prostate tumors. Biopsy samples were randomly taken from patients with prostate tumors. Tissue sections were prepared and stained for histopathology and immunohistochemistry. Out of the 100 patients with prostatic lesions, 25% had benign prostatic hyperplasia, 42% and 33% have a well differentiated and poorly differentiated adenocarcinoma, respectively. The results revealed a significant correlation between prostate cancer and tissue expression of CEA, P53 and HMW cytokeratin tumor markers. No significant relation was found between prostate cancer and tissues expression of Her2, PSA and Bcl2 tumor markers. A significant relation between tobacco usage and prostate cancer was observed. Factors such as family history, number of wives and alcohol consumption had insignificant relation with prostate cancer. The study concluded that PSA, BcL2, and Her2 tumor markers are invalid in the differential diagnosis of prostate tumors, whereas. P53, CEA and HMW cytokeratin tumor markers can be useful in the differential diagnosis of prostate tumors.

المستخلص : هدفت هذه الدراسة لتقويم صلاحية واسمات الاورام BcL2, Her2, P53, CEA, PSA والثايتوكيراتين ذو الوزن الجزيئي العالي في التعرف والتشخيص التفريقي لاورام البروستاتا.اخذت عينات الخزع النسيجية عشوائيا من مرضى يعانون من اورام البروستاتا . تم تحضير المقاطع النسيجية و صبغها للتشخيص بواسطة امراض الانسجة و تقنية الانسجة المناعية. من مجموع 100 مريض بافات البروستاتا كان هناك 25 % ورم البروستاتا الحميد ، 42 % و33 % سرطان البروستاتا الجيد التفريق وسرطان البروستاتا الضعيف التفريق على التوالى.كشفت الدراسة وجود ارتباط ذو دلالة بين سرطان البروستاتا والافراز النسيجى لواسمات الاورام CEA, P53 والثايتوكيراتين ذات الوزن الجزيئي العالي.ولا توجد علاقة ذات دلالة بين سرطان البروستاتا والافراز النسيجى لواسمات الاورام BcL2,Her2 وPSA . لوحظ وجود علاقة ذات دلالة بين استعمال التبغ وسرطان البروستاتا. بعض العوامل مثل التاريخ العائلي للسرطان وعدد الزوجات وتعاطي الكحول ليس له علاقة ذات دلالة مع سرطان البروستاتا. خلصت الدراسة الى ان تحديد واسمات الاورام BcL2,Her2 وPSA غير فعال في التشخيص التفريقي لأورام البروستاتا بينما تحديد واسمات الاورام CEA, P53 والثايتوكيراتين ذو الوزن الجزيئي العالي كان لها فاعلية في التشخيص التفريقي لاورام البروستاتا.

KEYWORDS: *Immunohistochemistry, Prostate cancer, Tumor markers.*

INTRODUCTION

Prostate cancer is one of the most serious cancers in men in many countries. In United Kingdom, it represented nearly a quarter of all new male diagnosed cancers [(1)]. In the United States of America 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 [(2)]. However, in Sudan, the number of prostatic cancer cases increases every year. Comprising 6.5%, 6.7%, and 7.8% in 2004, 2005, 2006, respectively of all new male diagnosed cancers [(3)]. Although some of the causes of prostatic cancer remain unclear, many etiological factors have been suggested, including environmental, hormonal and genetical factors, in addition to age, race and family history [(4)]. There is also a need for predicting how well the disease will respond to treatment [(5,6)].

Prostate cancers are high fat diet, which may act on the prostate through modification of circulating sex hormones[(7)], and sexual histories which include the number of sexual partners and frequency of intercourse[(8)].

Cancer diagnosis is the first step to its management [(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 essential in the diagnosis of prostate cancer [(11)]. Grading of the tumor influences the therapy and correlate well with prognosis [(4)].

Immunohistochemistry is an important tool, which confirms histological results. The identification of specific or highly selective cellular epitopes in formalin fixed paraffin wax embedded tissues with an antibody and appropriate labeling system, using immunohistochemistry, has a significant impact on histological diagnosis. Tumor markers are substances that can be detected higher than normal amounts in the blood, urine, or body tissues in certain types of tumors. Currently, the main use of tumor markers is to assess a cancer's response to treatment and to diagnose cancer recurrence. In some types of cancer, tumor marker levels may reflect the extent or stage of the disease and can be useful in predicting how well the disease will respond to treatment [(12)].

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Sample collection and staining: One hundred biopsies were obtained from patients with prostatic lesions. The size and depth of biopsy was measured to calculate the optimal time of fixation in neutral buffer formalin up to subsequent processing. The biopsies were processed in leica tissue processing machine; two sections of 5 µm in thickness were obtained from paraffin wax embedded tissues using leica rotary microtome. Sections were stained using haematoxylin and eosin by Mayer's procedure as described by Bancroft *etal*.[(2)]. Sections for immune-histochemistry examinations were retrieved by water bath heat retrieval technique, then immunostained using avidin. PSA, CEA, P53 BcL2, Her2 and HMW cytokeratin were detected using Biotin technique [(6)]. Chi square test was used to calculate the relation between histopathological diagnosis and the expression of markers.

RESULTS

The ages of patients investigated ranged between 35 to 104 years with a mean age of 69 years.

Fourty five patients had family history of prostatic cancer. The results revealed 25% benign prostatic hyperplasia and 75% prostatic carcinoma. Out of the 75 patients with adenocarcinoma, 42 (56%) were well differentiated and 33 (44%) were poorly differentiated (Table1).

Table1:Percentages of histopathological diagnosis among study group

Histopathological diagnosis	Frequency	Percentage (%)
Benign prostatic hyperplasia	25	25
Well differentiated adenocarcinoma	42	42
Poorly differentiated adenocarcinoma	33	33
Total	100	100

P. value< 0.01

Figures (1-3) show microphotographs for some samples obtained from a number of patients.

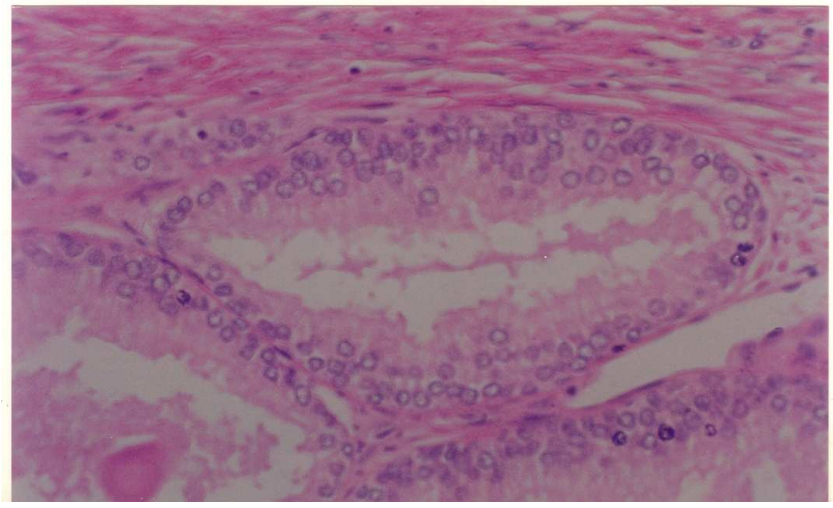

Figure 1: Haematoxylin & eosin stained section of benign prostatic hyperplasia of the prostate, obtained from 65 years old patient(x 40).

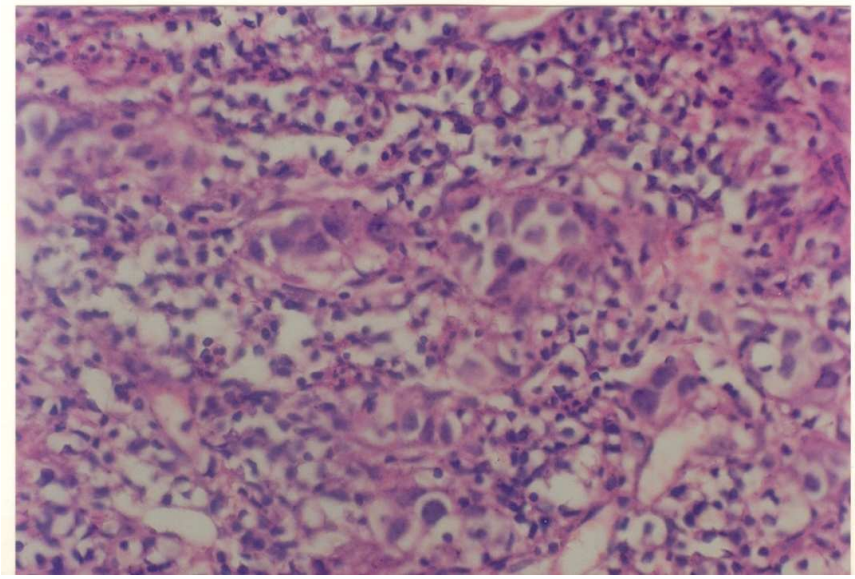

Figure 2: Haematoxylin & eosin stained section of well differentiate adenocarcinoma of the prostate, obtained from 60 years old patient (x 40).

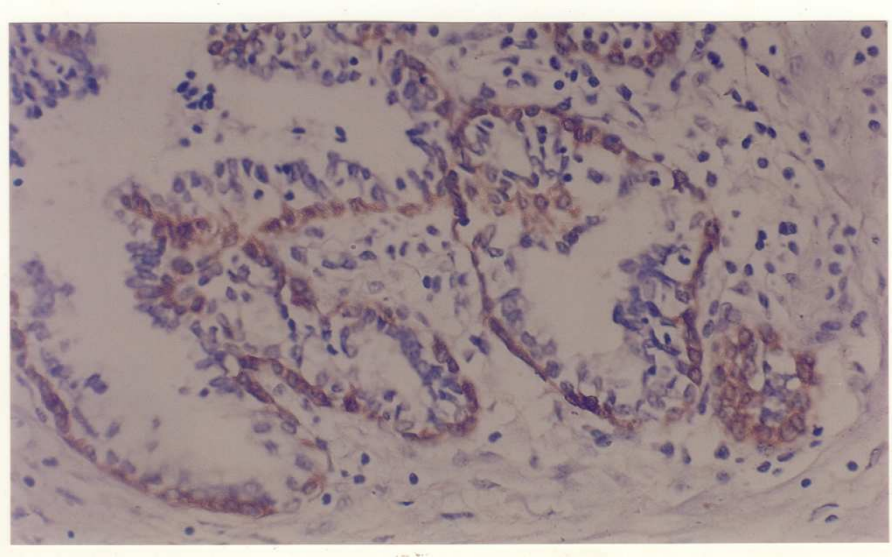

Figure 3: Formalin fixed paraffin wax embedded section of benign prostate hyperplasia of prostate immunostained for high molecular weight cytokeratin tumor marker using water bath heating and avidin biotin peroxidase diaminobenzidine labeling system, show cytoplasmic positivity (x 40).

The tissues expression of HMW, CEA and P53 and cytokeratin tumor markers were found to be correlated with prostate tumor (p value < 0.01) (Tables 2, 3 and 4).

Table 2: Relation between histopathological diagnosis and tissue HMW

Tissue HMW cytokeratin	Histopathological diagnosis			Total
	Benign prostatic hyperplasia	Well differentiated adenocarcinoma	Poor differentiated adenocarcinoma	
Positive	20	9	3	32
Negative	5	33	30	68
Total	25	42	33	100

P. value < 0.01

Table 3: Relation between histopathological diagnosis and tissue CEA cytokeratin

Tissue CEA	Histopathological diagnosis			Total
	Benign prostatic hyperplasia	Well differentiated adenocarcinoma	Poor differentiated adenocarcinoma	
Positive	16	11	01	28
Nnegative	09	31	32	72
Total	25	42	33	100

P. value < 0.01

Table 4: Relation between histopathological diagnosis and tissue P53

Tissue P53	Histopathological diagnosis		Total
	Benign prostatic hyperplasia	Prostatic adenocarcinoma	
Positive	3	46	62
Negative	22	29	38
Total	25	75	100

P. value< 0.01

No significant relation was detected between prostate tumor and tissues expression of Her2, and Bcl2, PSA tumor markers (p value > 0.01) (Tables 5, 6 and 7).

Table 5: Relation between histopathological diagnosis and tissue Her2

Tissue Her2	Histopathological diagnosis			Total
	Benign prostatic hyperplasia	Well differentiated adenocarcinoma	Poor differentiated adenocarcinoma	
Positive	0	1	1	2
Negative	25	41	32	98
Total	25	42	33	100

P. value>0.01

Table 6: Relation between histopathological diagnosis and tissue Bcl2

Tissue BCL2	Histopathological diagnosis			Total
	Benign prostatic hyperplasia	Well differentiated adenocarcinoma	Poor differentiated adenocarcinoma	
Positive	5	3	8	16
Negative	20	39	25	84
Total	25	42	33	100

P. value >0.01

Table 7: Relation between histopathological diagnosis and tissue PSA

Tissue PSA	Histopathological diagnosis			Total
	Benign prostatic hyperplasia	Well differentiated adenocarcinoma	Poor differentiated adenocarcinoma	
Positive	23	41	32	96
Negative	2	1	1	4
Total	25	42	33	100

P. value >0.01

No significant relation was detected between prostate tumor and family history, number of wives and alcohol consumption (p value > 0.01) (Tables 8 and 9).

Table 8: Relation between histopathological diagnosis and Family History

Family history	Histopathological diagnosis				Total
	Benign prostatic hyperplasia		Prostatic adenocarcinoma		
	N	%	N	Percentage %	
Present	10	40	35	47	45
Absent	15	60	40	53	55

Total	25	100	75	100	100

P. value>0.01

Table 9: Description of number of wives by histopathological diagnosis

Number of wives	Histopathological diagnosis				Total
	Benign prostatic hyperplasia		Prostatic adenocarcinoma		
	N	%	N	Percentage %	
1.00	21	84	61	81	45
≤2.00	4	16	14	19	55
Total	25	100	75	100	100

P. value >0.01

The correlation between histopathological diagnosis and tobacco usage was found statistically significant (p value < 0.01).

DISCUSSION

In the present study the expression of PSA was not associated with prostate cancer. Similar results were reported by Osegbe, *et al* [13], who found the expression of PSA was not associated with prostate cancer.

These findings are in contrast with that of Lora *et al.*[14], who found that the high expression and distribution of Her2 was retained in advanced stage of prostate cancer. The present study disagreed with the results of Zellweger *et al.* [15], who reported that Her2 was over expressed in hormone-refractory and early stage of prostate cancer.

In this study the results showed that the over expression of P53 is associated with high grade of prostate cancer (table 4). Such findings were previously reported by Petrescu *et al.* [16], who found an association between P53 oncogene and late stage of prostate cancer. Similar results were also reported by Salah *et al.* [17], who found that P53 was expressed in high grade prostatic adenocarcinoma. However, the findings disagree with Yamon *et al.* [18], who found that there was no relation between P53 oncogene and prostate cancer.

The study also revealed that the expression of Her2 was not associated with prostate cancer progression or with advanced stage prostate cancer.

Bcl2 had a low expression in the prostate tumors in this study and hence it was of little value in differential diagnosis of prostate lesions (table 6). This seems to agree with Gilvan *et al.* [9], who reported that Bcl2 was rarely expressed in adenocarcinoma of prostate, but disagrees with Parbhiot *et al.* [19], who found over expression of Bcl2 associated with primary and metastatic prostate cancer. It regulates cell proliferation and cell death.

The results in this study (Table 3) showed that the expression of CEA differentiate between benign and malignant a prostatic lesion, which agrees with Stephen *et al.* [20], who reported that CEA was expressed by many different tumor types, including poorly differentiated adenocarcinoma of prostate.

In the present study the over expression of HMW cytokeratin (Table 2) was associated with benign prostatic hyperplasia but not associated with prostatic adenocarcinoma. These findings are supported by the study of Thomas and Clayton. *et al* [21], which revealed that HMW cytokeratin was expressed in the basal cells in all cases of benign prostate hyperplasia, low-grade prostatic intra epithelial neoplasia (PIN) and high-grade PIN whereas, HMW cytokeratin was not seen in carcinoma foci. These findings disagreed with Varma *et al.* [22], who reported that HMW cytokeratin particularly when used with microwave heat retrieval was very sensitive positive marker for high grade invasive urothelial carcinoma from prostate cancer.

The study showed that smoking may increase the risk of prostate cancer. This finding is supported by the study of Lora *et al.*[14] , who demonstrated a modest positive association between cigarette smoking and risk of prostate cancer. In particular, current smokers, smokers of > 40 years duration, and those with > 40 pack-years of exposure have a 40–60% elevation in risk of prostate cancer relative to nonsmokers. Contrary to Doll *et al.* [7], who reported that prostate cancer has a relatively weak relationship with smoking. The present study showed no significant relation between prostate cancer and family history of patients.

This finding disagrees with Yen-Ching *et al.* [23], who reported that a family history of prostate cancer in a brother or father was associated with a 2.3-fold increased risk for developing prostate cancer. Similar results were reported by Kupelian *et al.* [12], who found that the presence of a family history of prostate cancer correlates with treatment outcome in a large unselected series of patients.

Further the study revealed no relation between alcohol consumption and prostate cancer. Contrary to that of Middleton *et al.* [24], who reported that prostate cancer incidence is positively linearly associated with heavier alcohol consumption, and Howard *et al.* [11], who found a positive association between moderate alcohol consumption and the risk of prostate cancer. Liquor, but not wine or beer, consumption was positively associated with prostate cancer.

Similarly, the results showed no significant association between number of wives and risk of prostate cancer. Dennis and Dawson,[8] observed no relation between prostate cancer and multiple marriages, age of first marriage or of first intercourse. However these authors found an increasing risk of prostate cancer in individuals sexually active in their 20s and 30s and also in those with increasing number of sexual partners.

On contrary, Giles *et al.* [10], observed no association between prostate cancer and number of sexual partners.

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