

The Role of Cytokeratin 5/6 in Differential Diagnosis of Prostate Tumors

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Abstract:

This is a hospital based analytical case control study aimed to evaluate the role of cytokeratin 5/6 (CK 5/6) in differential diagnosis of prostate tumors using immunohistochemical methods.

Sixty paraffin blocks were selected from patients samples affected with prostate tumor. Sections were cut then stained using immunohistochemical method (new indirect method) to detect cytokeratin5/6 expression in prostate tumors.

The age of study population ranged between 50 to 83 years with a mean age of 67 years. The study revealed that majority of patients were older than 60 years representing 48 (80%) and the remaining 12 (20%) were younger than 60 ± 7.75 years. The study includes 30 (50%) benign prostatic hyperplasia and 30 (50%) malignant prostate. Histopathological grading of cancer showed well differentiated tumor in 2 (3.3%) samples, moderately differentiated tumor in 10 (16.7%) samples and poorly differentiated tumor in 18 (30%) samples. CK5/6 was observed in 28 (46.7%) samples while 32 (53.3%) samples showed no expression of CK5/6. Histopathological diagnosis and CK5/6 expression showed that positive in 26 (43.3%) samples and negative in 4 (6.7%) samples among benign prostatic hyperplasia while 2 (3.3%) positive samples and 28 (46.6%) negative

samples among adenocarcinoma of prostate with significant statistical association ($P < 0.05$).

Conclusion: *The present study concludes that the expression of CK5/6 is associated with benign prostatic tumors while malignant forms of prostate tumors usually showed negative expression of CK 5/6.*

Key words: Prostate cancer, Prostate hyperplasia, CK5/6

INTRODUCTION:

Prostate cancer is the most common non-cutaneous cancer among men in worldwide, in 2005 estimated 232,090 new cases of prostate cancer in US and 30,350 deaths from prostate cancer. Prostate cancer remains the second leading cause of death in 2005 ⁽¹⁾. The incidence of prostate cancer has increased dramatically in the past 20 year, this likely due to combination of factors such as aging, family history and dietary factors ⁽²⁾. Ultrasound (US) and magnetic resonance imaging (MRI) are the two main imaging methods used for prostate cancer detection. Urologists use transrectal ultrasound during prostate biopsy and can sometimes see a hypoechoic area (tissues or structures that reflect relatively less of the ultrasound waves directed at them). But US have poor tissue resolution and thus, are generally not clinically used. Prostate MRI has better soft tissue resolution than ultrasound ⁽³⁾. Risk factors of prostate cancer are ages, races, ethnicity, alcohol consumption, genetic factors, farmers, a diet high in fat, also tire plant workers, and men who been around cadmium ⁽⁴⁾. Treatment of prostate gland depends on grading of cancer and overall health. For early-stage prostate cancer, this may include surgery (radical prostatectomy), radiation therapy, brachy therapy and proton therapy. An older age recommend simply monitoring the cancer with PSA test and biopsies. After prostate cancer spread,

treatment includes hormone therapy (medicines to reduce testosterone levels), surgery and chemotherapy ⁽⁵⁾.

Immunohistochemistry refers to the process of detecting antigens, e.g. proteins in cells of tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. Immunohistochemistry can play an important role in diagnostic surgical pathology of the prostate. Basal cell markers, such as the 34βE12 antibody and directed against cytokeratin5 and 6 or p63 are very useful for demonstration of basal cells as their presence argues against a diagnosis of invasive prostatic carcinoma. Several benign mimickers of prostatic carcinoma, including atrophy, atypical adenomatous hyperplasia, nephrogenic adenoma, and mesonephric hyperplasia, can stain negatively with these markers ⁽⁶⁾. CK 5/6 has an important criterion for diagnosis of prostatic carcinoma is the absence of basal cells in malignant glands. Ck5/6 is sensitive and specific marker for basal cell in prostatic acini ⁽⁷⁾.

MATERIALS AND METHODS:

Sample collection:

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

Slides preparation:

One section of 4μm thickness from each block was obtained by rotary microtome for immunohistochemistry which is then taken in thermal coated slides and dried in hot oven at 80°C for one hour.

Immunohistochemical staining:

Sections were brought to water and retrieved by water bath at 97°C, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffer saline (pH 7.4) for

five minutes, then treated with anti CK5/6 primary antibody for thirty minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for thirty minutes, then rinsed in phosphate buffer saline, then treated with DAB for seven minutes, then washed in phosphate buffer saline for five minutes, then counterstained in Mayer's haematoxylin for one minute, then washed in water and blued in 0.05% ammoniated water for sixteen second, then washed in water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in DPX mounting media ⁽⁸⁾.

RESULTS:

Out of sixty patients with prostate tumors the age ranged between 50-89 years with mean 67, 12 (20%) of the patient were less than 60 years, and 48 (80%) were older than 60 years (Table 1).

Among study subjects histopathological grading of cancer showed that well differentiated tumor in 2 (3.3%) samples, moderately differentiated tumor in 10 (16.7%) samples, and poorly differentiated tumor in 18 (30%) samples, and benign prostatic hyperplasia in 30 (50%) samples (Table 2). The relationship between the expression of CK5/6 among study population, 28 (46.7%) of samples were positively expressed CK5/6, and 32 (53.3%) were negatively for CK5/6 (Table 3).

Histopathological diagnosis and CK5/6 expression showed that positive in 26 (86.6%) samples, and negative in 4 (13.3%) samples among benign prostatic hyperplasia. 2 (6.6%) positive samples and 28 (93.3%) negative samples were adenocarcinoma of prostate with significant statistical association ($P < 0.05$) (Table 4).

Table (1): shows the distribution of age group among study population:

Age group	Frequency	Percent (%)
Younger than 60 years	12	20
Older than 60 years	48	80
Total	60	100%

Table (2): shows the histopathological grading of prostate cancer among the study population:

Grade of cancer	Frequency	Percent (%)
Well differentiated tumor	2	3.3
Moderately differentiated tumor	10	16.7
Poorly differentiated tumor	18	30
Benign prostatic hyperplasia	30	50
Total	60	100%

Table (3): shows the CK5/6 expression in prostate tumors among the study group:

CK5/6 result	Frequency	Percent
Positive	28	46.7
Negative	32	53.3
Total	60	100%

Table (4): shows the relation between histopathological diagnosis and CK5/6 expression:

Diagnosis	CK5/6		Total
	Positive	Negative	
Benign	26	4	30
Malignant	2	28	30
Total	28	32	60

P. value= 0.00

DISCUSSION:

Prostate cancer is one of the most common cancers affecting older men in developed countries, especially African-American men. It most commonly occurs in men over 65 years of age and is rare in men under 50 years of age⁽⁹⁾.

Immunohistochemistry can play an important role in diagnostic surgical pathology of the prostate. Basal cell markers, such as cytokeratin 5 and 6 are useful for

demonstration of basal cells in benign prostate tumor and absent in adenocarcinoma. CK5/6 is an antibody combination of HMWCKs 5 and 6 the overall sensitivity, specificity, and diagnostic utility of CK5/6 in prostate needle biopsies ⁽¹⁰⁾.

In this study sixty patients samples affected with prostate tumor were investigated by immunohistochemistry methods. Their ages ranged between 50 to 89 years with a mean age of 67 ± 7.75 years. Majority of patients were at age ranges older than 60 years representing 48 (80%) and the remaining 12 (20%) were younger than 60 years.

This study found that prostate cancer was common among older men whose age after sixty which agree with study by Arthur ⁽¹¹⁾ who reported that from 1998 to 2002 by median age at diagnosis was 68 years.

The present study showed that most tumor grade was poorly differentiated representing 30%, this finding is compatible with the result of Masahiro *et al.*, ⁽¹²⁾ who reported that among prostate cancers with multiple grades, the most common finding is poorly differentiated representing 53%.

In this study 26 (86.6%) benign samples were positive for CK5/6 stain cytoplasm brown color and 28 (93.3%) malignant samples were negative for CK5/6 ($P= 0.00$), this indicates that there was relation between CK5/6 and behavior of prostate tumor, this result is supported by Trpkov, et al. (2009) ⁽¹³⁾ who were evaluated the usefulness of cytokeratin 5/6 for diagnostic assessment of problematic prostate specimens, found all cancers lacked CK5/6 staining (100% specificity). Also the study agree with study by Abrahams *et al.*, ⁽¹⁰⁾ who found that 29/30 cases (97%) showed staining in >95% of benign glands with CK5/6.

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