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Detection of Human Papilloma Virus (HPV) Type 16 and 18 using Immunohistochemistry among Sudanese Patients with Prostate Cancer

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Abstract:

This study aimed to detect the expression of HPV16 E6/18 E6 in Prostate tumors using immunohistochemical method. Fifty formalin fixed paraffin blocks (FFPB) previously diagnosed as Prostate tumors (18 of them were malignant Prostate tumors and 32 were benign Prostate tumors) were used in this study. Blocks were cut and stained by immunohistochemical method for detection of HPV16 E6/18 E6. The age of patients ranged between 45 and 90 years with mean age 67.5 years. immunohistochemical analysis showed that HPV16 E6/18 E6 expression was positive in 7(14%) samples and negative in 43(86%) samples. (6) Positive samples were malignant and (1) were benign

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tumor and there was statistical association between HPV16 E6/18 E6 expression and histological diagnosis of the tumors (P=0.003). The relation between histological differentiation and HPV16 E6/18 E6 expression revealed that 1/33sample was well-differentiated tumor, 4/12 samples were moderate differentiated tumor and 2/5 sample was poor differentiated tumor with significant statistical association (P=0.007, 0.009, 0.003 respectively for the differentiation). The study concluded that the HPV16 E6/18 E6 expression in Prostate tumors was correlated with histological diagnosis and tumor differentiation.

Key words: Human Papilloma virus (HPV), prostate tumor, Adenocarcinoma, Benign prostatic hyperplasia (BPH), Early protein 6 (E6), Immunohistochemistry (IHC), Phosphate buffer saline (PBS).

INTRODUCTION:

Prostate cancer is a leading cause of illness and death among men in the United States and Western Europe [(1)] also it is the fourth most common male malignant neoplasm worldwide, 18% of American men were affected with causing death in 3% in 2005. [(2)] In Japan death from prostatic cancer was one- fifth to one-half the rate in United States and Europe in the 1990s. [(3)]

As of 2012, prostate cancer is the second most frequently diagnosed cancer (at 15% of all male cancers)[(4)] and the sixth leading cause of cancer death in males worldwide. [(5)] In 2010 it resulted in 256,000 deaths up from 156,000 deaths in 1990. [(6)] Rates of prostate cancer vary widely across the world. Although the rates vary widely between countries, it is least common in South and East Asia, and more common in Europe, North America, Australia and New Zealand. [(7)] Prostate cancer is least common among Asian men and most common among black men, with figures for white men in between. [(8)(9)]

Prostate cancer ranked fourth among all cancer sites in Khartoum. However; by gender it ranked first among Sudanese

men. It had the highest Age-Specific Rate (ASR) in seniors aged 65 years and older. [10]

Prostate cancer gained increased attention from Sudanese urologists owing to its rapidly increasing incidence as recent reports have indicated. [11] Prostatic cancers vary widely across the world, with the south and west Asia detecting less frequently than in Europe, and especially in United States. Prostatic cancer tend to develop in men over the age of fifty, and it's the second leading cause of cancer related death in men in the United States. [12] Prostatic cancer never has symptoms, undergo no therapy, and eventually die of other unrelated causes. Many factors including genetic diets, have been implicated in the development of prostatic cancer. The presence of the prostatic cancer may be indicated by symptoms, physical examination, prostatic specific antigen (PSA) and biopsy. Prostatic-specific antigen increases the cancer detection but does not decrease mortality. [13] The American cancer society believes that men should not be tested without learning about we know and don't know about the risks and possible benefit of testing and treatment Starting at age 50, if African American or brother or father suffered from condition before age of 65 he would know pros and cons of testing so you can decide if testing is the choice for you. [14] The only test that can fully confirm the diagnosis of prostatic cancer is biopsy, the removal of small pieces of the prostate for microscopic examination.

The human papillomavirus (HPV) is a DNA virus from the papilloma virus family that is capable of infecting humans. Like all papillomaviruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. While the majority of the known types of HPV cause no symptoms in most people, some types can cause warts (verrucae), while others can – in a minority of cases – lead to cancers of the cervix, vulva, vagina, penis, oro- pharynx and anus. Recently, HPV has also been linked to an increased risk of cardiovascular

disease. In addition, HPV 16 and 18 infections, apart from being responsible for cervical cancer, are strongly associated with an increased risk of oropharyngeal (throat) cancer [(15)].

The Human Papillomavirus (HPV) E6 protein is one of three oncoproteins encoded by the virus. It has long been recognized as a potent oncogene and is intimately associated with the events that result in the malignant conversion of virally infected cells [(16)].

MATERIAL AND METHODS

Samples collection:

Paraffin embedded tissue blocks previously diagnosed as Prostate tumors were collected for this study from different hospitals in Khartoum state.

Slide preparation and staining:

Sections (3µm) from formalin-fixed, paraffin-embedded blocks was cut and mounted onto salinized slides (Fisher brand). Following deparaffinization in xylene, slides were rehydrated through a graded series of ethanol (100%, 95%, 70% and 50%) and were washed in deionized H_2O. Samples were steamed for antigen retrieval using water bath. Briefly, slides were placed in slide container and covered with 10mMsodium citrate buffer (pH 6.0) to cover the sections, then were heated at 95-97c for 15 minutes then were allow sections to cool at RT 20 minutes, then washed in deionized water three times. Endogenous peroxidase activity was blocked with peroxidase block (provided by Santa Cruz Biotechnology INC) for 5 minutes, then washed by Phosphate buffer saline (PBS), then slides were incubate for 20 minutes with serum block (provided by Santa Cruz Biotechnology INC), After that slides were incubated with primary anti HPV16 E6/18 E6 to each slide overnight at room temperature in a moisture chamber, and then were rinsed in

phosphate buffer saline (PBS), then slides were incubate for 30 minutes with biotinylated secondary Antibody (Ab), then with AvidinHRP complex also for 30 minutes (provided by Santa Cruz Biotechnology INC.) Finally, the sections were washed in three changes of PBS, followed by adding 3,3- diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 10 min. then counterstained in Gill's haematoxylin for 30seconds, then washed in deionized water and blued in running tap water for 10 minutes, then dehydrated through ascending of ethanol (50%, 70%, 95% and 100%) rinse 10 second for each, then cleared in 3 change of xylene 2 minutes for each, and mounted in DPX mounting media.

Positive HPV16 E6/18 E6staining was identified in form of brown cytoplasmic staining.

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.

Data analysis:

The data were analyzed using version 20.0 SPSS computer program, frequencies, means and chi-square values were calculated.

RESULTS:

The study includes fifty samples; 18 (36%) samples were malignant tumor (Adenocarcinoma) and 32(64%) samples were benign tumor (benign hyperplasia) table (1). The age of study subjects from (45-60) years were 17 (34%) patients, and from (61-90) years were 33(66%) patients table (2), figure (2).

Out of fifty samples graded, 33(66.0%) samples were well-differentiated tumors, 12 (24%) samples were moderately differentiated tumors and 5(10.0%) samples were poorly differentiated tumors, table (3) and figure (3).

Table (4), from thirty-three well-differentiated samples (1) was adenocarcinoma and (32) was benign hyperplasia, also from twelve moderately differentiated adenocarcinoma was (12) and for poorly differentiated (5) was adenocarcinoma with significant statistical association (P=0.000).

Table (5), figure (5) the positive expression of HPV16 E6/18E6 marker among the population was 7 (14%) and the negative expression was 43 (86%). From eighteen adenocarcinoma samples (6) was been **HPV16 E6/18E6** positive while (12) was **HPV16 E6/18E6** negative with significant statistical association (P=0.003)

Table (6), from eighteen Adenocarcinoma samples (6) Positive expression, (12) negative expression; on the other hand from the thirty- tow (32) benign hyperplasia samples (1) was been Positive expression and (31) was negative with significant statistical association (P=0.003).

Table (7), the **HPV16 E6/18E6** was been positive in (1) well differentiated samples and (32) was negative, also it was been positive in (4) moderately differentiated samples and negative in (8), finally it was been positive in (2) poorly differentiated and (3) was negative with significant statistical association (P=0.007, 0.009 and 0.003) respectively.

Table (1): Distribution of sample among study population:

	Frequency	**Percent**
Adenocarcinoma	**18**	**36%**
Benign hyperplasia	**32**	**64%**
Total	**50**	**100%**

Table (2): distribution of age among study population:

Age	Frequency	Percent
45-45-60	17	34.0%
61-90	33	66.0%
Total	50	100.0%

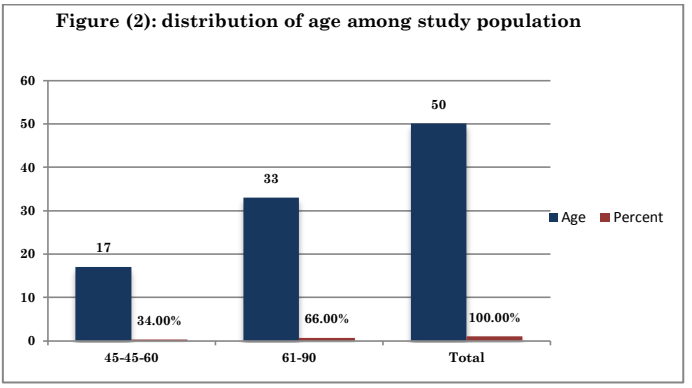


Table (3): Tumors grades of samples:

Tumors grades	Frequency	Percent
Well differentiated	33	66.0%
Moderate differentiated	12	24.0%
Poorly differentiated	5	10.0%
Total	50	100.0%

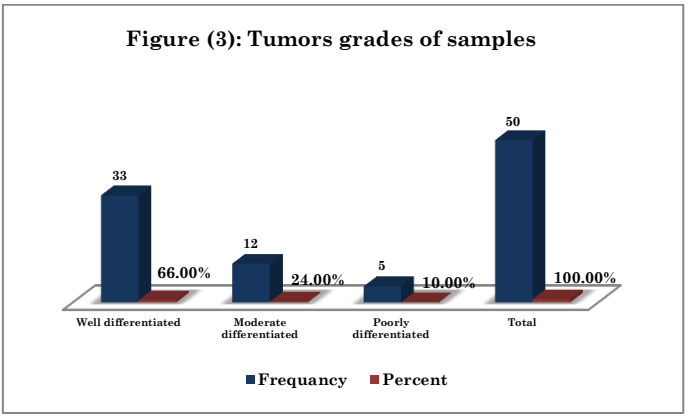


Table (4): relationship between tumors gradesand diagnosis:

Grade	Diagnosis		Total
	Adenocarcinoma	Benign hyperplasia	
Well differentiated	1	32	33
Moderate differentiated	12	0	12
Poorly differentiated	5	0	5
Total	18	32	50
P= .000			

Table (5): distribution of HPV16 E6/18E6 marker among study population:

HPV16 E6/18 E6	Frequency	Percent
Positive	7	14.0%
Negative	43	86.0%
Total	50	100.0%

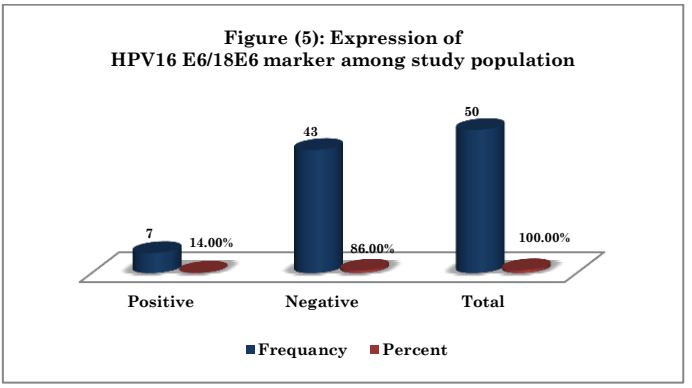
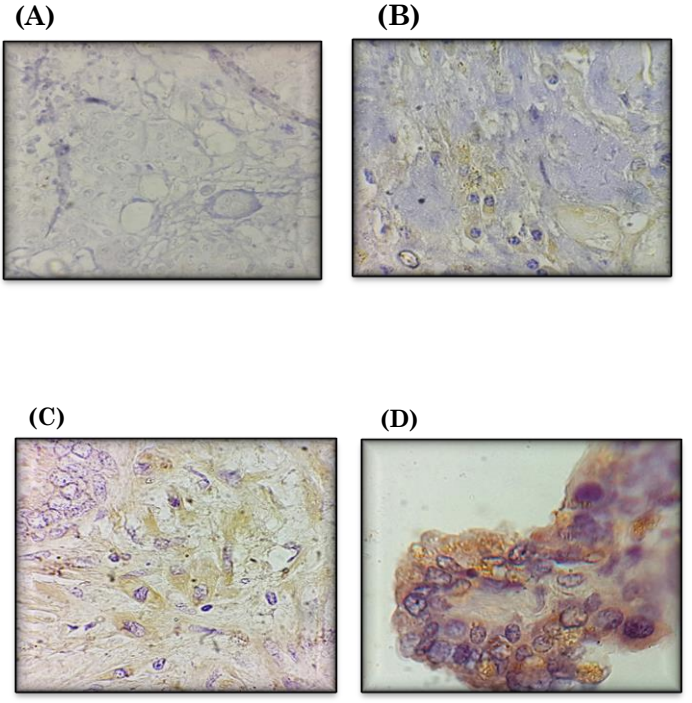


Table (6): relationship between histological diagnosis and HPV16 E6/18 E6 expression:

Histological diagnosis	HPV16 E6/18 E6		Total
	Positive	Negative	
Adenocarcinoma	6	12	18
Benign hyperplasia	1	31	32
Total	7	43	50
P= 0.003			

Table (7): Relationship between tumors grades and HPV16 E6/18E6 expression:

Grade	HPV16 E6/18E6		Total
	Positive	Negative	
Well differentiated	1	32	33
Moderate differentiated	4	8	12
Poorly differentiated	2	3	5
Total P = 0.007 = 0.009 = 0.003	7	43	50



(A): Negative control, (B): Positive control, (C): positive cytoplasmic HPV16 E6/18 E6 on prostatic adenocarcinoma, (D): positive cytoplasmic expression of HPV16 E6/18 E6 on group of prostatic adenocarcinoma cells.

DISCUSSION:

Prostate cancer is a leading cause of illness and death among men in the United States and Western Europe [(1)]. The present study includes 50 samples previously diagnosed as Prostate cancer, 18(36%) of them were malignant adenocarcinoma of prostate and 32(64%) were benign prostatic hyperplasia (BPH) tumor. In current work the age group showed from45-60 years were 17(34%) patients while from61-90 years were 33(66%) patients.

In this study according to differentiation of malignant tumor (Adenocarcinoma), moderately differentiation is predominant.

In our work we found that the expression of **HPV16 E6/18 E6** with Adenocarcinoma of the prostate is more than benign prostatic hyperplasia (BPH).

In this study we found that there is statistical significant association between **HPV16 E6/18 E6** expression and histological diagnosis and tumor differentiation, and this in accordance with several studies have investigated the relationship between HPV and Prostate cancer; MOKHTARI et al., [(17)] which they reported the correlation between prostate cancer and HPV was explored in Iran, they observed that HPV was positive in 10% of patients with prostate adenocarcinoma. In the BPH group, however, only 1.1% was found to be positive. They concluded that HPV was a risk factor for the occurrence of prostate adenocarcinoma [(17)].

CONCLUSION:

The study concluded that the **HPV16 E6/18 E6** expression in prostate carcinoma was correlated with histological diagnosis and tumor differentiation.

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