Molecular Detection of *Human Papilloma Virus* Type 16 and 18 among Sudanese Patients with Prostate Mass

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Abstract:

Background and Objective: Despite the rising number of research recently conducted to assess the association between *Human Papillomavirus* **(***HPV)* infections and prostate cancer, the results remain disputed; therefore, we try to investigate the possible role of HPV 16 and 18 as a risk factor for prostate cancer and benign prostatic hyperplasia in Sudanese patients. **Method:** This is a cross-sectional retrospective study conducted on formalin fixed paraffin wax embedded (FFPE) tissues, retrieved from the archives of histopathology Laboratory at Omdurman teaching hospital (Sudan). 200 samples of FFPE prostate tissue blocks from 2020 to 2022 were selected for this study. A 100 were prostate adenocarcinoma and 100 were benign prostatic hyperplasia. All samples were of Sudanese ethnic, sections of 10 µm prepared by sterile microtome blade, and then deparaffinized and the DNA extracted chemically and stored at - 20 °C. HPV 16 and 18 was investigated on all samples using PCR. The data analyzed by Statistical Package for the Social Sciences (SPSS) software version 19. **Results:** The results showed that *HPV*-16 was positive in 6(6%) of the adenocarcinoma and 4(4%) of the hyperplasia. The difference of *HPV*-16 infection between prostate adenocarcinoma and benign prostatic hyperplasia was statistically in significant (*P. Value* 0.374). *HPV*-18 was positive in 2(2%) of the adenocarcinoma and 4(4%) of the hyperplasia also with no statistical significant difference *(P. Value* 0.341), also there was in significant statistical correlation between HPV type 16 and 18 with prostate cancer grade as the p. value was (0.60 and 0.925) respectively**. Conclusion:** These findings excluded the role of *HPV* 16 and 18 in prostate cancer in Sudanese.

Keywords: Molecular detection; HPV 16 and 18 ; prostate masses ; Sudanese men

1. Introduction:

Prostate Cancer (PCa) is the commonest male cancer worldwide and represents a significant healthcare challenge. Many epidemiological studies on prostate cancer reported a positive

association between risk of prostate cancer and history of sexually transmitted diseases. [1, 2] However, sexual history is not related directly to exposure to specific microorganism and this association may vary between populations. An involvement of specific sexually transmitted diseases in prostate cancer has been sought for decades. Positive associations are reported with gonorrhea, syphilis, and *Human Papillomavirus* (*HPV*) infections.[1, 3-6] Studies suggested a possible links between HPV infections and prostate cancer risk factor, because of their link to cervical carcinoma. In addition to female's specific cancers, *HPV* associated with the risk of male anogenital and urinary cancers, such as penis, bladder and anus, [7] thus it hypothesized that the prostate gland may be infected by *HPV* for its anatomic proximity.

Human papillomavirus (*HPV*) are a group of genetically related viruses that infect epithelium leading to proliferative changes result in both benign and malignant tumors. [8] *HPV* are small non enveloped viruses with 55-nm-diameter icosahedral capsids contain double-stranded DNA of approximately 8,000 bp. [9] More than 200 different HPVs now have been characterized, and new types are regularly added to this list.[9] It reported that 15% of all cancers are due to viral infection. [10] Evidence for the etiological role of *HPV* in urological malignancy is squamous cell carcinoma of the penis [11]. Approximately 40 types associated with anogenital tract lesions. Infection with high-risk (oncogenic) types of *HPV* (HPV type 16, -18, -31, -33, -35, -39, -45, -52, -56, -58, and -68) is a well established risk factor for cervical and other anogenital malignancies while low-risk HPVs associated only with benign genital warts[12].

There are three regions in *HPV* genome. The first region is long regulatory region contains the promoter and the DNA replication regulator sequences. The second region involved in virus replication and carcinogenesis it contains E5, E6 and E7 that have been identified as oncoproteins. The third region encoding the L1 and L2 structural proteins for virus encapsulation[13], the proliferation of infected cells regulated by the action of *HPV E5* with a transmembrane lipoprotein of the endoplasmic reticulum [14]. *HPV E6* destroys the p53 tumor suppressor, whereas *HPV E7* destroys the *RB* tumor suppressor [15]. Oncoproteins *E6* and *E7* in *HPV* type 16 and 18 are the main cause of prostate epithelial cell accumulation [16]. Although several studies suggested that there is no association between HPV and prostate cancer because high-risk HPVs have a similar prevalence in benign and malignant prostate tissues, various studies reported differences in *HPV* expression. Conversely, Serth *et al*. [17] reported a significantly high number of *HPV*-16-E6 sequence copies in prostate cancer compared with prostate adenomas suggesting that *HPV*- 16 play an important role in

tumorigenesis. Thus considering *HPV* as an etiological factor for PCa still remains controversial not substantiated a firm link yet. In Sudan Ahmed Abdulmajed Merghani *et al* [18] in their inspiring paper reported significant association between *HPV* 16 and *HPV* 18 and Prostate cancer by immunohistochemical methods on 50 formalin fixed paraffin embedded biopsies, 18 of them were PCa tumors and 32 were benign prostatic hyperplasia (BPH). Therefore we try to investigate the possible role of *HPV* 16 and 18 as risk factor for prostate cancer by using polymerase chain reaction (PCR).

2. Methodology:

2.1. Study design and population:

This is a cross-sectional retrospective study on formalin fixed paraffin wax embedded (FFPE) tissues retrieved from the archives of Laboratory of Omdurman teaching hospital (Sudan). A well saved 200 samples of FFPE prostate tissue blocks in the period from 2020 to 2022 were selected randomly for this study. Based on their accompanied medical records 100 are prostate adenocarcinoma and 100 are benign prostatic hyperplasia. All samples were of Sudanese ethnic origin. None of them had received radiotherapy or chemotherapy.

Five serial sections of 10 µm thick were taken from each biopsy using a new blade between each sample to avoid cross-contamination. The three internal sections placed in an Eppendorf tube for DNA extraction while the two outer sections were stained with Hematoxylin (Harris) and Eosin and reexamined by a consultant pathologist to confirm diagnosis. The ethical permission was granted from Omdurman teaching hospital.

2.2.DNA extraction:

On Day 1 of the DNA extraction, tissues were incubated in xylene at 45°C for 15 minutes allowing the wax to dissolve. Samples were then centrifuged for 10 min at 14,000 rpm. The resultant supernatant is pipetted off, leaving the wax in the bottom of the tube. The latter steps were repeated once more. Then 1 ml 100% ethanol was added to the tissue pellet, vortexed and then centrifuged for 10 min at 14,000 rpm, and the supernatant pipetted off. 1 ml 90% ethanol was added to the tissue pellet, vortexed, centrifuged for 10 min at 14,000 rpm, and the supernatant pipette off. The same step was repeated, with 70% ethanol this time. The resultant pellet was then dried in a speed vac. Pellet is resuspended in 400 µl of DNA extraction buffer. 40 µl of Proteinase K (10 mg/ml) was added, vortexed briefly, and incubated at 55°C overnight to get rid of the protein. On Day 2 of DNA extraction experiments, 440 µl of phenol was added and was shaken vigorously by hand for 5 min, and centrifuged for 5 min at 8000 rpm. Supernatant was transferred into a new tube, a solution of

220µl phenol plus 220µl chloroform/isoamyl alcohol (24:1) was then added, shaken vigorously by hand for 5 min, and centrifuged for 5 min at 8000 rpm. The aim was to get rid of the Phenol. Again, the supernatant was transferred into a new tube, 440µl chloroform/isoamyl alcohol (24:1) was added, shaken vigorously by hand for 5 min, and centrifuged for 5 min at 8000 rpm, to get rid of chloroform/isoamyl alcohol. Then, the resultant supernatant was transferred into a new tube (2 ml Eppendorf tube), and 1/10 volume of sodium acetate (pH 5.2) was added. In addition, 3 volumes of ice-cold 100 % ethanol were added, and the tube was kept overnight at –20°C. By day 3 of the DNA extraction experiments, the tubes were centrifuged for 30 min at 4°C and the supernatant was removed. The pellet was dried in a speed vac. 20-50 µl sterile water was then added. Tubes were then shaken gently in a thermo mixer at 37°C for 2 hours.

Then Nanodrop (ND-1000) spectrophotometer was used to quantify the DNA concentration as follows the device was adjusted to 40ng /ul and was firstly blanked using 1ul of the same solvent in which DNA was dissolved. After good vortexing and mixing up and down using a pipette, 1ul of DNA was taken. The Nanodrop stages were cleaned well by wiping them with a clean dry tissue between each reading. For each sample, three readings were taken by increasing the taken amount from DNA by 0.5 ul each time than the average for the three readings was calculated.

2.3.Detection of *HPV*16 and *HPV*18 by using PCR :

The PCR was performed in 25 µl reaction mixtures. Thermal cycling of the amplification mixture was performed in the gradient PCR for a total of 35-40 cycles. The cycle represents denaturing for 1 minute at 94˚C, annealing for 1 min at 49˚C for *HPV*16 and 44˚C for *HPV*18, and a primer extension for 1 min at 72˚C. The name and sequences of primers as well as the length of PCR products are shown in Table 2.1. The thermal cycler was programmed for 40 cycles with an initial denaturation at 94°C for 4 min. At the end of the last cycle, the mixtures were incubated at 72°C for 10 min. The amplified products assessed by electrophoresis on a 1% (w/v) agarose gel in 1× Tris-borate-EDTA buffer at a pH of 8.6. The gel was stained with ethidium bromide of 10 mg/ml 2 µL in 100 mL of 1× Tris-borate-EDTA, and the amplified bands were visualized on an ultraviolet transilluminator.

Table 2.1. Name and Sequences of Primers Used in PCR Tests, the Target Gene, and the Length of PCR Products in Base Pairs (Bp). [19]

Primer Name	Primer Sequence	Target	Length of PCR Product in Base Pairs (bp)
F - 16	*TTATGAGCAATTAAATGACAGCTCAG*	*HPV* - 16 (E7 gene)	215 bp
R - 16	*TGAGAACAGATGGGGCACACAAT*		
F - 18	*GACCTTCTATGTCACGAGCAATTA*	*HPV* - 18 (E7 gene)	236 bp
R - 18	*TGCACACCACGGACACACAAAG*		

2.4. Statistical Analysis:

The data were revised then entered into the Statistical Package for the Social Sciences (SPSS) software version 19 and analyzed by Chi -square. P values less than 0.05 were considered statistically significant.

3. Results:

Table 3.1: HPV-16 detection by PCR in prostatic adenocarcinoma and benign prostatic hyperplasia groups (N= 200)

HPV-16	Adenocarcinoma	Hyperplasia	Total
Positive	6 (6%)	4 (4%)	10 (5%)
Negative	94 (94%)	96 (96%)	190 (95%)
Total			200

P. Value **0.374**

Table 3.2: HPV-18 detection by PCR in prostatic adenocarcinoma and benign prostatic hyperplasia groups (N= 200)

HPV-18	Adenocarcinoma	Hyperplasia	Total
Positive	2 (2%)	4 (4%)	6 (3%)
Negative	98 (98%)	96 (96%)	194 (97%)
Total			200

P. Value **0.341**

Table 3.3: correlation between HPV-16 and adenocarcinoma grade (N=100)

Grade	HPV-16	Total

	Positive	Negative	
3+3	3(3%)	26(26%)	29(29%)
3+4	0(0%)	3(3%)	3(3%)
3+5	0(0%)	2(2%)	2(2%)
4+3	1(1%)	20(20%)	21(21%)
4+4	2(2%)	13(13%)	15(15%)
4+5	0(0%)	12(12%)	12(12%)
5+2	0(0%)	1(1%)	1(1%)
5+3	0(0%)	3(3%)	3(3%)
5+4	0(0%)	8(8%)	8(8%)
5+5	0(0%)	6(6%)	6(6%)
P. Value	**0.860**		

Table 3.4: correlation between HPV-18 and adenocarcinoma grade (N=100)

Grade	HPV-18		Total
	Positive	Negative	
3+3	0(0%)	29(29%)	29(29%)
3+4	0(0%)	3(3%)	3(3%)
3+5	0(0%)	2(2%)	2(2%)
4+3	1(1%)	20(20%)	21(21%)
4+4	1(1%)	14(14%)	15(15%)
4+5	0(0%)	12(12%)	12(12%)
5+2	0(0%)	1(1%)	1(1%)
5+3	0(0%)	3(3%)	3(3%)
5+4	0(0%)	8(8%)	8(8%)
5+5	0(0%)	6(6%)	6(6%)
P. Value	**0.925**		

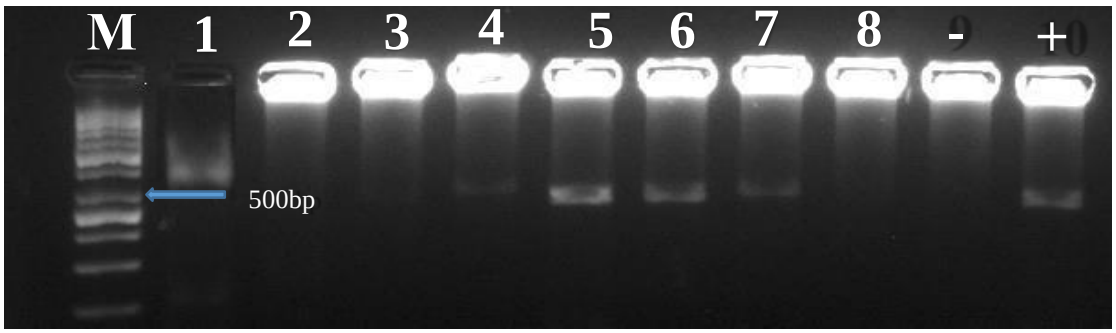


Figure 3.1: *HPV*16 PCR assay (215 bp). M = DNA molecular weight marker. Sample of positive PCR- reactions are seen in specimens 5, 6, and + = positive control, - = negative control

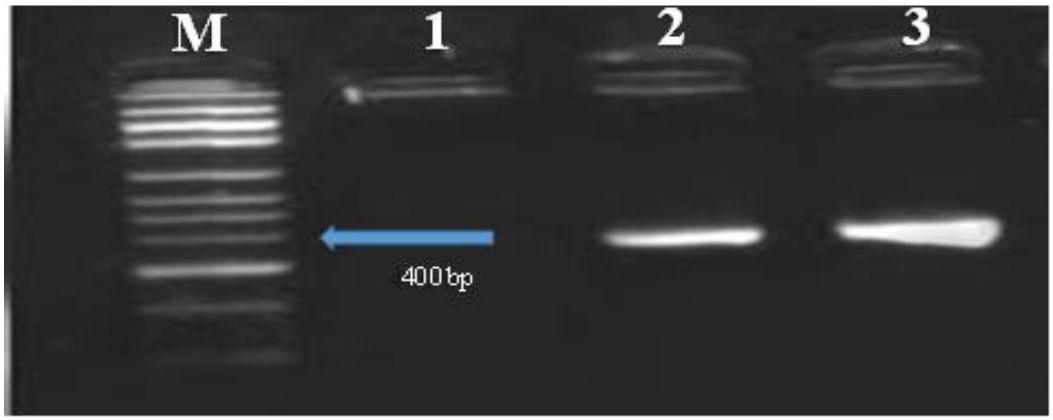


Figure 3.2: *HPV*18PCR assay (236 bp). M = DNA molecular weight marker. Sample of positive PCR- reactions are seen in specimens 3 and 1 = negative control, 2 = positive control.

4. Discussion:

Prostate cancer is the second most common cancer in men worldwide. [20] The incidence rate is the highest in African men followed by European and Asian. [21] In Sudan (Khartoum) among all cancer sites, prostate cancer ranked fourth and first among Sudanese men aged 65 years and older. [22] Despite all research efforts the risk factors for prostate cancer is still unclear, it has a multi-factorial origin with environmental, dietary and genetic factors reflecting its complex pathogenesis. Hamad, [23] 2011 in Gezira state, Sudan found the risk factors for prostate cancer include age, education level, occupation, unhealthy habit such as smoking and obesity. In addition to these factors, sexually transmitted disease considered one of the more consistently risk factors.[24] Recently, *HPV* types 16 and 18 specific DNA

sequences detected in prostate cancer biopsy. Since HPV types 16 and 18 are known oncogenic potential, an association between *HPV* infection and prostate cancer has been suggested. [25] Its oncogenicity is due to persistent infection and expression of viral proteins, E6 and E7, that inhibit cellular tumor suppressor gene such as *Rb*, and *p53*. [26]

According to available published literature this is the first study in Sudan to investigate the possible role of *HPV* 16 and 18 in prostate cancer through DNA detection using polymerase chain reaction (PCR). The study included 200 samples of FFPE prostate tissue blocks, 100 are prostate adenocarcinoma and 100 are benign prostatic hyperplasia (BPH). In the present study, the histological type of prostate cancer was adenocarcinoma which is compatible with the result of a meta analysis by Yang L, *et al*. [27] also from 16071 cases of prostate cancer in Iran during (2003 - 2008), (95.2 %) of them were adenocarcinoma [28]

The results of the current study showed that HPV-16 was positive in 6(6%) of the adenocarcinoma patients and 4(4%) of the hyperplasia patients. The difference of *HPV*-16 infection between groups was not significant (*P. Value* 0.374). HPV-18 was positive in 2(2%) of the adenocarcinoma patients and 4(4%) of the hyperplasia patients, with no significant difference *(P. Value* 0.341). However these findings were supported by Noshin Abdolmaleki, *et al* [29] , Adami HO, *et al* [30] , and Merve Aydin, *et al* [31] in their studies which concluded that no association between *HPV*-16 and 18 and prostate cancer. Two Meta - analyses studies reported significant association between *HPV* infection and the risk of prostate cancer, these results were not consistent with our findings [32, 33], these discrepancies may be due to varied distribution of *HPV* among different populations, differences in methods of prostate tissue sampling, the sample size, PCR analysis, in situ hybridization, immunohistochemistry or southern blot hybridization analysis. Also duration of specimen storage before fixation, the age of the tissue and blocks can all influence the integrity of stored DNA. In conclusion, these findings excluded the role of *HPV* 16 and 18 in prostate cancer in Sudanese. Also may reflect low prevalence of *HPV*.

Limitations:

As in all research, there are certain limitations in this study; the most important is that comparative group consisted of samples with BPH; however it should be normal prostate tissue to assess the role of HPV infection in prostate carcinogenesis appropriately. We did not have access to these samples.

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Conflicts of interest

No conflicts of interest.

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