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Genotyping Detection of Human Papillomavirus in Benign Prostate Hyperplasia in Ramadi City

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ABSTRACT

Background: The presence of Human Papillomavirus (HPV) in Benign Prostate Hyperplasia (BPH) could suggest an important role of viral infection in the pathogenesis of BPH, potentially influencing treatment strategies. If a correlation is established, it could lead to increased awareness and screening for HPV in male populations, similar to cervical cancer screening in women.

Objectives: To detect HPV serotypes in patients with BPH and to disclose their potential roles in the pathophysiology of BPH.

Patients and methods: A case control study, involve collection of 75 specimens from male individuals with BPH and compared to 75 specimens from age-matched healthy male volunteers. The samples were processed at College of Medicine laboratories- University of Anbar and Private Specialized laboratories in Ramadi city from the period December 2023 to April 2024, patient samples were collected based on physicians' recommendations, suspecting a viral infection with human Papillomavirus. In both groups, a quantitative test was conducted for specific HPV IgM16 and HPV IgM18 in serum using an Enzyme Linked Immunosorbant Assay (ELISA), Genotyping of HPV in the urine were detected by conventional PCR technique.

Results: The statistic results indicated that the mean value of specific HPV16 IgM-is 11.02±0.968 compared to control group value 0.647±0.132 at significant differences P= 0.0001, while mean value of –specific HPV18 IgM is 0.752±0.235 compared to control group value 0.165±0.015 at significant differences P=0.019. The molecular screening results indicated that the HPV16 serotype is most frequent in patients with BPH than HPV18, (78.7%) compared (20%) respectively.

Conclusion: HPVs, mainly serotype 16 and 18 were detected in patients with BPH, the study also shows human immune response to HPV 16 and HPV 18 in men with BPH which provide valuable information about immune state, understand how BPH develop, find new ways for diagnoses and development of BPH treatments. Urine sampling appears to be a suitable surrogate sample for DNA HPV serotype testing in male with limited access to healthcare.

Keywords: *HPV-16; HPV-18; prostate cancer, benign prostatic hyperplasia.*

1. INTRODUCTION

Viral factors are the most important class of infectious agents associated with human cancers. It was estimated that 17-20% of all worldwide incidence of cancers are attributable to a viral etiology. Human papilloma virus is sexually transmitted in adults [1]. Human papilloma viruses (HPVs) are regarded as specific epitheliotropic DNA viruses. HPVs can persistently infect prostate epithelium in non immunocomprised hosts. There are more than 200 HPV types which have been known, HPVs are classified on the basis of their epidemiological relationship with cervical cancer into carcinogenic (high risk) non carcinogenic (low risk) subtypes[2]. Out of these, 14 are classified as high-risk (HR) HPV types, they include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73[3]. Although HR HPV types account for mostly all HPV-associated cancers, HPV 16 and 18 are the major causes of 70% of cases globally [4].

Benign Prostate Hyperplasia (BPH) is the primary condition that affects males as they grow older[5]. The enlarged prostatic gland's symptomatology, another description of BPH, presents with various urinary tract problems and some kidney issues[6]. Benign prostatic hyperplasia (BPH) refers to the nonmalignant growth or hyperplasia of prostate tissue and is a common cause of lower urinary tract symptoms (LUTS) in older men. Disease prevalence has been shown to increase with advancing age. The histological prevalence of BPH at autopsy is as high as 50% to 60% for males in their 60, increasing to 80% to 90% of those older than 70 years of age[7,8]. The co-existence of prostate cancer (PC) in the living being treated for BPH is between 5 – 20%. This has been found in chippings from transurethral resection of the prostate (TURP) for BPH or simple prostatectomy specimens for BPH. Patients after TURP or simple prostatectomy can develop PC years after the procedure from the peripheral zone which is left behind as false capsule[9]. Although these two conditions, BPH and PC, are major urological conditions within the prostate gland and can co-exist, currently, there is no data to suggest a causal link. As BPH occur mostly in the transitional zone, PC occurs mainly in the peripheral zone although PC can occur in the transitional and the central zones [9]. Many studies suggest an association between HPV infection and increased the risk of prostate cancer. [10,11].

The association between HPV infection and BPH is still unclear so the current study sought to address this knowledge gap by systematically collecting and analyzing urine and blood samples from BPH patients for detection of HPV serotypes and their potential roles in the pathophysiology of BPH, this ultimately advancing our knowledge of prostate disease mechanisms and guiding future therapeutic approaches.

2. MATERIALS AND METHODS

A case control study design for understanding the role of Humanpapilloma virus in benign prostate hyperplasia in Ramadi city patients/ Iraq. The study conducted in College of Medicine laboratories- University of Anbar and Private Specialized laboratorires from November 2023 until September -2024. The study involved collection of 75 specimens from male individuals with BPH and assigned as group A and compared to 25 specimens from age-matched healthy male volunteers which served as control group and assigned as group B. Inclusion criteria include any patient with lower urinary tract symptoms with enlarged prostate by ultrasonography and normal prostate consistency on digital rectal examination and normal prostatic specific antigen level, any patient with prostatic cancer, urinary tract infection, previous instrumentation or previous prostatic biopsy or surgery was excluded from this study. Patient samples were collected based on physicians' recommendations, suspecting a viral infection with human Papillomavirus. Five milliliters of venous blood were drawn from patients and control groups member using a five-milliliter disposable syringe. The blood sample was immediately transferred into gel tube, mixed well and left at room temperature or 5 minutes in water bath at 370^{C} then centrifugation at 4000 rpm for 10 min. to separate serum for detection of immune-parameter including specific HPV IgM16, IgM18 serotyping. A midstream urine sample were collected using a sterile labeled container, the container was filled to at least 2/3 with sealed the container tightly. The sample was stored at 4°C (refrigerator temperature) immediately after collection, the sample could be frozen at -20°C for month if not be tested at the same day. Urine sample have been used for HPV serotypes isolation.

A quantitative test was conducted for specific HPV IgM16 and HPV IgM18 in serum using an Enzyme Linked Immunosorbant Assay (ELISA) device. The procedure details conducted according to the manufacturer's protocols (Cusabio, China). For DNA Extraction, the *Quick*-DNA™ Viral Kit from Zymo Research provides a streamlined method for rapid isolation of high-quality viral DNA from urine sources. The procedure done according to Vorsters, et al [12]. Polymerase chain reaction (PCR) was carried out using primer for gene target their details was listed in Table (1). All PCR reactions were done in Applied Bio-system 2720 thermo cyclers, and amplification of target gene. Twenty-five microliter of PCR amplification reaction contained 12.5 µl from OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1.5µl 10 pmol/µl from each primer and 6.5 µl of free-nuclease water.

Table (1): Primer Sequences of HPV 16 and 18 Serotype with their Gene Size

Gene		Sequence of forward and reverse Primer (5′- 3′)	PCR Product Size bp	Annealing Temp.	Reference
HPV16	F	5-AGCTTTGCAATATCCCCTGTGA -3	353 bp	60	NCBI
	R	3-CCAAATAGAAGTCACGTCGAGGA-5			
HPV18	F	5- TCTAAACCTGCCAAGCGTGT -3	681 bp	64	
	R	3-AAGGGTAGACAGAATGTTGGACA -			

		3			

All statistical analyses were performed using SPSS, version 26.0 (SPSS Inc., NY, USA). The study was approved by Ethics Committee of the Al Anbar Medical Research University (approval number 155 in December 12, 2023) before starting of the study. All individuals have been given consent to participate in the current study, in addition that there is no individuals under 16 years old.

3. RESULTS:

The age of patients in group A was ranged from 30 -76 with mean 51.6±2.23 and the age in group B ranged from 35-74 To with mean 54.8±2.41 and no statistical difference was observed among two groups (t = -1.330, p = 0.196).

The levels of IgM -16 are more in Group A (11.02 ± 0.968) compared to Group B (0.647 ± 0.132), with statistically significant difference (t = 10.466, p = 0.0001), also the levels of IgM-18 are more in Group A (0.752 ± 0.235) compared to Group B (0.165 ± 0.015), with statistically significant difference (t = 2.508, p = 0.019). The levels of IgG antibodies are higher in Group A (125.67 ± 13.67) compared to Group B (24.76 ± 2.908), with highly significant difference difference (t = 7.760, p = 0.0001). Table 2

Table 2: Mean ± SE and comparison of study parameters between patients and controls group.

Variables	Group A Mean±SE (n=75)	Group B Mean±SE (n=75)	Differences vs Controls	t	p-value
Age	51.6±2.23	54.8±2.41	↓ 3.2	-1.330	0.196
Specific IgM for HPV serotype16	11.02±0.968	0.647±0.132	↑ 10.382	10.466	0.0001
Specific IgM for HPV serotype18	0.752±0.235	0.165±0.015	↑ 0.587	2.508	0.019
Total IgG	125.67±13.67	24.76±2.908	↑ 100.912	7.760	0.0001
Group A: Patients Group B: Control SE: Standard deviation n: Number Significant differences between group (P-value ≤ 0.05)					

The distribution or HPV-16 is slightly left-skewed, where most individuals have positive test. The values of both skewness and kurtosis test are within the generally acceptable range, but the skewness test suggests some asymmetry.

There is a rightward skew for HPV-18, with the majority have negative test. The distribution is with acceptable kurtosis, and the skewness is outside the optimal range. And serotypes show statistically significant distributions (p-values < 0.05). Table 3

Table 3 : The frequency and percent with Skewness and kurtosis test value for specific IgM for serotype-16 and specific IgM for serotype-18

HPV serotype		Frequency (n)	Percent (%)	Skewness	Kurtosis	p-value
16	Negative	16	21.3	- 1.428	0.040	0.003
	Positive	59	78.7			
18	Negative	60	80	1.531	0.352	0.002
	Positive	15	20			
	Positive	0	0			
Total		75	100			
Skewness: The value between -1 and +1 is excellent, while -2 to +2 is generally acceptable						

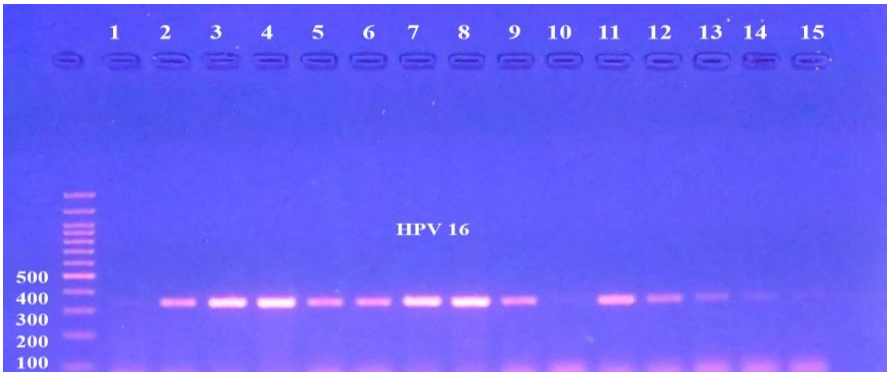

Figure 1. PCR amplification fragments for the detection of *HPV16* (1.5%agarose, 10 V/cm^{2}for 90min). Lane L: 100-bp DNA ladder. Lane 1-15: *HPV16 gene* bands with 353 bp.

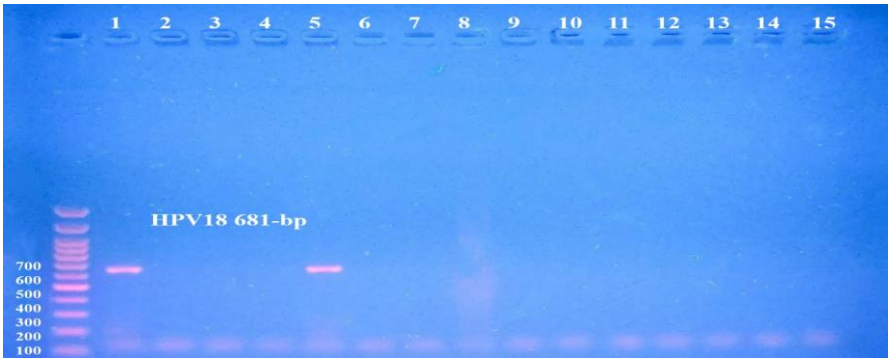

Figure 2. PCR amplification fragments for the detection of *HPV18* (1.5%agarose, 10 V/cm^{2}for 90min). Lane L: 100-bp DNA ladder. Lane 1, Lane5: *HPV18 gene* bands with 681 bp.

4. DISCUSSION:

The exploration of Human Papillomavirus (HPV) serotypes in patients with Benign Prostate Hyperplasia (BPH) presents a crucial intersection of virology and urology, particularly in understanding potential viral contributions to prostate health. Recent studies have indicated a possible correlation between HPV infection and the development of various prostate conditions, prompting the need for comprehensive sampling and analysis of HPV serotypes within BPH populations [13]. Our study sought to address this knowledge gap by systematically collecting and analyzing urine samples from BPH patients for detection of HPV serotype and their potential roles in the pathophysiology of BPH, ultimately advancing our knowledge of prostate disease mechanisms and guiding future therapeutic approaches in the future.

Serotyping detection involves identifying of specific HPV 16 and 18 in urine sample. This is crucial for understanding the epidemiology of HPV in Benign Prostate Hyperplasia (BPH). A total of 75 case study taken to investigate the presence of HPV serotypes 16 and 18. The observation that HPV serotype 16 values are often higher than those for serotype 18 in Benign Prostate Hyperplasia (BPH) could be attributed to several factors such higher prevalence: HPV 16 which is consider one of the most prevalent high-risk HPV types globally that is commonly associated with urology disease [4]. Individuals may be more frequently exposed to HPV 16, leading to a higher seroprevalence compared to HPV 18. On the other hand, the immune system's response to different HPV types can vary, HPV 16 may elicit a stronger or more detectable immune response than HPV 18, resulting in higher antibody levels in serological tests like ELISA test can be detected. Also, the chronic infection and persistence of HPV 16 may be more likely to persist in the body than HPV 18, leading to chronic immune stimulation and thus higher serological values [14].

The immune detection of HPV 16 and HPV 18 in the context of BPH presents a promising area for further research. To understand how the immune system responds to these viruses, we may uncover new insights into the pathophysiology of BPH and explore innovative therapeutic strategies to manage this common condition [15].

The nucleic acid (DNA) of HPV submitted to genomic extraction successfully from the urine samples. The purity and concentration of extracted DNA was measured by using Nano Drop device and the results ranged among (30.6 -65.4) ng/µl as a concentration while the purity was ranged from (1.7 to 2.0). Extracted DNA were confirmed and analyzed by gel electrophoresis to more ensure the presence of target DNA.

The molecular screening results indicate that the HPV16 serotype is most common frequency in patients with Benign Prostate Hyperplasia (BPH) than HPV18, this results consider a confirmation identify and support serotype foundation. PCR amplification results show that 59/75(78.75) of patients with Benign Prostate Hyperplasia infected with HPV16 (Fig.1), while 15/75 (20%) of case infected with HPV18 (Fig.2). The current study results are in agreement with Bordigoni, et al who found that HPV16 frequency is most common serotype in cases [16].

The results of this study were in agreement with the findings of Hisada, et al [17] who found 50% of HPV was serotyping 16 in BPH by using PCR method and also consistent with the findings of Hisada, et al who found 20% of HPV18 common in BPH. However ,our results are lower than those reported by McNicol et al and Hind et al [18] who found HPV16 and HPV18 in BPH in a percentage rate of (93.3% and 20%) respectively. PCR detection of HPV serotypes in patients with BPH is a valuable tool for exploring the potential relationship between HPV infections and prostate health. This methodology not only enhances understanding of the epidemiology of HPV in older male populations but also opens avenues for future research into prevention and treatment strategies for BPH and HPV-related diseases also vaccine preparation.

The roles of HPV 16 and HPV 18 in Benign Prostate Hyperplasia (BPH) are emerging areas of interest in urological investigation. HPV 16 is notably prevalent and highly oncogenic, with studies suggesting that its presence may lead to chronic inflammation in prostate tissue, potentially exacerbating hyperplastic changes. While HPV 18 is less common, it can still cause cancer and may contribute to inflammation in the prostate. Both HPV 16 and 18 can trigger immune reactions that could impact how BPH develops, potentially affecting cell growth and tissue changes. Understanding the relationship between these HPV types and BPH may not only enhance our knowledge of prostate health but also inform clinical strategies for prevention and management, particularly in populations at higher risk for both HPV infection and prostate-related diseases [19]. Glycine also plays a part in preventing oxidative stress [20], and has been demonstrated to be a successful therapy for benign prostatic hyperplasia [21]. The limitations of this study include small sample size and the lack of long term follow up of patients, other studies recommended the use of large sample size as screening test for study virus outbreak [22,23].

5. CONCLUSION:

HPVs, mainly serotype 16 and 18 were detected in patients with BPH, highlighting a potential association between viral infection and BPH development. the study also show human immune response to HPV 16 and HPV 18 in men with BPH which provide valuable information about immune state, understand how BPH develop , find new ways for diagnoses and development of BPH treatments. Urine sampling appears to be a suitable surrogate sample for DNA HPV serotype testing in male with limited access to healthcare

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