***Original Research Article***

**Detection of BK Virus Cytopathic Effect in Urine Samples of Sudanese Patients with Prostatic Cancer**

**ABSTRACT**

**Background**: Prostate cancer is one of the most common cancers among men. Some studies suggest a potential link between BK virus infection and the development of this cancer, highlighting the need for further investigation. **Objectives**: This study aims to detect BK virus in the urine of prostate cancer patients, examine associated cytomorphological changes, identify predisposing factors for infection, and evaluate the diagnostic value of urine cytology in benign prostatic hyperplasia (BPH). **Methods**: A case–control study was conducted from January to April 2025 at the Shendi Town Oncology and Cancer Treatment Center and Al Mak Nimir Hospital, enrolling 45 men (15 prostate cancer, 15 BPH, 15 healthy controls). Midstream urine samples (50–100 mL) were collected, processed within two hours (or fixed with 50% ethanol), centrifuged, and smears stained by Papanicolaou (with H&E as needed) to detect decoy cells and cytomorphological changes. Clinical and demographic data were gathered through a questionnaire, and statistical analysis (including frequencies, percentages, and p-values) was performed using SPSS. **Results**: Decoy cells were identified in 86.7% of prostate cancer patients, 40% of BPH cases, and 0% of healthy controls, demonstrating a strong association with malignancy. Their presence significantly correlated with hyperkeratosis (60%), inflammatory cells (83.3%), and bacterial infection (93.3%) (all P < 0.05). Clinically, decoy cells were linked to recurrent UTIs in 87% of prostate cancer patients but showed no relationship with family history. These data underscore the potential impact of BK virus–related and infectious factors over genetic predisposition in prostatic disease. **Conclusion**: This study highlights a strong connection between BK virus (BKV) and prostate cancer, with decoy cells frequently observed in cancer cases. These cells were associated with cytological abnormalities and infections, suggesting viral reactivation. Urine cytology proved more effective than PCR in some instances. No genetic link was found, emphasizing environmental factors. Findings support prior studies and advocate for further research into BKV as a diagnostic or therapeutic target.

**Keywords**: BKV, Urine, Prostatic Cancer, Cancer, Cytopathic Effect, Hyperplasia

1. **INTRODUCTION**

One of the most vital exocrine glands in men is the prostate. It is susceptible to several pathological illnesses, the most prevalent of which are benign and malignant diseases **[1].** Benign Prostatic Hyperplasia (BPH), which is common in older men and is not cancer, is caused by the prostate gland growing larger than usual. This condition can "squeeze" the urethra, resulting in several symptoms, including frequent urination and difficulty urinating during the day **[2].** One of the main causes of cancer-related mortality in men is prostate cancer (PCa), a physiologically homogeneous tumour **[3].** According to the GLOBOCAN 2018 report, prostate cancer was the second most common cancer and the fifth largest cause of cancer-related deaths in males worldwide in 2018, with an estimated 1.3 million new cases and 359,000 related deaths **[4].** Additionally, the survey showed that approximately 18% of the world's male population lives in nations with a very high developing index, where almost two-thirds of newly diagnosed cases will be identified **[5, 6].** In several nations at different stages of development, the incidence rates of PCa have sharply grown over the last forty years. The incidence rates of PCa vary by more than 100 times globally, with the lowest rates found in Asia and the highest rates found in several Caribbean islands, Australia/New Zealand, Northern and Western Europe, and North America, according to the GLOBOCAN 2018 report. In 46 countries, especially in Sub-Saharan Africa and the Caribbean, the disease is regarded as the primary cause of cancer-related deaths among men **[7].** In many countries, such as those in North America, Oceania, Northern and Western Europe, developed Asia, and the United States, the death rate from prostate cancer has been declining. However, in several Central and South American, Asian, and Central and Eastern European countries, such as Cuba, Brazil, the Philippines, Singapore, Bulgaria, Belarus, and Russia, the mortality rate is increasing **[5].** According to the GLOBOCAN 2018 study, the incidence rates of PCa in Africa varied from 66.9 to 111.8 per 100,000 people in Southern Africa to less than 16.3 per 100,000 people in Northern Africa nations, including Egypt, Libya, and Algeria, as well as in select Middle Africa countries like Sudan. Additionally, according to the GLOBOCAN 2018 study, the death rate from PCa per 100,000 people in Africa varied from 24.4 in southern Africa to 18.7 in eastern Africa, with Northern Africa having the lowest mortality rate at 7.0 **[8,9].** According to the Radiation and Isotopes Centre of Khartoum (RICK) report form, PCa is the most frequent cancer in Sudanese males (3.3%) and is currently acknowledged as one of the main medical issues affecting the country's male population **[8].** Prostate cancer has been much more common in the last 20 years, and Sudanese urologists are paying more attention to the condition **[10].** Additionally, there are roughly 600 Sudanese men who receive a PCa diagnosis each year **[1],** and the mortality rate is 8.7 per 100,000. The majority of cases (85.4%) had stage III and IV symptoms, and the disease was found to be evenly dispersed across the various tribes **[9].** Prostate cancer can be caused by several risk factors, such as age, race, ethnicity, alcohol usage, genetics, farming, eating a lot of fat, working in tire factories, and being around cadmium, in addition to viral infections **[11].** The objective of this study is to detect Bk Virus Cytopathic Effect in Urine Samples of Sudanese Patients with Prostate Cancer.

**2. METHODOLOGY**

This case-control study was conducted at the Shendi Town Oncology and Cancer Treatment Center and Al Maknimir University Hospital in Shendi town (Shendi locality, River Nile State, Sudan) – a historic trading center on the east bank of the Nile, 150 km northeast of Khartoum and 45 km southwest of Meroe. The study spanned from late January to April 26, 2025, and included men with prostate cancer (cases), healthy men, and men with benign prostatic hyperplasia (controls) who provided consent and attended the hospitals during the study period. Participants with other malignancies or immunocompromised conditions were excluded.

**Study sample**

Urine sample: fresh midstream urine (50-100 ml) collected in a sterile container urine The study sample included participants selected according to specific criteria. Samples were collected during a period extending from the end of January to the end of February.

**Sample size**

The study involved 45 men: 15 with histologically confirmed prostate cancer (cases), 15 with clinically diagnosed benign prostatic hyperplasia (BPH controls), and 15 age-matched healthy male volunteers with no history of prostatic disease (healthy controls). All participants provided written informed consent.

**Study variable and method of detection.**

This study focuses on several key variables, including the presence of decoy cells, which are abnormal urinary cells indicating BK virus infection. Urinary tract infections (UTIs) and recurrent bacterial infections are also considered, as they can influence urinary results and inflammatory responses. Additionally, cytomorphological changes, such as hyperkeratosis and inflammatory cells, are examined for their potential association with viral or bacterial infections. These variables are interconnected: the presence of decoy cells is closely linked with UTIs or viral infections, and recurrent bacterial infections may increase the likelihood of decoy cells in urine. Cytomorphological changes like hyperkeratosis are associated with both UTIs and viral infections, highlighting their role in diagnosing these conditions.

**Sample collection and preparation**

Urine samples for cytological examination are collected following the guidelines described in Bancroft’s histopathological techniques. The preferred sample is the first-morning voided urine, as it contains a higher concentration of exfoliated cells. Approximately 30 to 50 mL of urine is collected in a clean, sterile, wide-mouthed container. The sample should be processed within 1 to 2 hours to prevent cellular degeneration. If immediate processing is not possible, an equal volume of a cytology fixative, such as 50% ethanol, should be added to preserve the cells. For preparation, about 10 to 15 mL of urine is centrifuged at 1500 to 3000 rpm for 5 to 10 minutes. The supernatant is carefully discarded, and the sediment is resuspended in a small amount of the remaining fluid. A drop of this sediment is placed on a clean glass slide and gently spread to form a smear. The slide is then immediately fixed using 95% ethanol or a spray fixative. Air-drying is generally avoided, especially if Papanicolaou staining is intended. After fixation, the smears are stained—commonly with the Papanicolaou stain for routine cytological evaluation, or with hematoxylin and eosin (H&E) if additional diagnostic detail is required. Special stains may also be used in cases of suspected viral, fungal, or bacterial infections **[12].**

**Papanicolau staining technique**

Each fixed slide was rehydrated in descending grades of ethanol 2 minutes in each grade, after that slide was stained in Harris’s Haematoxylin solution for 1 minutes progressively, then slid was blued in running tap water for 10 minutes, after that slide was dehydrated in ascending grade ethanol 2 minute in each grade, then orange G6 (OG6) was applied onto slidfor 3 minutes followed by rinsed in 95% ethanol for 2 minutes, then eosin azure 50 (EA50) solution was applied onto slide for 3 minutes followed by rinsed in 95% ethanol for 2 minutes, after that slide was rinsed in absolute ethanol, dried at room temperature, cleared in xylene and mounted in Disterene A Plasticizer and Xylene (DPX). The smear was then screened under a light microscope **[13].**

**2.3 Data Analysis**

After examination of the sections, the results of the laboratory investigation, as well as the demographic data from the patient’s records, were processed using the Statistical Packages for Social Sciences (SPSS) computer program. Frequency, mean, and chi-square test values were calculated at <0.05 and considered statistically significant.

**3. RESULTS**

This study was conducted at Elmak Nimir University Hospital and the Oncology Hospital in Shendi locality to evaluate the relationship between the appearance of decoy cells in urine and various cytological and clinical features in prostatic disease patients. These findings suggest that environmental and infectious factors, particularly BK virus, may play a more influential role than genetic predisposition. Clinically, decoy cells correlated strongly with recurrent urinary tract infections, particularly among prostate cancer patients (87%), while no significant association was found with family history. The findings showed that decoy cells were present in 86.7% of prostate cancer cases, 40% of BPH cases, and absent in healthy individuals, indicating a strong association with malignancy. The presence of decoy cells was significantly associated with cytomorphological changes such as hyperkeratosis (60%), inflammatory cells (83.3%), and bacterial infection (93.3%), all with statistically significant p-values (P<0.05).

**Table 1. The cytological changes associated with viral changes were observed among the test group.**

|  |  |  |
| --- | --- | --- |
| **Test group** | **Frequency** | **Percentage %** |
| Decoy cell | 19 | 63.3 |
| hyperkeratosis | 18 | 60% |
| Inflammatory cells | 25 | 83.3% |
| Bacterial infection | 28 | 93.3% |

**Table 2. The frequencies of clinical data of the test group compared to the control group.**

|  |  |  |
| --- | --- | --- |
| **Control group** | **Frequency** | **Percentage %** |
| Recurrent UTI | 28 | 93.3% |
| Family history of prostate cancer | 2 | 6.6% |
| Renal diseases | 0| | 0|% |
| Immunity disease | 0 | 0% |

**Table 3. The compare the appearance of decoy cells and the cytopathic effect of polyomavirus (BK virus) among study groups.**

|  |  |  |
| --- | --- | --- |
| **Variables**  | **Decoy cell** | **Total** |
| **Absent** | **present** |
| Healthy | 15 | 0 | 15 |
| Benign prostatic | 9 | 6 | 15 |
| Prostate cancer | 2 | 13 | 15 |
| Total | 26 | 19 | 45 |

**Table 4. The correlation between decoy cells in urine smear appearance and other cytomorphological criteria among study groups.**

|  |  |  |
| --- | --- | --- |
| **Variables**  | **Decoy cell** | ***P. value***  |
| **Healthy** | **BPH** | **Prostate cancer** |
| Hyperkeratosis | 0% | 40% | 46% | 0.014 |
| Inflammatory cells | 0% | 40% | 80% | 0.00 |
| Bacterial infection | 0% | 40% | 73% | 0.00 |

**Table 5. The correlation between decoy cell appearance and the clinical data among study groups.**

|  |  |  |
| --- | --- | --- |
| **Variables**  | **Decoy cell** | ***P. value***  |
| **Healthy** | **BPH** | **Prostate cancer** |
| Recurrent UTI | 0% | 40% | 87% | 0.00 |
| Family history | 0% | 0% | 13% | 0.13 |

**4. DISCUSSION**

**The study supports the hypothesis that BK virus (BKV) infection is associated with prostatic diseases, particularly prostate cancer (PCa). The presence of decoy cells—a cytological hallmark of BKV reactivation—was observed in 86.7% of PCa cases, 40% of benign prostatic hyperplasia (BPH) cases, and was completely absent in healthy controls. This striking difference indicates a strong correlation between BKV and malignancy, highlighting the virus’s potential role in carcinogenesis. These findings align with Monini et al (2015) [14], who reported a higher prevalence of BKV DNA in prostate cancer tissues compared to BPH and healthy samples, suggesting that the virus may persist in a latent form and contribute to oncogenic transformation. Similarly, our results are supported by Gorish et al (2019) [1], who detected the BKV large T antigen (LTAg) in 30.9% of PCa samples versus 7.2% in BPH, with significantly higher viral loads in cancerous tissues. Gorish et al. also observed greater LTAg expression in samples with higher Gleason scores, suggesting a link between viral activity and tumor aggressiveness. Further support comes from the Ahvaz study (2021) [7], which found BKV DNA in 66% of PCa tissues and 36% of BPH samples (P = 0.003) using semi-nested PCR. Like our findings, the Ahvaz study did not find a significant correlation between age and viral presence (P = 0.086), nor between Gleason score and BKV positivity, reinforcing the idea that BKV might act as a cofactor in cancer development, independent of patient age or histological grade. Cytologically, our study revealed a strong association between decoy cells and features like hyperkeratosis (60%), inflammatory infiltrates (83.3%), and bacterial infections (93.3%). These findings indicate a highly active microenvironment conducive to viral reactivation. This aligns with previous reports suggesting that inflammation and chronic infection may promote viral activity and transformation. Clinically, we found a significant association between decoy cells and recurrent urinary tract infections (UTIs), particularly in PCa patients (87%). While Gorish et al. did not explore UTIs directly, they reported environmental factors like alcohol intake and geographical variation as influencing infection rates, suggesting that both local and systemic factors may contribute to viral reactivation and disease progression. Interestingly, our urine cytology method proved more sensitive than PCR in detecting BKV activity. While decoy cells were prevalent in 86.7% of PCa cases, Gorish et al. only detected BKV DNA in 1 of 17 urine samples from PCa patients (5.8%), though 75% of BPH urine samples were PCR-positive. This highlights potential differences in sampling techniques and detection sensitivity, emphasizing the value of cytological screening in non-invasive BKV monitoring. All studies, including ours, found no significant association between BKV infection and genetic predisposition (e.g., family history), supporting the theory that infectious and environmental factors may outweigh hereditary influences in the pathogenesis of prostate diseases. BK and JC polyomaviruses (PyV) have been linked to the development of various human cancers [15].**

## **5. CONCLUSION**

There is a strong association between BK virus (BKV) and prostate cancer, as indicated by the high prevalence of decoy cells in PCa cases. Decoy cells were associated with cytological changes (e.g., hyperkeratosis, inflammation), bacterial infections, and recurrent urinary tract infections (UTIs), suggesting a reactive environment that may support viral reactivation. Urine cytology proved to be an effective non-invasive method for detecting BKV activity, outperforming conventional PCR in some cases.

## **6. RECOMMENDATION**

1. Integrate cytological screening into the routine evaluation of prostatic and urinary tract conditions.
2. Use decoy cell detection as an early indicator of BK virus infection.
3. Promote cytology as a cost-effective tool, especially in low-resource settings.
4. Encourage further research on the role of BK virus in prostate disease progression.
5. Consider combining cytology with clinical and microbiological data for comprehensive diagnosis

**CONSENT**

The patient’s written consent has been collected.

**ETHICAL APPROVAL**

The study was approved by the Department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, and the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area of study. The aims and the benefits of this study were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

As a result, the Author (s) declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

**COMPETING INTERESTS DISCLAIMER**

Authors have declared that they have no known competing financial interests OR non-financial interests, OR personal relationships that could have appeared to influence the work reported in this paper.

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