The prevalence of JC and BK viruses among prostate cancer patients in Al-Najaf Al-Ashraf province

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Abstract. A total of 74 clinical samples Formalin-Fixed Paraffin-Embedded (FFPE) were collected from patients diagnosed with prostate cancer (PCa) aged between 41 and 90 years and these samples were obtained from patients treated at notable medical institutions like Al-Sadr Medical City and leading clinical laboratories in Al-Najaf City, Iraq, during the period of January to December 2023. Prostate cancer is considered a common malignant tumor in males, and studies are still investigating the possible relationship of the appearance of this disease with other factors, including viral infections, which were investigated in the current study in search of possible links between the presence of human BK oncolytic viruses (BKV). The JC (JCV) and the emergence of this disease. The current study indicated the potential role of the JCV virus in provoking prostatitis, which may lead to the emergence and development of prostate cancer in males compared to males who do not suffer from viral infection. The present study showed the absence of BKV virus DNA in prostate cancer tissue samples compared to the presence of JCV virus DNA, as the percentage of positive samples reached (11, 14.864%) compared to negative samples (63, 85.135%).

1 Introduction

That viral infections can cause inflammation in the prostate, potentially leading to the development of PC [1]. Oncogenic viruses contribute to around 20% of cancer cases globally [2] and among these viruses, the human polyomavirus (HPV) is notably significant in the etiology of PC [3]. The impact of the large tumor antigen from this polyomavirus on cell cycle regulation can lead to cellular transformation by interfering with the functions of tumor suppressor p53 and the pRB family [4]. Various studies have highlighted the undeniable involvement of the human polyomavirus in the development of several types of cancer like Merkel cell carcinoma (MCC), brain tumors, kidney cancer, urothelial carcinoma and prostate cancer [5,6,7,8]. The Polyomaviridae family, which includes the human BK virus (BKV), JC virus (JCV), and simian virus (SV40) along with human papillomaviruses (HPV), are frequently encountered pathogens in the urinary tract and they are often linked to the development of urinary tract malignancies like prostate cancer [9].

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The genetic material of polyomaviruses is characterized by a circular double-stranded DNA molecule spanning approximately 5 kilobases. [10] and the genomes include a segment that encodes the T-antigens (both large T and small t) early on, followed by regions that code for late expression proteins (agno and capsid proteins) at both early and late stages [11] and there is a regulatory region that does not code for proteins [12]. JCV can be categorized into two forms based on the structure of its noncoding regulatory region which are the archetype and rearranged variants [13]. The archetypal form of JCV includes a single copy of the promoter and enhancer [14] while rearranged variants including alterations in their genetic sequences that can affect various aspects of viral biology, including infectivity, replication, and pathogenicity and these variants may arise due to genetic mutations, recombination events, or other mechanisms [15]. In terms of nucleotide homology, JCV and BKV exhibit around 72% similarity with each other, and they both share 70% nucleotide homology with SV40 [16]. The JCV and BKV viruses have the potential to induce genetic and immune alterations in host cells, which could result in the onset of cancer [17] and nevertheless, the available evidence is inconclusive, highlighting the necessity for further research to establish a definitive causal link between these viruses and prostate cancer [18].

2 Material and Methods

2.1 Sample Collection

A total of 74 prostate tumor tissue blocks were from the archives of AL-Sadder Teaching Hospital and private histopathology laboratories in AL-Najaf city. The samples encompassed a timeline from January 2023 to December of the same year, providing a diverse representation of prostate cancer cases during this period.

2.2. DNA Extraction from FFPE Samples

For the efficient extraction of DNA from Formalin-fixed, paraffin-embedded (FFPE) tissue, the HiPure FFPE DNA Kit was employed. This kit utilizes silica gel column purification technology, obviating the need for labor-intensive methods such as phenol-chloroform extraction or alcohol precipitation. The entire extraction process was completed in a short span of 20 minutes, excluding digestion time.

Transfer of Samples: Approximately 150 (or 300) μl virus-infected tissue or cells were transferred into 1.5ml microcentrifuge tubes.

Lysis and Digestion: To each sample, 250 (or 500) μl of Lysis buffer was added. After a 15-second vertexing, the samples were incubated at room temperature (15-25 °C) for 10 minutes. Subsequently, 20μl of Proteinase K Solution (20mg/ml) was added, followed by an incubation step at 55°C for an additional 10 minutes.

Binding and Purification: Following the digestion step, 350 (or 700) μl of Binding buffer was added to each sample, and thorough mixing was achieved by gentle vertexing. The lysates were loaded onto spin columns, and centrifugation at 13,000 rpm for 1 minute facilitated binding and purification.

Washing Steps: The columns underwent two washing steps: first with 500μl of Washing buffer-A and then with 500μl of Washing buffer-B. Each washing step involved centrifugation for 1 minute at 13,000 rpm.

Elution of DNA: The purified DNA was eluted from the column by adding 30-60μl of Elution buffer directly onto the membrane. After a 1-minute incubation at room temperature, centrifugation for 1 minute at 13,000 rpm yielded the final eluted DNA.
2.3 Viral DNA Extraction

Viral DNA extraction was carried out using the Viral Gene-spin™ Viral DNA/RNA Extraction Kit.

Sample Preparation: A 150μl volume virus-infected tissue or cells was transferred to a 1.5ml microcentrifuge tube.

Lysis and Binding: To each sample, the appropriate volume of lysis buffer was added, and the mixture was incubated. Following incubation, the lysates were loaded onto spin columns, and centrifugation facilitated binding.

Washing Steps: Two washing steps were performed with specific washing buffers, each followed by centrifugation.

Elution of DNA/RNA: The final elution of viral DNA/RNA was achieved by adding an elution buffer, and the resulting solution was ready for downstream applications.

The extracted DNA samples were then subjected to molecular detection of JCV DNA using the Probe RT-qPCR assay.

Statistical Analysis

All values are expressed as mean ± standard deviation of mean and the study results were statistically analyzed using the software statistical package for the social sciences (SPSS) version 24. The Chi-square test (χ²) was employed for categorical data analysis A P ≤ 0.05 was considered significant [19].

3 Results

In present study 74 Formalin-Fixed Paraffin-Embedded (FFPE) samples from patients with prostate cancer the BK virus was investigated by using Real Time qPCR (table 1). The results showed there where none of the prostate cancer tissues analyzed showed any presence of the virus and the outcomes, illustrated in figure (1) indicated a consistent absence of the virus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primer name</th>
<th>5'-3'</th>
<th>Size</th>
<th>Number</th>
<th>Type of application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BKV</td>
<td>BKV-UF</td>
<td>CCTCAATGGATGGTTGCCTTTAC</td>
<td>141</td>
<td>AB3010 99</td>
<td>RT-qPCR</td>
<td>UGene, 2023</td>
</tr>
<tr>
<td>BKV</td>
<td>BKV-UR</td>
<td>ACTTGACGGGGTCCCTTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKV</td>
<td>BKV-VIC</td>
<td>ACGGGACTGTAACACCTGCTCTTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BKV</td>
<td>F</td>
<td>TTGCTTCCATCAGGCAA</td>
<td>84</td>
<td>AB3010 99</td>
<td>Conventional PCR + Sequencing</td>
<td>Ryschkewitsch et al., 2004</td>
</tr>
<tr>
<td>BKV</td>
<td>R</td>
<td>AGTCTGGATGAGTCCCTTAATG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Amplification plots for detection BKV in FFPE PCa by Real-time qPCR.

The study including examination 74 FFPE samples from prostate cancer patients, JCV was investigated by using Real Time qPCR (table 4-2) and 11 samples were positive for the presence of JCV viral DNA as shown in figure (4-2).

Table 2. JCV virus primers.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Primer name</th>
<th>5′-3′</th>
<th>size</th>
<th>Accession number</th>
<th>Type of application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCV</td>
<td>JCV-UF</td>
<td>CCAATGTGCAATCTGGTGAAT</td>
<td>114 bp</td>
<td>NC_001699</td>
<td>RT-qPCR</td>
<td>UGene, 2023</td>
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<tr>
<td></td>
<td>JCV-UR</td>
<td>TACAGTCCGTACACCCTAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JCV-FAM</td>
<td>TCTGCTCCTCAATGGATGTTGCTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCV</td>
<td>F</td>
<td>TCTTCTGGTTCCTCTGGGTAAAA</td>
<td>530 bp</td>
<td>NC_001699</td>
<td>Conventional PCR + Sequencing</td>
<td>UGene, 2023</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GAATGGGAAATCCTGGTGGAATA</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Fig. 2. Amplification plots for detection JCV in FFPE PCa by Real-time qPCR.

Based on the information present in the provided data table (3), it is evident that there exists a notable connection between the presence of the JC virus (JCV) and the development of prostate cancer. Among the 74 prostate cancer patients who were examined, 11 individuals (14.864%) were found to have tested positive for the JC virus, while the remaining 63 individuals (85.135%) tested negative and these results were highly significant (P≤0.0001).

<table>
<thead>
<tr>
<th>JCV</th>
<th>NO. of examination of prostate cancer patients blocks (74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
</tr>
</tbody>
</table>

Table 3. Incidence of viral infection (JCV) in prostate cancer patients.

$X^2 = 36.541 \ P. \ value: 0.0001 \ H. \ Significant \ (S)$

4 Discussions

Prostate cancer (PC) is the most commonly diagnosed cancer in men and the potential impact of infectious agents in triggering prostatic inflammation and leading to cancer development has been a subject of debate and ambiguous findings are present regarding the involvement of human polyomaviruses BK (BKV) and JC (JCV) in the causes of prostate cancer [20,21]. The findings illustrated in table (1) and figure (1), aligns with the findings [22], who inspected samples preserved in paraffin wax and similarly found no DNA traces. Upon comparison, [23] documented a minimal and statistically insignificant occurrence of BKV DNA, unlike our investigation. The association of the BKV virus with prostate cancer was confirmed by [24]. The discovery of JCV DNA in samples of prostate cancer initiates a conversation about the possible link between JCV infection and the development of prostate cancer [25,26,27] indicates there were a potential connection between JCV and different types of cancer, such as prostate cancer. The JCV virus is recognized for its capacity to induce cancer in various situations, mainly by disrupting the regulation of the
cell cycle and preventing programmed cell death [28,29]. The outcomes of present study are improved by evidence from other studies that indicated that JCV may disrupt cellular signaling pathways in prostate cells and this disruption can affect cell proliferation and survival, potentially initiating tumorigenesis [30,31]. Exploring the connection between JCV status and treatment response might influence the decisions made in therapy [32,33] and certain researchers [34,35] have indicated that detecting the JCV virus in tumor samples has led medical professionals to implement more rigorous treatment plans for affected individuals, than in infected without virus as they opt for less aggressive therapies to mitigate potential treatment-related toxicity and those studies further reinforces the findings from our investigation into identifying the virus. JCV infection in prostate cancer could hinder the body's natural defense against tumors, enabling cancer cells to go undetected by the immune system. this evasion of the immune response may aid in the advancement and spread of the tumor [36,37]. In general, although the clinical implications of finding JCV in patients with prostate cancer are still being understood and additional research is necessary to completely grasp the prognostic and predictive importance of JCV status and its possible influence on treatment choices and clinical management approaches. These results indicate a robust relationship between the existence of the JC virus and the occurrence of prostate cancer and essentially, those individuals who received a positive result for the JC virus are significantly more inclined to have prostate cancer compared to those who tested negative. These discoveries could have important implications in the field of clinical diagnosis and the treatment of prostate cancer and these screening for the JC virus might potentially act as an indicator or a risk element for the development or progression of prostate cancer. Nevertheless, additional research and large samples may be necessary to comprehend the underlying mechanisms of this connection and its detailed clinical repercussions. The findings indicate a robust connection between JC virus infection and prostate cancer and the notably higher percentage of positive JC virus tests in prostate cancer patients, as opposed to negative tests, suggests that JC virus infection could be a contributing factor to the development of prostate cancer [38]. The results could have important clinical consequences and checking for JC virus infection might be a valuable sign or risk factor in pinpointing people with a greater likelihood of developing prostate cancer and detecting and intervening early in these instances could enhance clinical results as mention by [39,40]. The significance of taking viral infections, like the JC virus, into account in prostate cancer diagnosis and treatment cannot be underestimated and including JC virus screening as a standard procedure for patients with prostate cancer might assist in detecting the disease early and tailoring treatment plans to individuals [41,42].The research presents convincing evidence of a notable link between JC virus infection and prostate cancer and these findings underscore the importance of screening for JC virus infection in prostate cancer patients and indicating the necessity for additional research to unravel the underlying mechanisms and clinical significance of this connection.

References

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34. Ó. Fernández, Therapeutic advances in neurological disorders, 6(2), 69-79 (2013).