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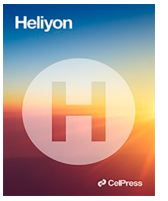

Case report

Concurrent high risk HPV35, HPV45, and HPV59 infections in prostate and bladder cancer tissues of a single patient: A case report

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ABSTRACT

Human papillomavirus (HPV) infections, primarily transmitted through sexual contact, have been linked to various cancers, including cervical, penile, anal, oropharynx, breast, and prostate cancers. This study presents a unique case of concurrent high-risk HPV35, HPV45, and HPV59 infections in both prostate and bladder cancer tissues from a single patient, representing the first documented instance worldwide with identical HPV types detected in two adjacent organs of the same individual. Employing a multiplex-PCR approach, gel electrophoresis, and Sanger sequencing, we confirmed the presence of these high-risk HPV types. Additionally, Western blot analysis using an HPV E7 antibody demonstrated the active expression of HPV oncoproteins in both cancer types. This discovery underscores the potential for HPV intra-organ transmission and necessitates further exploration of alternative transmission routes. The implications of our results offer new insights into the complex dynamics of HPV transmission in cancer pathogenesis. In conclusion our study reveals concurrent HPV infections in both prostate and bladder cancers within a single patient and highlights the potential intra-organ spread of HPV and the need for further investigation of alternative transmission routes.

1. Introduction

Human papillomavirus (HPV) is a sexually transmitted virus that can cause infections through abrasion of the skin and sexual intercourse [1,2] Over 200 HPV types have been identified and categorised as low-risk and high-risk based on their association with neoplastic growth. Infections by high-risk HPVs have been implicated in various cancers, such as cervical, penile, anal, oropharynx cancers, as well as breast and prostate cancers, emphasizing the oncogenic potential of this viral family as suggested by existing literature [3–10]. The relationship between bladder cancers and HPV as possible carcinogen was first proposed by Li et al. (2011) stating that high-risk HPV may play a contributing role in bladder carcinogenesis [11].

While the detection of HPV in various cancers raises questions about its potential role, direct evidence of HPV transmission from the site of initial infection to adjacent organs within the same individual remains an unexplored aspect. The metastatic spread of HPV-associated cancers to distant organs has been documented in rare cases, highlighting the need for further investigation into the

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mechanisms underlying viral transportation and its implications for cancer development [12,13].

In this study, we presented a unique case of concurrent high-risk HPV35, HPV45, and HPV59 infections in prostate and bladder cancer tissues of a single patient. To the best of our knowledge, this is the first documented case worldwide demonstrating the presence of identical HPV types in two adjacent organs of the same patient, shedding light on the potential transport of HPV from an organ of initial infection to a nearby organ. Therefore, this discovery not only expands our understanding of HPV-related oncogenesis but also provides an insight into the mechanism of HPV transmission within human body.

2. Case presentation and methodology

2.1. Case presentation

An 87-year-old Caucasian male patient, a smoker, with a history of multiple health conditions including recurrent visible haematuria and extended-spectrum beta-lactamases (ESBL) in urine, was admitted to Kingston Hospital. Initial cystoscopy examination revealed suspected bladder lesions, which upon resection pathology displayed a low-grade, non-muscle-invasive bladder carcinoma with a clinical stage of (pT1B). Diagnostic imaging, including abdominal and pelvic computed tomography scans (CT), was negative for metastatic lesions. One year later, this progressed to a metastatic transitional cell carcinoma (TCC) of the bladder involving the prostate and Pallative care was administered. Bladder and Prostate specimens were obtained from this patient following a written informed consent, approved by IRAS (Project ID 204705 – Leicester Central Research Ethics Committee) to investigate the presence and the expression of high-risk (HR) HPV genotypes in bladder and prostate cancer biopsies.

2.2. Detection and genotyping of HPV DNA

Cellular DNA was extracted from the collected bladder and prostate cancer tissue samples by using Gen Elute RNA/DNA/Protein Purification Plus kit (Sigma-Aldrich, UK) in accordance with the manufacturer protocol. The concentration and purity of the extracted DNA were assessed using NanoVue plus spectrophotometer (GE Lifesciences, Chicago, Illionis, USA). A multiplex-PCR approach was employed for simultaneous amplification of four targeted regions of the HPV gene using the Amplisens PV-HCR Genotype-Eph kit (AmpliSens, Bratislava, Slovak Republic). The HPV DNA amplification was carried out in three separate reactions as per manufacturer protocol, each distinctly amplifying the following HPV types: 16/31/33/35, 18/39/45/59, and 52/56/58/66. This methodology enabled the identification of HPV infections and co-infections of 12 distinct high-risk HPV subtypes in each sample. The PCR experiment was repeated in triplicate for each sample to ensure data accuracy. Positive amplification of the internal control β-globin gene (fragment size 723 bp) was observed in all samples analysed, indicating the quality of the extracted DNA. For the analysis and determination of HPV genotypes, amplified PCR products were electrophoresed on a 3 % (w/v) agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, CA, USA). β-globin amplification in each sample served as an internal control to confirm the adequacy of the extracted DNA for amplification. Gel visualization under UV was conducted using a Gel Doc XR + System (Bio-Rad, Hercules, CA, USA).

2.3. HPV DNA sequencing

To confirm the presence of HPV genes, PCR-amplified products from positive samples were subjected to direct sequencing following purification with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Sequencing was performed with an Applied Biosystems 3730xL analyser. The resulting data were analysed using the NCBI BLAST software [13,14]. This method served as an additional means of validating the obtained results.

2.4. Western Blotting

To investigate the expression of HPV oncoproteins in bladder and prostate cancer tissue samples, a standard semi-dry Western blotting technique was performed. Total proteins extracted using Gen Elute Protein Purification kit were electrophoresed in equal amounts. The membrane was blocked with 5 % bovine serum albumin (BSA) in TBST (10 mM Tris-HCI, pH 7.5, 150 mM NaCI, and 1 % (v/v) Tween 20) at room temperature for 2 hours to reduce nonspecific binding and incubated with anti-HPV E7 monoclonal antibody (Cervimax) – Valdospan GmbH, Austria, which reacts with a wide range of HPV subtypes, followed by Donkey anti-mouse (1:10,000) and actin antibody (1:10,000). The membrane was then visualized using the OdysseyClx Imaging System (Li-COR).

3. Results

3.1. Detection of HPVs 35,45 and 59 in bladder and prostate cancer

To identify high-risk HPV types in prostate and bladder cancer specimens, targeted regions of HPV genes were subjected to PCR amplification. The amplification of the β-globin gene, with a fragment size of 723 bp yielded positive results for all analysed samples. To ensure the reliability of the obtained data, the PCR experiment was meticulously repeated in triplicate, confirming the accuracy of the results across all samples. As a standardised protocol, each sample run in gel electrophoresis included both HPV positive controls and negative controls, ensuring the validity of the experimental setup.

3.2. Presence HPV35 in bladder and prostate cancer

The gel electrophoresis pattern of four high-risk HPV types (16/31/33/35) for a fresh bladder cancer sample (ID89)(Fig. 1A) and prostate cancer sample (ID129) (Fig. 1B) from same patient revealed molecular weights of the amplified DNA products comparable to the internal positive control β-Globin (723bp), confirming the adequacy of the extracted DNA. Notably, samples ID89 and ID129 exhibited a DNA band analogous to the HPV35 positive control, while no DNA band was observed for the negative control, confirming the absence of contaminations (Fig. 1). This finding showed that bladder and prostate cancer samples from same patient have a positive for HPV35 infection.

3.3. Presence of HPV45 and HPV59 in bladder and prostate cancer

The gel electrophoresis pattern of four high-risk HPV types (18/39/45/59) for the bladder cancer sample (ID89) (Fig. 2A) and prostate cancer sample (ID129) (Fig. 2B) demonstrated molecular weights of the amplified DNA product comparable to the internal positive control β-Globin (723bp), validating the quality of the extracted DNA. Samples ID89 and ID129 exhibited DNA bands akin to the HPV45 and HPV59 positive controls, respectively. As previously stated, the absence of a DNA band in the negative control confirmed the absence of contaminations. This finding also revealed that bladder and prostate cancer samples from same patient have positive for HPV45 and HPV59 infections.

3.4. Sanger sequencing of HPV DNA

Sanger sequencing was employed to confirm and enhance the validity of the PCR results. The four-colour chromatogram depicts the partial results of the sequencing in Figs. 3–5. Blast analysis revealed a robust concordance of over 90 % in HPV DNA sequences across all samples. This consistent data further affirms the accuracy and reliability of our findings.

3.5. The expression of HPV E7 oncoprotein in prostate and bladder cancer samples

To investigate the active expression of HPV DNA detected in both bladder and prostate cancer tissues, a Western blot analysis was conducted using an HPV E7 antibody. The Western blot analysis confirmed the expression of HPV E7 protein in both bladder and prostate cancer tissues (Fig. 6).

4. Discussion

Prostate and bladder cancers pose significant challenges to global public health, contributing substantial morbidity and mortality [15,16]. Despite extensive research efforts, the precise aetiology of these cancers remains elusive. It has been shown previously that different HR-HPV types, such as HPV16, HPV18, HPV31, HPV35, HPV39, HPV59 have the capacity to cause the development of certain cancers, such as cervical, oral, breast cancers [17]. Hence, it is possible that HPV may also play a major role in the development of prostate and bladder cancers. Indeed, several studies have found a significant number of samples of bladder and prostate cancers expressing HPV oncoproteins [10,18–20]. The identification of HPV types in urinary cancer tissues, along with the viral involvement in the deregulation of tumour suppressor proteins and certain cellular miRNAs (known as oncomiRs), can serve as a potential predictor of individual treatment. This approach aids in identifying patients suitable for different therapeutic regimens [21].

Interestingly, our investigation focuses on a patient diagnosed with concurrent prostate and bladder cancers, providing a unique opportunity to explore the presence and expression of different HPV types. Our results revealed the presence and expression of HPV types 35, 45, and 59 in both organs (Figs. 1–5), a novel finding and according to our knowledge this has not been previously

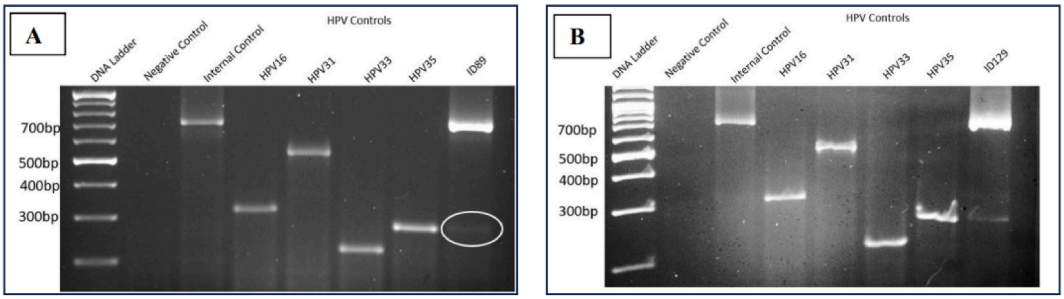

Fig. 1. Gel electrophoresis pattern of HR-HPV types 16/31/33/35 for Bladder (A) and Prostate (B) samples. The figure is a gel electrophoresis pattern of the analysis of four HR-HPV types (16, 31, 33 and 35) using multiplex DNA PCR of bladder tissue (ID89) and prostate tissue (ID129). DNA ladder 100bp plus (100bp-3000bp), HPV 16/31/33/35 = HPV positive DNA controls; HPV16 (325bp), HPV31 (520bp), HPV33 (227bp) and HPV35 (280bp), respectively. ID89 = HPV35 positive bladder cancer sample. ID129 = HPV35 positive prostate cancer sample Internal Control = Internal human DNA amplification control β-Globin (723bp).

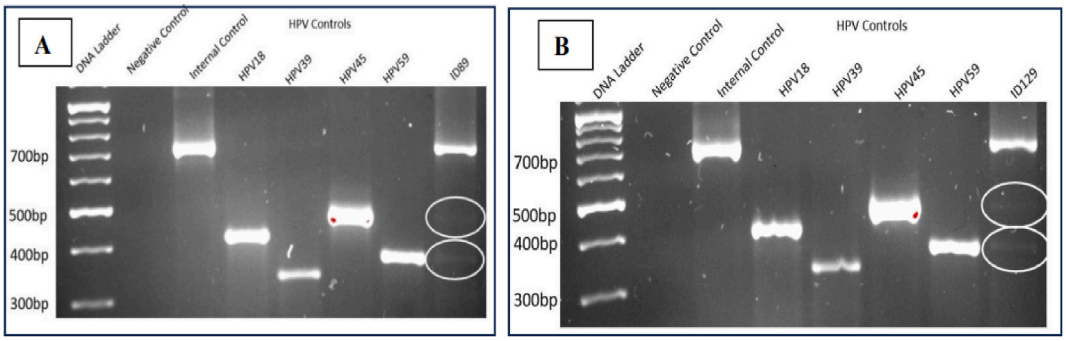

Fig. 2. Gel electrophoresis pattern of HR-HPV types 18/39/45/59 for Bladder (A) and Prostate (B) samples. The figure is a gel electrophoresis pattern of the analysis of four HR-HPV types (18, 39, 45 and 59) using multiplex DNA PCR of bladder tissue (ID89) and prostate tissue (ID129). DNA ladder 100bp plus (100pb-3000bp), HPV 18/39/45/59 = HPV positive DNA controls; HPV18 (425bp), HPV39 (340bp), HPV45 (475bp) and HPV59 (395bp), respectively. ID89 = HPV45 and HPV59 positive bladder cancer sample. ID129 = HPV45 and HPV59 positive prostate cancer sample. Internal Control = Internal human DNA amplification control β-Globin (723bp).

demonstrated in any study worldwide. This significant discovery suggests that HPV may not only be transmitted from person to person through sexual or skin-to-skin contact but also from one organ to another within the same individual. Specifically, our patient exhibits three distinct HPV infections concurrently in two different organs, highlighting the potential for intra-organ transmission.

Contrary to the widely accepted notion that HPV lacks a viraemic phase during infection, our findings prompt consideration of alternative transmission routes. The potential transmission of HPV in peripheral blood, raises the possibility that HPV may transmit haematogenously under certain conditions [22–26]. This alternative route could explain the simultaneous presence of HPV types 35, 45, and 59 in both prostate and bladder cancers in this patient. The exceptional outcome of this case report not only challenges conventional understanding but also contributes substantially to our knowledge of HPV transportation and transmission between different organs.

Finally, our study provides compelling evidence of the presence and expression of diverse HPV types in both prostate and bladder cancers within the same individual. This groundbreaking finding underscores the potential for HPV intra-organ transmission and necessitates further exploration of alternative transmission routes. The implications of our results offer new insights into the complex dynamics of viral transmission in cancer pathogenesis.

5. Conclusion

In summary, our study reveals concurrent HPV infections in both prostate and bladder cancers within a single patient, challenging conventional understanding of HPV transmission. This groundbreaking finding highlights the potential for intra-organ spread and underscores the need for further exploration of alternative transmission routes. Our research sheds new light on the complexity of HPV-associated cancers and informs future efforts in prevention and treatment strategies.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki. Bladder and Prostate specimens were obtained from this patient following a written informed consent, approved by IRAS (Project ID 204705 – Leicester Central Research Ethics Committee) to investigate the presence and the expression of high-risk (HR) HPV genotypes in bladder and prostate cancer biopsies. Written informed consent was obtained from the patient for the publication of all images, clinical data, and other related experimental data.

CRediT authorship contribution statement

M. Yahya Ahmed: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation. **Muharrem Okan Cakir:** Writing – review & editing, Writing – original draft, Validation. **Nadia Aziz Salman:** Writing – review & editing, Writing – original draft, Conceptualization. **Sarbjinder Sandhu:** Writing – review & editing, Resources, Methodology. **G. Hossein Ashrafi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

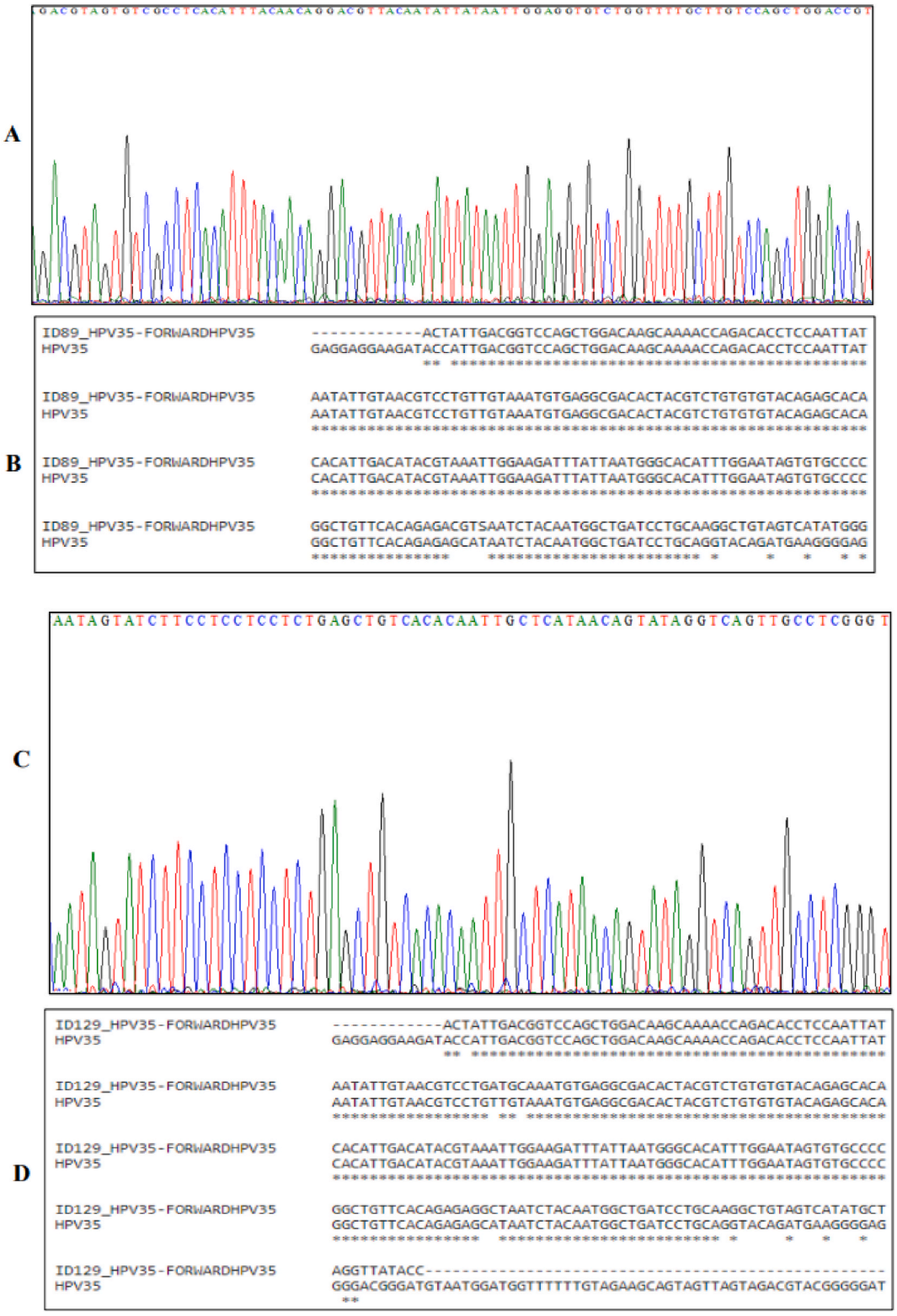

Fig. 3. Sanger sequencing and alignment for HPV35. The four-colour chromatogram showing partial sequence results in bladder sample (A) and prostate sample (C). The sequencing alignment with the program ClustalW after obtaining the corresponding HPV sequences from NCBI BLAST in bladder sample (C) and prostate sample (D).

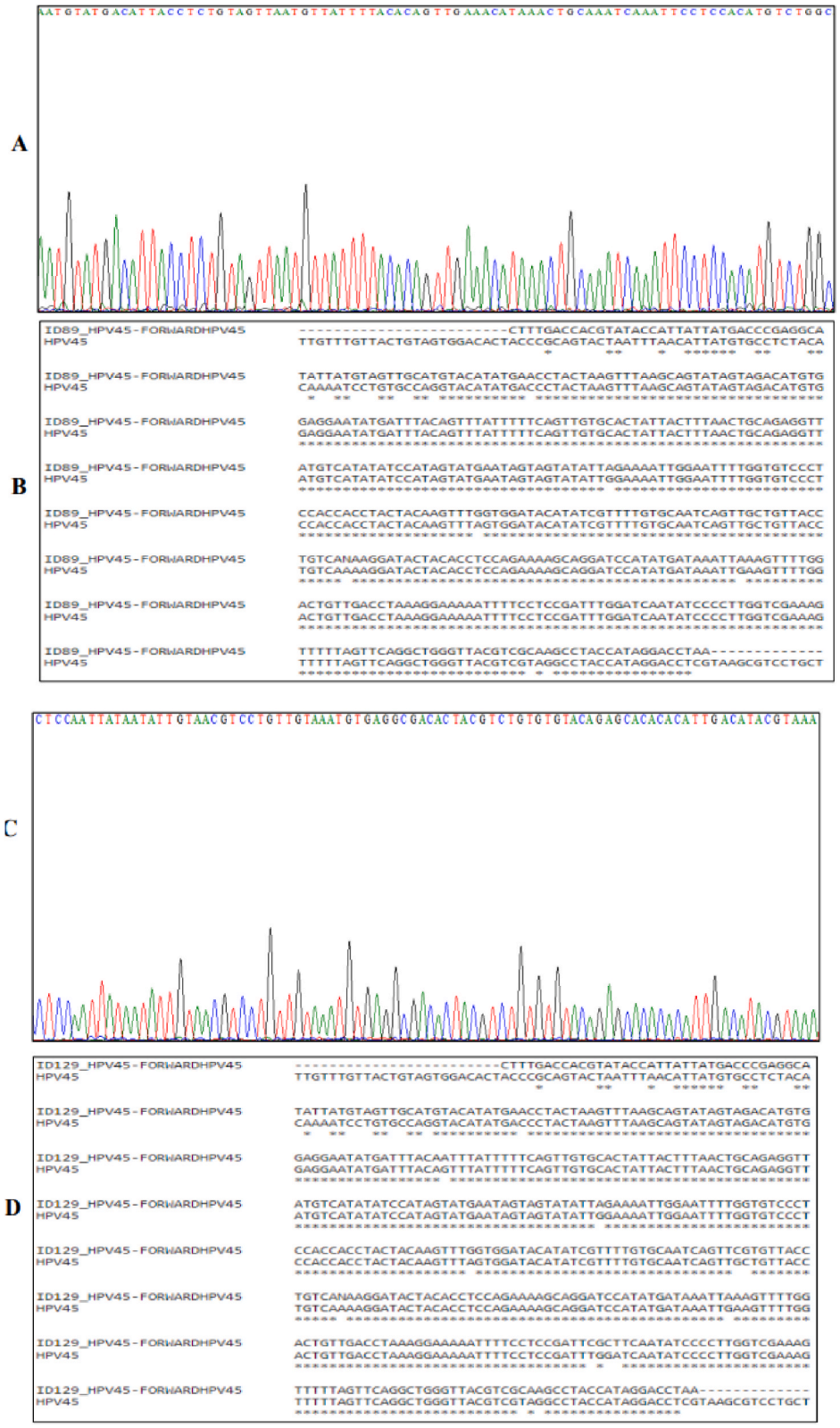

Fig. 4. Sanger sequencing and alignment for HPV45. The four-colour chromatogram showing partial sequence results in bladder sample (A) and prostate sample (C). The sequencing alignment with the program ClustalW after obtaining the corresponding HPV sequences from NCBI BLAST in bladder sample (C) and prostate sample (D).

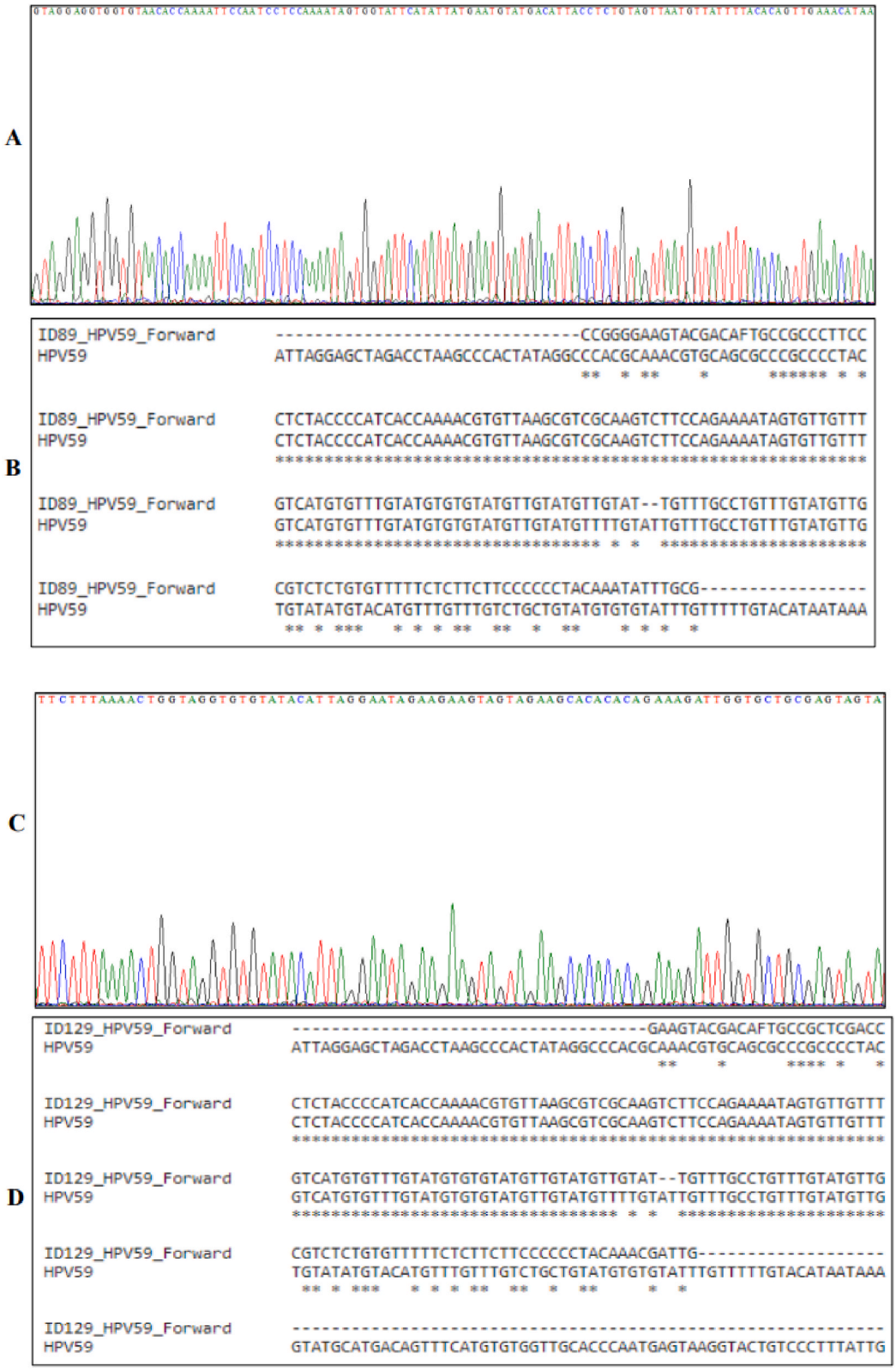

Fig. 5. Sanger sequencing and alignment for HPV59. The four-colour chromatogram showing partial sequence results in bladder sample (A) and prostate sample (C). The sequencing alignment with the program ClustalW after obtaining the corresponding HPV sequences from NCBI BLAST in bladder sample (C) and prostate sample (D).

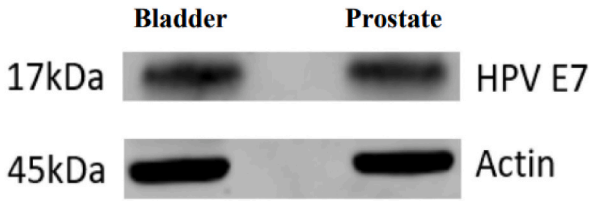

Fig. 6. Western Blot analysis of HPV E7 oncoprotein in bladder and prostate cancer samples of the same patient infected with HPV35, HPV45 and HPV59. The expression of beta-actin was used as a loading control. Western blotting results are representative of the results obtained in three separate experiments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35074.

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