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Urinary shedding of common DNA viruses and their possible association with bladder cancer: a qPCR-based study

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After a decade of human urinary microbiota research, little is known about the composition of the urinary virome and its association with health and disease. This study aimed to investigate the presence of 10 common DNA viruses in human urine and their putative association with bladder cancer (BC). Catheterized urine samples were collected from patients undergoing endoscopic urological procedures under anesthesia. After DNA extraction from the samples, viral DNA sequences were detected using real-time PCR. Viruria rates were compared between BC patients and controls. A total of 106 patients (89 males and 17 females) were included in the study. Fifty-seven (53.8%) were BC patients and 49 (46.2%) had upper urinary tract stones or bladder outlet obstruction. The viruses detected in the urine were human cytomegalovirus (2.0%), Epstein-Barr virus (6.0%), human herpesvirus-6 (12.5%), human papillomavirus (15.2%), BK polyomavirus (15.5%), torque teno virus (44.2%), and JC polyomavirus (47.6%), while no adenoviruses, herpes simplex virus 1 and 2, or parvoviruses were found. There were statistically significant differences in HPV viruria rates between cancer patients and controls (24.5% vs. 4.3%, p=0.032 after adjustment for age and gender). Viruria rates increased from benign to non-muscle-invasive and muscle-invasive tumors. Patients with a history of BC have higher HPV viruria rates than controls. Whether this relationship is a causal one remains to be established by further research.

Key words: bladder cancer; herpesviruses; human papillomavirus; torque teno virus; polyomaviruses

The clinical assumption of healthy urine being sterile has been challenged in the past decade when live bacteria were cultured and 16s rDNA of many others was sequenced from urine samples of healthy asymptomatic individuals [1, 2]. There is also evidence of viral DNA presence in human urine. Most viruses detected in urine are bacteriophages (bacteria-infecting viruses) [3] but some eukaryotic viruses have also been found [4]. There has been an in-depth study of some of the viruses present in human urine, e.g., BK polyomavirus (BKPyV), which is associated with renal allograft failure [5]. The shedding of other viruses [6] has been addressed only marginally or not at all. The biological significance of viruria remains unclear as does its potential clinical use.

Bladder cancer (BC) ranks as the 10th most common neoplasm in humans (worldwide age-standardized incidence rate is 9.5 for men and 2.4 for women per 100,000 person/years) [7]. While tobacco smoking is the leading etiological factor for urothelial BC, squamous cell BC is associated with *Schistosoma haematobium* and with chronic urinary tract infection [7]. Several studies have suggested a potential role for viruses in the etiopathogenesis of BC [8] whereas others reported contradictory results [9]. Seventy-five percent of BC patients present with a non-muscle invasive stage of the disease (NMIBC) and up to 40% of them may progress to muscle-invasive bladder cancer (MIBC) within five years, which is associated with markedly worse overall survival [7].

The Cancer Genome Atlas project of urothelial bladder carcinoma indicates that viral infections may have a role in the development of a small percentage of urothelial carcinomas. Viral DNA was found in seven of 122 tumors (5.7%), and viral transcripts were detected in five of 122 (4.1%) analyzed samples. Three tumors expressed human cytomegalovirus (HCMV) transcripts, and BKPyV expression or human papillomavirus (HPV) 16 expression was found in one tumor each [10].

The aim of the present study was to investigate the association between a history of urinary BC and the presence of viral DNA in human urine. A secondary objective was to establish the prevalence of common DNA viruses – a subset of the urinary virome – in urine.

Patients and methods

Study population. The study participants were recruited among consecutive patients undergoing endoscopic procedures for benign or malignant conditions of the urinary tract at the Department of Urology, 3rd Medical Faculty of Charles University and Thomayer Hospital, Prague, Czech Republic between August 2019 and February 2021. The hospital covers the respective catchment area and also serves as a tertiary referral center. Inclusion criteria were a negative result of standard urine culture preoperatively, no foreign body in the urinary bladder (such as an indwelling catheter, ureteric stent, or bladder stones), and no antibiotic treatment for any medical condition in the past six weeks. Patients with a preoperative culture positive for microbial growth exceeding 10^3 colony forming units/ml were excluded, as were those not consenting to participate. Urine specimens were collected in the theatre at the beginning of the procedure after disinfection of the genital area and surgical draping, immediately upon endoscope insertion. A water-based lubricant without disinfectant was used. Samples were stored at –20 °C until DNA extraction.

Cancer patients (cases) were those with a history of histopathologically confirmed NMIBC who attended for transurethral resection of tumor recurrence and those with a primary occurrence of BC in whom the tumor was histopathologically confirmed after this primary resection. Tumor recurrence is a common feature of BC reported in up to 78% of patients at five years after diagnosis and is likely the result of genomic instability [11] of the affected individual's urothelium [7]; clinically speaking, this is a chronic illness. Patients with a previous history of histopathologically proven BC were therefore included among cases even when the current resection did not yield a positive result. Controls were subjects without a previous history of BC who were undergoing endoscopic procedures for benign disease.

The study was conducted in accordance with the Declaration of Helsinki after previous approval by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital No. G-19-01, and informed consent was obtained from all participants prior to the enrollment.

DNA extraction and PCR. Deoxyribonucleic acids were extracted from urine using the Eligene Urine Isolation Kit (Elisabeth Pharmacon, Brno, Czech Republic) according to the manufacturer's instructions. Briefly, 10 ml urine was vortexed for 15 s and centrifuged at 6,000×g for 20 min, the supernatant was discarded, the pellet was resuspended in 200 µl of molecular grade water and 200 µl of MI3 solution, and 20 µl of proteinase K were added. After vortexing for 15 s, the mixture was incubated for 15 min at 65 °C. The lysate was centrifuged at 6,000×g for 5 min. The supernatant was transferred to a microtube and 210 µl of MI4 solution was added. The lysate was centrifuged for 1 min at 13,000×g. The presence of PCR inhibitors was checked by amplification of the reference beta-globin gene as described previously [12].

Viral DNA was detected using real-time PCR technology (qPCR) with the previously published primers and probes (Supplementary Table 1). For all qPCR assays, the fluorescent 5 6-FAM/3 BHQ-1 DNA probes were used, and the reactions were performed with HotStart Taq Polymerase (Qiagen, Germany) on the Biorad CFX96 Real-Time PCR system (Biorad Laboratories, California, USA). The temperature profile for all qPCR assays was 15 min at 95 °C followed by 45 cycles of 15 s at 94 °C and 1 min at 60 °C. The protocols were optimized to achieve maximum sensitivity and specificity for each viral target. The reaction consisted of 1×PCR buffer, 5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM concentration of each forward and reverse primers and 0.25 µM probe, 0.25 U HotStart Taq Polymerase (Qiagen, Germany), and DNA template. The analytical sensitivities of qPCRs were one copy of the plasmid standard per assay. Protocols were validated using clinical specimens and plasmids with viral target sequences. All PCR assays were performed in duplicate.

HPV DNA was detected by a combination of two PCR assays with primer sets specific for the L1 region MY09/11 and BS GP5+/6+ bio [12]. The genotyping was done using the amplicons of PCR with primers BS GP5+/6+ bio by the reverse line blot hybridization (RLB) with probes specific for 37 types as specified in detail in Tachezy et al. [12]. Furthermore, positive samples from both PCR assays, not genotyped by RLB, were further analyzed by Sanger sequencing.

Statistical analyses. Clinical and demographic data were analyzed and reported as median ± interquartile range (IQR) for continuous variables and counts with percentages for categorical variables. The Mann-Whitney and Chi-square tests were used to compare clinical variables between cases and controls. The Chi-square test or Fisher's exact test was used to test the association between viruria and BC. Multiple logistic regression was performed to adjust viral positivity for age and gender. No formal sample size calculation was performed because little is known about viruria prevalence and the expected effect size. IBM SPSS Statistics software version 28 (IBM Corp, Armonk, NY, USA) was used for statistical calculations. An alpha level of $p<0.05$ was considered statistically significant.

Next Generation Sequencing (NGS). Two urine samples were subjected to NGS as described previously [13]: 40 ml of urine samples were centrifuged at 180×g for 15 min at 4 °C to pellet, and the mammalian cells were removed. The supernatant was ultra-centrifuged at 100,000×g for 2 h at 4 °C to reduce the volume to 1 ml. The reduced samples

were processed using the NetoVir protocol and sequenced on NextSeq 500 (Illumina, San Diego, CA, USA) with paired end sequencing of 2×150 bp (IMG, Czech Republic). In the second protocol, the WTA2 kit was replaced by a rolling circle reaction done by Repli-G (Qiagen, Hilden, Germany), and Nextera tagmentation was extended to 5 min.

Reads were trimmed with Trimmomatic and checked before and after trimming with FastQC. Assembly was done with SPAdes --meta, -k 21,33,55,77, and the obtained contigs were blasted against nr database (downloaded on 08.24.2021) with Diamond. Reads were mapped back to the contigs with bwa-mem, and Kronatools were used to visualize the taxonomic classification for each sample. To improve the assembly of reads, the MIRA sequence assembler was also applied, two scaffold files (SPAdes and MIRA) with Minimus2 were merged, and the resulting scaffold was further processed as specified before.

Results

Demographic and clinical data. A total of 106 subjects (89 males and 17 females) were included in the study. Fifty-seven (53.8%) of them were BC patients undergoing transurethral resection of bladder tumor and 49 (46.2%) were treated for upper tract urinary stone disease or bladder outlet obstruction. The non-cancer patients served as controls. Bladder tumors were classified as NMIBC except for five cases of MIBC. The ratio between sexes reflects the reality of BC incidence. To avoid misinterpretation, all statistical results were adjusted for age and gender.

BC patients were older than controls (71.7 vs. 57.8 years, p<0.0001) and more likely to be hypertensive (p=0.042) and hyperlipidemic (p=0.003). No statistically significant differences were found for other clinical or demographic variables including smoking history and body mass index (Table 1).

Viruria rates of DNA viruses. HCMV DNA was only detected in 2.0% of the urine specimens and Epstein-Barr virus (EBV) DNA in 6.0%. Human herpesvirus-6 (HHV-6), HPV, and BKPyV DNA was detected in 12.5%, 15.2%, and 15.5% of specimens, respectively. The highest viruria rates were found for torque teno virus (TTV) and JC polyomavirus (JCPyV): 44.2% and 47.6%, respectively. No DNA of adenoviruses, parvoviruses, or human herpesvirus 1 and 2 (HSV-1 and HSV-2) was detected. Table 2 shows the rates of DNA viruria in BC patients and cancer-free controls.

Of 86 samples screened for all 10 DNA viruses, 20.9% remained negative for any viral DNA, 34.9% tested positive for a single virus, and 44.2% showed co-infection by two or more (up to five) DNA viruses (Figure 1). Co-infection with four or five viruses was only detected in BC patients. The number of viruses did not vary significantly between the BC patients and the controls (p=0.385).

Association of viruria with BC. Patients with a history of BC were more likely to harbor HPV (any type, 24.5% vs. 4.3%, p=0.005) and TTV (53.6% vs. 33.3%, p=0.038) in their urine than controls. After adjustment for age and gender, the statistical difference remained significant only for HPV (p=0.032). Out of 15 subjects in whom HPV DNA was detected, eight were classified as high-risk (HR) and seven as low-risk (LR) HPV. Seven HR (13.2%) and six LR HPV (11.3%) were found in cancer patients and one HR HPV (2.2%) and LR HPV (2.2%) each were detected in the control group. Three HPV types (18, 32, and 107) were identified in MIBC (Table 3).

The viruria rate increased from controls to NMIBC to MIBC for all viruses except polyomaviruses (Figure 2). No association was demonstrated between histopathological grading of the tumor and viruria rate (data not shown).

Next Generation Sequencing (NGS). NGS yielded a total of 19 and 20 million reads, respectively. Assembling the viral reads into contigs resulted in 370,833 contigs for one sample,

Table 1. Clinical and demographic variables of the study cohorts (n=106).

Variable	cancer patients n=57	cancer-free controls n=49	p-value*
Sex, male (%)	44/57 (77.2)	45/49 (91.8)	0.062
Age, median (IQR)	74.1 (68.9-76.1)	59.9 (46.4–71.3)	<0.0001
BMI, median (IQR)	28.1 (25.0-29.8)	27.6 (24.8–30.7)	0.753
Hypertension, n (%)	38/57 (66.7)	21/45† (46.7)	0.042
Diabetes, n (%)	18/57 (31.6)	9/45 (20.0)	0.188
Hyperlipidemia, n (%)	26/57 (45.6)	8/45 (17.8)	0.003
Chronic kidney disease[1], n (%)	12/43 (27.9)	7/38 (18.4)	0.315
History of instillation treatment for bladder cancer[2], n (%)	8/47 (17.0)	NA	
History of pelvic radiotherapy, n (%)	4/57 (7.0)	0/46 (0.0)	0.126
Current smoker, n (%)	14/47 (29.8)	9/38 (23.7)	0.529

Notes: [1]estimated glomerular filtration rate (CKD-EPI eGFR) <60 ml/min; [2]mitomycin-C, epirubicin, or Bacillus Calmette-Guérin; *chi-square, Mann-Whitney or Fisher's exact test as appropriate; †not all clinical variables were available for all study subjects.
Abbreviations: IQR-interquartile range; BMI-body mass index

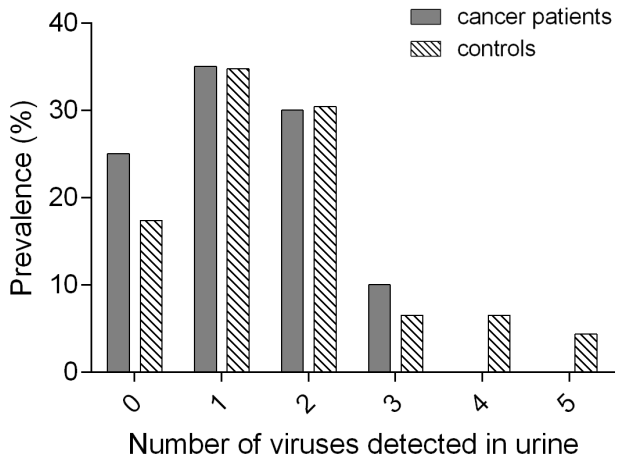

Figure 1. The number of DNA viruses detected in the urine of bladder cancer patients and cancer-free controls. No viral DNA was detected in 20.9%, a single virus was only found in 34.9%, and co-infection by two or more (up to five) DNA viruses was observed in 44.2%. The numbers of viruses did not vary significantly between bladder cancer patients and controls.

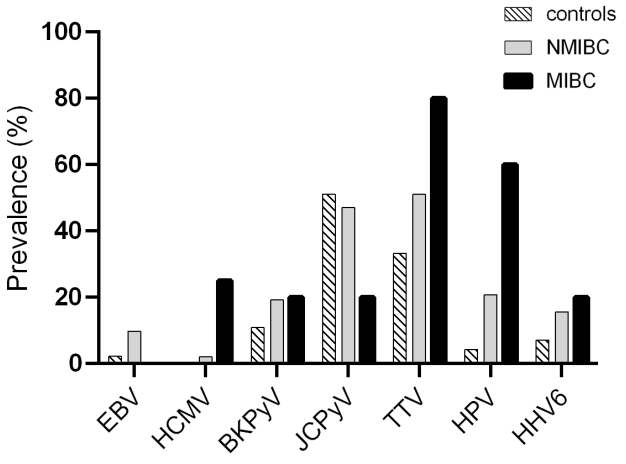

Figure 2. Viruria rate (%) according to cancer status. Viruria in cancer-free controls, non-muscle invasive bladder cancer (NMIBC), and muscle-invasive bladder cancer (MIBC) patients. Viruria rates increase from controls to NMIBC to MIBC except for polyomaviruses. Abbreviations: EBV-Epstein-Barr virus; HCMV-human cytomegalovirus; BKPyV-BK polyomavirus; JCPyV-JC polyomavirus; TTV-torque teno virus; HPV-human papillomavirus; HHV6-human herpes virus-6

Table 2. Prevalence of viral DNA in urine samples of cancer patients and controls.

Virus	n tested total	Number of positive (%) cancer patients	Number of positive (%) cancer-free controls	p-value	p-value*
Epstein-Barr virus	100	5/55 (9.1)	1/45 (2.2)	0.219	
Human cytomegalovirus	100	2/54 (3.7)	0/46 (0.0)	0.498	
BK polyomavirus	103	11/57 (19.3)	5/46 (10.9)	0.284	
JC polyomavirus	103	25/56 (44.6)	24/47 (51.1)	0.557	
Torque teno virus	104	30/56 (53.6)	16/48 (33.3)	**0.038**	0.086
Adenoviruses	100	0/55 (0.0)	0/45 (0.0)	NA	
Human papillomavirus	99	13/53 (24.5)	2/46 (4.3)	**0.005**	**0.032**
Parvoviruses	91	0/50 (0.0)	0/41 (0.0)	NA	
Herpes simplex virus 1/2	93	0/51 (0.0)	0/42 (0.0)	NA	
Human herpesvirus-6	92	8/50 (16.0)	3/42 (7.1)	0.218	

Note: due to varying amount of biological material available, not all viruses were tested in all samples; *adjusted for age and gender

Table 3. HPV types detected in the urine of bladder cancer patients and controls.

HPV	Type of HPV	Number of positive (%) cancer patients	Number of positive (%) cancer-free controls
HR HPV		7/53 (13.2%)	1/46 (2.2%)
	HPV 53	2	1
	HPV 33	2	
	HPV 18	1	
	HPV 31	1	
	HPV 52	1	
LR HPV		6/53 (11.3%)	1/46 (2.2%)
	HPV 32	3	
	HPV 6	1	
	HPV 67	1	
	HPV 107	1	
	HPV 42		1

only 394 of them longer than 1 kbp and two over 5 kbp. Assembly of the second sample resulted in 340,044 contigs, with 107 of them longer than 1 kbp and of 5 kbp or more. The N50 (weighted median statistics of the length of contigs) of contigs were 293 bp and 281 bp, respectively. Combining two assemblers (SPAdes and MIRA, see Methods) did not substantially improve the outcomes: the N50 of the length of contigs were 336 bp and 317 bp, respectively. Despite this fragmentation, hits to JCPyV were found for four contigs (none over 500 bp) in one sample and for five contigs (four over 500 bp but under 1 kbp) in another sample. Along with them, we detected several short bacteriophage sequences. To improve the quality of contigs, rolling circle amplification was used in sample processing instead of the WTA2 kit. Even though longer contigs were generated (N50 of 6,688 kbp and four contigs longer than 50 kbp), the sample lacked sequences of viral origin.

Discussion

Human urinary microbiota research has revealed differences in bacterial community composition between BC patients and cancer-free controls. In the present study, we investigated the putative association between BC and the urinary shedding of common DNA viruses. A statistically significant association was observed for the presence of HPV in urine and a history of BC. For others, we established the rates of viral shedding, some of which have not been previously reported. Of note, catheterized urine samples were used, thereby minimizing the risk of contamination from the prostate and urethra.

HPV. HPV is an established etiological agent of most anogenital cancers; however, the role of HPV in BC is controversial [14]. The conflicting results are likely due to the differences in methodological approach and type of samples used. A meta-analysis of 17 tissue-based studies reported HPV prevalence in tumor tissue of 16.9% and an odds ratio of 2.84 (95% confidence interval 1.39–5.80) of BC being associated with HPV (any type) [15]. This strength of association is similar to that of HPV and breast cancer. Most of the original manuscripts evaluated in this meta-analysis detected both LR and HR HPV types in BC tissues [15]. In our study, HR and LR HPV viruria was significantly more common in BC patients than in controls (24.5% vs. 4.3%, respectively; p=0.005). These rates of HPV shedding in cancer patients and controls are in line with those reported by a recent study (28.9% and 8.7% in cases and controls, respectively) [16]. The rare presence of exclusively HR HPV types in our dataset suggests that HR HPV is unlikely to be the main factor involved in BC. Still, a small fraction of BC cases might be attributed to HPV infection, as HPV was found to be integrated in BC tissue according to the Cancer Genome Atlas project [14]. This might happen probably due to the close anatomic proximity of the urinary and genital tracts and the infection of urinary epithelial cells. Papillomaviral oncoproteins E6 and E7 play an essential role in carcinogenesis via binding to the cellular tumor suppression proteins p53 and pRb [17]. Nevertheless, the role of HPV in BC pathogenesis remains far from proven as evidenced by two latest meta-analyses coming to opposite conclusions [18, 19].

Polyomaviruses. BKPyV and JCPyV asymptomatically infect the pediatric population causing no harm in immunocompetent individuals and persist in renal tissue and uroepithelium [20]. However, BKPyV may cause renal allograft nephropathy in kidney transplant recipients and hemorrhagic cystitis in bone marrow transplant recipients [5, 21, 22]. Reactivation of JCPyV in HIV-positive individuals, patients with hematological malignancies, or those treated with immunomodulatory drugs for chronic inflammatory disorders may result in progressive multifocal leukoencephalopathy. BKPyV and JCPyV urinary shedding was reported in 7.0% and 19.0% of 400 healthy blood donors, respectively [23], and viruria prevalence increases with age [20]. The JCPyV viruria rate in our study is comparable to the age-matched data from the previously mentioned Japanese study [20]. As for BKPyV, our data show a 15.5% prevalence, which is just in the middle of the range (0.8% to 32.0%) reported to date [20–22].

Both polyomaviruses are classified as potential carcinogens by the International Agency for Research on Cancer due to their transforming activity of early gene products *in vitro* in cells and animal models [24]. However, their role in human carcinogenesis has not been proven yet. One urine-based study and several tissue-based studies have investigated the association of polyomaviruses with BC by PCR or qPCR with negative results [8, 21, 25]. Contrary to this, an association between polyomavirus nephropathy and high-grade urothelial carcinomas has been reported worldwide in 20 cases since 2002 [26].

Herpesviruses (EBV, HCMV, HHV-6). Most adults have been exposed to EBV infection by the age of 30. EBV persists in B-lymphocytes lifelong and is periodically asymptomatically shed to saliva. It is associated with Hodgkin lymphoma, Burkitt's lymphoma, and nasopharyngeal cancer. A recent study has shown the presence of EBV-encoded RNA in lymphocytes infiltrating BC stromal tissue in 67.0% of samples while non-neoplastic bladder tissues remained negative. EBV RNA-expressing lymphocytes were detected more frequently in advanced cancer stages, which indicates there might be a role for EBV in BC progression [27]. In our study, the EBV viruria rate was 6/100 (6.0%) with no obvious difference between BC patients and controls. No comparison can be made with the literature data since to the best of our knowledge, this is the first report of EBV urinary shedding so far.

HCMV seroprevalence ranges from 56% to 94% worldwide. After infection at an early age, HCMV may persist in various tissues including the kidneys. Despite the high seroprevalence, urinary shedding rarely occurs except in subjects with sexually-transmitted infection or in immunosuppressed patients [28]. In a large population-based US study of 6,800 subjects, HCMV DNA was detected in 1.4% of urinary samples, similar to the 2.0% prevalence seen in our study participants [29].

HHV-6 has been detected in 37.0% of both cancer and normal bladder tissues [8]. Another study reported only a 6.8% rate of HHV-6 positivity in 74 bladder tumors [30]. In our population, HHV-6 was detected in 11/92 (12.5%) of urine samples with a non-significant difference between cancer cases and controls (p=0.218).

Torque teno virus. TTV is the most abundant eukaryotic virus of the human virome, and TTV DNA is detected in the blood of up to 90% of the population. No human disease has been associated with TTV infection to date. TTV persistently replicates in host cells, with the viral load reflecting the balance between viral reproduction and the host's clearance rate [31]. Immunosuppressed patients have higher viral loads than immunocompetent subjects, and the measuring of TTV

viral loads has been suggested to evaluate the function of the immune system [32]. TTV viral load in blood was higher in patients with various malignancies compared to healthy blood donors [33]. Similarly, TTV viruria was more common in BC patients than in controls in our study, albeit without statistical difference after adjustment for age and gender. It has been shown by us and others that TTV prevalence increases with age and is sex-dependent [34, 35].

TTV DNA was detected by Illumina sequencing in the urine of 12/22 (54.5%) kidney transplant patients [4] and in 66.6% of liver transplant recipients [36]. This is consistent with the 53.6% viruria rate in cancer patients in our study rather than TTV viruria in controls (33.3%) in our study, further upholding the observation of higher TTV presence in cancer and immunosuppression as compared to cancer-free, non-immunosuppressed individuals.

Adenoviruses, parvoviruses, and HSV. Relatively low attention has been paid to the detection of adenoviruses in human urine; while 32 of 398 sexually transmitted diseases clinic patients had adenovirus DNA detectable in their urine samples, only 1/124 (0.8%) controls did [6]. This is in line with the absence of adenoviral DNA in our study population. Most children are infected by adenoviruses before the age of 10, and after primary infection, adenoviruses reside in lymphoid tissue and renal parenchyma. Their reactivation in healthy subjects has mild clinical symptoms but may be lethal in immunosuppressed patients (18.0% mortality in the renal transplant population) [37].

Parvovirus B19 has been detected in the urine of acutely infected children and adults for short periods of time, but shedding is prolonged in immunosuppressed patients [38]. HSV1 was occasionally found in urethral swabs in men with acute urethritis. Neither parvoviruses nor HSV have been implicated in human carcinogenesis; on the contrary, these oncolytic viruses have been studied as a potential cancer treatment.

NGS analysis was performed to determine the composition of the whole virome. Although two protocols were used, the contigs obtained had a small average length and only a few of them matched one virus – JCPyV. Few literature data are available on the urinary virome. One study has come across a similar problem of short contig length [39]. Several subtypes of BKPyV, JCPyV, and TTVs as well as human papillomaviruses were identified in other studies. However, they differ in patients enrolled and methods used for virome analysis.

The limitations of the current study include the absence of information on the study subjects' serostatus relevant to the viruses investigated. It also needs to be acknowledged that the presence of a virus does not imply a causal role of the pathogen in a disease. In fact, the cause and effect of viral presence might be inverted as the tumor microenvironment impairs the function of the immune system [40] and might make the uroepithelium more susceptible to infection by viral pathogens: an increasing trend in viruria rates can be seen from cancer-free controls to NMIBC to MIBC patients although the number of MIBC cases is too small to assess this finding statistically.

Furthermore, the effect on carcinogenesis may be a result of an interplay among several microorganisms, both viral and bacterial, and their metabolome. Future studies might want to look for such a complex relationship using improved next-generation methods (multinomics), considering the whole of urinary virome and bacteriome and clinical variables (such as exposure to environmental carcinogens).

Our study explores viral urinary shedding of 10 DNA viruses in patients with BC and controls with benign disease and provides the first complex overview of urinary viral nucleic acids detected in BC patients. Catheterized urine samples were used to minimize the risk of contamination from the urethra and perimeatal flora. Subjects with a history of BC have higher HPV viruria rates than controls. Whether this relationship is a causal one remains to be elucidated by future research. No association between the history of BC and viruria has been demonstrated for other viruses under investigation. For most viruses, viruria rates increased from controls to NMIBC to MIBC patients.

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