

Epstein-Barr virus and human adenovirus viremia in renal tumors is associated with histological features of kidney cancer

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Abstract

Background. There is a growing evidence that viral infections may impact the risk and clinical course of malignancies, including solid tumors. The aim of this study was to assess the possible association of selected chronic / latent viral infections with the clinical course of renal cell carcinoma (RCC).

Methods. In this prospective study we enrolled 27 patients undergoing partial or radical nephrectomy due to the histologically confirmed RCC and followed them up for one year post-operation. Isolation of the nucleic acids of human adenovirus (ADV), herpes simplex virus HSV-1 and HSV-2, Epstein-Barr virus (EBV), cytomegalovirus (CMV), BK virus (BKV) and John Cunningham virus (JCV) from tumor tissue was performed using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) or EZ1 Virus Mini Kit (Qiagen, Hilden, Germany). The number of viral copies in the tissue was assessed with the real-time PCR.

Results. Viral infections were diagnosed in ten patients (37.0%), including three ADV cases (11.1%) and eight EBV cases (29.6%). Infected patients tended to be significantly older (71.3 vs. 57.6 years, $p < 0.05$), more commonly presented with chronic renal disease (OR 2.4, $p < 0.05$), diabetes (OR 4.2, $p < 0.05$) and overweight (OR 2.0, $p < 0.05$). Regarding oncological data, infected patients were found to have a higher rate of high-grade cancers (OR 5.0, $p < 0.05$) and a higher rate of papillary RCCs (OR 8.3, $p < 0.05$). Status of viral infections had no influence on the clinical cancer stage, surgical procedure or survival.

Conclusions. EBV and ADV infections are common in renal cancer patients and increase the risk of high-grade RCC presence. While there is no significant impact on short term survival, further studies are needed to assess the relevance of these findings in a long run.

Trial registration. Medical University of Warsaw KB/37/2017

Background

Renal cancer is the sixteenth most common cancer worldwide [1]. Among established risk factors, one should list smoking, arterial hypertension and obesity [2–4]. The most common histological type is renal cell carcinoma (RCC), arising from renal tubular epithelium and further divided into three main subtypes, namely clear cell, papillary and chromophobe RCC [5].

The role of latent viral infections in carcinogenesis of RCC remains a matter of debate. Some authors confirmed viral infection in RCC and suggest its role as a risk factor, a predictor of cancer histology and biological behavior or a consequence of immunocompromised tumor environment [6–8]. However, relation between renal cell carcinogenesis, RCC and viral infections is still to be defined. In the meantime, viral infections were linked to several malignancies, both in immunocompromised and immunocompetent patients.

Methods

The aim of this study was to assess the possible association of selected chronic / latent viral infections of RCC tumors with the clinical course of renal cancer.

Patients

In this prospective study we enrolled 27 patients undergoing the surgery due to renal tumor. Their mean age was 62.7 years, male to female ratio was 2.7:1. Twelve (44.4%) and fifteen (55.6%) patients underwent partial and radical nephrectomy, respectively. All patients gave informed written consent to participate in the study. Before study initiation, local review board have approved study protocol.

Apart from the experimental methods described below, all surgical specimens were examined routinely by urological pathologist, who eventually diagnosed RCCs in all patients, including clear cell type in 18 patients (66.7%), papillary type in 6 patients (22.2%) and chromophobe type in 3 patients (11.1%). After the surgery patients were followed-up for one year,

including clinical visits and laboratory tests every three months, as well as chest-abdominal CT scan at 6 and 12 months. As one patient was lost to follow-up, final survival analysis was based on 26 out of 27 patients.

Methods

Before the surgery, blood samples were taken from all participants and sera were frozen at -80°C. After the surgery, tissue homogenates from tumor specimens were tested for the presence of human adenovirus (ADV), herpes simplex virus HSV-1 and HSV-2, Epstein-Barr virus (EBV), cytomegalovirus (CMV), BK virus (BKV) and John Cunningham virus (JCV). After diagnosing EBV and ADV infections in tissue specimens, ADV and EBV nucleic acids were sought in the corresponding serum samples.

Number of ADV, HSV-1/2 and EBV virus copies in renal tumor tissue

DNA isolation from tumor tissue was performed using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. The number of ADV virus copies in the tissue was assessed with the real-time PCR method, using the primer sets and probes described previously [9]. The number of HSV-1/2 and EBV virus copies in the tissue was assessed with the real-time PCR method, using the R-gene Kits kit (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

Number of CMV, BKV and JCV virus copies in renal tumor tissue

Isolation of viral nucleic acids from tumor tissue was performed using the EZ1 Virus Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions with the EZ1 BioRobot device (Qiagen, Hilden, Germany). The number of CMV, BKV and JCV virus copies in the tissue were assessed with the real-time PCR method, using the GeneProof PCR Kit (GeneProof, Brno, Czech Republic) according to the manufacturer's instructions.

Number of EBV and ADV virus copies in serum

Methods described above for CMV, BKV and JCV diagnosis were adopted also for assessment of EBV and ADV virus copies in the serum.

Statistical analysis

Results are presented as absolute values, percentages and mean or median values for variables with or without normal distribution, respectively. Normal distribution was tested with Shapiro-Wilk test. Levene test was applied to assess the equality of variances. For comparisons between study groups, unpaired t-test and Pearson test were used for quantitative and qualitative variables, respectively. P value of < 0.05 was considered statistically significant.

Results

Viral sequences within tumors were diagnosed in tissue specimens from ten patients (37.0%), including eight cases of EBV (29.6%) and three cases of ADV (11.1%). In one patient concomitant EBV and ADV viral sequences were found. For all other examined infections, the results were negative. Also serum tests were negative for viral sequences in all patients. Results of viral tests are presented in Table 1.

Table 1
Results of viral tests in kidney specimens

	No of patients with positive result	Mean number of viral copies / ul (in positive patients)
EBV	8	58.7
ADV	3	68.9
HSV-1	0	-
HSV-2	0	-
CMV	0	-
BKV	0	-
JCV	0	-

Of note, infected patients tended to be significantly older (71.3 vs. 57.6 years, $p < 0.05$), more commonly presented chronic renal disease (70% vs. 29%, OR 2.4, $p < 0.05$), diabetes (50% vs. 12%, OR 4.2, $p < 0.05$) and overweight (80% vs. 41%, OR 2.0, $p < 0.05$). Table 2 presents a comparison of patients depending on status and etiology of viral infection.

Table 2
Comparison of patients depending on status and etiology of viral infection

Parameter	Non-infected patients (controls) (n = 17)	Infected patients (ADV or/and EBV infection) (n = 10)	P value (controls vs. ADV/EBV)	Patients with ADV infection (n = 3)	P value (ADV positive vs. ADV negative patients)	Patients with EBV infection (n = 8)	P value (EBV positive vs. EBV negative patients)
Percentage of women	41.2%	30.0%	> 0.05	66.7%	> 0.05	25.0%	> 0.05
Mean age (years)	57.6	71.3	0.02	73.3	> 0.05	69.9	> 0.05
Mean BMI (kg/m ²)	24.5	27.9	0.01	26.7	> 0.05	28.3	0.01
Percentage of overweight patients (BMI > 25 kg/m ²)	41.2%	80.0%	0.0499	66.7%	> 0.05	87.5%	0.03
Percentage of patients with diabetes	11.8%	50.0%	0.02	33.3%	> 0.05	62.5%	0.006
Percentage of patients with uncontrolled dyslipidemia	41.2%	10.0%	> 0.05	0%	> 0.05	12.5%	> 0.05
Median white blood cell count (k/ul)	8.79	6.14	> 0.05	10.04	> 0.05	5.97	0.049
Median hemoglobin concentration (g/dl)	13.8	13.1	> 0.05	13.3	> 0.05	13.1	> 0.05
Percentage of abnormal CRP values (> 5 mg/l)	23.5%	30.0%	> 0.05	66.7%	> 0.05	12.5%	> 0.05
Median creatinine serum concentration (mg/dl)	0.76	1.09	0.047	1.11	> 0.05	1.08	> 0.05
Percentage of patients with chronic kidney disease (GFR < 60 ml/min/1.73 m ²)	29.4%	70.0%	0.04	100%	0.04	62.5%	> 0.05
Percentage of patients with proteinuria (> 30 mg/dL)	23.5%	20.0%	> 0.05	33.3%	> 0.05	12.5%	> 0.05

Parameter	Non-infected patients (controls) (n = 17)	Infected patients (ADV or/and EBV infection) (n = 10)	P value (controls vs. ADV/EBV)	Patients with ADV infection (n = 3)	P value (ADV positive vs. ADV negative patients)	Patients with EBV infection (n = 8)	P value (EBV positive vs. EBV negative patients)
Percentage of patients with hematuria (> 3 erythrocytes/HPF)	41.2%	50.0%	> 0.05	66.7%	> 0.05	50.0%	> 0.05
Percentage of patients with pyuria (> 5 leukocytes/HPF)	35.3%	50.0%	> 0.05	66.7%	> 0.05	37.5%	> 0.05
Pathological stage of cancer	pT1a – 58.8% pT1b – 23.5% pT2a – 5.9% pT2b – 5.9% pT3a – 5.9% pT3b-4–0% Missing – 0%	pT1a – 20% pT1b – 20% pT2a – 20% pT2b – 10% pT3a – 20% pT3b-4–0% Missing – 10%	> 0.05	pT1a – 0% pT1b – 33.3% pT2a – 33.3% pT2b – 33.3% pT3a – 0% pT3b-4–0% Missing – 0%	> 0.05	pT1a – 25.0% pT1b – 12.5% pT2a – 12.5% pT2b – 12.5% pT3a – 25.0% pT3b-4–0% Missing – 12.5%	> 0.05
Histological grade of cancer	Low grade – 76.5% High grade – 11.8% Missing – 11.8%	Low grade – 40.0% High grade – 60.0% Missing – 0%	0.01	Low grade – 33.3% High grade – 66.7%	> 0.05	Low grade – 37.5% High grade – 62.5%	0.02
Histological RCC subtype	Clear cell – 76.5% Papillary – 5.9% Chromophobe – 17.6% Other – 0%	Clear cell – 40.0% Papillary – 50.0% Chromophobe – 0% Other – 10.0%	0.004	Clear cell – 33.3% Papillary – 66.7% Chromophobe – 0% Other – 0%	> 0.05	Clear cell – 50.0% Papillary – 37.5% Chromophobe – 0% Other – 12.5%	> 0.05
Surgical procedure	Partial nephrectomy – 47.1% Radical nephrectomy – 52.9%	Partial nephrectomy – 40.0% Radical nephrectomy – 60.0%	> 0.05	Partial nephrectomy – 33.3% Radical nephrectomy – 66.7%	> 0.05	Partial nephrectomy – 37.5% Radical nephrectomy – 62.5%	> 0.05
12-month cancer recurrence rate	12.5%	10.0%	> 0.05	0%	> 0.05	12.5%	> 0.05

Regarding oncological data, infected patients were found to have higher rate of poorly differentiated cancers defined as high-grade (60% vs. 12%, OR 5.0, $p < 0.05$). Simultaneously, there was a higher rate of papillary renal cell carcinoma in this group of patients (50% vs. 6%, OR 8.3, $p < 0.05$). Status of viral infection had no influence on clinical stage of renal cancer or surgical procedure (partial vs. radical nephrectomy).

When analyzing separately ADV and EBV infections, only the impact on the rate of chronic kidney disease remained statistically significant for ADV (100% vs. 29%, OR 3.4, $p < 0.05$), while EBV infection increased the rate of high-grade cancers (63% vs. 12%, OR 5.3, $p < 0.05$) with simultaneous higher rate of diabetes (63% vs. 12%, OR 5.3, $p < 0.05$) and overweight (88% vs. 41%, OR 2.1, $p < 0.05$).

Discussion

With increasing evidence on the impact of variety of viral infections on cancer development and limited data on relation between viral infections and RCC, we have conducted a prospective study aimed at assessment of the incidence of selected viral infections within renal tumors. We have found that EBV and ADV tumor infections are common and are associated with different histological cancer features.

While there are some data regarding the link between EBV infection and renal malignancy, to the best of our knowledge the association of ADV with renal tumors is raised for the first time. ADV usually causes acute self-limiting infections with mild clinical symptoms within eyes, respiratory or gastrointestinal tract. However, in some cases ADV can establish a latency within T lymphocytes [10]. For this reason, it is advised to differentiate ADV infection from disease [11].

The ADV infections and reinfections are more common and have more severe clinical course in immunocompromised patients, i.e. after organ transplantation [12–13]. Cancer related alterations within immune system can explain high rate of ADV presence in renal tumors, that we have noted in our study. The role of ADV infection in cancers is poorly understood and our study highlights the need of future research.

Much more is known in the field of EBV and cancer. After primary infection, EBV causes a lifelong asymptomatic latent infection within memory B lymphocytes [14]. It is assumed that 95% of healthy adult population is infected [15]. This can be associated with latent gene heterogeneity and deletions. A special interest was paid to the loss of function of LMP1, EBNA3B, EBNA2 and B95-8 suppressor genes [16–19]. In some cases, especially in the context of immunodeficiency, such infection can promote carcinogenesis, i.e. Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease and others [20]. Latent EBV infection also increases the risk of gastric cancer, so called EBV-associated gastric carcinoma, which nowadays accounts for 2–20% of gastric cancer cases and is associated with relatively good prognosis [21–23]. Simultaneously, EBV was detected in numerous tumors, including lymphoid, epithelial and mesenchymal tumors [20]. The first report on the causative role of EBV infection in kidney carcinogenesis in transgenic mice was published in 1997 by Tornell et al [24].

The relation between EBV infection and renal cancer was previously reported [6–8, 25–26]. What we did find is that EBV infection within renal tumors is frequent and associated with high-grade tumors. Shimakage et al. noted EBV infection within 100% of renal tumors [7]. On contrary, Salehipoor et al. did not find any case of EBV infection among 49 renal cancer patients [8]. Kim et al. found that EBV virus could be a marker of sarcomatoid RCC, as it is present in tumor-infiltrating B cells due to local modulation of immune response [6]. On the other hand, Karaarslan et al. observed EBV infection in 48% of RCCs, including infected tumor cells in 22% of cases [25]. Also Kang et al. noticed EBV presence in both tumor cells and tumor-infiltrating lymphocytes in 34% of RCC patients and the later phenomenon was found to be independent prognostic factor of poor patients survival [26]. Finally, Becker et al. showed that EBV infection of renal proximal tubular cells may participate in evoking a cellular immune response that results in a damaged renal interstitium in patients with chronic interstitial nephritis [27]. Taking all these data together, it remains unclear whether EBV infection is a cause or a result of RCC development and whether the infection is specific for tumor cells, B lymphocytes, renal parenchyma or all of them.

Apart from the finding that RCCs are infected with EBV and ADV, we did show that these infections lead to higher rate of high-grade cancers. Therefore, one can expect shorter survival in these patients as cancer grade is one the most important prognostic factors in postoperative follow-up [28–29]. This was already proven by Kang et al., who noticed significantly shorter overall survival in RCC patients and EBV infected tumor-infiltrating lymphocytes [26].

We have also noted that patients with viral infections have higher rate of chronic renal disease. However, this fact can be at least partially attributed not to virus, but to other differences in patient characteristics, including older age, higher rate of diabetes and overweight. All these facts are well known risk factors for renal disease. This explanation is supported by study from Blazquez-Navarro et al., which showed that EBV has no significant impact on the risk of renal failure in patients after renal transplantation [30].

This study is not free from limitations. First, study population is limited. However, for a pilot study with seven viruses tested, this limitation is justified to some extent. For future studies, one should plan to focus on EBV and ADV and enroll more patients. Second, as study methods clearly diagnosed or excluded viral infections in tissue homogenates, no information was gathered whether viral genetic material comes from cancer cells, infiltrative lymphocytes or other cells. This doubt does not change substantially clinical meaning of our findings. However, it needs to be addressed in the future. Third, selection of tested viruses was subjective and does not rule out the importance of other viral infections in renal malignancies.

Conclusions

EBV and ADV infections are common in renal cancer patients and increase the risk of high-grade RCC presence. While there is no significant impact on short term survival, further studies are needed to assess the relevance of these findings in a long run.

List Of Abbreviations

ADV - human adenovirus

BKV – BK virus

CMV - cytomegalovirus

EBV - Epstein-Barr virus

HSV-1 - herpes simplex virus 1,

HSV-2 - herpes simplex virus 2

JCV - John Cunningham virus

PCR - Polymerase Chain Reaction

RCC - renal cell carcinoma

Declarations

Ethics approval and consent to participate

The study protocol was accepted by Review Board at Medical University of Warsaw.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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None

Authors' contributions

PK – conception, acquisition, analysis, manuscript revision; SP – analysis, interpretation of data, manuscript preparation; AWT - design of the work, acquisition, interpretation of data; SG - acquisition, analysis; MW - design of the work, acquisition, interpretation of data; RK - design of the work, acquisition, interpretation of data; WZ – design of the work, acquisition, interpretation of data, manuscript revision; LP - design of the work, acquisition, interpretation of data, manuscript revision

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