WU and KI polyomaviruses in respiratory, blood and urine samples from renal transplant patients

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Abstract

Background: It is suggested that immunosuppression due to transplantation might be a risk for human polyomavirus KI (KIPyV) and WU (WUPyV) infection. Most of the publications report data about stem cell transplant patients, little is known about these virus infections in renal transplant patients.

Objectives: To study the presence of KIPyV and WUPyV in upper respiratory, plasma and urine samples from renal transplant patients. To analyse clinical and personal data.

Study design: 532 respiratory, 503 plasma and 464 urine samples were collected from 77 renal transplant patients. KIPyV and WUPyV were detected by nested and quantitative real-time PCR. Patient and clinical data from medical records were analyzed.

Results: KIPyV was detected in respiratory, plasma and urine samples from 14.3 %, 3.9 % and 4.1 % of renal transplant patients. WUPyV was found in respiratory and plasma specimens from 9.1 % and 5.3 % of the patients. Significant association was revealed between the detection of KIPyV and WUPyV and the time of samples collection and the age of the patients. KIPyV was presented in respiratory and plasma sample at the same time. KIPyV was detected in plasma samples from two patients and in urine samples of three other patients providing also KIPyV positive respiratory samples at the same time. No clinical consequences of KIPyV or WUPyV infection were found.

Conclusion: Although no clinical consequences of KIPyV and WUPyV infections were found in renal transplant patients, it is suggested that renal transplantation might result in higher susceptibility or reactivation of these infection.

1. Background

KI and WU polyomaviruses (KIPyV and WUPyV) were discovered in respiratory samples from children suffering from respiratory symptoms in 2007 [1, 2]. Subsequent studies using PCR methods revealed the presence of viral DNA in many different samples: respiratory, blood, stool, cerebrospinal fluid, lymphoid tissue, lung [3] and urine samples [4,

5]. Seroepidemiological studies showed that both viruses are widespread, the seropositivity of KIPyV and WUPyV are 55-100 % in the adult population [6-9]. Since similarly high seropositivity was also found among young children, it is suggested that primary infections by KI and WU viruses occur during childhood [6, 7]. This is also strengthened by the finding that the viruses were detected more frequently in samples from children [10]. Respiratory and/or fecal-oral transmission is suggested for both KI and WU viruses [11]. Despite the numerous prevalence data published, little is known about the pathogenesis of KIPyV and WUPyV, and none of them is associated with any disease. Primary infection by WU and KI viruses might be mild or asymptomatic. The higher prevalence in samples (respiratory samples, lymphoid tissues, blood and urine) from immunocompromised patients suggests that immunosuppression might result in higher susceptibility or reactivation of KIPyV and WUPyV [3-5, 12-15]. Transplantation associated with immunosuppression might be a risk for these infections, since higher frequencies of these viruses were found in stem cell transplant patients compared with other immunocompromised groups. Increased pathogenic potential was also suggested, even if not higher prevalence, but higher viral loads were detected in immunocompromised patients [16]. Most of these studies focused on haematopoietic cell transplant patients, and we published previously data about renal transplant patients [4].

In this study we detected KIPyV in respiratory, blood and urine samples from renal transplant patients, frequency was 14.3, 3.9 and 4.1%. WUPyV was found in 9.1% of respiratory and 5.3% of blood samples. No clinical consequence was found.

2. Objectives

Based on the above mentioned data it is suggested that renal transplant patients receiving immunosuppressive therapy might be more susceptible for these infections or reactivation of these viruses may occur. To examine the prevalence of these viruses in renal

transplant patients, to find potential site of viral replication and/or latency the presence of KIPyV and WUPyV was studied by PCR in respiratory, blood and urine samples from renal transplant patients from transplantation until 18 month. Clinical and personal data of the patients were analyzed to study the potential pathogenic role of KIPyV and WUPyV.

3. Study design

3.1. Patients and samples

The study was approved by Regional and Institutional Ethics Committee of University of Debrecen.

Throat swab, plasma (from EDTA blood samples) and urine samples were collected from 77 renal transplant patients receiving kidney between September 2008 and September 2012 at University of Debrecen as described previously [4]. Samples were collected from patients visiting the outpatient clinic of the renal transplant centre at University of Debrecen. Table 1. summarizes the number of samples and the data of the patients. It was preferred to collect blood and urine sample together with respiratory samples at the same time, but beside 532 respiratory samples, 503 plasma and 464 urine samples were provided. Samples were collected 1-16 times from the following number of patients: 2, 10, 9, 3, 5, 3, 6, 9, 11, 9, 4, 2, 1, 2, 1 patients at the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 16 time points.

Immediately after collection, nucleic acid was isolated from 200 µL of each sample using High Pure Viral Nucleic Acid Kit (Roche, Switzerland) according to the manufacturer's instructions. DNA eluted in 50 µL volume was stored at -20 ºC until use.

Clinical and laboratory data were obtained from medical records.

3.2. Nested and real-time PCR for WUPyV and KIPyV

Nested PCR was performed as described previously [17]. For quantitative detection of KIPyV and WUPyV DNA, multiplex real-time PCR (qPCR) was carried out with primers and probes published previously [14] in a final volume of 50 µL using TaqMan Universal PCR Master Mix in an Applied Biosystem 7500 real-time PCR instrument (Applied Biosystems, USA). Standard curves generated by PCR amplification of 10-fold dilutions of plasmids containing WU and KI polyomavirus DNA were used for absolute quantification. All samples were tested by nested and qPCR, 10 µL nucleic acid was amplified in each PCR. Nested PCR positivity was confirmed by sequencing if qPCR was negative.

3.3. Statistical analysis

Difference in frequency for categorical variables was analysed by Fisher's exact and χ^2 test. For continuous variables Mann-Whitney test was applied. Logistic regression was used to analyze the relationship between KIPyV and/or WUPyV detection and different variables. Cox regression models were performed to analyse the risk factors for detection of KI or WU viruses, including age, month of examination, sex, CMV reactivation, respiratory symptoms, leukopaenia, monocytopaenia, neutropaenia, leukocytosis, monocytosis and neutrophilia. Analyses were performed using SigmaPlot version 10.0 (Systat Software, Inc., USA) and XLSTAT 2014 software (Addinsoft, USA) , p value < 0.05 was considered significant.

4. Results

In our study group 20.7 % of the renal transplant patients (16/77) provided KIPyV and/or WUPyV positive respiratory samples during the examination period. KIPyV DNA was detected in 17 respiratory samples (17/532; 3.2 %) taken from 11 patients (11/77; 14.3 %). Three patients had two or three positive respiratory samples within 7-21 days, and one patient provided a KIPyV positive sample 158 days after his first positive sample. WUPyV was

found in 8 respiratory samples (8/532; 1.5%) from 7 patients (7/77; 9.1%). Two WUPyV positive samples were collected within 14 days from one patient. Both viruses was detected in one sample at the same time (Table 2.). The frequency of KIPyV was significantly higher than that of WUPyV in positive respiratory samples (17/24 vs. 8/24; p=0.019). Only three respiratory samples were qPCR negative and nested PCR positive, but sequencing proved the presence of the polyomaviruses. Additionally, one of these patients had KIPyV qPCR positive plasma, and one provided KIPyV positive urine at the same time. Although KIPyV viral loads were higher in respiratory samples (range: 2.2E+02-1.04E+07 copies/mL; median: 1.39E+03 copies/mL), it was not significant difference (p=0.056) comparing with WUPyV viral loads (range: 4.6E+02-2.18E+04 copies/mL; median: 7.31E+02 copies/mL). Significant difference was found regarding the sample collection time points between KI and/or WU viral DNA positive and negative samples (p=0.001; Table 2.). Although the number of positive samples/patients is low, patients provided WUPyV positive respiratory samples mainly during summer (6/7), seasonality was not apparent for KIPyV (Figure 1.). Logistic regression showed significant relationship between the presence of KIPyV and/or WUPyV in respiratory samples and the age of the patients (odds ratio 0.963, 95% CI 0.935-0.991, p = 0.01) Age was a significant risk factor for detecting KIPyV and/or WUPyV (hazard ratio 0.963 CI 0.936-0.991 p=0.009). KIPyV was detected in samples from patients 20-59 years of age, while WUPyV was found also in respiratory samples from a patient < 20 years of age. All patients > 60 years of age provided respiratory samples negative for KI and WU virus, respectively (Figure 2.). Most of the samples positive for KIPyV and WUPyV were collected 1-5 months after transplantation (Figure 3A), logistic regression significant relationship between the presence of KIPyV and/or WUPyV in respiratory samples and the time of sampling (odds ratio 0.992, 95% CI 0.988-0.996, p<0.001)..

Relationship between detection of KI and/or WU viruses in respiratory samples and sex, CMV reactivation, leukopaenia, monocytopaenia, neutropaenia, leukocytosis, monocytosis, neutrophilia was not revealed. Mild, upper respiratory symptoms were recorded only for three patients provided KIPyV/WUPyV positive respiratory samples, but similar symptoms were also existed when 26 negative samples were collected from patients (3/24 vs. 26/508; p=0.11).

Plasma samples were also collected together with the 19 respiratory samples positive for KIPyV and/or WUPyV from 13 patients. In two out of these 18 plasma samples KIPyV, and in one sample WUPyV was also detected, while the respiratory samples of these three patients were KIPyV positive. Therewith, in three additional plasmas out of the 503 samples WUPyV DNA was found, and in one of them KIPyV was also detected at the same time. The respiratory samples of these three patients were negative for viral DNA at that time point. Altogether, 7.9 % of the patients (6/76) had viraemia, the frequency of KIPyV and WUPyV was 0.6% and 0.8%, respectively (3/503 and 4/503). The viral loads were low, ≤ 260 copies /mL. Significant difference was found regarding the sample collection time points between KI and/or WU viral DNA positive and negative samples (p=0.006; Table 2.). Figure 3B shows the time points of sampling for KIPyV and WUPyv positive samples. KIPyV and/or WUPyV viraemia was significantly more frequent in patients providing also positive respiratory sample at the same time (3/19 vs. 3/484; p=0.0009). Logistic regression model found association between KIPyV/WUPyV viraemia and the presence of these viruses in respiratory samples at the same time (odds ratio 0.008 95 % CI 0.015-0.441, p=0.004). Not any other parameter (age, sex, CMV reactivation, leukopaenia, monocytopaenia, neutropaenia, leukocytosis, monocytosis, neutrophilia) was associated with KIPyv/WUPyV viraemia based on Cox regression model.

At the time point of WU or KI viral DNA positive respiratory samples 6 patients provided also urine samples, out of which KIPyV was detected in three samples (3/11). All other urine samples were negative for KIPyV and WUPyV, the frequency of KIPyV in urine samples was 0.6 % (3/464; Table 2.). Positivity was confirmed with sequencing of the nested PCR product. Viruria was revealed in 4.1 % of the patients, and viraemia was not detected at that time point. Figure 3C shows the time points of sampling.

5. Discussion

Previous studies with haematopoietic transplant patients suggest that immunosuppression related to transplantation may result in higher frequencies of KIPyV and WUPyV infection [14, 16, 18, 19]. At the same time, little is known about renal transplant patients [4]. In this study the presence of KIPyV and WUPyV was studied in upper respiratory tract, plasma and urine samples from 77 renal transplant patients from transplantation until 18 months after it. KIPyV was revealed in all sample types, WUPyV was found in respiratory and plasma samples. KIPyV was detected in respiratory samples from 14.3 % of the patients, while 9.1 % of the patients provided WUPyV positive respiratory sample during this study. The frequency in respiratory samples was 3.2 % and 1.5 % for KIPyV and WUPyV, respectively. KIPyV was detected in 0.8-17.8 % of respiratory specimens from stem cell transplant patients meaning 0.8-22 % KIPyV prevalence in these patients. Lower prevalence of WUPyV was reported, 0-7 % in stem cell transplant patients. [14, 16, 18, 19]. Our data from renal transplant patients are in accordance with these publications.

We found that most of the KIPyV and WUPyV positive respiratory samples were provided 1-5 month after transplantation. The detection of the viruses was significantly associated with the time point of sample collection. All KIPyV positive respiratory samples

were collected from patients at 21-44 years of age (median 29 years), while WUPyV was detected mainly in patients at 12-68 years of age (median 45 years), the difference was not statistically significant (p=0.058). Seroprevalence studies found high KIPyV and WUPyV seropositivity in young children similar to that of the adults suggesting that primary infections occur mainly during childhood [6, 7]. Additionally, in most of the publications higher PCR prevalence data for KIPyV and WUPyV were reported in respiratory samples compared with adults [3, 20, 21]. These findings suggest that the detected KIPyV and WUPyV infections might be reactivations or re-infections.

Seasonality of KIPyV in respiratory samples was not found in this study. Although the number of the positive samples is low, most of the WUPyV positive samples were provided during summer. A previous study also found a small peak of WUPyV detection during summer, however they found WUPyV throughout the year [22]. Further studies are required to determine whether seasonality of WUPyV occurs.

In our study group respiratory symptoms were not associated with the detection of KIPyV and/or WUPyV in the upper respiratory specimens. Only three patients provided KIPyV (1 patient) or WUPyV (2 patients) positive respiratory samples had mild, upper respiratory symptom (coughing, sore throat without fever). Not any risk factor was associated with KIPyV/WUPyV detection, although the available clinical data were analysed (CMV reactivation, haematologic clinical data, symptoms of patients).

In a study high viral loads of KIPyV in respiratory samples from paediatric stem cell transplant patients were found suggesting higher pathological role for KI virus in these patients, but not for WUPyV [16]. In our study KIPyV was not only more frequent, but the viral loads also were higher then that of WUPyV in respiratory samples. Although the KIPyV viral loads in respiratory samples from our patients were lower then was detected in paediatric patients [16], it may refer to active viral replication.

We detected KIPyV and WUPyV in plasma samples from 3.9 % and 5.2 % of patients. Previous publications also reported that KIPyV and WUPyV can be detected in blood [22]. At the same time, in a study with stem cell transplant patients KIPyV and WUPyV was not detected in plasma samples suggesting that these viruses have no effect on post-transplant clinical course [23]. Recently, detection of KIPyV antigen in lung and spleen tissues has been published. It was also determined that CD68+ cells in lung harbour KIPyV antigens, hence alveolar macrophages may be infected by KIPyV [24]. In our study we found co-existence of KIPyV in respiratory and blood specimen from two patients, and one patient provided KIPyV positive respiratory and WUPyV positive blood samples at the same time point. The viral loads in all plasma specimens were low, there is no evidence for active virus replication in blood circulation, but it also cannot be excluded. Blood cells might be the site of active infection, non productive infection, the site of latency, as it was hypothesized for CD68+ cells [24]. We detected KIPyV in urine samples from three patients provided also KIPyV positive respiratory specimen at the same time. Analysis of clinical data did not reveal any risk factor or association with clinical parameters.

Different hypotheses arise from the co-detection of KIPyV in respiratory and blood or urine specimens. The respiratory tract infection may enter the blood circulation directly or by circulating blood derived cells, as was suggested [24]. By the blood circulation the virus can reach other organs, maybe kidney, urinary tract. It cannot be excluded that KIPyV/WUpyV detected in respiratory, blood and urine samples from adult, renal transplant patients originated from reactivation of latency, or latent infections (blood, urinary tract) were found, if these viruses are able to establish latency.

In conclusion, we detected KIPyV and WUPyV in respiratory specimens from renal transplant patients with a frequency similar to published data about other immunocompromised patients. Age and the time point of sample collection were associated

with detection. KIPyV and WUPyV were also found in plasma samples, co-occurrence of KIPyV in blood and respiratory specimens were detected. Presence of KIPyV in urine samples were also revealed, these patients had KIPyV positive respiratory samples at the same time. No clinical consequences of KIPyV or WUPyV infection were found. Further studies are required to clarify many questions in connection with these viruses.

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Competing interests:

The authors have no competing interest.

Ethical approval :

The study was approved by Regional and Institutional Ethics Committee of University of Debrecen (number: 2740-2008, 2917-2009, IX-R-052/00876-2/2012).

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