Prevalence of WU and KI Polyomaviruses in Plasma, Urine and Respiratory Samples from Renal Transplant Patients

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WUPyV and KIPyV in Renal Transplant Patients

Keywords

WU polyomavirus, KI polyomavirus, renal transplantation

ABSTRACT

WU and KI polyomaviruses (WUPyV, KIPyV) have been detected in respiratory, blood, stool and lymphoid tissue, but not in urine samples. PCR based detection revealed higher frequency in immunocompromised individuals. In this study the prevalence of WUPyV and KIPyV was analyzed in respiratory, urine and blood samples from renal transplant patients compared with healthy individuals. WUPyV and KIPyV were detected by nested PCR. The PCR products were sequenced and viral DNA loads were determined by quantitative real-time PCR. WUPyV and KIPyV were found in plasma (3.6 %; 7/195), urine (14 %; 7/50) and respiratory samples (10 %; 9/90) of renal transplant patients, but not in plasma (0/200) and urine (0/36) specimens from healthy blood donors. WUPyV and KIPyV were detected mainly early after renal transplantation and the viral loads were low. A higher prevalence of WUPyV was found in plasma and urine samples, KIPyV was found more frequently in respiratory samples from renal transplant patients. It is hypothesized that immunosuppression due to the transplantation may result in reactivation of these viruses or may establish greater susceptibility to infection with KIPyV and WUPyV.

INTRODUCTION

Serological studies suggest that KI and WU polyomaviruses (KIPyV, WUPyV) are widespread. It is thought that primary infection may occur in childhood because the seropositivity for both viruses is high in children and reaches 70-80 % in adults [Neske et al., 2010; Nguyen et al., 2009]. Both WUPyV and KIPyV have been identified from respiratory specimens of patients with respiratory symptoms [Allander et al., 2007; Gaynor et al., 2007]. Although the pathogenic roles of these viruses have not been clarified, PCR based detection revealed 0.4-9 % prevalence in respiratory specimens of immunocompetent patients and higher frequency in children and immunocompromised individuals [Bialasiewicz et al., 2009; Dalianis et al., 2009; Mourez et al., 2009]. Viral DNA was also detected in blood samples from immunocompromised patients and children [Miller et al., 2009; Neske et al., 2009], in stool samples of children with gastroenteritis [Bialasiewicz et al., 2009; Neske et al., 2009], in lymphoid tissues from immunocompromised patients [Sharp et al., 2009], but not in urine samples from immunocompromised and immunocompetent patients [Bialasiewicz et al., 2009; Bofill-Mas et al., 2010; Gaynor et al., 2007]. The higher prevalence in immunocompromised patients suggest that these viruses may cause more severe problems in these individuals in a manner similar to the effect of BK and JC virus (BKV, JCV) [Jiang et al., 2009].

The prevalence of WUPyV and KIPyV has been studied and examined in respiratory, urine and blood samples from renal transplant patients.

MATERIALS AND METHODS

Test specimens. 195 blood samples from 195 patients (82 women, 113 men; median age 45.7 years; range 7-68.8 years) were collected at different times after renal transplantation (median 1188 days, range 3-7108). For control measurements, 200 blood samples from 200 healthy blood donors were taken (75 men, 125 women, median age 39 years, range 10-74 years). Fifty urine samples from 50 transplant patients were also collected after transplantation (range 5-6230 days; median 141 days). Thirty six urine specimens from healthy blood donors were used as controls. Ninety upper respiratory tract specimens using throat swabs from 90 renal transplant individuals were obtained 18-6230 days after the transplantation (median 1177 days).

Nucleic acids from 200 µL plasma, centrifuged for 10 min at 180×*g* at 4 ºC, 200 µl urine specimen and throat swab sample washed in 200 µL buffer were isolated using High Pure Viral Nucleic Acid Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Nucleic acid was eluted in 50 µl and stored at -20 ºC until use.

The Regional and Institutional Ethics Committee of University of Debrecen approved all of the studies. All patients gave their written informed consent.

Qualitative and quantitative detection of KIPyV and WUPyV DNA. To detect WUPyV and KIPyV DNA, the first round of WUKI nested-PCR was carried out with WUKI_OS and WUKI_OAS primers as described previously [Sharp et al., 2009] in a final volume of 20 µL containing 5 µL DNA solution, GenAmp Fast PCR Master Mix (Applied Bisystems, Foster City, CA, USA) and 10-10 pmol of each primer. For the second round, 4 µL of the PCR product from the first round was amplified in 20 µL final volume using GenAmp Fast PCR Master Mix and 10-10 pmol WUKI_IS and WUKI_IAS primers [Sharp et al., 2009]. The annealing temperature was 60 ºC in both rounds. Plasmids containing the genome of WUPyV and KIPyV were used as positive controls [Gaynor et al., 2007; Lindau et al., 2009]. The

sensitivity of this nested-PCR was <100 genome equivalent/mL (GEq/mL). Since this PCR method amplifies both WUPyV and KIPyV DNA and does not differentiate between them, the PCR products were sequenced by using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). At the same time, real-time PCR for WUPyV and also for KIPyV was performed with primers and probes described previously [Lindau et al., 2009] with 5 μL DNA to confirm the result of the nested-PCR and to quantify the viral loads in the samples.

χ^2 and Fisher's exact test was used to assess the difference in frequency for categorical variables. Mann-Whitney U test was applied for continuous variables. A difference was considered significant if p value was less then 0.05.

RESULTS

PCR prevalence of WUPyV and KIPyV in plasma samples. Seven (3.6 %) of 195 plasma samples from transplant patients and none from 200 healthy blood donors were positive by WUKI PCR ($p<0.01$; Table 1.). Sequencing of the PCR products revealed that two samples were positive for KIPyV and five for WUPyV DNA. The level of DNA load in six plasma samples was less then 250 GEq/mL urine, below the limit of detection, and 2.5 X 10^2 KIPyV GEq/mL in one specimen. Significant difference was found between polyoma-positive and negative samples regarding the time after renal transplantation ($p=0.001$) (Table 2.).

PCR prevalence of WUPyV and KIPyV in urine samples. Seven (14 %) urine specimens from transplanted patients and none from 36 healthy blood donors were positive for WUKI by PCR ($p<0.05$; Table 1.). One sample was KIPyV DNA positive (viral load was < 250 GEq/mL plasma) and six samples were WUPyV DNA positive by sequencing. The viral loads of four samples were < 250 GEq/mL, and two samples had 5 X 10^2 and 1.1 X 10^3 GEq / mL plasma. PCR positive samples were collected significantly earlier after transplantation then the negative samples ($p=0.001$) (Table 2.). In the case of two patients whose urine samples were WUPyV DNA positive (28.6 %), WUPyV viremia was also detected.

Prevalence of WUPyV and KIPyV by PCR in respiratory samples. Nine (10 %) of 90 respiratory samples were WUKI PCR positive (Table 1.). Six samples were KIPyV DNA positive (66.7 %) with viral loads ranging from 2.8 X10^2 to 3.7 X 10^5 GEq/mL (median 4.2 X 10^4; in two samples the viral load was below the limit of detection). Only one out of the three WUPyV positive samples had detectable viral load of 6.3 X 10^2 GEq/mL. Statistical analysis revealed that the PCR positive samples were collected significantly earlier after transplantation then the PCR negative samples ($p=0.002$; Table 2.). The plasma sample of one patient with a KIPyV positive respiratory specimen was positive for WUPyV DNA, the plasma samples of the others were PCR negative. All of the patients with a positive

113 respiratory specimen had acute upper respiratory tract infection, but none of these samples
114 were tested for any respiratory virus; a significantly higher frequency compared with the PCR
115 negative patients (9/9 vs. 47/81; p=0.01).
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DISCUSSION

This study revealed that WUPyV and KIPyV can be detected in blood, urine and respiratory samples from renal transplant patients, but these viruses were not found in blood and urine specimens from healthy blood donors.

Viremia was detected in 3.6 % of the renal transplant patients, mostly early after transplantation, but not in healthy blood donors. Other studies have found only WUPyV in blood samples [Bialasiewicz et al., 2009; Miller et al., 2009; Neske et al., 2009], but apart from WUPyV (2.6 %), KIPyV (1 %) was detected in the current study in renal transplant patients. The viral loads were very low, ≤ 250 GEq /mL plasma. Previously, WU and KI polyomaviruses were not found in urine samples from immunocompromised, renal transplant patients, immunocompetent patients and pregnant women [Bialasiewicz et al., 2009; Bofill-Mas et al., 2010; Gaynor et al., 2007]. In this study these viruses were not detected in urine specimens from healthy blood donors, but 14 % of the samples from renal transplant patients were positive for viral DNA. A higher prevalence of WUPyV was observed compared with KIPyV (6/7 vs. 1/7). The viruses appeared early after renal transplantation, and all of the positive samples were collected within two months after the transplantation. The viral loads in these urine samples were low (≤1.1 X 10^3 GEq/mL), but in two patients WUPyV viremia was also found at the same time. A different DNA isolation method in which samples were not stored and DNA was isolated immediately after the collection of the urine samples, primers and PCR conditions may be the reasons why these viruses were found while other investigator did not detect these viruses in urine samples [Bialasiewicz et al., 2009; Bofill-Mas et al., 2010; Gaynor et al., 2007].

In immunocompetent individuals a slightly higher prevalence of WUPyV DNA was observed in the respiratory samples [Dalianis et al., 2009] and also the seroprevalence of WUPyV was found to be greater [Neske et al., 2010; Nguyen et al., 2009] compared with KIPyV. In the

throat swab samples from renal transplant patients a higher prevalence of KIPyV (6/9; 6.7 %) then WUPyV (3/9; 3.3 %) was observed. This is in accordance with the results of Murez et al. [2009] who found a higher frequency of KIPyV in respiratory samples from immunocompromised patients. A significant difference was found between the PCR positive and the negative group of the patients regarding the date of samples collection. Viral DNA was detected mostly early after renal transplantation.

The results of previous studies suggest that primary infection with WU and KI viruses occurs during childhood with subclinical or mild illness [Abedi Kiasari et al., 2008; Bialasiewicz et al., 2007; Gaynor et al., 2007; Neske et al., 2010]. Transmission can be fecal-oral and/or via the respiratory route [Dalianis et al., 2009]. Presumably, in a manner similar to BKV and JCV, it may establish lifelong persistence [Jiang et al., 2009]. This study revealed that viruria with WUPyV and KIPyV can occur, so urine can also be a source of infection. The higher prevalence of WUPyV and KIPyV in respiratory samples from immunocompromised patients, and the findings that these viruses are present in blood and urine specimens from renal transplant patients, but not in healthy blood donors, suggest that similar to BKV and JCV, WUPyV and KIPyV might cause significant disease primarily in immunocompromised individuals. At the same time, because of the high rates of coinfections with other respiratory viruses, the pathological role and the clinical consequences of the KI and WU respiratory tract infections are not clean. Based on the knowledge of other human pathogen polyomaviruses it is hypothesized that immunosuppression due to transplantation may result in reactivation of these viruses, or may establish greater susceptibility to KIPyV and WUPyV [Jiang et al., 2009]. Although viremia and viruria were found in the current study in renal transplant patients, the viral loads were low. In the case of BKV the level of viruria correlates with the degree of immunosuppression, and the higher viral loads in urine and blood samples can result in more severe clinical consequences [Ahsan and Shah, 2006]. Further follow up

studies of renal transplant patients may help to clarify whether the presence of WUPyV and KIPyV in urine and blood samples can result in severe disease as observed with BKV.

REFERENCES

Abedi Kiasari B, Vallely PJ, Corless CE, Al-Hammadi M, Klapper PE. 2008. Age-related pattern of KI and WU polyomavirus infection. J Clin Virol 43:123-125.

Ahsan N, Shah KV. 2006. Polyomaviruses and human diseases. Adv Exp Med Biol 577:1-18.

Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T, Andersson B. 2007. Identification of a third human polyomavirus. J Virol 81:4130-4136.

Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP. 2009. Detection of BK, JC, WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. J Clin Virol 45:249-254.

Bialasiewicz S, Whiley DM, Lambert SB, Wang D, Nissen MD, Sloots TP. 2007. A newly reported human polyomavirus, KI virus, is present in the respiratory tract of Australian children. J Clin Virol 40:15-18.

Bofill-Mas S, Rodriguez-Manzano J, Calgua B, Carratala A, Girones R. 2010. Newly described human polyomaviruses Merkel cell, KI and WU are present in urban sewage and may represent potential environmental contaminants. Virol J 7:141.

Dalianis T, Ramqvist T, Andreasson K, Kean JM, Garcea RL. 2009. KI, WU and Merkel cell polyomaviruses: a new era for human polyomavirus research. Semin Cancer Biol 19:270-275.

Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP, Wang D. 2007. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog 3:e64.

Jiang M, Abend JR, Johnson SF, Imperiale MJ. 2009. The role of polyomaviruses in human disease. Virology 384:266-273.

Lindau C, Tiveljung-Lindell A, Goh S, Ramqvist T, Allander T. 2009. A single-tube, real-time PCR assay for detection of the two newly characterized human KI and WU polyomaviruses. J Clin Virol 44:24-26.

Miller MA, Weibel C, Ferguson D, Landry ML, Kahn JS. 2009. WU polyomavirus in patients infected with HIV or hepatitis C virus, Connecticut, USA, 2007. Emerg Infect Dis 15:1095-1097.

Mourez T, Bergeron A, Ribaud P, Scieux C, de Latour RP, Tazi A, Socie G, Simon F, LeGoff J. 2009. Polyomaviruses KI and WU in immunocompromised patients with respiratory disease. Emerg Infect Dis 15:107-109.

Neske F, Blessing K, Prottel A, Ullrich F, Kreth HW, Weissbrich B. 2009. Detection of WU polyomavirus DNA by real-time PCR in nasopharyngeal aspirates, serum, and stool samples. J Clin Virol 44:115-118.

Neske F, Prifert C, Scheiner B, Ewald M, Schubert J, Opitz A, Weissbrich B. 2010. High prevalence of antibodies against polyomavirus WU, polyomavirus KI, and human bocavirus in German blood donors. BMC Infect Dis 10:215.

Nguyen NL, Le BM, Wang D. 2009. Serologic evidence of frequent human infection with WU and KI polyomaviruses. Emerg Infect Dis 15:1199-1205.

Sharp CP, Norja P, Anthony I, Bell JE, Simmonds P. 2009. Reactivation and mutation of newly discovered WU, KI, and Merkel cell carcinoma polyomaviruses in immunosuppressed individuals. J Infect Dis 199:398-404.

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Conflict of interest

The authors have no conflict of interest.

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226 Tables

227 Table 1. Prevalence of WU and KI polyomaviruses in different samples from renal transplant

228 patients and healthy blood donors

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		KI and WU polyomavirus DNA in samples, number (%)			
Sample source	Sample	KIPyV positive	WUPyV positive	Negative	Total number of samples (patients)
Renal transplant patients	plasma	2 (1)	5 (2.6)	188 (96.4)	195 (195)
Renal transplant patients	urine	1 (2)	6 (12)	43 (86)	50 (50)
Renal transplant patients	throat swab	6 (6.6)	3 (3.3)	81 (90)	90 (90)
Healthy blood donors	plasma	0 (0)	0 (0)	200 (100)	200 (200)
Healthy blood donors	urine	0 (0)	0 (0)	36 (100)	36 (36)

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Table 2. Detection of WU and KI polyomaviruses after renal transplantation

Samples from renal transplant patients	Days after renal transplantation, range (median)	
	WUPyV and KIPyV positive	Negative
plasma	8-2122 (24) *	3-7108 (1271)
urine	8-58 (30) *	7-6230 (745)
throat swab	21-822 (101) #	18-6230 (1177)

* p=0.001 vs. negative

p=0.002 vs. negative