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

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Keywords:	WU polyomavirus, KI polyomavirus, renal transplantation

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Table 1. Prevalence of WU and KI polyomaviruses in different samples from renal transplant patients and healthy blood donors

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)

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

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3 1 Prevalence of WU and KI Polyomaviruses in Plasma, Urine and Respiratory Samples from
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6 2 Renal Transplant Patients

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27 11 Running head

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29 12 WUPyV and KIPyV in Renal Transplant Patients
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31 13 Keywords

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34 14 WU polyomavirus, KI polyomavirus, renal transplantation
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3 **15 ABSTRACT**
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6 **16** WU and KI polyomaviruses (WUPyV, KIPyV) have been detected in respiratory, blood, stool
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8 **17** and lymphoid tissue, but not in urine samples. PCR based detection revealed higher frequency
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10 **18** in immunocompromised individuals. In this study the prevalence of WUPyV and KIPyV was
11
12 **19** analyzed in respiratory, urine and blood samples from renal transplant patients compared with
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14 **20** healthy individuals. WUPyV and KIPyV were detected by nested PCR. The PCR products
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16 **21** were sequenced and viral DNA loads were determined by quantitative real-time PCR.
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18 **22** WUPyV and KIPyV were found in plasma (3.6 %; 7/195), urine (14 %; 7/50) and respiratory
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20 **23** samples (10 %; 9/90) of renal transplant patients, but not in plasma (0/200) and urine (0/36)
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22 **24** specimens from healthy blood donors. WUPyV and KIPyV were detected mainly early after
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24 **25** renal transplantation and the viral loads were low. A higher prevalence of WUPyV was found
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26 **26** in plasma and urine samples, KIPyV was found more frequently in respiratory samples from
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28 **27** renal transplant patients. It is hypothesized that immunosuppression due to the transplantation
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30 **28** may result in reactivation of these viruses or may establish greater susceptibility to infection
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32 **29** with KIPyV and WUPyV.
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30 INTRODUCTION

31 Serological studies suggest that KI and WU polyomaviruses (KIPyV, WUPyV) are
32 widespread. It is thought that primary infection may occur in childhood because the
33 seropositivity for both viruses is high in children and reaches 70-80 % in adults [Neske et al.,
34 2010; Nguyen et al., 2009]. Both WUPyV and KIPyV have been identified from respiratory
35 specimens of patients with respiratory symptoms [Allander et al., 2007; Gaynor et al., 2007].
36 Although the pathogenic roles of these viruses have not been clarified, PCR based detection
37 revealed 0.4-9 % prevalence in respiratory specimens of immunocompetent patients and
38 higher frequency in children and immunocompromised individuals [Bialasiewicz et al., 2009;
39 Dalianis et al., 2009; Mourez et al., 2009]. Viral DNA was also detected in blood samples
40 from immunocompromised patients and children [Miller et al., 2009; Neske et al., 2009], in
41 stool samples of children with gastroenteritis [Bialasiewicz et al., 2009; Neske et al., 2009], in
42 lymphoid tissues from immunocompromised patients [Sharp et al., 2009], but not in urine
43 samples from immunocompromised and immunocompetent patients [Bialasiewicz et al.,
44 2009; Bofill-Mas et al., 2010; Gaynor et al., 2007]. The higher prevalence in
45 immunocompromised patients suggest that these viruses may cause more severe problems in
46 these individuals in a manner similar to the effect of BK and JC virus (BKV, JCV) [Jiang et
47 al., 2009].

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

50 MATERIALS AND METHODS

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

60 Nucleic acids from 200 μ L plasma, centrifuged for 10 min at $180\times g$ at 4 °C, 200 μ l urine
61 specimen and throat swab sample washed in 200 μ L buffer were isolated using High Pure
62 Viral Nucleic Acid Kit (Roche, Basel, Switzerland) according to the manufacturer's
63 instructions. Nucleic acid was eluted in 50 μ l and stored at -20 °C until use.

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

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

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3 75 sensitivity of this nested-PCR was <100 genome equivalent/mL (GEq/mL). Since this PCR
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5 76 method amplifies both WUPyV and KIPyV DNA and does not differentiate between them,
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8 77 the PCR products were sequenced by using the ABI PRISM 3100 Genetic Analyzer (Applied
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10 78 Biosystems). At the same time, real-time PCR for WUPyV and also for KIPyV was
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12 79 performed with primers and probes described previously [Lindau et al., 2009] with 5 μ L DNA
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15 80 to confirm the result of the nested-PCR and to quantify the viral loads in the samples.
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17 81 χ^2 and Fisher's exact test was used to assess the difference in frequency for categorical
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19 82 variables. Mann-Whitney U test was applied for continuous variables. A difference was
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22 83 considered significant if p value was less than 0.05.
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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 than 250 GEq/mL urine, below the limit of detection, and 2.5×10^2 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 5×10^2 and 1.1×10^3 GEq / mL plasma. PCR positive samples were collected significantly earlier after transplantation than 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 2.8×10^2 to 3.7×10^5 GEq/mL (median 4.2×10^4 ; 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 6.3×10^2 GEq/mL. Statistical analysis revealed that the PCR positive samples were collected significantly earlier after transplantation than 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

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3 109 respiratory specimen had acute upper respiratory tract infection, but none of these samples
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6 110 were tested for any respiratory virus; a significantly higher frequency compared with the PCR
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8 111 negative patients (9/9 vs. 47/81; $p=0.01$).
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3 112 **DISCUSSION**
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5 113 This study revealed that WUPyV and KIPyV can be detected in blood, urine and respiratory
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7 114 samples from renal transplant patients, but these viruses were not found in blood and urine
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9 115 specimens from healthy blood donors.

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12 116 Viremia was detected in 3.6 % of the renal transplant patients, mostly early after
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14 117 transplantation, but not in healthy blood donors. Other studies have found only WUPyV in
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16 118 blood samples [Bialasiewicz et al., 2009; Miller et al., 2009; Neske et al., 2009], but apart
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18 119 from WUPyV (2.6 %), KIPyV (1 %) was detected in the current study in renal transplant
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20 120 patients. The viral loads were very low, ≤ 250 GEq /mL plasma. Previously, WU and KI
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22 121 polyomaviruses were not found in urine samples from immunocompromised, renal transplant
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24 122 patients, immunocompetent patients and pregnant women [Bialasiewicz et al., 2009; Bofill-
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26 123 Mas et al., 2010; Gaynor et al., 2007]. In this study these viruses were not detected in urine
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28 124 specimens from healthy blood donors, but 14 % of the samples from renal transplant patients
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30 125 were positive for viral DNA. A higher prevalence of WUPyV was observed compared with
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32 126 KIPyV (6/7 vs. 1/7). The viruses appeared early after renal transplantation, and all of the
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34 127 positive samples were collected within two months after the transplantation. The viral loads in
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36 128 these urine samples were low ($\leq 1.1 \times 10^3$ GEq/mL), but in two patients WUPyV viremia was
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38 129 also found at the same time. A different DNA isolation method in which samples were not
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40 130 stored and DNA was isolated immediately after the collection of the urine samples, primers
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42 131 and PCR conditions may be the reasons why these viruses were found while other
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44 132 investigators did not detect these viruses in urine samples [Bialasiewicz et al., 2009; Bofill-
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46 133 Mas et al., 2010; Gaynor et al., 2007].
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48 134 In immunocompetent individuals a slightly higher prevalence of WUPyV DNA was observed
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50 135 in the respiratory samples [Dalianis et al., 2009] and also the seroprevalence of WUPyV was
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52 136 found to be greater [Neske et al., 2010; Nguyen et al., 2009] compared with KIPyV. In the
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3 137 throat swab samples from renal transplant patients a higher prevalence of KIPyV (6/9; 6.7 %)
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5 138 then WUPyV (3/9; 3.3 %) was observed. This is in accordance with the results of Murez et al.
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8 139 [2009] who found a higher frequency of KIPyV in respiratory samples from
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10 140 immunocompromised patients. A significant difference was found between the PCR positive
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12 141 and the negative group of the patients regarding the date of samples collection. Viral DNA
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14 142 was detected mostly early after renal transplantation.
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17 143 The results of previous studies suggest that primary infection with WU and KI viruses occurs
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19 144 during childhood with subclinical or mild illness [Abedi Kiasari et al., 2008; Bialasiewicz et
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21 145 al., 2007; Gaynor et al., 2007; Neske et al., 2010]. Transmission can be fecal-oral and/or via
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23 146 the respiratory route [Dalianis et al., 2009]. Presumably, in a manner similar to BKV and
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25 147 JCV, it may establish lifelong persistence [Jiang et al., 2009]. This study revealed that viruria
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27 148 with WUPyV and KIPyV can occur, so urine can also be a source of infection. The higher
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29 149 prevalence of WUPyV and KIPyV in respiratory samples from immunocompromised
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31 150 patients, and the findings that these viruses are present in blood and urine specimens from
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33 151 renal transplant patients, but not in healthy blood donors, suggest that similar to BKV and
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35 152 JCV, WUPyV and KIPyV might cause significant disease primarily in immunocompromised
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37 153 individuals. At the same time, because of the high rates of coinfections with other respiratory
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39 154 viruses, the pathological role and the clinical consequences of the KI and WU respiratory tract
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41 155 infections are not clear. Based on the knowledge of other human pathogen polyomaviruses it
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43 156 is hypothesized that immunosuppression due to transplantation may result in reactivation of
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45 157 these viruses, or may establish greater susceptibility to KIPyV and WUPyV [Jiang et al.,
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47 158 2009]. Although viremia and viruria were found in the current study in renal transplant
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49 159 patients, the viral loads were low. In the case of BKV the level of viruria correlates with the
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51 160 degree of immunosuppression, and the higher viral loads in urine and blood samples can
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53 161 result in more severe clinical consequences [Ahsan and Shah, 2006]. Further follow up
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3 162 studies of renal transplant patients may help to clarify whether the presence of WUPyV and
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5 163 KIPyV in urine and blood samples can result in severe disease as observed with BKV.
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215

216 **Conflict of interest**

217 The authors have no conflict of interest.

For Peer Review

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 219 Tables
 220 Table 1. Prevalence of WU and KI polyomaviruses in different samples from renal transplant
 221 patients and healthy blood donors
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KI and WU polyomavirus					
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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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225 Table 2. Detection of WU and KI polyomaviruses after renal transplantation

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Samples from renal transplant patients	Days after renal transplantation, range (median)	
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