Novel human polyomaviruses in pregnancy: higher prevalence of BKPyV, but no WUPyV, KIPyV and HPyV9

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Abbreviations:

WU polyomavirus (WUPyV), KI polyomavirus (KIPyV), human polyomavirus 9 (HPyV9), BK polyomavirus (BKPyV), genome equivalent (GEq), polymerase chain reaction (PCR)

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Abstract

Background: Immunosuppression due to pregnancy may lead to higher susceptibility to infections and reactivation of latent infections, such as BK polyomavirus (BKPyV). There is lack of information about the prevalence of novel human polyomavirus 9 (HPyV9), WU (WUPyV) and KI (KIPyV) during pregnancy.

Objectives: To study whether pregnancy results in higher prevalence of HPyV9, WUPyV, KIPyV and their correlation with BKPyV.

Study design: Plasma, urine and throat swab samples from 100 pregnant and 100 non pregnant women were screened for the presence of WUPyV, KIPyV, HPyV9 and BKPyV by PCR.

Results: No WUPyV DNA was detected in plasma, urine and respiratory samples from pregnant and non pregnant women. KIPyV DNA was found in two plasma samples from non pregnant women (2 %) and not detected in other samples from neither pregnant nor non pregnant women. HPyV9 DNA was determined in all sample types of pregnant and non pregnant women, respectively. There were no significant differences between pregnant and non pregnant women in HPyV9 DNA frequencies for plasma (2 % vs. 6 %), urine (3 % vs. 2 %) and respiratory samples (2 % vs. 2 %). Prevalence of BKPyV in urine samples was significantly higher (p=0.039) in pregnant women (13 %) then in non pregnant women (4 %); co nfection with KIPyV and/or HPyV9 was not detected.

Conclusions: In contrast with BKPyV, infection with WUPyV, KIPyV and HPyV9 was not detected more frequently during pregnancy. To our knowledge HPyV9 was detected first in respiratory samples in our study.

Key words: human polyomaviruses, pregnancy

1. Background

Human polyomavirus BK (BKPyV) seroprevalence increases with age reaching high, 80-90 % in adult population.[1] Similarly high, 55-90 % adult seropositivities were observed for recently discovered KI[2] and WU[3] polyomaviruses (KIPyV, WUPyV).[4-6] Investigation of seropositivity against the newly discovered human polyomavirus 9 (HPyV9)[7] revealed 47 % positivity for healthy adults.[8] It is well known that after the childhood primary infection with BKPyV, lifelong persistent infection is established mainly in renal and urinary tract cells.[1] Transient immunosuppression due to pregnancy may lead to reactivation of BKPyV resulting in generally asymptomatic viruria with frequency of 3 to 54 %.[1, 9-11] Beside viruria, BKPyV viraemia was also detected in pregnant women. [11] The pathogenic role of the novel WUPyV, KIPyV and HPyV9 is far from clear, only speculative. WU and KI viruses were found in various sample types – respiratory samples, blood, faeces, cerebrospinal fluid, lymphoid tissues, urine – and higher prevalence was observed in children and immunocompromised patients.[12-16] HPyV9 was described from blood and urine samples of kidney transplant patients, then it was found in skin samples, but no in respiratory and fecal samples.[7, 17] The higher frequency of these viruses in immunocompromised patients suggests higher susceptibility or reactivation due to immunosuppression. Up to now only four urine samples from pregnant women were investigated for the presence of KIPyV and WUPyV DNA with negative result.[18] The genetic and possible transmission similarities to BKPyV, and the higher PCR prevalence data among immunocopromised patients may suggest that immunosuppression, thus pregnancy may lead to higher susceptibility to infection with WUPyV , KIPyV and HPyV9 or may result in reactivation of possible latent infections.

2. Objective

The aim of the present study was to evaluate the prevalence of three new human polyomaviruses (WUPyV, KIPyV and HPyV9) during pregnancy, to study whether immunosuppression due to pregnancy may lead to higher prevalence as it was found in case of BKPyV. The possible correlations of these viruses were also investigated.

3. Study design

3.1. Patients and samples

Urine, plasma (from EDTA blood samples) and throat swab samples were collected on the same day from 100 healthy pregnant women (age 16.5-41.9 years, median 32.1 years; pregnancy 5-39 weeks; median 26 weeks) and 100 non pregnant women (age 18-44.3 years, median 31.6 years) between September 2011 and December 2011. Samples from pregnant women were collected in all three trimesters: first trimester n=28; second trimester n=27; third trimester n=45. The control samples were taken from healthy, non pregnant, fertility exam visitor women.

Immediately after collection, nucleic acid was isolated from samples using High Pure Viral Nucleic Acid Kit (Roche, Switzerland) according to the manufacturer's instructions. Briefly, nucleic acids from 200 µl plasma, 200 µl urine specimen and throat swab sample washed in 200 µl buffer were eluted in 50 µl and stored at -20 °C until use.

The study was approved by Regional and Institutional Ethics Committee of University of Debrecen. All patients were asked to sign written informed consent.

3.2. Nested and real-time PCR for WUPyV, KIPyV, HPyV9 and BKPyV

All PCR methods were carried out with 10 µl nucleic acid in a final volume of 25 µl. For nested PCR AmpliTaq Gold 360 Master Mix, for WUPyV and KIPyV real-time PCR TaqMan Universal PCR Master Mix (Applied Biosystems, USA) were used. The calibrants for quantitative PCRs were serial dilutions of KIPyV plasmid (in which the genome of KI polyomavirus isolate Stockholm 60 was incorporated) and AP-p003 plasmid (containing the 2228 bp half genome of WU polyomavirus) kindly provided by Tobias Allander and David Wang. WUKI nested PCR and real-time PCR for WU and KI virus were performed as described previously.[16] HPyV9 PCR was carried out with diagnostic primers and annealing temperature published by Scuda et al.[7] For the first round of BKV nested PCR, k1 (5' TGAAGCATATGAAGATGGCC 3') and k2 (5' GTTACAGCCTCCCACATC 3') primers were used with 60 ºC annealing temperature, while for the second round b1 (5' GATGGCCCCAACCAAAAG 3') and b2 (5' CTAGAACTTCTACTCCTCC 3') primers and 56 ºC annealing temperature were applied. PCR products were visualized by electrophoresis in 1.5 % agarose gel containing ethidium bromide (0.5 μg/mL). The amplified PCR products from WUKI and HPyV9 nested PCR were cut, purified with QIAquick Gel Extraction Kit (Qiagen) according to the instructions and sequenced by using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). To determine BKPyV viral load BKV virus R-gene quantification kit was used (Argene, USA) according to the manufacturer's instructions.

3.3. Statistical analysis

Difference in frequency for categorical variables was analysed by Fisher's exact test. For continuous variables Mann-Whitney U test was applied. Difference was considered significant if p value was less then 0.05.

4. Results

4.1. Detection of WUPyV, KIPyV and HPyV9 DNA in plasma, urine and respiratory samples

Table 1 shows the results of PCR detections for the various samples. WUPyV DNA was not detected in plasma, urine and respiratory samples neither from pregnant nor from non pregnant women. KIPyV was found in two plasma samples of non pregnant women, but was not determined in any other samples. To confirm the positive PCR results and to determine KI or WU virus DNA was detected, PCR products were sequenced. The viral loads were below the limit of detection (< 250 GEq/mL; genome equivalent/mL) by real-time PCR. HPyV9 DNA was detected in urine, plasma and respiratory samples from both studied groups. To prove the results from PCR, all PCR products were sequenced. In details, the prevalence of HPyV9 DNA in plasma samples was higher in control, non pregnant group then in pregnant women (6/100; 6 % vs. 2/100; 2%), but the difference was not statistically significant. The two positive samples were taken in the second trimester of pregnancy. Two samples from control, non pregnant women with HPyV9 viraemia were also positive for KIPyV DNA. In respiratory samples the frequency of HPyV9 DNA was the same in both studied groups (2/100; 2% and 2/100; 2%). Both of the positive samples in pregnant women group were collected in the first trimester. Three urine samples from pregnant women were HPyV9 PCR positive (3/100; 3%), while in control group 2 samples were positive (2/100; 2%) which is not statistically significant difference.

4.2. Prevalence of BKPyV in urine and plasma samples

BKPyV was not detected in plasma samples. Frequency for BKPyV viruria was 13 % (13/100) in pregnant women and 4 % (4/100) in non pregnant, control group (Table 1.). The difference is statistically significant (p=0.039). The BKPyV viral load in samples from pregnant women (range 50-1.86 x 10^8; median 11.82 x 10^3 GEq/mL) did not show statistically significant difference from the viral load in control samples (range 2.25 x 10^2-3.58 x 10^5; median 2.98 x 10^2). BKPyV presence in urine samples was found in all trimesters.

5. Discussion

In our study significantly higher prevalence of BK viruria was observed in pregnant women in contrast with non pregnant women. Human polyomavirus 9 was found in plasma, urine and respiratory samples from pregnant women but not more frequently then in samples from non pregnant women. WU and KI viruses were not detected in any of the studied samples from pregnant women.

BK polyomavirus is ubiquitous in the human population, the primary infection generally occurs during childhood without significant clinical consequences, respiratory diseases might occur. Transmission of the viruses is not well clarified, but it is suggested that these viruses are acquired mainly through respiratory, faecal-oral and urinary routes, alternatively by blood transfusion and organ transplantation.[1] After the primary infection, lifelong persistence of the virus is established mainly in kidney and urinary tract.[19, 20] Lytic infection with viruria occurs in 5-10 % of immunocompetent individuals[21], but more frequently in immunocompromised patients.[22] During pregnancy immunologic changes together with hormonal effects may result in viral infections, reactivations. Viruria was detected for 3-54 % of pregnant women, while viraemia was found to be less frequent.[1, 10, 11, 23] In accordance with literature, in this study 13 % of pregnant women had active BKPyV replication resulting in viruria, but no viraemia. The possible effect of BK virus replication during pregnancy is not clarified. Although viral DNA was demonstrated in fetal tissues [24] the hypothesis of transplacental transmission was not confirmed [10, 11]. Recently serological evidence for vertical transmission of BKPyV was published.[9]

Hitherto, there are no prevalence data about the novel WU, KI and human polyomavirus 9 during pregnancy. Bofill-Mas et al. investigated 4 urine samples from pregnant women, but WU and KI viruses were not found. [18] Foetal tissues were also negative for WU and KI viruses. [25] In this study WU and KI viruses were not found in urine, plasma and respiratory samples collected during pregnancy. KIPyV DNA was detected in two plasma samples, but not in urine and respiratory samples from control, non pregnant women. The high, 55-90 % seropositivity in adult population, and the higher PCR prevalence in samples from children suggest childhood primary KI and WU virus infection. [6, 15] Viruses were found with frequency 0.4-14 % in various samples types including respiratory samples, blood, faeces, cerebrospinal fluid, lymphoid tissues and urine samples, with generally higher frequency in immunocompromised patients. The possible way of transmission might be respiratory and/or faecal-oral.[2, 3, 12-16] The higher PCR prevalence data of immunocompromised patients suggests that immunosuppression might result in reactivation of these viruses, or might establish higher susceptibility to KIPyV and WUPyV infection.[1] It was hypothesized that similarly to BKPyV, transient immunosuppression due to pregnancy might result in higher frequency of WU and/or KI viral infections, but no evidence for it was found during this study. However it is important to note, that it was not a follow up study, samples were collected once randomly during pregnancy.

Human polyomavirus 9 was described in 2011.[7] Up to now, viral DNA was found in blood and urine samples from immunocompromised patients and skin samples, but neither in respiratory samples from patients with respiratory failure nor in faeces from children with gastroenteritis.[7, 17] Based on these data and the recently published 47 % adulthood seropositivity [8] , Van Ghelue et al. hypothesized that HPyV9 is less frequent in the human population[6]. We found HPyV9 DNA is all studied samples from pregnant and non pregnant women with frequency of 2-6 %. There was no or not statistically significant difference between the PCR prevalence in the respiratory (2 vs. 2 %), urine (3 vs. 2%) and plasma samples (2 vs. 6 %) between pregnant and non pregnant women. To our knowledge we published first HPyV9 presence in respiratory samples which may suggest respiratory transmission of this virus. In this study higher prevalence of HPyV9 was not found during pregnancy, but the viral loads were not examined which might have been different. Since mother to foetus transmission of polyomaviruses BK and JC are suggested, this way of transmission cannot excluded in case of the novel WUPyV, KIPyV and HPyV9. Even if this study could not support evidence for higher susceptibility of infection by these viruses, further, follow up study of pregnant women during the whole period of pregnancy might answer this question.

In conclusion, KI and WU viruses were not found in urine, respiratory and blood samples from pregnant women, while HPyV9 was detected in all sample types but with no significantly higher frequency then it was observed for non pregnant women.

Conflict of interest

The authors have no conflict of interest.

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