

## PREVALENCE OF HUMAN HERPESVIRUS 6 A AND B DURING PREGNANCY

BEÁTA MÉSZÁROS<sup>1</sup>, TAMÁS SÁPY<sup>2</sup>, LAJOS GERGELY<sup>1</sup>, ESZTER CSOMA<sup>1\*</sup>

<sup>1</sup> Department of Medical Microbiology, University of Debrecen, Debrecen, Hungary

<sup>2</sup> Department of Obstetrics and Gynecology, University of Debrecen, Debrecen, Hungary

\* corresponding author

The aim of the present study was to assess the frequency of human herpesvirus 6A (HHV-6A) and human herpesvirus 6B (HHV-6B) infection during pregnancy. 100-100 blood samples were collected from pregnant and non pregnant women, then nucleic acid was isolated from both plasma and leukocytes fraction. Nested and real-time PCR were used to detect and differentiate HHV-6A and HHV-6B DNA and to determine viral loads. Reverse transcription PCR (RT-PCR) for HHV-6 U79/80 mRNA was performed in order to reveal active HHV-6 replication.

HHV-6A and HHV-6B active infection was not detected in blood samples neither from pregnant nor from non pregnant women. Frequency of HHV-6B and HHV-6A latency did not show difference between the studied groups (15 % vs. 16%). HHV-6B latency was dominant in both studied groups (14/15 and 15/16). Beside these results, in leukocyte samples of one pregnant and three non pregnant women high HHV-6A viral loads ( $1.28 \times 10^5$  -  $5.07 \times 10^5$  GEq /  $1.5 \times 10^6$  leukocytes) were detected, and viral DNA was also found in plasma samples. Although RT-PCR did not confirm virus replication, but chromosomal integration was also not proved unequivocally, the number of 0.08-0.33 HHV-6 copy/ 1 leukocyte refers more to postnatal infection.

Keywords: HHV-6A, HHV-6B, blood, PCR, pregnancy, healthy women

## **Introduction**

Human herpesvirus 6A and 6B were classified as distinct viruses in 2012 by the International Committee on Taxonomy of Viruses [1]. Based on seroepidemiologic studies seropositivity in adult population exceeds 95 %, however it is important to note that most of these studies did not discriminate HHV-6A and HHV-6B [2]. Spreading via saliva is suggested as the most probable way of horizontal transmission [3], but vertical, transplacental transmission of maternal infections (primary infection or reactivation) is also possible, the frequency is about 1 % [4-7]. Chromosomal integration of HHV-6A and HHV-6B (ciHHV6) in germ line also enables the vertical transmission via a Mendelian manner, the frequency is 0.2-2 % [8]. Although HHV-6A or HHV-6B can be detected in the genital tract of a fifth of pregnant women, perinatal transmission is suggested to be unlikely [9, 10].

Primary infection occurs in children between 6 months and 2 years, almost exclusively with HHV-6B, after which latency is established [11]. Specific clinical symptoms are not realised in most of the cases, but exanthema subitum is a characteristic disease in 1/3 of the infections [2]. Little is known about HHV-6A infection. It may cause symptomatic, even primary [12] infection in children, but it is also suggested that asymptomatic HHV-6A infection may occur after the HHV-6B childhood infection, later in life [2]. Reactivation of latent HHV-6B and HHV-6A may occur in mostly immunocompromised patients and rarely in immunocompetent individuals [8]. Transient immunosuppression during pregnancy may result in reactivation of latent infections or may result in higher susceptibility to infections. Seroprevalence studies revealed that the rate of HHV-6 seropositivity in pregnant and non pregnant women are not different and 97-100 %, but the antibody titer is significantly lower in pregnant women, and low avidity IgG was detected in 5 % of pregnant women [13, 14]. Since only some publications are available about HHV-6 infection during pregnancy, and no data is published from Hungary, the aim of this study was to determine the prevalence of

HHV-6A and HHV-6B in peripheral blood from pregnant and non pregnant women in Hungary, to study whether active infection occurs during pregnancy.

## **Materials and methods**

### *Study groups and samples*

EDTA blood samples were collected from 100 healthy pregnant (age 16.5–41.9 years, median 32.1 years) women. 28 of them were in the first trimester, 27 in the second, and 45 in the third trimester of pregnancy. As control, EDTA blood samples were collected from healthy, non pregnant women (age 18–44.3 years, median 31.6 years). Regional and Institutional Ethics Committee of University of Debrecen approved the study.

Nucleic acid was isolated immediately after blood taken from samples using High Pure Viral Nucleic Acid Kit (Roche, Switzerland) according to the manufacturer's instructions. Plasma fraction was obtained from EDTA blood sample after centrifugation, then 200  $\mu$ L plasma was used for nucleic acid isolation. Red blood cells from the cell pellet were lysed with sterile, nuclease free lysis buffer containing ammonium chloride, potassium carbonate and EDTA. Leukocytes (white blood cells, WBCs) were washed with sterile, nuclease free phosphate buffered saline, then nucleic acid was isolated from  $1.5 \times 10^6$  WBCs. Nucleic acids were stored at  $-20^\circ\text{C}$  until use.

### *Detection of HHV-6A and HHV-6B DNA in samples*

Nested HHV-6 PCR which able to differentiate HHV-6A and HHV-6B based on the size of the PCR amplicons was performed as described previously [15]. Quantitative HHV-6 real-time PCR (Q-PCR) was carried out with HHV6 ELITe MGB® Kit (ELITech Group, France) using 7500 Real-Time PCR System (Applied Biosystems, USA) according to the instructions. The multiplex real-time kit ensures detection of human beta globin gene beside

the HHV-6 U67, hence effectiveness of nucleic acid isolation was also tested. Chi square test and Fisher's exact test were used to assess the difference in frequency for categorical variables. Difference was considered significant if  $p < 0.05$ .

#### *HHV-6 reverse transcription-PCR (RT-PCR)*

To synthesize cDNA High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) with random hexamers were used after DNaseI treatment of nucleic acid isolated from WBCs (Sigma-Aldrich, USA). To control the effectiveness of RNA isolation, GAPDH RT-PCR was carried out as detailed previously [16]. HHV-6 RT-PCR was performed as described previously [15].

## **Results**

HHV-6B was detected in 14 WBC samples from pregnant women and 15 WBC samples from non pregnant women (14/100 vs. 15/100;  $p > 0.05$ ), while HHV-6A was revealed in WBCs from one pregnant and one non pregnant woman (1/100 vs. 1/100). The leukocytes from this non pregnant woman carried both HHV-6A and HHV-6B DNA. Viral DNA was detected in WBCs with nested PCR, but HHV-6 DNA was found using real-time PCR only in four control WBC samples with less than 10 genome equivalent (GEq) per reaction (Table I). All the plasma samples from these patients were negative for HHV-6B and HHV-6A DNA, suggesting that HHV-6B and HHV-6A latency was detected.

One plasma sample from a 35 week pregnant woman was positive for HHV-6A DNA, the viral load was  $2 \times 10^2$  GEq / mL plasma. HHV-6A DNA with  $5.07 \times 10^5$  GEq /  $1.5 \times 10^6$  WBCs viral load was detected also in the leukocytes (Table II.). HHV-6 RT-PCR was negative, but GAPDH RT-PCR proved that RNA isolation was successful. HHV-6A DNA was detected by nested PCR in plasma samples from four non pregnant women, and in

leukocytes from three of them. In plasma sample with HHV-6 negative WBCs Q-PCR did not detect viral DNA. The viral loads are detailed in Table II. HHV-6 RT PCR did not prove active HHV-6 infection, GAPDH RT-PCR confirmed the presence of amplifiable RNA, all the samples were positive for GAPDH RNA.

## **Discussion**

The present study examined HHV-6A and HHV-6B prevalence in peripheral blood from pregnant and non pregnant women. HHV-6B and HHV-6A positivity in leukocytes referring to latency was found both in pregnant and non pregnant women; the prevalence did not show difference between the two groups (15/100 vs. 16/100). HHV-6B past infection was dominant in both pregnant (14/15) and non pregnant (15/16) study group. Latency was found in all three trimesters. One pregnant woman carried latent HHV-6A and HHV-6B DNA together in her peripheral leukocytes. Our data strengthen the suggestion that primary infections occur mainly with HHV-6B [2]. The frequency of DNA positivity found in peripheral blood samples depends on the methods used [4, 11, 17]. The sensitivity of the nested PCR used in this study was better than the Q-PCR. The commercially available kit validated for clinical diagnosis and used in this study found only 4 samples to be positive for latent HHV-6 DNA, even the sensitivity of the Q-PCR is 10 GEq / PCR.

HHV-6A DNA in plasma was detected by nested PCR in samples from pregnant (2/100) and non pregnant women (4/100), the difference is not significant statistically. In one pregnant woman HHV-6 DNA was found only in plasma and not in WBCs, and the viral load was less than the sensitivity of the Q-PCR. In that case HHV-6 might replicate in other tissue and not in blood. HHV-6B in plasma samples was not detected. HHV-6 RT PCR did not prove active HHV-6 replication, since HHV-6 U79/80 mRNA could not be detected. Beta globin DNA, hence the release of genomic DNA from dead cells was detected in nucleic acids

isolated from plasma using HHV6 ELITe MGB® Kit. Not any of these patients had clinical symptoms. We have no data about the previous HHV-6 serostatus of the patients. It cannot be excluded whether any of the studied patients have ciHHV-6, but Q-PCR did not confirm it. Despite the HHV-6 viral loads in plasma were  $2.03 \times 10^2 - 2.5 \times 10^3$  GEq / mL plasma, RT-PCR did not prove active HHV-6 infection. Presence of genomic DNA from dead cells was revealed suggesting that HHV-6 DNA might be released from cells. In patients with ciHHV-6 HHV-6 genome exists in all nucleated cell, the ratio of viral genome to human genome is 1:1 [8]. Although HHV-6 viral loads in WBC samples were high in these patients, the HHV-6 copy number / 1 leukocyte did not prove ciHHV-6 (0.08-0.33 GEq/ 1 leukocyte) unequivocally, hence postnatal infections are most likely [8, 18]. Infection or reactivation shortly before the sample taken cannot be excluded. Since the sample from a pregnant woman was collected in the 35<sup>th</sup> week of pregnancy, it might occur during pregnancy. Reactivation of latent infection during pregnancy might be transmitted through the placenta, but antibodies against the known virus might prevent congenital infections. At the same time, antibody from previous HHV-6B or HHV-6A infection might not prevent from reinfection with the other HHV-6 virus or from different genotypes [13].

In conclusion, active HHV-6A or HHV-6B infection was not detected during pregnancy. Frequency of HHV-6B and HHV-6A latency did not show difference between pregnant and non pregnant study group, HHV-6B virus was found to be dominant. In blood samples from one pregnant woman and three non pregnant women high viral loads of HHV-6A were found, RT-PCR did not confirm active HHV-6 replication. The result of the Q-PCR refers more to postnatal infection than ciHHV-6.

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**Address of the authors**

Beáta Mészáros e-mail: [mesz.beata@gmail.com](mailto:mesz.beata@gmail.com)

Tamás Sápó e-mail: [tsapy@med.unideb.hu](mailto:tsapy@med.unideb.hu)

Lajos Gergely e-mail: [gl@med.unideb.hu](mailto:gl@med.unideb.hu)

Eszter Csoma (corresponding author) e-mail: [csomae@freemail.hu](mailto:csomae@freemail.hu); address: Department of Medical Microbiology, University of Debrecen, 4032 Debrecen, Hungary, Nagyerdei krt. 98.;

fax: +36 52 255 424