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Exploring Maternal-Fetal Transmission of Human Herpesvirus 6: A Study of Placental, Embryonic, and Autopsy Samples

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ABSTRACT

Background: Considering the limited studies conducted on the possibility of vertical transmission of HHV-6 (human herpesvirus 6) in humans at different stages of pregnancy, the objective of this research was to examine the vertical transmission of HHV-6 in various tissues of aborted fetuses at different months of pregnancy. .

Materials & Methods: This research was conducted on 58 formalin-fixed paraffin-embedded tissues (FFPE) from 26 fetopsies. DNA extraction was performed using the phenol-chloroform technique. The quantity of extracted DNA samples was measured using the NanoDrop spectrophotometric method. PCR of the beta-globin gene confirmed the quality of the extracted DNA, and then the presence of the HHV-6 genome was tested using the real-time PCR method.

Findings: Of the 26 fetuses examined, 22 (84.6%) were negative for HHV-6, and four (15.4%) were positive. All six first-trimester fetuses were negative. Among 13 second-trimester fetuses (29 FFPE tissues), two (7.7%) tested positive. While none of the seven third-trimester fetuses (22 FFPE tissues) had positive placentas, HHV-6 was detected in non-placental fetal tissues, including the liver of fetus No. 16 and the heart of fetus No. 22, both of which were in the third trimester.

Conclusion: These findings suggest that while vertical transmission of HHV-6 may occur, particularly in later stages of pregnancy and in specific fetal tissues, the overall prevalence in the sample studied was relatively low. Further investigation is needed to understand the implications of these results for maternal and fetal health.

Keywords: HHV-6, Pregnancy, Vertical transmission, Placenta

CITATION LINKS

[1] Pantry SN, Medveczky PG. Latency, integration, and reactivation of human... [2] Arbuckle JH, Medveczky PG. The molecular biology of... [3] Campadelli-Fiume G, Mirandola P, Menotti L. Human herpesvirus... [4] Agut H, Bonnafous P, Gautheret-Dejean A. Update on infections with human... [5] Komaroff AL, Pellett PE, Jacobson S. Human herpesviruses 6A and 6B in brain diseases... [6] Caserta MT, Hall CB, Schnabel K, Lofthus G, McDermott MP. Human herpesvirus... [7] Komaroff AL, Rizzo R, Ecker JL. Human herpesviruses 6A and 6B in reproductive diseases... [8] Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. Clin Microbiol Rev... [9] Levy JA, Ferro F, Greenspan D, Lennette E. Frequent isolation of... [10] Caserta MT, Mock DJ, Dewhurst S. Human.... [11] Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of... [12] Baillargeon J, Piper J, Leach CT. Epidemiology of human herpesvirus 6 (HHV-6) infection in pregnant and... [13] Bortolotti D, et al. HHV-6A infection of endometrial epithelial cells affects miRNA... [14] Ashshi AM, Cooper RJ, Klapper PE, Al-Jiffri O, Moore L. Detection of human herpes virus 6 DNA in fetal hydrops.... [15] Aubin JT, et al. Intrauterine transmission of human herpesvirus... [16] Shokoofeh M, Malekshahi SS, Soltanghoraee H. The molecular footprints of BK virus in... [17] Hall CB, et al. Transplacental congenital... [18] Fjaertoft G, Dahl H, Linde A. Transmission of human herpesvirus 6 (HHV-6) from... [19] Dahl H, Fjaertoft G, Norsted T, Wang FZ, Mousavi-Jazi M, Linde A. Reactivation of human herpesvirus...

Introduction:

Human herpesvirus type 6 (HHV-6) was initially identified in 1986 in the blood of patients suffering from different lymphoproliferative disorders, including some cases associated with AIDS (acquired immunodeficiency syndrome). HHV-6 is a DNA virus of the *herpesviridae* family, beta-*herpesvirinae* subfamily, and *Roseolovirus* genus [1]. After initial infection, HHV-6 establishes a latent state in the host, particularly in lymphocytes and monocytes [2]. HHV-6 is most commonly recognized for causing roseola infantum, also known as exanthema subitum or sixth disease, in young children.

The clinical presentation typically includes a high fever lasting for a few days, followed by a mild maculopapular rash [3]. In addition, HHV-6 has been implicated as a source of opportunistic infections in immunocompromised patients, which could manifest as encephalitis, hepatitis, colitis, and pneumonitis [4]. HHV-6 has a broad tropism, infecting various cell types across different body systems, including the immune and nervous systems. Other tissue cells such as endothelial cells, epithelial cells, human fibroblasts, and liver cells could also be infected, along with various cells of the reproductive system [4, 5]. Following primary infection, HHV-6 establishes latency in the central nervous system (CNS), peripheral blood mononuclear cells (PBMCs), salivary glands, and female reproductive tract [6]. Recent research has indicated that HHV-6 may contribute to primary infertility and be involved in the pathogenesis of preeclampsia (PE) in some cases [7]. The exact mechanism of HHV-6 transmission is still under investigation; several studies have shown transmission of the virus through saliva [8, 9]. The HHV-6 genome has also been identified in the CSF (cerebrospinal fluid) of children during both primary and latent infections as well as in brain material of adults, implicating both the CNS and salivary glands as reservoirs for viral latency [10]. HHV-6 has been detected in various reproductive tissues, including the vagina, cervix, and uterus. Research has shown that HHV-6 could be found in the genital secretions of about 4% of non-pregnant women, while its prevalence among pregnant women ranges from 2 to 18% [6, 11, 12]. HHV-6 could infect endometrial cells and syncytiotrophoblasts, which are crucial for implantation and placental development.

The virus reaches these reproductive organs primarily through the bloodstream, often via infected lymphocytes [13]. Some research suggests that HHV-6 may be transmitted from mother to fetus.

In 2000, the HHV-6 genome was detected in paraffin-embedded tissue samples (heart and lung, kidney, liver, and placenta) from eight fetuses with hydrops fetalis (at 16 and 17 weeks of pregnancy) but in none of the 10 stillborn fetuses without hydrops [14]. A study involving thymus samples from 52 fetuses acquired via induced abortion from HIV-1-seropositive women found HHV-6 in one specific case. The fetus also exhibited HHV-6 DNA in various tissues, including PBMCs, liver, spleen, brain, and CSF. Their study results provided evidence of intrauterine transmission of HHV-6 [15].

Given that HHV-6 could infect a wide range of host cells and is associated with diseases such as preeclampsia, infertility, and endometrial infections, some researchers have explored the possibility of vertical transmission of HHV-6 in humans. Although some studies have demonstrated its transmission from mother to fetus, further research is needed.

Objectives: Therefore, considering the limited studies conducted in this field at different stages of pregnancy, the objective of this research was to examine the vertical transmission of HHV-6 in various tissues

of aborted fetuses at different months of pregnancy.

Materials and Methods

Study population: This research was conducted on 58 formalin-fixed paraffin-embedded tissues (FFPE) obtained from 26 fetopsies. These tissues included 26 placental tissue samples (six from the first trimester, 13 from the second trimester, and seven from the third trimester), 14 heart tissue samples (seven from the second trimester and seven from the third trimester), 13 liver tissue samples (eight from the second trimester and five from the third trimester), two gonadal tissue samples, two thymus tissue samples, and one kidney tissue sample. The tissue samples were chosen from the pathology laboratory archives at Avicenna Infertility Clinic in Tehran, Iran. An experienced pathologist examined histological slides of the first trimester specimens before laboratory testing. Only samples containing embryonic, fetal, or placental villous tissue were included; those containing maternal or macerated tissue were excluded from the study. Ethical approval for the research was obtained from the Medical Ethics Committee of Tarbiat Modares University (IR.MODARES.REC.1402.173).

DNA extraction: A 10-µm section was carefully cut from paraffin-embedded fetal and placental tissues using single-used blades, placed in sterile 1.5 mL tubes, and handled with frequent changes of gloves and blades to prevent cross-contamination. The sections underwent deparaffinization with xylene, washed with absolute ethanol, and digested overnight at 37 °C in lysis buffer (50 mM Tris-HCl, pH 8.5; 1 mM EDTA; 150 mM NaCl; 1% SDS) containing proteinase K. DNA was extracted using the phenol-chloroform technique, precipitated with cold ethanol, and then dissolved in TE buffer. DNA concentration was measured by a Nanodrop spectrophotometer, and the quality of the extracted DNA was confirmed by PCR amplification of the beta-globin gene [16], which was successful in all 58 samples. PCR was performed in 20 µL reaction mixtures containing 10 µL of Taq 2x Master Mix RED (2 mM $MgCl_2$, Ampliqon), 100–300 ng of DNA, 0.5 µL of each primer (10 pmol), and 7 µL of double-distilled water. Cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 20 seconds, 57 °C for 20 seconds, and 72 °C for 30 seconds.

Real-time PCR amplification: HHV-6 DNA was measured using a STAT-NAT HHV-6 kit (Sentinel CH. SpA) and run on the StepOnePlus real-time PCR system. The kit comprises 6x8-well strips pre-filled with a complete master mix containing enzymes, dNTPs, $MgCl_2$, target-specific oligonucleotides, and primers to perform the reaction. The internal control (beta globin) serves to indicate the functionality of the system and to confirm the absence of polymerase activity inhibitors that could lead to false negatives. Samples were tested in a 25 µL reaction mixture, which included 15 µL of STAT-NAT reconstitution buffer and 10 µL of either extracted DNA or none template control (NTC). Positive and negative controls were run along with each test. The PCR protocol consisted of an initial denaturation step of 2 min at 95 °C, followed by 10 cycles of 15 s at 95 °C and 60 s at 60 °C, succeeded by 35 cycles of 15 s at 95 °C and 60 s at 60 °C.

Findings

The median age of the 26 pregnant women was 32 years (interquartile range: 28–35). Among the studied mothers, 22% had blood type A+, 4.3% had blood type A-, 30% had blood type B+, 30% had blood type O+, and 13% had blood type O-. However, there was no significant association between maternal

Table 1) The 26 studied fetuses and their associated information

Case	Pregnancy trimester	Tissues obtained from each fetus	Mother's age (year)	Gestational age (week)	HHV-6 PCR result
1	First	Placenta	29	12	Negative
2	First	Placenta	37	10	Negative
3	First	Placenta	28	12	Negative
4	First	Placenta	N/A	12	Negative
5	First	Placenta	31	12	Negative
6	First	Placenta	34	12	Negative
7	Second	Placenta	32	18	Negative
8	Second	Placenta	34	21	Negative
9	Second	Placenta	39	27	Negative
10	Second	Placenta	43	17	Negative
11	Second	Placenta	29	23	Negative
12	Third	Placenta/Liver/Heart	28	28	Negative
13	Third	Placenta/Liver/Heart	34	40	Negative
14	Third	Placenta/Liver/Heart/Thymus	28	29	Negative
15	Third	Placenta/Liver/Heart/Kidney	35	31	Negative
16	Second	Placenta/Liver/Heart/Thymus	38	15	Positive in Liver
17	Second	Placenta/Liver	40	17	Negative
18	Second	Placenta/Liver/Heart	36	16	Negative
19	Second	Placenta/Liver/Heart		15	Negative
20	Third	Placenta/Liver/Heart/Gonad	40	28	Negative
21	Third	Placenta/Heart	29	39	Negative
22	Third	Placenta/Heart	28	28	Positive in heart
23	Second	Placenta/Liver/Heart	25	21	Positive in placenta
24	Second	Placenta/Liver/Heart	27	26	Positive in placenta
25	Second	Placenta/Liver/Heart	35	21	Negative
26	Second	Placenta/Liver/Heart	18	19	Negative

blood type and the presence of the virus (p= .091). Additionally, the variables of parental family relationship (p> .9), method of conception (p= .7), and method of abortion (p= .2) were not significantly associated with the presence of the virus. The characteristics of the 26 fetuses are detailed in Table 1, including data on the tissues examined, maternal age, gestational week, trimester of pregnancy, and the tissues that tested positive for the presence of HHV-6. Among the 26 fetuses, six fetuses with a mean age of 12 weeks were in the first trimester of pregnancy, and none of them tested positive for HHV-6. Also, 13 fetuses (with 29 FFPE tissues) with a mean age of 18 weeks were in the second trimester, while seven fetuses (with 22 FFPE tissues) with a mean age of 29 weeks were in the third trimester. Among the placental materials of the second-trimester fetuses, only two out of the 13 (7.7%) cases tested positive for HHV-6. Notably, there were instances where no virus was detected in placental tissues, but HHV-6 was found in

other tissues, including fetus No.16, which tested positive for HHV-6 in liver tissue, and fetus No. 22, which tested positive for HHV-6 in heart tissue, both of which were in the third trimester. Out of the 26 fetuses examined, 22 (84.6%) were negative for HHV-6, while four (15.4%) were positive.

Discussion

This study results provide valuable insights into vertical transmission of HHV-6 during pregnancy. Previous research has demonstrated that HHV-6 could be transmitted to the fetus via the placenta. However, the extent and conditions under which this virus is transmitted, especially at different stages of pregnancy, remain poorly understood. The findings suggest that vertical transmission of HHV-6 to the fetus is rare and occurs mainly in the second and third trimesters of pregnancy. This may be attributed to physiological changes in both the placenta and the maternal immune system during these stages, which may facilitate viral transmission. In this study, 84.6% of fetuses tested negative for HHV-6, indicating a low rate of vertical transmission. These results align with the findings of similar studies; for example, Hall et al. (2010) [17] also reported that fetal transmission of HHV-6 was a relatively uncommon occurrence. The present study findings suggest that the virus could be detected in various fetal tissues, even when the placenta is negative for the virus. This suggests that the placenta may not be the sole route of transmission, and that alternative mechanisms are likely involved in vertical transmission of HHV-6. Notably, two cases of HHV-6 transmission were identified in the second trimester, which may reflect the influence of immunological changes and increased invasiveness of viral cells during this period. Furthermore, in the third trimester, the detection of HHV-6 in fetal liver and heart tissues indicates the ability of the virus to infect specific fetal organs. This may highlight the importance of a broader investigation into various fetal tissues to identify infection patterns and transmission routes. Given the possible involvement of HHV-6 in pregnancy-related diseases such as preeclampsia and endometrial infections as well as its effects on fetal health, further studies on vertical transmission of this virus and its effects are warranted. In a study involving 107 newborns, HHV-6 DNA was detected in 5.6% of cord blood samples. The analysis showed that the presence of HHV-6 DNA was significantly higher in mothers compared to healthy controls, indicating that maternal infection may lead to fetal exposure during pregnancy or delivery [18]. Research has indicated that HHV-6 tends to reactivate during pregnancy, with HHV-6 DNA detected in 41-44% of maternal blood samples during the second trimester and only in 1% of cord blood samples, suggesting that while reactivation is common, actual transmission to the fetus remains relatively rare [19].

One of the findings of this study is the detection of HHV-6 in specific fetal tissues such as the liver and heart without detection of the virus in the placenta. This finding suggests that the placenta is not the only route of virus transmission, and that the virus may be transmitted to the fetus through other routes such as maternal blood. Previous studies have focused less on this issue and more on the role of the placenta and have not typically examined HHV-6 transmission throughout pregnancy, while this study shows that virus transmission occurs mainly in the second and third trimesters of pregnancy compared to the first trimester. This difference could be due to physiological changes in the placenta and the maternal immune system. While confirming previous findings on the low rate of vertical

transmission of HHV-6, the present study results provide valuable insights into the role of nonplacental tissues and differences in the timing of vertical transmission. These differences emphasize the need for a more comprehensive study of the transmission mechanisms and effects of HHV-6 in different fetal tissues. The limitation of the present study was the small sample size; thus, future studies with larger sample sizes should investigate these mechanisms and the impact of HHV-6 infection on pregnancy outcomes and fetal development.

Conclusion

These findings suggest that although vertical transmission of HHV-6 may occur, particularly in later stages of pregnancy and in specific fetal tissues, the overall prevalence in the studied sample was relatively low. Further investigation is needed to understand the implications of these results for maternal and fetal health.

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Ethical permissions: This study was approved by the Ethics Committee of Tarbiat Modares University (IR.MODARES.REC.1402.173).

Authors' contributions: SSM designed and supervised the work. MSH contributed to sample collection and genome extraction. MGH performed laboratory tests. SSM wrote the manuscript.

Conflicts of interests: The authors declare no competing interests

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References

1. Pantry SN, Medveczky PG. Latency, integration, and reactivation of human herpesvirus-6. Viruses. 2017;9(7):194.
2. Arbuckle JH, Medveczky PG. The molecular biology of human herpesvirus-6 latency and telomere integration. Microbes Infect. 2011;13(8-9):731-41.
3. Campadelli-Fiume G, Mirandola P, Menotti L. Human herpesvirus 6: An emerging pathogen. Emerg Infect Dis. 1999;5(3):353-66.
4. Agut H, Bonnafous P, Gautheret-Dejean A. Update on infections with human herpesviruses 6A, 6B, and 7. Med Mal Infect. 2017;47(2):83-91.
5. Komaroff AL, Pellett PE, Jacobson S. Human herpesviruses 6A and 6B in brain diseases: Association versus causation. Clin Microbiol Rev. 2020;34(1):10-128.
6. Caserta MT, Hall CB, Schnabel K, Lofthus G, McDermott MP. Human herpesvirus (HHV)-6 and HHV-7 infections in pregnant women. J Infect Dis. 2007;196(9):1296-303.
7. Komaroff AL, Rizzo R, Ecker JL. Human herpesviruses 6A and 6B in reproductive diseases. Front Immunol. 2021;12:648945.
8. Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. Clin Microbiol Rev. 1997;10(3):521-67.
9. Levy JA, Ferro F, Greenspan D, Lennette E. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. Lancet. 1990;335(8697):1047-50.
10. Caserta MT, Mock DJ, Dewhurst S. Human herpesvirus 6. Clin Infect Dis. 2001;33(6):829-33.
11. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of pregnant women. J Clin Microbiol. 1995;33(7):1968-70.
12. Baillargeon J, Piper J, Leach CT. Epidemiology of human herpesvirus 6 (HHV-6) infection in pregnant and nonpregnant women. J Clin Virol. 2000;16(3):149-57.
13. Bortolotti D, Soffritti I, D'Accolti M, Gentili V, Di Luca D, Rizzo R, et al. HHV-6A infection of endometrial epithelial cells affects miRNA expression and trophoblast cell attachment. Reprod Sci. 2020;27:779-86.
14. Ashshi AM, Cooper RJ, Klapper PE, Al-Jiffri O, Moore L. Detection of human herpes

virus 6 DNA in fetal hydrops. Lancet. 2000;355(9214):1519-20.
15. Aubin JT, Poirel L, Agut H, Huraux JM, Bignozzi C, Brossard Y, et al. Intrauterine transmission of human herpesvirus 6. Lancet. 1992;340(8817):482-3.
16. Shokoofeh M, Malekshahi SS, Soltanghoraee H. The molecular footprints of BK virus in the product of conception over the second and third gestational trimesters. BMC Res Notes. 2023;16(1):367.
17. Hall CB, Caserta MT, Schnabel KC, Shelley LM, Carnahan JA, Marino AS, et al. Transplacental congenital human herpesvirus 6 infection caused by maternal chromosomally integrated virus. J Infect Dis. 2010;201(4):505-7.
18. Fjaertoft G, Dahl H, Linde A. Transmission of human herpesvirus 6 (HHV-6) from mother to infant during pregnancy and/or delivery. Reactivation of HHV-6 during pregnancy.• 1310. Pediatr Res. 1997;41(4):221.
19. Dahl H, Fjaertoft G, Norsted T, Wang FZ, Mousavi-Jazi M, Linde A. Reactivation of human herpesvirus 6 during pregnancy. J Infect Dis. 1999;180(6):2035-8.