SEROLOGICAL MARKERS OF SELECTED SEXUALLY AND BLOOD TRANSMITTED INFECTIONS IN PREGNANT WOMEN AND IN NEWBORNS OF HIV-POSITIVE MOTHERS IN THE SLOVAK REPUBLIC

Danica Staneková[1], **Jarmila Adamčáková**[1], Tana Kopilcová[3], Ján Kotuliak[2], Elena Vaculiková[2], Monika Hábeková[1], Miloš Mokráš[2]

[1]National Reference Center, Slovak Medical University, Bratislava, Slovak Republic
[2]Department of Microbiology, Derer´s Hospital, Bratislava, Slovak Republic
[3]Department of Infectology and Geographical Medicine, Derer´s Hospital, Bratislava, Slovak Republic
[4]Hospital of the Ministry of Defence SR, Bratislava, Slovak Republic

SUMMARY

Aim of the study was to investigate serological markers of selected sexually and blood transmitted infections (HBV, HCV, CMV, and *Treponema pallidum*) in the group of pregnant women and in newborns of HIV-positive mothers in Slovakia.

IgG antibodies to CMV were found in 78 of 97 women, from them in 6 of 7 HIV-positive and in 72 of 90 HIV-negative persons. Occurrence of HbsAg and HCV was significantly higher in the group of HIV-positive women (1/7 and 2/7) comparing to the HIV-negative one (4/90 and 0/92, respectively). Antibodies to *T.pallidum* were found only in one HIV-negative woman from 92 women tested.

Five of seven children born to HIV-1 infected mothers were found HIV-1 negative, two children are still under control. Four of 7 newborns were found anti-CMV IgG positive. Although one woman, IDU, was confirmed HbsAg and anti-HCV positive, vertical transmission of HBV and HCV to her newborn was not observed by molecular-biological methods. Similarly, HCV antibodies were found in one more women and neither in her child HCV infection was confirmed. In no one of mother – child pair's antibodies to *T.pallidum* were observed.

Due to these findings strong attention should be paid to health education and prophylaxis of mother to child HIV and other STIs transmission in Slovakia.

Key words: pregnant women, newborns, HIV, STIs

Address for correspondence: Danica Staneková, NRC HIV/AIDS, Slovak Medical University, Limbová 14, 833 01 Bratislava, SR.
E-mail: danica.stanekova@szu.sk

INTRODUCTION

Slovakia (SR) ranks among the countries with relatively low HIV (human immunodeficiency virus) prevalence. From 1985 when the first case of HIV-infection was found in former Czechoslovakia till December 31 2004, 216 HIV-infected people were found in Slovakia, out of them 78 were foreigners. From 138 Slovak citizens 24 were women. According to the rules embedded in the "Expert Guidelines for providing HIV prevention in SR" (Gazette of Slovak Ministry of Health 2000, part 6-9) pregnant women are voluntarily tested for antibodies to HIV (HIV-Ab) in the first trimester of pregnancy and repeatedly one month before the delivery. In Slovakia mandatory HIV testing of pregnant women was provided only from 1991 to 1992. During this period no one pregnant women was found HIV-positive. Later on from 1997 to 2003, 7 pregnant women were found HIV-1 positive in Slovakia.

High rates of STIs were reported where HIV epidemic spread rapidly. Reports from earlier studies suggest that untreated STIs that disrupt epithelial surfaces increase the efficiency of sexual transmission of HIV. Therefore preventing, diagnosing and treating of STIs are essential components of an effective HIV prevention strategy. The seroprevalence of HIV and other sexually and blood transmitted diseases in pregnant women differs from country to country. For example in Greece 2.907% and 0.16% of pregnant Greek women were tested positive for HbsAg and HCV, respectively (1). In Northeast Italy the seroprevalence of HbsAg was found to 1.0%, anti-HCV was 1.9%, and the prevalence of anti-HIV was 0.3% (2). In Bali 1.9% and 0.04% of pregnant women were tested positive for HbsAg and HCV, respectively (3). Similarly, low prevalence of HbsAg and antibodies to *T.pallidum* (0.4% and 0%) was observed in studies of pregnant women in Salamanca, Spain (4).

Abbreviations: Ab – antibodies, AZT – Azidothymidine, AIDS – acquired immunodeficiency syndrome, ART – antiretroviral therapy, CDC – Center for Disease Control, CMV – cytomegalovirus, HBV – hepatitis B virus, HCV – hepatitis C virus, HIV – human immunodeficiency virus, HSV – herpes simplex virus, ND – not done, IDU – injecting drug use, IDUs – injecting drug users, PEP – post exposure prophylaxis, PCR – polymerase chain reaction, p.o. – per os, STIs – sexually transmitted infections, TPHA – *Treponema pallidum* hemagglutination assay

Most of sexually transmitted infections could be also transmitted vertically from mother to child. Daily more than 1600 newborns are infected by HIV from their mothers from all over the world (5). Probability of HIV-vertical transmission differs according to the geographical region. In EU to the end of 2003 2066 newborns became HIV-infected by mother-to child transmission (6). From 1994 when antiretroviral therapy (ART) was introduced into the prophylaxis of neonates of HIV-positive mothers significant decrease of HIV-vertical transmission has been observed in USA (to 3%), in Europe (to 5.4%) (7).

The aim of our study was to investigate the prevalence of serological markers of other sexually and blood transmitted infections in the groups of HIV-negative and HIV-positive pregnant women and newborns born to HIV-positive mothers in Slovakia.

MATERIALS AND METHODS

Patients: In the study blood samples of 97 pregnant women and 7 newborns of HIV-1 positive mothers were collected at the Department of Microbiology, Bratislava and in the National Reference Center for HIV (NRC HIV/AIDS) in Bratislava in the period of 2000–2004. Pregnant women were tested for HIV and other infections in the 1st trimester of pregnancy. Five of seven HIV-infected pregnant women (No. 2, 3, 4, 5, 7) took antiretroviral therapy from 28 weeks of expectancy (AZT 2 x 250 mg daily) and AZT infusion during Caesarean section. HIV-status of two of these women (No. 1 and No. 6) was known only soon after the delivery therefore these women were not treated during the pregnancy. After the delivery lactation of all women was stopped. Post exposure prophylaxis of all newborns of HIV-positive mothers (AZT / Lamivudin p. o.) endured 6 weeks after birth and breastfeeding was avoided.

Laboratory testing in pregnant women: Confirmation of HIV-reactive serum samples was done by ELISA HIV-1/2 Ag/Ab test, Western Blot HIV-1/2 and RT-PCR. HIV-positive women were tested also for CMV IgG antibodies several times during the pregnancy and at the 1st month after the delivery. Samples of HIV-negative pregnant women were tested retrospectively for the presence of hepatitis B surface antigen (HbsAg) and antibodies against HCV, CMV, *T. pallidum*. Antibodies against HCV and *T. pallidum* were tested only in 92 of 97 women, because of insufficient volume of sera collected from 5 HIV-negative women.

Laboratory testing in newborns: HIV testing of newborns of HIV-positive mothers was performed twice a year after the delivery by ELISA HIV-1/2 Ag/Ab test, Western Blot HIV-1/2 and RT-PCR. Confirmation of newborns´ HIV status was completed after 2 years of follow up using serological and molecular-biological methods. Sera of newborns of HIV- positive mother were screened for the presence of antibodies to HIV and other infections at the 1st month after the delivery. Collection of blood of neonates of HIV-negative mothers was not provided on Paediatric Clinics therefore these samples were not available for testing.

Testing of serological markers: In the study only commercial diagnostic kits were used for the serological diagnostic: For screening testing of HIV Ag/Ab the ELISA tests GENSCREEN PLUS HIV Ag-Ab (Bio-RAD) and ABBOTT HIV 1/2 gO EIA (ABBOTT) were used. Samples with reactive results in screening testing were confirmed with Western Blot HIV Blot 2.2 (ABBOTT). Antibodies to HCV were tested with MONOLISA anti-HCV PLUS Version 2 (BIO-RAD). For testing of acute HBV infection Murex HBsAg Version 3 (ABBOTT) was used. CMV IgG were tested by the use of ETI-CYTOK – G PLUS (DiaSorin). Serological response to *T.pallidum* was tested by the test TPHA 200 (Newmarket Laboratories Ltd).

Testing of molecular biological markers: Following molecular biological tests were used for the confirmatory testing of infectious agents: Cobas Amplicor HIV-1 Monitor™ Test version 1.5 (Roche Diagnostics Systems) RT-PCR was used for the confirmation of HIV-reactive samples and samples of newborns of HIV-positive mothers, Cobas Amplicor HCV RNA v. 2.0 (Roche Diagnostics Systems) for the confirmation of presence of HCV in anti-HCV positive samples.

RESULTS

Testing of serological markers of selected infections – HIV, HBV, HCV, CMV and *T.pallidum* – was performed in the group of 97 pregnant women, including 7 HIV-positive women. Seven newborns of HIV-1 positive mothers were tested for the presence of HBV, HIV and of antibodies to CMV, HCV and *T.pallidum* after the delivery. Following results were obtained by testing of pregnant women (Table 1).

IgG antibodies to CMV were found in 78 of all 97 women, in 6 of 7 HIV-1 positive and in 72 of 90 HIV-1/2 negative persons.

The presence of HBsAg was confirmed in 5 of 97 women (5/97), in 1 of 7 HIV-1 positive and in 4 of 90 HIV-1/2 negative women.

Two of 92 women were found IgM/IgG HCV-positive. Both women belonged to the group of 7 HIV-1 positive persons, one of them was dependent on injecting drug use (IDU). One of these women was confirmed HCV positive by RNA-PCR. HCV testing of the other woman (No. 6) with molecular-biological methods was not done because of lack of her blood sample.

Antibodies to *T.pallidum* were found only in one HIV-1/2 negative women from all 92 women (85 HIV-1 negative and 7 HIV-1 positive) tested.

In this study prevalence of CMV IgG was found to be significantly higher when compared to the prevalence of other serological markers tested in pregnant women. Occurrence of HbsAg and anti- HCV was significantly higher in the group of HIV-positive women (14.3 % and 28.6 %) compared to the HIV-negative ones (4.4 % and 0 %).

To conclude, in all tested persons low prevalence of HBV, antibodies to HCV and *T.pallidum* infection was found, while prevalence of chronic CMV infection seemed to be relatively high in comparison with other infections. Occurrence of HbsAg and antibodies to HCV was found significantly higher in the group of HIV-positive women compared to HIV-negative one.

After 2 years of follow up from the delivery 5 of 7 babies born to HIV-1 infected mothers were confirmed HIV-1/2 negative. Confirmation of HIV status of one child (No. 7) is still not completed. Women No. 6 and her child left Slovakia soon after the childbirth, therefore further testing of this pair could not be done.

Seven pairs (HIV-1 positive mother and her HIV-1/2 negative newborn) were tested for the presence HBsAg, CMV IgG, CMV IgM, antibodies against HCV and *T.pallidum* (Table 2). Six of 7

Table 1. *Prevalence of HbsAg and antibodies to HCV, CMV and T.pallidum infection in pregnant women*

HIV-1 positive	Total	No. of positive	(%)	No. of negative	(%)
CMV (IgG)	7	6	85.7	1	14.3
HBsAg	7	1	14.3	6	85.7
HCV (Ab)	7	2	28.6	5	71.4
T.pallidum (Ab)	7	0	0	7	100
HIV-negative					
CMV(IgG)	90	72	80.0	18	20.0
HBsAg	90	4	4.4	86	95.6
HCV (Ab)	85	0	0	85	100
T.pallidum (Ab)	85	1	1.2	84	98.8
Total tested					
CMV (IgG)	97	78	81.4	19	19.6
HbsAg	97	5	5.2	92	94.8
HCV (IgG)	92	2	2.2	90	97.8
T.pallidum (IgG)	92	1	1.1	91	98.9

HIV-positive pregnant women and 4 from 7 newborns were found positive for IgG antibodies to CMV. Women were tested several times (during the pregnancy, before and soon after the delivery) with negative results for CMV IgM.

One woman (No. 2) dependent on IDU was confirmed HbsAg and HCV positive. Nevertheless vertical HBV mother to child transmission was not confirmed in this pair. However, in this newborn HCV antibodies were found, but HCV infection was not confirmed by the use of HCV PCR. HCV antibodies were found also in one woman (No. 6). This pair left Slovakia soon after the delivery therefore diagnostic of vertical transmission of HIV infection and other STI in this pair could not be completed.

Antibodies to *T.pallidum* were detected in none of mother – child pair.

Table 2. *Results of HIV and selected sexually and blood transmitted infections in HIV-positive mothers and their newborns*

Person tested	PEP	Risk behav.	HIV (PCR)	HVB HBsAg	CMV (IgG)	CMV (IgM)	HCV (AB)	HCV (PCR)	*T. palladium* (TPHA)
Mother No.1	–	–	+	–	–	–	–	–	–
Newborn No.1	–	–	–	–	–	–	–	–	–
Mother No.2	+	IDU	+	+	+	–	+	+	–
Newborn No.2	+	–	–	–	+	–	+	–	–
Mother No.3	+	–	+	–	+	–	–	–	–
Newborn No.3	+	–	–	–	–	–	–	–	–
Mother No.4	+	–	+	–	+	–	–	–	–
Newborn No.4	+	–	–	–	+	–	–	–	–
Mother No.5	+	–	+	–	+	–	–	–	–
Newborn No.5	+	–	–	–	+	–	–	–	–
Mother No.6	–	–	+	–	+	–	+	ND	–
Newborn No.6	–	–	ND	–	+	–	–	ND	–
Mother No.7	+	–	+	–	–	–	–	–	–
Newborn No.7	+	–	NK	–	–	–	–	–	–

PEP = postexposure prophylaxis, ND = not done, NK= not known, IDU = injecting drug usage, behav. = behaviour, Ab = antibodies, TPHA = *Treponema pallidum* hemagglutination assay, PCR = polymerase chain reaction

DISCUSSION

Study of serological markers of HBV, HCV, CMV and *T.pallidum* was accomplished in the group of 97 pregnant women, including 7 HIV-positive, and subsequently in 7 babies born to HIV-infected mothers. All studied infections can be transmitted by sexual contact, by the blood and/or vertically from mother to child.

Testing of other STI enables to predict the trend of long-term asymptomatic HIV infection in the population. Infections with cytomegalovirus are widespread among populations. In our study the prevalence of IgG CMV antibodies in all tested pregnant women was also high. Our findings correlate with the results of seroprevalence studies of CMV infection in the group of 31–40 years old women (79.1%) made in Madrid in 1999 (8) and in pregnant women (83.9%) in Sapporo, Japan (9). Reactivation of CMV infection could be accompanied by the synthesis of IgM antibodies (10). In our study all HIV-1 positive women were investigated for CMV IgM several times during the pregnancy and also soon after the delivery. In our study CMV reactivation was not observed in any case.

Generally the prevalence of HCV infection in pregnant women in Europe is bellow 2% (11). In our study seroprevalence of HCV and HBsAg was low in pregnant women, too. Similarly, low prevalence of HBsAg (6.2%) was observed in studies of pregnant women in Sierra Leone (12). Significantly higher occurrence of HbsAg positivity in our group of HIV-positive pregnant women compared to HIV-negative one could be due to the risk behaviour of HIV-positive women dependent on intravenous drug usage. IDU belongs to high-risk behaviour linked to HIV and other sexually and blood transmitted infections. In our study simultaneous appearance of HbsAg and HCV antibodies was observed in one HIV-positive pregnant woman dependent on IDU.

During the last years the prevalence of syphilis worldwide has been increasing (13). In our study positive result in TPHA was found only in one of 92 tested women. Similarly low results (1.3%) were observed in the group of young pregnant women in Addis Abeba (14). In our study presence of IgG antibodies could be due to the foregoing as well as recent *T.pallidum* infection. Therefore additional tests should be performed in this person.

Slovakia belongs to the countries with relatively low HIV-prevalence. Because of small number of HIV-positive women tested results of our study expressed in percentage could be burden with a real mistake and more precise results can be shown only by real numbers in tables.

In addition, in our study serological markers of selected sexually and blood transmitted infections were tested in newborns born to HIV-infected mothers. All these infections could be transmitted from mother to child via the placenta or perinataly (15). In our study after two years of follow up 5 of 7 children were confirmed HIV-1 negative by serological as well as molecular-biological methods. One of HIV-negative children was born to mother who did not take HIV mother to child prophylaxis either during the pregnancy or during delivery. HIV-status of 2 children is not known because one mother–child pair left Slovakia soon after delivery and testing of the last born child was not finished by now. In comparison with other countries the number of childbirths of HIV-positive women is relatively small in SR. In 2003 estimated 630, 000 children worldwide became infected with HIV – the vast majority of them during their mother's pregnancy and delivery, or as a results of breastfeeding (16).

In the world by now HIV, CMV and HBV vertical transmission through breast-feeding has been documented (17). Risk for postnatal transmission of HCV cannot be excluded but is likely to be low for most of HCV infected women. In our study children born to HIV-infected mothers were not breastfed therefore this type of transmission of HIV and other infections was avoided.

HCV is transmitted mainly parenterally. Generally, HCV mother to child transmission is very rare and it appears in about 5% of women who are positive for viral RNA at the end of pregnancy (18). In our study antibodies to HCV were observed in two HIV-1 infected mothers, but in no one HIV-negative pregnant woman. One of these two women, dependent on IDU, was found HbsAg positive, though her child was confirmed HBV negative. It is assumed that mandatory vaccination of this child against HBV infection in 24 hours after the delivery could be the cause of successful prophylaxis of HBV mother to child transmission. RNA-PCR did not confirm vertical HCV infection in this child, too, although mothers with high levels of HCV-RNA and co-infected for HIV are documented to have risk factors for vertical transmission of HCV (19). Increased risk of HCV transmission is associated with parallel HIV infection in at least 15% (20). In our study the other one women, HIV 1- and anti-HCV-positive, left with her child Slovakia soon after the delivery therefore the diagnostic of infections in this pair could not be completed.

CMV infection is the frequent congenital infection worldwide with the incidence 0.3-2.2% of live births. In our study in 5 of 7 pregnant women and in 4 from 7 neonates IgG CMV antibodies were found. However, testing of CMV antibodies did not reveal acute infection in any mother – child pair. In spite of these results other laboratory tools used in the diagnosis of neonates are recommended (21).

T.pallidum co-infection of HIV-positive women is also significantly associated with vertical transmission of HIV-infection (22). Investigation of vertical transmission of *T.pallidum* seemed to be irrelevant in our study, because of negative results of TPHA testing of all HIV-positive pregnant women.

In our study newborns of HIV-negative mothers could not be tested because their sera were not available. Therefore comparative study of seromarkers of other infections in the group of HIV-positive vs. HIV-negative newborns could not be provided.

Due to all these findings strong attention should by paid to health education and prophylaxis of mother to child HIV and other STI transmission in Slovakia.

Acknowledgements

The authors thank Mrs. Jarmila Präsensová and Ms. Maria Sochanová for their skilful technical assistance.

REFERENCES

1. Panagopoulos P, Economou A, Kasimi A, Spyropoulou P, Kanellopoulos N, Dadiotis L, et al. Prevalence of hepatitis B and C in the maternity department of a Greek district hospital. J Matern Fetal Neonatal Med. 2004 Aug;16(2):106-10.
2. Baldo V, Floreani A, Menegon T, Grella P, Paternoster DM, Trivello R. Hepatitis C virus, hepatitis B virus and human immunodeficiency virus infection in pregnant women in North-East Italy: a seroepidemiological study. Eur J Epidemiol. 2000 Jan;16(1):87-91.

3. Surya IG, Kornia K, Suwardewa TG, Mulyanto, Tsuda F, Mishiro S. Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. J Med Virol. 2005 Apr;75(4):499-503.
4. Gutierrez-Zufiaurre N, Sanchez-Hernandez J, Munoz S, Marin R, Delgado N, Saenz MC, et al. Seroprevalence of antibodies against Treponema pallidum, Toxoplasma gondii, rubella virus, hepatitis B and C virus, and HIV in pregnant women. Enferm Infecc Microbiol Clin. 2004 Nov;22(9):512-6. (In Spanish.)
5. Mofenson LM, Munderi P. Safety of antiretroviral prophylaxis of perinatal transmission for HIV-infected pregnant women and their infants. J Acquir Immune Defic Syndr. 2002 Jun 1;30(2):200-15.
6. EuroHIV. HIV/AIDS Surveillance in Europe. End-year report 2003. Saint-Maurice: Institut de Veille Sanitaire; 2004. No.: 70.
7. McGowan JP, Shah SS. Prevention of perinatal HIV transmission during pregnancy. J Antimicrob Chemother. 2000 Nov;46(5):657-68 .
8. de Ory F, Ramirez R, Garcia Comas L, Leon P, Sagues MJ, Sanz JC. Is there a change in cytomegalovirus seroepidemiology in Spain? Eur J Epidemiol. 2004;19(1):85-9.
9. Numazaki K, Fujikawa T, Asanuma H. Immunological evaluation and clinical aspects of children with congenital cytomegalovirus infection. Congenit Anom (Kyoto). 2002 Sep;42(3):181-6.
10. Treponema, Borrelia and Leptospira. In: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002. p. 378-83.
11. Newell ML, Pembrey L. Mother-to-child transmission of hepatitis C virus infection. Drugs Today (Barc). 2002 May;38(5):321-37.
12. Wurie IM, Wurie AT, Gevao SM. Sero-prevalence of hepatitis B virus among middle to high socio-economic antenatal population in Sierra Leone. West Afr J Med. 2005 Jan-Mar;24(1):18-20.
13. Lee MJ, Hallmark RJ, Frenkel LM, Del Priore G. Maternal syphilis and vertical perinatal transmission of human immunodeficiency virus type-1 infection. Int J Gynaecol Obstet. 1998 Dec;63(3):247-52.
14. Nicoll A, Hamers FF. Are trends in HIV, gonorrhea, and syphilis worsening in western Europe? BMJ. 2002 Jun 1;324(7349):1324-7.
15. Tsegaye A, Rinke de Wit TF, Mekonnen Y, Beyene A, Aklilu M, Messele T et al. Decline in prevalence of HIV-1 infection and syphilis among young women attending antenatal care clinics in Addis Ababa, Ethiopia: results from sentinel surveillance, 1995-2001. Ethiop Med J. 2003 Jun;41 Suppl 1:31-4.
16. Friedberg M. Congenital syphilis: need for adequate antenatal care. Harefuah. 1999 Nov 15;137(10),457-9, 510. (In Hebrew.)
17. 2004 report on the global HIV/AIDS epidemic: 4th global report. Geneva: UNAIDS; 2004.
18. Pool R, Nyanzi S, Whitworth JA. Breastfeeding practices and attitudes relevant to the vertical transmission of HIV in rural south-west Uganda. Ann Trop Paediatr. 2001 Jun; 21(2):119-25.
19. Mok J, Pembrey L, Tovo PA, Newell ML; European Pediatric Hepatitis C Virus Network. When does mother to child transmission of hepatitis virus C occur? Arch Dis Child Fetal Neonatal Ed. 2005 Mar;90(2):F156-60.
20. Batallan A, Faucher P, Poncelet C, Demaria F, Benifla JL, Maedelenat P. Mother-to- child transmission of hepatitis C virus: recent news about the benefit of caesarean sections. Gynecol Obstet Fertil. 2003 Nov;31(11):964-8. (In French.)
21. Ranger-Rogez S, Alain S, Denis F. Hepatitis viruses: mother to child transmission. Pathol Biol (Paris). 2002 Nov:50(9):568-575. (In French.)
22. Fedorova NE, Madzhidova MG, Vorontsova ON, Degtiarev DN, Pustowoit B, Asadi Mobarkhan SM, et al. Quantitative laboratory methods for the diagnosis of cytomegalovirus infection in premature infants.Vopr Virusol. 2005 Jan-Feb;50(1):9-14. (In Russian.)

Received December 13, 2005
Received in revised form and accepted March 29, 2006