



## **The prevalence of TTV and SEN-V among children who borned from HIV-infected mothers**

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### **ABSTRACT**

Women may have persistent risk of HIV acquisition during pregnancy and postpartum . Children acquire infection through mother-to-child transmission (MTCT) either during pregnancy, delivery, or by breast-feeding .evaluating risk of HIV during these periods is important to inform optimal prevention approaches. The aim of this study was to determine the prevalence of TTV and SEN-V among children who borned from HIV-infected mothers by multiplex-nested-RT-PCR protocol and ELISA test. Samples were obtained from 100 infants born with HIV- infected mothers. Infants were followed to 18 months of age and plasma was stored within 7 days of birth, at 2, 5, 6, and 8 weeks, and 3, 6, 9, 12 and 18 months. Mothers and their Infants were tested for HIV ,SEN Virus and TTV using multiplex-nested-RT-PCR protocol. In this study, Of 100 children ( Female=59, Male=41 ) were born from mothers with HIV who samples were collected after birth. Examined using PCR technique showed of 150 mother who were infected with HIV, 72/150(48%) SENV and 63/150 (42%) TTV . While PCR results showed that infants were infected with HIV,58%TTV positive and 48% SENV positive. In weeks 5 and 10 samples were collected from all infants and were analyzed by PCR technique. There was no difference in the results compared to the seventh day. If babies born with HIV were treated with the ART drugs. There was a high frequency of late diagnosis in this patient cohort. Earlier diagnosis is an important measure for controlling HIV among children and also the results showed that pregnant women with HIV, if, according to a regular schedule ART drugs are prescribed. Reduced risk of transmission to infants.

**Keywords:** HIV, MTCT, SENV, TTV.

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### **INTRODUCTION**

Mother-to-child transmission [MTCT] of HIV resulted in approximately 370,000 infant infections worldwide in 2009 [1]. This same year, an estimated 2.5 million children worldwide were living with HIV, mostly a consequence of MTCT, and more than 90% of these children are in sub-Saharan Africa[1]. In the absence of any intervention, the combined risk of MTCT of HIV in utero and intrapartum is 15-30%. The risk is increased in breastfed children to 20- 45%[2,3]. It has been proven that antiretroviral [ARV] drugs given to HIV-positive pregnant women and their newborn babies reduce the risk of MTCT[4-6]. In resource-limited countries, about 35% and 52% of HIV infected infants without any therapeutic intervention die by age one and two respectively[7]. Observational studies in developed countries and a randomized clinical trial in South Africa demonstrated that early initiation of Highly Active Anti- Retroviral Therapy [HAART] in infants considerably reduces morbidity and mortality[8-11].

Human immunodeficiency virus [HIV] and hepatitis viruses are the most common chronic viral infections throughout the world. Patients infected with HIV are often co-infected with other pathogens such as SEN Virus and TT[12,13]. As with HIV, these viruses can be transmitted efficiently through blood transfusion, sexual intercourse, and parenteral routes. Available reports suggest that SEN Virus and TT are more prevalent in high-risk groups such as hemodialysis patients, hemophiliacs, intravenous drug abusers, homosexuals, and heterosexuals[14,15].

These viruses are prevalent worldwide in healthy populations, such as blood donors and also in certain groups of patients, such as HIV-infected subjects. In recent years, each time a new virus is discovered, studies on its effects on mothers and fetuses have received great attention.

SEN Virus [SEN-V] and TT virus [TTV] were classified in the circoviridae family. TTV and SEN-V are assumed to be responsible for post-transfusion non A-E hepatitis[16].

TT virus is a small single-stranded DNA that was first discovered in the sera of three Japanese patients with post-transfusion hepatitis in 1997[17].

Several studies have shown that TTV is distributed widely throughout the world. Approximately 50% of patients with non-A-non-G chronic hepatitis or fulminant hepatitis are TTV-positive and more than 50% of high-risk patients.

SEN virus is a single-stranded DNA virus and distantly related to the TT virus. Among the nine genotypes, SEN-V-D and SEN-V-H are more prevalent in serum samples from patients with transfusion-associated non A-E hepatitis [18]. Parenteral transmission through blood and blood products is clearly evidenced by the higher detection rates of these viruses among multiple transfused individuals[19].

In obstetrics, the hepatitis B and C viruses have received greatest attention in terms of mother-child transmissions. Infection routes regarding mother-child transmission and preventive methods have been extensively studied with regard to HIV, but there is not great report about the transmission of TTV, SENV from mother to child, while they are very important in regard of pathology and diseases [20].

HIV-infected patients are at high risk for blood-borne infections because of getting regular blood for a long time and potential for exposure to contaminated equipment[21].

Information regarding TTV and SEN-V infections in the Iran population is less among HIV-infected mother and transmission to their's child[22]. The aim of this study was to determine the prevalence of TTV and SEN-V among children who borned from HIV-infected mothers.

## MATERIALS AND METHODS

This study was performed in the Gerash Research Center affiliated with the Shiraz University of Medical Sciences in Southern Iran between 2010 and 2015. Serum samples were collected from HIV-infected Mother and their children. This study included 250 blood samples that were obtained from 150 HIV-infected Mother and 100 their children who were referred to the Gerash and Namazee Hospitals. Samples were centrifuged and stored at -70°C within hours of collection. Five ml of blood was collected from all study participants (HIV positive), and sera were screened with anti-HIV antibody (Dia lab @HIV Ab, Austria) in duplicate.

Total nucleic acids from 100 µL of serum were isolated with the QIAamp blood kit, the nucleic acid was resuspended in 50 µl of distilled water. Polymerase chain reaction (PCR) assays were performed using 3 µL of the DNA sample, 1× PCR buffer (10 mM Tris-HCl(pH 9.0), 50 mM KCl, 1 mM MgCl<sub>2</sub>, 0.01% gelatin, and 0.1% Triton X-100), a 200 µM concentration of each dNTP, 100 ng of each primer in a final volume of 25 µl

Total DNA were amplified by semi-nested PCR using primers NG059, NG061, and NG063 in order to detection of TTV. The first round PCR program consisted of 35 cycles of denaturation for 30 s at 94°C, annealing for 45s at 60°C, and extension for 45s at 72°C, with the sense primers NG059 (5'-ACA GAC AGA GGA GAA GGC AAC ATG -3') and anti-sense primer NG063 (5'-CTG GCA TTT TAC CAT TTC CAA AGT T-3'). The second round of PCR was performed with the sense primer NG061 (5'-GGC AAC ATG TTA TGG ATA GAC TGG-3') and the anti-sense primer NG063 for 25 cycles, under the same conditions as used for the first round of PCR. The amplified DNA products were analyzed by 3% agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light (22-25).

SEN-V-D/H DNA was determined by PCR. Nucleic acids were isolated from 100 µL of serum with QIAamp blood kit and resuspended in 50 µl of elution buffer. DNA was amplified by nested-PCR, with forward primer AI-1F (5'-TWC YCM AAC GAC CAG CTA GAC CT -3'; W =A or T, Y =C or T, M =A or C) and reverse primer AI-1R (5'-GTT TGT GGT GAG CAG AAC GGA-3') for first round for all the SEN-V genotypes . The PCR Conditions in the first round were: Denaturation for 45s at 94 °C, primer annealing for 45s at 56°C and extension for 45s at 72°C, with a final extension step for 7 min at 72 °C for 35 cycles. Second-round PCR amplification were done with specific primers. forward and reverse primers for SEN-V-D including D-1148F(5'-CTA AGC AGC CCT AAC ACT CAT CCA G-3') and D-1341R (5'-GCA GTT GAC CGC AAA GTT ACA AGA G-3') and for SEN-V-H including H-1020F (5'-TTT GGC TGC ACC TTC TGG TT-3') and H-1138R (5'-AGA AAT GAT GGG TGA GTG TTA GGG-3'). The amplification products were separated by 3% agarose gel electrophoresis, stained with ethidium bromide, and photographed under UV light(25-27).

### Statistical Analysis

Statistical analyses were conducted by SPSS software version 15.0 (SPSS Inc., USA). Also, the differences between prevalence of ,HIV,TTV and SEN-V infection in HIV-infected Mother and theirs children were examined by T-test ,Chi-Square and One-away ANOVA statistical analysis. P values <0.05 were considered significant.

## RESULTS

One hundred and fifty HIV-infected mothers and one hundred their childrens( Female=59, Male=41 ) were included in this study. Mothers were categorized into five different groups: Intravenous drug users (n=63), Liver disease (n=83), Kidney disease (n=57), With history of blood transfusion (n=77) and With on history of transfusion (n=77) . RNA was extracted from 100 µlof serum or plasma using RNX plus kit. Reverse transcription polymerase chain reaction (RT-PCR) and nested PCR were used to detect HIV,TTV and SENV.

### *1-Positive SENV response rates in pregnant women*

SENV DNA was detected in 63 (42%) of 150 mothers included in the study. Thirty-eight (60.32%) were SENV-D positive and five (7.95%) were SENV-H positive. twenty (31.75%) mothers were positive for both SENV-D and -H. The mothers were divided in groups according to the presence, in their clinical history, of risk conditions for bloodborne infection. 87 mothers (58%) had been IDUs, 63 mothers (42 %) had no risk factor for bloodborne infection .

56% children were born by vaginal delivery and 44% were born by Caesarean section. All Caesarean sections were done for obstetrical reasons independent of maternal HCV infection. As a whole, 42% babies born to SENV-infected mothers were found to be SENV positive also. None of the SENV-infected infants were born from SENV-uninfected mothers.

Eighteen out of 48 children(SENV+) born to SENV-D-positive mothers (60.32%) were infected by SENV-D. Twenty of the children born to SENV-H . Ten out of forty eight (10%) children born to SENV-D and H co-infected mothers was infected by SENV-D and -H; thus, genotype was always concordant between mothers and children.

Twenty six of 56 ( 54.17%) infected children were SENV positive at delivery (eight were SENV-D positive, Twelve SENV-H and Six SENV- and H co-infected ).While, Twenty two of 44 (45.83%) infected children were SENV positive at Caesarean section (Ten were SENV-D positive, Eight SENV-H and Four SENV- and H co-infected ).Table1

All the infants included in the study had normal values of conjugated bilirubin throughout the study and unconjugated bilirubin levels were increased only in the first days of life, because of physiological hyperbilirubinemia of the newborn .

### *2-The history of Blood transfusion among HIV-infected mothers*

Seventy seven of the 150 pregnant women were with history of blood transfusion and seventy three of them were with no history blood transfusion.

According to Table-2, positive TTV response rates were significantly higher among women with history of blood transfusion (p=0.01).

There was no significant difference in the TTV-positive rate according to the age of the pregnant women or the length of pregnancy. Representative results of the TTV-DNA detection are shown in Table2.

**3- Positive TTV response rates in children born from TTV positive mothers**

All of children born by vaginal and abdominal sesarean section were TTV and SENV DNA negative within 7 days of birth while all 40 children born by vaginal delivery, all 45 children born by abdominal cesarean section were TTV DNA negative 10 days after birth(Table3) .

**DISCUSSION**

The majority of new cases of HIV-infected children below 5 years old worldwide are either from maternal-infant transmission in utero or occur during labor and vaginal delivery or through caesarean section [1] Since the study of Connors et al. [2] showing a 67% reduction in the risk of human immunodeficiency virus [HIV] maternal-to-child transmission [MTCT] by offering zidovudine to women during pregnancy and childbirth and to the newborns for 6 weeks, it became clear that the MTCT of HIV could be reduced to levels as low as 1% with the use of combined antiretroviral therapy and cesarean sections [2,3] . The main indicator for monitoring HIV infection in children is the incidence rate of acquired immunodeficiency syndrome [AIDS] in children under 5 years old. This rate is used as a proxy for the rate of MTCT because it represents almost 90% of all cases [28].

The strongest evidence for SENV transmission between mother and child derives from molecular linkage experiments.

The delayed identification of SENV in the ten babies who were found to be SENV several months after delivery may be due to subclinical SENV infections in samples obtained sooner after delivery, with SENV DNA levels that cannot be detected by PCR performed with degenerate primers. This may also explain the undetectable levels of SENV found in mothers whose children acquired SENV after birth and may explain why some samples from the children tested negative at some points of the follow-up[29].

The present study demonstrates that 40% women with HIV infection are co-infected with SENV-D or -H or both, and that SENV infection is associated with maternal history of drug abuse. We also demonstrate the prevalence can be transmitted to the offspring with an overall transmission rate of 47%; and that vertical infection of HIV does not facilitate SENV infection of offspring.

SENV infection seem to be responsible for significant liver disease in mothers, because most of SENV-infected mothers indicated the history of liver disease . Co-infection with HIV is commonly considered to be a risk factor for SENV infection[30,31].

**Table1: The frequency of TTV/SENV in mothers with Blood transfusion**

Women with TTV <sup>+</sup> (N=72)		Women with TTV <sup>-</sup> (N=78)		Women with SENV <sup>+</sup> (N=63)		Women with SENV <sup>-</sup> (N=87)	
Blood transfusion	No Blood transfusion	Blood transfusion	No Blood transfusion	Blood transfusion	No Blood transfusion	Blood transfusion	No Blood transfusion
62	10	15	63	52	11	25	62
%86.12	%13.88	%19.33	%80.77	%82.54	%17.46	%27.58	%72.42

**Table2: The frequency of TTV/SENV in the mothers included in this study**

TTV/SENV Kind of birth	TTV <sup>+</sup> (N=58)	TTV <sup>-</sup> (N=42)	SENV <sup>+</sup> (N=48)	SENV <sup>-</sup> (N=72)
Vaginal delivery (N=56)	31(%53.44)	25(%59.52)	26(%54.17)	<b>30(%57.69)</b>
Caesarean section (N=44)	27(%46.56)	17(%40.47)	22(%45.83)	<b>22(%42.31)</b>

**Table3: The history of disease in the mothers included in this study**

The history of disease	Number (%)	TTV <sup>+</sup> N (%)	SENV <sup>+</sup> N (%)
<b>Liver disease</b>	83(55.33)	(69.88%) <b>58</b>	<b>25(30.12%)</b>
<b>Kidney disease</b>	57(38)	32(56.15%)	<b>58(43.86%)</b>
<b>Blood transfusion</b>	77(51.33)	(80.52%) <b>62</b>	<b>52(67.54%)</b>
<b>With out history of Blood transfusion</b>	73(48.67)	(13.70%) <b>10</b>	<b>11(15.06%)</b>
<b>IDU</b>	63(42)	(71.43%) <b>45</b>	<b>40(63.50%)</b>

These data are supported by the results reported above. Actually the prevalence of SENV infection in the group studied, consisting of HIV positive, HIV-1-Negative, nonimmunocompromised women is significantly higher than the prevalence in blood donors, even if it does not reach the highest rate found in HIV-1-infected patients[32] and in other Western countries. The data confirm what has been described in other countries[33].

This study we determined whether SENV and TTV strains can be transferred from mothers to infants? we selected 150 HIV-infected mothers. Also children born by vaginal and abdominal sesarean section.

First, SENV infection is not rare in healthy individuals and, therefore, an epidemiological study on mother-to-child transmission would have required a small number of samples to be tested [34]. Furthermore, this screening is complicated by the many SENV strains and by the need of PCR assay to detect SENV-DNA. Second, because studies of infants with HIV infection demonstrated that these viruses may not be identified until a few months from birth[35], we believed that it might be extremely difficult to obtain blood from HIV-infected children in order to TTV and SENV determination. Finally, clinical and immunological follow-up is easier in babies who are already monitored for known diseases, such as HIV or HCV infection. As observed for HIV infections[36], in our selected population, most children were probably infected at birth, or shortly afterward All of children born by vaginal and abdominal sesarean section were TTV and SENV DNA negative within 7 days of birth while all 40 children born by vaginal delivery, all 45 children born by abdominal cesarean section were TTV DNA negative 10 days after birth. These data are supported by the results reported above. Actually the prevalence of SENV infection in the group studied, consisting of HIV positive, with history of blood transfusion women is significantly higher than the prevalence in Iranian blood donors. SENV-D and SENV-H were the most represented strains in both mothers and children. Despite the fact that SENV may be transmitted via parenteral routes or by sexual contact[37], our knowledge of SENV strain transmission still remains incomplete.

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