



## Evaluation of Hepatitis C Virus Infection in Antibody Positive Orphan Newborns

Mazyar Ziyaeyan<sup>1\*</sup>, Marziyeh Jamalidoust<sup>1</sup>, Mahsa Moeini<sup>1</sup>

<sup>1</sup> Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, IR Iran

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### ABSTRACT

**Background:** The rate of mother-to-infant hepatitis C virus (HCV) transmission is relatively low, however, this mode of infection is the most important route for childhood HCV involvement. The chances of transmission are increased by the level of maternal HCV viraemia and maternal human immunodeficiency virus (HIV) co infection.

**Objectives:** The objective of the present study was to determine the presence of HCV infection in orphan newborns of infected mothers.

**Patients and Methods:** A total of 29, two to seven month old, orphan infants were included in the study. The tests performed consisted of the detection of; anti-HCV and anti-HIV antibodies, HCV RNA in the plasma and peripheral blood mononuclear cells by real-time polymerase chain reaction (PCR), and finally the detection of HCV core antigens (HCV core Ag).

**Results:** Anti-HCV antibodies were detectable in all of the infants. However HCV RNA was undetectable in both plasma and peripheral blood mononuclear cells (PBMCs) and there were also no anti-HIV 1/2 antibodies present in the infants.

**Conclusions:** Few studies have addressed the issue of mother-to-infant transmission of HCV among orphan newborns. According to the present study, carried out with orphan babies, the use of a HCV core Ag assay and evaluation of HCV RNA in PBMCs together with HCV RNA real-time PCR on the plasma, could benefit the prediction or exclusion of HCV transmission from mother to child.

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### ► Implication for health policy/practice/research/medical education:

Comparing diagnostic methods of vertical transmission of HCV infection can serve as a cornerstone for better understanding of the capability of each methods in more accurate diagnosis of HCV infection in the newborns.

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## 1. Background

Hepatitis C virus (HCV) infection is a major cause of; chronic hepatitis, liver cirrhosis and liver cancer. It represents an enormous global health problem, affecting 130–170 million people worldwide. In Iran, the prevalence of HCV in the general population is 0.5% (1), but the rate is higher in certain at risk populations (2-4).

HCV is spread through contaminated blood and blood products with percutaneous exposure. This disease also poses a risk to injection drug users (IDU) and hemodialysis patients. However, sexual transmission of HCV is comparably low in Iran, and in addition the risk through occupational exposure is also very low. Mother-to-infant transmission rates vary widely (5-9). Usually, a mother

\* Corresponding author: Mazyar Ziyaeyan, Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Namazi Hospital, 7193711351, Shiraz, IR Iran. Tel: +98-7116474304, Fax: +98-7116474303, E-mail: ziyayeanm@sums.ac.ir

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with negative HCV RNA does not transmit HCV to her infant (10), and the infection has rarely been reported in children born to mothers with undetectable HCV RNA (11, 12). This seems to be related to the fluctuation of HCV viral loads above and below the sensitivity level of the test, or resulting from the use of testing methods that are not sensitive enough to detect low levels of HCV RNA. Depending on the load of the virus, the risk of transmission from positive HCV RNA mothers may become greater (13), although a specific cut-off value predicting or excluding transmission, has yet to be defined (14, 15).

HCV and HIV coinfection in mothers can increase the risk of transmission substantially (16). The importance of the genetic background has also been taken into consideration. The roles of several genes, most of which are involved in the host immune response, have been considered in HCV vertical transmission (17). There is no evidence that a caesarean section delivery reduces the risk of vertical infection, compared to a vaginal delivery. Moreover, breast-feeding is rarely associated with mother-to-child transmission, as revealed by other studies (12, 13).

## 2. Objectives

The goal of the present study was to determine the presence of HCV infection in orphan newborns of mothers with anti-HCV antibodies. A real-time PCR assay was used to assess the presence or absence of HCV RNA in plasma and peripheral blood mononuclear cells (PBMCs). In addition, anti-HCV, anti-HIV antibodies and the presence of HCV core antigens (HCVcoreAg) were investigated using an enzyme-linked immunosorbent assay (ELISA) test.

## 3. Patients and Methods

### 3.1. Patients and Samples

From January 2009 to December 2010, 29 newborns (16 females and 13 males), born to mothers with anti-HCV antibodies were enrolled in the study. They were among orphans placed in a care center in Shiraz, run by the Iranian Ministry of Health. Their ages ranged from two to seven months and no information was available about their birth settings. The preliminary study disclosed HCV antibodies present in the babies. However, to further assess the presence or absence of HCV infection, they were referred to the Professor Alborzi Clinical Microbiology Research Center. Five milliliters of whole, EDTA-preserved, blood samples were collected from the patients. The collected samples were processed within four hours. The data obtained in this study were used to rule out or confirm the presence of a HCV infection in the newborns. Those in charge of the previously mentioned care center, gave us permission to publish this information, on the condition that the participants' identities were not disclosed.

### 3.2. PBMC and Plasma

Five milliliters of EDTA-treated peripheral blood samples were layered onto an equal volume of Lymphoprep (Axis-Shield PoC AS, Oslo, Norway). The peripheral blood mononuclear cell (PBMC) layer was obtained by centrifugation at 800 g for 45 minutes. The cells were washed twice in phosphate-buffered saline at pH 7.3, centrifuged at 600 g for 20 minutes, and counted. Plasma was obtained from the supernatants of the undiluted upper layer of Lymphoprep density gradient centrifuge tubes. A second centrifugation was completed at 800 g for 10 minutes to remove cellular debris and remaining platelets. Antibodies against HCV were analyzed by a third-generation ELISA (DIAPRO, Diagnostic BioProbes Srl. Milano, Italy), and a third generation ELISA was used to determine the antibodies to the HIV types 1/2 (DIAPRO, Diagnostic BioProbes Srl. Milano, Italy). Processing and cut-off values were defined according to the manufacturer's instructions, respectively. A HCVcoreAg assay was performed using a commercially available kit (Ortho-Clinical Diagnostics, Amersham, Bucks, UK), according to the manufacturer's recommendations.

### 3.3. Sample Extraction and RNA Purification

Total RNA was isolated from plasma and PBMC samples by a modified version of the acid guanidinium thiocyanate phenol-chloroform method (18). Briefly, 200  $\mu$ L of serum or  $1 \times 10^6$  PBMC (400  $\mu$ L) was mixed with 600  $\mu$ L of RNX solution (CinnaGen, Tehran, Iran). Then, 200  $\mu$ L of chloroform was added and the total RNA mixture was precipitated from the aqueous phase using 600  $\mu$ L of isopropanol. Finally, the RNA pellet was briefly air-dried, dissolved in diethyl pyrocarbonate-treated water. Six microliters of internal control RNA, supplied with the real-time PCR kit, were added to each sample to monitor the efficiency of extraction, the elimination of reverse transcription and PCR inhibitors, and the cDNA synthesis process. Negative and positive controls were included in the extraction process.

### 3.4. Real-Time PCR

We used a commercially available real-time PCR kit to detect and quantify HCV RNA. All procedures were performed according to the recommendations in the manufacturer's manual (Quantification of Hepatitis C Virus Advanced kit, PrimerDesign Ltd., Millbrook Technology Campus, Southampton, UK). The kit amplified a highly conserved target sequence within the 5' non-coding region of HCV. The amplification process was performed using TaqMan one-step real-time PCR master mix reagents (Roche, Branchburg, New Jersey, USA) in a 7500 real-time PCR system instrument (Applied Biosystems, USA) as follows; 48° C for 30 minutes (reverse transcription) and 95° C for 10 minutes (DNA polymerase activation), followed

by 45 cycles of 94° C for 10 seconds (denaturation), and finally 60° C for 60 seconds (annealing and extension).

#### 4. Results

Anti-HCV antibodies were detected in all of the 29 newborns' plasmas, but no anti-HIV 1/2 antibodies were present. Furthermore, all of the plasmas tested negative for HCVcoreAg. It is worth noting that the serological tests were performed twice at different intervals and the results were consistent. HCV RNA was not detected in any of the newborns' plasmas and HCV RNA real-time PCR results in their PBMCs were found to be negative.

#### 5. Discussion

Some reported studies have demonstrated that HCV can infect lymphocyte and bone marrow cells. It has also been shown that HCV E2 protein, binds to common receptors on hepatocytes and PBMC (19). The presence of HCV RNA in maternal PBMCs is highly associated with the transmission of HCV to newborns. Transmission from mothers to infants is greater in mothers with identified positive strands of HCV RNA in their PBMCs, than those with HCV RNA negative strands (20). Taking into account the undetectability of HCV RNA in the newborns' plasmas, and the important role of PBMCs in HCV infections (21, 22), we performed a real-time PCR assay on the newborns' PBMCs to detect HCV RNA and to investigate the role of these cells in HCV RNA negative newborns. As the results show, HCV RNA was not detected in the cells, nor was it found in the plasma.

The above mentioned orphan care center in Shiraz, Iran, is a major referral one which admits newborns left without parents or guardians. Although gestational age influences the rate of infection, the mother's age and weight of the newborn at birth had no effect on that rate. According to a report by the care center, none of the newborns were suffering from any deformity or immaturity and their weights after birth were within normal range. However, no information about the respective mothers' age range was available. Nevertheless, it seems reasonable to claim that the mothers were among high risk groups. HCV transmission is probable during the lactation period, but the orphan newborns in the present study were deprived of this period.

It is worth mentioning that all of the orphan newborns' guardians were advised to send the babies for a repeated qualitative PCR check at nine months of age, and in the case of negative results, an anti-HCV antibodies check by an ELISA at 18 month age, to ensure that they were not affected by a HCV infection, as suggested in a previous study (23, 24). Considering the finding that all of the newborns were anti-HCV positive, we can conclude that their respective mothers were also anti-HCV positive. But no estimation could be made about HCV RNA among the

mothers due to their unavailability. Since the newborns were HCV RNA negative on examination, they were adopted by families with appropriate living conditions. Therefore, more specific follow-up of the newborns after adoption was not possible because the adoptive families were living far from Shiraz or they preferred the follow-up for their children to be conducted privately. Therefore, performing a second test for the removal of maternal antibodies from the newborns was not possible, either.

The importance of HCVcoreAg in the early diagnosis of HCV infection has been established in many studies (25-27). Also, the monitoring of HCVcoreAg during the treatment course can help to predict responses to anti-viral therapy (28, 29). In the present study, a HCVcoreAg assay was used to diagnose a probable infection in the orphan newborns, because it could be indirectly indicative of HCV viral replication. HCV viral RNA real-time PCR, together with a HCVcoreAg assay can serve as strong evidence for predicting or excluding a HCV infection. Moreover, as some reported studies have shown, HCVcoreAg can be detected earlier than the anti-HCV antibodies produced by an individual (30). This may have implications for the newborns with maternal HCV antibodies against HCV.

In conclusion, the present study may be among the few investigating the probable transmission of HCV infection from mothers to orphan newborns. Based on these results, the use of a HCVcoreAg assay and evaluation of HCV RNA in the newborns' PBMCs along with HCV real-time PCR on the plasma, might prove helpful in the diagnosis of mother-to-child transmission of a HCV infection.

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#### Financial Disclosure

All authors declare that they have no conflict of interest.

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#### Authors' Contribution

None declared.

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