ORIGINAL ARTICLE

Mother to child transmission of Hepatitis C Virus in a province of Northern Italy

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Key words

HCV infection • Vertical transmission • Follow-up

Summary

Introduction. *Study reports of mother to child transmission of hepatitis C virus (HCV) have shown transmission rates ranging from 3 to 37%, according to maternal viremia and HIV-1 coinfection. The present study evaluated the prevalence of the HCV infection in the general population and the incidence of vertical transmission, from women who delivered in the Obstetric Clinic of the Hospital of Parma from January 1st 1996 to 31st 2001 December.*
Methods. *Mothers and children were tested for the presence of HCV-RNA within one week after delivery. Children were considered to be infected when they were found positive at least twice for viral RNA or antibodies were still detectable at the end of the follow-up period (18 months) in blood.*
Results. *Out of 13,025 women, 110 (0.8%) were found positive for anti-HCV antibodies; 72 of them (65.4%) were HCV-RNA positive. All 110 children were positive for anti-HCV antibodies in the first blood sample (time 0); 8 of them were HCV-RNA positive. Three children were still viremic at the end of the follow-up whereas 5 showed a clearance. No significant differences were found between viremic and nonviremic children with respect to gestational week, maternal alanine aminotransferase (ALT) levels and newborns weight at birth.*
Conclusion. *This investigation shows that vertical transmission may occur in a general obstetric population despite a low prevalence of HCV-positive subjects.*

Introduction

The prevalence of hepatitis C (HCV) infection in the general Italian population is between 1% and 3% in adults and up to 16% in elderly subjects (8-36%). Studies carried out in Italy during the '90s showed viral replication in 54%-85% of subjects with anti-HCV antibodies according to the presence of HCV-RNA [1-9]. The particular distribution by age of anti-HCV-positive subjects may be explained by a cohort effect. The high prevalence observed in the older population might represent the outcome of an epidemic that in Italy was particularly virulent between the '50s and the early '80s. Hepatitis C virus is mainly transmitted by the parenteral route through blood and blood products, so that in the past uncontrolled transfusions and medical procedures contributed to the spread of the disease [10, 11].
Recently, the use of intravenous drugs was found to be the major risk factor related to seroprevalence.
Since 1990, onward, utilization of new diagnostic tests, disposable medical devices, a better knowledge of ways of transmission, and the use of *standard precautions* in general medicine and in dentistry have dramatically reduced the incidence of the disease (from 5 to 0.5 per 100,000 inhabitants in 2004) [12]. Infection is still asymptomatic in 90% of cases. These data represent an underestimate of the actual number of new cases that may realistically range between 14 and 50 per 100,000 person-year in the general population [13] and 2,4 person-year in blood donors [14].
The vertical transmission seems to occur in 3-7% of cases in the presence of high viraemia and HIV co-infection in HCV-RNA positive women with known risk factors.
Maternal viremia represents the most important risk factor [15] even though the possible transmission from HCV-RNA negative mothers has been also reported in the literature [16, 17]. This might be attributed to the typical trend of chronic infections that may alternate blood clearance phases to viremic phases. Concerning breast feeding and type of delivery, available data are uncertain [18].
In the present study we evaluated the prevalence of HCV infections in a general obstetric population of a northern Italian city and the incidence of vertical transmission in the relative cohort of newborne. All women who delivered at the Gynaecologic and Obstetric Clinic of the Hospital of Parma from January 1st 1996 to December 31st 2001 was enrolled. In the study, follow-up of children born to HCV-positive mothers was carried out over 18-months after birth.

Methods

All women admitted at the Gynaecologic and Obstetric Clinic of the Hospital of Parma for delivery from January 1st 1996 to December 31st 2001 underwent an anti-HCV antibody screening. Subject showing positive values were prospective enrolled in this 18 months follow-up with their children. All mothers gave the informed consent to attend at the scheduled visits at time 0, 3, 6, 10, and 18 months. Mothers and children were assessed for liver enzymes (ALT and AST), measurement of antibodies (C100, HC-34, HCr-43, NS5) in the blood with a third generation ELISA test, ABBOTT HCV EIA 3.0®, followed by a RIBA™ test (Ortho Chiron RIBA HCV 3.0 SIA®) and tested for the presence

of HCV-RNA within one week from delivery. Viral nucleic acid extraction was performed with TRIZOL® (LIFE TECHNOLOGIES) followed by a qualitative RT-PCR with a sensitivity higher than 100 bp.
Nucleic acids were reverse transcripted into c-DNA and amplified by using specific nested primers from the most conserved 5' untranslated region of the HCV virus genome. (For further details, see previously published notes) [19-21]. At the moment of enrollement, were collected information about mother age at time of delivery, gestational week, type of delivery, newbornes' weight, type of feed, history of past or recent drug addition, blood transfusions and occupation.
Type-specific primers were used for genotyping of viral RNA of the core region. In particular, cDNA was amplified with a universal primer (No 23, 467 to 486) to obtain a fragment of 285 bp. Such fragment was then amplified by specific primers for the genotypes 1a, 1b, 2 and 3a [22].
Children were considered to be infected (a) when they were positive for viral RNA at least twice or (b) if they did not became seronegative, showing demonstrable blood antibodies at the end of the follow-up period (18 months).
Statistical analysis: differences in frequencies were assessed by the X^2 Test, using the Fisher correction factor and T Student's Test for variance analysis as appropriate.
Major risk factors were evaluated with a univariate model for the calculation of raw odds ratio, followed by a multivariate analysis by using the statistical software programme SPSS 12.0.

Results

Out of the 13,025 pregnant women delivered at the Gynaecologic and Obstetric Clinic of the Hospital of Parma from January 1st 1996 to December 31st 2001 (mean/year: 2,170) 110 (0.8%), showed detectable anti-HCV antibodies. Seventy-two of them (65.4%) showed HCV-RNA.
The average age at the time of delivery was 32.2 years (range 20-41); 36.4% were primiparae (average age 29.9 years). No statistically significant differences were observed between HCV viremic and nonviremic mothers for average age, ALT, gestational week, and newborns' weight.
Histories of past or recent (during pregnancy) drug addiction and blood transfusions were taken into account as possible risk factors related to mother's viremia. None of these factors, assessed either individually or in correlation, was found to be related to maternal viremia.
Jobs of 14.7% of women were associated with risk factors in the medical field (doctors, nurses, attendants). This factor was also unrelated to the viremic status. During the observational period, scalp electrodes were not frequently used; However, in view of the mother serologic status the use of scalp electrodes became contraindicated. Maternal feed was recommended in all children.
All 110 children showed anti-HCV antibodies in the first blood sample (time 0). In addition, eight of them, (all born by mothers with detectable HCV-RNA at delivery), were also found to be HCV-RNA positive in at least one test during the follow-up. Only two children were found HCV-RNA positive at time 0. Six children became HCV-RNA positive at second scheduled visit (3rd month of life).
Out of the 8 children, 3 were still viremic at the end of the follow-up period (2.7%), 3 RNA positive children in at least 1 blood sample, showed a clearance for HCV-RNA at the end of the follow-up period, the remaining 2 children abandoned the study after follow-up time 3 when they became HCV-RNA negative. Concerning gestational week, maternal ALT and weight at birth, no significant differences were observed between viremic and nonviremic children.
One of the 3 children definitely infected was HIV positive. Concerning infant gender, a greater prevalence of females with respect to males was found (6 *vs.* 2 [OR 2.7 IC95% 0.5-14.2]). No significant differences were found for type of delivery and type of feeding. In nonviremic children, seroreversion was preferentially observed in the 3rd or in the 4th blood samples (between the 6th and the 10th month of life).
The RNA identified in children and mothers was genotyped and compared. In the 72 viremic mothers group the most frequent genotype was the 1b (32%), followed by 3a (25%). The 2a genotype was identified in 4 mothers and in their children, followed by the 1b genotype (2 children) and by the 3a genotype (1 child), whereas one of the viruses could not be genotyped.

Discussion and conclusions

The data presented here agree with the results of a previous study performed in the early '90s. In that study, the prevalence of HCV-positive women in the cohort of parturients was 1.3% with respect to the 0.8% prevalence detected in the present study. Therefore, in both studies, prevalence was within the lowest range of the overall prevalence in Italy [21].
The search for viral RNA in maternal blood was performed in a sample taken within 1 week after delivery. This method provided reliable data concerning the viremic or nonviremic status of the mothers. According to recent studies [23] the percentage of HCV-RNA positive mothers resulted to be high (65.45%). During the 5 years of investigation, 110 children born to HCV-positive mothers were monitored with the planned follow-up until their 18th month of life. Eight of them were found to be HCV-RNA positive, at least in one follow-up visit, but only two of them showed this condition at time 0. The hypothesis is that the delay observed could be associated to different timing in infection transmission (transplacental or perinatal route). Overall, the presence of viral RNA in at least one blood sample taken within a few weeks from delivery led the authors of the study to deem the actual occurrence of the vertical transmission. Therefore, the eight children were considered a homogeneous sample for statistical analysis. Females showed a higher (even though non significant) prevalence (6 *vs.* 2); this observation is in agreement with the reports of others [24]. One of the 3 infants with a positive status at the end of the follow-up period was born to HIV positive mother and resulted to be HIV

positive. The mothers of the others two children who were still viremic (both with genotype 2a), did not differ in term of risk factors, but according to clinic specialists, their condition was classificated as "active chronic hepatitis C". Two children abandoned the study after 2 consecutive HCV-RNA negative tests; three children, who became HCV-RNA negative at time 4, were tested until the end of the follow-up and remained negative. These children did not show any clinical symptoms of infections during the scheduled follow-up visits; their transaminese tests were normal. Concerning maternal genotypes in agreement with other studies we observed the highest frequency of the 1b genotype, followed by the 3a genotype. In contrast in viremic children and in their mothers the 2a genotype was found to be the most frequent (4/8).

This investigation confirms that vertical transmission of hepatitis C virus infection may occur despite a low prevalence of HCV-positive subjects in a population of women in fertile age. The serologic tests, performed during pregnancy as screening, appear to be highly helpful to distinguish between women with or without risk factors. The major aim of the screening programs is represented by investigations of infants born to HCV positive mother [25] in order to establish appropriate treatments in an early stage of life.

References

[1] VI Updating Seminar. HCV hepatitis virus and new putative hepatitis viruses: diagnosis, epidemiology, prevention and therapy. Istituto Superiore di Sanità. Rome, November 27-28, 2003. Proceedings edited by Maria Rapicetta 2003, iii, 139 p. Rapporti ISTISAN 03/29 (in Italian and English).

[2] Maio G, D'Argenio P, Stroffolini T, Bozza A, Sacco L, Tosti ML, et al. *Hepatitis C virus infection and alanine transaminase levels in the general population: a survey in a southern Italian town*. J Hepatol 2000;33:116-20.

[3] Guadagnino V, Stroffolini T, Rapicetta M, Costantini A, Condili LA, Menniti-Ippolito F, et al. *Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy*. Hepatology 1997;26:1006-11.

[4] Di Stefano R, Stroffolini T, Ferraro D, Usticano A, Valenza LM, Montalbano L, et al. *Endemic hepatits C virus infection in a Sicilian town: further evidence for iatrogenic transmission*. J Med Virol 2002;67:339-44.

[5] Stroffolini T, Menchinelli M, Taliani G, Dambruoso V, Poliandri G, Bozza A, et al. *High prevalence of hepatitis C virus infection in a small central Italian town: lack of evidence of parenteral exposure*. Ital J Gastroenterol 1995;27:235-8.

[6] Campello C, Poli A, Dal Molin G, Besozzi-Valentini. *Seroprevalence, viremia and genotype distribution of hepatits C virus: a community-based population study in northern Italy*. Infection 2002;30:7-12.

[7] Bellentani S, Pozzato G, Saccoccio G, Crovatto M, Croce LS, Mazzoran L, et al. *Clinical course and risk factors of hepatitis C virus related liver disease in the general population: a report from the Dionysos study*. Gut 1999;44:874-80.

[8] Coppola RC, Masia G, Pradat P, Trepò C, Carboni G, Argiolas F, et al. *Impact of hepatitis C virus infection on healthy subjects on an Italian island*. J Viral Hepatitis 2000;7:130-7.

[9] Ansaldi F, Bruzzone B, Salmaso S, Rota MC, Durando P, Gasparini R, et al. *Different Seroprevalence and Molecular Epidemiology Patterns of Hepatitis C Virus Infection in Italy*. J Med Virol 2005;76:327-32.

[10] Chiaramonte M, Stroffolini T, Lorenzoni V, Minniti F, Conti S, Floreali A, et al. *Risk factors in community acquired hepatitis C virus chronic infection: a case-control study in Italy*. J Hepatol 1996;24:129-34.

[11] Gaeta GB, Stroffolini T, Taliani G, Ippolito FM, Giusti G, De Bac C. *Surgical procedures as a major risk factor of chronic hepatitis C virus infection in Italy: evidence from a case-control study*. Int J Infect Dis 1999;4:207-10.

[12] Available at http://www.iss.it/seie/index.php

[13] Kondili LA, Chionne P, Costantino A, Villano U, Lo Noce C, Pannozzo F, et al. *Infection rate and spontaneous seroreversion of antihepatitis C virus during the natural course of hepatitis C virus infection in the general population*. Gut 2002;50:693-6.

[14] Tosti ME, Solinas S, Prati D, Salvaneschi L, Manca M, Francesconi M, et al. *An estimate of the current risk of transmitting blood-borne infections through blood transfusion in Italy*. Br J Haematol 2002;117:215-9.

[15] Ferrero S, Lungarno P, Buzzone BM, Gotta C, Bentivoglio G, Ragni N. *Prospective study of mother-to-infant transmission of hepatitis C virus: a 10-year survey (1990-2000)*. Acta Obstet Gynecol Scand 2003;82:229-34.

[16] Mok J, Pembrey L, Tovo PA, Newell ML, and the European Paediatric Hepatitis C Virus Network. *When does mother to child transmission of hepatitis C virus occur?* Arch Dis Child Fetal Neonatal Ed 2005;90:156-60.

[17] Steininger C, Kundi M, Jatzko G, Kiss H, Lischka A, Holzmann H. *Increased Risk of Mother-to-Infant Transmission of Hepatitis C Virus By Intrapartum Infantile Exposure to Maternal Blood*. J Infect Dis 2003;187:345-51.

[18] Roberts EA, Yeung L. *Maternal-infant transmission of hepatitis C virus infection*. Hepatology 2002;36:S106-13.

[19] Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, et al. *Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources*. J Gen Virol 1992;73:673-9.

[20] Tagger A, Ribero ML, Tremolada F, Casarin C, Rapicetta M, Cristiano K, et al. *Hepatitis C viremia and serologic profile in post transfusion non-A, non-B hepatitis*. In: Nishioka K, Suzuki H, Mishiro S, eds. *Viral hepatitis and liver disease*. Tokio: Sprinter Verlag 1994, p. 565-8.

[21] Tanzi ML, Bellelli E, Benaglia G, Cavatorta E, Merialdi A, Mordacci E, et al. *The prevalence of HCV infection in a cohort of pregnant women, the related risk factors and the possibility of vertical transmission*. Eur J Epidemiol 1997;13:517-21.

[22] Simmonds P, Holmes EC, Cha TA. *Classification of Hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region*. J Gen Virol 1993;74:2391-9.

[23] Mast EE, Hwang LY, Seto DSY, Nolte FS, Nainan OV, Wurtzel H, et al. *Risk Factors for Perinatal Transmission of Hepatitis C Virus (HCV) and the Natural History of HCV Infection Acquired in Infancy*. J Infect Dis 2005;192:1880-9.

[24] European Paediatric Hepatitis C Virus Network. *A significant sex – but not elective cesarean section – effect on mother – to-child transmission of hepatitis C virus infection*. J Infect Dis 2005;192:1872-9.

[25] Hardikar W, Elliott JE, Jones AC. *The silent infection: should we be testing for perinatal hepatitis C and, if so, how?* Med J Aust 2006;184:54-5.

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