Diagnosis of hepatitis C in pregnant mothers and its transfer pattern in neonates

Nosheen Aslam[1], Abdul Ghaffar[1], Mohsin Khurshid[2], Syed Makhdoom Hussain[3] and Muhammad Afzal[4]

[1]Department of Applied Chemistry & Biochemistry, Government College University, Faisalabad, Pakistan
[2]College of Allied Health Professional, Directorate of Medical Sciences, Government College University, Faisalabad, Pakistan
[3]Fish Nutrition Lab, Department of Zoology, Government College University, Faisalabad, Pakistan
[4]Health Care Center, Government College University, Faisalabad, Pakistan

Abstract: Hepatitis is the most common liver diseases in the Pakistan caused by blood-borne infection of HCV. Viral transmission is frequent through blood contact. Vertical transmission is transfer of disease from mother to infant. The women who are infected with hepatitis C virus RNA are at high risk of infecting their babies. Actual transmission occurs during labor and at time of delivery when blood of both mother and neonate is in contact with each other. Vertical transmission rate is lowered when mother is HCV RN Anegative. The project was designed to determine the percentage of transmission and prevalence of Hepatitis C virus from mother to neonates. Assessment of the quantitative analysis of RNA levels in mother blood and viraemic status from the early postpartum period onwards of children born to HCV-infected mothers. For the diagnosis of hepatitis C in mothers, blood samples of fifty HCV pregnant women between 23-41 years old were taken. The blood samples were centrifuged at 8,000 rpm and serum was separated and stored at 4^0C. The values of the Alanine Aminotransferase was determined at 2nd and 3rd trimester of pregnancy. After extraction HCV-RN Awere transcribed and amplified by PCR. The samples were further authenticated through the Agarose Gel Electrophoresis system and bands were obtained. Nested reverse transcription PCR (RT-PCR) was conducted for the quantitative analysis of HCV-RNA. The results showed that in 66% cases, the mothers had high level of ALT at 2nd trimester of pregnancy. Their ALT level was decreased in the 3rd trimester of pregnancy. PCR results showed that 40% pregnant women had quantity of HCV-RNA in the range of 1000-10,000 IU/mL and in 18% women were above 100000 The results of spectrophotometer showed that 80% infants had the antibodies against HCV-RNA while only 20% of the neonates did not have antibody right after birth. The 29% babies got HCV-RNA in their serum and became positive for HCV-RNA.

Keywords: Alanine aminotransferase, Hepatitis C virus, maternal cirrhosis, hypertension and gestational diabetes.

INTRODUCTION

Hepatitis is the most common liver diseases in the Pakistan caused by blood-borne infection of HCV. (Ferreros *et al*., 2003) An estimated 170 million people worldwide are chronically infected with hepatitis C virus, 35 % of these are women of child bearing age (20-40 years) and it shows an annual fertility rate of 2%. Viral transmission is frequent through blood contact (Ghany *et al*., 2009). Vertical transmission is transfer of disease from mother to infant. The women who are infected with hepatitis C virus RNA are at high risk of infecting their babies (RESTI *et al*., 1998). It is transmitted during gestation and delivery (Ponde, 2011).

Actual transmission occurs during labor and at time of delivery when blood of both mother and neonate is in contact with each other (Alter, 2007). Vertical transmission rate is lowered when mother is HCV RNA-negetive (Ziyaenyan *et al*., 2013). Vertical transmission is correlated with higher maternal viral titer when mother has elevated level of alanine aminotransferase in the year before pregnanacy (Indolfi & Resti, 2006). It is estimated that 10,000 to 60,000 newborn babies are infected with hepatitis C virus (HCV) each year. (Campion *et al*., 2012) Hepatitis C may be acute or chronic, acute hepatitis C is mild and often asymptomatic while chronic hepatitis C is an indolent course but may progress to cirrhosis. Therefore, early diagnosis of HCV becomes more important for possible treatment of the child. (Zanetti *et al*., 1998

Pregnancy, itself do not hasten the disease process but the cirrhosis of liver affects newborn because the immune system of hepatitis C virus (HCV) infected newborn children is very weak so the death rate is very high among these children. (Polywka *et al*., 1997; Granovsky *et al*., 1998) Therefore, conventional methods based upon antibody detection through kit are not reliable. This necessitates the screening of HCV through PCR for precise detection in neonates. Gibb *et al*., 2000)

The presence of maternal cirrhosis and maternal intravenous drug use also increases the vertical transmission (Kamary *et al*., 2003). HCV may causes the

**Corresponding author* Email: afzal_nosheen@yahoo.com

Cirrhosis of liver that affects newborn babies because the immune system of hepatitis C virus (HCV) infected newborn children is very weak so the death rate is high among these children. In many cases, viremia has been demonstrated in newborn serum, where in most cases, HCV-RNA was only detectable after a few week of birth (Nesrine, *et al.,* 2012).

Multiple studies suggested that breastfeeding to neonate does not transfer the HCV from mother to neonate. (Conte *et al.,* 2000) It is observed that gastric acid rapidly inactivates the HCV so breastfeeding poorly transfer the HCV. However the mothers who have chronic HCV infection with jaundice or develop cracked bleeding nipples are suggested to stop feeding to their neonates (Mast *et al*., 2005).

The project was designed to determine the percentage of transmission and prevalence of Hepatitis C virus from mother to neonates. Assessment of the quantitative analysis of RNA levels in mother blood and viraemic status from the early postpartum period onwards of children born to HCV-infected mothers.

MATERIALS AND METHODS

In the present study, Hepatitis C virus in mothers was diagnosis and transfer ratio of Hepatitis C from mother to neonates was determined in Bioinformatics and Biotechnology Laboratories of GC University, Faisalabad. For the diagnosis of hepatitis C in mothers, fifty HCV pregnant women between 23-41 years old were enrolled. All the mothers were enrolled at the end of first trimester and studies were carried up to three months of delivery. Pregnant women were enrolled from different hospitals in Faisalabad and Lahore. WHO and Helsinki protocols were followed for sampling ethics. 2 ml blood sample from baby and mother was taken in heparinized tubes and was distributed accordingly for biochemical and genetic study. Blood samples were collected at the sample collection rooms of hospitals for the detection of HCV during pregnancy. The blood was stored at 4^{o}C in the eppendr of and heparin was added so that blood could not coagulate. Repeatedly serological-testing for the presence of HCV-RNA in the mother's serum was carried out. The blood samples were centrifuged at 8,000 rpm and serum was separated and stored at 4^{o}C. The values of the Alanine Aminotransferase was determined at 2^{nd} and 3^{rd} trimester of pregnancy.[17]For extraction of viral RNA, the GF-1 viral Nucleic Acid kit was used**.** The extracted HCV-RNA was stored and Conditions were optimized for PCR. The collected samples of extracted RNA were transcribed and amplified by using Ampli Sens HCV-FRT PCR kit The samples amplified by PCR were further authenticated through the Agarose Gel Electrophoresis system and bands were obtained which was the most important indication for the presence of HCV genotype with the help of HCV genotype-specific primers.. Nested reverse transcription PCR (RT-PCR) was conducted for the quantitative analysis of HCV-RNA. Blood samples of babies were taken immediately after birth. The serum was separated out and preserved in Eppendorf at 4^{o}C. Their blood was analyzed to check the presence of antibodies in the blood of neonates against HCV-RNA.HCV-RNA third-generation ELISA kit (SD-HCV ELISA 3.0) was used to check the presence of antibodies. Neonates were also screened for HCV. After screening data was analysed

STATISTICAL ANALYSIS

The ranges, means ±SD, correlation values and significance of differences in means were calculated by ANOVA following Steel *et al.* (1997).

RESULTS

In the present study, 50 pregnant women were tested for the presence of the hepatitis C virus. All the patients enrolled were infected with HCV before pregnancy. They were positive for serological markers of HCV infection. Eligibility criteria comprised on repeatedly positive results of serological testing for HCV antibodies and for the presence of HCV-RNA in the mother`s serum. The patients enrolled were positive for HCV-RNA before pregnancy and blood samples were taken immediately after labor. All the patients under study were about 23-41 years old.

No case of abortion was observed in the chronic hepatitis C patients. There was no case of obstetric complications such as hypertension and gestational diabetes. Only nine pre-term deliveries were observed in anti-HCV positive women. It was found that all the mothers had HCV infection during the whole period of pregnancy. The blood of infants of all the mothers was also tested repeatedly after their birth and after 6 months of their birth.

The change in the values of the Alanine Aminotransferase was determined at 2^{nd} and 3^{rd} trimester of pregnancy and compared with normal range of ALT values as shown in table 1. There was a significant difference ($p<0.05$) in ALT level at 2^{nd} trimester of pregnancy. The ALT level at 2^{nd} trimester of pregnancy increased in all the 5 age groups as normal range of ALT level was about 9-52 U/L. The ALT level was decreased in the 3^{rd} trimester of pregnancyin all the 5 age groups. The decreased ALT level is showed in the table 1.

The results of PCR showed that all the mothers were positive for HCV-RNA and had high quantity of HCV-RNA.PCR results showed that groups 1, 4 and 5 had high quantity of HCV-RNA as compared to group 2 and 3. It was observed that one infectious woman having higher values of HCV-RNA in group 5 was died after 5 months of her delivery. The samples amplified by PCR were further authenticated through the Gel Electrophoresis

system and bands were obtained which was the most important indication of presence of HCV genotype with the help of HCV genotype-specific primers. The genotype-specific band sizes were compared with a 50 bp DNA ladder. The samples contained most of the genotype which was present in the ladder. The bands of all the samples are showed in the fig that was taken from the gel documentation system. The gel electrophoresis results of present studies showed that all of the samples had 3a genotypes (220 bp).

Mode of delivery in mothers

With the completion of pregnancy period, the mothers gave birth to their infants. It was found that 36% women had the C-Section delivery while all the remaining 64% had normal deliveriesin groups 1, 2 and 4. There was 39% normal deliveries and 61% women had the C-Section delivery in group 5 while in group 3 all the delivered their babies in normal way.

Presence of antibodies in neonates after delivery

When babies were weighted after birth they all were of normal weight. The results of spectrophotometer showed that 80% infants had the antibodies against HCV-RNA while only 20% of the neonates did not have antibody right after birth.

For the detection of HCV-RNA in the babies, the serum of all the babies was analyzed again by polymerase chain reactions after the three months of the birth. The 29% babies got HCV-RNA in their serum and became positive for HCV-RNA while rests of the infants remained negative for HCV-RNA till 3 months of the birth.

DISCUSSION

Transmission of Hepatitis C from infected mothers has become the most important mode of HCV infection among children. It is generally reported that the risk of HCV infection in mothers without HCV viremia is very low (Wejstal *et al*., 2005; Roberts *et al*., 2002 and Qawi *et al*., 2010). We also found HCV viremia to be a precondition for transmission (Wilkins *et al*., 2010 and Marine *et al*., 2007) because none of the HCV RNA-negative mothers transmitted the virus to her child. In the present study, the mean maternal HCV load was higher in mothers whose children were HCV infected than in those whose children were not infected (Paternoster *et al*., 2001).Death of one infectious woman having higher values of HCV-RNA might be due to the contraction of uterine muscles (Dal Molin *et al*., 2002).

ALT determination, a simple, widely available and inexpensive test, may help in identifying mothers with an increased risk of HCV vertical transmission. (Tassopoulos., 1999). The risk of transmission from mothers with constantly raised ALT levels was more evident than that from mothers with changing ALT levels. Increased ALT levels may reflect a more severe liver disease and a higher viral load, factors known to be associated with vertical transmission. (Marcellin *et al*., 1997 and Carter, 1990). The mothers had high level of ALT at 2nd trimester of pregnancy. The reason behind the increased level of ALT at 2nd trimester was the contraction of uterine muscles and this was the major indication of the liver diseases found in the patients (Bacq *et al*., 1996). All the mothers either having normal or abnormal ALT level at 2nd trimester, their ALT level was decreased in the 3rd trimester due to the contraction of the uterine muscles at the time of delivery due to labor, (Guntupalli and Steingrub 2005 and Loganathan *et al*., 2000) environmental and physiologic changes associated with pregnancy including high plasma concentrations of estrogen, expansion in plasma volume and changes in immune reactivity.

The genotype does not guess the consequence of infection it does predict the possibility of treatment response and also determines the duration of treatment. The gel electrophoresis results of present studies showed that all of the samples had 3a genotypes except 4 which had 1a genotype. (Attaullah *et al*., 2011; Jara &Hierro 2010; Simmonds *et al*., 1993 and Zuccotti 1995) Second more frequently genotype was genotype 1 in Punjab province. Our results showed reduced range of subtypes in Faisalabad. This is similar to other studies done by (Ijaz *et al*., 2008 and Ahmad *et al.,* 2007). High prevalence of HCV genotype 3 in Pakistan is a good hope for cure as well as control of HCV infection (Mumtaz *et al*., 2005).

After delivery it was found that 66% mothers had normal deliveries while 33% gave birth through C-Section. (Samdal, 2000 and Cottrell *et al*., 2013) reported that mode of delivery was not the main route of transmission of HCV-RNA from mother to neonate (McIntyre *et al*., 2006). Our results however, compatible with the usual reported because of difference in designing the experiment, characteristics of mothers, criteria of diagnosis, and time duration of infected neonates.

Normal weight of the babies after birth showed that chronic hepatitis C infection did not affect the development of pregnancy, birth and weight of the newborn baby. Mostly, the babies were positive for anti-HCV antibodies because all the mothers were anti-HCV positive and they passed these antibodies to their offspring before birth (Steininger *et al*., 2003). The number of these antibodies started to decrease with the passage of time. Presence of HCV-RNA after the three months of the birth in the serum of babies showed that infection occurred during pregnancy because if it occurred at the time of delivery it would not be measurable after several weeks (Paccagnini *et al*., 1995 and Mok *et al*., 2005).

Table 1: Biochemical profile of mothers at the time of delivery

Sr. #	group	age	2nd trimester ALT results U/L	3rd trimester ALT results U/L	Nested RT-PCR Results (IU/ml	Mode of Delivery		Genotype
						normal	c-section	
1	21-25	24±1.11803	69.4±2.1190	24.3±1.3964	31,953±1.0166	5	4	3a
2	26-30	27.7±1.340	72.4±2.082	17.9±1.4141	22,243±2.635	10	6	3a
3	31-35	32.4±1.164	68.1±2.36	17.3±1.373	14,898±2.22	12	0	3a
4	36-40	38±1.414	80±1.984	20.3±1.592	33,432±2.958	8	3	3a
5	41-45	41.5±0.577	66±0.9660	16.8±2.98	38,936±3.627	2	5	3a

CONCLUSION

During elective caesarean section there is a lack of protective effect, so this is the main reason of intrauterine transmission of Hepatitis C virus (perinatal). But inspite of this, the threshold level of viremia and increased level of alanine aminotransferase also attitudes a higher risk for HCV transmission from mother to neonates. Antiviral therapy of high risk mothers may decrease the risk of maternal HCV transmission.

ACKNOWLEDGEMENTS

The first author acknowledges the financial grant from Higher Education Commission, Islamabad Pakistan.

REFERENCES

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

Ghany M, Strader DB, Thomas DL and Seef LB (2009). Diagnosis, management and treatment of hepatitis C: an update. *Hepatology,* **49**: 1335-1374.

Resti M, Bortolotti F, Vajro, P, Maggiore G. Guidelines for the screening and follow-up of infants born to anti-HCV positive mothers. *Journal of Digestive and Liver Disease*, **5**(7): 453-457.

Ponde RA AH (2011). Hazards of HCV transmission. Medical. *Microbiology and Immunology*, **200**(1): 7-11.

Alter MJ (2007). Epidemiology of hepatitis C virus infection. *World J. Gastroenterol.,* **2013**(17): 2436-2441.

Ziyaenyan M, Jamalidout M, Moeini M (2013). Evaluation of Hepatitis C infection in antibody positive orphan newborns. *Jundishapur Microbiology,* **6**(1): 2-75.

Indolfi G and Resti M (2009). Perinatal transmission of hepatitis C virus infection. *J. Med. Virol.,* **81**(5): 836-843.

Campion A, LaroucheA, Fauteux-Daniel S,Soudeyns S. Pathogenesis of Hepatitis C During Pregnancy and Childhood. *Viruses,* **4**(12): 3531-3550.

Zanetti AR, Tanzi E, Romano L, Zuin G, Minola E, Vecchi L and Principi N (1998). A prospective study on mother-to-infant transmission of hepatitis C virus. *Intervirology,* **41**: 208-212.

Polywka S, Feucht H, Zollner B, Laufs R. Hepatitis C virus infection in pregnancy and the risk of mother to-child transmission. *Eur. J. Clin. Microbiol. Infect Dis*., **16**: 121-24.

Granovsky MO, Minkoff HL, Tess BH, Waters D, Hatzakis A, Devoid DE and Landesman SH (1998). Hepatitis C virus infection in the mothers and infants cohort study. *Pediatrics,* **102**: 355-339.

Gibb DM, Goodall RL, Dunn DT, Healy M, Neave P, Cafferkey M and Butler K (2000). Mother-to-child transmission of hepatitis C virus: Evidence for preventable peripartum transmission. *Lancet,* **356**: 904-990.

Kamary SS, Serwint JR, Joffe A, Santosham M and Duggan AK (2003). Prevalence of hepatitis C virus infection in urban children. *J. Pediatr.,* **143**(1): 54-59.

Nesrine F and Saleh H (2012). Hepatitis C virus (HCV) status in newborns born to HCV positive women performing intracytoplasmic sperm injection. *Afr Health Sci*, **12**(1): 58-62.

Conte D, Fraquelli M, Prati D, Colucci A and Minola E (2000). Prevalence and clinical course of chronic hepatitis C virus (HCV) infection and rate of HCV vertical transmission in a cohort of 15,250 pregnant women. *Hepatology*, **31**: 751-55.

Mast EE, Hwang LY, Seto DS, Nolte FS, Nainan OV, Wurtzel H and Alter MJ (2005). Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. *J. Infect. Dis.,* **192**(11): 1880-1889.

Bergmeyer HU and Horden M (1980). Alanine aminotransferase test. *Journal of Clinical Chemistry*, **18**: 521-524.

Steel RGD, Torrie MJH and Dickey DA (1997). *Principles and Procedures of Statistics:* A Biometrical Approach. McGraw HillBook Co., Inc. New York, USA

Wejstal R, Hermodsson S, Lwarson G and Norkrans S (2005). Mother to infant transfer of hepatitis C virus. *J. Med. Virol.,* 30(3): 178-80.

Roberts EA and Yeung L (2002). Meterna-infant transmission of hepatitis C virus infection. *Hepatology,* **36**(5): 106-113.

Qawi K, Youssef A, Metwally MA, Ragih I, Abdul Hamid M and Shaheen A (2010). Prospective study of prevalence and risk factors for hepatitis C in pregnant Egyptian women and its transmission to their infants. *Croat. Med. J.*, **51**(3): 219-228.

Wilkins T, Malcolm JK, Raina D and Schade RR (2010). Hepatitis C: Diagnosis and treatment. *Am. Fam. Physician*, **81**(11): 1351-1357.

Marine B E, Berrebi A and Giordanengo V (2007). HCV/HIV co-infection, HCV viral load and mode of delivery: risk factors for mother-to-child transmission of hepatitis C virus? *AIDS.,* **21**: 1811-1815.

Paternoster DM, Santarossa C, Grella PC, Grella P, Palu G, Baldo V, Boccagni P and Floreani A (2001). Viral load in HCV RNA-positive pregnant women. *Am. J. Gastroenterol.*, **96**: 2751-2754.

Dal Molin GD, Agaro P, Ansaldi F,Ciana G, Fertz C, Alberico S and Campello C (2002). Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti-HCV as risk factors. *J. Med. Virol.*, **67**: 137-142.

Tassopoulos NC. Treatment of patients with chronic hepatitis C and normal ALT levels. *J. Hepatol.*, **31**(1): 193-196.

Marcellin P, Lévy S and Erlinger S (1997). Therapy of hepatitis C: patients with normal aminotransferase levels. *Hepatology*, **26**(1): 133-136.

Carter J (1990). Liver function in normal pregnancy. *Aust. N. Z. J. Obstet. Gynaecol.*, **30**(4): 296-302.

Bacq Y, Zarka O and Brechot JF (1996). Liver Function tests in normal pregnancy: A prospective study of 103 pregnant women and 103 matched controls. *Hepatology*, **23**(5): 1030-1034.

Guntupalli RS and Steingrub J (2005). Hepatic disease and pregnancy: An overview of diagnosis and management. *Critical Care Medicines*, **33**(10): 332-339.

Loganathan G, Rachel G and Eapen CE (2000). Liver function tests in normal pregnancy: A study from Southern India. *Indian J. Gastroenterol.*, **24**(6): 268-69.

Attaullah S, Sanaullah K and Ijaz A (2011). Hepatitis C virus genotypes in Pakistan: A systemic review. *Virology Journal*, **8**: 433.

Jara P and Hierro L (2010). Treatment of hepatitis C in children. *Expert Review of Gastroenterology & Hepatology*, **4**(1): 51-61.

Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL and Kolberg J (1993). 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.*, **74**(11): 2391-2399.

Zuccotti GV, Ribero ML, Giovannini M, Fasola M, Riva E, Portera G, Biasucci G, Decarlis S, Profeta ML and Tagger A (1995). Effect of hepatitis C genotype on mother-to-infant transmission of virus. *Journal of Pediatrition*, **127**(2): 278-280.

Ijaz T, Shahzad MK, Sarfraz N and Khan MA (2008). Prevalence of Genotype 3a Hepatitis C Virus (HCV) In the Infected Population of Lahore, Pakistan. *IJAVMS* **2**: 14-17.

Ahmad N, Asgher M, Shafique M and Qureshi JA (2007). An evidence of high prevalence of Hepatitis C virus in Faisalabad, Pakistan. *Saudi Med. J.*, **28**(3): 390-395.

Mumtaz K, Hamid SS, Moatter T, Abid S, Shah HA and Jafri W (2005). Distribution of hepatitis C virus genotypes and its response to treatment in Pakistani patients. *Saudi Med. J*., **29**(11): 1671-1673.

Samdal HH (2000). Hepatitis C virus infection among pregnant women and their children in Norway. *Tidsskr Nor Laegeforen*, **120**(9): 1047-1050.

Cottrell E.B, Chou R, Wasson N, Rahman B and Guise JM (2013). Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. *Ann. Intern. Med.*, **158**(2): 109-113.

McIntyre PG, Tosh K and McGuire W (2006). Caesarean section versus vaginal delivery for preventing mother to infant hepatitis C virus transmission. *Cochrane Database Syst. Rev.*, pp.54-56.

Steininger C, Kundi M, Jatzko G, Kiss H, Lischka A and Holzmann H (2003). Increased risk of mother-to-infant transmission of hepatitis C virus by intrapartum infantile exposure to maternal blood. *J. Infect Dis.*, **187**: 345-351.

Paccagnini S, Principi N and Massironi El (1995). Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis. J.*, **14**: 195-199.

Mok J, Pembrey L, Tovo P and Newell M (2005). When does mother to child transmission of hepatitis C virus occur. *Arch. Dis. Child Fetal Neonatal. Ed.*, **90**(2): 156-160.