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The mode of delivery-based estimate of the mother to fetus vertical infection of HCV: A multicenter observational study.

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ABSTRACT

HCV is the primary cause of chronic viral hepatitis, and it is estimated that one-third of those infected will develop liver cancer. Mother-to-infant transmission may become the primary route of HCV infection in the future, as there is no current method to prevent vertical transmission. The study included 400 pregnant women and their neonates, with 98 infants born to 98 HCV-positive mothers, resulting in a 100% vertical transmission rate for HCV-NS4 antigen. The mode of delivery did not affect the transmission rate of HCV-NS4 antigen, with 64 cases (22.9%) of vaginal delivery and 34 cases (28.3%) of cesarean section. The transmission rate of HCV antigen did not differ significantly between children born via vaginal delivery or cesarean section after membrane rupture.

1. INTRODUCTION

The Hepatitis C virus (HCV) is a highly mutable RNA virus with a strong inclination for chronic infection, impacting over 3% of the global population. Chronic infection can result in long-term hepatitis, which may progress to cirrhosis and hepatocellular carcinoma after several years of infection [6]. Since its discovery in 1989, there has been significant progress in comprehending the virology, epidemiology, natural history, diagnosis, and therapy of HCV. Globally, more than 170 million people are chronically infected with HCV, which is a common cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma [7]. Unlike other hepatotropic viruses, only a small percentage of acute HCV infections are eliminated, and many infected individuals develop persistent HCV infection in the absence of effective treatment. It is believed that a combination of viral and host factors contributes to the inability of the host immune system to clear the initial

infection and lead to the high likelihood of chronic HCV infection [21]. Epidemiological investigations have revealed two primary modes of HCV transmission, namely, obvious parenteral transmission through blood transfusions and intravenous drug use, as well as less apparent forms of parenteral or mucosal exposure, such as medical procedures, tattooing, acupuncture, accidental needlestick injuries, and household transmission [11]. The prevalence of HCV in Egypt is the highest in the world, likely due to mass parenteral anti-schistosomal therapy [9]. The risk of mother-to-infant transmission of HCV varies depending on the population studied and the tests used (Sabatino et al., 1996). In Egyptian HIV-negative pregnant women, there is a high prevalence of HCV seropositivity and a significant rate of vertical transmission of HCV [15]. This study aimed to explore the possibility of vertical transmission of HCV-NS4 antigen from infected mothers to their newborns through vaginal delivery versus cesarean section.

2. Materials and Methods

Blood samples were obtained from 400 pregnant women, and the cord blood samples comprised 280 samples from newborns born via normal vaginal delivery and 120 samples from newborns born via cesarean section. The blood samples were subjected to the following procedures:

* Determination of anti-HCV antibodies using ELISA.
* Techniques of Molecular Biology:

A. Extraction of HCV-RNA from serum

B. Boom method for RNA extraction using reagents and buffers.

C. RNA extraction from serum

E. PCR amplification and Agarose Gel Electrophoresis

* Detection of HCV-NS4 antigen using ELISA, involving the binding of diluted serum samples in coating buffer to the wells of ELISA plates overnight.
* Immunoblotting Technique (Western Blot) to determine the HCV-NS4 antigen in serum and cord samples.
* Immunostaining using anti-HCV-specific antibody.
* Purification of HCV-NS4 antigen from mothers' serum samples and cord samples.
* Biochemical characterization of HCV-NS4 antigen from serum mothers' samples and cord.

3. Statistical Analyses

The statistical analysis was performed using SPSS software for windows, and statistical analysis in R version 4.3.1 was used. The Fisher's exact test, Welch two-sample t-test, and Wilcoxon rank sum test were used. A p-value < 0.05 is considered significant.

4. Results

In the present study: Serum sample of 400 pregnant women were included in the present study. Serum samples were screened for anti-HCV antibody using ELISA. Of the 400 samples, 140 cases were positive for anti-HCV antibody and 260 cases were negative for anti-HCV antibody.

- Selected sera positive for anti-HCV antibodies (n=55) were tested for HCV-RNA using RT-PCR assay as gold standard for diagnosis of HCV infection. Of the 55 serum samples positive for anti-HCV antibodies, 39 (71%) were positive for HCV-RNA and 16 (29 %) were negative for HCV-RNA.
- Serum samples of the 39 cases positive for RT-PCR were tested for HCV- NS4 antigen using ELISA. 38 cases (97.4%) were positive and only one case (2.6%) was negative for HCV- NS4 antigen. HCV-NS4 was identified in HCV infected pregnant women with high sensitivity (97.4%). Specificity, Efficiency, positive predictive value, and negative predictive value were 93.8 %, 96.4 %, 97.4%, 93.8 % respectively in Fig.2-4.
- Western blot analysis revealed that specific anti-HCV-NS4 antibody reacted against HCV- NS4 antigen at an apparent molecular weight of 27 kDa in serum samples of infected pregnant women and their

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umbilical cord but no reaction was observed in serum samples of control as shown in Fig. 1.

- The 27–kDa HCV- NS4 antigen was purified from serum sample of infected pregnant women and their cord using electroelution from polyacrylamide preparative slab gels. Purified antigen showed a single band in comassie blue stained SDS-PAGE and one peak when analyzed by capillary zone electrophoresis at 7.5 min. The reactivity of the serum and cord HCV-NS4 antigen was lost after exposure to 56 ºC and higher degrees of temperature, acid, and alkali, β-mercaptoethanol treatment, but it was maintained after periodate. The purified antigen was precipitated with 40% TCA and reconstituted in PBS, pH 7.2. The reconstituted precipitated of purified antigen showed high reactivity toward HCV-NS4 antibody. In contrast, the supernatant of purified antigen showed no reactivity. Also, the reactivity of the serum and cord purified HCV-NS4 antigen was decreased after treatment with α-chymotrypsin enzyme, and it was completely lost after 60 minutes.

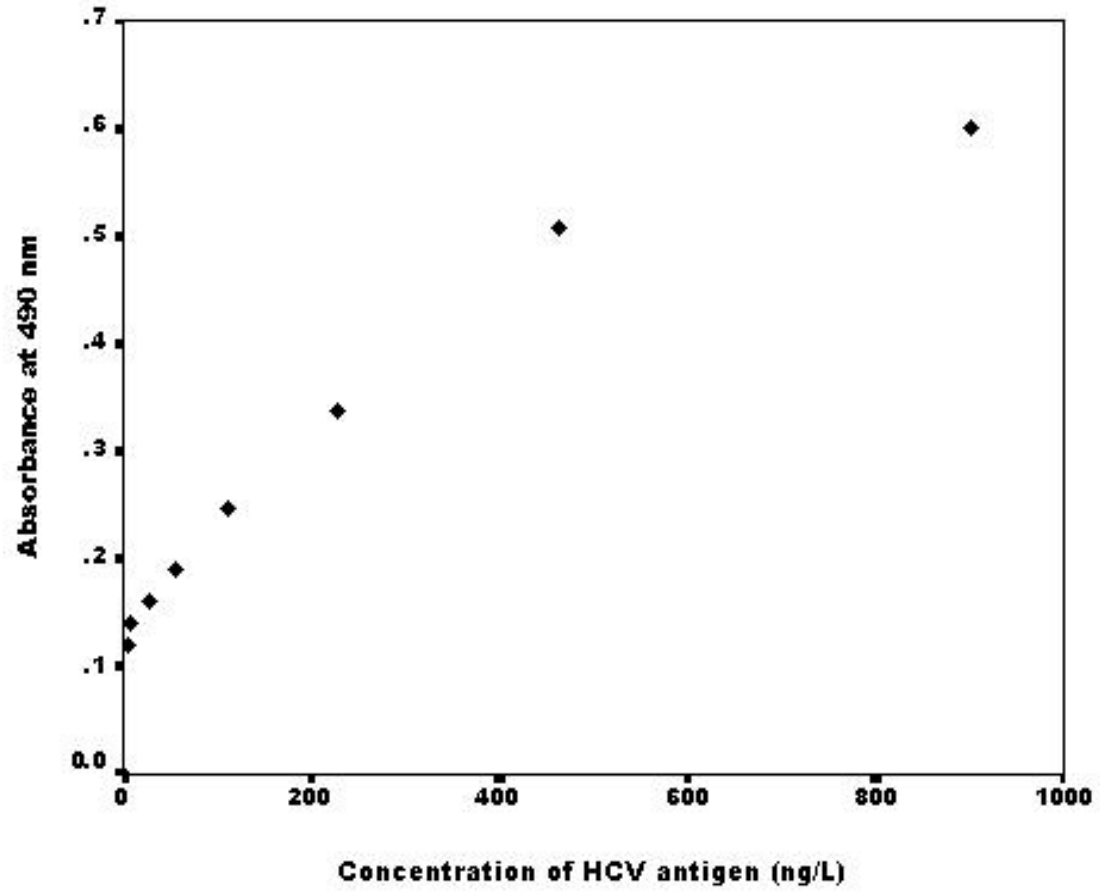

Figure 1. Dose-response curve for HCV-NS4 antigen in the ELISA showing OD at 490 nm as a function of the concentration of antigen (ng/ L) in serum and cord.

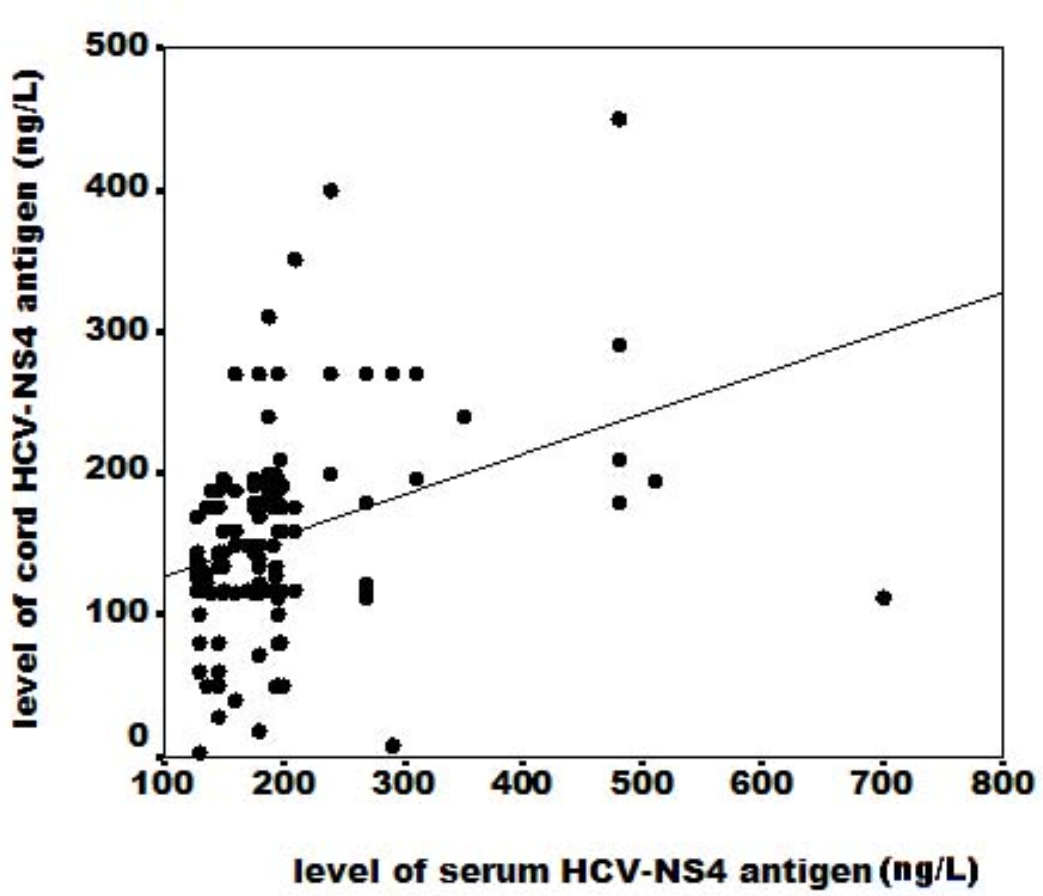

Figure 2. Correlation between levels of HCV-NS4 antigen in sera of mothers and cord samples from their neonates (r=0.344; p < 0.0001).

Table 1. Relation between detection rate of serum HCV-NS4 antigen and mode of mothers' delivery

		Serum HCV-NS4 using ELISA		Total
Mode of delivery		- ve	+ ve	
	Vaginal	216 (77.1 %)	64 (22.9 %)	280
	Cesarean	86 (71.7 %)	34 (28.3%)	120
Total		302 (75.5 %)	98 (24.5 %)	400
X^2	1.2			
P value	0.212			

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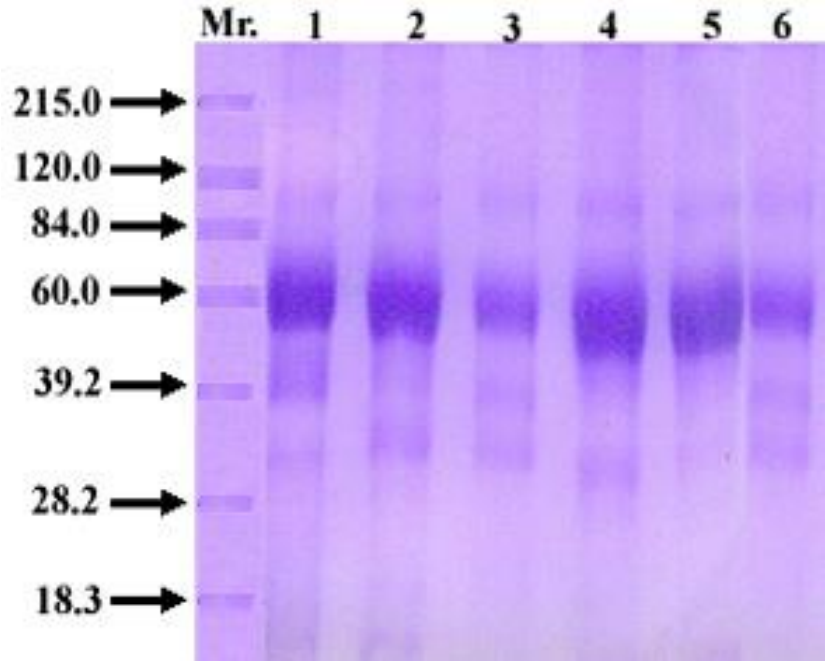

Figure 3. Coomassie blue stained SDS-PAGE showing the polypeptide pattern of cord samples from their newborns.

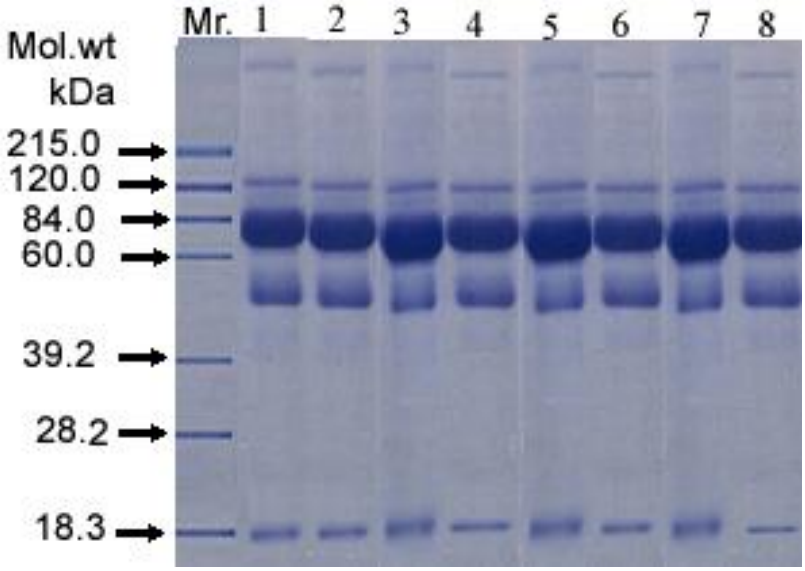

Figure 4. Coomassie blue stained SDS-PAGE showing the polypeptide pattern of serum of infected and non-infected pregnant women.

The present study analyzed serum and cord samples from 400 pregnant women and their neonates. ELISA was used to screen serum samples from 400 pregnant women for HCV-NS4 antigen, and 98 out of the total 400 serum samples were found to be positive with a detection rate of 24.5%, while 302 samples were negative with a rate of 75.5%. The cord samples of 400 neonates were tested for HCV-NS4 antigen using ELISA, with 98 out of the total 400 samples testing positive for HCV antigen, indicating a detection rate of 24.5%. All 98 neonates born to infected mothers tested positive for HCV antigen, indicating a vertical transmission rate of 100%. The mode of delivery did not impact the transmission rate of HCV-NS4 antigen, with 64 (22.9%) vaginal deliveries and 34 (28.3%) cesarean sections being included in the study, and no significant difference

in HCV antigen transmission rates was observed between children born via vaginal delivery or cesarean section after membrane rupture. The results showed no significant correlation between the detection rate of serum HCV-NS4 antigen and the mode of delivery.

5. Discussion

HCV infection is a significant public health issue with a wide range of health, social, and economic consequences. It is the leading cause of chronic viral hepatitis and is estimated to cause liver cancer in 25-30% of those with cirrhosis [1], [13]. In developed countries, the primary mode of transmission for HCV is changing, and it is primarily spread through parenteral or percutaneous routes. However, nonparenteral transmission, such as through sexual activity, household contact, and vertical or perinatal exposure to body fluids or secretions, can also occur [4], [3]. Vertical transmission, or the transfer of HCV from mother to child, is the most common mode of transmission in children, and it is influenced by maternal virus load [19], [14]. The purpose of this study was to determine the prevalence of HCV in pregnant women and assess the burden of HCV-NS4 antigen vertical transmission in relation to mode of delivery. Among 400 pregnant women, 140 cases (35%) were positive for anti-HCV antibody, and 260 cases (65%) were negative. HCV RNA was detected in 39/55 (71%) of the women who tested positive for anti-HCV antibodies using RT-PCR as the gold standard for HCV infection diagnosis.

Several authors confirm the presence of anti-HCV antibodies using immunoassay and reverse transcriptase-polymerase chain reaction as a gold standard [8]. In this study, the HCV-NS4 antigen was detected in female sera positive for HCV-RNA using the enzyme-linked immunosorbent assay (ELISA). The HCV-NS4 antigen was found in 38 out of 39 (97.4%) of the serum samples positive for HCV-RNA, while it was not detected in one sample. The sensitivity, specificity, efficiency, and negative predictive value of the HCV-NS4 antigen for identifying pregnant women with chronic HCV infection were 97.4%, 93.8%, 96.4%, and 93.8%, respectively.

Several methods for detecting viral antigens in sera have been developed by applying monoclonal antibodies to the HCV core antigen (HCVc Ag) [2], [22], [12], [5]. Similarly, [17] developed a method for detecting viral core protein in the plasma of HCV-infected individuals using monoclonal antibodies. In their study, 27 anti-HCV-positive donor plasmas were analyzed, of which 21 contained HCV RNA and 6 were negative. The core protein was found in 19 (90.5%) of the 21 RNA-positive plasmas.

In this study, Western blot analysis disclosed that a specific HCV antibody reacted with the HCV-NS4 antigen at a molecular weight of 27 kDa in both the selected sera and cord samples of HCV-infected mothers. No reaction was observed in sera and cord samples from non-infected mothers. [10] conducted a study using Western blot analysis to show the presence of core and E1 target antigens in serum samples. The analysis based on monospecific antibodies against core and E1 recognized the 38-kDa and 88-kDa bands, respectively, in the sera of all infected patients. However, no specific reaction was observed in the sera of uninfected individuals. In the present study, the HCV-NS4 target antigen was purified from the serum of HCV-infected mothers and their umbilical cord using electroelution from polyacrylamide preparative slab gels. The results showed that the TCA precipitate of the purified antigen from the serum of HCV-infected mothers and their umbilical cord revealed a single polypeptide chain at 27 kDa and showed a single peak when analyzed by capillary zone electrophoresis at 7.5 minutes. The reactivity of the serum and cord purified HCV-NS4 antigen was lost after exposure to high temperatures, acid, alkali, and β-mercaptoethanol treatment, but it was maintained after periodate treatment. The serum and cord purified antigen (HCV-NS4) were precipitated with 40% TCA and reconstituted in PBS, pH 7.2. The reconstituted precipitated of serum and cord purified antigen showed high reactivity toward specific anti-HCV antibody, whereas the supernatant of serum and cord

purified antigen (HCV-NS4) showed no reactivity.

The serum and cord purified HCV-NS4 antigen reactivity was decreased after treatment with α-chymotrypsin enzyme and was completely lost after 60 minutes. The biochemical characters of serum HCV-NS4 antigen did not change when transmitted to neonates via the placenta. The rate of mother-to-infant transmission is 4% to 7% per pregnancy in women with HCV viremia, and co-infection with HIV increases the rate of transmission 4 to 5 fold. The actual time and mode of transmission are not known, but the vertical transmission of HCV-NS4 antigen should be an important way of HCV transmission. In the present study, 400 pregnant women's serum was tested for HCV-NS4 antigen with ELISA, and neonatal cord blood was similarly tested. ELISA assay detected HCV-NS4 antigen in 98/400 (24.5%) pregnant women and 302/400 (75.5%) pregnant women were negative. In neonate samples, HCV-NS4 antigen was detected in 98/400 (24.5%) cord samples. There were 98 neonates of 98 infected mothers with vertical transmission, with a rate of 100%. These results showed a high prevalence of HCV infection in pregnant women living in Nile village, and vertical transmission of HCV-NS4 antigen occurs during pregnancy (100%). The mode of delivery for mothers in the present study was studied, and the results showed no significant difference between vertical transmission of HCV-NS4 antigen and mode of delivery ($p > 0.05$). 64 (22.9%) of 280 neonates delivered vaginally had HCV-NS4 antigen, while 34 (28.3%) of 120 neonates delivered by cesarean section had HCV-NS4 antigen.

Many studies have demonstrated that the rate of transmission does not significantly differ between children born via vaginal delivery or cesarean section after membrane rupture [20], [16]. Additionally, this study revealed that the transmission of HCV-NS4 antigen does not differ significantly between these two groups of children, and that the mode of delivery does not affect the transmission rate of HCV NS4 antigen.

6. Conclusion

Using the western blot technique, we identified the presence of the 27-kDa HCV-NS4 antigen in infected pregnant women and their cord sera. The successful purification of the 27-kDa HCV-NS4 antigen from serum and cord samples was demonstrated through the display of a single band in Comassie blue stained SDS-PAGE and a single peak in capillary zone electrophoresis analysis at 7.5 minutes. Vertical transmission of the HCV-NS4 antigen during pregnancy was confirmed, and the biochemical characteristics of the transmitted antigen did not differ when transmitted to neonates via the placenta. ELISA detected the 27-kDa HCV-NS4 antigen in 98 infected mothers and their cord sera, with a detection rate of 24.5% in both serum and cord samples. The vertical transmission rate was 100%, and the mode of delivery did not impact vertical transmission of the HCV-NS4 antigen.

7. References

[1] Ahmetagic S, Muminhodzic K, Cickusic E, Stojic V, Petrovic J and Tihic N. 2006. Hepatitis C infection in risk groups. Bosn J Basic Med Sci. 6(4):13-17.

[2] Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K and Yagi S. 1999. Development of a simple and higly sensitive enzyme immuno assay for hepatitis C virus core antigen. J Clin Microbiol, 37:1802-1808.

[3] Bayindir Y, Kalcioglu MT, Durmaz R and Ozturan O. 2005. Detection of HCV-RNA in cerumen of chronically HCV-infected patients. Laryngoscope, 115(3):508-511.

[4] Bortolotti F, Resti M, Giacchino R, Crivellaro C, Zancan L, Azzari C and Gussetti N. 1998. Changing epidemiologic pattern of chronic hepatitis C virus infection in Italian children. J Pediatr, 133:378-381.

[5] Bouvier AM, Patel K, Dahari H, Beaucourt S, Larderie P, Blatt L, Hezode C and Picchio Dhumeaux D. 2002. Clinical utility of total HCV core antigen quantification: a new indirect marker of HCV replication. Hepato, 36(1): 211.

[6] Chang KM. 2007. Regulatory T cells in hepatitis C virus infection. Hepatol Res, 37 Suppl 3:S327-330.

[7] Cheney CP, Chopra S and Graham C. 2000. Hepatitis C virus. Inet Dis Clin North Am, 14 (3):633- 667.

[8] Davoren A, Dillon AD, Power JP, Donnellan J, Quinn JM, Willis JW, Lawlor EM and O Riordan JM. 2002. Outcome of an optional HCV screening program of blood transfusion recipients in Ireland. Transfusion, 42(11):1501-1506.

[9] Deuffic – Burban S, Mohamed MK, Larouze B, Carrat F and Valleron AJ. 2006. Expected increase in hepatitis C- related mortality in Egypt due to pre -2000 infections. J Hepatol, 44 (3): 455-461.

[10] El- Awady MK, El Abd YS, Shoeb HA, Tabll AA, Hosny Ael D, El Shenawy RM, Atef K, Bader El Din NG and Bahgat MM. 2006. Circulating viral core and E1 antigen levels as supplemental markers for HCV chronic hepatitis. Virol J, 1:63-67.

[11] Esteban JI, Gonzales A and Hernandez JM. 1991. Open prospective efficacy trial of anti-HCV screening of blood donors to prevent post-tranfusion hepatitis in: Hollinger FB, Lemon SM, Margolis HS (eds.): Viral Hepatitis and Liver Disease. Baltimore, MD, Williams and Wilkins, 431-433.

[12] Grant PR, Sims CM and Tedder RS, 2002. Quantification of HCV RNA Levels and detection of core antigen in donations before seroconversion Transfusion, 42 (8) 1032.

[13] Iancu LS. 2001. Diagnostic strategies in Hepatitis C virus infection. Rev Med Chir Soc Med Nat Iasi, 105(1):37-42.

[14] Indolfi G, Azzari C, Moriondo M, Lippi F,de Martino M and Resti M. 2006. Alanine transaminase levels in the year before pregnancy predict the risk of hepatitis C virus vertical transmission. J Med Virol, 78(7):911-914.

[15] Kassem AS, el-Nawawy AA, Massoud MN, el-Nazar SY and Sobhi EM. 2000. Prevalence of hepatitis C virus (HCV) infection and its vertical transmission in Egyptian pregnant women and their newborns. J Trop Pediatr, 46(4):231-233.

[16] Mariné-Barjoan E, Berrébi A, Giordanengo V, Favre SF, Haas H, Moreigne M, Izopet J, Tricoire J, Tran A, Pradier C and Bongain A. 2007. HCV/HIV co-infection, HCV viral load and mode of delivery: risk factors for mother-to-child transmission of hepatitis C virus? AIDS, 20;21(13):1811-1815.

[17] Masalova OV, Atanadze SN, Samokhvalov EI, Petrakova NV, Kalinina TI, Smirnov VD, Khudyakov YE, Fields HA and Kushch AA. 1998. Detection of hepatitis C virus core protein circulating within different virus particle populations. J Med Virol, 55(1):1-6.

[18] Roberts EA and Yeung L. 2002. Maternal-infant transmission of hepatitis C virus infection. Hepatology, 36(5 Suppl 1):S106-113.

[19] Resti M, Bortolotti F, Azzari C, Giacchino R, Zancan L, Gussetti N. 2000 Transmission of hepatitis C virus from infected mother to offspring during subsequent pregnancies. J Pediatr Gastroenterol Nutr, 30:491-493.

[20] Syriopoulou V, Nikolopoulou G, Daikos GL, Theodoridou M, Pavlopoulou I, Nicolaidou P and Manolaki N. 2005. Mother to child transmission of hepatitis C virus: rate of infection and risk factors. Scand J Infect Dis, 37(5):350-353.

[21] Szabo G and Dolganiuc A. 2006. HCV immunopathogenesis: virus –induced strategies against host immunity. Clin Liver Dis, 10 (4): 753-771.

[22] Widell A, Molnegren V, pieksma F, Calmann M, Peterson j and Lee SR, 2002. Detection of hepatitis C core antigen in serum or plasma as a marker of hepatitis C viraemia in the serological window–phase. Transfus Med, 36(1):211-218.