

Assesment of the Materno-fetal transmission of HCV infections; a propspective observational study.

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Keywords:

Hepatitis C Virus, Assessment, materno-fetal transmission.

ABSTRACT

Today, the main source of pediatric HCV infection is the transmission of the virus from mother to infant. Here, a particular HCV-NS4 antibody and Western blot at 27-kDa were used to detect the HCV-NS4 antigen in serum samples from pregnant women who were infected and their cords. From the serum and cord samples, the HCV-NS4 antigen was isolated and identified as a protein. HCV-NS4 antigen vertical transmission is in place, and when this antigen is passed to newborns, its biochemical characteristics are unaltered. The ELISA found the HCV-NS4 antigen in the sera of pregnant women who were infected with a detection rate of 24.5% in serum, 24.5% in cord samples, and 100% in vertical transmission. The mechanism of delivery had no effect on vertical transmission.



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1. INTRODUCTION

Over 3% of the world's population is affected by the highly changeable RNA virus known as hepatitis C virus (HCV), which has a high propensity for chronic infection. Chronic hepatitis is linked to persistent infection, and after many years of infection, cirrhosis and hepatocellular cancer may develop. Rapid advancements have been achieved in our comprehension of the virology, epidemiology, natural history, diagnosis, and therapy of HCV since its initial identification in 1989 [1], [2].

More than 170 million people around the world have a chronic HCV infection, which is a common cause of cirrhosis, hepatocellular cancer, and chronic hepatitis. Only a small portion of acute HCV infections are eliminated, in contrast to infections with other hepatotropic viruses, and most infected people experience lifetime HCV infection in the absence of effective treatment. It is thought that both viral and host variables

play a role in the host immune system's incapacity to eradicate the initial infection and hence the high likelihood of chronic HCV infection [3].

Inapparent parenteral or per mucosal exposures like medical intervention, tattooing, acupuncture, vertical, sexual, accidental needlestick, and household transmission are examples of inapparent parenteral or per mucosal exposures. Epidemiological studies have established that there are two routes of transmission for HCV [4].

According to reports, mass parenteral anti-schistosomal medication is the reason why Egypt has the highest prevalence of HCV in the entire world. The probability of passing the HCV virus from mother to child varies depending on the population being investigated and the tests used. Pregnant Egyptian women in good health have a high anti-HCV prevalence, and vertical transmission is a significant danger for chronic HCV carriers. pregnant Egyptian women who tested negative for HIV had a high prevalence of HCV seropositivity and a substantially high risk of HCV vertical transmission. In the early detection and treatment of HCV infection, the identification of native HCV antigens may prove to be highly helpful. A natural HCV-NS4 antigen with a molecular weight of 27 kDa was found in the serum of HCV-infected individuals by [5- 9].

In this study, two objectives were investigated: (1) the potential for vertical transmission of the HCV-NS4 antigen from infected mothers to their newborns; and (2) the biochemical properties of cord-purified HCV-NS4 antigen in comparison to serum-purified HCV-NS4 antigen from infected pregnant women.

2. Materials and Methods

1. Samples:-

Blood samples were collected from 400 pregnant women. Their mean age was 25 years. The minimum and maximum ages were 20 and 40 years; respectively. Samples were obtained from Kafr saad General Hospital, Damietta, Egypt. Cord blood samples were collected at delivery from all 400 pregnant women. Blood and cord blood sera were separated and stored at -20°C until used. Cord blood samples included 280 samples from newborns delivered by normal vaginal and 120 samples from newborns delivered by cesarean section.

2. Determination of anti-HCV antibodies using ELISA:

Principle:

The HCV-Ab ELISA (Biotec Laboratories Ltd., Suffolk, UK) is an immuno-enzymatic technique in which HCV-specific synthetic antigens derived from core and NS regions (NS3, NS4 and NS5) representing HCV epitopes are coated in the wells of a microtiter plate. If HCV-specific antibodies are present in the sample, they will be captured by the HCV antigen in the wells and diluted serum samples will be added. A rabbit anti-human IgG/IgM labeled with horseradish peroxidase (HRP) is added after removing all of the other components of the sample, and if the antigen/antibody complex is present, the conjugate will bind to the complex. The enzyme immobilized on the solid phase reacts with the mixture of substrate and chromogen to produce an optical signal.

Assay technique

- 1) Before starting the test, all reagents were given time to come to room temperature. Each liquid reagent was carefully combined. 50 ml of the concentrated washing solution and 450 ml of distilled water were combined to cover the entire microtiter plate.
- 2) Five wells were left unfilled for the blank and controls, and 240 ml of sample diluent was added to each well before 10 ml of each serum sample was added to each well.

- 3) 250 ml of a negative control and 250 ml of a positive control were added to two wells, respectively (without dilution).
- 4) The plate was covered and heated to 37 C for an hour of incubation.
- 5) The wells were thoroughly filled (>300ml) with the diluted solution after the supernatants were discarded.

Validity of Assay

- 6) The blanking well's OD (at 450 nm) should be smaller than 0.1.
- 7) The mean OD at 450 nm value for the negative control (NC) should be less than 0.2 after blanking the A1.
- 8) The positive control's (PC) mean OD at 450 nm ought to be higher than 0.800.

Check the kit's expiration date, the functionality of the equipment, and the distribution of controls and samples protocol before repeating the test if the data above do not match the proper values.

Statistical Analysis:

"SPSS 28.0 for Windows, SPSS Inc." was used to perform all statistical analyses. The arithmetic mean and standard deviation (X SD) were used to express the data. With the help of X², the parameters of the various groups were compared. Calculations were made to determine the diagnostic sensitivity, specificity, effectiveness, and positive and negative predictive values (PPV and NPV).

3. Results

3.1 HCV-RNA detection in anti- HCV positive females using RT-PCR):

In this study, 400 pregnant Egyptian women at random had their serum samples obtained. They were 25 years old on average. The age ranges were 20 years for the lowest and 40 years for the maximum. There were 140 out of 400 positive results for HCV antibodies after testing all of these samples. However, 260 were utilized as negative controls because they had no HCV antibodies. As the gold standard for the diagnosis of HCV infection, RT-PCR assays were used to detect HCV-RNA in selected sera positive for anti-HCV antibodies (n=55). A primer set from the 5'-UTR was used in PCR to first extract the viral RNA from the serum. With the aid of DNA markers and agarose gel electrophoresis, the size of the amplified PCR products was assessed. The cases of HCV-RNA positivity displayed distinct bands at 150 bp, as illustrated in Figure (1). 39 (71%) of the 55 serum samples with anti-HCV antibody positivity also tested positive for HCV-RNA, while 16 (29%) tested negative for HCV-RNA.

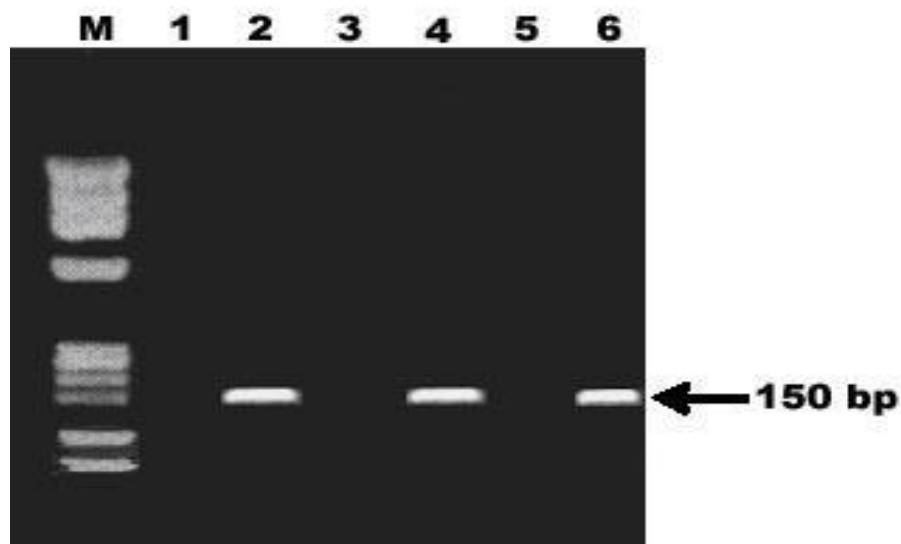


Figure 1. Agarose gel electrophoresis of HCV–RNA PCR products.

M: DNA marker.

Lanes 1, 3, 5: Serum samples of 3 HCV noninfected pregnant women.

Lanes 2, 4, 6: Serum samples of 3 HCV-infected pregnant women.

bp: base pair.

3.2 Detection of HCV-NS4 antigen in pregnant women sera using ELISA:

ELISA technique of [9] HCV-NS4 antigen was found in serum using a sensitive and precise test. In the ELISA, a particular anti-HCV-NS4 antibody was employed as a probe. Overnight, coating buffer-diluted serum samples were allowed to bond to the wells of ELISA plates. Following the addition of particular antibodies to the HCV-NS4 antigen, goat anti-rabbit IgG was conjugated with alkaline phosphatase. By incubating the linked conjugate with 1 mg/ml P-Nitrophenyl phosphate in substrate buffer and reading the absorbance at 490 nm on a microtiter plate reader, the amount of coupled conjugate was ascertained. Using blood samples from 16 negative controls (-ve HCV- RAN), the ELISA cut-off level (mean 3 standard deviations) was chosen at 0.30. (figure 2).

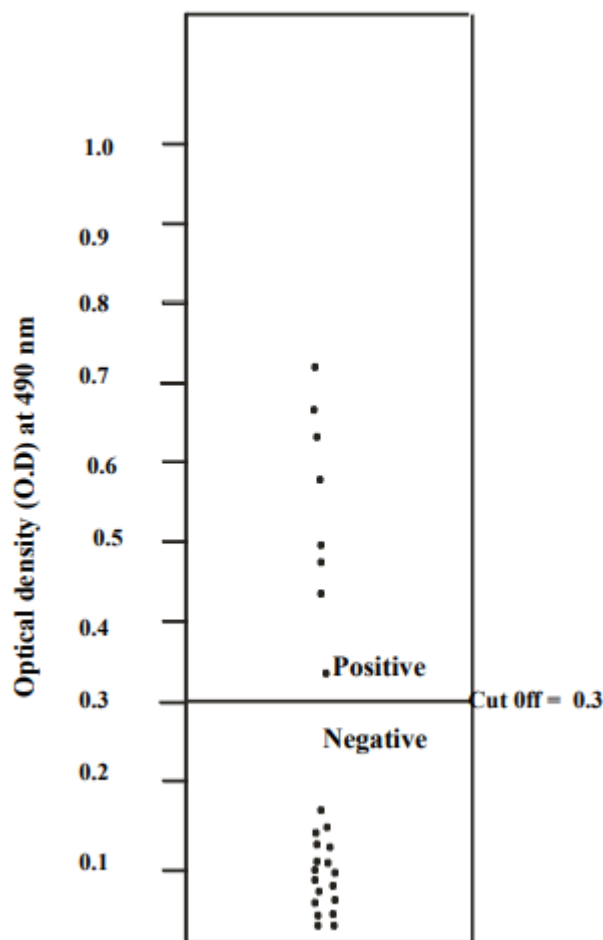


Figure 2. The cut off level of HCV-NS4 antigen using ELISA above or below which the tested sample is considered positive or negative.

3.2.1 Performance characteristics of HCV-NS4 antigen for diagnosis of HCV infection:

ELISA was used to find HCV-NS4 antigen expression in serum from pregnant women who had HCV-RNA-positive positivepositive results. Table 1 lists the 39 serum samples that tested positive for HCV-RNA and the 38 serum samples in which the HCV-NS4 antigen was found. The HCV-NS4 antigen has a positive predictive value of 97.4% for detecting pregnant women with chronic HCV infection. 97.4%, 93.8%, 96.4%, and 93.8% for sensitivity, specificity, efficiency, and negative predictive value, respectively; figure For the detection of HCV-NS4 antigen in serum, a sensitive and focused immunoassay was created. This test can be used to diagnose HCV infection in the lab.

Table 1. Performance characteristics of HCV-NS4 antigen for diagnosis of HCV infection:

HCV-RNA using RT- PCR	HCV-NS4 using ELISA		Total
	Positive	Negative	
Positive	38	1	39
Negative	1	15	16
Total	39	16	55

Sensitivity = $38/39 = 97.4 \%$
 Specificity = $15/16 = 93.8 \%$
 Efficiency = $38+15/55 = 96.4 \%$
 Positive predictive value = $38/39 = 97.4\%$
 Negative predictive value = $15/16 = 93.8 \%$

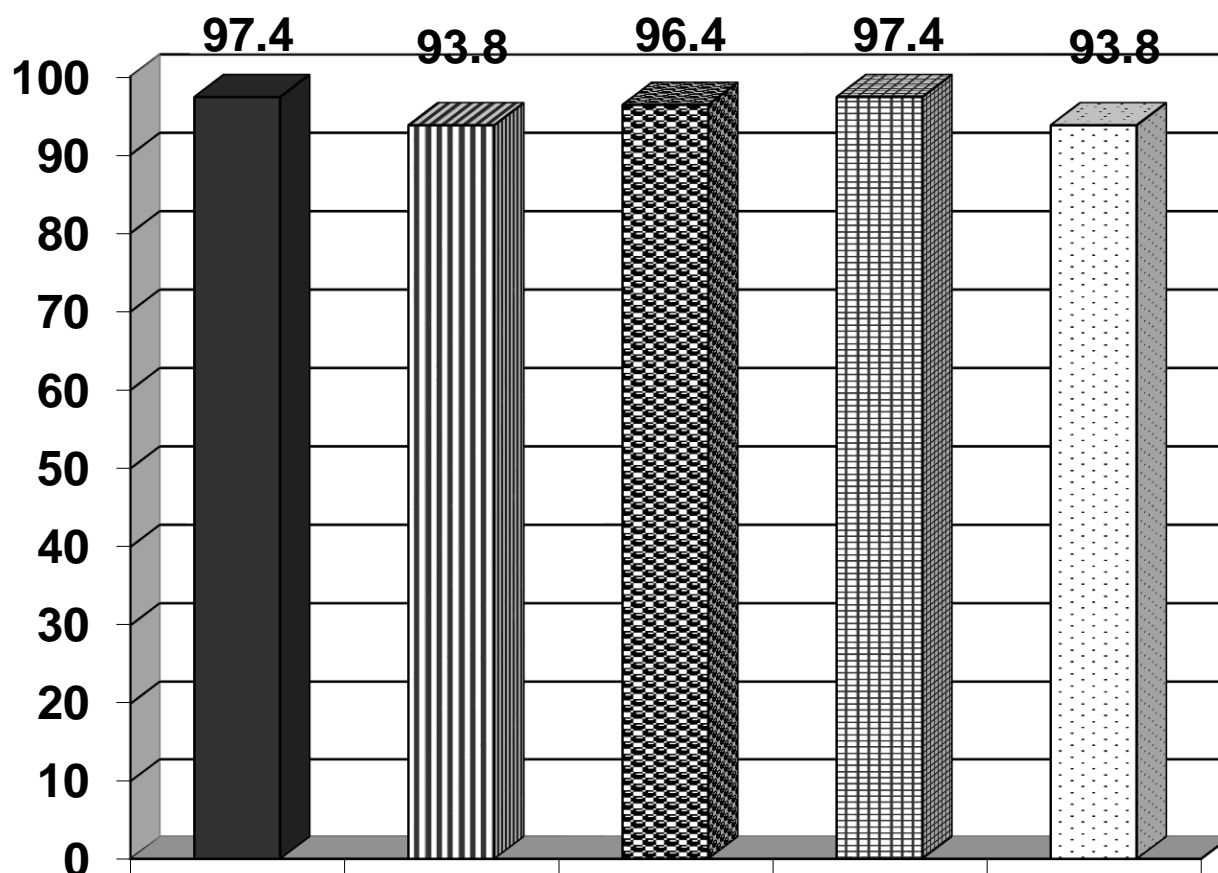


Figure 3. Performance characteristics of HCV-NS4 antigen for diagnosis of HCV infection.

PPV= Positive predictive value

NPV= Negative predictive value

3.3 Identification of HCV-NS4 antigen in pregnant women sera and cord samples collected from their neonates at delivery:

3.3.1 SDS-polyacrylamide gel electrophoresis:

Small molecules can flow through the gel's molecular sieve relatively easily, while the mobility of bigger molecules is constrained. Higher concentrations of acrylamide were employed for the best separation of tiny proteins whereas lower quantities were needed for larger proteins. Proteins are only separated by size using the SDS-PAGE method. Because it has a negative charge, sodium dodecyl sulfate is an ionic detergent that binds to proteins in direct proportion to their molecular weight. A 12% one-dimensional SDS-PAGE gel is filled with serum and cord samples from HCV-infected moms and healthy individuals. Prior to loading, cord, and serum samples were heated in sample buffers containing SDS and -mercaptoethanol as a reducing agent that destroys the protein's tertiary structure and breaks the disulfide connections, allowing the separation of the individual polypeptide subunits.

30 l/lane of chosen serum and cord samples were put into each well before electrophoresis. After running the gel, stain it with the coomassie blue to inspect the sizes and proportions of the protein on the gel.

The coomassie blue stain revealed that distinct polypeptides with a wide range of molecular weights from 215 kDa to 18.3 kDa were present in both the serum and cord samples.

3.3.2 Identification of HCV-NS4 antigen in pregnant women sera and cord samples using western blot.

Protein detection and characterization can both be done using the western blot approach. Protein mixture is first electrophoretically separated on a gel before blotting. The nitrocellulose membrane was positioned up against the gel, and the proteins were transferred there by an electrical field. Through non-covalent interactions, the transferred proteins adhere to the nitrocellulose membrane in an essentially irreversible manner. The proteins are exactly oriented the same way on the nitrocellulose as they were on the gel after being electrophoretically transferred from gel to membrane. Following transfer, the nitrocellulose is blocked with a protein-containing solution to stop a primary antibody from attaching non-specifically to it. A second antibody is employed to make the location of the complexes evident as it is not yet apparent. This secondary antibody has an enzyme that hydrolyzes substrate covalently linked (BCIP/NBT system).

Both chosen sera and cord samples from women who had HCV infection were found to contain a distinct band by Western blot analysis using a particular antibody against HCV-NS4. In samples from pregnant women who were not infected with HCV, no band could be seen.

3.3.3 Determination of the molecular weight of the reactive band using relative mobility:

The term "relative mobility" (R_f) refers to the ratio between the distance a protein has gone compared to the distance traveled by the tracking dye since its point of origin (top of the separating gel) (gel front). To establish a relationship between the molecular weight of the protein standards mixture and their flow rates on SDS-PAGE, Table 2 was created in order to calculate the molecular weight of the reactive epitope for anti HCV-NS4 in pregnant women serum samples infected with HCV. The linear calibration was used to quantify the reactive band's flow rate and estimate its molecular weight.

The reactive band had a molecular weight of 27 kDa in both the serum and the cord samples from the women who had HCV infection. This indicates that when the HCV-NS4 antigen was transferred to neonates, its molecular weight was unaltered.

Table 2. R_f values of unknown antigen and of standards protein mixture:

Molecular weight (kDa)	Log Molecular weight	R_f values
215.0	2.33	0.12
120.0	1.82	0.24
84.0	1.74	0.31
60.0	1.63	0.45
39.2	1.6	0.50
28.2	1.45	0.71
18.3	1.30	0.96
Unknown antigen (27 kDa)	1.43	0.84

4.1 Isolation and Purification of the 27 kDa HCV-NS4 antigen from serum and cord:

to determine whether the HCV-NS4 antigen's nature changed when it was transferred to newborns through the placenta. Using the electroelution technique from preparative slab gels, the HCV-NS4 antigen was identified and purified from selected serum and cord samples that were infected with the virus.

4.4.1 SDS-PAGE:

The 27 kDa HCV-NS4 antigen, which had been isolated from serum and cord, was examined using 12% SDS-PAGE and stained with Coomassie blue. Results demonstrated that in both serum and cord samples from pregnant moms who were HCV-infected, the precipitate showed a polypeptide chain at 27 kDa.

4.4.2 Capillary electrophoresis (CE):

Using CE, the purified antigen was examined. At 7.5 minutes, only one peak was visible. This outcome verified the purity of the single polypeptide band at 27 kDa that was eluted from the serum and cord samples of HCV-infected pregnant women.

4.4.3 Reactivity of the specific anti- HCV-NS4 antibody against the 27 kDa purified from pregnant women sera and cord from their neonates using western blot:

The purified 27 kDa antigen was recognized as a strong band by the specific HCV-NS4 antibody in serum and cord samples from HCV-infected expectant mothers. It was noticed in the TCA reconstituted precipitate but not in the soluble fraction of the supernatant.

5.1 Quantitation of HCV-NS4 antigen using ELISA.

By analyzing serial dilutions of the isolated antigen and computing the result from a standard curve carried out with each experiment, the HCV-NS4 antigen was quantified in unidentified samples. Both mothers who were infected and those who were not, as well as cord samples from the babies they gave birth to, had HCV-NS4 antigen concentrations.

6.1 Characterization of the reactive epitope of the HCV- NS4 antigen before and after transmission to neonates:

By subjecting the purified antigen from serum and cord to various physicochemical modifications, such as heat and chemical reagents, the reactive epitope identified in serum and cord samples by a particular anti-HCV antibody was characterized. Then, an ELISA test was performed to determine how well the specific antibody responded to treated serum and cord antigen. As a negative control, bovine serum albumin (BSA) was utilized.

6.6.1 Summary of partial characterization of serum and cord HCV-NS4 antigen using ELISA:

After exposure to temperatures of 56 oC and higher, treatments with acids, alkalis, and -mercaptoethanol, and after periodate, the serum and cord HCV-NS4 antigen's responsiveness was lost.

Contrary to the supernatant, which did not exhibit reactivity, cord HCV-NS4 antigen that was precipitated with TCA reacted with a particular anti-HCV-NS4 antibody. Additionally, the -chymotrypsin enzyme was used to treat the cord HCV-NS4 antigen at a steady quantity. At various times, the enzymatic reaction was interrupted (15, 30, 45, and 60 minutes). As the enzyme's incubation time was extended, the reactivity dropped until it vanished entirely after 60 minutes.

Table 3 showed that when serum HCV-NS4 antigen was passed from mother to fetus via the placenta, its biochemical characteristics did not alter.

Table 3. Comparison between the serum and cord HCV-NS4 antigen before and after transmission vertically to neonates:

Treatment	Serum HCV-NS4Antigen	Cord HCV-NS4 Antigen
1. Heat: a) -20, Zero, 24, 37 °C b) 56 °C	+ve -ve	+ve -ve
2. Acid and Alkali: a) 0.2 M HCl b) 0.2 M NaOH	-ve -ve	-ve -ve
3. Periodate oxidation:	+ve	+ve
4. TCA treatment: a) Precipitate b) Supernatant	+ve -ve	+ve -ve
β-mercaptoethanol: a) Zero, 20, 60 mM b) 180 mM	+ve -ve	+ve -ve
α-chymotrypsin: a) 15, 30 minutes b) 45 minutes	+ve -ve	+ve -ve

-ve: Reactivity lost.

+ve: Reactivity maintained.

7.1 Screening of HCV-NS4 antigen in pregnant women using ELISA:

Our goal was to investigate the vertical transmission of the HCV-NS4 antigen and determine whether or not its characteristics alter when it goes from the mother to the newborn. Thus, 400 serum samples from unrelated pregnant women were obtained, and 400 cord samples from the newborns' cords were obtained.

These women's modes and numbers of deliveries were examined; 280 moms gave birth vaginally, while the remaining 120 underwent cesarean delivery.

Pregnant women were tested for the presence of the HCV-NS4 antigen using ELISA, a quick and accurate method. We discovered that 98 out of 400 serum samples tested positive for the antigen with a detection rate of 24.5%, while 302 samples tested negative for the antigen (75.5%). There was a strong connection ($r=0.334$; $p\ 0.0001$) between the amounts of HCV-NS4 antigen in the sera of infected and uninfected women and cord

samples from their newborns.

With a detection rate of 24.5%, 98 out of 400 cord samples from newborns were positive for HCV-NS4 antigen. 98 neonates with HCV-NS4 antigen and 100% vertical transmission rate were born to 98 infected moms (table 4).

The detection rate of blood HCV-NS4 antigen and administration method do not differ significantly ($p > 0.05$). Of the 280 pregnant women with normal vaginal health, 64 (22.9%) have HCV-NS4 antigen. HCV-NS4 antigen is present in 34 (28.3%) of 120 pregnant women who underwent cesarean delivery (table 4).

Table 4. Relation between detection rate of serum HCV-NS4 antigen and mode of mothers delivery

Mode of delivery	Cord HCV-NS4 using ELISA			Total
		- 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			

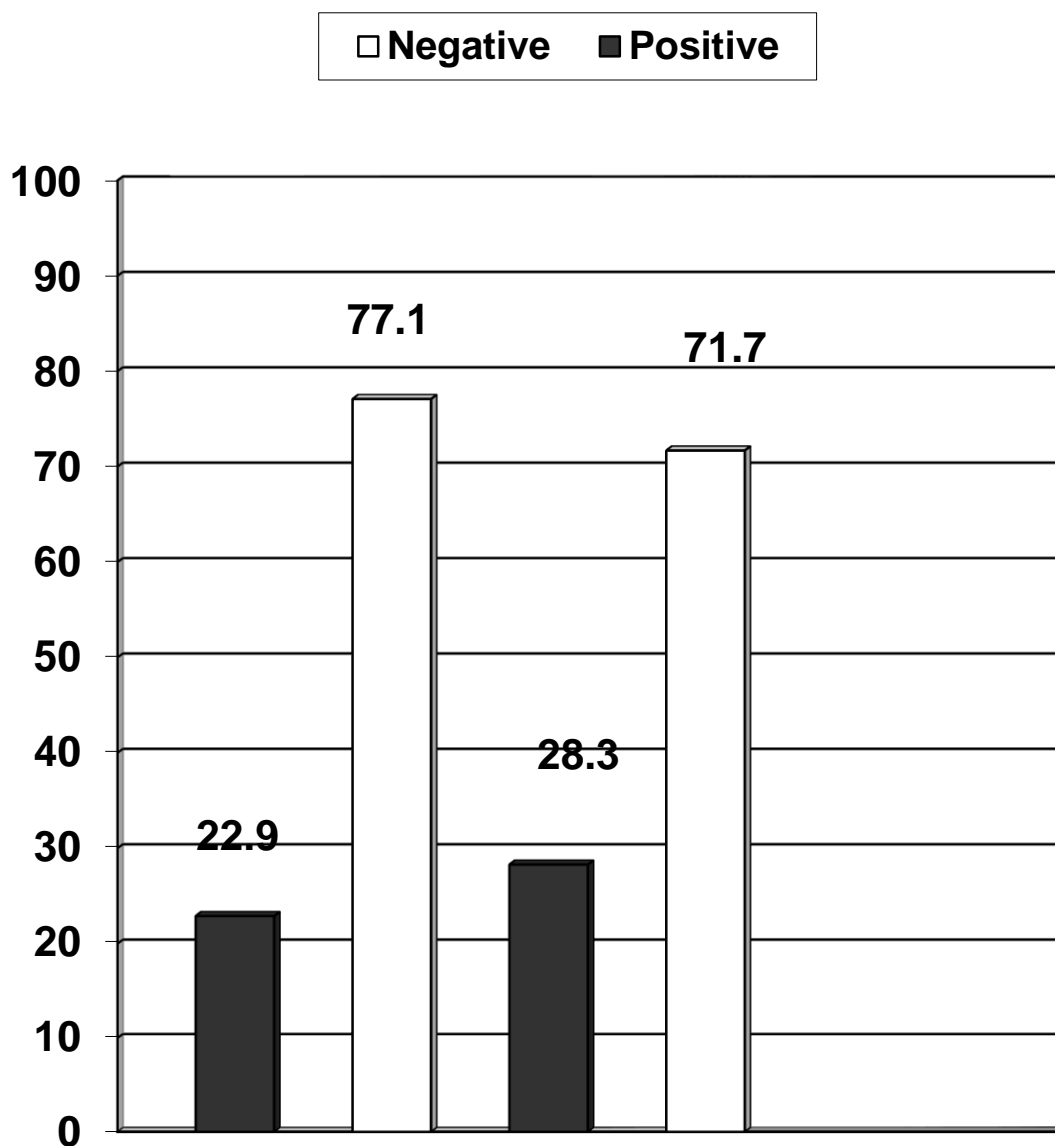


Figure 4. Relation between detection rate of cord HCV-NS4 antigen and mode of mothers delivery. There is no significant difference between detection of cord HCV-NS4 antigen and mode of delivery ($p > 0.05$).

4. Discussion

HCV infection is a significant global health issue with numerous negative effects on one's physical, psychological, and social well-being. The most frequent cause of chronic viral hepatitis is HCV. According to the World Health Organization, 170 million people worldwide are infected with HCV; in 10 to 30 years, 70% of them will experience chronic hepatitis and 20 to 30% may acquire cirrhosis. An estimated 25–30% of people with cirrhosis will develop liver cancer [10], [11].

The aim of this study was to examine the burden of HCV-NS4 antigen vertical transmission in Egypt and Saudi Arabia as well as the prevalence of HCV in pregnant Egyptian and Saudi women. In the current study, out of 400 pregnant women, 140 (35%) tested positive for anti-HCV antibodies, while 260 (65%) tested negative. Using RT-PCR assay as the gold standard for HCV infection detection, HCV RNA was found in 39/55 (71%) serum of women with anti-HCV antibodies positive.

14,917 people, 85% of whom were women, had their HCV status tested, according to [12]. 61 persons had their HCV status confirmed as positive by RIBA 3.0. The HCV-positive rate for blood component transfusion users (n=46) was 0.3% after excluding people with additional risk factors (n=15). 38 of 46 (83%) anti-HCV seropositive transfusion patients who were examined had HCV-RNA that could be detected by PCR.

In the current investigation, female sera positive for HCV-RNA were tested for HCV-NS4 antigen using ELISA. When serum samples were tested and found to be positive for HCV-RNA, the HCV-NS4 antigen was found in 38 out of 39 (97.4%), but not in one sample. With a positive predictive value of 97.4%, the hepatitis C viral non-structural 4 antigens (HCV-NS4) can detect pregnant women who have chronic HCV infection. 97.4%, 93.8%, 96.4%, and 93.8%, respectively, were the values for sensitivity, specificity, efficiency, and negative predictive value.

Using samples from the initial stages of HCV infection, [13] tested a prototype technique for the serological detection of HCV core antigen. Serial samples from 24 people who were experiencing HCV seroconversion were examined for anti-HCV, HCV RNA, and HCV core antigen levels. In 83% (20/24) of the cases, HCV antigen and HCV RNA were both found. HCV antigen was often detected 1 day after HCV RNA in the meantime to the first detection. In total, 87% of HCV-RNA-positive specimens had HCV core antigens that could be detected.

To effectively detect viral core and E1 antigens in circulating immune complexes precipitated from 65 serum samples of HCV patients, [14] evaluated the performance of polyclonal monospecific several rabbit anti-sera raised against synthetic peptides derived from conserved HCV sequences of genotype 4. The discovery of HCV RNA in the patient's serum established the infection. HCV core and E1 antigen are found using ELISA in serum samples.

In individuals with chronic hepatitis C, [9]. were able to identify and describe the native HCV-NS4 antigen. The native antigen in the serum of HCV serotype 4 infected individuals was found using the western blot and ELISA methods. The serum's native NS4 antigen was found to have a molecular weight of 27 kDa. SDS-PAGE staining of the isolated HCV antigen revealed a polypeptide band at 27 kDa, and capillary zone electrophoresis revealed a single peak at 7.6 min.

In the current investigation, Western blot analysis showed that specific HCV antibodies responded against HCV- NS4 antigen in both chosen sera and cord samples from women who had the virus, with an apparent molecular weight of 27 kDa. Sera and cord samples from non-infected moms showed no reactivity.

Western blot studies were utilized by [14] to prove that serum samples contained the core and E1 target antigens. All infected patients' sera were found to have the 38-kDa and 88-kDa bands, respectively, by Western blot analysis using monospecific antibodies against the core and E1. The sera from healthy individuals showed no particular response. A number of researchers isolated, purified and described the HCV-NS4 antigen, which was recognized by particular antibodies and may be used in immunodiagnosis [9].

Using electroelution from polyacrylamide preparative slab gels, the molecular weight 27-kDa HCV-NS4 target antigen was isolated from the umbilical cord and serum of HCV-infected mothers in the current investigation. The findings demonstrated a single polypeptide chain of 27 kDa in the TCA precipitate of purified antigen from serum of HCV-infected mothers and their umbilical cord, which also produced a single peak upon analysis by capillary zone electrophoresis at 7.5 minutes. The blood and cord-purified HCV-NS4 antigen's reactivity was lost after exposure to temperatures of 56 oC and higher, to acids and alkalis, and to -

mercaptoethanol treatment, while it was preserved following periodate. 40% TCA was used to precipitate the serum and cord-purified antigen (HCV-NS4), which was then reconstituted in PBS, pH 7.2. The cord-purified antigen in the reconstituted precipitate of serum and serum shown high reactivity (i.e., a positive ELISA result) toward a particular anti-HCV antibody. Contrarily, the serum and cord purified antigen (HCV-NS4) supernatant displayed no reaction (i.e. showing negative result using ELISA). Additionally, upon treatment with the -chymotrypsin enzyme, the reactivity of the serum- and cord-purified HCV-NS4 antigen was reduced, and it was completely gone after 60 minutes. Results showed that placental transmission of serum HCV-NS4 antigen to newborns did not alter its biochemical characteristics.

An infectious adenovirus recombinant with core-E1-E2 HCV genes was characterized by [15]. The early stages of viral infection are when the core protein was primarily generated. An immunoprecipitation using patient sera who were HCV-positive allowed for the detection of the expression of HCV E1 and E2 envelope proteins. According to prior findings in other mammalian expression methods, the purified E1 and E2 proteins appeared to be primarily constituted of a heterodimeric form. A trace amount of E1 and E2 monomers and inter disulfide-linked E1E2 aggregates were found. Evidently, heterodimeric E1E2 complexes were reactive in serology.

Anti-HCV prevalence ranges from 0.1% to 2.4% in pregnant women, while it might be substantially higher in some endemic regions. 60% to 70% of women with anti-HCV have viremia that is actively infected. Only when measurable serum HCV RNA is present and may be associated with greater levels can HCV transmission take place. In women with HCV viremia, the rate of mother-to-infant transmission ranges from 4% to 7% every pregnancy. HIV co-infection causes a 4- to 5-fold increase in the rate of transmission. Unknown are the actual transmission timing and method [16].

A key method of HCV transmission should be vertical transmission. Therefore, in the current investigation, we investigated vertical transmission of the HCV-NS4 antigen and identified the prevalence of the antigen in samples of pregnant women from the Nile river delta (Damietta governorate) and Saudi Arabia. In the current investigation, 400 Egyptian pregnant women's serum samples underwent ELISA testing for HCV-NS4 antigen. The HCV-NS4 antigen was similarly examined in newborn cord blood. In 98/400 (24.5%) pregnant women who underwent an ELISA assay, the HCV-NS4 antigen was found, while 302/400 (75.5%) pregnant women tested negative. HCV-NS4 antigen was found in 98/400 (24.5%) cord samples from newborns. HCV antigen was present in 98 neonates with a vertical transmission rate of 100% and 98 infected mothers. These findings demonstrated a high prevalence of HCV in pregnant women living in Nile village and Saudi Arabia and vertical transmission of HCV-NS4 antigen occurs during pregnancy (100%).

Many research have looked at how common HCV is among expectant women. The size of the research, regional factors, and the quality of laboratory testing varied widely between the studies. Based on the studies with at least 3,000 participants, the prevalence of detectable anti-HCV is generally around 1%. In the six trials with more than 10,000 women combined, the mean prevalence of anti-HCV was 1.26%, with almost 60% of these women having detectable HCV RNA. Early, smaller American investigations discovered a 4%–5% prevalence of anti-HCV. Small Egyptian investigations found a fairly high prevalence, ranging from 10% to 20%. Mother-to-infant transmission rates ranged from 5.6% in Italian research with viremic mothers to 6.9% in comparable Japanese studies and 3.1% in trials with viremic mothers from other countries [16].

According to [8], there is a considerable increase in the vertical transmission of HCV among Egyptian pregnant women who are HIV-negative. ELISA and polymerase chain reaction testing of HCV-RNA to determine the prevalence of HCV antibody seropositivity in pregnant women and their offspring. 14 of the

19 pregnant women who tested positive for HCV (14/19) had circulating HCV-RNA, as determined by PCR. Nine of the 19 kids born to HCV-positive mothers showed circulating antibodies, although only five of them had HCV-RNA. Accordingly, the chance of vertical transmission is 5/14 (36%) for moms who have HCV-RNA and 5/19 (26%) for those who have HCV antibodies in their blood.

In 30 HCV-positive/HIV-negative pregnant women in Italy, [6] studied the HCV vertical transmission rate. A second-generation ELISA was used to check for anti-HCV antibodies in 2,980 consecutive pregnant women, and a second-generation RIBA was used to re-test the results. 32 mothers in all (1.07%) tested positive for HCV on the RIBA2 confirmatory test, with 30 of 32 having a reactive result. Mothers who tested positive for anti-HCV did not have HIV. 10 of the 30 moms who tested positive for anti-HCV also tested positive by PCR for serum HCV-RNA. 10% of those who test positive for anti-HCV and 33% of moms who test positive for HCV-RNA can vertically transmit the virus.

Researchers [16] examined vertical HCV transmission. 50 pregnant women who were in their third trimester or nearing delivery joined the study. ELISA tests of the second and third generations were used to find anti-HCV antibodies. A commercial immunoblot was used to validate reactivity, and nested PCR was used to find HCV-RNA. At delivery, 28 out of 50 women (or 56%) tested positive for HCV-RNA, while in 17 out of 50 cases, no risk indicators for HCV infection were discovered. When compared to 22 non-viremic women, only 3/28 infants delivered to viremic mothers (10.7%) showed evidence of vertical transmission of the illness.

According to maternal viremia and HIV-1 coinfection, [17] studied mother-to-child transmission of the hepatitis C virus and found transmission rates ranging from 3 to 37%. 110 (1%) of the 13,025 females tested positive for anti-HCV antibodies, and 72 of them (65.4%) also tested positive for HCV RNA. In the initial blood sample, all 110 kids tested positive for anti-HCV antibodies; 8 of them also tested positive for HCV-RNA. Despite a small percentage of people with HCV in the general obstetric community, vertical transmission can nonetheless happen.

In the current study, the method of birth for mothers was examined. There were 280 vaginal deliveries and 120 cesarean sections included in the study. The mode of delivery and vertical transmission of the HCV-NS4 antigen are not significantly different ($p > 0.05$). Of the 280 newborns delivered via normal vaginal delivery, 64 (22.9%) carry HCV-NS4 antigen. Of the 120 neonates born by cesarean section, 34 (28.3%) have HCV-NS4 antigen. Furthermore, this study demonstrated that the transmission rate of HCV-NS4 antigen was unaffected by the manner of delivery and did not significantly differ between children born vaginally or via cesarean surgery following membrane rupture. Numerous investigations revealed that the rate of transmission did not significantly differ between infants delivered vaginally vs those delivered via cesarean section following membrane rupture [18], [19].

5. Conclusion

Using the western blot approach, we were able to detect the 27-kDa HCV-NS4 antigen in the cord sera of infected pregnant mothers. The 27-kDa HCV-NS4 antigen was isolated from serum and cord samples, and it displayed one band on an SDS-PAGE stained with coomassie blue and one peak when it was examined by capillary zone electrophoresis at 7.5 minutes. During pregnancy, there is vertical transmission of the HCV-NS4 antigen, and when it is passed to neonates via the placenta, its biochemical characteristics are unaltered. In 98 infected women and their cord sera, ELISA identified the 27-kDa HCV-NS4 antigen with detection rates of 24.5% in the serum, 24.5% in the cord, and 100% in vertical transmission. The mode of distribution had no effect on the vertical transmission of the HCV-NS4 antigen.

6. References

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