Preliminary screening of hepatitis C virus in pregnant females and their neonates

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Introduction

Hepatitis C virus is a spherical, enveloped, single stranded RNA virus.(1) Six major HCV genotypes and more than 90 subtypes have been identified based on molecular relatedness.(2) Genotype 1,2 &3 have a worldwide distribution, while genotype 4,5&6 are localized to specific geographic location.(3)

In Egypt, HCV is a major cause of liver disease & leading cause of death, with an estimated HCV infection rate of 15% to 20%. Egypt has a higher incidence of HCV infection than any other country in the world.(4) Most Egyptian cases of HCV are due to genotype S, which is uncommon in the west. (5)

Diagnosis of HCV infection involves the presence of the virus and assessment of the severity of liver infection. The diagnosis workup should include investigations that may help to predict prognosis and response to treatment. Diagnosis of chronic HCV infection based upon the presence of elevated serum aminotransferase and anti-HCV antibodies or the presence of HCV RNA in the serum. The disease is said to be chronic if amniotransferases were elevated for more than 6 months or the liver biopsy shows chronic pathological changes of hepatitis.(6)

Aim of the work

The aim of this work was to detect the prevalence of hepatitis C virus infection among pregnant women and their neonates at El-Shatby maternity university hospital.

Patients

This work is a random cross-sectional study performed at El-Shatby maternity university hospital in Alexandria, Egypt. The study was conducted over one thousand pregnant females in the third trimester attending for labor. Counseling and explanation of all aspects of the study to the participants and their consent was taken prior to their participation. Withdrawal of the samples started in May and ended in December 2005 with the completion of the one thousand cases included in the study. There were no exclusion criteria in this study.

Methods

All patients were subjected to the following:

1. Full history taking including:
   - Personal history: age, occupation, residence and special habits.
   - History of medical illness: diabetes, liver disease & or treatment
   - Surgical history: previous surgery & or blood transfusion.

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- History of dental manipulation & tattooing.
- Positive family history of liver diseases and any relevant history.
- Obstetrical & gynecological history.

2. Complete general examination.
3. Complete obstetrical examination.

Blood sampling and laboratory analysis:

Blood samples (3ml each) were withdrawn from the pregnant females & umbilical cord of their neonates. Sera were separated by centrifugation and stored at (-20°C) till testing time. At that time the sera were taken out of the freezer, left to thaw, remixed and centrifuged.

The supernatant of the maternal samples were tested for hepatitis C antibodies using enzyme linked immunosorbent assay (ELISA).

The supernatant of the neonates of HCV-Antibodies positive mothers were tested for HCV-Antibodies by enzyme linked immunosorbent assay (ELISA).

HCV-Antibodies positive samples were examined for liver function tests namely:

(a) ALT (serum alanine aminotransferase).
(b) AST (serum aspartate aminotransferase).

Fifty HCV-Antibodies negative cases were examined for liver function tests as a control group.

Results

In this study a total number of one thousand pregnant females in the third trimester were randomly selected from attendance of shatby maternity university hospital for delivery, they were screened for hepatitis C virus infection using the third generation ELISA. Blood samples were taken from the selected cases and the umbilical cord of their neonates immediately after birth, the umbilical cord blood samples of the HCV-Ab's positive mothers were investigated for the presence of HCV-Ab's.

Forty five pregnant females tested positive for HCV-Ab's representing 4.5% of the studied cases and all their 47 neonates (including two sets of twins) tested positive immediately after birth.

Liver function tests (AST & ALT) were done to the HCV-Ab's positive cases and compared with (AST & ALT) of fifty HCV-Ab's negative cases which was used as a control group.

Table 1: Distribution of HCV-positive pregnant females among the studied sample

Age group in years	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	NO	%	NO	%	NO	%
<20	65	6.8	2	4.4	67	6.7
20-<25	287	30	15	33.3	302	30.2
25-<30	344	36.1	11	24.5	355	35.5
30-<35	149	15.6	9	20	158	15.8
35-<40	110	11.5	8	17.8	118	11.8
Total	955	100	45	100	1000	100
Range Mean ± S.D	16-40 26.65±5.18		16-40 27.56±6.06		16-40 26.61±5.22	
Significance	X^2=4.086 P=0.394=NS					

X2=Chi square, P=probability, NS=non significant

According to age group

The mean age of the studied sample was 26.61 years ranging from 16 to 40 years with the highest frequency seen in the 25 to less than 30 years segment amounting to 35.5% of the whole sample. Forty five pregnant females were tested positive for HCV- Ab's. The mean age of the HCV-Ab's positive mothers were 27.56 years ranging from 16 to 40 with the highest frequency seen in the 20 to less than 25 years segment representing 33.3% of the HCV-Ab's positive group.

Table 2: Distribution of HCV-positive pregnant females among the studied samples according to their residence

Residence	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
Rural	457	47.9	19	42.2	476	47.6
Urban	498	52.1	26	57.8	524	52.4
Total	955	100	45	100	1000	100
Significance	X^2=0.546 P=0.460=NS OR=1.256 (95% CI=0.686-2.300)					

X^2=Chi square, P=probability, NS=non significant, OR=odds ratio, CI=confidence interval

Most of the cases enrolled in this study were from the urban areas (52.4%) and 26 of them tested positive for HCV=Ab's forming 57.8% of the HCV-Ab's positive group. On the other hand, 47.6% of the cases were from the rural areas and 19 of them tested positive making 42.2 of the HCV-Ab's positive group.

Table 3: Distribution of HCV-positive pregnant females among the studied samples according to gravidity

Gravidity	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
Primigravidae	336	35.2	11	24.4	347	34.7
Multigravidae 2-4 >4	 503 116	 52.7 12.1	 28 6	 62.3 13.3	 531 122	 53.1 12.2
Total	955	100	45	100	1000	100
Range Mean S.D.	1-12 2.47 1.74		1-12 3.11 2.29		1-12 2.50 1.77	

The number of the primigravidae was 347 of the studied sample 34.7% and 11 of them tested positive for HCV representing 24.4% of the HCV-positive cases. On the other hand, the number of multigravidae was 653 and 34 of them tested positive for HCV with the highest frequency seen in multigravidae 2 to 4 making 62.3% of the HCV-positive cases. The mean vidity among the studied sample was 2.50 ranging from 1 to 12. On the other hand, the mean gravidity among the HCV-Ab's positive cases was 3.11 as compared to 2.47 among the non infected cases.

Table 4: Distribution of HCV-positive pregnant females among the studied samples according to the mode of delivery

Mode of delivery	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
Vaginal	694	72.2	32	71.1	726	72.6
Cesarean	261	27.4	13	28.9	274	27.4
Total	955	100	45	100	1000	100
Significance	X^2=0.053 P=0.819=NS OR=1.080 (95% CI=0.558-2.090)=NS					

X^2=Chi square, P=probability, NS=non significant, OR=odds ratio, CI=confidence interval

Total number 726 cases of the studied sample 72.6% were delivered vaginally and 32 of them tested positive for HCV-Ab's representing 71.1% of the HCV-positive cases as compared to 72.6% of the HCV-negative cases. On the other hand, 274 cases 27.4% delivered by cesarean section and 13 of them tested positive for HCV-Ab's representing 28.9% of the HCV-positive cases as compared to 27.4% of the HCV-negative cases.

Table 5: Distribution of HCV-positive pregnant females among the studied samples according to fetal outcomes

Fetal outcome	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
Normal	936	98	45	100	981	98.1
Still birth or IUFD	12	1.3	-	-	12	1.2
Congenital malformations	7	0.7	-	-	7	0.7
Total	955	100	45	100	1000	100
Significance	X^2=1.757 P=0.415=NS					

X^2=Chi square, P=probability, NS=non significant, OR=odds ratio, CI=confidence interval

A total number of 981 pregnant females of the studied sample delivered normal living babies with no congenital fetal malformations and 45 of them tested positive for HCV-Ab's.

Table 6: Distribution of HCV-positive pregnant females among the studied samples according to number of delivered neonates

Number of babies	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
Single	943	98.7	43	95.6	986	98.6
Twin	11	1.2	2	4.4	13	1.3
Triplet	1	0.1	-	-	1	0.1
Total	955	100	45	100	1000	100
Significance	X^2=3.675 P=0.159=NS					

X^2=Chi square, P=probability, NS=non significant

A total number of 986 pregnant females in this study delivered single neonates representing 98.6% of the studied sample and 43 of them tested positive for HCV-Ab's representing 95.6% of the HCV-positive cases as compared to 98.7% of the HCV-negative group. On the other hand, 13 delivered twins representing 1.3% of the studied sample and 2 of them tested positive representing 4.4% of the HCV-positive cases as compared to HCV-negative group. Only one female delivered triplet representing 0.1% of the studied sample. All the 47 neonates of the 45 HCV-positive mothers (including two sets of twins) tested positive for HCV-Ab's immediately after birth.

Table 7: Distribution of HCV-positive pregnant females among the studied samples according to history of blood transfusion

History of blood transfusion	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)	
	No	%	No	%	No	%
	79	8.3	13	28.9	92	9.2
Significance	X^2=21.866, P=0.000*, OR=4.505, 95% CI=2.272-8.932					

X^2=Chi square, P=probability, *=significant difference, OR=odds ratio, CI=confidence interval

92 pregnant females of the studied sample 9.2% gave history of at least one episode of transfusion of blood or blood products and 13 of them tested positive for HCV-Ab's representing 28.9% of the HCV-positive cases as compared to 8.3% of the HCV-negative group, i.e. nearly about one third of the infected individuals received at least a single transfusion of blood or blood products.

Table 8: Distribution of HCV-positive pregnant females among the studied samples according to history of operative intervention

Risk factor	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)		Significance
	No	%	No	%	No	%	
Previous obstetric operations (C.S, forceps, ventose)	171	17.9	8	17.8	179	17.9	X^2=0.000 P=0.983, NS OR=0.991,NS
Previous gynecologic operations (D&C, laparoscope, myomectomy, repair oper.)	212	22.1	10	22.2	222	22.2	X^2=0.137 P=0.711, NS OR=1.141,NS
Previous surgical operations (tonsillectomy, appendectomy, thyroidectomy, hernia repair)	365	38.2	27	60	392	39.2	X^2=8.554 P=0.003* OR=2.425* 95%CI=1.317-4.465
Total	747	78.2	45	100	793	79.3	

X^2=Chi square, P=probability, NS=non significant, *=significant difference, OR=odds ratio, CI=confidence interval

*some cases had more than one operative intervention.

A total number of 793 pregnant females had a positive history of previous operative intervention regardless the indications, representing 79.3% of the studied sample. Around 179 of the studied population 17.9% gave a history of either cesarean section or assisted delivery using forceps or ventose and 8 of them tested positive for HCV representing 17.8% of the HCV-positive cases. ON the other hand, 22.2% gave a history of at least one episode of invasive gynecologic procedure including laparoscopy, dilatation and curettage and a variety of major gynecologic surgeries including myomectomies and repair procedures and 10 of them tested positive for HCV representing 22.2% of the HCV-Ab's positive cases. Finally, 392 of the tested females 39.2% had other surgical (non-obstetric, non-gynecologic) operations ranging from tonsillectomy, appendectomy to hernia repair and thyroidectomy and 27 of them tested positive for HCV representing 60% of the HCV-Ab's positive cases.

Table 9: Distribution of HCV-positive pregnant females among the studied samples according to history of dental procedures and unsafe injections

Risk factor	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)		Significance
	No	%	No	%	No	%	
Previous dental procedures	133	13.9	30	66.7	163	16.3	X^2=87.616 P=0.000* 95%CI=6.477-23.590
Unsafe injections (using non-disposable syringe)	17	1.8	1	2.2	18	1.8	X^2=0.048 P=0.827, NS 95%CI=0.163-9.638

X^2=Chi square, P=probability, NS=non significant, *=significant difference, OR=odds ratio, CI=confidence interval

Certain factors were shown to be associated with a high prevalence of HCV-Ab's. History of previous dental procedures were positively correlated with a high prevalence of HCV-Ab's; around 16.3% of the studied females gave a history of a t least one dental visit and 30 of them tested positive for HCV-Ab's representing 66.7% of the HCV-positive cases as compared to 13.9% of the HCV-negative group; this difference is statistically significant. On the other hand, the difference between infected and non-infected individuals as regards history of unsafe injection using non-disposable syringe was not statistically significant.

Table 10: Distribution of HCV-positive pregnant females among the studied samples according to presence of some other risk factors

Risk factors	HCV-negative (n=955)		HCV-positive (n=45)		Total (n=1000)		Significance
	No	%	No	%	No	%	
History of bilharziasis and treatment	47	4.9	-	-	47	4.7	X^2=2.324, P=0.127,NS OR=0.953,NS 95%CI=0.939-966
• Oral treatment	3	0.3	4	8.9	7	0.7	
• Parenteral treatment	3	0.3	4	8.9	7	0.7	X^2=45.458, P=0.000* OR=30.959* 95%CI=6.709-142.86
High risk occupation	4	0.4	2	4.4	6	0.6	X^2=11.677, P=0.001* OR=11.058* 95%CI=1.971-62.043

X^2=Chi square, P=probability, NS=non significant, *=significant difference, OR=odds ratio, CI=confidence interval

Fifty four pregnant females of the studied sample gave a history of bilharziasis and treatment; 47 of them were treated with oral therapy and non-of them tested positive for HCV. On the other hand, 7 pregnant females were treated with parenteral therapy and 4 of them tested positive for HCV representing 8.9% of the HCV-Ab's positive cases as compared to 0.3% of the HCV-negative cases; this difference was statistically significant. Only 6 of the whole studied population gave a history of a high risk occupation including nurses, healthcare workers, employees in laboratories & dialysis units and 2 of them tested positive for tested positive for HCV representing 4.4% of the HCV-positive cases as compared to 0.4% of the HCV-negative cases; this difference was also statistically significant.

Table 11: Multiple regression analysis (linear model) for some predictor risk factors for HCV-positive cases

Predictor	Unstandardized coefficients		Standardized coefficients	T	Significant
	B	Std.error	Beta		
Constant	0.019	0.008		2.435	0.15
History of blood transfusion	0.0467	0.023	0.065	2.015	0.044*
Previous obstetric operations	-0.075	0.020	-0.138	-3.663	0.000*
Previous gynecologic operations	0.108	0.022	-0.216	-4.955	0.000*
Previous surgical operations	0.0645	0.023	0.152	2.817	0.005*
Previous dental procedures	0.175	0.019	0.313	9.354	0.000*
Unsafe injections	-0.127	0.047	-0.082	-2.696	0.007*
High risk occupation	0.213	0.079	0.079	2.695	0.007*
Parentral treatment of bilharziasis	0.482	0.074	0.194	6.519	0.000*

For regression model: F=110168, P=0.000*

On doing multiple regression analysis (=multivariate analysis) using linear model it was found that history of blood transfusion, previous obstetric operations, previous gynecologic operations, previous surgical operations, dental procedures, unsafe injections using un-disposable syringes, high risk occupations as well as parenteral therapy for bilharziasis were all statistically significant risk factors for HCV.

Table 12: Comparison of liver function tests (AST & ALT) in HCV-positive and HCV-negative cases (control group).

SGOT (AST)	HCV-negative (n=50)		HCV-positive (n=45)		Total (n=95)	
	No	%	No	%	No	%
Normal	43	86	37	82.2	80	84.2
Elevated	7	14	8	17.8	15	15.8
Total	50	100	45	100	95	100
Range Mean S.D.	17-127 39.44 1.79		18-223 48.04 37.12		-	
Significant	X^2=0.254 P=0.614, NS					
SGPT (ALT)	HCV-negative (n=50)		HCV-positive (n=45)		Total (n=95)	
	No	%	No	%	No	%
Normal	46	92	40	88.9	86	90.5
Elevated	4	8	5	11.1	9	9.5
Total	50	100	45	100	95	100
Range Mean S.D.	13-123 39.44 23.47		15-65 24.73 13.10		-	
Significant	X^2=0.267 P=0.605, NS					

X^2=Chi square, P=probability, NS=non significant

AST was elevated in 8 females 17.8% of the HCV-positive cases with the serum level ranging from 18 to 233 and the mean level was 48.04. On the other hand, it was elevated in 7 cases 14% of the HCV-negative control group with the serum level ranging from 17 to 127 and the mean level was 39.44. ALT was elevated in 5 cases 11.1% of the HCV-positive group with the serum level ranging from 15 to 65 and the mean level was 24.73. On the other hand, it was elevated in 4 cases, 8% of the HCV-negative control group with the serum level ranging from 13 to 123 and the mean level was 23.47.

Discussion

Hepatitis C virus is a major health problem throughout the world. It is estimated that 3% or nearly about one hundred and seventy million people worldwide are HCV antibodies positive; of those, 35% are women in childbearing period. (7)

Our work is cross sectional study depending on the screening of blood samples withdrawn from one thousand pregnant women and their neonates immediately after birth. The withdrawn samples were screened for the presence of HXC antibodies using the 3rd generation ELISA technique.

The results of this study revealed that the seroprevalence rate of HCV antibodies among all the tested pregnant females were 4.5%. This goes with the most of the United States and international studies that reported that the seroprevalence rate of HCV antibodies among the pregnant women to be between 0.7 and 4.4%. (8,9,10) Umbilical cord samples of the HCV antibodies positive mothers were all HCV antibodies positive at birth. Further confirmation of these results is by detection of HCV-RNA using the PCR. (11)

In 1994, a prospective study was performed in blood transfusion service, Fukushima medical college, Japan, to assess the vertical transmission of HCV. It included 7698 parturient women. They were tested for anti HCV antibodies; 53 were positive and 31 of them only were HCV-RNA positive. The 54 infants of the HCV antibodies positive mothers (including one st of twins) were HCV antibodies positive at birth and were followed for 6 months. They were tested for HCV-RNA. Three of the 54 babies (5.6%) became HCV-RNA positive during the follow up period. None of the

babies of the 22 HCV antibodies positive but HCV-RNA negative mothers became positive for HCV_ RNA. (12)

In May 2001, Sophie P. et al in France, published a study to evaluate the risk factors for vertical transmission using a case control design. This study demonstrated an increase HCV vertical transmission risk during vaginal delivery and with the use of forceps compared with caesarean section. Episiotomy did not appear as a risk factor during vaginal delivery. Maternal breast feeding was not found to be a risk factor. Furthermore; amniocentesis did not increase the risk of vertical transmission.(13)

In 2005, two separate studies were done to estimate the vertical transmission of HCV and investigate the effect of mode of delivery and infant feed in on the risk of HCV transmission. In the 1st study, the overall rate of vertical transmission from mothers who wee HCV RNA positive at delivery was 4.7%. In the 2nd study, the overall rate of HCV vertical transmission was 6.2%. Membrane rupture for more than 6 hours and internal fetal monitoring were associated with transmission of HCV to infants in both studies. (14)

In our study, the HCV antibodies positive cases were increased with age and gravidity. This may be attributed to increased exposure to other risk factors as increased operative interference and transfusion of blood and blood products. Nearly one third of the HCV antibodies positive mothers (28.9%) received at least a single transfusion of blood or blood products. This is in agree with Arthur et al (1997) who reported a rate of 24.8% among blood donors from different Egyptian governorates. (4)

As regards the fetal outcome in our study, all the neonates of the HCV positive mothers were normal, with no congenital anomalies. This is in agreement with Kudo T (1997) who reported no association between maternal HCV infection with abortion, still birth, premature birth or congenital anomalies. (15)

In the present study, there were no significant difference between HCV antibodies positive cases and the control group as regards liver enzymes. This goes with Gervais et al (2000). (16).

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This study was done in 2012 before the era of sofosbuvir for treatment of hepatitis C. our recommendation is to do the study again to find if there is any change in the prevalence of hepatitis C in Egypt after introduction of this line of treatment.