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Prospective Study of Prevalence and Risk Factors for Hepatitis C in Pregnant Egyptian Women and Its Transmission to Their Infants

Khaled AbdulQawi[1], Ahmed Youssef[2], Mohamed A Metwally[3], Ibrahim Ragih[4], Mohamed AbdulHamid[5,6], AbdulAziz Shaheen[5]

[1]Pediatrics Department, Benha University, Benha, Egypt

[2]Gynecology and Obstetrics Department, Benha University, Benha, Egypt

[3]Hepatology, Gastroenterology, and Infectious Diseases Department, Benha University, Benha, Egypt

[4]Clinical Pathology Department, Benha University, Benha, Egypt

[5]National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt

[6]Hepatology, Gastroenterology, and Infectious Diseases Department, Minia Faculty of Medicine, Minia, Egypt

Aim To estimate the hepatitis C virus (HCV) vertical transmission rate, the effect of potential risk factors, and the pattern of HCV antibody response and viremia in HCV-infected infants in Benha, Egypt.

Methods A total of 1224 pregnant women who were treated at Benha University Hospital, Egypt, were included in the study. They completed a questionnaire about risk factors for HCV acquisition and suspected risk factors for mother-to-infant transmission and were tested for HCV antibody using a third-generation ELISA test. Women positive for HCV antibody were tested for HCV RNA by polymerase chain reaction. Peripheral blood of infants of positive HCV-RNA women was tested for HCV antibody and HCV-RNA at 1 and after 6 months of age.

Results Out of 1224 pregnant women, 105 (8.6%; 95% confidence interval, 7.05-10.17) were positive for HCV antibody. Only 83 (6.8%; 5.39-7.21) were positive for HCV-RNA. HCV infection was associated with older age (1.16; 1.1-1.2, $P=0.001$), blood transfusion (2.69; 1.2-6.0, $P=0.016$), and HCV infection of the husband (5.47; 1.4-21, $P=0.014$) or other household members (2.29; 1.2-4.6, $P=0.019$). Out of 53 infants tested at first month, 43 (81%; 71-92%) were positive for HCV antibody, but only 7 (13%; 4.1-22%) were positive for HCV-RNA. After 6 months, only 2 (3.8%; 0-8.95%) remained positive for HCV RNA.

Conclusions The prevalence of HCV in pregnant women in Egypt is lower than previously reported and the potential risk factors associated with HCV infection suggest intra-familial transmission. The frequency of vertical transmission of HCV in Egypt is not substantially different from other countries and does not play a role in the high prevalence of HCV in Egypt.

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Correspondence to:
Khaled Abdulqawi
Department of Pediatrics
Benha University
2 Saad Zaghloul st
Benha, PO Box:113
Qualyabia governorate, Egypt
khaledqawi@yahoo.com

Worldwide, hepatitis C virus (HCV) infection is one of the most prevalent causes of liver diseases. There are estimated 300 million carriers of the virus all over the world (1). In the USA, the overall prevalence of HCV antibodies in the general population is 1.8%, the prevalence in children 6-11 years is 0.2%, and in adolescents 12-19 years old is 0.4% (2). In most developed countries, HCV infection is associated with percutaneous blood exposure, primarily as a result of blood transfusion and intravenous drug addiction (3).

Egypt has the highest prevalence of hepatitis C in the world. Studies have found widely varying levels (10-50%) of the prevalence, depending on the populations covered; overall, estimates of the HCV rate in the general population range between 10 and 20% (4,5). Geographically, hepatitis C prevalence is higher in Lower Egypt (Nile delta) than Upper Egypt, and it is lower in urban than rural areas (6).

In Egypt, the use of contaminated needles and syringes during mass schistosomiasis treatment campaigns during the period the 1960s-1980s has been identified as a key mode of transmission for HCV infection, suggesting that parenteral exposure continues to cause infections (7). Evidence of high interfamilial HCV transmission was found in a study in a rural community in the Nile Delta in the 1990s (5-8), although the exact modes of transmission were not identified.

Vertical transmission may help to explain the high prevalence in Egypt. This type of transmission in France is estimated to be less than 6% in HIV- negative patients (9). Indeed, a review of 13 studies on vertical transmission of HCV showed that the overall rate was 5.2% (10). However, a brief report from Egypt showed that vertical transmission of HCV was 36% (11), but this study looked at a small sample of 19 out of 100 pregnant women positive for HCV antibody, and only 14 of them were positive for HCV RNA. The sample of Kassem et al (11) comprised 100 randomly selected HIV-negative pregnant women and was too small for a valid estimation of the proportion of vertical transmission. It also used a limited definition of vertical transmission, defining it as the presence of HCV RNA in cord blood, and it did not repeat the polymerase chain reaction (PCR) test for HCV-RNA for infants after 6 months.

The aim of the present article was to perform a more extensive study to estimate the HCV vertical transmission rate, the effect of potential risk factors, and the pattern of HCV antibody response and viremia in HCV-infected infants in Benha, Egypt.

METHODS

Site

The study was conducted in Benha, the capital of Qualyabia governorate, a small city 35 km north of Cairo. It is a semi-urban area with irrigated farmlands and surrounded by canals, a feature typical of the Nile delta. It has the characteristics typical of a Lower Egyptian community: a mixture of urban and rural areas, with significant influence of Egyptian traditions and attitudes. Benha University Hospital is one of the largest hospitals in Benha city. This hospital accepts patients from Benha city and the rural areas from Menufia and Sharkia governorates nearby (village households, inhabited mainly by farmers and their families). Most of the community studies on HCV in Nile delta have been conducted in Qualyabia and Menoufia.

Study design and population

This prospective study was conducted in two stages: the first stage was a cross-sectional study to identify the prevalence of HCV among pregnant women and the second stage was a longitudinal study of the infants of infected women to identify the rate of vertical transmission. The study population included all pregnant women who were admitted at the obstetric emergency department at Benha University Hospital (Benha, Egypt) for delivery between October 2003 and July 2008. The women who gave birth more than once during the study period were not included in the study. We did not test for HIV, which is exceedingly rare in rural Egyptian communities.

The study protocol was approved by the clinical research committee of Benha University Hospital, the Institutional Review Board of the Egyptian Ministry of Health and Population, and the National Hepatology and Tropical Medicine Research Institute (Cairo). Patients were asked to sign a written consent for the study.

Data collection

After signing the consent form, the authors conducted interviews with the women using a standardized questionnaire designed by a team of sociologists, epidemiologists, and clinicians familiar with the risk factors for HCV acquisition and suspected risk factors for mother-to-infant transmission of HCV (older age, history of blood transfusion, duration of marriage, parity of more than two, husband and other household members positive for HCV). This ques-

tionnaire assessed sociodemographic characteristics, present and past health, and potential risk factors for exposure to HCV and mother-to-infant transmission of HCV. The address and phone number of the patients and the name and phone number of close relatives were taken to facilitate follow-up and decrease patient loss during the study.

Specimen collection and serological testing of pregnant women. After the questionnaire was filled out, blood samples (10 mL) were taken from the pregnant women and sent to the laboratory. Serum alanine transaminase level (ALT) was assessed within 6 hours of sampling using ALT FLEX™, AR model of the DIMENSION™ system (Dade Behring Inc., Newark, DE, USA). When ALT was found to be elevated, additional tests were performed to exclude metabolic and viral liver disease other than hepatitis C. The serum was separated and aliquoted into 3 cryotubes, one aliquot was sent in an ice bag to the HCV Reference Laboratory at the National Hepatology and Tropical Medicine Research Institute (Cairo), where the serum was tested for HCV antibodies using a third-generation ELISA test (Axsym System HCV, version 3.0, Abbott Diagnostics Division; Wiesbaden, Germany) as recommended by the manufacturer. The other two aliquots were stored in -70°C freezers to be tested later if needed. Infected pregnant women were identified by testing serum for the presence of HCV antibody. Serological samples that were positive for HCV antibody were tested for the presence of HCV-RNA using a procedure of whole-serum amplification of DNA based on an in-house reverse transcription-nested polymerase chain reaction (RT-PCR). Pregnant women were considered infected only if both the HCV antibody and HCV-RNA tests were positive.

Serological testing of infants and classification of results

Infected patients who tested positive were called back to get a peripheral blood sample from their infants. HCV antibody testing was done first on the infants and then positive HCV antibody samples were tested for HCV-RNA. Infants were considered uninfected if they had never been positive for HCV RNA or if they cleared anti-HCV antibodies after 6 months of age. Infants were considered to have perinatal mother-to-infant transmission if they were HCV-RNA positive at any time following birth or showed anti-HCV antibodies after 6 months of age. They were considered to have transient perinatal HCV infection if they were positive for HCV RNA at the 6-month visit, but negative for both anti-HCV and HCV-RNA after the 6-month visit. The children continuing to have HCV-RNA after the 6-month visit were considered to have persistent perinatal HCV infections. Anti-HCV antibodies detected in the blood of children whose mothers tested positive for anti-HCV antibodies 2-6 months after delivery were considered to be maternally acquired (12).

PCR-based detection of HCV-RNA

The protocol was based on a previously published procedure (13) modified to increase the sensitivity of the assay. HCV RNA was detected by PCR (HCV AMPLICOR™, Roche Diagnostic systems, Inc., Branchburg, NJ, USA) and quantified by the branched DNA signal amplification test (b-DNA) (Quantiplex™ HCV RNA 2.0, Chiron diagnostics, Emeryville, CA, USA). Samples were prepared as a 3:10 dilution using 3 µL of serum and 7 µL of phosphate buffered saline in thin-walled PCR tubes. Tubes were incubated at 95°C for 4 minutes and chilled on ice for 10 minutes, prior to the addition of RT-PCR master mix (Promega, Madison, WI, USA). RT-PCR reactions were carried out in a total volume of 100 µL containing 1X Taq buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Promega), 20 pmol each of primer 1 (PSEA-HCV-1, 5′ HEX- AAG GAC CCG GTC GTC CT 3′; Sigma-Genosys, Woodlands, TX, USA) and primer 2 (PSEA-HCV-2, 5′ FAM-TAT CCA AGA AAG GAC CCA 3′; Sigma-Genosys), 20 units of ribonuclease inhibitor (RNasin; Promega), 10 units of MV Reverse Transcriptase (RT; Promega), and 2.5 units of Taq DNA polymerase (Roche Diagnostic Systems). Master mix (90 µL) (Promega) was added to each sample and the mixture was incubated at 42°C for 30 minutes and at 95°C for 4 minutes followed immediately by 35 cycles at the following conditions: 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute, and a final cycle of 72°C for 10 minutes. The second PCR, using the inner primer 3 (PSEA-HCV-3, 5′ FAM-CAA CAC TAC TCG GCT AGT 3′; Sigma-Genosys) and primer 4 (PSEA-HCV-4, 5′ HEX- CAT GGC GTT AGT ATG AGT GTT 3′; Sigma-Genosys), was performed by transferring 10 µL from the initial reaction to 90 µL of master mix (1X Taq buffer, 0.2 mM dNTPs, 20 pmol of each nested primer, and 2.5 units of Taq polymerase). The samples were incubated for 35 cycles as in step 2 without the RT step. The PCR products were analyzed on 3% agarose in 0.5X TBE buffer.

The primers were derived from the highly conserved 5′-untranslated region of the HCV genome to allow nested amplification of a 237-base pair product. Negative samples were retested for PCR after RNA extraction using the QIAamp Viral RNA kit (catalog No. 52906, Qiagen, Hilden, Germany). The sensitivity of the assay was 50 IU/mL according to the manufacturer's information. Despite

its greater sensitivity, an in-house nested RT-PCR invites problems with contamination and should be used with extreme care in the clinical setting.

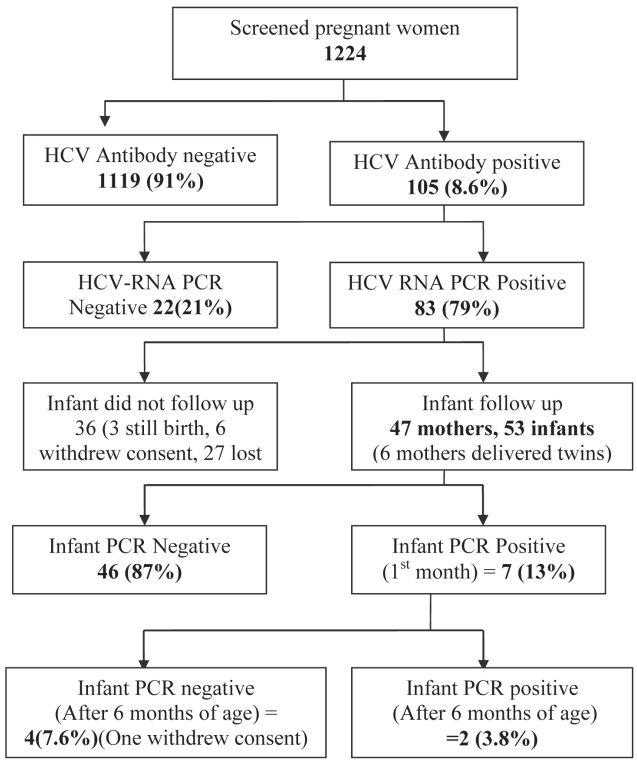
Statistical analysis

The sample size for this study was calculated according to the primary aim (assess the prevalence of HCV among pregnant women). Demographic and laboratory data were compared for the infected pregnant women with non-infected group using χ^2 and Fischer exact tests for categorical or dichotomous variables, and the unpaired independent *t*-test for continuous variables. Differences were considered significant for *P* values of 0.05 or less. Significant parameters were included in a multivariate logistic regression analysis to identify independent predictors of HCV positivity among pregnant women, and the odds ratio was calculated for each significant parameter. Rate of vertical transmission was calculated based on the frequencies of HCV-RNA PCR positivity in mothers and their infants.

RESULTS

Design and follow up are shown in Figure 1. The study was carried out among 1224 pregnant women (mean age, 25.3 ± 5.1 years; range, 16-45 years) who came for delivery to the obstetric emergency department at Benha University Hospital. Pregnant women with metabolic or viral diseases other than hepatitis C (114 women) and women who gave birth more than once during the study period (67 women) were not included in the study. Of 1224 pregnant women, 105 (8.6%) were positive for HCV antibodies. Only 83 (6.8%) were positive for HCV-RNA by PCR, corresponding to 79% of women positive for HCV antibodies. Table 1 shows the results of univariate analysis of risk factors for HCV infection among pregnant women. There was an association between HCV infection and the following variables: older age ($P < 0.001$), history of blood transfusion ($P = 0.016$), marriage lasting more than 3 years ($P < 0.001$), a former or current husband with HCV ($P = 0.014$), other household members infected with HCV ($P = 0.019$), and parity of more than two ($P < 0.001$). Although the proportion of HCV-posi-

Figure 1.



Design and follow up of the study. Abbreviations: HCV – hepatitis C virus; PCR – polymerase chain reaction.

tive women living in rural areas (72 of 83, 87%) was slightly higher than that of uninfected women (919 of 1141, 80%), this difference was not significant ($P=0.16$).

Table 2 shows the results of multivariate analysis. Independent risk factors for being positive for HCV-RNA by PCR were the following: older age ($P=0.001$), history of blood transfusion ($P=0.016$), a former or current husband infected with HCV ($P=0.014$), and other household members positive for HCV ($P=0.019$).

Out of the 83 infected mothers, samples of only 47 mothers and 53 children (6 mothers delivered twins) were available. Of these 83 infected mothers, 3 (3.5%) mothers had still births, 27 (33%) were lost to follow-up, 6 (7%) withdrew consent, and 6 (7%) had twins. Out of the 53 infants tested in the first month of life, 43 (81%) were positive for HCV antibodies, and 10 (19%) were negative. The latter group was considered as non-infected infants without maternally-acquired antibodies. Of the 43 infants positive for HCV antibodies, only 7 (13%) were found positive for HCV-RNA by PCR; these were considered to be HCV-infected infants. HCV-RNA was not detected in 36 of 43 (87%) of infants born to HCV-RNA positive mothers; this group was considered as non-infected infants with maternally-acquired antibodies. At 6 months of age, 6 children who had been found positive for HCV-RNA by PCR were examined again; one mother withdrew consent for her children. Only two of the 53 (3.8%) remained positive for HCV-RNA; this pair was considered to show persistent HCV infection. The other 4 infants (7.6%) cleared their HCV-RNA and sero-reverted to become negative for HCV antibodies; this group was considered to show clearance of perinatal HCV infection.

Patients lost to follow-up did not show significant demographic differences in comparison with patients who continued to participate. Loss of follow-up in infants who were tested during the first month was not high (14.2%).

TABLE 1. Univariate analysis for risk factors for HCV infection among pregnant women

	Pregnant women		
Parameters	negative for HCV (n=1141)	positive for HCV (n=83)	*P**
Age (years, mean±SD)	25.1±4.8	29.5±6.1	<0.001
Rural residency, No. (%)	919 (81)	72 (87)	0.16
Blood transfusion, No. (%)	45 (3.9)	12 (15)	<0.001
Hospitalization, No. (%)	317 (28)	25 (30)	0.6
Major operation, No. (%)	319 (28)	24 (29)	0.9
Major accidents, No. (%)	9 (0.8)	1 (1.2)	0.7
Schistosomiasis history, No. (%)	22 (1.9)	3 (3.6)	0.3
Other household members who are HCV-positive, No. (%)	7 (0.6)	4 (4.8)	<0.001
HCV positive husband, No. (%)	90 (7.9)	13(16)	0.01
Parity >2, No. (%)	290 (25)	41 (49)	<0.001
Duration of marriage in years (mean±SD)	4.4±4.4	7.4±6.7	<0.001
Number of pregnancies, No. (%)	2±1.3	3.3±3.5	<0.001
Alanine transaminase (international units, mean±SD)	18±19	21±14	0.2

*Pearson χ^2 test and Fisher exact test. SD – standard deviation.

TABLE 2. Multivariate logistic regression analysis for risk factors with HCV infection among pregnant women

Parameter	Odds ratio (95% confidence interval)	*P*
Age (years)	1.16 (1.1-1.2)	0.001
HCV-positive husband	5.47 (1.4-21)	0.014
Blood transfusion	2.69 (1.2- 6.0)	0.016
Other household members who are HCV positive	2.29 (1.2-4.6)	0.019

DISCUSSION

Our study showed that the risk factors for HCV infection among pregnant women in an Egypt regions were older age, HCV-positive husband, administration of blood transfusion, and HCV-positive other member of the household members. Egypt is considered one of the countries with the highest prevalence of HCV in the world (5), and a country with a high prevalence among children. There some reports claim that vertical transmission is higher in Egypt than in other countries (11), although these studies show some limitations in sample size and diagnostic method for determining vertical transmission. In the present study, we aimed to assess the prevalence and risk factors of HCV among pregnant women in a small city in the Nile Delta, Egypt. We also sought to determine whether vertical transmission plays a major role in HCV endemicity in Egypt.

Prevalence of HCV among pregnant women

We found the prevalence of pregnant women positive for HCV-RNA to be 6.8%. This study included women ranging in age from 16 to 45 years, with a mean age of 25.3. The prevalence in this age group was lower than reported in Egypt before: 37.5% among those older than 30 years, with a marked increase among those in their thirties and forties, and a peak of over 60% among those in their sixties (5). Our lower prevalence may be explained by

a cohort phenomenon among patients treated by parenteral anti-schistosoma therapy (PAT) in Egypt from 1960s-1980s. Our study group contained a smaller proportion of PAT-treated cohort than did the studies conducted more than 10 years ago, which may explain the lower prevalence of HCV.

Our study also found a slightly higher rate of infection among women living in rural areas than in urban areas, though this difference was not significant. As reported by Frank et al (7), higher infection rates among older women and rural residents may be partially explained by the differential exposure of these groups to schistosomiasis campaigns in Egypt, and the use of contaminated needles or syringes during treatment campaigns, suggesting that parenteral exposure continues to be a major transmission route for HCV infection in Egypt (7).

Evidence suggests that our data indeed reflect a decrease in HCV prevalence. Our patients came from the same areas as in the studies reporting higher prevalence of HCV (5). In addition, other studies published recently have shown a decrease in HCV prevalence in Egypt (14). Although the prevalence of HCV in Egypt appears to have decreased, our prevalence of 6.8% is still higher than in other countries such as the USA (3.2%), Taiwan (1.5%), Zaire (6%), and Saudi Arabia (0.6%) (2,15,16). Risk factors for this high prevalence should be studied, especially the avoidable ones.

Risk factors for HCV infection

Although in this study there were many factors associated with HCV infection in univariate analysis, multivariate analysis found only 4 independent risk factors. Old age was the first independent factor, which suggests the same cohort phenomenon described above and the cumulative effect of exposure to HCV due to the long period of viral exposure over one's lifetime, as well as exposure to other potential HCV risk factors. Our results are in agreement with Costa et al (17), who indicated that HCV is associated with older age and not associated with ethnicity or greater number of pregnancies. Also, a previous community-based study in Egypt has found that older age patients have a higher prevalence of HCV (5,18).

Further independent risk factors were having a husband or another household member positive for HCV. This association suggests that the significance of intrafamilial transmission of HCV is comparable with that of sexual transmission. Intra-familial transmission of HCV has also been reported by Mohamed et al (8).

Another risk factor was blood transfusion. Several patients in our group had received blood transfusions before blood donors in Egypt underwent routine screening for HCV. These patients also had other risk factors, like hospitalization and major operations. Although blood transfusion is now considered a less important risk factor, it should be considered carefully, especially in a country with such a high prevalence of the disease. Our results are in agreement with those of Sangha et al (19), who found that a history of surgery, blood transfusion, or injection to treat schistosomiasis increased the risk of active infection with hepatitis C, independent of the other characteristics.

We did not test for other hepatitis viruses or for HIV, which is exceedingly rare in rural Egyptian communities. A UNAIDS/WHO report from 2008 (20) estimated that HIV/AIDS was present in 9000 Egyptians, predominately men with high risk behaviors, who account for less than 0.1% of the total population. No HIV-positive pregnant women were reported during sentinel site surveillance outside of "major urban areas" from 1992 to 1996 and in 2004.

Mother-to-infant transmission for HCV

We found that only 13% of the 43 infants who were positive for HCV antibodies in the first month of life were also positive for HCV-RNA. We considered this group as HCV-infected infants. This result is compatible with previous studies, which have found that most infants born to HCV-positive mothers have HCV antibodies in their blood and that we cannot use the presence of these antibodies to diagnose vertical transmission until after 18 months (21). Although 43 of 53 (81%) infants had HCV antibodies in the first month of life, only 7 (13%) were positive for HCV-RNA at the same time. At 6 months of life, only 2 (3.8%) were positive for HCV-RNA, indicating persistent HCV infection, while the other 4 infants had cleared their HCV-RNA indicating clearance of perinatal HCV infection. A similar figure for vertical transmission of HCV (4.6%) was reported recently from Egypt at one year of age (12). These results show that a large proportion of infants were only temporarily positive for HCV-RNA during the first weeks of life and the PCR test should be repeated again at 6 months of life. Studies that do not test infants when they are older may lead to overestimates of HCV prevalence and this may be the case with community-based study of perina-

tal HCV transmission in 3 rural Egyptian villages, where the overall HCV prevalence of more than 20% was found (5,22-24).

Frequent clearances of perinatal HCV infection may explain the previous reports of a high incidence of vertical transmission in Egypt. These reports have relied on cord blood samples or PCR results taken only once within a few weeks after delivery (11). Together with previous studies, the present study confirms that the incidence of vertical transmission of HCV in Egypt is similar to that in other parts of the world, where it varies from 4.5% to 6.0% (10,25-27), and that vertical transmission does not play a major role in the high endemicity of HCV in Egypt.

Our definition of perinatal transmission of HCV was that infants had to be positive for both anti-HCV antibodies and HCV RNA. HCV-RNA was not detected in 36 of 43 (87%) of infants born to HCV-RNA positive mothers; this group was considered as non-infected infants with maternally-acquired antibodies. Only 2 of the 53 infants (3.8%) remained positive for HCV-RNA; this pair was considered to show persistent HCV infection. The other 4 infants (7.6%) cleared their HCV-RNA and sero-reverted to become negative for HCV antibodies; this group was considered to show clearance of perinatal HCV infection. Consistent with our approach, the European Pediatric HCV Network (EPHN) criterion for perinatal transmission of HCV in their multicenter trial of 1787 mother-child pairs was two or more positive HCV-RNA PCR test results and/or anti-HCV antibody positivity after 18 months of age (28,29). The EPHN trial did not include a transient infection category. Their study focused on risk factors for infection and did not show data about HCV-RNA positive children who did not meet their criteria for infection. However, they did describe 279 (16%) children classified as having "indeterminate infection status," who did not meet their criteria for either infected or none infected, and they classified the infants with fewer than two positive HCV-RNA results as non-infected (28). Members of the EPHN group subsequently reported on accuracy of HCV-RNA PCR testing for the diagnosis of vertically acquired HCV infections (30). Since they used the presence of anti-HCV at 18 months as their "gold standard" for mother-to-infant transmission, it is not surprising that they concluded the test was not reliable during the first few months of life. Sensitivity and positive predictive value of the PCR testing was poor, since children who had positive tests for HCV-RNA without persistence of HCV antibodies and RNA were considered to be false positives.

We suggest that a higher proportion of infants born to HCV-infected mothers have infections and then clear their infections than is generally reported. In our study, this proportion was 13.2% in infants whose mothers were HCV-RNA positive, but it may have been greater if we had sampled the infants earlier and more frequently. When Ketzinel-Gilad et al (31) did this, they detected transient HCV-RNA in 5 of 23 (22%) infants born to 22 HCV-infected women. Ceci et al (31) conducted a 2-year follow-up of 60 HCV-infected infants born to HCV-RNA-positive mothers and reported that 75% cleared their infections. Ruiz-Extremera et al (32) reported that 7 of 8 Spanish infants born to anti-HCV-positive mothers followed for an average of 29 months had detectable HCV-RNA and anti-HCV, both of which cleared. Others reported that 2 of 3 infants with perinatal HCV cleared their viremia by 2 months of age (33).

It is unlikely that the clearance observed in our study is due to cross-contamination or laboratory error leading to false-positive RT-PCR testing for RT-PCR results for HCV-RNA. Firstly, mothers were tested during the last trimester of pregnancy; in contrast, children were tested after they were born; thus, the samples were not processed in parallel, reducing the chance of cross-contamination. In addition, in most cases there was more than one positive blood sample; thus, anti-HCV antibody tests were performed in duplicate on most mothers and children. Lastly, none of the 1119 children born to mothers who did not have HCV antibodies had HCV infections.

Our study detected transient infections in 2 (9%) of 22 children whose mothers had HCV antibodies in the absence of RNA. Although most studies have reported transmission of HCV to infants only from mothers who had detectable HCV-RNA (31,34-37), a large multicenter study has reported 5 infections in infants from mothers who had only HCV antibodies (38). This may be due to fluctuating viremia that is undetectable at the time of testing.

For this reason, we used used the persistence of HCV antibodies for more than 18-24 months in the absence of detectable HCV-RNA as criterion for mother-to-infant transmission of HCV (37,39-41). Gibb et al (40) has reported that among infants born to 441 HCV-infected mothers, half of uninfected infants cleared their anti-HCV antibodies by 8 months and 95% were negative for anti-HCV antibodies at 13 months. Others have reported that anti-HCV antibodies transmitted passively, disappeared in infants by 6 or 8 months (42-44), and 4 cases who were in-

fected perinatally lost anti-HCV antibodies transferred passively from their mothers by 6-12 months before making their own anti-HCV antibodies (37). All these results suggest some infants with anti-HCV antibodies persisting longer than 12 months could be infected cases with temporarily undetectable viremia.

Our 4 transiently infected infants cleared their HCV-RNA and sero-reverted to become negative for HCV antibodies; this group was considered to show clearance of perinatal HCV infection. Others have reported that infants cleared their viruses without having persistent antibodies (33,42,43), which could have several explanations. One of these could be the passage of neutralizing antibodies along with low titers of viral particles from mothers to their newborns; these antibodies could clear the infection and either abort or diminish the child's antibody production (45).

The fact that children are more likely to clear HCV infections than adults is supported by the finding that only 8 of 33 children sampled in years 2 and/or 3 continued to be HCV-RNA positive (32). The 76% (25 of 33) HCV clearance rate was much greater than the 23% clearance rate among the children in the EPHN multicenter project (29). However, these authors had different criteria for classifying initial infection and only considered the clearance occurring in children who remained infected until their first birthday. Using their criteria, about half the children in this report with HCV-RNA cleared their infections by their third birthday.

Potential limitations of this study need to be mentioned. Although the prevalence of HCV was assessed in a large number of pregnant women, it cannot exactly apply to the general population of Egypt because the selected sample consisted solely of women of childbearing age from only one area of Egypt. However, Roberts and Yeung (21) reviewed 12 studies from different countries that included more than 3000 pregnant women and concluded that prevalence of HCV among pregnant women was not different from that of the age-matched general population (21). In addition, our results are consistent with those from previously published studies on the prevalence of HCV in Egypt from the same area that we studied; therefore, we believe that our results reliably indicate a change in the prevalence of HCV in Egypt. The sample size is inadequate for statistically identifying some potential risk factors for infant infection. The study was unable to show that most maternal or infant characteristics, including the infant's sex and source of nutrition, were a risk for infection. The effect of breast-feeding upon the infants' infection status has been unclear and variable (28,31,34,38,41), so further study is needed to address this. The second shortcoming is the high dropout rate and loss to follow-up in our groups, despite the precautions that we took to avoid the loss of patients. Patients who were lost to follow-up were not different from patients who continued to participate in the study regarding demographic features. Loss to follow-up in infants who were tested at the first month was not high (14.2%). The third shortcoming is that the relatively small sample size made the confidence interval of the estimated incidence of vertical transmission relatively wide. The short follow-up of our patients of merely 6 months is unsatisfactory; during the first year or two of life, serum HCV RNA may become positive, fluctuate between positive and negative, and in some cases seropositive cases can exhibit spontaneous clearance. Because serum HCV RNA levels fluctuate over time and longitudinal data have clearly shown that a baby initially negative for HCV-RNA may subsequently test positive, the 43 babies initially negative for HCV-RNA should have been retested – for HCV-RNA at 6 months and for anti-HCV antibodies at 12-18 months.

In conclusion, the prevalence of HCV in Egypt is lower than previously reported. Risk factors for vertical transmission suggest that intra-familial transmission is an important concern, and further studies are needed to explore this issue further. Incidence of vertical transmission of HCV in Egypt is not different than in other countries and it plays no role in the high endemicity in Egypt.

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