TRANSMISSION OF HEPATITIS C VIRUS BETWEEN PARENTS AND CHILDREN

MOSTAFA K. MOHAMED, LAURENCE S. MAGDER, MOHAMED ABDEL-HAMID, MAY EL-DALY, NABIEL N. MIKHAIL, FATMA ABDEL-AZIZ, AHMED MEDHAT, VALERIE THIERS, AND G. THOMAS STRICKLAND*  
National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt; Department of Community Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt; Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland; South Egypt Cancer Institute and Faculty of Medicine, Assiut University, Assiut, Egypt; Center for Field and Applied Research, Qalyub, Egypt; Centre National de la Recherche Hépatites Virales B et C, Laboratoire Mixte Pasteur-Necker, Faculté de Necker, Faculté de Necker, Paris, France

Abstract. Egyptian children with infected parents are at high risk of infection with hepatitis C (HCV). Analysis of data collected during surveys of rural communities show children whose parents had antibodies to HCV (anti-HCV) were at higher risk for having anti-HCV than children whose parents did not. The association was greater with mothers than fathers and when the parent had HCV RNA. For instance, 87 (14%) of 612 children had anti-HCV whose mothers had HCV RNA compared with 28 (7%) of 401 whose mothers only had anti-HCV and 79 (2.6%) of 3,086 whose mothers were seronegative. These associations persisted after controlling for age, parenteral exposures, and serologic status of the other parent. Sequencing isolates from 13 families with parent(s) and children having HCV RNA showed 10 of 18 had genetically similar viruses. These findings suggest Egyptian children are at high risk of being infected with HCV by their parents and identification of the transmission routes would allow for preventive measures.

INTRODUCTION

Prevalence of antibody to hepatitis C virus (anti-HCV) in rural Egypt is among the highest in the world, with rates in many communities in the range of 15% to 30%.1–4 Although mass campaigns to control schistosomiasis that took place decades earlier may have been responsible for much of current seroprevalence,5,6 it is also high in children too young to have been involved in those campaigns. Among these infected children, the source and route of transmission is generally unknown.7–9 Recently, we reported results suggesting one source of infection is from the child’s parents, either directly through household contact or indirectly through shared needles or other possible fomites.10

To further explore this hypothesis, we analyzed cross-sectional data from large serologic surveys performed in two communities in Egypt. Our results confirm that HCV is frequently transmitted between infected parents and their children.

MATERIALS AND METHODS

Community surveys. In 1997 we conducted surveys in villages in the Nile Delta (Lower Egypt) and in the Assiut Governorate (Upper Egypt) after obtaining approval from the Institutional Review Boards of the Egyptian Ministry of Health and Population, Assiut University, and the University of Maryland.2,5,7,8 Each community had a population of approximately 11,000 inhabitants. The Nile Delta community was mapped and a systematic sample of half of the households was selected for the survey. In the Upper Egyptian community, all households were selected for the survey. Sampled households were visited and residents were invited to participate. Those who provided informed consent were interviewed with a structured questionnaire to identify potential exposures that might be related to HCV acquisition. Household participation was 70% among those targeted in the Nile Delta community, and 87% of households participated in the Upper Egyptian village. Subjects ≥ 5 years of age provided a serum sample for analysis for anti-HCV and HCV RNA. This report is based upon information provided during the surveys, anti-HCV and HCV RNA results from 5–18-year-old children and their parents, and the virus sequences from members of 13 households.

Laboratory methods. All samples were tested for anti-HCV by a second-generation enzyme immunoassay (ELA) for anti-HCV (Abbott HCV EIA 2.0; Abbott Laboratories, Chicago, IL) according to the manufacturer’s instructions. Seropositivity for HCV was defined by a ratio of optical density to the cutoff value ≥ 1. Samples with a ratio between 0.8 and 1.0 (gray zone) were retested in duplicate, and those repeatedly > 1.0 were considered positive. HCV RNA was assessed in the ELA-seropositive samples using a direct reverse transcription–polymerase chain reaction (PCR) method as previously described.11 Briefly, HCV RNA was amplified by nested PCR directly from the serum using primers specific for the 5’ untranslated region.12

Phylogenetic analysis of HCV. A phylogenetic analysis of HCV sequences was performed to determine if HCV RNA–positive children and their family members were infected with closely related strains. Partial amplification of NS5B gene (330 nucleotides) was performed as described elsewhere.13 Amplified fragments were purified, and bidirectional sequence analysis was performed. Sequence alignment and phylogenetic analysis were performed using the neighbor joining program in the Mega package (Bootstrap support 1,000 random resamplings of the sequences).14

Statistical methods. Contingency tables were used to compare the anti-HCV seroprevalence in subgroups of children defined by community, age, sex, community exposures, and parental HCV status. The association between a predictor and seroprevalence while controlling for other variables was estimated by logistic regression. In all analyses, P values were based on the generalized estimating equation (GEE) approach15 to appropriately account for the correlation between multiple children from the same household, using the generalized score statistics provided in Proc Genmod (SAS Institute, Cary, NC).16
### RESULTS

**Study sample.** Questionnaire data were collected from 5,792 children 5–18 years of age. Serologic results were available for 4,631 (80%) of these children. The percentages providing sera were 77% in the Nile Delta and 82% in Upper Egyptian communities. Because it was more difficult to obtain blood from younger children, the proportion among 5–9-year-old children was only 69%.

**Seroprevalence associations with demographic and exposure characteristics.** The prevalence of anti-HCV in the 5–18-year-old children was 8.2% in the Nile Delta community, and 2.5% in the Upper Egypt community (Table 1). It did not differ by sex, but it increased by age in both communities: from 3.5% among those 5–9 years of age to 6.3% among those 15–18 years of age. After adjusting for age, circumcision by traditional health practitioners was significantly associated with anti-HCV for males ($P = 0.004$) but not for females ($P = 0.41$). A history of an invasive hospital procedure (i.e., surgery, urinary or venous catheterization, blood transfusion, or endoscopy) was also associated with a higher risk of anti-HCV ($P = 0.02$). Each village had similar trends. However, the Nile Delta village had a higher overall prevalence of anti-HCV.

**Seroprevalence associations with parental HCV status.** The HCV status (anti-HCV and HCV RNA) of at least one parent was known for 92% of the 4,631 children. It was known for both parents for 2,562 children (55%), for the mother only for 1,537 children (33%), for the father only for 181 children (4%), and for 351 children (8%), the HCV status of both parents was unknown.

The proportion of children with anti-HCV was significantly related to the HCV status of both mothers and fathers (Table 2). For each parent and for each village, the children’s seropreva-

### Table 1

<table>
<thead>
<tr>
<th>Demographic variables and risk factors</th>
<th>Overall</th>
<th>Nile Delta village</th>
<th>Upper Egypt village</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion (%) positive</td>
<td>$P^*$</td>
<td>Proportion (%) positive</td>
</tr>
<tr>
<td>Total sample</td>
<td>220/4,631 (4.8)</td>
<td>–</td>
<td>149/1,823 (8.2)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>5–9</td>
<td>51/1,458 (3.5)</td>
<td></td>
<td>38/600 (6.3)</td>
</tr>
<tr>
<td>10–14</td>
<td>92/1,942 (4.7)</td>
<td></td>
<td>60/755 (7.9)</td>
</tr>
<tr>
<td>15–18</td>
<td>77/1,231 (6.3)</td>
<td></td>
<td>51/468 (10.9)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>118/2,311 (5.1)</td>
<td></td>
<td>80/926 (8.6)</td>
</tr>
<tr>
<td>Female</td>
<td>102/2,320 (4.4)</td>
<td></td>
<td>69/897 (7.7)</td>
</tr>
<tr>
<td>Circumcision (males)</td>
<td></td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6/206 (2.9)</td>
<td></td>
<td>1/45 (2.2)</td>
</tr>
<tr>
<td>Traditional</td>
<td>86/1,275 (6.8)</td>
<td></td>
<td>63/630 (10.0)</td>
</tr>
<tr>
<td>Health professional</td>
<td>26/826 (3.2)</td>
<td></td>
<td>16/251 (6.4)</td>
</tr>
<tr>
<td>Circumcision (females)</td>
<td></td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>27/780 (3.5)</td>
<td></td>
<td>24/370 (6.5)</td>
</tr>
<tr>
<td>Traditional</td>
<td>71/1,399 (5.1)</td>
<td></td>
<td>43/495 (8.7)</td>
</tr>
<tr>
<td>Health professional</td>
<td>4/139 (2.9)</td>
<td></td>
<td>2/32 (6.3)</td>
</tr>
<tr>
<td>Invasive medical procedure</td>
<td></td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>187/4,172 (4.5)</td>
<td></td>
<td>136/1,684 (8.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>33/459 (7.2)</td>
<td></td>
<td>13/139 (9.4)</td>
</tr>
</tbody>
</table>

$^a P$ values (with the exception of the $P$ values for age group) are based on a model that adjusts for age and is fit using generalized estimating equations.

### Table 2

<table>
<thead>
<tr>
<th>Parent’s HCV status</th>
<th>Overall</th>
<th>Nile Delta</th>
<th>Upper Egypt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion (%) positive</td>
<td>$P^*$</td>
<td>Proportion (%) positive</td>
</tr>
<tr>
<td>Fathers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>38/1,537 (2.5)</td>
<td></td>
<td>18/425 (4.2)</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>29/430 (6.7)</td>
<td></td>
<td>21/222 (9.5)</td>
</tr>
<tr>
<td>RNA positive</td>
<td>72/776 (9.3)</td>
<td></td>
<td>56/418 (13.4)</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>79/3,086 (2.6)</td>
<td></td>
<td>42/923 (4.6)</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>28/401 (7.0)</td>
<td></td>
<td>23/284 (8.1)</td>
</tr>
<tr>
<td>RNA positive</td>
<td>87/612 (14.2)</td>
<td></td>
<td>67/419 (16.0)</td>
</tr>
<tr>
<td>Fathers (with anti-HCV-negative mothers)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>15/1,178 (1.3)</td>
<td></td>
<td>7/250 (2.8)</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>10/258 (3.9)</td>
<td></td>
<td>6/106 (5.7)</td>
</tr>
<tr>
<td>RNA positive</td>
<td>26/462 (5.6)</td>
<td></td>
<td>16/183 (8.7)</td>
</tr>
<tr>
<td>Mothers (with anti-HCV-negative fathers)</td>
<td></td>
<td>0.0066</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>15/1,178 (1.3)</td>
<td></td>
<td>7/250 (2.8)</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>7/124 (5.7)</td>
<td></td>
<td>5/79 (6.3)</td>
</tr>
<tr>
<td>RNA positive</td>
<td>14/130 (10.8)</td>
<td></td>
<td>6/76 (7.9)</td>
</tr>
</tbody>
</table>

$^a P$ values are based on a model that adjusts for age and is fit using generalized estimating equations.
ence was highest for those with parents having HCV RNA, and lowest when the parent(s) did not have anti-HCV. In the Nile Delta village, children with HCV RNA–positive fathers had a higher risk of having anti-HCV (13.4%) than those with anti-HCV–negative fathers (4.2%). The association with maternal HCV status was even higher: 16% and 4.6% for children of mothers with HCV RNA and without anti-HCV, respectively. This same three-fold increased level of risk for HCV infection was also present in children from the Upper Egypt village (Table 2). The association between a father’s HCV status and his child’s seroprevalence persisted in the subgroup of children with anti-HCV negative mothers and vice versa.

Multivariable analysis results. Table 3 shows the results of a logistic regression model to assess the association between parental HCV status and their children’s seroprevalence. This model controlled for the HCV status of the other parent as well as age, community, circumcision, and exposure to invasive hospital exposures. These results are only based on the subset of children with information about the HCV status of both parents. After controlling for the other variables, both maternal and paternal HCV status was strongly and independently associated with anti-HCV in the child. The age adjusted odds ratio estimate was 2.5 (95% confidence interval [CI] = 1.5–4.2) for HCV RNA–positive fathers compared with anti-HCV–negative fathers. For HCV RNA–positive mothers, the age adjusted relative risk estimate was 3.9 (95% CI = 2.2–6.9; Table 3).

Genetic sequence analysis. The HCV RNA isolates were sequenced from members of 13 families having HCV RNA–positive children. Of the 18 HCV RNA–positive children in these families, 10 (56%) had an HCV genetic sequence that matched the sequence of HCV in one or more parent. Four families had multiple HCV RNA–positive children. In one of these families, three children had sequences that matched each other and their mother. In two families there were two HCV RNA–positive children whose sequences did not match each other; however, one of these matched the mother’s sequence. In the fourth family the two children and the father all were positive but had different sequences.

### DISCUSSION

We observed a strong association between the prevalence of anti-HCV in rural Egyptian children and HCV status of their parents. These results have been corroborated by our follow-up study in these communities in which we found that the strongest risk factor for incident infection in children in these communities was the parent’s serologic status. Boys, who are often traditionally circumcised as small children in groups by non-medically trained persons during ceremonies in rural villages, were at increased risk for HCV infection. However, girls, who are traditionally circumcised individually when older, showed no increased risk.

Several mechanisms could explain an association between a parent and child’s HCV infection status. First, vertical transmission or transmission during breast-feeding could account for some concordance between a mother and child, as well as indirectly between a father and child (if the mother and father’s isolates are concordant). Several recent studies estimated rates of perinatal transmission of HCV ranging from 2% to 8% among women not coinfected with human immunodeficiency virus. However, the strong associations between fathers’ and children’s HCV status we observed even when the mother was anti-HCV negative, suggests our observed parent-child associations are not totally explained by neonatal transmission from the mother or from breast-feeding.

A second possible explanation for an observed association between a parent and child’s HCV status is that particular community exposures are experienced in common by a family. However, if this were the total explanation, we would not expect the association to vary depending on whether the parents were viremic. The fact that we observed highest seroprevalence among children whose parents had HCV RNA is more consistent with person-to-person transmission.

A third possible explanation for the observed concordance is confounding by age. This could occur because seroprevalence increases by age and older parents are more likely to have older children. However, our observed association persists after controlling for age, which shows that it is not totally due to the confounding of age.

Given that none of these possible explanations can totally account for the high risk of anti-HCV in children whose parents have antibodies to HCV and/or HCV RNA, our observations suggest a fourth possible explanation: concordance could be the result of person-to-person transmission, either within the household, sharing of needles in a health care setting, or through other possible settings. This explanation is further supported by the fact that the genetic sequence of the virus isolated from 10 of 18 children matched the sequence of a virus found in one of the parents.

Some previous studies of household spread of HCV have arrived at differing conclusions regarding the possibility, and importance, of household spread. Most of these report small

### Table 3

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>1.0 (Reference group)</td>
<td>0.0036</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>1.8 (0.9, 3.4)</td>
<td></td>
</tr>
<tr>
<td>RNA positive</td>
<td>2.5 (1.5, 4.2)</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HCV negative</td>
<td>1.0 (Reference group)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Anti-HCV positive, RNA negative</td>
<td>1.9 (1.0, 3.9)</td>
<td></td>
</tr>
<tr>
<td>RNA positive</td>
<td>3.9 (2.2, 6.9)</td>
<td></td>
</tr>
<tr>
<td>Exposure to invasive hospital procedure</td>
<td>1.3 (0.8, 2.31)</td>
<td>0.35</td>
</tr>
<tr>
<td>Age (per five years)</td>
<td>1.2 (0.9, 1.6)</td>
<td>0.23</td>
</tr>
<tr>
<td>Circumcision among males</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>None</td>
<td>1.0 (Reference group)</td>
<td></td>
</tr>
<tr>
<td>Traditional provider</td>
<td>1.3 (0.7, 2.4)</td>
<td></td>
</tr>
<tr>
<td>Trained health professional</td>
<td>0.7 (0.4, 1.5)</td>
<td></td>
</tr>
<tr>
<td>Circumcision among females</td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>None</td>
<td>1.0 (Reference group)</td>
<td></td>
</tr>
<tr>
<td>Traditional provider</td>
<td>1.1 (0.6, 2.1)</td>
<td></td>
</tr>
<tr>
<td>Trained health professional</td>
<td>0.8 (0.2, 3.1)</td>
<td></td>
</tr>
<tr>
<td>Community</td>
<td></td>
<td>0.052</td>
</tr>
<tr>
<td>Upper Egypt</td>
<td>1.0 (Reference group)</td>
<td></td>
</tr>
<tr>
<td>Nile Delta</td>
<td>1.7 (1.0, 2.9)</td>
<td></td>
</tr>
</tbody>
</table>
rates of household spread.21–27 Napoli and others found that among family contacts of patients chronically infected with HCV, excluding spouses, 5 of 76 were anti-HCV positive, in comparison with to 0 of 45 among members of control families.22 They concluded intrafamilial transmission is an important route of HCV infection. In contrast, based upon finding that only 8 (3.3%) of 250 family contacts of anti-HCV–positive patients were positive, in comparison with 3 (1.8%) of 170 among control families, Kim and others concluded that familial transmission “if it occurs is rare.”23 However, their reported two-fold increase in rate of anti-HCV in family members of anti-HCV–positive persons is compatible with household transmission.

This high risk of HCV infection in children living in rural Egyptian communities having a very high reservoir of infection may not be externally valid to other developed and less developed countries. The minimal published data as noted above on intrafamilial transmission reflects upon the difficulty in conducting these prospective community-based studies in other sites where population prevalence of HCV is less. In our opinion, with the exception of transmission risks that are low or absent in our communities, e.g., intravenous drug abuse, promiscuous sexual activity, and similar household behaviors, habits and activities are present in many developing countries.

The plausibility of household transmission by other than parenteral means is supported by studies showing that HCV RNA has often been detected in saliva of patients with HCV RNA in serum, even in some patients without circulating HCV RNA.28–30 Pooling studies in a systematic review, Ackerman and others reported that HCV RNA was found in the saliva of 79 (47%) of 168 patients with circulating HCV RNA and in 7 (7%) of 54 of patients with anti-HCV without circulating RNA.31 HCV RNA has also been reported in semen, breast milk, vaginal fluids, and urine.31 Transmission might also occur during a common exposure from outside the household. All children in this study were too young to have been infected during the mass treatment campaigns to control schistosomiasis that transmitted HCV to a large proportion of the rural population of Egypt.4 A common parental exposure experienced by members of these communities is injections by formal or traditional health care providers.7,8 We have been informed that some health care providers reuse needles and multiple members of a family receive injections from the same needle; this could explain some of the concordances within the family.

In summary, 1 in every 6–7.5 children in the Nile Delta village whose mothers or fathers had HCV RNA had been infected with HCV. Children whose parents had HCV RNA had increased odds of infection to 4.9 and 2.7 times that of children whose mothers and fathers did not have anti-HCV. Our data does not specifically define how the transmission is occurring, but HCV sequencing suggests that more than half of infections in these children are transmitted from their parents. We suspect some infections may be transmitted by non-parenteral familial contacts that involve inconsiderate exposures to contaminated body fluids and blood. These children having parents infected with HCV are at high risk of HCV infection. It is very important to focus preventive strategies upon them and they would particularly benefit from an HCV vaccine when one becomes available.

Received January 31, 2006. Accepted for publication March 22, 2006.

Acknowledgments: We thank the field teams from the Center for Field and Applied Research and the Department of Tropical Medicine at Assuit University who collected the data and blood samples; the technologists in the Viral Hepatitis Reference Laboratory who performed the laboratory tests; the members of the Data Management Team at the National Hepatology and Tropical Medicine Research Institute who managed the data; and Professor Alaa Ismail (Dean of the National Hepatology and Tropical Medicine Research Institute) and Mar Jan Ostrowski and his administrative staff who supported these efforts.

Financial support: This research was supported by United States Agency for International Development grant 263-G-00-96-00043-00, Wellcome Trust-Burroughs Wellcome Fund grants 059113/Z/99/A (Egypt) and 059113/Z/99/Z (USA), and National Institute of Allergy and Infectious Diseases/National Institute of Child Health and Human Development International Collaborations in Infectious Disease Research grant U01-AI058372.

Authors’ addresses: Mostafa K. Mohamed, Department of Community Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt, Telephone: 20-2-483-7888, Fax: 20-2-486-3822, E-mail: ecge@internetegypt.com. Laurence S. Magder and G. Thomas Strickland, Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, Telephone: 410-706-5253 and 410-706-7550, Fax: 410-706-8380 and 410-706-8013, E-mails: lmagder@epi.umaryland.edu and tstrick@epi.umaryland.edu. Mohamed Abdel-Hamid and May El-Daly, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt, Telephone: 20-2-368-6275, Fax: 20-2-368-2774, E-mail: heplab@link.com.eg. Nabil M. Mikhail, South Egypt Cancer Institute, Assiut University, Assiut, Egypt, Telephone: 20-2-368-6275, Fax: 20-2-368-2774, E-mail: nabilm@hcpp-egypt.com. Fatma Abdel-Aziz, Center for Field and Applied Research, Qalyub, Egypt, Telephone: 20-2-540-5129, Fax: 20-2-541-9403, E-mail: hpcfar@umegypt.com. Ahmed Medhat, Department of Tropical Medicine and Hepatology, Faculty of Medicine, Assiut University, Assiut, Egypt, Telephone: 20-2-368-6275, Fax: 20-2-368-2774, E-mail: a_medhat_nasr@yahoo.com. Valerie Thiers, Centre National de la Recherche Hepatitiques and Infectious Diseases, Laboratoire Mixte Pasteur-Necker, Faculté de Medicine, Paris, France, Telephone: 33-1-40-61-55-44, Fax: 33-1-40-61-55-81, E-mail: vthieres@pasteur.fr.

Reprint requests: G. Thomas Strickland, International Health Division, Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, 660 W. Redwood Street, Baltimore, MD 21201, E-mail: tstrick@epi.umaryland.edu.

REFERENCES


