Prevalence of hepatitis C Antibody in Human Immunodeficiency Virus infected children

Abstract: Background: Hepatitis C virus (HCV) is a major public health problem for Human Immunodeficiency virus (HIV) infected population. Both infections share same routes of transmission, and quite often co-exist, with dual infections associated with reciprocal and mutually more rapid progression than either infection alone. Co-infection also adversely impacts on the course and management of both infections. This study was carried out to document the prevalence and determinants of HCV sero-positivity in HIV-infected children.

Methodology: A total of 132 HIV-infected children attending the Paediatric Antiretroviral Clinic were recruited as subjects. Another 132 HIV negative children matched for age and sex were recruited as controls. Relevant demographic data was taken from each child. Blood samples were also obtained from each child and from their mothers when available, and assayed for the presence of anti-HCV using a membrane-based immune-assay kit.

Results: The sero-prevalence of HCV antibodies was 9.8% among HIV-infected children and 3.0% among the controls. This was a statistically significant difference (p = 0.042, Fisher exact). HCV sero-positivity was more frequent in children after 5 years of age in both subjects (92.3%) and controls (100.0%). Injection at patent medicine vendor (PMV) was noted to be the most risky practice leading to HCV in children, with more than thrice the chances of HCV sero-positivity than in those who didn’t receive injections at PMV. Four mothers of the HIV-infected children were co-infected with HCV and none in the control group. All 4 children of these dually infected mothers were also co-infected. Controlling for other factors, children of HIV infected mothers were more than twice as likely to have HCV antibody as those whose mothers were HIV negative (RR = 2.67). Similarly, HCV infected mothers have 12% greater chance of transmitting HCV to their children than non-infected mothers and children delivered vaginally were 1.6 times more likely to have HCV antibody than those delivered via caesarean section.

Conclusions: The prevalence of anti-HCV in HIV-infected children is significantly higher than that of HIV uninfected peers. Factors strongly associated with HCV sero-positivity identified are maternal HIV and HCV infections, vaginal delivery and injections at patent medicine vendor.

Key words: HCV; HIV; children

Introduction

Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) co-infection is a growing public health concern.\(^1\) It has gained importance with decreasing AIDS-related morbidity and mortality due to HIV treatment. HCV/HIV co-infection is associated with higher HCV viral load and a more rapid progression of HCV-related liver disease, leading to an increased risk of cirrhosis and hepatocellular carcinoma (HCC).\(^2,3\) HCV infection acts as an opportunistic disease in HIV-infected persons because of the increased incidence and accelerated natural history in co-infected persons.\(^4\) HCV infection may also impact the course and management of HIV disease, particularly by increasing the risk of antiretroviral drug-induced hepatotoxicity.\(^5,6\) It is estimated that four to five million persons are co-infected with HIV and HCV worldwide.\(^7\) According to
the World Health Organization (WHO), sub-Saharan Africa has the highest prevalence of both infections,\(^5\) being home to almost two thirds (63\%) of all persons infected with HIV and the majority of the 2.3 million children living with HIV worldwide\(^9\) and having estimated 5.3\% average prevalence for HCV.\(^10\)

Nigeria, with an overall HIV prevalence of 4.4\%\(^11\) in 2006 and also appears to have a high incidence of HCV infection with estimated prevalence rates of up to 14\%.\(^12\) This suggests a very high burden of co-infection with both viruses and makes the country a potential source of an emerging epidemic of HIV/HCV co-infection.\(^13\)

Prevalence rates of HIV/HCV co-infection depend on the mode of acquisition of both infections and the population studied. Although both HIV and HCV share similar modes of transmission, the relative efficiency of transmission of the two viruses by different routes varies.\(^14\) and mother to child transmission is the main route of acquiring both infections among children under the age of 15 years.\(^15\)

Only a few studies focussed on HIV/HCV coinfection among the paediatric population even in most parts of Africa and Nigeria where the potential for high disease burden exists.\(^16\)-\(^19\) Whether or not prevalence rates and other observations about hepatitis C virus and HIV co-infection in several adult populations can be projected to the paediatric population remains to be seen. This study therefore is aimed at determining the seroprevalence of HCV/HIV co-infection in children aged 2 years to 15 years.

**Subjects and methods**

**Study area**

The study was conducted at the Paediatric Antiretroviral (PARV) Clinic. The clinic is situated in a tertiary referral hospital, and sees an average of 50 patients a week from across the northern states.

**Study population and design**

A hospital-based prospective cross-sectional descriptive study of children between the ages of two years and fifteen years attending the clinic was carried out. HIV negative children, matched for age and sex, attending other paediatric specialist clinics, were recruited as controls. Children were included in the study as cases if aged between 2 and 15 years, have a HIV positivity and are attending the PARV clinic. Inclusion in the study as control was if they were aged between 2 and 15 years and are HIV negative. Children were excluded from the study if parents or caregivers declined consent for the study.

**Ethical Consideration**

Informed consent of each of the children’s parents or caregivers was obtained before recruitment into the study. Pre-test and post-test counselling was done as appropriate and test results were communicated to the primary paediatrician managing the case. Approval of the Scientific and Ethical committee was obtained before the commencement of the study.

**Data Collection**

Relevant data from all children enrolled for the study was obtained and a detailed physical examination was conducted at recruitment. Data obtained from history, physical examination and laboratory results was recorded into a specifically designed proforma.

**Laboratory Methods**

Five millimetres of venous blood was taken each from all patients and their mothers and centrifuged by the investigator. Sera obtained were assayed for the presence of antibodies to hepatitis C virus (Anti-HCV). Detection of Anti-HCV was carried out using the HCV One Step test kit for the qualitative detection of antibodies to hepatitis C virus in serum or plasma. It is a membrane-based immunoassay that is commercially available. The kit has a relative sensitivity of 96.8\% and specificity of 98.9\%. Manufacturer’s instructions were strictly followed to determine the serum samples that would be seropositive for HCV antibody.

**Data Analysis**

Data obtained from the study was analyzed using the computer SPSS version 15.0.0. Results are presented in figures, tables and graphs as appropriate. Differences between proportions were evaluated by the Chi-square test with Yate’s correction applied as appropriate or Fishers exact test was used where appropriate. A p-value of less than 0.05 was considered to be statistically significant in comparative analyses. Relative risks with 95\% confidence intervals were calculated.

**Case Management of children**

All children recruited for the study were routinely additionally investigated as appropriate based on their other presenting symptoms and signs to establish the existence or otherwise of concomitant disease according to the standard of care in the hospital. All the children recruited for the study were then managed accordingly either in the Paediatric ARV Clinic, or the Paediatric Gastro-enterology, Hepatology and Nutrition clinic or in any of the other paediatric units as appropriate.

**Results**

A total of one hundred and thirty two HIV infected children who fulfilled the criteria for inclusion were prospectively studied for presence of HCV antibody between November 2008 and August 2010 as study subjects. Another 132 HIV negative children matched for age and sex were studied as controls during the same period.
Prevalence of antibody to HCV in study group and Controls

Out of the 132 study subjects, 13 tested positive to HCV antibody. This gives the prevalence of antibody to HCV of 9.8% among the study subjects. Among the 132 controls tested, 4 had antibody to HCV, giving a prevalence of 3.0% as shown in Table 1. The difference between the two groups was statistically significant (p = 0.0142, Fisher’s Exact test).

### Table 1: Prevalence of HCV antibody in study subjects and controls

<table>
<thead>
<tr>
<th>Anti-HCV</th>
<th>HIV</th>
<th>Controls</th>
<th>p-value (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>13  (9.8)</td>
<td>4 (3.0)</td>
<td>0.042</td>
</tr>
<tr>
<td>Negative</td>
<td>119 (90.2)</td>
<td>128 (97.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>132 (100.0)</td>
<td>132 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

### Age prevalence

Fig 1 shows the prevalence of antibody to HCV by age among both the study subjects and the controls. Anti-HCV positivity was detected more in children aged above five years in both study subjects and controls. This difference was statistically significant. (χ² = 1.000 with Yates correction, p = 0.00).

### Sex prevalence

The distribution of antibody to HCV by gender is shown in Table 2. Eight (6.1%) of the children among the study subjects who had positive antibody to HCV were males as against 3 (2.3%) among the controls. Five (3.8%) among the study subjects with positive anti-HCV were females as against 1 (0.8%) among the controls. This difference was not statistically significant (p = 1.000, Fisher Exact test).

### Table 2: Distribution of antibody to HCV by gender in study subjects and controls

<table>
<thead>
<tr>
<th>Sex</th>
<th>Study subjects, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-HCV positive, n = 13</td>
<td>Anti-HCV negative, n = 119</td>
</tr>
<tr>
<td>Male</td>
<td>8 (6.1)</td>
<td>73 (55.3)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (3.8)</td>
<td>46 (34.8)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (9.8)</td>
<td>119 (90.2)</td>
</tr>
</tbody>
</table>

(p = 1.000, Fisher Exact test)

### Risk factors for anti-HCV positivity:

Table 3 shows risk factors of mode of delivery, early breastfeeding status, and maternal anti-HCV, HIV status of children with a positive anti-HCV in both study subjects and the controls. Of the 13 study subjects with a positive anti-HCV, 12 (92.3%) were delivered vaginally while 3 (75.0%) of the 4 controls with positive anti-HCV were delivered vaginally. The relative risk of a positive anti-HCV by vaginal delivery was 1.60 (95% CI = 0.76 – 31.57) which was not statistically significant. Eleven children with positive anti-HCV among the study subjects (84.6%) had been breastfed while among the controls, 1 (25.0%) of those with positive anti-HCV had been breastfed as shown in table 3. The relative risk was 1.18 (95% CI = 0.73– 6.10), which was also was not a statistically significant difference.

Table 3 also shows HIV and HCV status of mothers of children with a positive anti-HCV among both study subjects and controls. Not all mothers in both groups were available for testing. Four (44.4%) of the nine mothers of children in the study group also tested positive for anti-HCV as against a mother (33.3%) out of 3 in the control group. The relative risk for a positive anti-HCV was 1.12 (95% CI = 0.52 – 1.71). This however was not a statistically significant increased risk. Similarly, 8 (88.9%) of 9 mothers of children in the study subjects with positive anti-HCV were also HIV infected as against a mother out of the 3 (33.3) in the control group. The relative risk was 2.67 (95% CI = 0.83 – 33.18), which again was not statistically significant. Four mothers of the HIV-infected children were co-infected with HCV and none in the control group. All 4 children of these dually infected mothers were also co-infected.

### Table 3: Maternally-associated risk factors in children with positive anti-HCV among study subjects and controls

<table>
<thead>
<tr>
<th>Maternally-associated risk factors</th>
<th>Anti-HCV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study subjects, n = 13</td>
<td>Controls, n = 4</td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>12 (92.3)</td>
</tr>
<tr>
<td>Caesarean</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>Early infant feeding:</td>
<td></td>
</tr>
<tr>
<td>Ever breastfed</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>Never breastfed</td>
<td>2 (15.4)</td>
</tr>
<tr>
<td>Maternal HIV and HCV status:</td>
<td></td>
</tr>
<tr>
<td>Anti-HCV +ve</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>HIV +ve</td>
<td>8 (88.9)</td>
</tr>
</tbody>
</table>

* n is the number of mothers of children with positive anti-HCV available for assay

### Other Risk factors

Other potential risk factors evaluated for positive HCV antibody in both study subjects and controls including certain cosmetic, religious and cultural practices, unsafe injections and exposure to blood or blood products as shown in table 4.

Table 4 depicts the cosmetic, religious or cultural prac-
tices that may be potential risk factors for HCV infection in children found to be anti-HCV positive among study subjects and control groups.

Of the 13 study subjects with positive HCV antibody, 8 had tribal marks as against 2 out of 4 controls with positive HCV antibody. This gives a relative risk of a positive anti-HCV in study subjects and controls for tribal marks, tattoos, uvulectomy, ear piercing, circumcision and transfusion and their corresponding 95% confidence interval were 1.12 (95% CI = 0.68 – 1.98), 1.07 (95% CI = 0.45 – 1.47), 1.22 (95% CI = 0.63 – 1.64), 0.89, (95% CI = 0.52 – 2.55), 1.39 (95% CI = 0.62 – 2.41) and 1.15 (95% CI = 0.55 – 1.55) respectively. All these were found not to be statistically significant as shown in table IV. However, 12 of 13 study subjects with positive HCV antibody had received injections at patent medicine stores as against a child among the control group. This was statistically significant, (RR = 3.69; 95% CI =1.03 – 44.55).

### Table 4: Risk factors for positive HCV antibody among study subjects and controls

<table>
<thead>
<tr>
<th>Potential risk factors</th>
<th>Anti-HCV positive (n = 13)</th>
<th>Controls (n = 4)</th>
<th>Relative risk</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cosmetic, religious/cultural practices:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tribal marks</td>
<td>8</td>
<td>2</td>
<td>1.12</td>
<td>0.68 – 1.98</td>
</tr>
<tr>
<td>Tattoos</td>
<td>4</td>
<td>1</td>
<td>1.07</td>
<td>0.45 – 1.47</td>
</tr>
<tr>
<td>Uvulectomy</td>
<td>6</td>
<td>1</td>
<td>1.22</td>
<td>0.63 – 1.64</td>
</tr>
<tr>
<td>Ear piercing*</td>
<td>4</td>
<td>2</td>
<td>0.89</td>
<td>0.52 – 2.55</td>
</tr>
<tr>
<td>Circumcision*</td>
<td>5</td>
<td>2</td>
<td>1.39</td>
<td>0.62 – 2.41</td>
</tr>
<tr>
<td><strong>Use of blood/blood products:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusion</td>
<td>5</td>
<td>1</td>
<td>1.15</td>
<td>0.55 – 1.55</td>
</tr>
<tr>
<td><strong>Unsafe use of sharps:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection at PMV</td>
<td>12</td>
<td>1</td>
<td>3.69</td>
<td>1.03 – 44.55</td>
</tr>
</tbody>
</table>

* Ear piercing was seen only in girls

PMV = Patent medicine vendor

A cultural practice done by traditional barber

### Discussion

This study has shown the presence of HCV antibodies in HIV infected children aged 2 to 15 years as well as in age and sex-matched controls. The prevalence of HCV antibodies in HIV infected children aged 2 to 15 years was 9.8%. The prevalence of HCV antibodies in HIV negative children aged 2 to 15 years was 3%. This difference in the prevalence rates between the study subjects and controls is statistically significant, and suggests an increased rate of HCV sero-positivity among HIV infected children. This has been previously documented by other workers in Tanzania and also across Africa among different adult populations. The 9.8% HIV/HCV co-infection rate found in this study is far lower than the 20.2% rate reported in Jos among adult population, but much higher than rates of 0.02% - 3.3% reported by other workers in paediatric population and elsewhere among adult populations. These differences may reflect differences in methods of HCV antibody assay used and the differences in risk factors depending on the age variations of populations studied.

It was observed in this study that HCV antibody positivity was more frequent after the age of five years. This was the case among both the study subjects and the Controls, with no statistically significant differences between study subjects and Controls. There was however a statistically significant difference between rates of HCV antibodies positivity and age less than or five years and beyond. The increased rate of HCV sero-positivity beyond five years found in this study may suggest the relative importance of post-partum transmission due to greater cumulative opportunities for contact via continuous or repeated exposure to risk factors as the child grows compared to perinatal transmission. This is contrary to findings reported among children across eleven tertiary care centres in Nigeria where the median age was 3.4 years, and elsewhere in which age was found not to be significantly associated with anti-HCV sero-positivity. The reasons for these differences however are unclear. More epidemiologic data on HCV infection in HIV-infected children are required to make comparison on the role of age as an independent risk factor. Sex was not noted in this study to be of any significance in influencing the rates of HIV/HCV co-infection or HCV infection alone. Again this has been previously noted in adult population. This is contrary to the report from Tanzania among HIV-infected children in which the prevalence of HCV antibody positivity was significantly higher among girls than boys. On the other hand, the study across 11 care centres in Nigeria found 76% of HCV co-infected children to be males. The reason for these differences is not clear.

One aim of this study was to document risk factors of transmission. The various potential risk factors of transmission of HCV in both HIV infected subjects and controls were therefore also explored. The possible role of mode of delivery as a risk factor was examined. Observations in this study showed that children who were vaginally delivered were 1.6 times more likely to have positive HCV antibodies than children delivered via caesarean section. This however was not statistically significant. There is a similar report of no statistically significant difference between different modes of delivery and rates of HCV transmission or infection. The present study was not able to further segregate delivery methods into normal vaginal or instrumental and assisted vaginal deliveries on one hand, and elective or emergency caesarean section on the other hand because of inconclusive information from caregivers as to what type of vaginal delivery or section was used in some cases. There is nevertheless a case of a clinically increased risk of HCV antibody positivity when delivered via vaginal route over caesarean section.

Breastfeeding was another potential risk factor explored in this study. It was observed that breastfed children in the study were 1.18 times as likely to have a positive
HCV antibody as their non-breastfed peers. Again, this was not of statistical significance. The marginal increase in risk was in keeping with other studies that showed that transmission via breast milk was not a significant mode of transmission in spite of detection of HCV RNA in the breast milk of viraemic mothers.\textsuperscript{15, 20} It was suggested that acquisition of HCV by children through breastfeeding will be dependent on the viral load in the breast milk, which is usually said to be highest soon after delivery.\textsuperscript{26} It was not possible to substantiate this in the present study because maternal HCV viral load in blood or breast milk was not determined.

This study however determined the maternal HCV antibody status and how it relates to HCV antibody positivity in both the study subjects and the controls. It was noted that HCV seropositive mothers with HIV infection had a 12% increased chance of having children with HCV antibody positivity than HCV sero-negative mothers. Although a mother with HCV infection could transmit it vertically to her children, this however does not conclusively prove route of transmission.

This study was not designed to determine the route of HCV transmission for co-infected children. Further tests would be required to confirm if transmission was mother-to-child when mother-child pair is found to have HCV infection, since both may have acquired the HCV infection from the same source. It was not possible from the design of the present study therefore to speculate on possible vertical transmission of HCV infection among study subjects and controls.

Maternal HIV infection was observed to enhance HCV antibody positivity in children in this study. Eighty eight percent (8/9) of mothers of children with positive HCV antibodies available for assay were HIV infected among the cases as against one of three (33.3%) corresponding mothers among the controls. Children whose mothers were HIV infected were found to be more than twice as likely to have HCV antibody positivity as children whose mothers were HIV negative. This is in conformity with reports that vertical transmission rates for HCV were 8 to 40% higher for women who were also HIV infected.\textsuperscript{15}

The role of some other potential risk factors for transmission was also explored. Results from this study suggested that unsafe therapeutic injection practice by patent medicine vendors was a very significant risk factor for HCV transmission in this environment. Twelve of thirteen (92.3%) children with positive HCV antibodies available for assay were HIV infected among the cases as against one of three (33.3%) corresponding mothers among the controls. This was a statistically significant difference between the groups. This was much higher than the 39% of co-infected patients reported in one cohort of having had a history of needle injection at patent medicine stores.\textsuperscript{23}

Blood transfusion was yet another risk factor usually associated with HCV infection explored in the present study. Five of the 13 children (38.5%) among the study subjects with a positive HCV antibody had a history of previous blood transfusion as against one of four (25%) among the controls with positive HCV antibodies. This was not a statistically significant difference. The finding in this study was not entirely unexpected as transfusion of blood or blood products carried out in standard health facilities offering transfusion services such as this centre usually screen the blood or blood component for HCV. Significant HCV nosocomial transmission through transfusion of blood or its components would only occur in settings with absence of blood screening services. That was not the setting in this environment.

Certain cosmetic, cultural or religious practices were identified in the present study to marginally raise the risk of positive HCV antibodies. These included tribal marks and tattoos, which increased the chances of transmission by 1.12 and 1.07 times more than those without these procedures respectively. These figures however did not reach statistical significance. Other risky practices of note in this study were uvulectomy and circumcision, which increased the probability of HCV antibody positivity by 1.22 and 1.39 times respectively more than in children who did not have them. Again, these were not statistically significant.

All these practices, especially tribal marks, tattoos and uvulectomy, and to a less extent circumcision, in our environment are carried out by traditional barbers, often using the same blade for multiple subjects with little or no sterilization before re-use. This setting would highly favour HCV transmission.\textsuperscript{10} This study has demonstrated weak associations of these very common practices in our environment with HCV antibody positivity, which because of their widespread practice in the community cannot be overlooked as potentially risky procedures.

Surprisingly, it is noteworthy that this study found no association between ear piercing and HCV antibody positivity. It was expected that this very common cosmetic and cultural practice in the community would also be potentially risky considering that non-professionals commonly perform it. In this study however, it was observed that children who had their ear pierced had 0.89 times the probability of HCV antibody positivity than girls who did not have ear piercing done. Perhaps a plausible reason for this relative decreased risk may be because of the less likelihood of re-use of needle used for the procedure than in other cultural procedures such as circumcision or uvulectomy.

Overall, this study has demonstrated the prevalence of HCV antibody in HIV infected children to be statistically different from that in HIV negative children. This underscores the importance of routine screening of all HIV-infected children for HCV infection, to avail them with prompt and adequate treatment. The study has also documented some possible risk factors for HCV/HIV co-infection in these children and highlights the need to provide safer injection practices especially to HIV-infected children through a combination of education, legislation and regulation of patent medicine vendors.
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