Haemophilia A patients are not at increased risk of hepatitis A virus infection: An Egyptian experience

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Received 14 October 2011; accepted 19 November 2011
Available online 21 February 2012

KEYWORDS
Hepatitis A virus; Haemophilia A; Hepatitis A vaccine

Abstract

**Background:** Hepatitis A virus (HAV) infection is endemic in Egypt. Haemophiliacs are at risk of transmission through exposure to blood products. We evaluated the seroprevalence of hepatitis A in Egyptian patients with haemophilia A as well as the safety and immunogenicity of subcutaneous hepatitis A vaccine in haemophiliacs.

**Methods:** 182 male children and adolescents were studied (82 patients with moderate and severe haemophilia A and 100 healthy controls). Screening for anti-HAV antibody was done and seronegative subjects received hepatitis A vaccine (Havrix™) at a dose of 720 Elisa Units at 0 and 6 months, given subcutaneously in haemophiliacs and intramuscularly for controls. Seroconversion was assessed 2 months after the second vaccine dose by anti-HAV IgG.

**Results:** Seroprevalence of HAV antibodies was 87.6% among haemophiliacs and 90% among the control group. Seronegative children (mean age 4.4 ± 3.71 years) were significantly younger than seropositive children (mean age 10.2 ± 3.86 years). Hepatitis A vaccine was given to 10 non immune haemophilia patients and 10 controls. The vaccine was well tolerated with local side effects including pain in 40% and erythema in 20% of haemophiliacs versus 20% for pain and erythema in the control group. All patients and controls developed seroconversion 2 months after the second
1. Introduction

Infection with hepatitis A virus (HAV) occurs worldwide and is the most common cause of acute viral hepatitis [1]. The highest prevalence of this infection is seen in developing countries, where low standards of sanitation promote the transmission of the virus [2].

Patients with haemophilia require long-life intravenous infusion of factor concentrates to treat bleedings, which increases the risk of transmission of blood-borne infections mainly human immunodeficiency virus (HIV) and hepatitis C virus (HCV) [3]. Although transmission of HAV infection is primarily via the faecal-oral route, transmission may also occur in individuals with haemophilia through clotting factor concentrate, particularly products inactivated by solvent–detergent purification [4].

Egypt has the highest prevalence of antibodies to hepatitis C virus (HCV) in the world, estimated nationally at 14.7% [5], it is also classified as hepatitis A virus endemic country [6]. Acute hepatitis A virus (HAV) super infection on the top of chronic liver disease causes progressive disease, acute hepatic failure and higher fatality rates, specifically in chronic hepatitis B (HBV) and chronic hepatitis C (HCV) infections [7,8]. Both the American Academy of Pediatrics (AAP) [9] and the Centres for Disease Control (CDC) [10] recommend hepatitis A virus (HAV) vaccine for persons with clotting-function disorders.

Hepatitis A vaccines licensed in the United States and used in Egypt, including Havrix™, are inactivated whole-cell virus vaccines grown in human diploid fibroblast cells [11]. The recommended route of administration for the vaccine is intramuscular with the potential risk of bleeding in patients with congenital bleeding disorders especially in severe deficiencies and in the presence of inhibitors [12,13]. A previous study revealed that proper factor replacement in haemophilia patients in Egypt is lacking [14] increasing their risk of bleeding with intramuscular vaccination.

This study aimed to evaluate the seroprevalence of hepatitis A virus infection in Egyptian children and adolescents with moderate/severe haemophilia A compared to the general population, and to assess the efficacy and safety of subcutaneous hepatitis A vaccination in haemophiliacs.

2. Patients and methods

The study had a two-step design. The first step was a cross sectional study to determine the proportion of Egyptian children and adolescents with moderate and severe haemophilia A who had HAV protective antibody, compared to healthy age and sex matched control group. Children who had no HAV protective antibody received two doses of HAV vaccine in the second step, given subcutaneous in haemophiliacs and intramuscular in healthy controls.

The study was conducted in the Paediatric Haematology Unit, Children’s Hospital, Ain Shams University, in Cairo, Egypt, in the period from January 2009 to May 2010. The study included 182 male children and adolescents, aged 2–18 years. They were divided into two groups: 82 patients with moderate/severe haemophilia A (mean age 9.49 ± 4.26 years) and 100 healthy controls (mean age 8.67 ± 3.97 years). The diagnosis of haemophilia A and severity were based on factor VIII level assays [15]. Exclusion criteria included infants aged less than two years, patients with a known immunologic defect, HIV positive patients and patients with history suggestive of previous hepatitis infection or previous vaccination with hepatitis A vaccine.

The study was approved by the Ethical Committee of the Paediatric Department, Ain Shams University. All patients and/or their guardians signed informed consent forms.

All the included children and adolescents were subjected to complete history taking including age, type and severity of haemophilia, type of treatment received, age at first factor VIII infusion, and history suggestive of hepatitis infection (jaundice, changing colour of urine, isolation in fever hospital, vomiting, previous abnormal liver function tests). Children were given a thorough medical examination to exclude the presence of any current liver disease.

Blood samples were obtained at enrollment from all children to measure total HAV antibody level by competitive enzyme immunoassay test (ELISA) using commercially available kits (DiaPro Diagnostic Bioprobes Srl., Milan, Italy). Initially all patients were also tested for factor VIII-inhibitory activity by a quantitative Bethesda method [16].

Children who had no HAV protective antibody, defined as anti-HAV antibody < 20 mIU/mL [17], were given two 0.5 mL doses of 720 ELISA (enzyme linked immunosorbant assay) units of inactivated HAV vaccine, Havrix™ (Glaxo Smith Kline, Marly-Le-Roi, France) 6 months apart. Haemophilia patients received the vaccine subcutaneously; while non-haemophilic controls received the vaccine intramuscularly in the deltoid. No pre-vaccination FVIII replacement therapy was given to haemophilia patients. After vaccination all participants were asked to record any local or systemic adverse events for 3 days after each inoculation.

All vaccinated patients and controls had blood drawn 2 months after the second vaccine dose (month 8) to assess total HAV antibody by ELISA kits (DiaPro Diagnostic Bioprobes Srl., Milan, Italy). Assessment of HAV IgM was done by Enzyme Immuno Assay kits (DiaPro Diagnostic Bioprobes Milano, Italy) one month after the 1st and 2nd vaccination doses (at month 1 and 7) to exclude concurrent active HAV infection.

3. Statistical analysis

Statistical analysis was done using SPSS software version 15. Descriptive statistics were presented as means, standard deviation (SD), number and percentage (frequency distributions).
The chi-square test was used to assess the association between groups. The Fisher exact test was performed in tables containing values less than five. Student’s t-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples. The level \( p < 0.05 \) was considered the cut-off value for significance.

### 4. Results

This study included 182 male children and adolescents classified into two age groups: 2–6 years (28.6%) and >6–18 years (71.4%) (Table 1).

Among the studied children and adolescents, HAV seropositivity was 87.8% in the haemophilia patients and 90% in healthy controls with an overall prevalence of 89%, with non-significant difference between patients and control group (Table 2). The seroprevalence of HAV was 90.7% and 84.6% in severe and moderate haemophilia, respectively, the difference was not significant (Table 3).

In our work we found that unvaccinated children (patients and controls) who were seronegative for HAV were significantly younger than seropositive children (Table 4). The mean age of seronegative children was 4.4 ± 3.71 years compared to 10.2 ± 3.86 years in seropositive children (\( p < 0.01 \)).

Ten seronegative haemophilia patients received Havrix™ vaccine subcutaneously while the 10 seronegative controls received the vaccine intramuscularly at 0 and 6 months. The vaccine was well tolerated with minor local adverse events. Pain/or soreness was the most common local non serious event in both patients and controls (occurring in 40% and 20% of both

### Table 1  Age distribution of the studied groups of haemophiliac children and control group.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Patients (( n = 82 ))</th>
<th>Controls (( n = 100 ))</th>
<th>Total</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>2–6 years</td>
<td>20</td>
<td>24.4</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>&gt;6–18 years</td>
<td>62</td>
<td>75.6</td>
<td>68</td>
<td>68</td>
</tr>
</tbody>
</table>

* Fisher exact test (two-tailed).

### Table 2  Seroprevalence of anti-hepatitis A virus (anti-HAV) antibodies among patients with haemophilia and control group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Haemophilia (( n = 82 ))</th>
<th>Control (( n = 100 ))</th>
<th>Total (( n = 182 ))</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Anti-HAV negative</td>
<td>10</td>
<td>12.2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Anti-HAV positive</td>
<td>72</td>
<td>87.8</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

* Fisher exact test (two-tailed).

### Table 3  Classification of the studied haemophilic children as regards severity of haemophilia and hepatitis A virus seroprevalence.

<table>
<thead>
<tr>
<th>Haemophilia severity</th>
<th>HAV antibody seroprevalence</th>
<th>( P ) value***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Severe( ^* )</td>
<td>4</td>
<td>9.3</td>
</tr>
<tr>
<td>Moderate( ^** )</td>
<td>6</td>
<td>15.4</td>
</tr>
</tbody>
</table>

\( ^* \) Factor VIII level <1%.

\( ^** \) Factor VIII level 1–5%.

*** Fisher exact test (two-tailed).

### Table 4  Seroprevalence of anti-hepatitis A virus antibodies (anti-HAV Ab) in different age groups of the studied patients and controls.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Anti-HAV Ab negative (( n = 20 ))</th>
<th>Anti-HAV Ab positive (( n = 162 ))</th>
<th>Total</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>2–6 years</td>
<td>16</td>
<td>80</td>
<td>36</td>
<td>22.2</td>
</tr>
<tr>
<td>&gt;6–18 years</td>
<td>4</td>
<td>20</td>
<td>126</td>
<td>77.8</td>
</tr>
</tbody>
</table>

* Fisher exact test (two-tailed).
groups, respectively), followed by erythema (occurring in 20% of either group), no swelling or bruising was reported in patients or controls.

After subcutaneous injection of the hepatitis A vaccine, the seroconversion rate two months after the second dose was 100%, similar to the results in the control group given intramuscular vaccine. As regards immunogenicity, there was a statistically significant difference between the mean titres of total anti-HAV antibody in haemophilia children compared to control group ($p > 0.05$) (Table 5). No patients developed anti-HAV IgM antibody or signs of acute hepatitis during the study interval.

Among the studied 82 haemophilia patients, five patients had FVIII inhibitors (6.1%), with titre varying between 2 and 4 BU/ml, considered as low titre [18], all had severe haemophilia and all were seropositive for HAV antibodies, so did not receive the vaccine.

### 5. Discussion

Hepatitis A virus infection is endemic in Egypt [6]. In this study, HAV overall seroprevalence was 89% in children aged 2–18 years with no significant difference between haemophiliacs and controls. Previous studies [19,20], reported higher HAV seroprevalence up to 100% in Egyptian children. Another Egyptian study in 2004 recorded a HAV prevalence of 86.2% in children aged 3–18 years [21]. A more recent study [22] assessed the seroprevalence of HAV in 296 Egyptian children aged 2.5–18 years of different social classes, and they found that 61.4% were seropositive with a significant increase in seroprevalence (87.5%) in the low social class group. Similarly, Salama et al. [23] described HAV seroprevalence of 50% in high social class and 90% in low social class children in Egypt. The high seroprevalence among our patients could be related to the socioeconomic status as most of our studied patients belong to the unprivileged social class. The prevalence of HAV infection is reported to closely correlate with environmental sanitation and the prevailing socioeconomic and hygienic conditions [24].

In our work we found that unvaccinated children (patients and controls) who were seropositive for HAV were significantly older than seronegative children. This is presumably due to increased likelihood of exposure to HAV with advancing age [22]. Similar age related increased HAV seroprevalence has been reported in Egypt [21,25] and other HAV endemic countries [26,27]. In Iran, Sofian et al. [28] reported HAV seroprevalence in Tehran rising from 51.7% in children aged 2–5 years to 85% in the age group 16–20 years, while Kaya et al. [29] reported the seroprevalence of HAV in an endemic area in Turkey to vary from 19.2% in 2–5 years old children to 92.8% in 15–18 years old group.

The possibility of HAV transmission via plasma derived factor VIII concentrate is documented [30–32]. However in the present study as well as in other studies [33], there was no significant difference between haemophiliacs and healthy subjects regarding the seroprevalence of HAV, in spite of the fact that haemophiliacs in our centre receive mainly on demand plasma derived factor VIII products including cryoprecipitate and intermediate purity FVIII concentrate. Presumably in the present study, the herd immunity conferred by the poor sanitation has eliminated transfusion as a risk factor.

In this study subcutaneous HAV vaccine was well tolerated with minor local adverse events, similar to previous results [7,31]. Soreness and erythema were the most common adverse events in both patients and controls in our study, no swelling or bruising was reported. However, Ragni et al. [32] reported bruising and swelling occurring in 7% and 24%, respectively, with no difference between haemophiliacs and controls.

As regards efficacy after subcutaneous Havrix™ vaccine, the present study showed 100% seroconversion rate two months after the second dose, similar to control group given the intramuscular vaccine. This is in agreement with Ragni et al. [32] who found that the anti-HAV IgG titres peaked at eight months, with 100% seroconversion rate.

As regards vaccine immunogenicity, there was no statistically significant difference between the mean titres of anti-HAV in haemophilia children compared to the control group. This is in agreement with Zuckerman et al. [13] who compared the immunogenicity, reactogenicity and safety of an inactivated hepatitis A vaccine administered subcutaneously to patients with congenital coagulation disorders and found no significant difference in the geometric mean titres between the two groups.

However, Ragni et al. [32] found that the HAV IgG geometric mean titres were lower in the haemophilic children compared to their non-haemophilic siblings. They suggested that the potential reasons for these differences in HAV antibody titre may be the possibility that the immunogenicity of a vaccine given by the subcutaneous route is inferior to that given by the intramuscular route, which disagree with our results.

An important limitation of the study was the small number of hepatitis A naive individuals in both haemophiliacs and controls who were subjected to vaccination as well as the heterogeneity of route of vaccine administration of both groups.

In conclusion, these results show that the seroprevalence of HAV infection in haemophiliacs in Egypt is similar to that in the general population and there is no significant excess of HAV infections among haemophiliacs with high exposure to coagulation factor concentrates in spite of high HAV endemicity. Although the WHO postulated that in countries with high HAV endemicity large-scale vaccination programmes are not recommended [34], vaccination of high risk groups like haemo-

### Table 5

Comparison between patients with haemophilia and control group as regards total HAV antibody titre 2 months after 2nd dose of Havrix™.

<table>
<thead>
<tr>
<th></th>
<th>Haemophiliacs ($n = 10$)</th>
<th>Controls ($n = 10$)</th>
<th>$t$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>Anti-HAV titre (mIU/ml)</td>
<td>275–450</td>
<td>351.4</td>
<td>65.0</td>
<td>175–525</td>
</tr>
</tbody>
</table>

95% confidence interval (−113.5 to 82.3).
philia is mandatory and prevaccination testing for antibodies is recommended and cost-effective. Subcutaneous hepatitis A vaccine given as standard two doses 6 months apart is recommended in children as it is safe and immunogenic. Prevaccination factor replacement is generally not necessary with subcutaneous vaccination.

References