Seroprevalence of hepatitis A virus in Mumbai, and immunogenicity and safety of hepatitis A vaccine

P S DHAWAN, S S SHAH, J F ALVARES, A KHER,* SHANKARAN K, P W KANDOTH,* P N SHETH,*** H KAMATH,** A KAMATH,** G V KOPPIKAR,** R H KALRO

Departments of Gastroenterology, *Pediatrics and **Microbiology, BYL Nair Hospital and ***Department of Pediatrics, Bombay Hospital Institute of Medical Sciences, Mumbai

Objectives: Since epidemiologic trends of hepatitis A are changing worldwide, we studied its seroprevalence in Mumbai, which is thought to be a high-endemicity area. The immunogenicity and safety of a hepatitis A vaccine were also studied.

Methods: Six hundred and seventy subjects (456 men; age range 6 mo-60 y) answered a questionnaire on social and medical history. Qualitative analysis of total anti-HAV was performed in all subjects by ELISA. One hundred and seven of 147 anti-HAV negative subjects received hepatitis A vaccine at months 0, 1 and 6. Subjects were followed up (months 1, 2, 6, 7) to look for side-effects and seroconversion. Results: The seroprevalence of HAV was 525/670 (78%); 38% of children <5 years were anti-HAV negative. Seroprevalence rates of 80% were reached by 18 years. Prevalence was lower in the higher socio-economic group (151/234; 64.5%) compared with the lower socio-economic group (372/436; 85%) (p < 0.001). One month after doses 1, 2 and 3 of the hepatitis A vaccine, seropositivity was 92%, 99% and 100%, respectively. Minor self-limited side-effects occurred in 19.5% of subjects; there were no major side-effects. Conclusions: The seroprevalence of anti-HAV is high in Mumbai. Seroprevalence is lower in the higher socio-economic groups. The hepatitis A vaccine is safe and immunogenic. [Indian J Gastroenterol 1998; 17: 16-18]

Key words: Epidemiology, prevention, control

Transmission of hepatitis A virus (HAV) is mainly by the feco-oral route. Amongst the important risk factors are improper sanitation, inadequate access to clean drinking water, overcrowding and poor living conditions. Incidence figures are unreliable because most cases are subclinical or mild and unreported. Hence the epidemiology of HAV infection is best defined by measuring humoral antibodies.

See editorial on page 2

Epidemiologically, areas of HAV endemicity have been described as high, intermediate and low. In the high-endemicity areas (underdeveloped countries), infection in childhood (<5 y) is common, so that by adulthood almost all persons have antibodies. On the other hand, in low-endemic industrialized nations, the percentage of seropositive persons is low in childhood, increases during adolescence and early adulthood, and reaches a high level by late adulthood (sigmoid-shaped curve). With increasing socio-economic development, the epidemiology of HAV is changing in developing countries.

For prevention of HAV infection, general measures of hygiene, passive immunization (immunoglobulin) and active immunization can be used. Whereas general public health measures can retard transmission they do not decrease the disease burden since infection-to-disease ratio decreases as age at acquisition increases. The protection conferred by immunoglobulin is short-lasting (3-6 months). Active immunization thus remains the best modality of protection against HAV. Since HAV exists as a single serotype and humans are the only source, it can theoretically be eradicated. Recently an inactivated hepatitis A vaccine has been licensed in many countries and is found to be highly immunogenic and safe.

We undertook this study to determine the current age-related seroprevalence of HAV in subjects from Mumbai; we also studied the various factors that influence seropositivity. Also, the immunogenicity and safety of the inactivated hepatitis A vaccine in subjects who were susceptible was studied.

Methods

Two centers participated in this study from April 1995 to June 1996 — one, a private hospital catering to the middle and upper socio-economic classes, and the other a public hospital catering to predominantly the lower socio-economic class. Six hundred and seventy subjects (456 males; 6 mo-60 years), who were not suffering from any obvious hepatic or gastrointestinal disease were prospectively studied. All subjects were investigated with a uniform social and medical history questionnaire. Socio-economic groups were divided on the monthly income as high (HSG; more than Rs 5000) or low (LSG). Past history of jaundice, family size, and whether drinking water at home is boiled and/or filtered were specifically recorded.

Of the 670 subjects, 107 subjects (including 72 who were <15 years) seronegative for anti-HAV were offered the hepatitis A vaccine. Subjects who had received any other vaccine preparation within one month of the first scheduled dose of the study vaccine were excluded.

Copyright © 1998 by Indian Society of Gastroenterology
The project was approved by the hospital's ethics committee. All subjects gave informed consent for participation in the study.

**Vaccine**

All vaccinees received the HM 175 (RIT 4380) inactivated HAV vaccine (Havrix®; SmithKline Beecham Biologicals, Rixensart, Belgium). The vaccine virus was grown on MRC5 cells and after purification by ultrafiltration, ion-exchange chromatography and gradient centrifugation, was inactivated with formaldehyde (250 μg/mL for 15 days at 37°C). It was adsorbed on aluminium hydroxide (0.5 mg/mL), giving a final concentration of 720 ELISA units (EU) of antigen per mL for the adult dose. The vaccine was stored at a temperature of 2°C-8°C.

Subjects less than 15 years of age received 0.5 mL and adults 1 mL of the vaccine intramuscularly in the deltoid region at months 0, 1 and 6. Subjects were observed for 3 hours post-vaccination. A diary card was given to each subject to be maintained for the first 3 days after each injection. Local side-effects such as tenderness, swelling or redness and systemic side-effects such as fever and bodyache were recorded. Each symptom or sign was graded from 0 to 3 (0 - absent, 1 - mild, 2 - moderate, 3 - severe).

Patients were asked to follow up at one month after each dose. During these visits the diary card was examined, findings were recorded and blood collected for testing for antibodies to HAV.

Serum was separated from the blood, collected and stored at -20°C. Liver biochemistry (ALT, AST, serum bilirubin, alkaline phosphatase) was done using an autoanalyzer. Sera were tested for qualitative detection of antibody to HAV (total anti-HAV) using enzyme immunoassay (HAVAB-ELISA; Abbott Diagnostics, Illinois, USA); an automated Abbott ELISA reader was used for this purpose. The difference between negative and positive control mean absorbance value was at least 0.4.

**Statistical analysis**

Seroprevalence rates in different age groups were compared using $\chi^2$ test. A probability (p) value less than 0.05 was considered significant.

### Results

#### Seroprevalence

Of 670 subjects tested, 523 (78%) were positive for anti-HAV. There was no difference in the gender ratio between subjects who were anti-HAV positive (M:F 368:155) or negative (88:59). Age-related seroprevalence is given in the Table. Anti-HAV antibody was positive in 386/527 (71.5%) subjects <40 years of age, and in 137/143 (96%) subjects >40 years of age (p<0.01). Seropositivity of 80% was reached in the 11-15 years age range.

Seropositivity was higher in the LSG (85.3%) as compared with the HSG (64.5%; p<0.001). Past history of jaundice was present in 16.1% and 14.2%, and boiling and/or filtering drinking water was done by 21.1% and 14.2%, of the anti-HAV positive and negative groups, respectively; the latter values were similar in the HSG and LSG.

#### Immunogenicity of vaccine

One hundred and seven subjects (65 males; age range 1.5 - 40 y) who were anti-HAV negative were enrolled to receive hepatitis A vaccine. Two of these subjects did not come for the second dose, and eight for the third dose. Anti-HAV antibody appeared in 97/105 (92.4%) one month after the first injection, and in 103/104 (99%) one month after the second injection. At the time of the third dose (month 6) all 97 subjects who followed up had seroconverted.

No major side-effect occurred in any patient. Minor local side-effects such as redness and swelling occurred in 18/92 (19.5%). Most reactions were mild (grade 1-2-3) and self-limited. Systemic side-effects such as headache and fever occurred in 3 (3.3%) subjects.

#### Discussion

The overall seroprevalence rate of about 80% makes Mumbai an area of high endemicity for HAV; 38% of children under 5 years were anti-HAV negative and 80% seropositivity was present in the 11-15 years age group.

The epidemiology of HAV infection worldwide is changing; in many countries the graph of age-related seroprevalence has shifted to the right. In Malaysia, the seroprevalence in 1985 was 43% and 64% at 10 years and 11-20 years, respectively and fell to 15% and 28% in 1994. Similar changes have been reported in Singapore as well. This change in epidemiologic pattern would increase the disease burden (more clinical/icteric cases, more fulminant hepatitis), cause large community outbreaks and lead to increased health-care costs.

For analyzing economic status, subjects in our study were divided into HSG and LSG depending on the monthly income being more than or less than

---

**Table: Age-related seroprevalence (anti-HAV positivity)**

<table>
<thead>
<tr>
<th>Years</th>
<th>Total</th>
<th>+ve</th>
<th>%</th>
<th>Total</th>
<th>+ve</th>
<th>%</th>
<th>Total</th>
<th>+ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>75</td>
<td>38</td>
<td>50.7</td>
<td>26</td>
<td>7</td>
<td>26.9</td>
<td>49</td>
<td>31</td>
<td>63.2*</td>
</tr>
<tr>
<td>4-5</td>
<td>45</td>
<td>28</td>
<td>62.2</td>
<td>18</td>
<td>6</td>
<td>33.3</td>
<td>27</td>
<td>22</td>
<td>81.5*</td>
</tr>
<tr>
<td>6-10</td>
<td>98</td>
<td>76</td>
<td>77.5</td>
<td>32</td>
<td>24</td>
<td>75</td>
<td>66</td>
<td>52</td>
<td>78.8</td>
</tr>
<tr>
<td>11-15</td>
<td>66</td>
<td>55</td>
<td>83.3</td>
<td>20</td>
<td>13</td>
<td>65</td>
<td>46</td>
<td>42</td>
<td>91.3*</td>
</tr>
<tr>
<td>16-20</td>
<td>37</td>
<td>25</td>
<td>67.6</td>
<td>14</td>
<td>7</td>
<td>50</td>
<td>23</td>
<td>18</td>
<td>78.2</td>
</tr>
<tr>
<td>21-30</td>
<td>121</td>
<td>96</td>
<td>79.3</td>
<td>31</td>
<td>13</td>
<td>42</td>
<td>90</td>
<td>63</td>
<td>93.2*</td>
</tr>
<tr>
<td>31-40</td>
<td>85</td>
<td>68</td>
<td>80</td>
<td>27</td>
<td>17</td>
<td>63</td>
<td>58</td>
<td>51</td>
<td>87.9*</td>
</tr>
<tr>
<td>&gt;40</td>
<td>145</td>
<td>137</td>
<td>95.8</td>
<td>66</td>
<td>64</td>
<td>97</td>
<td>77</td>
<td>73</td>
<td>94.8</td>
</tr>
<tr>
<td>Total</td>
<td>670</td>
<td>523</td>
<td>78</td>
<td>234</td>
<td>151</td>
<td>64.5**</td>
<td>436</td>
<td>372</td>
<td>85.3</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.001 HSG vs LSG*
Hepatitis A seroprevalence and vaccination

Rs 5000. This was based on the per capita monthly income of Indians (Rs 5670; 1991-92 census). The seroprevalence rates were significantly lower in the HSG, probably due to better hygiene, sanitation and intake of clean water. Similar patterns were seen in Bangkok, Thailand which is a country with high endemicity. In another study a negative correlation of seroprevalence was noted with educational status.

There was no correlation of anti-HAV antibody status with past history of jaundice because a majority of episodes of HAV infection in childhood are asymptomatic. We also found no relation with the method of treating drinking water at home, i.e., boiling and/or filtration. However, this was only a one-point analysis and we did not study the total duration of these practices.

This study confirms the immunogenicity as well as safety of the inactivated hepatitis A vaccine. A high seroconversion was noted after the first dose and almost all patients seroconverted after the second dose. This can obviate the use of immunoglobulins in most instances; the protection may last for over 10 years. Similar results have been obtained by other investigators.

Clemens et al analyzed 104 studies from 27 countries involving 50,677 subjects and >120,000 vaccine doses. The vaccine was found to be highly immunogenic at all ages. Innis et al evaluated the protective efficacy of hepatitis A vaccine in over 40,000 children and found it to be 94% after two doses at 8 months' follow-up. Westblom et al used different dosages and schedules: after one dose using 720 EU and 1440 EU, 90% and 100%, respectively seroconverted. Hence, further studies need to be done to study dosages and a schedule which is practical.

The use of a vaccine depends on the epidemiology of the disease, disease burden, resources (vaccine cost, operational feasibility) and other health priorities. For HAV infection, despite changing epidemiologic patterns and the availability of a safe and effective vaccine, other health priorities are paramount and universal immunization is likely to prove unacceptably costly at present.

In conclusion, although the overall seroprevalence rates of HAV in Indian subjects are high, about 40% of children less than 5 years are susceptible; high (>80%) positivity rates are seen only by age of 15 years. The prevalence is lower in high compared with low socio-economic groups. The inactivated hepatitis A vaccine is safe, clinically well tolerated and immunogenic. Further epidemiologic studies to target specific risk groups on a regional basis are needed.

References

Correspondence to: Dr Dhawan, S/1 Mitra Kunj, 16 Pedder Road, Mumbai 400 026

Acknowledgements: The authors thank the Research Society, BYL Nair Hospital for financial support; Dr HH Gill for help during the preparation and conduct of the study; and SmithKline Beecham Pharmaceuticals, Bangalore for providing the vaccine.