Serological Studies of Hepatitis A Virus (HAV) on Jeddah Population, Saudi Arabia

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Abstract: Hepatitis A virus (HAV) infection is often a self limiting disease that can be associated with fulminant hepatic failure (FHF). The objective of this study was prevalence of Hepatitis A virus (HAV) on Jeddah population, Saudi Arabia. Also find out the relationship of result with age, gender and ethnicity. For all age groups, there was no significant difference between genders regarding anti-HAV sero-positivity. The prevalence of IgG antibodies to HAV was 33.1% overall, 33.6% among males and 32.5% among females with no statistically significant difference (P>0.05). However, it increased significantly with the age. Prevalence of Hepatitis A virus by nationality, The IgG antibodies to HAV were found in 20.3 % of Saudi population and 49% of non Saudi population (p<0.05). A higher proportion of non-Saudi population were found anti HAV sero-positive than Saudi population. There was significant difference between nationalities regarding anti-HAV seropositivity. The prevalence of HAV among adult population (39.3%) was higher than among children population (22.4). The chi-square test showed significant differences in the prevalence of HAV between adult and children population (p<0.05).

Key words: Prevalence • HAV • Serology • ELAZA

INTRODUCTION

Hepatitis is an inflammatory disease of the liver that can severely damage the organ. The disease can result from non-infectious causes or from infectious viral and bacterial agents. The hepatitis A virus (HAV), a member of the family picornaviridae, is a non enveloped virus with a linear, single-stranded, positive-sense RNA genome of approximately 7.5 kb in length. There is only one HAV serotype, which is organized into six genotypes [1].

HAV is transmitted almost exclusively by the fecal-oral route and transmission is enhanced by poor hygiene, overcrowding and contaminated food or water. The presence and severity of symptoms with hepatitis A virus infection is related to the patient’s age. Approximately 70 percent of infected adults develop symptoms, including jaundice. In contrast, only 30 percent of children younger than six years of age develop symptoms, which usually are nonspecific and flu-like without jaundice. Because even asymptomatic infected children may shed virus in their stools for up to six months, infection in children often initiates and perpetuates community-wide outbreaks. On rare occasions, hepatitis A virus has been transmitted by transfusion of blood products collected during the donor’s viremic phase. The potential for transmission via intravenous drug use is unknown [2].

The incubation period is 15 to 50 days, with an average of 25 to 30 days. Common symptoms include malaise, loss of appetite, nausea and icterus. Symptoms, which are often mild and even subclinical, usually resolve within two to four weeks of onset. Recovery is complete, with no chronic sequelae and long-lasting, usually lifelong, immunity results. Although the disease is usually mild in young children, its severity increases with age. Peak infectivity correlates with the greatest viral excretion in the stool during the two weeks before the onset of jaundice or elevation of liver enzyme levels. Viremia occurs soon after infection and persists through the period of liver enzyme elevation [3].
Highly effective vaccines against hepatitis A have been available since the mid 1990s and have proven to reduce disease burden and offer protection to at-risk populations. However, vaccination is expensive and there are numerous reports worldwide on the changing epidemiology of hepatitis A and its prevalence. Therefore, information on the prevalence of immunity in each population would be required for any national hepatitis vaccination programme Levy et al. [4]. HAV infection in childhood decreases in many developing countries including Saudi Arabia; the pool of susceptible adults increases. The prevalence of HAV is closely linked to the level of development in socio-economic status and sanitation, it is expected that further decline in HAV infection may occur in Saudi Arabia following the improvement in the living conditions.

The objective of this study was prevalence of Hepatitis A Virus (HAV) on Jeddah population, Saudi Arabia. Also find out the relationship of result with age, gender and ethnicity.

**MATERIALS AND METHODS**

The present work was carried out at the Biological Science Dept. college of Science. The procedures for this work as follows:

**Serum Samples Collection:** A total of 1050 serum samples; 581 Saudi samples and 469 non Saudi samples were collected some diagnostic laboratories throughout Jeddah. Saudi samples were collected from medical laboratory of King Fahd hospital, medical laboratory of King Abdul-Aziz University hospital, medical laboratory of University street hospital. Non Saudi samples collected from the Medical Laboratory of Ghulail Dispensary, Medical Laboratory of Badr Al deen Dispensary, Medical laboratory of AL-Quds dispensary and Medical laboratory of University street hospital. These laboratories supplied remnant sera from samples that had been submitted for serological testing and would otherwise have been discarded. Sera from subjects who were known to be infected with human immunodeficiency virus, hepatitis B virus and hepatitis C virus were excluded. Sera were identified at the referring laboratory by the sex of the subject, age or date of birth, date of collection and nationality. The samples were coded by date of collection, nationality/territory of origin, sample number and referring laboratory. All serum samples were stored at -20°C until use.

**Study Population:** Serum samples were collected from studied population between 2 and 35 or more years of age and stratified into the following age groups: 2 to 5, 6 to 9, 10 to14, 15 to19, 20 to 24, 25 to 29, 30 to 34 and more than 35 year, Serum samples were not available for less than 2 year of age. Serum samples also were stratified according to nationality into Saudi and non Saudi. And according to sex serum samples were stratified into male and female. Prevalence was calculated separately for each age group, for male and female separately and for studied population as a whole. Sample sizes were calculated to achieve a 95% confidence interval (CI) of approximately ±5% for each age group.

**Serological Testing:** Serum samples were tested for HAV-specific immunoglobulin G (IgG) using a HAV IgG enzyme-linked immunosorbent assay (ELISA) technique and procedure using Dia Sorin kit.

**Calculation of Cut-Off Value:** The cut-off value is determined by the mean absorbance for the 20 mIU/mL calibrator values (Cal).

**Interpretation of the Result:** The unknown samples with absorbance values below the grey zone should be considered reactive for anti-HAV antibodies. The unknown samples with absorbance values above the grey zone should be considered non-reactive. Samples with absorbance values within ± 20% of the cut-off value (grey zone) must be retested in duplicate in order to confirm the initial result. Samples which are repeatedly reactive in at least one replicate should be considered positive. Samples which are non reactive at the second test should be considered negative.

**Statistical Analysis:** The percentages of individuals with positive, negative and equivocal results were determined for each age group and sex. SPSS program version 15 was used for the analysis and comparison of sero-statuses among age groups, male and female and each nationality. Ninety-five-percent confidence intervals were calculated where appropriate and P values of < 0.05 were considered statistically significant.

**RESULTS**

A total of 1050 cases (559 male and 491 female) between ages of 2 years and over 35 years were enrolled in the study. The studied populations according to age
were stratified to eight age groups: Group 1 (2-5 years; n= 124), Group 2 (6-9 years; n=128), Group 3 (10-14 years; n= 132), Group 4 (15-19 years; n=136), Group 5 (20-24 years; n=134). Group 6 (25-29 years; n=131), Group 7 (30-34 years; n= 133), Group 8 (>35 years; n= 132).

Demographic Characteristics of the Studied Population:
Prevalence of HAV IgG in each group were tested using enzyme linked immunosorbent assay and calculated separately for each age group, for male and female separately and for studied population as a whole using chi square test. The demographic characteristic of population tested was age, sex, race and nationality (Saudi and non Saudi) as shown in Table 4.1.

Prevalence of Hepatitis a Virus According Sex (Males and Females): For all age groups, there was no significant difference between genders regarding anti-HAV seropositivity. The prevalence of IgG antibodies to HAV was 33.1% overall, 33.6% among males and 32.5% among females with no statistically significant difference (P>0.05). However, it increased significantly with the age. These were shown at Table 4.2.and Figure 1.

Prevalence of Hepatitis a Virus According Nationality: Table 4.3.and Figure 2. show prevalence of Hepatitis A virus by nationality. The IgG antibodies to HAV were found in 20.3 % of Saudi population and 49% of non Saudi population (P<0.05). A higher proportion of non-Saudi population were found anti HAV seropositive than Saudi population. There was significant difference between nationalities regarding anti-HAV seropositivity.

Prevalence of Hepatitis a Virus by Age Groups from 2 to > 35 Years Old: The overall prevalence of total anti-HAV was 33.1% (95% CI: 31.9% - 34.1%). Total anti-HAV seroprevalence was significantly different between age groups (P<0.05). HAV prevalence rates according to age groups were as follows: Group 1; 17% (95% CI: 15.8% - 21.4%), Group 2; 21.9%(95% CI: 16.5% - 26.1%), Group 3; 28.8%(95% CI: 25.1% - 32.3%), Group 4; 27.2%(95% CI: 25.5% - 28.9%), Group 5; 34.3%(95% CI: 25.2% - 44.6%), Group 6; 38.2%(95% CI: 33.1% - 43.5 %), Group 7; 47.7% (95% CI: 42.7% - 52.5%), Group 8; 49.2%(95% CI: 46.1% - 52.5%). The prevalence of HAV by age groups the results were shown at Table 4.4. and Figure 3.

5. & 6. Prevalence of Hepatitis a Virus in Females and Males by Age Groups: The female were stratified to eight age groups: Group 1 (2-5 years; n= 58), Group 2 (6-9 years;
The overall prevalence of total anti-HAV in males was 33.6% (95% CI: 31.9%-34.1%). HAV prevalence rates according to age groups were as follows: Group 1; 16.6% (95% CI: 10.4% - 23%), Group 2; 17.9% (95% CI: 15.5% - 25.4%), Group 3; 28.8% (95% CI: 19.9% - 36.9%), Group 4; 26.6% (95% CI: 17.7% - 35.7%), Group 5; 35.2% (95% CI: 24.2% - 53.4%), Group 6; 39.7% (95% CI: 29.3% - 48.5%), Group 7; 49.2% (95% CI: 38.6% - 58%), Group 8; 53.6% (95% CI: 37.7% - 67.1%).

Table 4.6 and Figure 5 show the results of HAV prevalence in males according age group.

Prevalence of Cytomegalovirus in Females and Males According Age Groups: The prevalence of HAV among adult population (39.3%) was higher than among children population (22.4%). The chi-square test showed significant
Table 5: Prevalence of Hepatitis A Virus in Females according age group:

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of Samples</th>
<th>No.</th>
<th>%</th>
<th>χ² (p)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - 5</td>
<td>58</td>
<td>10</td>
<td>17.2</td>
<td>Reference</td>
<td>18.5-25.5</td>
</tr>
<tr>
<td>6-9</td>
<td>62</td>
<td>16</td>
<td>25.8</td>
<td>0.316 (&lt;0.05)</td>
<td>22.0-30.0</td>
</tr>
<tr>
<td>10-14</td>
<td>59</td>
<td>17</td>
<td>28.8</td>
<td>2.206 (&lt;0.05)</td>
<td>18.8-40.6</td>
</tr>
<tr>
<td>15-19</td>
<td>61</td>
<td>17</td>
<td>27.8</td>
<td>1.914 (&lt;0.05)</td>
<td>26.4-34.4</td>
</tr>
<tr>
<td>20-24</td>
<td>63</td>
<td>21</td>
<td>33.3</td>
<td>4.104 (&lt;0.05)</td>
<td>32.2-38.2</td>
</tr>
<tr>
<td>25-29</td>
<td>63</td>
<td>23</td>
<td>38.2</td>
<td>5.652 (&lt;0.05)</td>
<td>31.9-41.5</td>
</tr>
<tr>
<td>30-34</td>
<td>62</td>
<td>28</td>
<td>45.1</td>
<td>10.795 (&lt;0.05)</td>
<td>38.4-51.4</td>
</tr>
<tr>
<td>&gt;35</td>
<td>63</td>
<td>28</td>
<td>44.4</td>
<td>10.374 (&lt;0.05)</td>
<td>37.1-55.5</td>
</tr>
</tbody>
</table>

Table 6: Prevalence of Hepatitis A Virus in Males according age group:

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of Samples</th>
<th>No.</th>
<th>%</th>
<th>χ² (p)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - 5</td>
<td>66</td>
<td>11</td>
<td>16.6</td>
<td>Reference</td>
<td>10.4-23</td>
</tr>
<tr>
<td>6-9</td>
<td>66</td>
<td>12</td>
<td>17.9</td>
<td>0.390 (&lt;0.05)</td>
<td>15.5-23.4</td>
</tr>
<tr>
<td>10-14</td>
<td>73</td>
<td>21</td>
<td>28.8</td>
<td>2.864 (&lt;0.05)</td>
<td>19.9-36.9</td>
</tr>
<tr>
<td>15-19</td>
<td>75</td>
<td>20</td>
<td>26.6</td>
<td>2.047 (&lt;0.05)</td>
<td>17.7-35.7</td>
</tr>
<tr>
<td>20-24</td>
<td>71</td>
<td>25</td>
<td>35.2</td>
<td>6.072 (&lt;0.05)</td>
<td>24.2-53.4</td>
</tr>
<tr>
<td>25-29</td>
<td>68</td>
<td>27</td>
<td>39.7</td>
<td>8.751 (&lt;0.05)</td>
<td>29.3-48.5</td>
</tr>
<tr>
<td>30-34</td>
<td>71</td>
<td>35</td>
<td>49.2</td>
<td>16.33 (&lt;0.05)</td>
<td>38.6-58</td>
</tr>
<tr>
<td>&gt;35</td>
<td>69</td>
<td>37</td>
<td>53.6</td>
<td>20.107 (&lt;0.05)</td>
<td>37.7-67.1</td>
</tr>
</tbody>
</table>

Table 7: Prevalence of Hepatitis A Virus by age

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>No.</th>
<th>%</th>
<th>χ² (p)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>384</td>
<td>86</td>
<td>22.4</td>
<td>31.56 (&lt;0.05)</td>
<td>14.0-48.0</td>
</tr>
<tr>
<td>Adult</td>
<td>666</td>
<td>262</td>
<td>39.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1050</td>
<td>348</td>
<td>33.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

differences in the prevalence of HAV between adult and children population (p<0.05). The prevalence of HAV among children and adult population as shown at Table 4.7 and Figure 4.6.

**DISCUSSION**

Hepatitis A virus (HAV) is a major global public health problem especially in developing countries Hollinger & Ticehurst [5]. In these countries, most infections occur under the age of 10 and majority of these infections are asymptomatic. In contrast, the seroprevalence in several industrialized countries in pediatric ages are low and infection is usually acquired during late adolescence and early adulthood and accompanies with significant morbidity (CDC, 2005). The severity of acute hepatitis A increases with age. Most infections that occur in children younger than age 6 are asymptomatic and jaundice is rare. Infections in older children and adults are usually symptomatic, with the majority exhibiting jaundice [6].

The virus replicates in liver, is excreted in the bile and is found in high In our study, the enzyme linked immunosorbent assay was used for the detection of HAV IgG in studied population from different nationalities (Saudi and non Saudi) in Jeddah region. The seroprevalence of HAV IgG was 33.1% in studied population. The prevalence rate was suggestive of ubiquitous past exposure to infection. This perhaps could be related to socio-economic, environmental and climatic factors. The seroprevalence of antibodies to HAV varies in different population and in different places. The results of our study are generally consistent with those of other reports from other countries where HAV infection occurs early in life. Ataei et al found that the seroprevalence for anti-HAV IgG in children over 6 years from urban and rural areas of the Isfahan (Borkhar and Meimeh) was 28.6% [7]. Alkhalidi et al. [8] reported that the seroprevalence of HAV among healthy outpatients applying for civilian or military jobs in Kuwait was 28.8% [8]. Al Faleh et al. [9]. Recorded that the prevalence of HAV seropositivity Saudi children in Madinah was 27.4%. Moisseeva et al found that seroprevalence of HAV antibodies in persons aged 1 to 85 years, visiting four municipal healthcare centres in the Ukrainian capital, Kiev was 31.9% Moisseeva et al. [10] and Bell et al. [11] reported that seroprevalence of HAV among U.S. population was 31.3%. Nubling et al found that the seroprevalence for anti-HAV IgG among hospital employees adult between 19-50 in German was 28% [12]. Tseng et al found the seroprevalence of HAV among healthy residents in Taiwan was 25.2% [13].

The results of our study are different with those of other reports from other countries and this perhaps due to the influence of many factors, such as hygienic circumstances, ethnic and socioeconomic factors and sexual contacts. Ahmed et al found that the prevalence of HAV in Bangladesh was 74.8% [14], Gadgil reported in the Indian population indicating a rise in the rate of hepatitis A infection among adults. Positive samples were tested for HAV-RNA. Age wise anti-HAV positivity was significantly low in adults aged 18-25 years (90.4%) compared to those aged >25 years (97.4%) [15]. Salama et al determine seroprevalence of anti-hepatitis A virus (HAV) children aged 3-18 years from low socioeconomic status areas and 142 from high
socioeconomic status areas in Cairo Seropositivity to anti-HAV antibodies was significantly higher among children of low and very low socioeconomic status, 90%, compared to children of high socioeconomic status, 50% [16].

Our results are consistent with the result of other researches carried out in different parts of the world. Mehr et al. [17] found that the HAV seroprevalence among children in Iran was 22.2% in males and 22.5% in females. Almuneef et al. reported the prevalence was almost the same in male and female (28.2%, 29.5%, respectively). Among children in Saudi Arabia Almuneef et al. [18]. In another study, carried out in Saudi Arabia, anti-HAV IgG antibodies were detected in 51.3% of males and 53.5% of females. Yap et al. [19] reported that the seroprevalence of HAV within the healthy volunteers in Singapore between the year 1987 and 1991 in women subjects (26%) and men subjects (29%). Jindal and his colleagues also found that the prevalence of HAV among adult population in New Delhi was 64% in females and 60.9% in males [20].

The result of present study is also consistent to the result of another study carried out by Lafer et al. in the year 2001 to determine HAV prevalence in the Native Indians from Xingu National Park, Brazil, in five Kaiabi and Kuikuro villages was 93.7% (age 1-5), 98.2% (age 6-10), 100% (age 11-15), 100% (age 16-20), 100% (age 21+) [21]. El-Gilany et al. reported that the ethnicity appeared to be related to seroprevalence of Hepatitis A virus (HAV) among children between 1-6 years in Northern region, Saudi Arabia. A higher proportion of non-Saudi children 43.1% were found anti HAV seropositive than Saudi children 32.7% [22]. In the study reported herein, the seroprevalence of HAV IgG among the studied population varied with age ranging from 17% in 2 to 5 year age group, 21.9% in the 6 to 9 year age group, 28.8% in the 10 to 14 year age group, 27.2% in the 15 to 19 year age group, 34.3% in the 20 to 24 year age group, 38.2% in the 25 to 29 year age group, 47.7% in the 30 to 34 and 49.2% in >35 year age group. The prevalence increased significantly with age when all age groups compare with 2 to 5 year age group as reference (p< 0.05) except for the age group 6 to 9 year, 10 to 14 and 15 to 19 years (p>0.05). This perhaps due to the immune system gets weaker with age.

The results of the present study is consistent to the studies reported elsewhere which showed a significantly increased seropositivity with increasing age. Almuneef et al. found that an overall association identified between seroprevalence of HAV in Saudi Arabia and increasing age. For the groups of children aged < 8 years and 8-11 years, the recorded seroprevalence was 7% and 14% respectively. Seroprevalence continued to rise with age from 30% in 12- to 15-year-olds to 52% in over 16 years -year-olds [22]. In another study, carried out to study the seroprevalence of HAV among the healthy urban children in Jeddah, Makkah Province, 14.8% of the children aged between 4 to 6 years were seropositive for HAV, as against 38.1% in those aged 7-11 years [22]. Fathalla et al. reported that the seroprevalence in Dammam.

In conclusion, a 33.1% prevalence of HAV infection was identified in this and it confirms a moderate sero-prevalence of HAV infection in studied population in the Jeddah region. The seroprevalence of HAV IgG among the studied population varied with nationality and/or ethnicity, the highest rates of prevalence was found in non-Saudi (49.0 %) and the lowest rates of prevalence was found in Saudi (20.3 %). The high prevalence rate suggests of ubiquitous past exposure to infection and this perhaps could be related to socio-economic, environmental and climatic factors. This study found that prevalence of HAV was no significant difference in females (32.5%) and males (33.6%). The seroprevalence of HAV IgG among the studied population increased gradually with age from 17 % in 2 to 5 year age group to 49.2 % in the >35 year age groups.

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