Molecular Detection and Immunology Study of Hepatitis A Virus in Children under 6 Years Age

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ABSTRACT

Introduction: Hepatitis A virus (HAV) infection is the most common viral infection that causes hepatitis and remains a global health problem for both developed and non-developed countries.

Method: This study was done in Al Suwaira General Hospital for 70 current HAV infections (1–6 years old) children with the addition of 20 healthy children.

Result: Infection rates were higher in patients between (3–6) years than in (1–3) years by using both enzyme-linked immunoassay (ELISA) and polymerase chain reaction (PCR) techniques. Infection rates were also higher in patients in rural areas than urban areas. The interferon level was significantly higher in children who have current HAV infection than the healthy control children.

Keywords: Hepatitis A Virus, Immunology Study, Infection rate.

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INTRODUCTION

Hepatitis A virus causes acute liver disease, estimated to cause HAV to 10 million people worldwide each year. In Iraq, HAV infection is the most common cause of hepatitis after HBV. The time between infection and symptom appearance (incubation) is between 2-6 weeks, with an average incubation time of 28 days and the end-of-incubation of HAV on the faces. HAV is transmitted mainly through fecal and fecal oral routing and is gained by consuming contaminated food or water. HAV is usually autonomous, although symptoms may vary from asymptomatic hepatitis to fulminant hepatitis. HAV infection was chronic, even though hepatitis A has been reported to be prolonged or relapsed. The symptoms of acute viral hepatitis include nausea, vomiting, fatigue, malaise, abdominal pain, poor appetite and fever. HAV infections are not distinguished from the other types of acute viral hepatitis. Young kids generally become asymptomatic, although symptoms are more likely to occur with age. HAV is an unenveloped RNA virus in the Picornaviridae family classified as the genus Hepatovirus. For months, HAV can be environmentally stable. The virus is relatively stable at low pH and freezes at moderate temperatures. However, it can be inactivated with high temperatures or surfaces with a 1:100 sodium hypochlorite dilution into water ([85°C] or more for one minute). HAV endemicity depends largely on socio-economic development and is typically the highest in low sanitation and safe drinking water regions. The majority of adults are HAV-prone in high-income countries and dietary outbreaks are increasing.

Active hepatitis A diagnoses are most commonly made by detecting an anti-hepatitis A antibody, immunoglobulin M (IgM). The general preventive measures of treatment are generally beneficial, include improved hygiene standards, hygiene and vaccination. HAV is a disease prevention vaccine. The most effective method for eradicating and preventing transmission is universal childhood vaccination.

METHOD

The present study was conducted to understand the hepatitis A virus (HAV) infection status among young children (1–6 years age) in Suwaira, wasit, Iraq. Blood samples from 70 individuals, who visited Al- Suwaira General Hospital for different health complaints, including symptoms of acute hepatitis, were collected. The demographic information (age, gender, and residential region) of the participants was recorded. Blood samples from 20 healthy child collected (as a control). The blood samples were first subjected to immune-globulin M (IgM) based serological test to identify seropositivity (for current infection). Second, the samples were introduced to reverse transcription polymerase chain reaction (RT-PCR) test to determine current (active) infection.

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RESULT

The current study revealed that the infection rates were higher in the age range of 3–6 years old in both ELISA and PCR.

In ELISA (55/70 patients) at an infection rate of 78.6%, especially when compared to those from the age range of less than three years old (15/70 patients) at an infection rate of 21.4% (Table 1). In PCR (20/70 patients) at an infection rate of 28.6%, especially when compared to those from the age range of less than three years old (4/70 patients) at an infection rate of 5.7% (Table 2). Also, the rates of infection by HAV were higher in rural than those in urban areas (Table 3). β-IFN levels in the serum of HAV patients were significantly higher than those from the control subjects (Table 4).

DISCUSSION

One of the biggest frequent causes of acute hepatitis globally is HAV. The WHO statistics claim that there were around 13.7 million HAV infections and 28,000 HAV-related fatalities in 2010. HAV is most often spread via contact with an infected individual or via contaminated food or water. An increased level of cleanliness, along with HAV vaccination, correlates with lower levels of HAV endemicity. In nations with high, moderate, low, and extremely low HAV endemicity, the globe may be classified into countries with varying levels of HAV prevalence. Many individuals in countries with a high HAV prevalence get infected during childhood and remain HAV-immune for life. Opposite to that, adults from endemic regions are more likely to come into contact with HAV by travelling to or residing in an endemic area, or participating in high-risk activities, such as sexual contact with infected individuals.13-15 Gupta et al., 2019 have found that, in 1084 children, one third of the research subjects (31.7%) were between the ages of four and five years, another third (25.6%) were between the ages of two and less than three years, a third part of subjects (23.2%) were between the ages of three and four years, and a final third (19.6%) were between the ages of one and less than two years of old. Gupta et al.16 have recorded that the HAV total prevalence rate in children between the ages of one and five years ranged from 39% to 47%, and the rate for those aged one to less than two years of old was lower than for children ages four to five years of old. This agrees with our findings, in which the high rate of prevalence was in children age between 3 to 6 year old than that from the age range of 1 to less than 3 years old. In an agreement with our results, Behzadi et al.17 have found that the seropositivity of infection was in a significant positive correlation as age

Table 1: Frequency distribution of hepatitis A virus seropositive patients and control individuals based on age using IgM test.

<table>
<thead>
<tr>
<th>Control (n=20)</th>
<th>Patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 years old</td>
<td>3–6 years old</td>
</tr>
<tr>
<td>10 (50)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>¥ = 18.36</td>
<td>OR = 3.76</td>
</tr>
</tbody>
</table>

n: number of cases; S: Significant at p<0.05; ¥: Chi-square; OR: Odds ratio; different letters mean significant tests.

Table 2: Frequency distribution of hepatitis A virus positive patients and control individuals based on age using qPCR.

<table>
<thead>
<tr>
<th>Control (n=20)</th>
<th>Patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 years old</td>
<td>3–6 years old</td>
</tr>
<tr>
<td>QPCR n (%)</td>
<td>A</td>
</tr>
<tr>
<td>10 (50)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>¥=11.53</td>
<td>OR = 4.8</td>
</tr>
</tbody>
</table>

n: number of cases; S: Significant at p<0.05; ¥: Chi square; OR: Odds ratio; different letters mean significant tests.

Table 3: Frequency distribution of hepatitis A virus positive patients and control individuals based on area of residence using ELISA IgM.

<table>
<thead>
<tr>
<th>Residence</th>
<th>Control (n=20)</th>
<th>Patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Urban</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Rural</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>¥ = 8.3</td>
<td>OR = 2.3</td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; S: Significant at p<0.05; ¥: Chi square; OR: Odds ratio; different letters mean significant tests.

Table 4: Beta IFN levels of hepatitis A virus positive patients and control individuals.

<table>
<thead>
<tr>
<th>Control</th>
<th>Patients</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SE (pg/mL)</td>
<td>897.6 ± 54.1, n = 20</td>
<td>1307 ± 68.88, n = 70</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>

R square = 0.09784

n: number of subjects; S: Significant at p<0.05; different letters mean significant tests.* Significant link between Beta IFN and hepatitis A Virus.
increases. This may be related to water contamination, such as with inadequate or polluted wastewater disposal system or polluted water source. The water of the tested areas for the presence of HAV infection may be contaminated with HAV because of untreated sewage, which originates from human waste. HAV prevalence was found to be greatest in Bashaghard County (95.8%) and lowest in Bandar Abbas County (88.1%) from Iran. Human waste is more common in rural regions, and whereas in the city, the sewage is nearly handled correctly. The most important source of agricultural irrigation and illness in the human community is found in these waterways. Our findings concur with earlier studies, showing that the HAV Ab prevalence rises with age, further implying the long-term exposure to HAV. In several Eastern Mediterranean nations, HAV seroprevalence has been found to be above the world average. Most in Afghanistan (99%), Iraq (96%), and Palestine (93.7%), with the least reported incidence in the United Arab Emirates (UAE) and Kuwait, which has a frequency assessed to be below 50%. There were reported to be 62% HAV seroprevalence in Iran, which may be defined as moderate endemicity for HAV infection. HAV seroprevalence levels fluctuate greatly across Asia, ranging from high to moderate and then to low. Some nations with a low incidence, like as Japan, have seen their prevalence decline significantly in the past few years. While older studies found that in the 1970s more than 90% of adults had anti-HAV antibodies, afterward surveys demonstrate that in the Taipei metropolitan region, the prevalence was close to zero, and in rural areas, very few people, particularly adolescents and young adults, were found to have had the virus in the past. There has been a rise in the number of cases of adult hepatitis A during the last few generations in Korea. Moreover, age-specific HAV seroprevalence patterns have altered as a result of economic development. 10–50 year-olds have seen the prevalence of anti-HAV decrease significantly over the past three decades. Because of this, people in this age range have a significant risk of HAV infection and of developing clinical hepatitis A, which has been shown to be on the rise in adolescents and adults.

CONCLUSIONS

The present study conclude that the infection rates were higher in the age range of 3–6 years old compared to those from the age range of less than three years old. The rates of infection by HAV were higher in rural than those from urban areas. Not all IgM positive patients had the virus in their serum in the use of PCR technique.

REFERENCES


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