Research Article

Hepatitis A Seropositivity among Children and Students Under 18 Years Old in Sulaimani Governorate-KRG

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Abstract: To find the rates of anti-HAV IgG and IgM seropositivity among less than 18 years-old persons, 328 cases were tested by ELISA in Sulaimani Governorates, Iraqi Kurdistan region. It was observed that 47.86% cases were seropositive for anti-HAV IgG whereas only 16.15% were positive for anti-HAV IgM. Sex showed no significant effects on positive results for both IgG and IgM (p = 0.435, 0.891) respectively. The highest rate of anti-IgG seropositivity was among cases with more than 12 years-old (80.82%) while the highest rate for anti-IgM was among 5-8 years old (2.89%). The age showed noticeable effects on anti-IgG seropositivity (p = 0.000) unlike anti-IgM (p = 0.521). The child educational level also showed a significant effect on anti-IgG (p = 0.000) and anti-IgM (p = 0.0022). It was noticed that the well water has a significant effect on anti-IgM seropositivity (p = 0.027) among tested cases, while other sources showed no effects on the results (p>0.05). Family education showed significant effects on the anti-IgG seropositivity (p = 0.0022) unlike anti-IgG (p = 0.713). Moreover, it was concluded that the living places and family sizes were significantly effective on both anti-IgG and IgM seropositivity (p = 0.0022 and 0.0020) respectively for living places and (p = 0.0019 and 0.047) for family size). Socioeconomic status also significantly effective on anti-IgG (p = 0.0008) and anti-IgM seropositivity (p = 0.0078). The jaundice history showed valuable effects on anti-HAV IgG (p = 0.0207) and IgM seropositivity (p = 0.0017).

Keywords: Anti-IgG, anti IgM, HAV, oral-fecal route, socioeconomic status

INTRODUCTION

In 1967 the term of hepatitis A was, firstly, used for describing a liver infection due to a virus known as Hepatitis A virus (Krugman et al., 1967). Hepatitis A virus possesses a single stranded positive-sense RNA genome, non-enveloped, acid stable and it is a member of Picornaviridae family. However, it was formerly classified as Enterovirus-serotype 72-within the Picornaviridae family (Rueckert and Wimmer, 1984; Melnick, 1992), moreover, due to its unique feature among the genus Hepatovirus, it requires a long adaptation period to replicate in cell culture and rarely produces a cytopathic effect (Cromeans et al., 1987; Lemon, 1992). Hepatitis A virus can be transmitted by fecal-oral and the infection usually acquired by person-to-person contact or through ingestion of contaminated food or water (Cliver, 1985). Hepatitis A infection can induce life-long immunity whereas it can also produce effects ranging from asymptomatic to fulminant hepatic failure, unfortunately in some cases may lead to death (Ross and Anderson, 1991). However, it was previously reported that the fatality rates among Hepatitis A infected individual was lower than 0.1%, as well as it was noticed that young children and adults with underlying chronic liver disease were at risk (Akriviadis and Redeker, 1989).

The severity of hepatitis A infection and clinically apparent infection usually increases with age, as it was reported by Hadler et al. (1980) that more than 70% of HAV infection can be occurred among children aged less than six years with no symptoms, while between 70% of adults infected with hepatitis A virus suffered from more severe infections with clinical symptoms, including jaundice malaise, fever and dark urine in 70% of cases (Lednar et al., 1985). HAV is a worldwide infection and the rates of its prevalence are strongly related to the degree of sanitation and health hygiene (Hadler, 1991). In certain areas of endemic HAV infection, the percentage of anti HAV IgG seropositivity may reach 90% among adults, whereas in areas with intermediates endemicity the rates declined to 50-60%. At the same time, it was reported that in areas with lowest endemicity rates where hygienic improvement and overall rises in socioeconomic status have brought a fall in HAV infection during early childhood, the majority of adults remains susceptible to HAV infection and the chance of outbreaks is high (Mackiewicz et al., 2005).

The current study is aimed to show the percentage of seropositivity of Hepatitis A IgG and IgM rather than Viral RNA among distinctive age groups, including preschool, primary school and adults in some rural urban areas of Sulaimani Governorates, in addition to studying distinct factors, including socioeconomic status; water supplies sources...and their relation to the seropositivity of HAV.
MATERIALS AND METHODS

The samples were collected from (328) children and students from different villages, small towns and cities located in Sulaimani Governorate during February to June 2015. IgG and IgM for hepatitis A virus was detected using ELISA technique. Three ml of fresh blood was collected from each case and after separation of the serum the samples were stored in -45°C until further works performed. The ELISA protocol for detection of both IgG and IgM was done according to supplied company directions and the results were tabulated. Both anti-HAV IgG (EIA- 4233) and anti-HAV IgM(EIA-3889) kits (DRG Diagnostics, Germany) were uses for performing Enzyme-Linked Immunosorbent Assay.

Anti-HAV IgG detection: The assay is based on the principle of competition where the antibodies in the sample compete with anti-HAV specific antibodies, labeled with HRP, for a fixed amount of antigen on the solid phase. A purified and inactivated HAV is coated to the micro-wells. The patient’s serum is added to the micro-well and antibodies to HAV are captured by the solid phase. As the plates washed, the enzyme conjugate was added, which can bind to the free HAV antigen if still present. Before adding the chromogenic substrate, the plate also was washed. The intensity of color changes of the end-product was measured.

Anti-HAV IgM detection: The purified Anti-µ-chain is coated on the solid phase of multi-wells. Serum sample, HAVAg and Horseradish peroxidase labeled with Anti-HAV (conjugated) were added to coated wells. After incubation, (in the case of presence of HAV-IgM, a complex of Anti-µ-chain-HAV-IgM-HAVAg-Anti-HAV labeled with HRP will form. When the plate washed, the wells were incubated with the substrate (TMB). The intensity of colored end-product was measured at 450 nm.

Statistical analysis: SPSS V.17 software was used and Chi-square test was depended for statistical analysis.

RESULTS

From the current study in general out of (328) tested samples 157(47.86%) were positive for anti-Hepatitis A IgG, whereas the rate of seropositive anti-HAV IgM was lower, which was 53 (16.15%) tested samples. Only five (1.5%) cases were positive for both anti-HAV IgG and IgM. Moreover, it was noticed that sex has no significant effects upon the percentage rates of HAV seropositivity regarding IgG, IgM and both (p = 0.435, 0.588 and 0.891 respectively) (Table 1).

The tested cases were grouped into five sub-groups and it was noticed that the age showed weighty effects on the percentage rates of anti-HAV IgG seropositivity (p = 0.00) as it was also concluded that the higher the age, the higher the percentage rates of anti-HAV IgG and IgM seropositivity. Whereas age showed no significant effects on the percentage rates of positive results related to anti-HAV IgM and both IgG-IgM seropositivity (p = 0.169, 0.521) respectively (Table 2).

The education level of the tested children also was studied and it was concluded that the higher percentage rates of anti-HAV IgG and IgM were higher among those who are in primary schools and it was appeared that the education level of children was significantly effective on anti-HAV IgG and IgM seropositivity (p = 0.00, 0.002) respectively, whereas showed no significant effects on IgG-IgM seropositivity percentage rates (p = 0.269) (Table 3).

Moreover, the effects of water supply were studied. It was noticed that water supply has a weighty effect on the acute HVA infection expressed as anti-HAV IgM seropositivity (p = 0.027), whereas no significant effects were seen for anti-HAV IgG and IgG-IgM (p = 0.589, 0.594) respectively. It was concluded that the wells as water supply sources cause highest anti-HAV seropositivity (Table 4).

Family education level also was another factor, which was studied. It was appeared as that family education has a significant effect in anti-HAV IgM seropositivity (p = 0.0022) while no significant effects were observed in regard to anti-HAV IgG and IgG-IgM.

| Table 1: Anti-HAV IgG and IgM seropositivity related to gender | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|---------------------------------------------------------------|-------------|-------------|-----------------|
| Studied parameters                                            | Tested No.  | No (%)      | p-value         | No (%)      | p-value         | No. (%)     | p-value |
| Sex                                                          |             |             |                |             |                |             |         |
| Males                                                        | 187         | 93 (49.73)  | 0.435          | 32 (17.11)  | 0.588          | 3 (1.6)     | 0.891   |
| Females                                                      | 141         | 64 (45.39)  |                | 21 (14.89)  |                | 2 (1.41)    |          |
| Total (Mean)                                                 | 328         | 157 (47.86) |                | 53 (16.15)  |                | 5 (0.015)   |          |

| Table 2: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to age groups | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|-------------------------------------------------------------------------|-------------|-------------|-----------------|
| Studied parameters                                                        | Tested No.  | No (%)      | p-value         | No (%)      | p-value         | No. (%)     | p-value |
| Age groups                                                               |             |             |                |             |                |             |         |
| <3 years                                                                  | 41          | 9 (21.95)   | 0.000          | 3 (7.31)    | 0.167          | 0 (0.0)     | 0.521   |
| 3-5 years                                                                 | 77          | 22 (28.57)  |                | 9 (11.68)   |                | 2 (2.59)    |          |
| 5-8 years                                                                 | 69          | 31 (44.92)  |                | 12 (17.39)  |                | 2 (2.89)    |          |
| 8-12 years                                                                | 68          | 36 (52.94)  |                | 13 (19.11)  |                | 1 (1.47)    |          |
| >12 years                                                                 | 73          | 59 (80.82)  |                | 16 (21.91)  |                | 0 (0.0)     |          |

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Table 3: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to child education

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Child education    |            |              |              |                 |
| None               | 81         | 11 (13.58)   | 4 (4.93)     | 0 (0.0)         |
| Kindergarten       | 73         | 30 (41.09)   | 9 (12.32)    | 2 (2.73)        |
| Primary            | 112        | 81 (72.32)   | 27 (24.1)    | 3 (2.67)        |
| Secondary          | 62         | 35 (56.45)   | 13 (20.96)   | 0 (0.0)         |

Table 4: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to water supply sources

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Water supply       |            |              |              |                 |
| Natural springs    | 86         | 43 (50)      | 17 (19.7)    | 2 (2.32)        |
| Wells              | 39         | 21 (53.8)    | 11 (28.2)    | 1 (2.5)         |
| Chlorinated        | 203        | 93 (45.8)    | 25 (12.31)   | 2 (0.9)         |

Table 5: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to the family education

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Family education   |            |              |              |                 |
| Illiterate         | 70         | 41 (58.57)   | 17 (24.28)   | 2 (2.85)        |
| Primary*           | 101        | 49 (48.51)   | 24 (23.76)   | 2 (1.98)        |
| Secondary**        | 108        | 45 (41.66)   | 9 (8.33)     | 1 (0.92)        |
| University         | 37         | 16 (43.24)   | 3 (8.1)      | 0 (0.0)         |
| Postgraduate       | 12         | 6 (50)       | 0 (0.0)      | 0 (0.0)         |

Table 6: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to the lifestyle and living places

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Lifestyle (Living places) |        |              |              |                 |
| Urban              | 198        | 81 (40.9)    | 22 (11.11)   | 2 (1.01)        |
| Rural              | 130        | 76 (58.46)   | 31 (23.84)   | 3 (2.3)         |

Table 7: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to the family size

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Family size        |            |              |              |                 |
| 3-5                | 87         | 32 (36.78)   | 10 (11.49)   | 1 (1.49)        |
| 5-8                | 108        | 46 (42.59)   | 14 (12.96)   | 1 (0.92)        |
| < 8                | 133        | 79 (59.39)   | 30 (22.55)   | 3 (2.25)        |

Table 8: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to the socioeconomic status

| Studied parameters | Tested No. | Anti-HAV IgG | Anti-HAV IgM | Anti-IgG and IgM |
|--------------------|------------|--------------|--------------|-----------------|
| Socioeconomic status |          |              |              |                 |
| Very low           | 58         | 37 (63.79)   | 15 (25.86)   | 3 (5.17)        |
| Low                | 71         | 42 (59.15)   | 16 (22.53)   | 1 (14.08)       |
| Medium             | 90         | 41 (45.55)   | 15 (16.66)   | 1 (11.11)       |
| Good               | 68         | 21 (30.88)   | 4 (5.88)     | 0 (0.0)         |
| High               | 41         | 16 (39.02)   | 3 (7.31)     | 0 (0.0)         |

(P = 0.713, 0.738) respectively. The highest percentage rates on anti-HAV IgG was among those with postgraduate family education, while for IgM, it was among those whose families were illiterate (Table 5).

It was noticed that the percentage rates on anti-HAV seropositivity among rural inhabitants were higher than urban inhabitants. It was observed that the lifestyle and living places showed significant effects on anti-HAV IgG and IgM (p = 0.0022, 0.0020) respectively, while showed no effects on anti-HAV IgG-IgM seropositivity (p = 0.348) (Table 6).

The current study also revealed that the family size had significant effects of anti-HAV IgG and IgM seropositivity (p = 0.0019, 0.0047) respectively. The higher the family size the higher the percentage rates of seropositivity (Table 7).

Socioeconomic status also was another factor, which showed significant effects on both anti-HAV IgG and IgM seropositivity (p = 0.0008, 0.0078) respectively.

It was noticed that the lower the socioeconomic status, the higher the percentage rates of anti-HAV seropositivity (Table 8).

The effect of history of jaundice investigates as well and it was appeared that history and family history of jaundice were significantly effective on anti-HAV
seropositivity regarding both anti-HAV IgG and IgM (p = 0.0207, 0.0017) respectively (Table 9).

**DISCUSSION**

In General, the percentage rate of HAV seropositivity was relatively high among tested persons, although the rates of anti-HAV IgG were higher than anti-HAV IgM. Our results were agreed with observations recorded by Vitral et al. (2012) in Brazil who found that low socioeconomic status, age and parent’s education can act as a risk factor for HAV infection. Similar to our results, they found proportional relation between increasing the age and increasing the prevalence rate of HAV seropositivity where they found that the higher the age, the higher the rates of HAV seropositivity. Similarly, in a previous study, it was found significant relations between some risk factors and HAV seropositivity. It was noticed that the incidence of HAV infection and the prevalence of antibodies against HAV are closely associated with economic development and access to safe drinking water and sanitation. As individual income increases and access to safe drinking water and improved sanitation condition increases, the incidence of HAV infection decreases, the current results were agreed with those reported by Jacobsen and Weirsma (2010).

In a study done in Egypt in 2007 similar observations were reported regarding relations between socioeconomic status, age and poor sanitary areas that showed significant effects on HAV seropositivity like the results from this study (Salama et al., 2007). Unlike our observations, they found no weighty relations between HAV seropositivity with family size and history of jaundice. It was reported by other researchers in Palestine that socioeconomic status had significant effects of HAV seropositivity (Yassin et al., 2001), which was similar to the results observed during this study.

Antaki and Kebbewar (2000) in Syria found an elevated prevalence of HAV in their study on HAV seropositivity, which was similar to the current results obtained during this study. Investigators in India found that the prevalence of HAV seropositivity was relatively high among preschool children and reached (90.9%) (Das et al., 2000) which is in agreement with conclusions recorded during this study.

The family size was among the important efficient factors on HAV seropositivity in the present study, which agreed with observations reported other investigators who found significant higher prevalence of HAV among crowd families (Fix et al., 2002). Similarly, in a previous study done in Saudi Arabia, related results were noticed, which designated the family size as an important effective factor on HAV seropositivity (Khalil et al., 1998) which agreed with the results of the existing study. The results observed in the present study were disagreed with observations reported by Ciaccia et al. (2012) in city of Santos, who found that the general prevalence of anti-HAV IgG was 9.72%, among them 74.6% were reactive for anti-HAV IgM. Unlike their results, we noticed higher percentage rates as well as the percentage rates of IgG seropositivity were higher than IgM seropositivity. Whereas our results were agreed with some other results, including child education and sex, which showed similar effects on HAV seropositivity. Moreover, it was reported by them that parent education had no significant effect, which was disagreed with the results of the present study where parent’s education showed significant effects. The results of the existing study were agreed with observations recorded by other researchers in Mexico in 1997 who found that child and parent's education level were significantly effective on HAV seropositivity (Redlinger et al., 1997). Moreover, they found that the age and drinking water supply (source) were also having a significant effect on HAV seropositivity, which was similar to the current results. It was found in a recent study in Turkey that the percentage of HAV seropositivity was lower than 20% (Arvas et al., 2014), which was distinct from the results recorded in the current study. They found that the percentage of anti-HAV IgM was higher than anti-IgG, which was entirely different from our results, where we noticed that the percentage rates of anti-HAV seropositivity were higher than that of anti-IgM.

Moreover, in a study done in Iran, it was noticed that the percentage of HAV seropositivity was 44.3% (Kazemi et al., 2007), which was lower than the results obtained by the current study. They noticed that the percentage was lesser among 7-8 years-old children in comparison to older ones. Our results agreed with these obtained results regarding the age groups and distributions of HAV seropositivity among tested children.

**CONCLUSION**

- The highest rate of anti-IgG seropositivity was among cases with more than 12 years-old (80.82%) while the highest rate for anti-IgM was among 5-8 years old (2.89%).
- The age, child educational level showed noticeable effects on anti-IgG seropositivity and on anti-IgM for the latter.
It was noticed that the well water has a significant effect on anti-IgM seropositivity.

It was concluded that the family education, living places, family sizes, socioeconomic status and history of jaundice were significantly effective on the hepatitis A seropositivity results among studied cases.

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