Asian Journal of Medical Sciences 7(4): 36-40, 2015
ISSN: 2040-8765; e-ISSN: 2040-8773

Submitted: May 11, 2015 Accepted: July 2, 2015 Published: October 25, 2015

Research Article

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

Salih A. Hama
Biology Department, School of Science, University of Sulaimani, Tel.: 009647701495656

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 ($p = 435, 0.891$) 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-IgM 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 *Picornavaridae* family. However, it was formerly classified as *Enterovirus*-serotype 72-within the *Picornavaridae* 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-orally 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 ratesamong 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 and urban areas of Sulaimani Governorate, 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 ant-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 the 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

Studied parameters		Tested No.	Anti-HAV IgG No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM 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

Studied parameters		Tested No.	Anti-HAV IgG No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM 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)	

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

Studied parameters		Tested No.	Anti-HAV IgG No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Child education	None	81	11 (13.58)	0.000	4 (4.93)	0.0022	0 (0.0)	0.269
	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 No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Water supply	Natural springs	86	43 (50)	0.589	17 (19.7)	0.027	2 (2.32)	0.594
	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 No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Family education	Illiterate	70	41 (58.57)	0.713	17 (24.28)	0.0022	2 (2.85)	0.738
	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)	

* Primary school ** secondary school

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

Studied parameters		Tested No.	Anti-HAV IgG No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Life style (Living places)	Urban	198	81 (40.9)	0.0022	22 (11.11)	0.0020	2 (1.01)	0.348
	Rural	130	76 (58.46)		31 (23.84)		3 (2.3)	

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

Studied parameters		Tested No.	Anti-HAV IgG No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Family size	3-5	87	32 (36.78)	0.0019	10 (11.49)	0.047	1 (1.49)	0.666
	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 No (%)	p-value	Anti-HAV IgM No (%)	p-value	Anti-IgG and IgM No. (%)	p-value
Socioeconomic status	Very low	58	37 (63.79)	0.0008	15 (25.86)	0.0078	3 (5.17)	0.139
	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

Table 9: Anti-HAV IgG, IgM and IgG-IgM seropositivity related to the history of jaundice

			Anti-HAV IgG		Anti-HAV IgM		Anti-IgG and IgM	
Studied parameters		Tested No.	No (%)	p-value	No (%)	p-value	No. (%)	p-value
History of Jaundice	Yes	129	73 (56.58)	0.0207	22 (17.05)	0.0017	2 (1.55)	0.953
	No	148	59 (39.86)		15 (10.13)		2 (1.35)	
	Family history	51	25 (49.01)		16 (31.37)		1 (1.96)	

seropositivity regarding both ant-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 testes 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.

REFERENCES

Akriviadis, E.A. and A.G. Redeker, 1989. Fulminant hepatitis A in intravenous drug users with chronic liver disease. Ann. Intern. Med., 110: 838-839.

Antaki, N. and M.K. Kebbewar, 2000. Hepatitis A seroprevalence rates in Syria. Trop. Doct., 30(2): 99-101.

Arvas, G., A. Bora, T. Özsari, B. Kaya and G. Açikgöz, 2014. Investigation of Hepatitis A seropositivity in children with different age groups in Igdir, Turkey. Comp. Res. J. Med. Med. Sci., 2(1): 8-13.

Ciaccia, M.C.C., R.C. Moreira, A.A. Ferraro, M.F. Lemos, G. Porta and I.T. Oba, 2012. Epidemiological and serological aspects of hepatitis A among children and teenagers in the city of Santos: A cross-sectional study. Sao Paulo Med J.; 130(4):230-5

Cliver, D.O., 1985. Vehicular transmission of hepatitis A. Public Health Rev., 13: 235-292.

Cromeans, T., M.D. Sobsey and H.A. Fields, 1987. Development of a plaque assay for a cytopathic, rapidly replicating isolate of hepatitis A virus. J. Med. Virol., 22: 45-56.

Das, K., A. Jain, S. Gupta, S. Kapoor, R.K. Gupta, A. Chakravorty *et al*., 2000. The changing epidemiology pattern of Hepatitis A in an Urban population in India: Emergence of a trend similar to the European countries. Eur. J. Epidemiol., 16(6): 507-510.

Fix, A.D., O.S. Martin, L. Gallicchio, P.A. Vial and R. Lagos, 2002. Age-specific prevalence of antibodies to hepatitis A in Santiago, Chile: Risk factors and shift in age of infection among children and young adults. Am. J. Trop. Med. Hyg., 66(5): 628-632.

Hadler, S.C., 1991. Global Impact of Hepatitis A Infection: Changing Patterns. In: Hollinger, F.B., S.M. Lemon and H.S. Margolis (Eds.), Williams and Wilkins, Baltimer.

Hadler, S.C., H.M. Webster, J.J. Erben, J.E. Swanson and J.E. Maynard, 1980. Hepatitis A in day-care centers. A community-wide assessment. New Engl. J. Med., 302: 1222-1227.

Jacobsen, K.H. and S.T. Wiersma, 2010. Hepatitis A virus seroprevalence by age and world region (1990 and 2005). Vaccine, 41: 6653-6657.

Kazemi, S.A., M. Mahram, A. Koosha and H.R. Amirmoghaddami, 2007. Seroprevalence of Hepatitis A in 7-10 year-old children. Iran J. Ped., 17(1): 47-51.

Khalil, M., *et al*., 1998. Childhood epidemiology of Hepatitia A virus in Riyadh, Saudi Arabia. Anna. Saudi Med., 18(1): 18-21.

Krugman, S., J.P. Giles and J. Hammond, 1967. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological and immunological types of infection. J. Am. Med. Assoc., 200: 365-373.

Lednar, W.M., S.M. Lemon, J.W. Kirkpatrick, R.R. Redfield, M.L. Fields and P.W. Kelley, 1985. Frequency of illness associated with epidemic hepatitis A virus infections in adults. Am. J. Epidemiol., 122: 226-33.

Lemon, S.M., 1992. Hepatitis A virus: Current concepts of the molecular virology, immunobiology and approaches to vaccine development. Rev. Med. Virol., 2: 73-87.

Mackiewicz, V., A.M. Roque-Afonso, E. Marchadier, E. Nicand, L. Fki-Berrajah and E. Dussaix, 2005. Rapid investigation of hepatitis A virus outbreak by single strand conformation polymorphism analysis. J. Med. Virol., 76: 271-278.

Melnick, J.L., 1992. Properties and classification of hepatitis A virus. Vaccine, 10(Suppl. 1): S24-S26.

Redlinger, T., K. O'Rourke and J. Van Derslice, 1997. Hepatitis A among Schoolchildren in a US-Mexico border community. Am. J. Publ. Health, 87(10): 1715-1717.

Ross, B.C. and D.A. Anderson, 1991. Characterization of hepatitis A virus capsid proteins with antisera raised to recombinant antigens. J. Virol. Meth., 32: 213-220.

Rueckert, R.R. and E. Wimmer, 1984. Systematic nomenclature of picorna virus proteins. J. Virol., 50: 957-959.

Salama, I.I., S.M. Samy, A.I. Hassanin and A.L. Abou Ismael, 2007. Seroprevalence of hepatitis A among children of different socioeconomic status in Cairo. Health Rev. La Mediterranee Oreintale, 13(6): 1256-1264.

Vitral, C.L., F.L.N. Ospina, S. Artimos, J.G. Melgaço, O.G. Cruz, V.S. de Paula, S.B. Luz, M. Freire, L.P. Gaspar, L.A. Amado, E.M. Engstrom, C.D.F.M. Fortes, T.C. de Souza, M.N. Dias, A.M.C. Gaspar and F.J.D. Souto, 2012. Declining prevalence of hepatitis A virus antibodies among children from low socioeconomic groups reinforces the need for the implementation of hepatitis A vaccination in Brazil. Mem. Inst. Oswaldo. Cruz. Rio de Janeiro, 107(5): 652-658.

Yassin, K., R. Awad, A. Tebi, A. Queder and U. Laaser, 2001. The epidemiology of hepatitis A infection in Palestine: A universal vaccination programme is not yet needed. Epidemiol. Infect., 127(2): 335-339.