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Acute HAV Infected Patient's Distribution According to Age and Sex in Wasit Province/Iraq.

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Abstract

Hepatitis A virus infection occurs globally and is causing a public health concern, primarily in developing countries due to its persistent circulation in the environment. A total of 120 individuals were included in this study, anti-HAV IgM antibodies were detected in serum of 100 individuals of them (patients group) who attended the Wasit central public health laboratory from different cities in Wasit Province, Iraq, during the period from November 2013 to March 2014.While another 20 individuals were considered as control group which anti – HAV IgM antibody was negative in their serum.

Acute infection is confirmed by detection of IgM anti–hepatitis A virus (HAV), which appears early in the course of infection, anti-HAV IgM antibodies were significantly higher ($P<0.001$) in hepatitis A patients than control using a solid phase, two-step incubation, antibody capture ELISA kit .

Most of the HAV patients were located within the first decade (1-10 years) with a percentage of 91%, whereas 9% of all patients were within second decade (10-20 years). In this study, HAV infection is distributed equally among genders. Therefore, there was no significant difference between females than male with a ratio of (1 female: 0.786 male) and percentage of 56% females than 44% males.

The aim of the study is to determine the distribution of acute HAV infection with age and sex of the infected patient in Wasit province/ Iraq.

Keywords: Acute Hepatitis A Virus, anti-HAV IgM antibody, Age, Sex.

توزيع الاصابة بمرض التهاب الكبد الفايروسي الحاد نوع أ بين المرضى حسب العمر والجنس في محافظة واسط /العراق

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الخلاصة

يحدث التهاب الكبد الفيروسي نوع A عدوى على مستوى العالم مما يسبب مصدر قلق على الصحة العامة، ولا سيما في البلدان النامية بسبب التداول المستمر في البيئة. وأدرجت ما مجموعه ١٢٠ فردا في هذه الدراسة، حيث شخصت الأجسام المضادة للغلوبولين المناعي نوع M لفايروس التهاب الكبد نوع A في مصول ١٠٠ شخص منهم (مجموعة المرضى)، الذين حضروا الى مختبر الصحة العامة المركزي في محافظه واسط من مدن ومناطق مختلفة فيها خلال المدة من تشرين الثاني ٢٠١٣ الى اذار ٢٠١٤. واعد العشرون شخصا الباقين مجموعة السيطرة لعدم ويوجد الأجسام المضادة للغلوبولين المناعي نوع M لفايرس

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التهاب الكبد نوع A في مصولهم. كما تم التاكد من وجود الاصابه من النوع الحاد عن طريق قياس تركيز الاجسام المضادة للغلوبولين المناعي نوع M للفايرس وكانت عاليه معنويا (P <0.001) في مجموعه المرضى عن مجموعة السيطرة باستخدام طريقه ELISA. معظم الاشخاص المرضى في هذه الدراسة تقع اعمارهم ضمن العقد الأول (١-١٠ سنة) وبنسبة ٩١٪ بينما كانت ٩٪ نسبه المرضى في العقد الثاني (١٠-٢٠ سنة). كما انه توزيع إصابات التهاب الكبد الفايروسي الحاد نوع A بين الجنسين على حد سواء وبالتالي لم يكن هناك فرق معنوي في نسبه الاصابه بين الإناث و الذكور (١ أنثى : ٧٨٦,. ذكور) وتشكل نسبة الإناث ٥٦٪ بينما االذكور ٤٤٪.

Introduction

Viral Hepatitis is liver inflammation due to a viral infection. It may present in acute (recent infection, relatively rapid onset) or chronic forms. Seven viruses, hepatitis viruses A through G, are responsible for most cases of viral hepatitis [1]. Viral hepatitis is the most common cause of hepatitis worldwide [2].

Hepatitis A virus (HAV) is a member of the *Hepatovirus* genus of Picornaviridae family. HAV is a non-enveloped (naked), linear, single stranded RNA virus of an icosahedral symmetry [3]. Morphologically, HAV is an isometric particle with a diameter of 27-32nm and composed entirely of 70% viral protein and 30% ribonucleic acid [4].

Most HAV infections occur through fecal–oral transmission, either by direct contact with an infected person or by ingestion of contaminated food and water [5]. Transmission of HAV during sexual activity or can occur through serum, blood products rarely occurs [6]. The adaptive immune response to HAV is robust and extremely effective in elimination of the virus. Neutralizing antibodies to the virus anti-HAV generally appear in the serum concurrent with the earliest evidence of hepatocellular injury and aminotransferase elevation [7].

Hepatitis A occurs sporadically and in epidemic worldwide, with a tendency for cyclic recurrences. Every year there are an estimated 1.4 million cases of hepatitis A worldwide. Among patients with a clinical diagnosis of acute viral hepatitis, two fifths had serologic evidence of type A. Hepatitis A is hyper endemic in Iraq, after 2003 war situation in Iraq is disastrous, due to the damage that occurred to water supply infrastructure and its contamination with sewage. Based on the WHO-supported study in 2006, hepatitis A is hyper-endemic in Iraq, 96.4% of people at one stage have been exposed to hepatitis A [2].The aim of this study is determine the distribution of acute HAV infected patients according to age and sex in Wasit province.

Materials and Methods

Study groups:

A total of 120 individuals were included in this study, anti-HAV IgM antibody was detected in serum of 100 individuals of them (patients group) while others 20 individuals were considered as a control group where anti HAV-IgM antibody negative in their serum.

Patients group:

Patients with Hepatitis type A included in this study who attended the Wasit central public health laboratory from different cities in Wasit Province, Iraq, during the period from November 2013 to March 2014. The patients were 49 males and 51 females with age range 1-21 years. Specimens were collected by venipuncture, 5 ml of blood was drawn using disposable syringes. The blood was placed in plastic disposable tubes, then left to stand at room temperature (18-25°C) to clot. Sera were separated by centrifugation for 5 minutes at 3000 rpm, and kept at -20ºC until assayed.

Control group:

Twenty healthy individuals were included in the current study: 11 males and 9 females with age range similar with patient group.

To exclude other etiologies of hepatitis, serum specimens of patients and control groups were tested for hepatitis B surface antigen (HBsAg), for IgM antibodies directed against the HBV core protein (anti-HBc IgM) and presence of anti-HCV antibodies by central public health laboratory staff using enzyme immunoassay.

Biochemical Tests:

TSB determination:

In order to determine TSB, BILTS Total Bilirubin Special Kit, for quantitative TSB in human serum and plasma of adults and neonates were used by cobas^{TM} c 111 chemistry analyzer annd according to the manufacturer instructions [8].

ALT, AST and ALP determination:

Reflotron® GPT (ALT), GOT (AST) and ALP reagent strips were used for Quantitative determination of ALT, AST and ALP, respectively in blood, serum and plasma with Reflotron® Plus System by Roche Company and according to the manufacture instructions [9-11].

Detection of HAV IgM Antibodies:

A solid phase, two-step incubation, antibody capture ELISA kit for qualitative determination of IgM-class antibodies to hepatitis A virus in human serum or plasma was used and according to the manufacturer instructions [12].

Results and Discussion:

Anti-HAV IgM antibody:

As shown in table-1 and figure-1, the mean ± SD of anti-HAV IgM antibodies were significantly higher 4.844 ± 1.55 in hepatitis A patients than control 0.451 ± 0.141.

Table 1-anti-HAV IgM antibody index unit in Hepatitis A patients and control.

Specimen groups	Anti HAV IgM antibodies Mean ± SD
Hepatitis A patients	(4.844 ± 1.55)
Control	(0.451 ± 0.141)
P value	<0.001

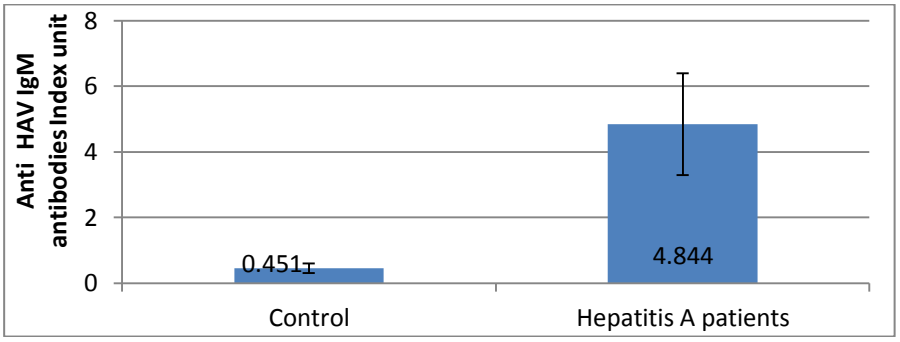

Figure 1-anti-HAV IgM antibody in Hepatitis A patients and control.

Acute infection is confirmed by detection of IgM anti–hepatitis A virus (HAV), which appears early in the course of infection while transaminase levels are still elevated and viral shedding is still occurring. IgG anti-HAV becomes the predominant antibody during convalescence; measurement of IgG anti-HAV serves little clinical purpose other than to confirm previous infection.

In acute hepatitis A, the presence of anti-HAV IgM is detectable about 3 weeks after exposure, its titre increases over 4 to 6 weeks, then declines to non detectable levels generally within 6 months of infection.

Anti-HAV IgA and IgG are detectable within a few days of the onset of symptoms. IgG antibodies persist for years after infection and provide lifelong immunity. The development of antibody to HAV coincides with a decrease in quantity of viremia and faecal shedding of virus [13].

HAV infection is hyper endemic in Iraq, the relative frequency of positive anti-HAV IgM antibodies was 41.0% of patients with a clinical suspicion of acute viral hepatitis [14].

The antibodies are usually directed against surface proteins. The capsid proteins VP1 and VP3 and the precursor protein VP0 may be recognized. Almost all patients expressed both IgG and IgM antibodies to VP1. The IgG response to VP3 was detectable for years after disease resolution. Antibodies to

nonstructural proteins are also induced. Although they are less abundant and lack neutralizing activity, they are produced in most individuals early in the infection [15].

Distribution of HAV patients according to age:

The distribution of HAV patients according to age is represented in table-2. It was found that the age of HAV patients ranged between 1-20 years with a mean± SD of 6.36 ± 2.97. Most of HAV patients in this study were located within the first decade (1-10 years) with a percentage of 91%; whereas 9% of all patients were within the second decade (10-20 years).

Table 2-The distribution of HAV patients according to the age.

Age groups (years)	No.	%
First decade (1- 10)	91	91%
Second decade (10-20)	9	9%
Total	100	100%
Mean ± SD = (6.36 ± 2.97)		

These results agreed with WHO report, 2014 which found that in the developing world about 90% of children have been infected by age of 10 years and thus are immune by adulthood [16] and with the results of Wheeler *et al*.,[17] who found that in developing nations, the age of HAV acquisition is before the first 2 years and the presence of disease manifestations and the severity of symptoms after HAV infection directly correlate with patient age while in Western societies, HAV acquisition is most frequent in persons aged 5-17 years . Also with Turky *et al*., [14] who reported that Hepatitis A is hyper-endemic in Iraq, serologic evidence of previous exposure to the infective agent is present in the majority of general population (96.4%). It is a disease of childhood. After the second decade of life no obvious increase in its prevalence was noticed.

Categorizing the seroprevalence by the age enables indirect measurement of age-specific incidence rates of HAV infection, and is considered as the best way to describe the hepatitis A situation in a country [18]. In Middle East and south Asia countries with high endemicity of HAV infection, Iraq [19], Syria [20], Pakistan [21], Turkey[22], Egypt [23] and Lebanon [24] more than 90% of subjects tested in these countries are reported to be sero-positive with significant infection in children aged less than 10 years of age. Studies from some countries in the 2000s show a lower rate in children, with less than 50% of the 15 years old individuals being immune in studies conducted in Kuwait [25], Saudi Arabia [26], and the United Arab Emirates [27]. In Iran, a study conducted on children referring to pediatric hospitals in Tehran; the observed trend of a lesser prevalence in younger subjects continues to rates as low as 26% for children aged 10 – 15 and 21% for those younger than 10 [28]. Furthermore, in Jordan, number of HAV cases within age groups differ significantly ($P<0.0001$). The highest number of cases 16.4% was reported for age group 5-14 years and the lowest number of cases 0.02%. The present results suggested a link between the age groups, year, month and occurrence of HAV infection [29].

Distribution of HAV patients according to gender:

The distribution of HAV patients according to gender is shown in table-3. The females (56%) significantly outnumbered males (44%) with a ratio of 1 female: 0.786 male.

Table 3-The distribution of HAV patients according to the gender.

Gender	**No.**	**%**
Male	44	44%
Female	56	56%
Total	100	100%
Male/ Female ratio = 0.786		P value =0.002

This result is similar to Cetinkaya *et al*., [30] result who reported that HAV infection is distributed equally among genders and also similar to AL Faleh *et al*., [31] in their study on changing patterns of HAV prevalence in the Saudi population over the 18 years observed with no difference occurred among males and females in the HAV causation. In Amman Jourdan, Battikhi and Battikhi,[29] found that Male to female ratio indicates no significant sex variation . The overall seroprevalence of hepatitis A virus in the general population of Iran was 86% and did not differ between the two genders [28]. In previous study in Iraq, No significant sex variation was observed for HAV, while males had a higher risk (by 15%) for HEV[14].

A study carried out in Canada by Wu *et al*. [32] reported that males have higher proportion than females in respect to HAV infection; however, the present study disagreed with their findings as well as with those of Barrientos-Gutierrez *et al*. [33]as they revealed that 77.2% of cases were males.

Biochemical Parameters:

As shown in table-4 there were significantly higher increase in the concentrations of TSB, ALT , AST and ALP mean ± SD (4.340 ± 2.698), (514.7 ± 394.1), (372.3 ± 337.5) and (318.3 ± 167.0), respectively in Hepatitis A patients than control mean ± SD (0.485 ± 0.156), (14.55 ± 3.486), (13.0 ± 2.316) and (55.9 ± 7.601), respectively.

Table 4-Biochemical tests in Hepatitis A patients and controls.

Biochemical test	Mean ± SD		P value
	Hepatitis A patients	Control	
TSB up to 1.4 mg/dl*	(4.340 ± 2.698)	(0.485 ± 0.156)	<0.001
ALT up to 41 U/L *	(514.7 ± 394.1)	(14.55 ± 3.486)	< 0.001
AST up to 40 U/L *	(372.3 ± 337.5)	(13.0 ± 2.316)	< 0.001
ALP 40-129 U/L*	(318.3 ± 167.0)	(55.9 ± 7.601)	< 0.001

*****Normal values [34].

In symptomatic HAV infection patients, elevations of ALT and AST, alkaline phosphatase and bilirubin occur frequently [35]. Typically, total serum bilirubin levels remain below 10 mg/dl similar to the result of this study, but levels of 20 mg/dl may occasionally be observed [36]. The concentrations of ALT and AST provide a quantitative assessment of liver damage during acute infection. ALT is located primarily in the liver, and is limited to the hepatocyte cytosol, while AST is found in the mitochondria (80%) and cytosol (20%). This compartmentalization of enzymes may partially explain the pattern of aminotransferases observed in many forms of liver disease, since during acute hepatitis, levels of ALT are significantly higher than the levels of AST, resulting in a high ratio of ALT/AST levels (>1.4) in most cases [37] which is similar to the result of this study the level of ALT was significantly higher than the level of ALT in a ratio of 1.38: 1. Hepatocellular injury becomes evident owing to the marked elevation in hepatic aminotransferase levels, often greater than 500 units/l, which peak shortly after the prodromal period [37], However, exceptions can occur in situations where severe tissue necrosis develops, resulting in an increased release of AST in the blood. The elevation of transaminases occurs in the prodromal phase, reaching a peak at the same time as the clinical symptoms, and concentrations above 1000 IU/l are common. In 2 months, 60% of patients have normal biochemical tests, reaching almost 100% in 6 months. Clinical and biochemical testing does not allow the differentiation of hepatitis A from other forms of acute hepatitis, hence serological tests are needed to identify the etiologic agent [6].

In this study, there was no significant difference in biochemical parameters level, which indicate the severity of disease between patients with first and second decade as shown in table-5. This result was not agreed with WHO, 2014 report which reported that the severity of the disease increases with age at time of infection [16].

Table 5-The biochemical test mean ± SD in acute hepatitis A patients with first and second decade.

Biochemical test	Hepatitis A patients Mean ± SD		P value
	First decade	Second decade	
TSB	(5.19 ± 2.6)	(6.42 ± 2.7)	0.3 NS
ALT	(812.2 ± 490.9)	(481.7 ± 370.0)	0.06 NS
AST	(616.8 ± 459.9)	(345.2± 314.0)	0.10 NS
ALP	(452.7 ± 707.7)	(303.4 ± 153.8)	0.056 NS

Also there was no significant difference between ALT and AST level with patient's sex, while there was significant difference between the level of TSB and ALP with patient sex, as shown in table-6; this may be due to the effect of sex hormones in the level of liver enzymes.

Table 6-The distribution of biochemical test mean ± SD in acute hepatitis A patients according to sex.

Biochemical test	Hepatitis A patients Mean ± SD		P value
	male	female	
TSB	(3.71 ± 2.16)	(4.95 ± 3.03)	0.02 S
ALT	(478.0 ± 333.46)	(559.77 ± 444.3)	0.3 NS
AST	(330.2 ± 261.0)	(417.9± 398.3)	0.19 NS
ALP	(269.3 ± 136.5)	(372.7 ± 175.0)	0.001 S

In a conclusion the distribution of acute hepatitis A infection was positively related to the age of patient and negatively related with patients sex in Wasit province/ Iraq.

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