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Correlation between of hepatitis A virus infection and vitamin B12 level in Iraqi patients

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Abstract: Hepatitis A is a public health problem all over the world, especially in developing countries, because the virus is so common in the environment. Anti-HAV IgM antibodies were detected in the serum of 100 of the 120 persons (patients group) collected from the Al-Zahra hospital in Al-rusafa/Baghdad between November 2021 and March 2022. Twenty individuals with negative blood levels of anti-HAV IgM antibodies were deemed a control group. Anti-HAV IgM antibodies were significantly higher (P<0.001) in hepatitis A patients than controls using a solid phase, two-step incubation, antibody capture ELISA kit, indicating acute infection. The majority of HAV patients (91% of all patients) were found during the first decade (1-10 years), whereas 9% were found within the second decade (10-20 years). HAV infection is spread evenly across genders in this research. As a result, females and males had no statistically considerable differences, with a ratio of (1 female: 0.786 male) and a proportion of 56% females vs 44% males. The study showed a considerable decrease in vitamin B12 in male patients with HAV (426.09 ± 35.61 pg/mL) comparing with healthy control (566.45 ± 28.06 pg/mL) (P= 0.001). While in females, the findings displayed a considerable decrease in the patient's group (595.01 ± 47.47 pg/mL) compared with the control group(721.04 ± 58.28 pg/mL)

Key word: HAV, hepatitis, vitamin b12, HAV IgM

1- Introduction

Hepatitis A virus (HAV) is a member of the Picornaviridae family and belongs to the Hepatovirus genus (Chironna et al., 2003). Infectious HAV particles are classified into two types: naked and quasi-enveloped virions. Naked virions are quasi-enveloped virions whose membrane is dissolved by bile acids in the biliary canaliculus before they are ejected in faeces. Semi-enveloped virions include a lipid membrane and are found in blood and culture supernatants (Antaki et al.,2000).

Positive-strand RNA at 7.5 kb in size, the HAV genome encodes a single large polyprotein via a single open reading frame (ORF) (Aziz et al., 2007). From this polyprotein, viral (protease 3C) and host cell proteases synthesise the mature structural (VP4, VP2, VP3, and VP1) and non-structural (VP4, VP2, VP3, and VP1) and RNA-dependent RNA polymerase (RNA-dependent RNA polymerase) proteins (Kaya et al 2007).

According to the most recent International Committee on Virus Taxonomy (ICTV) study, HAV is now divided into five genotypes (Salama et al., 2007). Humans are solely susceptible to infections produced by genotypes I, II, and III, which are further split into subtypes A and B.For genotype I, a third subgenotype, IC, was suggested but is not yet recognised by the ICTV (Chironna et al., 2003). Despite the presence of several genotypes, there is only a single serotype.

After the first 4-week incubation period, there is usually a vague prodromal phase through which an infected individual may have symptoms such as the flu and digestive issues for a few days. Icteric is the name given to the following stage, which is characterised by jaundice, death of liver cells, and elevated blood aminotransferase activity (Aziz et al., 2007).

Although the majority of children less than six years old (more than 90%) show no signs of infection, symptoms are far more prevalent in adults (more than 70%) (Salama et al., 2007). The risk of serious consequences, hospitalisation, and death is higher in older people (Antaki et al.,2000).

Unlike other hepatitis viruses, HAV does not cause infections that last for a long time. Acute hepatitis A rarely causes symptoms outside of the liver, but when it does, they can include "rash, pancreatitis, arthritis,

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myocarditis, acute kidney injury, and blood disorders like haemolysis and cryoglobulinemia" (Chironna et al., 2003).

After the first incubation period of four weeks, there is often a hazy prodromal phase in which an infected person may experience symptoms such as the flu and stomach difficulties for a few days. Their presence suggests previous, treated illnesses. Their existence suggests that previous infections have been resolved (Antaki et al.,2000). Although nucleic acid amplification assays are not often used for diagnosis, Chironna et al. (2003) found that these tests had the ability to detect HAV RNA in the faeces and plasma of infected individuals, in addition to contaminated water and food (Bizri et al., 2006). The majority of the time, sequencing and phylogenetic analysis are utilised to follow epidemics. These methods are very valuable for establishing how diseases spread from person to person. (Salama et al., 2007). The primary objective of this research is to analyse the prevalence of acute HAV infections in Baghdad according to age, gender, and the degree to which these factors are correlated with vitamin B12 levels.

2- Materials and Method

2:1 Study groups:

Anti-HAV IgM antibody was discovered in serum of 100 individuals among them (patients group), while other 20 persons were regarded to be a control group since anti-HAV-IgM antibody was not present in their blood. There were a total of 120 people who participated in this research.

2:2 Patients group:

Patients with Hepatitis type A included in this study who collected from Al-zahra hospital in Al-Rusafa /Baghdad, during the period from November 2021 to March 2022. The patients ranged in age from 1 to 21 years and included 49 men and 51 women. Venipuncture was used to collect specimens, and 5 mL of blood was extracted using disposable syringes. The blood was placed in disposable plastic tubes and coagulated at room temperature (18-25°C). The sera were centrifuged at 3000 rpm for 5 minutes and kept at -20ºC until analysis.

2:3 Control group:

The present research comprised twenty healthy people: 11 men and 9 women in the same age range as the sick group. The staff at the central public health laboratory examined serum samples from patients and control groups for the presence of hepatitis B surface antigen (HBsAg), IgM antibodies directed against the HBV core protein (anti-HBc IgM), and antibodies against hepatitis C virus in order to rule out the possibility of other causes of hepatitis (anti-HCV). These tests were performed using enzyme immunoassay.

2:4 Detection of HAV IgM Antibodies:

According to the manufacturer's instructions, a solid phase, two-step incubation antibody capture ELISA kit for qualitative measurement of IgM-class antibodies to hepatitis A virus in human serum or plasma was employed (Berge et al., 2020)

2:5 Measuring the concentration of vitamin B12

Using a test manufactured by Sigma Aldrich in the United States, the concentration of vitamin B12 in patient and healthy blood is determined. The fundamental concept is to add a specified concentration of dissolved vitamin B12 to the model and then quantify the concentration change by measuring the optical density using a spectrophotometer at a wavelength of 546 nm while maintaining a normal ratio (300-600 p mole / L) (Salama et al., 2007).

3- Results and Discussion:

3:1 Anti-HAV IgM antibody:

As it is clear in table-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)

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P value	**<0.001**

Acute infection is verified by the presence of IgM anti–hepatitis A virus (HAV), which emerges early in the course of illness when transaminase levels are still increased and viral shedding is ongoing. During convalescence, IgG anti-HAV becomes the predominate antibody; detection of IgG anti-HAV provides little therapeutic benefit beyond confirming past infection.
The presence of anti-HAV IgM is detected roughly 3 weeks after acute hepatitis A exposure; its titre increases over 4 to 6 weeks, then declines to undetectable levels after 6 months of infection.
Anti-HAV IgA and IgG antibodies may be detected only a few days after the first signs of illness appear. After an infection, IgG antibodies may be present in the body for years and offer immunity for a lifetime. The production of an antibody against HAV is accompanied with a reduction in the amount of virus that is shed in faeces and in the viremia of the patient (Lemon, 1997)
HAV infection is very common in Iraq; 41.0% of people with a clinical suspicion of acute viral hepatitis had anti-HAV IgM antibodies that were positive (Turky et al., 2011)
Typically, the antibodies are aimed towards surface proteins. It is possible to identify the precursor protein VP0, the capsid proteins VP1 and VP3, and both. The majority of patients had IgG and IgM antibodies to VP1. Years after the remission of the illness, the IgG response to VP3 was discovered. Additionally, antibodies are generated against nonstructural proteins. Although they are less plentiful and lack neutralising function, the majority of infected people develop these antibodies early on (Wang et al., 2011)

3:2 Distribution of HAV patients according to age:

Table-2 displays the age distribution of HAV patients. The ages of HAV patients varied from 1 to 20 years, with a mean ±SD of 6.36 ± 2.97 years. In table-2, you can see how the ages of HAV patients are spread out.The majority of HAV patients in this research (91% of all patients) were found during the first decade (1-10 years), whereas 9% were found 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)		

In a 2014 WHO report, it was found that 90% of children in the developing world have been infected by the time they are 10 years old, making them immune by the time they are adults. These results were the same. Wheeler *et al.* (2005) found that most people get HAV between the ages of 0 and 2 in developing countries, and that the presence of disease symptoms and the severity of symptoms after HAV infection are directly related to the patient's age. In contrast, most people get HAV between the ages of 5 and 17 in Western countries.
According to Turky et al. (2011), who asserted that Hepatitis A is hyperendemic in Iraq, 96.4% of the general population exhibited serologic evidence of previous exposure to the infectious agent. It is an illness that mostly affects children. There was no statistically significant increase in the prevalence of the condition beyond the second decade of life.

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Seroprevalence by age is thought to be the most accurate way to figure out how common hepatitis A is in a country because it can be used to figure out how often HAV infection happens at different ages.

Iraq, Syria, Pakistan, Turkey, Egypt, and Lebanon are all places in the Middle East and south Asia where HAV infections are common. More than 90% of people tested in these countries are sero-positive, and children younger than 10 years old are most likely to be infected. Some studies from the 2000s reveal a lower prevalence among children, with fewer than 50% of 15-year-olds immune in studies performed in Kuwait, Saudi Arabia, and the United Arab Emirates (MohdHanafiah et al., 2011).

In Iran, a research on children admitted to paediatric hospitals in Tehran found that the prevalence of the disease is decreasing with age, with rates as low as 26% for children between the ages of 10 and 15 and 21% for those under 10 years old (Mehr et al., 2004).

Furthermore, in Jordan, number of HAV cases within age groups differ significantly (P<0.0001). The age group 5-14 years old had the most cases, 16.4%, and the youngest age group, 0.02%, had the least. The findings of the present investigation displayed that there was a link between age groups, year, and month of infection with HAV (MohdHanafiah et al., 2011).

3:3 Distribution of HAV patients according to gender:

Table 3 displays the distribution of HAV patients by gender. With a ratio of 1 female to 0.786 male, females (56%) considerably outnumbered males (44%) in the sample.

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

These findings are comparable to (MohdHanafiah et al., 2011). result who reported that HAV infection is distributed evenly between sexes, and also in line with AL Faleh et al. (2008), who, over the course of an 18-year study of the fluctuating patterns of HAV prevalence in the Saudi population, found no difference between males and females in the HAV's aetiology.

According to Amman Jourdan, Mehr et.el, (2004), the ratio of males to females suggests no substantial sex differences.

The total seroprevalence of hepatitis A virus in Iran's general population was 86%, with no difference across genders (MohdHanafiah et al., 2011). Previous research in Iraq found no significant sex differences in regards to HAV, whereas men were at a greater risk (by 15%) for HEV (Barrientos-Gutierrez *et al*. 2011)

Wu *et al.* (2001) claimed that males had a larger prevalence of HAV infection than females in Canada; however, the current research conflicted with their results as well as those of Barrientos-Gutierrez *et al.* (2011), revealing that 77.2% of patients were males.

3-4- Estimation of vitamin B12

The study displayed a significant decrease in vitamin B12 in male patients with HAV (426.09 ± 35.61 pg/mL) comparing with healthy control (566.45 ± 28.06 pg/mL) (P= 0.001). While in females, the results showed a significant decrease in the patient's group (595.01 ± 47.47 pg/mL) compared with the control group(721.04 ± 58.28 pg/mL) as it is clear in table (3)

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Table (3): The mean values (±SD) of some vitamins between HAV patients and control subjects

Groups	Female-Control	Female-Patients	Male-Control	Male-Patients
Vitamin B12 pg/mL	721.04 ± 58.28	595.01 ± 47.47***	566.45 ± 28.06	426.09 ± 35.61***

*Significant (P<0.05), **highly significant (P<0.01), ***very highly significant (P<0.001)

These findings reveal a correlation between HCV replication and endogenous cobalamin levels. No link has been proven, to our knowledge, between HCV viral load and any other quantifiable parameter (34). In accordance with a previousinvestigations, we found that neither the HCV viral load nor the total blood cobalamin concentration had any correlation with alanine aminotransferase (ALT) levels in our investigation (the data are not being provided) (35). According to Lott et al. [28], it is possible that HCV has developed to take advantage of high quantities of vitamin B12 present in the hepatocytes in order to achieve maximal replications values.

References:

1. **Al Faleh, F., Al Shehri, S., Al Ansari, S., Al Jeffri, M., Al Mazrou, Y., Shaffi, A. and Abdo, A. A. (2008)**. Changing patterns of hepatitis A prevalence within the Saudi population over the last 18 years. *World J Gastroenterol*.14:7371-7375.
2. **Alkhalidi, J., Alenezi, B., Al-Mufti, S., Hussain, E., Askar, H., Kemmer, N.,and Neff, G.W. (2009).** Seroepidemiology of hepatitis A virus in Kuwait. *World J Gastroenterol*. 15:102-5.
3. **Almuneef, M. A., Memish, Z. A., Balkhy, H. H., Qahtani, M., Alotaibi, B., Hajeer, A., Qasim,L. and Al Knawe, B.(2006).** Epidemiologic shift in the prevalence of hepatitis A virus in Saudi Arabia: A case for routine Hepatitis A vaccination. *Vaccine*. 24:5599-5603.
4. **Antaki, N. and Kebbewar, M. K. (2000).** Hepatitis A seroprevalence rate in Syria. *Trop Doct*. 30:99-101.
5. **Aziz, S., Muzaffar, R., Hafiz, S., Abbas, Z., Zafar, M. N., Naqvi, S. A. and Rizvi, S. A. (2007).** Helicobacter pylori, hepatitis viruses A, C, E, antibodies and HBsAg-prevalence and associated risk factors in pediatric communities of Karachi. *J Coll Physicians Surg Pak*. 17:195-198.
6. **Barrientos-Gutierrez, T. , Brizuela-Alcantara, D. and Chavez-Tapia, G. (2011).** Hepatitis A Virus infection in high risk subjects. *Annais of Hepatology*. 10:578-579..
7. **Battikhi, M. N.and Battikhi, E. G. (2004).** The seroepidemiology of Hepatitis A virus in Amman, Jordan. *New Microbiol*. 27:215-220.
8. **Berge, J. J., Drennan, D. P., Jacobs, R. J., Jakins, A., Meyerhoff, A. S., Stubblefield, W. and Weinberg, M.(2000).** The cost of hepatitis A infections in American adolescents and adults in 1997. *Hepatology*. 31:469-473.
9. **Bizri, A. R., Nuwayhid, I. A., Hamadeh, G. N., Steitieh, S. W., Choukair, A. M. and Musharrafieh, U. M. (2006).** Association between hepatitis A virus and *Helicobacter pylori* in a developing country: The saga continues. *J Gastroenterol Hepatol*. 21:1615-1621.
10. **Cetinkaya, B. Tezer, H. Parlakay, A. O. and Saylı, T. R. (2014).** Evaluation of pediatric patients with hepatitis A. *J Infect Dev Ctries*. 8:326-330.
11. **Chironna, M., Germinario, C., Lopalco, P. L., Carrozzini, F., Barbuti, S. and Quarto, M. (2003).** Prevalence rates of viral hepatitis infections in refugee Kurds from Iraq and Turkey. *Infection*. 31:70-74.
12. **Kaya,D., Guler, E., Ekerbicer, H. C., Dilber, C., Karabiber, H., Guler, S., Davutoglu, M. and Ciragil, P. (2007).** Hepatitis A seroprevalence and its relationship with environmental factors in children of different age groups in Kahramanmaras, Eastern Mediterranean region of Turkey. *J Viral Hepat*. 14:830-834.
13. **Lemon, S. M. (1997).** Type A viral hepatitis: epidemiology, diagnosis, and prevention. *Clinical ChemistryAugust* .43: 1494-1499.

ISSN NO: 2771-8840
Date of Publication: 20-10-2022

14. **Mehr, A. J., Ardakani, M. J., Hedayati, M., Shahraz, S., Mehr, E. J. and Zali, M. R. (2004).** Age-specific seroprevalence of hepatitis A infection among children visited in pediatric hospitals of Tehran, Iran. *Eur J Epidemiol*. 19: 275 – 278.
15. **MohdHanafiah, K., Jacobsen, K.H. and Wiersma, S.T.(2011).** Challenges to mapping the health risk of hepatitis A virus infection. *Int J Health Geogr*. 10:57.
16. **Salama, I. I., Samy, S. M., Shaaban, F. A., Hassanin, A. I. and Abou Ismail, L. A. (2007).** Seroprevalence of hepatitis A among children of different socioeconomic status in Cairo. *East Mediterr Health J*. 13:1256-1264.
17. **Sharar, Z. A., Rajah, J. and Parsons, H. (2008).** Childhood seroprevalence of hepatitis A in the United Arab Emirates. *Trop Doct*. 38:65-66.
18. **Turky, A. M., Akram, W., Al-Naaimi, A. S., Omer, A. R. and Al- Rawi, J. R.(2011).** Analysis of acute viral hepatitis (A and E) in Iraq. *Global Journal of Health Science*. 3:70- 76.
19. **Wang, C. H., Tschen, S. Y., Heinricy, U., Weber, M. and Flehmig, B. (1996).** Immune response to hepatitis A virus capsid proteins after infection. *J. Clin. Microbiol*. 34:707-713.
20. **Wheeler, C., Vogt, T. M., Armstrong, G.L., Vaughan, G., Weltman, A., Nainan, O. V., Dato, V., Xia, G., Waller, K., Amon, J., Lee, T. M., Highbaugh-Battle, A., Hembree, C., Evenson, S., Ruta, M. A., Williams, I. T., Fiore, A. E. and Bell, B. P. (2005).** An Outbreak of Hepatitis A associated with green onions. *N Engl J Med*. 353:890-897.
21. **World Health Organization. (2014)** . Hepatitis A Fact sheet N°328. WHO.
22. **Wu, J., Zou, S. and Giulivi, A. (2001).** Current hepatitis A status in Canada. *Can J Infect Dis*. 12:341-344.