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The Role of HAV Infection Diagnostic Methods in Detection of Liver Function in Sample of Iraqi Patients

[1]Zahraa A. Hussein, [2]Saife D. Al-Ahmar

[1,2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

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Abstract: Hepatitis A virus infection is a health threat with multiple transmission patterns across areas. This study aimed to use HAV diagnostic methods, including reverse transcription polymerase chain reaction (RT-PCR) test, anti-HAV IgM antibodies, and biochemical analysis to assess liver function. A total of 110 Iraqi individuals participated in this study, comprising 60 HAV-infected patients (Patient group) and 50 healthy controls. The study was conducted from December 2022 to July 2023. Blood samples were collected for HAV antibody titers and RT-PCR confirmation of infection. Liver function tests measured alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total serum bilirubin (TSB) in serum. Our results showed that HAV RNA was detected in samples by RT-PCR, demonstrating the effectiveness of molecular methods in diagnosing HAV infection. Higher levels of HAV IgM antibodies in patients compared to healthy individuals indicated acute infection. Biochemical testing revealed abnormalities in liver function markers in HAV-infected individuals, including elevated ALT, AST, ALP, and total serum bilirubin. This study highlights the benefits of liver function tests in assessing liver damage and diagnosing hepatitis. It emphasizes the need for accurate diagnosis and management of HAV infection to better understand its prevalence and health impact.

Keywords: HAV, RT-PCR, IgMtiter, GOT, GPT, ALP, TSB.

Corresponding author: (E-mail: Zahraaahmeddd767@gmail.com)

Introduction

Hepatitis A virus (HAV) contamination is a main public health difficulty, affecting approximately 1.4 million people globally each 12 months(1). However, the actual incidence of infection can be more than 5times more (2). This viral infection is common in places with low socioeconomic stage, terrible cleanliness, and overcrowding; hygiene situations are inadequate (1,3,4). The time period hepatitis suggests infection or disease that affects hepatocytes and may be verified by using bizarre liver characteristic assessments (LFTs) (5). Viral hepatitis is as a result of as a minimum five exclusive viruses (6). Acute viral hepatitis is in particular caused by Hepatitis A virus (HAV), which happens worldwide however maximum commonly in developing nations as each epidemic and sporadic forms. HAV is a non-enveloped single stranded RNA virus classified within the Heparnavirus genus of the

Picornaviridae family (7). In Iraq HAV infection is hyperendemic with an estimated IgG seroprevalence rate of 98% among individuals aged fifty years and above (8). The primary method for transmitting the disease is through the fecal-oral route either via direct contact with an infected person or consumption of contaminated food or water (9,10).

The HAV infection presents itself as an acute viral illness accompanied by fever, raised ranges of aminotransferase enzymes, abdominal pain, and jaundice(11), Diagnosis of HAV infection basically based on clinical presentation is not practicable (12). However, testing for Immunoglobulin (Ig) antibodies to HAV is advised for diagnosis. IgM anti-HAV antibodies can identify the acute stage of HAV infection since they usually show up 1-2 weeks after infection and go away in 3-6 months. Hepatitis A Virus (HAV) RNA can also be found in clinical specimens using reverse transcription polymerase chain reaction (RT-PCR). RT-PCR is exceedingly sensitive and specific, allowing for the direct detection of viral nucleic acids, including HAV RNA, which aids in the diagnosis of HAV infection. This study sought to evaluate the diagnostic value of the disease more closely Multiple methods for detection of hepatitis A virus (HAV) infection. These methods include serological testing for anti-HAV IgM antibodies and molecular pathways such as reverse transcription polymerase chain reaction (RT-PCR) for the detection of viral RNA. In order to evaluate alterations in liver function markers in patients with acute HAV infection in Baghdad and Al-Diwanyah cities.

Materials and methods

One hundred and ten Iraqi individuals participated in this study, comprising 60 patients infected with the Hepatitis A virus (Patient group) and 50 apparently healthy individuals (Control group). Diagnosis of HAV infection in the patient group was confirmed using RT-PCR. Blood samples were collected from both groups at the Gastroenterology and Liver Hospital in the Gastroenterology and Liver Hospital in the Medical City and Al-Diwaniyah Hospital, following ethical considerations and hospital approvals. Participants in both groups ranged in age from ≤1 to ≥25years. For sample collection, 5 milliliters of blood were obtained from both HAV-infected patients and healthy individuals using disposable syringes. The samples have been allowed to stand at room temperature for 10 minutes to activate the clot activator included in the tubes, Blood samples were transferred into gel activator tubes for serum separation and then freeze the samples at -20°C for later virus detection by RT-PCR. Subsequently, the samples were centrifuged at 3500 rpm for 10 minutes to separate the serum. Biochemical tests and IgM titers were measured immediately following serum separation.

Extraction of RNA

The RNA extraction and purification from blood samples of the hepatitis A virus by using Addprep genomic DNA extraction kit (Addbio/ Korea) used according to manufacturer instructions and protocol. The amount

of RNA isolated from 200 µl of whole blood is 5 to 30 µg , the pellet was washed with 400 µl of 70% then in a RNase-free tube, 10 µl of DNase and 40µl of DNase I Reaction Buffer added to remove DNA traces during the extraction process and then the RNA kept in -70°C until RT-PCR reaction using (LineGeneK, China).

Detection of hepatitis A virus by one-step RT-PCR

The RT-PCR within one step Three primary operations are used in the HAV Real-TM test: real-time cDNA amplification, one-step reverse transcription of RNA, and HAV RNA separation from samples. The PCR amplification of a particular area of the pathogen's genome using unique primers and fluorescence dye detection are the two main components of HAV detection. These colors adhere to ligated oligonucleotide probes. in particular to the product that is inflating. Following a PCR run, accumulation of product can be detected without reopening reaction tubes thanks to real-time PCR fluorescence intensity monitoring. Conditions for the Real Time PCR Program's thermal cycling, followed by the application's thermal cycling configuration in (Table 1), The reaction mixture mix has been prepared for the necessary quantity of samples.

HAV IgM test device by VIDAS

VIDAS HAV IgM is an automated qualitative test for use on VIDAS family instruments detecting IgM directed against coronavirus A (HAV) after immunocapture, in human serum or plasma (heparin or EDTA), using ELFA (Enzyme Customized) technology. fluorescent examination). instruments detecting IgM directed against coronavirus A (HAV) after immunocapture, in human serum or plasma (heparin or EDTA), using ELFA (Enzyme Customized) technology. fluorescent examination).

Measurement levels biochemical tests for both patients and healthy people

All the serum samples that were collected from the HAV patients and healthy people were subjected to the biochemical test which alanine aminotransferase (ALT), aspartate aminotransferase (AST). They were tested by using kits from (Biosystem,Spain) and measured by Spectrophotometer (CE7200,England).

Statistical Analysis

Data were collected and analyzed using appropriate statistical methods , Statistics package for Social Science (SPSS), version 25 for windows software was used for statistics analysis, The data are normally distributed and expressed as mean ± standard deviation (SD) The data are normally distributed and analyzed by using frequency and percentage distribution.

$$\% = \text{frequency}/n * 100$$

Student’s t-test were performed to analyze the statistical significance of difference between group (1and 2), Significant was considered whenever the p value was equal or less than (0.05).

Table (1): Real Time PCR Program.

Stage	Temp,°C	Time	Fluorescence detection	Cycle repeats
Hold	50	30 min	-	1
Hold	95	15 min	-	1
Cycling	95	5s	-	10
Cycling	60	20s	-	10
Cycling	72	15s	-	10
Cycling 2	95	5s	-	40
Cycling 2	60	30s	FAM, JOE/HEX/Cy3	40
Cycling 2	72	15s	-	40

Results and Discussion

On a global scale HAV infection is a significant public health concern, and there are considerable regional variations in HAV infection patterns both within and between nations (13). Three methods were used in this study to identify HAV infection: biochemical investigation of serum tests as a confirmation test of HAV infection, including levels of serum TSB, ALT, AST, and ALP; molecular method by RT-PCR; and serological method by identifying anti-HAV IgM antibody. Likewise, the results of HAV detection showed that 60 of HAV patients by the one-step RT-PCR who include (25.88±1.87) males and (25.61±1.47) females out of HAV patients there are not significant difference for patients according the gender. The results of this study of the detection the HAV in acute HAV patients revealed that the RNA of the virus in patients was Positive. The detection of Hepatitis A Virus (HAV) using reverse transcription polymerase chain reaction (RT-PCR) represents a critical component of diagnostic strategies for HAV infection. RT-PCR is widely recognized for its high sensitivity and specificity in detecting viral nucleic acids, including HAV RNA, in clinical specimens (14). While, the serological result of HAV showed the mean and standard deviation for patients was (1.819±0.625) while the normal range for Titer of IgM is (0.4-0.5), by One-Sample T-test Statistics there are significant differences between patient and control the P value =0.00 it was less than sig = 0.05. This result is agreement with (15), who also discovered that patients with HAV have elevated IgM levels (Table 2), The serological and molecular tests are the indicated methodology to diagnosis hepatitis infection in patients(16). While the laboratory liver tests also serve as valuable tools in evaluating and managing patients with liver dysfunction. The liver plays a crucial role in metabolizing various substances such as carbohydrates, proteins, and fats. Consequently, the end products of these metabolic pathways, along with certain enzymes that exhibit high sensitivity to abnormalities, are often regarded biochemical markers indicative of liver dysfunction (17). the results in (Table 3) the level of ALT level it was found that there are significant differences P value =0.00 less than 0.05, as it was found that the (arithmetic mean ±Sd) is higher for patients, (170.18±117.67), while it is lower for healthy people, (21.25±7.41),

AST level it was found that there are significant differences P value =0.00 less than sig = 0.05, as it was found that the (arithmetic mean ±Sd) is higher for patients, (183.05±128.13), while it is lower for healthy people, (26±7.69), ALP level it was found that the (arithmetic mean ±Sd) is higher for patients, (607.68±214.93), while it is lower for healthy people, (202.02±121.35). While, the TSB level it was found that there are significant.

Table (2): Distribution of mean and Sd of the IgM titer for HAV patients.

Mean	Sd	Minimum	Maximum	Pvalue
1.819	0.625	0.59	2.67	0.00

Table (3): Distribution of mean and Sd for Liver function tests according to status.

Variables		ALT(GPT)	AST(GOT)	ALP	TSB
		(Mean ±Sd)	(Mean ±Sd)	(Mean ±Sd)	(Mean ±Sd)
Status	Patient	170.18±117.67	183.05±128.13	607.68±214.93	2.77±2.5
	Control	21.25±7.41	26±7.69	202.02±121.35	0.55±0.14
	P value	0.00*	0.00*	0.00*	0.00*

Differences P value =0.00 it was less than sig= 0.05, as it was found that the (arithmetic mean ±Sd) is higher for patients, (2.77±2.5), while it is lower for healthy people, (0.55±0.14). These results are in agreement with a study by (18). which found that viral hepatitis was associated with significantly elevated levels of AST, ALT, and ALP.

The elevation of liver enzymes strongly indicates hepatocellular injury (19). Additionally, the levels of ALP, GPT, GOT, and TSB in patients with undetected virus remain within the normal range compared to the reference values of liver function tests (20,21).

Conclusion

This study's findings underscore the need of using a variety of approaches to identify Hepatitis A Virus (HAV). HAV RNA can be detected in clinical samples using molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR), which shown good sensitivity and specificity in test results management strategies. Further research and validation studies are warranted to confirm these observations and explore their implications for clinical practice and public health interventions. We also compared the results of the molecular and serological testing, and our findings revealed that the sensitivity to the presence of the virus was comparable, since all infected persons tested positive in both tests. This indicates that they contributed important information needed to ascertain if the infection is present or not. Additionally, anti-HAVIgM antibodies were screened for by serological testing, which showed acute HAV infection; patients' IgM titers were much higher. contrasted with healthy controls, we noticed. These results are in line with other research that has indicated the value of IgM as a diagnostic marker for HAV infection. Serum evaluation of those with acute HVA infection revealed some noteworthy alterations in liver function parameters in addition to biochemical abnormalities. The levels of alanine

aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB) were greater in HVA patients compared to healthy persons. These changes suggest that there was hepatocyte damage and liver function degradation, which are common aspects shown viral hepatitis. The fact that there were significant differences between cases and controls concerning these indicators underlines further on their clinical significance. These findings contribute to our understanding of the epidemiology and pathogenesis of HAV infection, facilitating timely interventions and improved patient.

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