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Molecular Study of Hepatitis A Virus Strain Detected in Children Admitted to Babylon Hospital for Obstetric and Children in Babylon Province

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Abstract: Hepatitis A infection (HAV) is the best incessant reason for virus-related hepatitis worldwide and is known as quite possibly the most predominant foodborne microorganisms. HAV genotypes vary in their natural spread and the event of HAV disease contrasts fundamentally among nations, and is especially high in territories with helpless sterilization and cleanliness. The research was a Babylon hospital.a hundred and twenty children have been collected, One hundred children of sufferers have been recognized with the hepatitis virus primarily based totally at the scientific symptoms and symptoms of the disease and twenty children of healthful control during the duration from September 2018 to September 2019. Phylogenetic investigations are generally utilized in clinical microbiology for following the environmental birth place of HAV strains**.** The real-time TaqMan RT-PCR created in the current study is focused on polyprotein gene amplification for the quantification of HAV.For the quantification of (8) samples of HAV genotypes, the sequence alignment of the real-time TaqMan RT-PCR created in the current study is focused on polyprotein gene amplification for the quantification of HAV.

Key word: HAV, IgM, RT-PCR.

INTRODUCTION

A small single stranded positive sense hepatitis A virus (ss+ RNA virus) non-enveloped , member from the Picornaviridae viruses is an main virus that affects individuals globally[1]. It has been identified as an constituent of the genus Hepatovirus in the Picornaviridae viruses in 1991 . Replicates from HAV. In hepatocytes, and interfering with the role of the liver in sparking an immune response that causes inflammation of the liver[2]. A common catching aetiology of extreme hepatitis worldwide is the hepatitis A virus (HAV); HAV does not cause various hepatitis B or C chronic liver diseases. Extreme hepatitis is usually a self-limited illness and it is rare for fulminant hepatitis to occur [3]. Widespread areas, in addition to lower public socio-economic status , tend to be deprived of general sanitation and hygiene [4]. (Characteristic signs of extreme infection include the following signs nausea, vomiting, belly pain, weakness, malaise, decreased appetite, and fever. Managing is sympathetic. Peak HAV infections occur either through direct contact with an infected person or thru fecal-oral transmission of impureness diet and water intake..,). (Upon consumption, the pathogen movements thru the intestinal tract and gets. stuck there in the liver. It can be recurrent. It restricts with hepatic function and makes an induction of immune system, causing irritation of the liver[5].

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MATERIALS AND METHODS

Study Population

In this study, anti-HAV IgM antibodies were found in the serum in a total of 120 individuals (group of patients) who attended the Babylon hospital in Babylon-Iraq Province.,{throughout the duration (from September 2018}to September 2019 .While another 20 individuals were considered as a control group which the anti – HAV IgM antibody was negative in their serum. Use real time-qPCR to detection hepatitis A in stool sample.

Blood Sample

From each patient, three ml of venous blood samples and (healthy controls) were taken. Put(three ml of blood samples) in the (gel tube to obtain the serum) were separated for 3 minutes by centrifugation at 3000 rpm and HAV IgM was used for detection[6].

Stool Sample

The study including takeing a small amount of stool from each patient and collect the samples in a stool container and then exam to detect the hepatitis virus type A by rapid test(HAV IgM Test card) .

Viral RNA Extraction

Using the AccuZolTM Complete RNA Extraction Kit (Bioneer, Korea),

Estimation of Extracted RNA

The removed RNA was estimated using the (Nanodrop spectrophotometer) used as pure RNA to measure [the RNA concentration and purity at absorption 260/280 nm at ratio 1.8].

Reverse Transcription Step (cDNA Synthesis

Reverse Transcription was performed for converted extracted viral RNA molecules into cDNA templates by using**(NEXscript™ cDNA Synthesis Kit)**

Real-Time Polymerase Chain Reaction

This technique was carried out following steps that used for detection of Hepatitis A virus based polyprotein gene.

Real-Time PCR Master Mix Preparation

qPCR master mix was prepared by using (NEXpro™ qPCR Master Mix (Probe).

Real-Time PCR Thermocycler Conditions

Real-Time PCR thermocycler conditions was set according to primer annealing temperature and qPCRTaqMan kit instructions by Biorad Real-Time PCR thermocycler system as in the following table.

TABLE 1. Biorad Real-Time PCR thermocycler system

Step	Condition	Cycle
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	45
Annealing/Extension	60 °C 30 sec	
Detection (Scan)		

Real-Time PCR Data Analysis

qPCR data analysis was performed by(calculation the threshold cycle number) (CT value) that) presented the positive (amplification in Real-Time PCR cycle) number

RESULT AND DISCUSSION

Hepatitis A virus detection by quick test established anti-HAV IgM detection in acute hepatitis A infection Figure (1) is detectable approximately 3 weeks after exposure, rises over{ 4 to 6} weeks, then the levels decreases and become undetectable common(within 6 months of infection). In Iraq, HAV infection is (hyper-endemic), with a relative prevalence of positive anti-HAV IgM antibodies in patients with HAV IgM.. Previous HAV contamination detection observes reviews 20% of affected person signs discovered inside two to 4 weeks after being infected, that could final from every (week to greater than a month) anywhere. Around [15% of people with hepatitis A] have signs that final among (6 and 9) months (7). With age, the occurrence of this HAV contamination will increase and older age companies are much more likely to growth themorbidity and mortality of the disease.

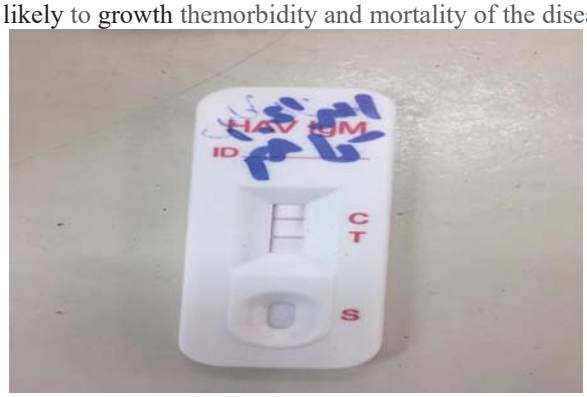

FIGURE 1. Hepatitis A infection confirmed by detection anti-HAV IgM

Detection of Hepatitis A Virus by Real Time-PCR Assay

In stool samples, HAV antigen has been identified by RT-qPCR assay. Blood antigen detection has been difficult because fibronectin can bind to HAV and mask the antigenic determinants required for immunological detection (8). Nucleic acid detection methods are more vulnerable to HAV detection in stool samples than viral antigen immunoassays. Viral RNA amplification.

The first step in RT-PCR (Robertson et al., 1991) is the purification of viral RNA from stool samples. Real-time PCR, which has revolutionized nucleic acid detection through its high speed, sensitivity, reproducibility and contamination minimization, has been extended to the detection and quantification of HAV (9),due to its [sensitivity, accuracy and capacity to deliver quantitative data in samples,] real-time reverse-transcription PCR has become one of the most promising methods of detection. The real-time TaqMan RT-PCR established in the current HAV quantification study is focused on the amplification of polyprotein gene genetics (10).. This area is the utmost preserved genome of the HAV and any other picornavirus and is therefore a good choice for quantification techniques to be developed (11). In the current analysis, Figure (2) shows the effects of quantitative HAV detection in stool

samples obtained from (100) infected children and viral concentrations found to be higher than the second decade in the first decade and represented sixteen positive for Hepatitis A Virus in the sample.

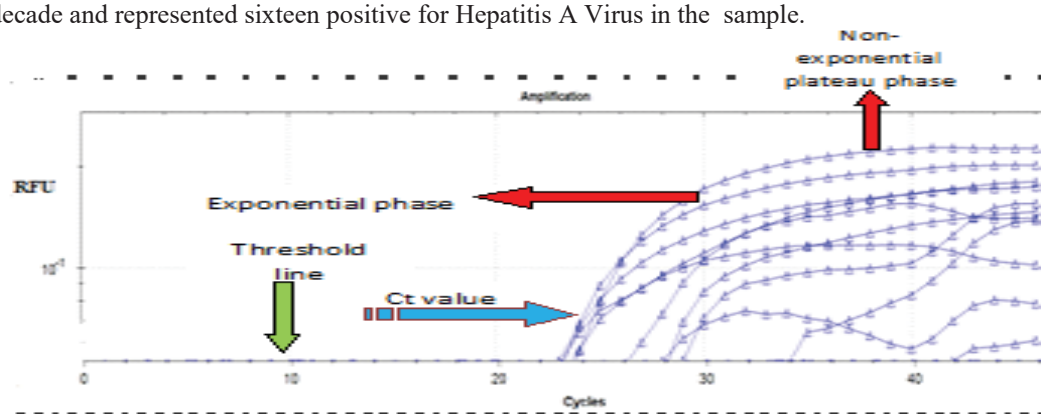

FIGURE 2. Amplification plots for hepatitis A virus

The PCR length number is proven on the x-axis on this plot, and the fluorescence from the amplification reaction is proven on the y-axis, which is proportional to the amount of amplified product inside the tube. The amplification plot reveals two levels, an exponential phase followed with the aid of the anon-exponential plateau phase. The sum of the PCR product roughly doubles in each step during the exponential process. However, as the reaction progresses, reaction components are consumed and one or more of the components are finally reduced. The reaction reduces and arrives the (plateau phase) at this phase.

Real time PCR 100 samples of stool were examined to be detected in patient samples where the RNA virus was extracted and then converted to CDNA by RT-PCR and upon obtaining the results of PCR we used RT-qPCR where 16 positive samples were detected, that is why Real time use of PCR is to obtain real-time results from amplifying the sample as the device draws a curve for each sample and through it we can know the amplified samples and that the value of CT is the number of PCR cycles

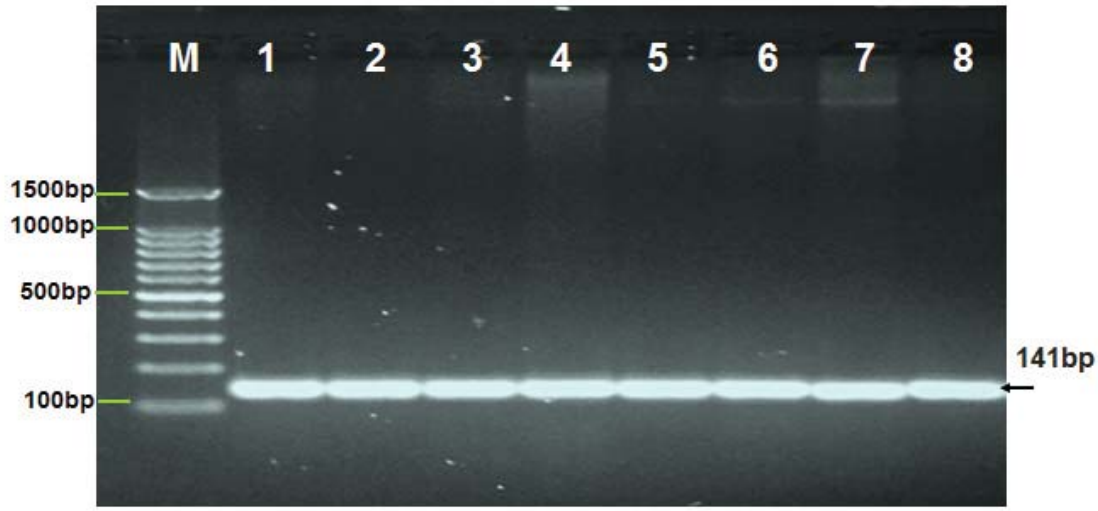

FIGURE 3. Agarose gel electrophoresis image that showed qPCR product analysis for polyprotein gene in Hepatovirus A isolates. M (Marker ladder 1500-100bp). Lane (1-8) only positive Hepatovirus A isolates at 141bp product size.

Sequencing for HAV Isolates from Infected Children

Sequencing of viral nucleic acid methods can be done on PCR yields to check their specificity, and this supply the best actual way to recognize and genotype the viral pathogen. Selected regions of the HAV genome as the nucleic acid sequencing has been used to limit the genetic interactions among its isolates(12, 13).

The target regions of sequence alignment of the(primers and probe) used in this research revealed them to be adequate for the quantification of eight samples of HAV genotypes as in Figures (3),(4) respectively.

FIGURE 4. Multiple collection alignment evaluation of polyprotein gene in neighborhood HepatovirusA Human isolates and NCBI-Genbank Hepatovirus A isolates. The more than one alignment evaluation turned into built the usage of Clustal W alignment device in (MEGA 6.zero version). That display the nucleotide alignment similarity as (*) with substitution mutations in polyprotein gene.

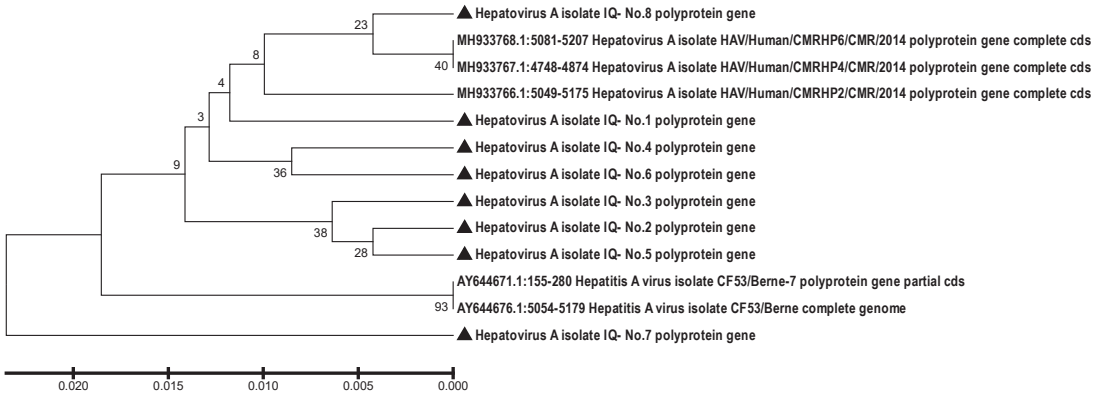

FIGURE 5. Phylogenetic tree evaluation primarily based totally on polyprotein gene partial sequence in neighborhood Hepatovirus A Human isolates that used for genetic Hepatovirus A Human genetic identification) .[The become built using: Un weighted Pair Group approach with Arithmetic Mean[(UPGMA tree)]]in (MEGA 6.zero version).at overall genetic changes (0.005-0.020%).

The genetic tree fee is to determine the genetic changes, the difference and similarity between local and global isolates recorded in the gene bank and the genetic tree analysis if the change is more than 0.1 this means polymorphism and if it is less than 0,1e it means mutation and hemology analysis Sequence identity using MEGA 6 Version.

Table 2 the NCBI-BLAST Homology Sequence identity (98-99%) between local Hepatovirus A isolate and NCBI-BLAST submitted Hepatovirus A isolate:

TABLE 2. The NCBI-BLAST Homology Sequence identity (98-99%) between local Hepatovirus

Hepatovirus Aisolate No.1	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)		
		Identical isolate	Genbank Accession number	Identity (%)
Hepatovirus A isolate No.1	MN313379	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	98.43%
Hepatovirus A isolate No.2	MN313380	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	99.17%
Hepatovirus A isolate No.3	MN313381	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	98.29%
Hepatovirus A isolate No.4	MN313382	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	98.39%
Hepatovirus Aisolate No.5	MN313383	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	99.14%
Hepatovirus Aisolate No.6	MN313384	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	98.33%
Hepatovirus Aisolate No.7	MN313385	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	96.67%
Hepatovirus Aisolate No.8	MN313386	Hepatovirus A isolate HAV/Human/CMRHP4/CMR/2014	MH933767.1	99.16%

This study analyzed of poly protein gene for HAV isolate, resultant in the classification of eight genotypes of hepatitis A virus as in table (1). A genotype is well-defined as a assemblage of viruses possessing {nucleotide sequences }that are more than (85%) identical (14).

Ethical Approval and Consent

All subjects involved in this work are informed and the agreement will obtained verbally from each one before the collection of samples. This study was approved by the committee on publication ethics at college of medicine, University of Babylon, Iraq, under the reference No. BMS/0231/016.

CONCLUSION

1-The correlation among viral load and precise serological markers are vital gear to the prognosis.

2-The prognosis in sufferers with out precise antibodies for hepatitis A need to be performed through the detection and quantification of HAV RNA, that's beneficial for following the contamination and to make clear the prognosis.

3-These RT-qPCR assays can be in particular beneficial for appropriately tracing HAV in low-degree infected samples

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