Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science

Department of Biology

Genotyping of Hepatitis C Virus As Forensic Evidence in Babylon province

A Research

Submitted to Council of the College of Science / University of Babylon in Partial Fulfillment to the Requirements for the Degree of High Diploma in Science /Forensic Evidence

By

Hussein Jasim Mohammed Terfah

B.Sc. Biology /University of Babylon(2001)

Supervised By

Professor

Dr. Shakir Hammad Mohammed Hassan

Ph.D Molecular Virology

2021 A. D **1443 A.H**

بسم الله الرحمن الرحيم

﴿ وَيُعَلِّمُكُمُ ٱلْكِتَٰبَ وَٱلْحِكْمَةَ وَيُعَلِّمُكُم مَّا لَمْ تَكُونُواْ تَعْلَمُونَ ﴿١٥١﴾ ﴾

صدق الله العلي العظيم

(البقرة١٥١)

certification

I certify that research is prepared under my supervision at the Department of Biology, College of Sciences, University of Babylon, as partial fulfillment of requirements for the Degree of Higher Diploma in Forensic Evidence.

Signature:

Name : Dr. Shakir Hammad Mohammed Hassan

Date:

In view of available recommendation, I forward this research for debate by the Examination Committee

Signature:

Name: Dr. Adi Jasim Abd AL-Razzak

Title: Head of Biology

Date:

Committee Certification

We, the examining committee, certify that we have read the research entitled(**Genotyping of Hepatitis C Virus As Forensic Evidence in Babylon province**) and examined the student (**Hussein Jasim Mohammed Terfah**) in its contents at 21 / 11 /2021, and that in our opinion it is accepted as a research for the degree of **High Diploma in Forensic Evidence** with estimation.

Signature:

Prof. Dr .Maysaa Adil Hadi

Data: / / 2021

(Chairman)

Signature:

Assist .Prof. Dr.Noor Salman Kadhim

Data: / / 2021

(Member)

Signature:

Prof. Dr. Shakir Hammad Mohammed Hassan

Depart of Biology

College of Science

University of Babylon

Data: / / 2021

(Member and Advisor)

Approved by the College Committee on Graduate Studies

Signature:

Prof. Dr. 1nas Mohammed Al-Rubyn

Dean of Collage of Sciences –University of Babylon

Data: / /2021

Dedication

To the reason being the creator of every things ,thanks and praise be to Allah for every things .

To my wife for her supports and helping me in my study.

To my lovely sons and dear daughter.

To all people who I love.

To every person who help me with my regards.

I dedicate this work.

Hussein 2021

ACKNOWLEDGEMENTS

At first, I thank my God "*Allah*" for providing me with strength, patience and willingness to establish this work. I would like to express my heartiest and sincerest sense of gratitude to my elder brother, my teacher, and my worthy supervisor ***Professor Dr. Shakir Hammad Mohammed*** for providing his valuable guidance, inspiration, supervision, and moral support from the very early stage till the end of my work.

My special thanks to the head of Biology Department and the dean of the Science College , University of Babylon for their assistance .

My sincere thanks and gratitude are to the teaching staff members , friends and Diploma students who helped me at the Biology Department in College of Science, University of Babylon**.**

My special thanks and gratitude for **Dr. Raheem Tuama Al-Mammori** , Ph.D Clinical immunology for his helping in my research.

Hussein 2021

Summary

Background: Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver; The virus persists in the liver in about 75% to 85% of those initially infected. Early on chronic infection typically has no symptoms .The classification of hepatitis C virus (HCV) genotypes is of clinical importance as it may help to predict drug therapy responses and estimate treatment duration. The classical method of HCV sub genotype classification is whole genome sequencing (WGS).

Objectives: Determine the infection rate of hepatitis C virus genotypes among patients infected with HCV ;blood donors attended to Blood Bank in Babylon province , Iraq.

Patients and Methods: Cross sectional study which conducted at patients infected with HCV ;blood donors attended to blood bank in Babylon province , Iraq. Database were collected from general health department at Babylon Health directorate in the last 5 years (June 2016-June 2021) and 12260 investigated person; they're aged from 17-65 years during a period from Dec. 2020 to June. 2021. Blood samples were collected from all participants then analyzed by use different enzyme linked immunesorrbent assay (ELISA) kits and real-time polymerase chain reaction (RT-PCR) to determine the HCV genotypes. Only 586 HCV patients was analysis by RT-PCR around the year 2020in Babylon province.

Results : Detection of HCV –RT-PCR reactions was observed in 48 out of 586 (8.2%). The genotype 4 pattern that displays 76.92% of untreated patients group and 8.7% of treated patients group. In general, there was no significant association between the different genotypes and some demographical factors, serological investigations, and liver function test.

Conclusions : The most common HCV genotype in Iraq was genotype 4. Further studies involving the sources of transmission and impact of hepatic biomarker are required to enhance the control measures of HCV infections.

CONTENTS

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Complete Name
Apo	Apolipoproteins
CD	Clusters of Differentiations
DNA	Deoxy riboNucleic Acid
ELISA	enzyme linked immune sorrbent assay
ER	Endoplasmic Reticulum
HBV	Hepatitis B Virus
HCC	hepatocellular carcinoma
HCV	Hepatitis C Virus
HSC	hepatic stellate cells
IC	Internal Control
IDU	injection drug use
IFN	Interferon
IgG	Immune globin G
IRES	internal ribosomal entrance site
LDL	low density lipoproteins
NK	natural killer
ORF	single open reading frame
RNA	Ribo Nucleic Acid
RT-PCR	Real Time Polymerase Chain Reaction
SVR	sustained viral response
Treg	regulatory T cells
UTR	untranslated regions
VLDL	very low density lipoproteins
WHO	World Health Organization

Chapter One
Introduction

1. Introduction

1.1.Introduction

Hepatitis C is a liver disease effects on the lives of 14 million people about one in every 50 people. This is caused by the hepatitis C virus (HCV) and may cause serious complications, including cirrhosis and liver cancer, both acute and chronic infections (WHO, 2019). Typically, it is spread by blood. Sexual intercourse may also happen, but there is a very small chance of this. The greatest important risk factor for HCV infection in the American United States is usage of injection medication (Jill, 2020).

About more than 120 million, or 3% of the world's were HCV-infected people. Depending to the World Health Organization (WHO), nearly 3 - 4 million new cases of Hepatitis C virus infection are reported yearly (Morozov and Lagaye, 2018). It is a major cause of hepatic morbidity and mortality by its predisposition to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Annually, HCV causes about more than 390,000 deaths world-wide, mostly from cirrhosis and hepatocellular carcinoma (Renau and Berenguer, 2018).

In Iraq, hepatitis C is considered of low endemicity. The prevalence of anti-HCV was little (0.4%) (Hanan *et al.,* 2019). HCV prevalence was with a range of 0.32 % to 7.1 % in Iraqi general people (Abdulghani *et al.,* 2016). There are six major genotypes with several subtypes, HCV dominance is inconstant in different areas of the world and among dissimilar population groups. For greatest countries, genotype-1 and genotype-3 are more predominant relative to other genotypes, whereas genotype-4 and 5 are predominant in less advanced countries. In Middle East and Africa, genotype 4 are the most predominant genotypes (Nouroz *et al.*, 2015). There have been several improvements in the management of HCV over the last decade, including the introduction of different and more powerful drugs.

Harvoni is the newly drug that is given once-daily, fixed-dose oral combination therapy that has been approved for HCV genotype 1. It is unusual in that it does not require co-administration of ribavirin and/or interferon and has shown higher levels of sustained viral response (SVR) at the end treatment duration relative to conventional controls (Diana and Gregory , 2015)

1.2. Aim of Study

This study was aimed to determine the prevalence rate of HCV infection for last year (2020-2021) in Babylon Province via the following objectives :

1. To investigate the prevalence of HCV genotypes, its correlation with demographic factors.
2. Obtaining data on the number HCV on the record kept at general health department at Babylon health directorate.

Chapter Two
Literature Review

2. Literature Review

2.1 HCV Virology

Hepatitis is liver inflammation, due to a variety of causes, such as viral infection, caused primarily by infection with one of the seven hepatitis viruses (A G), that use the liver as their primary replication site (Vijay *et al*., 2010). These viruses cause similar clinical symptoms during the time of acute infection such as weakness, malaise, low-grade fever, headache, myalgia, arthralgia, lack of appetite, nausea and vomiting, altered taste sensation and avoidance of fatty foods and smoking, but differ in their ability to induce chronic infectious disease (Hollinger and Emerson, 2007).

Hepatitis C virus (HCV) is one of the major causes of chronic liver disease worldwide. It was isolated in 1989; it's the main agent of so-called chronic hepatitis non A / non B (Clodoveo *et al*., 2015).

Hepatic injury with or without hepatocellular carcinoma (HCC) can range from minor histological changes to severe fibrosis and cirrhosis (Polaris, 2015). Approximately 71 million people worldwide are chronically infected, many of whom are unaware of their infection, with significant variations according to the geographical area (European Union, 2017). Hepatitis C causes about 399,000 deaths worldwide each year, most of them from cirrhosis and hepatocellular carcinoma (HCC) (WHO, 2017). The genome from the HCV has shown prominent genetic diversity. Seven genotypes were identified (gt 1-7), and 67 subtypes (a, b, c, etc.). Genotypes are defined by an distinct geographical distribution and clinical symptoms (Smith *et al*., 2014), and HCV mutants circulate in the blood as millions of quasi-organisms, several of which can shirk host immune responses and even direct antiviral treatment (Agata, 2017).

2.2 Morphology and Molecular biology of HCV

HCV is a small, enveloped, positive single-stranded ribose nucleic acid (ssRNA) virus which belongs to the Flaviviridae family genus Hepacivirus. Virus analysis shows that the enveloped particles are icosahedral and have a diameter of 56-65 nm, while the viral core is around 45 nm. Viral spikes on the virion membrane are about 6 nm and are formed by heterodimers of E1 and E2 glycoproteins (Gastaminza *et al*.,2010).

Large quantities of the particles are connected to cellular lipoproteins making this a hallmark of HCV (Moriishi and Matsuura, 2012). The sequence of lipoproteins associated with the virus may vary, and many of these are most commonly associated with HCV: low density lipoproteins (LDL), very low density lipoproteins (VLDL), and apolipoproteins (Apo) A1, B, C, and E (Vercauteren *et al*., 2014). The lipoprotein-related viral particles are called "lipoviral particles (LVP)" (Grassi *et al*.,2016).

The viral RNA genome is approximately 9600 nucleotides long. This involves two untranslated regions (UTR) 5'-UTR and 3'-UTR with a single open reading frame (ORF) coding a single precursor of poly protein 3010 to 3033 amino acids (aa) (Sharma, 2011). Translation occurs in the endoplasmic reticulum (ER) and is caused by the internal ribosomal entrance site (IRES) at the 5 'UTR (Fraser and Doudna, 2007). Cellular and viral proteases transform a single precursor of polyproteins into ten proteins (Vijay *et al*., 2010). Three structural proteins (core, E1, E2) are located on the polyprotein's amino-terminal portion, and are essential components of the virions. In the remaining part of the polyprotein, seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) are contributed in particle morphogenesis, RNA replication and cell function control (Vladimir and Sylvie, 2018).

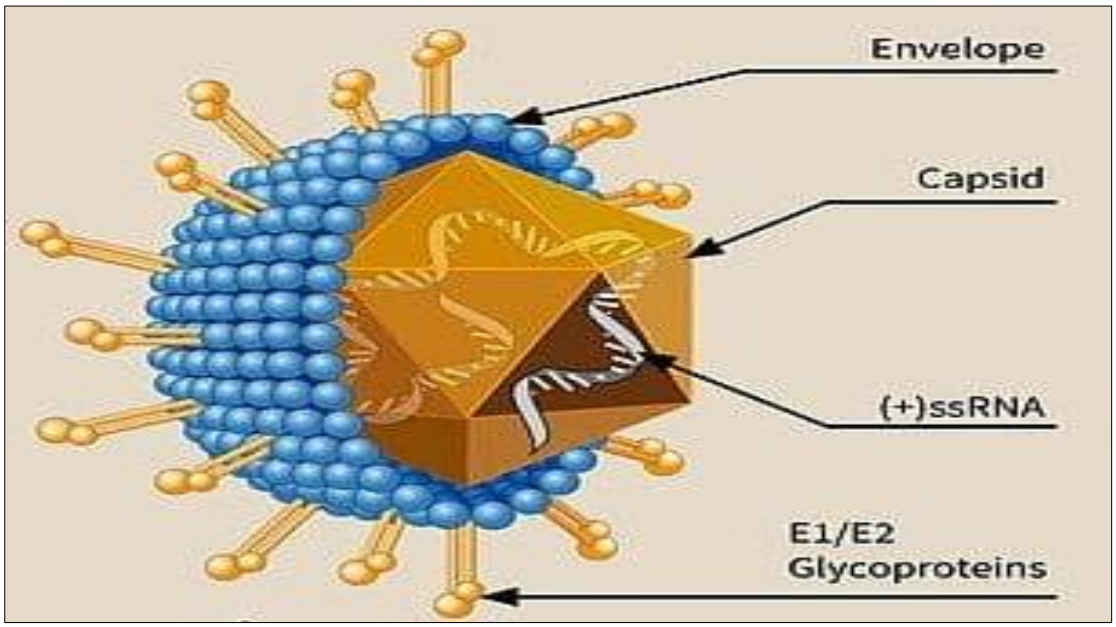

Figure (2.1):Structure of Hepatitis C Virus.

2.3 HCV Genotypes

Hepatitis C virus has a high degree of genetic diversity, the number of HCV genotypes and subtypes continues to increase. HCV has been categorized into seven distinct genotypes that vary by more than 30% at nucleotide level (Smith *et al*., 2014). A novel genotype of the HCV, genotype 8, was newly identified in four epidemiologically unlinked patients from the Indian state of Punjab, forming a distinct phylogenetic community from previously described sequences (Borgia *et al*., 2018). Genotypes are further classified into subtypes with more than 15 percent gene divergence (Smith *et al*., 2014). To date, it has described 86 confirmed HCV subtypes with probably even more genotypes and subtypes to be found (Widell *et al*., 2016). Hepatitis C virus genotype 1, 2 and 3 circulate around the world, though with variable distribution in diverse geographic areas. HCV genotype 1 is the most prevalent genotype in the world (46 %). HCV subtypes 1a and 1b predominate in North America, Europe and Australia, while in Japan, 73 percent of people diagnosed with HCV have subtype 1b infection. Genotype 3 is the world's second most prevalent (30%) genotype, predominantly distributed in South Asia, and reflects an disproportionately high

distribution among people, who inject drugs (PWIDs), regardless of geography (Messina *et al*. 2015). HCV genotype 4 infections occur mainly in Africa and the Middle East, with genotype 5 and genotype 6 being limited to South Africa and South East Asia, respectively (Asselah *et al*., 2017). Genotypes 1, 2, 3, 4 and 6 are made up of many subtypes, indicating a high degree of genetic variation. A patient from the Democratic Republic of Congo has been reported as having the HCV genotype 7a in 2006. Later another patient was found to be infected with genotype 7b from the same area. Genotype 5 and the recently defined genotype 8 have one subtype each (Schreiber *et al*., 2016).

2.4 Epidemiology of HCV

The disease is distributed unevenly in different nations, with a prevalence in the general population of between 0.5 and 6.5 percent. In Western countries and Australia, this rate ranges from 0.5 to 1.5 percent; it exceeds 2.3 percent in Southeast Asian countries and in the eastern Mediterranean regions (WHO, 2017), 3.2 percent in China, 0.9 percent in India, 2.2 per cent in Indonesia and 6.5 percent in Pakistan (Petruzziello *et al*., 2016). The global distribution of every genotype of HCV varies across different geographic regions. HCV genotype 1 is the world's most common genotype and has a wide geographic distribution of 46 per cent of all HCV infections. HCV genotype 3 is the second most prevalent genotype in South Asia, Australia and several European countries, responsible for 30 percent of global infections (Smith *et al*., 2014). HCV genotypes 2 and 4 represent 9-13 per cent of more broadly distributed HCV infections; HCV genotype 2 prevalence is higher in Asia and West Africa; HCV genotype 4 infection is high in Central and Eastern SubSaharan Africa, North Africa and the Middle East. HCV genotypes 5, 6, and 7 are the most geographically confined, with genotype 5 widespread in South Africa and genotype 6 prevalent in East and South East Asia, while genotype 7 has been reported in a small number in the Democratic Republic of Congo (Gower *et al*., 2014).

2.5 Pathogenesis

The HCV induces liver cell damage but the exact mechanism of this effect is unclear. The damage is thinking to be primarily mediated by the immune response of the host, including long-lasting inflammation (CDC., 2015). Although other subsets, such as CD4+T and natural killer (NK) cells and regulatory T cells (Treg) are identified (Spengler and Nattermann, 2007), a lympho-mononuclear infiltrate predominantly represented by CD8+T cells predicted to play a major role in viral thoroughness. Inflammatory cells of the cytokines and chemokines secreting the intrahepatic infiltrate induce liver fibrosis to trigger hepatic stellate cells (HSC) for collagen secretion. HSCs may exist as many different phenotypes with distinct molecular and cellular functions and characteristics, each contributing significantly to hepatic homeostasis and illness (Lee *et al*., 2015).

2.6 Acute Infection

Acute hepatitis C is asymptomatic for 90 percent of infected people (Modi and Liang, 2008). For certain cases there may be asthenia, fever, and muscle, as well as joint pain. And there aren't frequent jaundice signs. A transient increase in serum transaminase levels is known as acute hepatitis C. The first noticeable marker for viruses is the viral RNA, that occurs one to two weeks later after exposure. Seven-eight weeks later, the anti-HCV IgG response can then be identified (Modi and Liang 2008 ; Maasoumy and Wedemeyer 2012). Hepatitis C is normally healed by innate and adaptive immunity in 20 percent of cases. The viral RNA is undetectable within three to four months of infection. Several factors may be facilitating the viral clearance. Similarly, acute hepatitis symptoms will indicate a substantial immune response on the host (Thomas and Seeff, 2005).

2.7 Chronic Infection

For around 80 percent of cases, the immune system is unable to kill HCV during the acute period of infection. Hepatitis is seen as recurring (Maasoumy and Wedemeyer, 2012) when the viral replication occurs for more than six months after an acute infection. Most patients are asymptomatic at the chronic hepatitis stage, and may have no non-specific symptoms such as weakness, arthralgia, or myalgia. The levels of transaminase can increase moderately, or even be normal (Scott and Gretch 2007). The progression of chronic infection over the long term is complex. The factors which accelerate the progression of the disease are: gender, HIV co-infection, higher body mass index, fatty live and alcohol consumption (Thomas and Seeff, 2005).

After 10 to 30 years, about 20 to 30 percent of patients have cirrhosis. Cirrhosis after portal hypertension may be associated with liver failure (ascites, gastrointestinal bleeding, etc.) as a decompensation. For cirrhotic patients, the risk of death from complications is 4% per year, and their risk of developing hepatocellular carcinoma (HCC) is 1 to 5% per annum. Within one year of diagnosis 33 percent of HCC patients die (Thomas and Seeff, 2005 ; Scott and Gretch, 2007). Overall, cirrhosis patients have a 5-year survival rate of 50%. Decompensated cirrhosis is the leading cause of hepatic transplantation (Thomas and Seeff, 2005 ; Maasoumy and Wedemeyer, 2012).

Chapter Three

Materials and Methods

3.Materials and Methods

Raw database were collected from general health department at Babylon health directorate in the last 5 years (June 2016- June 2021) and (435949) case were investigated ; they're aged from 17-65 years during a period from Dec. 2020 to June. 2021.

3.1 Patients Selection

There were 586 of known cases of Hepatitis C infection recorded in the Babylon GIT center for last year, 48 patients have positive viral load , while other has undetectable level of nucleic acid or (Viral load < 10 IU/ml) in which that lower level can be detected in Cephid machine provided in the PCR unit of the center , Positive recoded cases were done in Babylon GIT center at the period between Dec. 2020 up to June 2021 **,** the statistical comparison in which done between the positive cases and undetectable cases instead of health control

3.2. Extraction of viral genome :-

The provided lists or material were listed individually according to manual part of procedures **,** in addition to the RT-PCR and other recommended materials.

3.2.1. Methods:-

By using user manual of RNA / DNA - Ribo Virus Kit. The Kit of Ribo Virus is designed for the rapid preparation of highly pure viral nucleic acids (e.g. HCV, HIV, HAV, HDV, Enteroviruses, CMV, HBV) from fluid biological samples e.g. plasma, serum, urine, bone marrow, swabs, liquor.

3.2.1.1. Principle of assay

With the **Ribo Virus**, RNA viruses are lysed quickly and efficiently by lysis buffer RAV1 which is a highly concentrated solution . Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane in the **Ribo Virus** columns**.** Carrier RNA improves binding and recovery of the low-concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and

soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers **RAW** and finally **RAV3**. The nucleic acids can be eluted in low salt buffer or water and are ready-for use in subsequent reactions. The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic manipulation.

3.2.1.2. Materials Provided .

· Buffer RAV1 , 2 x 35 ml;

· Buffer RAW , 2 x 30 ml;

· Buffer RAV3 (concentrate) , 2 x 12 ml;

· Buffer RE , 2 x 13 ml;

· Rnase -free H2O , 2 x 13 ml;

· Carrier RNA (lyophilized) , 2 x 1 mg;

· Proteinase K , 2 x 50 mg;

· Proteinase buffer , 8,0 ml;

· Ribo Virus columns with collecting tubes (2ml), 100 piece

· Collecting tubes (2ml), 8 x 50

3.2.1.3. Preparation of Working Solution :-

- **Ribo Virus** columns should be stored dry at room temperature (15–25°C) . All solutions should be stored at room temperature unless otherwise stated. **Ribo Virus** spin columns, all buffers and reagents can be stored for up to 2 years under the above conditions without showing any reduction in performance.

- Before use, add 1 ml lysis buffer **RAV1** to the complete contents of the carrier RNA tube. Dissolve the RNA and transfer it back to the RAV1 bottle.

- Before first use of the kit, add **2,25 ml of Proteinase Buffer** into the vial containing Proteinase K, to dissolve lyophilized proteinase K.

- Buffer RAV1 including carrier RNA can be stored at room temperature for 1-2 weeks. Also can be stored at 4°C for up to 4 weeks or aliquot and stored at –20°C for longer periods.
- Do not warm buffer RAV1 containing carrier RNA more than 4 times! Frequent warming, temperatures >80°C and extended heat incubation will accelerate the degradation of carrier RNA. This leads to reduced recovery of viral RNA and eventually false negative RT-PCR results, in particular if low titer samples are used.

· **Dissolved Proteinase** is stable for up to 6 months when stored at 2–8°C. Storage at –20°C is recommended to prolong the life of Proteinase, but repeated freezing and thawing should be avoided.

· **Buffer RAV3**: Add **48 ml ethanol** (96-100%) to **buffer RAV3**. Store buffer RAV3 at room temperature for up to one year.

3.2.1.4. Extraction Protocol

Viral RNA/DNA isolation from cell-free biological fluids (Plasma) with Ribo Virus. Before starting the viral RNA/DNA isolation, prepare a 70°C incubation block and preheat an aliquot of elution buffer/water. The steps were listed briefly in the following figure (3 - 1).

Step	Description
1. Lyse	 • 600 µl RAV1 • 150 µl sample • 20 µl Proteinase K • Internal Control (according to the PCR amplification manufacture's instruction) 70°C 5 min
2. Adjust DNA	600 µl Ethanol
3. Bind	 Load sample  1 min 8000 x g
4. Wash	 1st wash: 500 µl RAW 2nd wash: 600 µl RAV3 3rd wash: 200 µl RAV3  1st and 2nd 1 min 8000 x g  3rd 5 min 11000 x g
5. Elute	 50 µl Rnase-free H_2O or Buffer RE (70°C) 1-2 min  1 min 11000 x g

Figure (3 - 1) Manual procedure of RNA/DNA Extraction .

3.3 Determination of viral load by Sacace HCV Real-TM Quant Dx PCR Kit.

I. Principle:

HCV Real-TM Quant Dx Kit is a Real-Time test for the Quantitative detection of Hepatitis C Virus in human plasma. HCV RNA was extracted from plasma, amplified using real time amplification and detected using fluorescent reporter dye probes specific for HCV or HCV IC.

II. Reagents

A. RT-PCR reagent pack

• Ninety six vials (0.2 ml) with lyophilized amplification reagents.

B. Sacace HCV Real-TM Quant Dx Control Kit1

• **Control int.**: lyophilized reagent HCV-IC-L.

• **Control 1:** lyophilized reagent HCV-Pos1-L C+ - Sacace HCV RealTM Quant Dx High Positive Control.

• **Control 2:** lyophilized reagent HCV-Pos2-L C+ - Sacace HCV RealTM Quant Dx Low Positive Control.

• **Control, negative:** Negative Control.

C. Sacace HCV Real-TM Quant Dx Calibrator Kit

• **CAL 1:** lyophilized reagent HCV Quantitative Standard 1.

• **CAL 2:** lyophilized reagent HCV Quantitative Standard 2.

3.3.1Procedure

1. Six reaction tubes with lyophilized reagents were prepared to perform PCR of extracted calibrators.
2. Fifty µl of calibrator were added to the tube of reaction.
3. Requested quantity of reaction tubes with lyophilized reagents were prepared to perform PCR of extracted samples and control .
4. Fifty µl of RNA sample, negative control and positive control were added to reaction tube.
5. The tubes were closed and transferred them into the Real Time PCR instrument.
6. A temperature profile was created on Real-time instrument as follows.

- Ten µl of internal control was introduced into each sample at the beginning of sample preparation procedure.
- Each DNA amplification was associated with generation of a fluorescence signal measurable in FAM/Green channel (for IC) or in Joe/Yellow/HEX channel (for HCV RNA) resulting in a sigmoid growth curve (log scale).
- The linear range of the HCV Real-TM Quant Dx kit was determined by analyzing a dilution series (from 8,00 log IU/ml to 1,00 log IU/ml) of an HCV synthetic quantitative standard.
- HCV RNA concentration was expressed in IU/ml.

3.4 Genotype detection

I. Principle:

HCV Genotype Plus Real-TM is based on three major processes:

1. Isolation of HCV RNA from specimens.
2. Reverse transcription of the RNA
3. Real time PCR:

3.5 PCR-mix-1-FRT HCV 1b/3 with primers and probes for subtypes 1b,

- PCR-mix-1-FRT HCV 1a/2 with primers and probes for subtypes 1a, 2.
- PCR-mix-1-FRT HCV 4/IC with primers and probes for subtypes 4 and Internal Control
- PCR-mix-1-FRT HCV 5a/6 with primers and probes for subtypes 5a, 6.

3.5.1Procedure

1. PCR reagent were thawed and centrifuged briefly.
2. Four PCR tubes were prepared for each sample and marked them properly (1b/3, 1a/2, 4/IC, 5a/6).
3. Four master mixes were prepared 1b/3, 1a/2, 4/IC and 5a/6
4. The following reagent were added for each sample in sterile tube:

- Ten µl of PCR-mix-1-FRT HCV genotype 1b/3 (or 1a/2 or 4/IC or 5a/6) were added.
- Five µl of RT-PCR-mix-2-TM.
- Half (0.5) µl of polymerase (TaqF).

5. Fifteen µl of Master Mix and ten µl of cDNA sample were added to an appropriate tube.
6. Fifteen µl of Master Mix and ten µl of negative control were added to an appropriate tube .
7. Positive control was performed for each PCR-mix-1 as fallowing:

- Ten µl of positive control cDNA HCV genotype 1b/3, 1a/2, 4/IC and 5a/6 were added to the tubes with 1b/3, 1a/2, 4/IC and 5a/6

reaction mix labeled C+ve 1b/3, C+ve 1a/2, C+ve 4/IC and C+ve 5a/6 respectively.

3.6 Data analysis

Statistical analysis were done by using SPSS program. The fluorescence curves are analyzed with the software of Real Time PCR instruments on the 2 channels (FAM/Green and Joe/Yellow).

Chapter Four

Results

4.Results :-

4.1 Distribution and incidence rate of HCV infection among screened patients in Babylon province for past 5 years (June 2016 to June 2021).

Total of (435949) cases were investigated for IgG & IgM - HCV using rapid test and ELISA assays to confirm infections**.** The results revealed that , total positive cases were 2956 for HCV. The highest no. of cases for HCV was recorded in 2021 year 586 (0.878%).The incidence rate of HCV was varies worldwide.(Table 4.1).

Table 4.1: Distribution of incidence % of HCV infections in Babylon Province (2016-2021).

YEAR	Tested	IgG & IgM HCV Cases	HCV %
2016	82895	572	0.690
2017	77857	443	0.568
2018	78674	565	0.718
2019	76578	348	0.454
2020	53267	442	0.829
2021	66678	586	0.878
TOTAL	435949	2956	0.678

4.2 Distribution of Patients Infected with HCV in Regarding of Geographical Area of Babylon Province

The north area has higher number of patients 43% (1270 out of 2956 cases) comparing to the middle 15.2 % (450 out of 2956 cases) and south area 41.8% (1236 out of 2956 cases) as illustrated

in Table (4-2). The statistical analysis showed significant difference (P≤ 0.05) among the geographical Area of Babylon province.

Table 4.2: Distribution of Patients Infectious with HCV in Regarding of Geographical Area of Babylon Province

District	HCV POSTIVE	%	P-Value
North Babylon Mahaweel , Musaib and Al – Eskanderia	251	43	P≤ 0.05
Middle area , the center area of province	89	15.2	
South Babylon Al – Hashimia , Alkefel and Al –Shomaly	246	41.8	
Total	586	100	

4.3 Distribution of patients According Rural and Urban area.

The rural area has higher number of patients(43%) rather than urban (15.2%) in the recorded patients which have enrolled in this study as shown in table (4-3). The statistical analysis showed significant difference (P≤ 0.05) according rural and urban area of Babylon Province.

Table 4.3: Address distribution of Hepatitis C virus patients According to Residence(2021).

District	HCV POSTIVE	%	P-Value
Rural	335	57	
Urban	251	43	
Total	586	100	P≤ 0.05

4.4 Distribution of HCV patients According Gender.

The figure (4.1) was showed the gender distribution of HCV patients showed that female patients have higher number than male.

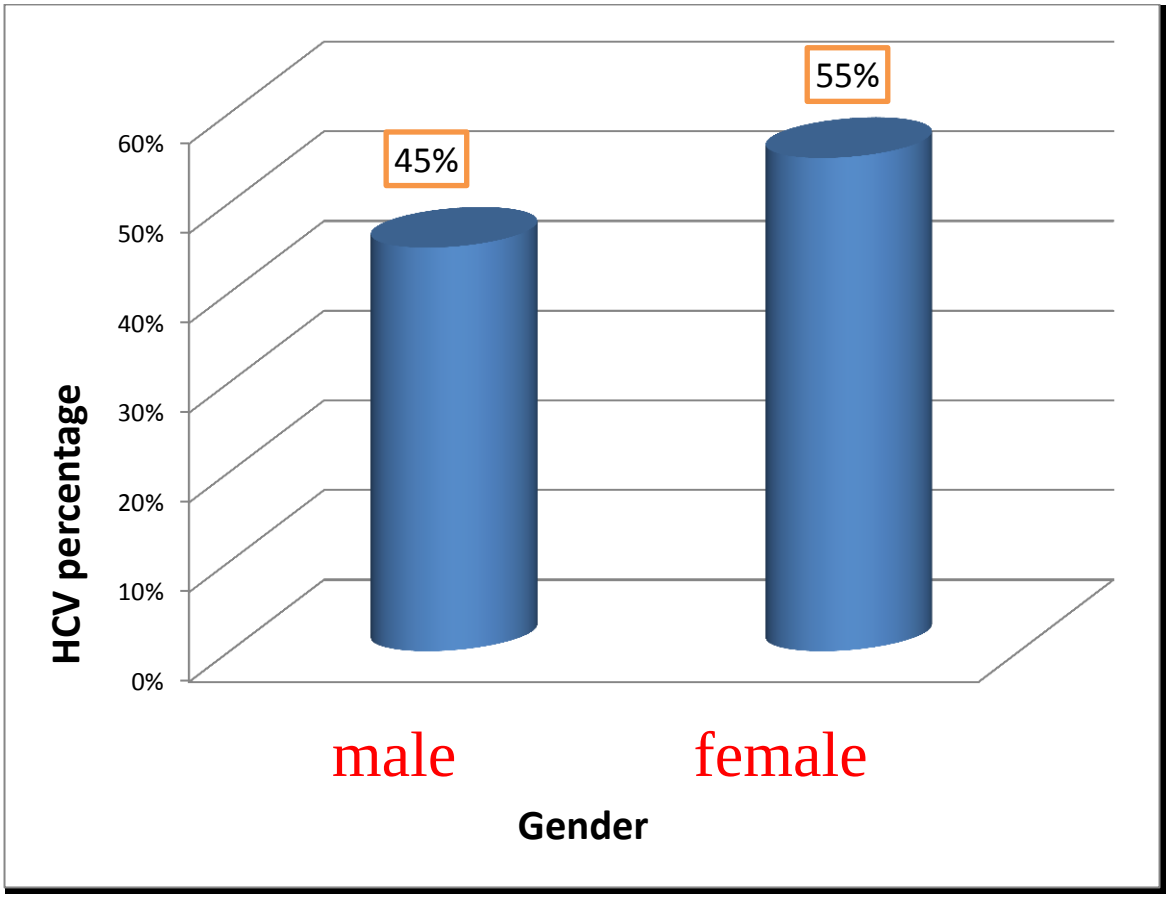

Figure 4.1: Gender Distribution of HCV Recorded Patients.

4.5 Detection of HCV Genome By RT.PCR:

The positive result according to RT-PCR shows 8.2% (48 out of 586 cases) as positive while 91.8%% (538 out of 586 cases) as negative, as shown in Table (4-4) as well as Figures (4-3-A, & B). Statistically significant differences (p = 0.04) among patients group.

Table 4.5: Percentage of HCV Positive Signals by Using qRT.PCR Technique.

Total Viral genome	No.	%	P value
Positive	**48**	8.2	**P≤ 0.05**
Negative	538	91.8	
Total	586	100	

Figure (4.2) both of them shows line positive control and line negative control and also positive samples and negative samples, according to detection kit (RT-PCR) of HCV which used in this study the curves that represent positive samples appear between the line positive control and line negative control.

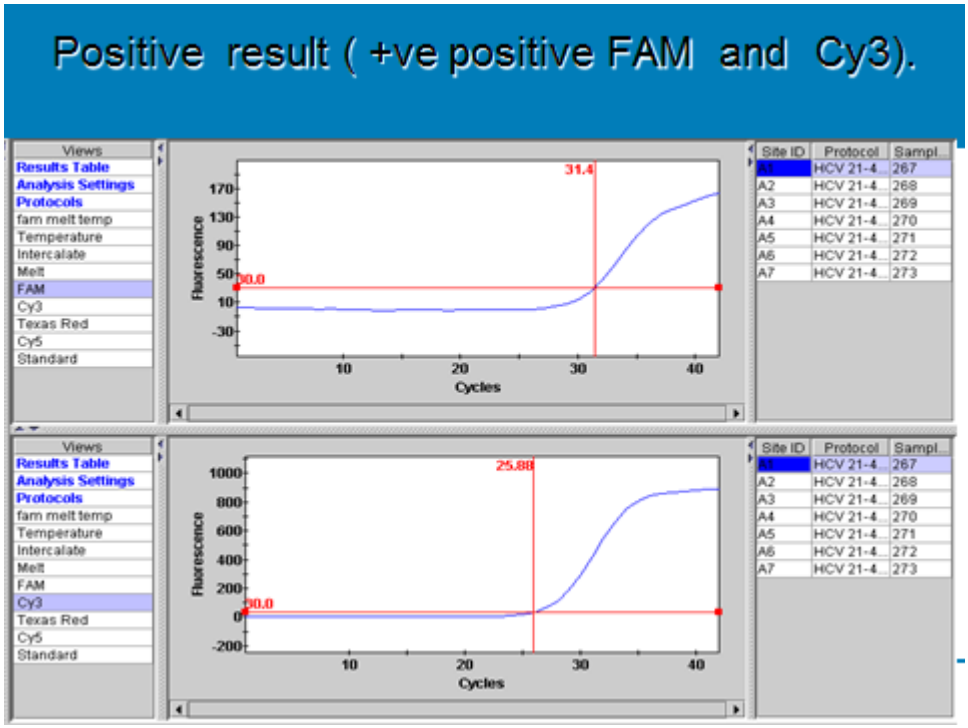

Figure 4.2: Detection of HCV by RT-PCR.

4.6 Typing of HCV genotypes

Table (4-5) revealed the percentage of positive hepatitis C virus of untreated patients group was 84.62%, treated patients group was 8.70% and healthy control group was 0.0% . In addition, the outcomes of Real Time poly chain reaction in the same table revealed three patterns of positive HCV in studied groups; genotype 1a of untreated patients group was 3.85% and treated patients group was 0.00% . While genotype 3 exposed in studied groups; of untreated patient group 3.85% and treated patients group was 0.00% .

The pattern 4 of genotypes which has been detected is illustrated in the same table that displays 76.92% of untreated patients group and 8.7% of treated patients group. Statistical analysis revealed ,that there were high significant differences among studied groups (untreated versus treated & healthy control); according to genotyping patterns ($P< 0.01$). In general genotype 4 percentage was greater in hepatitis C patients comparing with other patterns.

Table 4.5: Typing of HCV Genotypes

Genotypes	Control	With treatment	Without treatment	Chi-Square ($\chi 2$)
Negative	100%	91.3%	15.38%	13.4**
Ia	0.00%	0.00%	3.85%	NS 0.647
3	0.00%	0.00%	3.85%	NS 0.647
4	0.00%	8.70	76.92%	12.5

H.S**: High significant differences $P \leq 0.05$

Chapter Five

Discussion

5.Discussion

In the present study females appeared to be more affected than males, this results agree with previous Iraqi studies submitted by (Alsamarai *et al.,* 2016) and (Al-Safar,2015). The previous conclusion of Omosigho *et al.* (2010), Zhili *et al.*, (2016) and Daniele *et al.*,(2017) were agreed with our results who stated that women's risk of HCV is more than in men, Where the latter found that the frequency of HCV prevalence was significantly higher in female patients compared to males before the age of 60 years, whereas the adverse findings were reported after the age of 60 years.
Hamied *et al.*, (2010), who researched the prevalence of HBV and HCV among patients referred to Al-Karama hospital in Baghdad, published opposing findings, and found the disease to be more prevalent in males than females. In addition, Arabian populations, as well as other worldwide ethnicities, reported different findings Khairy *et al.*, (2013) who observed predominance of males with HCV infected patients in Egypt (72.2 percent) and with Shimizu *et al*., (2012) who reported higher risk of exposure to viral hepatitis among males. Tungtrongchitr *et al.*, (2006) have shared these last findings in patients from Thailand. This study was conducted in one city in Iraq, so the epidemiological conclusion may be very limited to exposure sources from a specific population even though it is based on known global risk factors for HCV infection.

This study agreed with Abdul- Sada, (2011) and Yahya *et al.*, (2013); The study reported that, the viral load ranged from 102 to 4.5 × 108 IU/ml of blood, whereas, the mean and median were 5.8 × 106 and 3.6 × 104 IU /ml of blood respectively. The second recorded that the viral units were ranged from 1.19×10^3 IU/ ml to 4.3×10^6 IU/ml and the mean was 5.9×105 IU/ml whilst, the median was 2.6×10^5 IU/ml of the blood. The connection between the genotypes of HCV and the viral load remains disputed. Large number viraemia was linked to advanced liver disease (Adinolfi *et al.*, 2001). Whereas others originate association with HCV genotypes (Shahraki *et al.*, 2010).

This extensive range can be established in the viral load due to the diversity of patients from unlike groups, ages, and disease phases. The immune response and the patient's recent status have also played an essential role in the viral load oscillation at the identical individual (Santantonio *et al.*, 2008 ; Jacobson *et al.*, 2010). However this search showed no variation in viral titer between patients sets due to the large ratio of infections belonged to the similar genotype (Genotype 4).

Viral load is the amount of viruses present in a certain volume of blood taken from an infected person. More precisely, it means the amount of genetic material for the viral hepatitis virus appear in the blood and therefore it indicates the number of virus particles in the blood also termed as viral titer or viral copies (Komurian-Pradel *et al.*, 2001). The WHO Using international standard, a viral load of two millions copies/ml (Predictive cut-off value significance for therapeutic efficacy in initial clinical IFN trials) was found to resemble to 800 000 IU/ml (Strader *et al.*, 2004). Approaches for reliable quantitative serum and plasma hepatitis C virus levels of RNA have become important resources for both understanding HCV infection biology and clinical management of patients under care. The ability to know patients responsiveness to treatment by measuring the rates of low levels of the hepatitis C virus, it provides a more accurate treatment management also classification of patients, who are not responding to therapy early in the treatment, which avoids them progressing the disease and the high cost (Florence *et al.*, 2001).

The present research display that there was high significant metamorphoses between treated and untreated groups in viral load levels, this support that the Harvoni drug (new antiviral drug) has potential effect in viral infection treatment.

One of the studies that It was conducted showed that the use of Harvoni as therapy was more effective and resulted in a sustained viral response rate of 97% or higher and the discontinuation were higher for 24 weeks of treatment versus 12 weeks. The study showed that 12 weeks of lidpasvir / sofosbuvir without ribavirin is an effective treatment

for patients with the hepatitis C virus genotype 1 infection (Abdul-Sada, 2011). The effectiveness of Harvoni may be belong to large effect on synthesis of virus components where it inhibited the HCV NS5B RNA-dependent RNA polymerase, which acts as a chain terminator. Inhibition of hyperphosphorylation of NS5A plays critical role in stopping virus synthesis and prevent viral assembly (Pawlotsky, 2013). This effectiveness can be confirmed with high sensitive analyses later 24 weeks of management, because patients with measurable HCV RNA at this period point only have a 1-2% chance of reaching sustained virological response (SVR). SVR, identified as the absence of noticeable HCV RNA 24 weeks after achievement of the treatment, must be estimated by an HCV RNA detection test with a minor threshold of 50 IU / ml or fewer to evaluate long-term success of the treatment (Manns *et al.*, 2006)**.**

Statistical analysis revealed, that there were high significant differences among studied groups (untreated versus treated & healthy control); according to genotyping patterns ($P< 0.01$). In general genotype 4 percentage was greater in hepatitis C patients paralleling with other patterns.

Knowledge of virus genotypes plays critical rule in identifying the source of outbreak and infection may be person to person spread or vertically (mother to baby) as well as sexual contact and needle twinge (Pekova *et al*., 2007).

Three genotypes 1a, 3 and 4 were observed, but the latter was the most commonly found. HCV genotype 1 is the most prevalent (46%) genotype globally and predominates in Europe, North America, and Australia fallowed by Genotype 3 which is the second most prevalent (30%) genotype worldwide primarily distributed in South Asia, particularly the Indian (Sergio *et al*., 2018). Infections with HCV genotype 4 are mainly found in Africa and the Middle East (Charlotte *et al*., 2019).

This study is agreed with four previous local studies in Iraqi population primary by Al-Kubaisy *et.al.*, (2006) have outcomes, who stated that genotype 4 was the greatest recurrent type followed by 1a and 1b, and exhibited mixed genotypes 1a and 4 infection. The second trial by Abdul-Sada, (2011) showed that the predominant genotype 4 was existing in 89.4% of infected patients followed by groups 6a, 3a, 2b and 1b with percentage 1.94%, 2.91%, 2.91% and 6.79% respectively, third research by Yahay *et al.*, (2013) that indicated three HCV genotype (1a, 1b and 4) were recognized, and the latter was the major (86.27 percent), followed by 1b (37.25 percent), and 1a (33.33 percent). And eventually by Jawad *et al.*, (2014) were exhibited that HCV genotype 4 is the main genotype (fifty sex percent) followed by genotype 1b (twenty three percent) and genotype 1a (twelve percent), while genotype 3 was found in only (nine percent). The prevalence of genotype 4 in Iraq is confirmed by these reports, including the current study. However, in Iraqi hemodialysis patients the numerousness of HCV genotype 4 is in line with supplementary studies on the genotyping of HCV isolates in various Middle Eastern countries such as Egypt (up to 80 percent) (Ray *et al.*, 2000).

Genotype 4 epidemiological profile consists of settlers from other nations, mostly Egyptian migrants in their home country being diagnosed with HCV-4. Owing to the usage of unsterile tools during mass care of the people with parenteral anti-schistosomal treatment from the 1920s to the 1980s, Egypt has the main prevalence of HCV contagion in the realm (Frank *et al.*, 2000). Around 90 percent of Egyptian patients are HCV-4 (Ray *et al.*, 2000). Genotype 1a epidemiology profile was related to injection drug use (IDU), and genotype 1b is more frequently associated with patients who received HCV via blood transfusion (Elghouzzi *et al.*, 2000).

The surprising the present of genotype 1 and 3 can be ascribable partially to the open human movement policy adopted by the Iraqi government over the last 15 years, chiefly in the population of the Baghdad city. In this meaning, the return of large number of Iraqi people living in foreign countries in addition to the influx of persons of dissimilar ethnicities (Asians, Europeans and Americans)

in search of better employment opportunities in Iraq may have likelihood of present the 1 and 3 genotype in Iraq.

Conclusions

1. High incidence rate was investigated among prisoners and foreigners so must focusing on those sectors and place regulation and strategies for mitigation.
2. Genotype 4 is the most commonly encountered genotype.
3. The north area of Babylon province had higher percentage than south and middle areas , especially in rural area than urban.
4. There was a relationship between inherited genotypes as a result of mutations and Hepatitis C infection.
5. High activity of treatment protocol was mentioned on HCV infected patients, where few of patients had final outcome as undetectable viral load after treatment with interferon or oral anti – viral drugs.

Recommendations

1. Studying the polymorphism in HCV patients in Babylon\ Iraq .
2. Studying the mortality rate of HCV .

References

References A

Abdulghani, M. A.; Gailan, A. and Amina, H. A. (2016). Seroprevalence of Hepatitis C Virus in Iraqi Population. J. Immuno Virology 1(3).

Abdul-Sada, K.M. (2011). Estimation of HCV genome genotyping and the role of mosquitoes in its transmission. Ph.D. Thesis, College of Medicine, University of Kufa. Iraq.

Aboud, R.S.(2015) .The relationship between hepatitis C virus infection and diabetes mellitus type 2. J. Pharm Chem Biol Sci. 3(1):73–78.

Adinolfi, L.E.; Gambardella, M.; Andreana, A.; Tripodi, M.F.; Utili, R. andRuggiero, G. (2001). Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatol., 33(6): 1358–64.

Afdhal, N.; Reddy, K.R.; Nelson, D.R.; Lawitz, E.; Gordon, S.C.; Schiff, E.; Nahass, R.; Ghalib, R.; Gitlin, N. and Herring, R. (2014) N. Engl. J. Med. 370:1483–1493.

Agata, B. (2017). heterogeneity of hepatitis c virus particles and their evolution during infection. cellular and molecular gastroenterology and hepatology 4:443– 444 .

Al-Kubaisy, W.A.; Al-Naib, K.T. and Habib, M.A. (2006). Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. Eas. Mediterr. Heal. J., 12(1-2):204-10.

Al-Saffar, O. (2015). The predictive significance of some cytokine genetic polymorphism in Iraqi patients with hepatitis B and C

viral infections. Ph.D.thesis College of science. University of Baghdad.

Alsamarai A., Abdulrazaq G., and Alobaidi A.(2016). Seroprevalence of Hepatitis C virus in Iraqi population. JoJ immune virology. 1(3):555565.

Asselah, T.; Hassanein, T.; Waked, I.; Mansouri, A.; Dusheiko, G. and Gane, E. (2017). Eliminating hepatitis C within low-income countries - The need to cure genotypes 4, 5, 6. J. Hepatol.

B

Borgia, S.M.; Hedskog, C.; Parhy, B.; Hyland, R.H.; Stamm, L.M.; Brainard, D.M.; et al. (2018). Identification of a Novel Hepatitis C Virus Genotype from Punjab, India - Expanding Classification of Hepatitis C Virus into 8 Genotypes. J Infect Dis.

C

CDC. (2015). Hepatitis C FAQs for the public. Available from: URL: http://www.cdc.gov/hepatitis/c/cfaq.htm.

Chakravarti A. (2001). To a future of genetic medicine. J. Nature 409: 822-3.

Charlotte, H.; Bandita, P.; Silvia, C.; Stefan, Z.; Christophe, M.; Stephen, D. S.; Sergio, M. B.; Tarik, A.; Laurent, A.; Armand, A.; Jyh-Jou, C.; Jane, C.; Dharmesh, K.; Robert, H. H.; Peter, S.; Hongmei, M. and Evguenia, S. S. (2019). Identification of 19 Novel Hepatitis C Virus Subtypes-Further Expanding HCV Classification Downloaded from https://academic.oup.com.

Clodoveo, F.; Marco, S.; Dilia, G.; Michele, C.; Poupak, F.; Alessia, P.; Alessandro, A. and Anna, L. Z. (2015). Hepatitis C virus syndrome: Aconstellation of organ- and non-organ specific

autoimmune disorders, B-cell non- Hodgkin's lymphoma, and cancer. J. Hepatol. 7(3): 327-343.

D

Daniele, B. O.; Patrícia, M. P.; António, L.; Guida, S.; Angela, M. G. S and Luís, C. P. (2017). Exposure source prevalence is associated with gender in hepatitis C virus patients from Rio de Janeiro, Brazil. Vol. 112(9): 632-639.

Diana, G. and Gregory, H. (2015). Ledipasvir/Sofosbuvir (Harvoni): Improving Options for Hepatitis C Virus Infection. J. P&T 40:4.

Diegelmann, J.; Beigel, F.; Zitzmann, K.; Kaul, A.; Goke, B.; Auernhammer, C.; Bartenschlager, R.; Diepolder, H. and Brand S. (2010). Comparative analysis ofthe lambda-interferons IL-28A and IL-29 regarding their transcriptome and their antiviral properties against hepatitis C virus. PLoS One; 5:e15200.

E

Elghouzzi, M.H.; Bouchardeau, F.; Pillonel, J.; Boiret, E.; Tirtaine, C.; Barlet, V.; Moncharmont, P.; Maisonneuve, P.; Puy-Montbrun, M.C.; Lyon-Caen, D. and Courouce, A.M. (2000). Hepatitis C virus: routes of infection and genotypes in a cohort of anti-HCV-positive French blood donors.Vox Sang.,79:138-44. European Union HCV Collaborators (2017). Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: Gastro enterol Hepatol. 2:325–336.

European Union HCV Collaborators (2017). Hepatitis C virus prevalence and level of intervention required to achieve the WHO targets for elimination in the European Union by 2030: Gastroenterol Hepatol. 2:325–336.

F

Florence, K.; Gla´ucia, P.; Mireille, S.; Philippe, C.; Bernard, M.; Vincent, L.; Patrice, A. (2001). Quantitation of HCV RNA using real-time PCR and fluorimetry. J. Virological Methods 95:111–119.

Food and Drug Administration. (2014). FDA approves first combination pill to treat hepatitis C. http://www.fda.gov/NewsEvents/Newsroom/ PressAnnouncements/ucm418365. htm.

Frank, C.; Mohamed, M.K.; Strickland, G.T.; Lavanchy, D.; Arthur, R.R.; Magder, L.S.; El Khoby, T.; Abdel-Wahab, Y.; Aly Ohn, E.S.; Anwar, W. andSallam, I. (2000). The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet., 355: 887–891.

Fraser, C.S and Doudna, J.A. (2007). Structural and mechanistic insights into hepatitis C viral translation initiation. J.Nat Rev Microbiol 5: 29-38.

Fricke, M.; Dünnes, N.; Zayas, M.; Bartenschlager, R.; Niepmann, M. and Marz M. (2015). Conserved RNA secondary structures and long-range interactions inhepatitis C viruses. J. RNA 21:1219–1232.

G

Gastaminza, P.; Dryden, K.A.; Boyd, B.; Wood, M.R.; Law, M.; Yeager, M. and Chisari, F.V. (2010). Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. J. Virol 84: 10999-11009.

Ge, D.; Fellay, J.; Thompson, A.J.; Simon, J.S.; Shianna, K.V.; Urban, T.J. et al. (2009). Genetic variation in IL28B predicts hepatitis C treatmentinduced viral clearance. J. Nature 461: 399–401.

Gebretsakan, G.; Mebrahtu, T.; Letebrhan, H.; Tuom, G.; Berihu, G. and Hailay, G.(2018). Hepatitis C virus infections and associated risk factors in patients with diabetes mellitus; case control study in North West Tigray, Ethiopia.

Goodbourn, S.; Didcock, L.and Randall, R. E. (2000). Interferons: cell signaling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol. 81:2341–2364.

Gower, E.; Estes, C.; Blach, S.; Razavi-Shearer, K. and Razavi H. (2014). Globalepidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol. 61:S45–57.

Grassi, G.; Di Caprio, G.; Fimia, G.M.; Ippolito, G.; Tripodi, M. and AlonziT.(2016). Hepatitis C virus relies on lipoproteins for its life cycle. J. Gastroenterol 22: 1953-1965.

H

Hamied L.; Abdulla R. M. and Abdulla A. M(2010). Seroprevalence of hepatitis B and C virus infection in Iraq. The N. Iraq J. Med; 6: 69-73.

Hanan Najem Najaf, Dheyaa J. Kadhim , Ahmed J. Alkofee , Dheyaa A. Al-Mashhadani(2019). Depression, Anxiety and Stress Among a Sample of Chronic Hepatitis C Patients in AL-Najaf Province /Iraq. Int. J. Res. Pharm. Sci., 10(4), 3170-3177.

Hollinger FB, Emerson SU(2007). Hepatitis A virus. In: Knipe DM, Howley PM, editors. Fields virology. Philadelphia: Lippincott Williams & Wilkins; p. 911–947 [chapter 27].

J

Jacobson, I. M.; Davis, G. L.; El-Serag, H.; Negro, F. and Trepo, C. (2010). Prevalence and challenges of liver diseases in patients with chronic hepatitis C virus infection. Clin.Gastro-enterol.Hepatol., 8(11):924-33**.**

Jawad, K. M.; Eman, Sh. and Omar, F. (2014). Distribution of Hepatitis C Virus (HCV) Genotype among Iraqi Hemodialysis Patients. J.Advanced Research V: 2(1): 442-445.

Jill, J. (2020). Screening for Hepatitis C Virus Infection. J. JAMA 323:10 Juan, R. L.; Elia, M.; Megha, U. L.; Silvia, G.; Alicia, L.; Joaquín, M.; Cristian, P. and Eduardo, S. (2014). Adaptive immune response during hepatitis C virus infection. J. Gastroenterology 20:13.

K

Khairy, M.; Fouad, R.; Mabrouk, M.; El-akel, W.; Awad, A.; Salama, R.; Elnegouly, M. and Shaker O. (2013). The impact of interleukin 28β gene polymorphism on the virological response to combined pegylated interferon and ribavirin therapy in chronic HCV Genotype 4 infected Egyptian patients Using Data Mining Analysis. Hepatitis Monthly. 13: e10509.

Komurian-Pradel, F.; Paranhos-Baccala,G.; Sodoyer, M.; Chevallier, P.; Mandrand, B.; Lotteau,V. and Andre, P. (2001). Quantitation of HCV RNA using real-time PCR and fuorimetry.Virol. methods, 95:111-119.

L

Lee, Y.A.; Wallace, M.C. and Friedman, S.L. (2015). Pathobiology of liver fibrosis: a translational success story. J. Gut 64: 830-841.

M

Maasoumy, B. and Wedemeyer, H. (2012). Natural history of acute and chronic hepatitis C. J. Clin Gastroenterol 26: 401-412.
Maher, S. G.; Sheikh, F.; Scarzello, A. J.; Romero-Weaver, A. L.; Baker,

Manns, M.P.; Wedemeyer, H. and Cornberg, M. (2006). Treating viral hepatitis C: efficacy, side effects, and complications. Gut., 55(9): 1350-9.

Messina, J.P.; Humphreys, I.; Flaxman, A.; Brown, A.; Cooke, G.S.; Pybus, O.G.; et al. (2015). Global distribution and prevalence of hepatitis C virus genotypes. J. Hepatol. 61(1):77-87.

Modi, A.A. and Liang T.J. (2008). Hepatitis C: a clinical review. J. Oral Dis14: 10-14.

Moriishi, K. and Matsuura, Y. (2012). Exploitation of lipid components by viraland host proteins for hepatitis C virus infection. J. Microbiol 3: 54.

Morozov, V.A. and Lagaye, S. (2018). Hepatitis C virus: morphogenesis, infection and therapy. J. of hepat. 10(2):186–186.

N

Niu Z., Zhang P. and Tong Y. (2016). Age and gender distribution of Hepatitis Cvirus prevalence and genotypes of individuals of physical examination in WuHan, Central China. Springerplus. 1:1557.

Nouroz, F.; Sidra, S.; Ghulam, M. and Shumaila, N. (2015). An overview on hepatitis C virus genotypes and its control. J. Med Hum Genet.

Nowak, M.A. and Bangham, C.R. (1996). Population dynamics of immune responses to persistent viruses. J. Sci. 272: 74-79.

O

Omosigho, S.; Mohammed , H.; Inyinbor, G.; Emumwen, S.; Ogedengbe, I.; Okorie, J.; Njab, A.; Dangana, O.; Oladejo, E. and Emumwen, S. (2010). Hepatitis B Surface antigen in human Immuno deficiency virus positive patients in Bida, Norh Central Nigeria. *Indian J. Gastroenternal*., 10: 187-195.

P

Pawlotsky J.M. (2013). NS5A inhibitors in the treatment of hepatitis C. J. Hepatol 59(2):375–382.

Pekova, L.M.; Teocharov, P. and Sakarev, A. (2007). Clinical course and outcome of a nosocomial outbreak of hepatitis C in a urology ward. J. Hosp. Infect., 67(1):86-91.

Petruzziello, A.; Marigliano, S.; Loquercio, G.; Cozzolino, A. and Cacciapuotion C. (2016). Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. J. Gastroenterol. 22(34):7824–40.

Polaris, Observatory HCV Collaborators (2015). Global prevalence andgenotype distribution of hepatitis C virus infection in 2015. Gastroenterol
Hepatol 2:161–176.

Priyanka , M. (2014). Interferons and Interferon Therapy. J. pharm. Sci. 6(12): 400-403.

R

Ray, S.C.; Arthur, R.R.; Carella, A.; Bukh, J. and Thomas, D.L. (2000). Genetic epidemiology of hepatitis C virus throughout Egypt. Infect. Dis.,182:698–707.

Renau, L. P. and Berenguer, M. (2018). Introduction to hepatitis C virus infection: Overview and history of hepatitis C virus therapies. Hemodialysis International, 22:8–21.

S

Santantonio, T.; Wiegand, J. and Gerlach, J.T. (2008). Acute hepatitis C: current status and remaining challenges. Hepatol. 49(4):625-33.

Schreiber, J.; McNally, J.; Chodavarapu, K.; Svarovskaia, E. and Moreno, C. (2016). Treatment of a patient with genotype 7 hepatitis C virus infection with sofosbuvir and velpatasvir. J. Hepatol. 64(3):983-5.

Scott, J.D. and Gretch, D.R. (2007). Molecular diagnostics of hepatitis C virus infection: a systematic review.J. JAMA 297: 724-732.

Sergio, M. B.; Charlotte, H.; Bandita, P.; Robert, H. H.; Luisa, M. S.; Diana, M. B.; Mani, G. S.; John, G. M.; Hongmei M.; E. S. and Stephen, D. S. (2018). Identification of a Novel Hepatitis C Virus Genotype from Punjab, India -Expanding Classification of Hepatitis C Virus into 8 Genotypes. Downloadedfrom https://academic.oup.com.

Shahraki, T.; Shahraki, M.; Moghaddam, E.; Najafi, M. and Bahari, A. (2010). Determination of Hepatitis C Genotypes and viral titer Distribution in childrenand adolescents with Major Thalassemia . Iran J Pediater., 20(1):75-81.

Sharma, N.R.; Mateu, G.; Dreux, M.; Grakoui, A.; Cosset, F.L. and Melikyan, G.B. (2011). Hepatitis C virus is primed by CD81 protein for low pH dependent fusion. J. Biol Chem 286: 30361-30376.

Shimizu, I.; Matsumoto, T.; Suzuki, N.; Sagara, C.; Koizumi, Y.; Asaki, T.; Katakure, Y. and Fukita, Y. (2012). Chronic liver diseases develops more slowly in females than males, p.3-18.In: Shimizu I (ed), preventive females sex factors against the development of chronic liver disease. Bentham Science publishers, Japan.

Smith, D.B.; Bukh, J.; Kuiken, C.; Muerhoff, A.S.; Rice, C.M.; Stapleton, J.Tand Simmonds P. (2014). Expanded classification of hepatitis C virus into 7genotypes and 67 subtypes. J. Hepatology 59: 318-327.

Spengler, U. and Nattermann, J. (2007). Immunopathogenesis in hepatitis Cvirus cirrhosis. J. Clin. Sci. 112: 141-155.

Strader, D.B.; Wright, T.L.; Thomas, D.L. and Seeff, L.B. (2004). Diagnosis, management and treatment of hepatitis C. AASLD practice guideline. J. Hepatol., 39:1147. Strader, D.B.; Wright, T.L.; Thomas, D.L. and Seeff, L.B. (2004). Diagnosis, management and treatment of hepatitis C. AASLD practice guideline. J. Hepatol., 39:1147.

T

Thomas, D.L. and Seeff, L.B. (2005). Natural history of hepatitis C. J. Clin LiverDis 9: 383-398.

Tungtrongchitr, R.; Treeprasertsuk, S. and Thepouyporn, A. (2006). Serumleptin concentration in chronic hepatitis. *J. Med Assoc Thai*., 89:490-499.

V

Vercauteren, K.; Mesalam, A.A.; Leroux-Roels, G.; and Meuleman P. (2014) .Impact of lipids and lipoproteins on hepatitis C virus infection and virus neutralization. J. Gastroenterol 20: 15975-15991.

Vijay, K.; Saumitra, D. and Shahid, J. (2010). The biology and pathogenesis of hepatitis viruses. J. current science, 98: 3-10.

Vladimir, A. M. and Sylvie, L. (2018). Hepatitis C virus: Morphogenesis, infection and therapy. J. Hepatol10(2): 186-212.

W

WHO(2017). HepatitisC. http://www.who.int/mediacentre/factsheets/fs164/en/.WHO, hepatitis C,2019.

Widell, A.; Forslund, O. and Medstrand, P. A. (2016). divergent hepatitis C virus (HCV) strain may represent a new major HCV genotype (Genotype 8). Abstract at 23rd International Symposium on Hepatitis C Virus and Related Viruses, Kyoto Japan. World Health Organization. 2017. Global Hepatitis Report, ISBN: 978– 92-4-156545-5 http://apps.who.int/iris/bitstream.

Y

Yahya A. A.; Adnan, H. A. and Bushra, J. H. (2013). Determination of HepatitisC Viral Load and Genotypes by Real-Time and RT-PCR at Thi_Qar Province. QMJ 9-15.

Z

ZhiLi Niu, PingAn Zhang and YongQing Tong(2016). Age and gender distribution of Hepatitis C virus prevalence and genotypes of individuals of physical examination in WuHan, Central China. SpringerPlus 5:1557 DOI 10.1186/s40064-016-3224-z.

الخلاصة

الخلفية:

التهاب الكبد C هو مرض معد يسببه فيروس التهاب الكبد نوع سي(HCV) ويصيب الكبد في المقام الأول. يستمر الفيروس في الكبد في حوالي ٧٥٪ إلى ٨٥٪ من المصابين في البداية. عادة لا تظهر أي أعراض للعدوى المزمنة في وقت مبكر ، وتصنيف الأنماط الجينية لفيروس التهاب الكبد الوبائي سي (HCV) له أهمية سريرية لأنه قد يساعد في التنبؤ باستجابات العلاج الدوائي وتقدير مدة العلاج. الطريقة الكلاسيكية لتصنيف النمط الجيني لـ HCV هي تسلسل الجينوم الكامل (WGS).

الأهداف:

تحديد معدل الإصابة بالأنماط الجينية لفيروس التهاب الكبد الوبائي سي بين المرضى المصابين بفيروس التهاب الكبد الوبائي ؛ المتبرعون بالدم حضروا إلى بنك الدم في محافظة بابل/ العراق.

المرضى والطرق:

دراسة مقطعية أجريت على مرضى مصابين بفيروس التهاب الكبد C ؛ متبرعون بالدم حضروا إلى بنك الدم في محافظة بابل ، العراق. تم جمع قاعدة البيانات من دائرة الصحة العامة في مديرية صحة بابل في السنوات الخمس الماضية (حزيران ٢٠١٦ - حزيران ٢٠٢١) و ١٢٢٦٠ شخص تم فحصهم. تتراوح أعمارهم بين ١٧ و ٦٥ عامًا خلال الفترة من كانون الاول ٢٠٢٠ إلى حزيران ٢٠٢١. تم جمع عينات الدم من جميع المشاركين ثم تم تحليلها باستخدام مجموعات مختلفة من مقايسة المناعي المرتبط بالإنزيم (ELISA) وتفاعل البوليميراز المتسلسل في الوقت الحقيقي (RT-PCR) لتحديد الأنماط الجينية لفيروس HCV. تم تحليل ٥٨٦ مريضاً فقط من مرضى التهاب الكبد الفيروسي HCV بواسطة RT-PCR في حوالي عام ٢٠٢١ في محافظة بابل.

النتائج:

لوحظ الكشف عن تفاعلات HCV -RT-PCR في ٤٨ من أصل ٥٨٦ (٨.٢٪). النمط الجيني ٤ الذي يظهر ٧٦.٩٢٪ من مجموعة المرضى غير المعالجين و ٨.٧٪ من مجموعة المرضى

المعالجين. بشكل عام ، لم يكن هناك ارتباط معنوي بين الطرز الوراثية المختلفة وبعض العوامل الديموغرافية ، والتحقيقات المصلية ، واختبار وظائف الكبد.

الاستنتاجات:

كان النمط الجيني الأكثر شيوعًا في العراق هو النمط الجيني ٤. هناك حاجة إلى مزيد من الدراسات التي تشمل مصادر انتقال وتأثير العلامات الحيوية الكبدية لتعزيز تدابير السيطرة على عدوى التهاب الكبد الفيروسي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم
قسم علوم الحياة

التنميط الوراثي لفايروس التهاب الكبد C كدليل عدلي في محافظة بابل

بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم/ أدلة جنائية

من قبل

حسين جاسم محمد طرفة

بكالوريوس علوم حياة/جامعة بابل (٢٠٠١)

اشراف

أ. د شاكر حماد محمد حسن

(دكتوراه في علم الفيروسات الجزيئي)

٢٠٢١ م **١٤٤٣هـ**