Thi-Qar Medical Journal (TQMJ):Vol.(25),No.(1),2023

Web Site: https://jmed.utq.edu **Email:** utjmed@utq.edu.iq

ISSN (Print):1992-9218, ISSN (Online):1992-9218

Immunological and Genetic Study for Chronic Hepatitis C Virus Infection

Aneeda Khraibet Abed, Department of Microbiology, College of Medicine, University of Babylon, Iraq, Email: anakabd78@gmail.com

Zaytoon Abdulridha Ighewish Alkhafaji, Department of Microbiology, College of Medicine, University of Babylon, Iraq, E-mail: dr.zaitoonalkhafaji@gmail.com

Ali Jaber Abood, Department of Internal Medicine, College of Medicine, University of Babylon, Iraq, E-mail: minaaligaber@gmail.com

Abstract

Background: Acute and chronic hepatitis, both of which can be brought on by the hepatitis C virus, can range in severity from a mild condition to a devastating, life-long sickness that can result in liver cancer and cirrhosis. A liver inflammatory disease called hepatitis C. Cytokines is chemical mediators that control immune cells' development, division, and function. Evidence suggests that inadequate immune responses contribute to the persistence of HCV.

Objective:

The goal of this study was to immunogenetic of hepatitis C to determine the prevalence of [hepatitis C virus (HCV Ag)] among HCV surface antigen-positive (HCV Ag+) persons by analyzing the association between age and gender and the severity of hepatitis C virus (HCV) infection, identify and analyze the UTR-polyprotein gene of local Hepatitis C viru

Materials and Methods:

hire 48 individuals From August 2022 to November 2022, a total of persons with acute or chronic HCV infection were recruited and 5 sample for genetic sequence. Healthy controls and research participants ranged in age from 21 to 80, and all of them were analyzed using ELISA RT-PCR and using statistical analysis SPSS and ANOVA.

Results:

48 affirmatives out of 48 Using an ELISA (enzyme-linked immunosorbent test) double-antibody sandwich. we determined that HCV participants met the inclusion criteria. The findings of the HCV IgM ELISA Kit indicated that the prevalence of HCV was greatest in those aged 41 to 60 (45.8%), lowest in those aged 21 to 40 (16.7%) and HCV Ag The use of an ELISA kit enabled the discovery. Of the 48 individuals who tested positive for HCV Ag, 21 (56.2%) were female and 27 (43.8%) were male. This indicates that males are more likely than females to have HCV Ag infection. And 5 sample in DNA sequencing method was used to genotype identify and analyze the UTR-polyprotein gene of local Hepatitis C virus isolates (IQ.No.1 - IQ.No.5) and related Hepatitis C virus genotype isolates from NCBI-Blast.

The local Hepatitis C virus isolates were revealed to be closely linked to NCBI-BLAST Hepatitis C virus subtype 1a (HQ113638.1) at total genetic alterations (0.3-0.05%), according to the phylogenetic tree genetic relationship study.

The genetic homology sequence identity between the local isolates of the Hepatitis C virus (IQ.No. 1 through IQ.No. 5) and the Hepatitis C virus identified by NCBI BLAST ranged from (99.41% to 99.61%).

The local isolates of the Hepatitis C virus (IQ.No.1 to IQK.No.5) were then submitted to the NCBI Genbank and given accession numbers (OQ843893, OQ843894, OQ843895, OQ843896, OQ843897).

Conclusion: TNF levels in patients with chronic HCV were noticeably higher than average, New strains of the virus in the gene bank, and therefore we did not find any Iraqi strains registered can compare with them

Keywords: HCV Ag, UTR Gen, TNF- α.

Introduction:

56.8 million persons worldwide are estimated to have persistently active hepatitis C virus (HCV) [1]. A persistent liver inflammation that eventually leads to cirrhosis, which is the

primary risk factor for the formation of hepatocellular carcinoma (HCC), characterizes the natural course of chronic hepatitis C (CHC) [2].

The natural outcome of HCV infection varies dramatically among individuals. HCV infection is self-limited in a fortunate minority, while the majority of subjects develop persistent chronic infection. Its outcome is determined by various kinds of host factors and viral factors. The former includes race, age, genes, the combination of other chronic diseases, the appropriateness of treatment, and so on. Although the reasons leading to HCV infection have not been fully clearly understood yet, more and more evidence showed that cytokines play important roles in perpetuating the chronic inflammatory state. TNF-α and are also important mediators in the antiviral response. In addition, data from related studies indicated the ability and degree to clear the virus, and the strength of T-cell response (such as TNF-α production), which correlated with the result of HCV infection. There are significant differences in the ability to produce cytokines among individuals. [3]

People with cirrhosis remain at risk of getting HCC even after their HCV has been treated [4]. In previous study found that the assessment of serum epidermal growth factor receptor 3 in HCC patients with an HCV diagnosis was able to predict overall survival, regardless of the patient's age, liver function, or tumor stage [5]. Compromised immunity, repeated hospital visits, and the need for surgical treatments all contribute to the elevated risk of infection in hemodialysis patients. The extracorporeal circuit, vascular access, proximity to other patients while getting dialysis, interactions with medical professionals, and replacing the dialysis machine all contribute to the fact that hemodialysis itself exposes the patient to blood frequently and/or for a long time. Hepatitis C virus (HCV) infection is a blood-borne viral disease that is frequently contracted by patients receiving hemodialysis [6]. The link between chronic hepatitis with HCV and a higher morbidity-mortality rate among recipients of organ transplants and hemodialysis patients has been the subject of numerous studies in the specialized literature. Furthermore, according to statistical research, between 5 and 60% of CKD patients (in industrialized nations) have an infection, with certain patients receiving chronic hemodialysis exhibiting a predominance [7]. Additionally, data collected up to 2006 shows that the prevalence of HCV is rising internationally, with 1.47 cases per 100 patient years, with industrialized and undeveloped countries showing a distinct difference [8].

Hepatitis B and C virus infections cause an innate type I interferon response, as well as an innate (NK cells) and adaptive cellular immunological response (T cells), which together make up the antiviral immune response. The cellular response kills infected hepatocytes by inducing apoptosis, which is activated either by granzyme B and perforin or by TNF superfamily members like Fas ligand or TNF-related apoptosis-inducing ligand (TRAIL). activated CD8+ effectors When T cells recognize an antigen, they also release TNF and other cytokines that help regulate viral infection. Although in theory protective, as viruses do not themselves cause cytopenia, a prolonged antiviral immune response underlies the immunological pathogenesis of chronic viral hepatitis. Polymorphisms in the TNF promoter region affect the susceptibility to HBV infection [9].

TNF alters nephron transport and renal hemodynamics, which impacts the expression and activity of transporters. It also plays a mediating role in organ injury by promoting the infiltration and death of immune cells. Tumor necrosis factor-alpha (TNF) is a pleiotropic cytokine that mediates both rises and drops in blood pressure and is upregulated in chronic inflammatory conditions including hypertension and diabetes. Yet, modest elevations in TNF have been linked to increased NaCl retention and hypertension, whereas high amounts of TNF reduce blood pressure. Different levels of TNF inside the kidney, the subject's physiological condition, or the sort of stimuli inducing the inflammatory response may all play a role in explaining the varying effects seenTNF alters the expression and activity of transporters, which has an impact on renal hemodynamics and nephron transport. It also promotes immune cell infiltration and cell death, which contributes to mediating organ damage. Below, we will discuss the existing research and try to explain the disparities we found [10].

HCV is a ribovirus with a lipoprotein envelope and a 9.600 nucleotide 5-3 UTR [11]. The RNA genome is composed of a UTR, three structural genes (core, E1, and E2), and seven nonstructural genes (p7, NS2-NS5). The nonstructural protein (NS5B), which has a rather variable region, is widely used for HCV subtyping [12]. In terms of both primary sequence and secondary structures, the 5′ and 3′ UTRs are the most conserved regions of the HCV RNA. One of the more variable 5' UTR regions is found in the NS5A gene, which codes for a nonstructural protein. [13]. The 5' UTR, which includes 341 nucleotides, is widely used to identify a genotype due to its 90% sequence identity. The 5'-UTR stem-loop structure

contains entry sites (IRES) [14]. The 5-UTR seldom changes, however, on occasion, compensatory mutations are made to preserve the base-pairing form and structural characteristics associated with translation efficiency. Recent research has revealed that the first 145 sequences of the 5'-UTR are essential for the replication of HCV RNA [15].

Materials and Procedures

Sample collection: The Thi-Qar and Babylon Health Department of the Ministry of Health gave its consent to the research using a tailored approval template. 48 people had either a recent or long-term HCV infection.

Collection place: The study was at an Al-Hussein Teaching Hospital infectious diseases Department the Main Blood Bank of Thi-Qar Province and Al-Mousawi Private Laboratory in AL Nasiriya city, Medical Marjan city in Babylon province for sequence.

Collection period: Between August 2022 and November 2022.

Sample type: (53) Serum samples from study participants were frozen at -20 degrees Celsius to prevent repeated thawing and freezing.

Examination: An anti-HCV ELISA kit was used to determine the concentration of the antibody in the plasma sample. The assay was carried out by the manufacturer's instructions, and the microplates were read using an ELISA reader (human) at 450 nm.). The absorbance of the occulted sample might be compared to the cutoff value to identify the presence or absence of HCV Ag. Using 3 ccs of serum and the manufacturer's recommendations from Sun Long/China, the HCV Ag was determined. For this purpose, we employed SPSS and ANOVA Statistics. The (SPSS) test was used to analyze the demographics of the HCV Ag-seropositive population, and significant differences in prevalence across sexes and throughout the age spectrum were defined as[p values of 0.05 or below]. Then, the positive Human Hepatitis C virus UTR region of the polyprotein gene was subjected to viral RNA extraction, estimation of extracted RNA, reverse transcription PCR (RT-PCR), RT-PCR product analysis, and DNA sequencing method by RT-PCR products. These samples (Serum) were sent to Macrogen Company in Korea, where the DNA sequencing was carried out using an AB DNA sequencing system. The phylogenetic tree UPGMA method (MEGA 6.0 version), multiple alignment analysis based on Clustal W alignment analysis, and NCBI-BLAST for homologous sequence identity were used in the DNA sequencing analysis.

Primers

The one-step RT-PCR primers utilized for the UTR region of the polyprotein gene-based genotyping of the Human Hepatitis C virus were created by [16]. The following tables were provided by (Scientific Researcher Co. Ltd, Iraq) for this table:

Primer		Sequence 5'-3'	Amplicon
UTR-Polyprotein Gene Primer	F	Gtctagccatggcgttagtatgagtg	374bp
	R	Acaagtaaactccaccaacgatctg	

Ethics-approved: Before beginning sampling, patients gave their permission after being explained the study's goals, To guarantee that the participants weren't placed in needless danger, the Declaration of Helsinki was scrupulously followed. Before collecting the sample, we asked the patient for both their verbal and written consent. Document BMS 510/16 (with the number and date of 27/06/2022) demonstrates that a local ethics committee evaluated and approved the study methodology, subject information, and consent form.

Results:

The concentration of TNF α in patients with the Hepatitis C virus

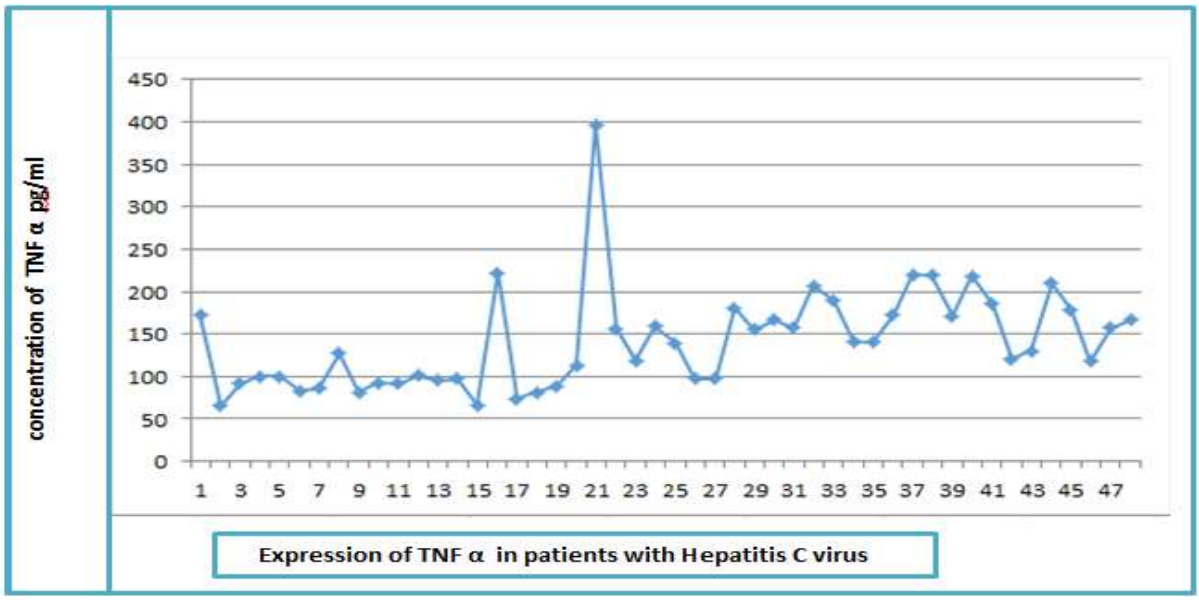

Figure (1): Concentration of TNF α in patients with Hepatitis C virus

Distribution of patients with Hepatitis C virus in blood samples according to their age

Thi-Qar Medical Journal (TQMJ):Vol.(25),No.(1),2023

Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq

ISSN (Print):1992-9218, ISSN (Online):1992-9218

The findings of testing blood for HCV Ag show a history of recent HCV infection. Table 1 shows the prevalence of (anti-HCV) antibodies and the trend for a decline or increase in prevalence with age. The distribution of HCV patients by age is shown in Table (1). There were substantial disparities between the ages. Patients with HCV ranged in age from 21 to 80, with a mean age of 55.52.

Table (1): Distribution of patients with Hepatitis C virus in blood samples according to their age by SPSS

	Patients Of *Hepatitis C*	No.	Mean Of Age	S.D	S.E	Minimum	Maximum	P-Value
	Blood Sample	**48**	**55.52**	**14.500**	**2.093**	**21**	**80**	**<0.05**

Table 1 displays the distribution of individuals with hepatitis C virus in blood samples by age, with a mean and standard deviation of (14.500). S.E. at (2.093), the domestic analysis yields a P value of 0.05.

Frequency of HCV and blood samples according to the age stratum

The findings showed that the age group (41-60) had the highest rate of infection at 45.8%, while the age group (21-40) had the lowest rate of infection at 16.7%. At a p-value of 0.05, the results indicate a significant difference.

Table (2): Frequency of HCV and blood samples according to the age stratum By SPSS

Age Stratum	Years	Hcv		P Value
		No.	%	
	21-40	8	16.7	<0.05
	41-60	22	45.8	
	61-80	18	37.5	
Total		48	100	

Distribution of patients with hepatitis C virus according to Gender and age

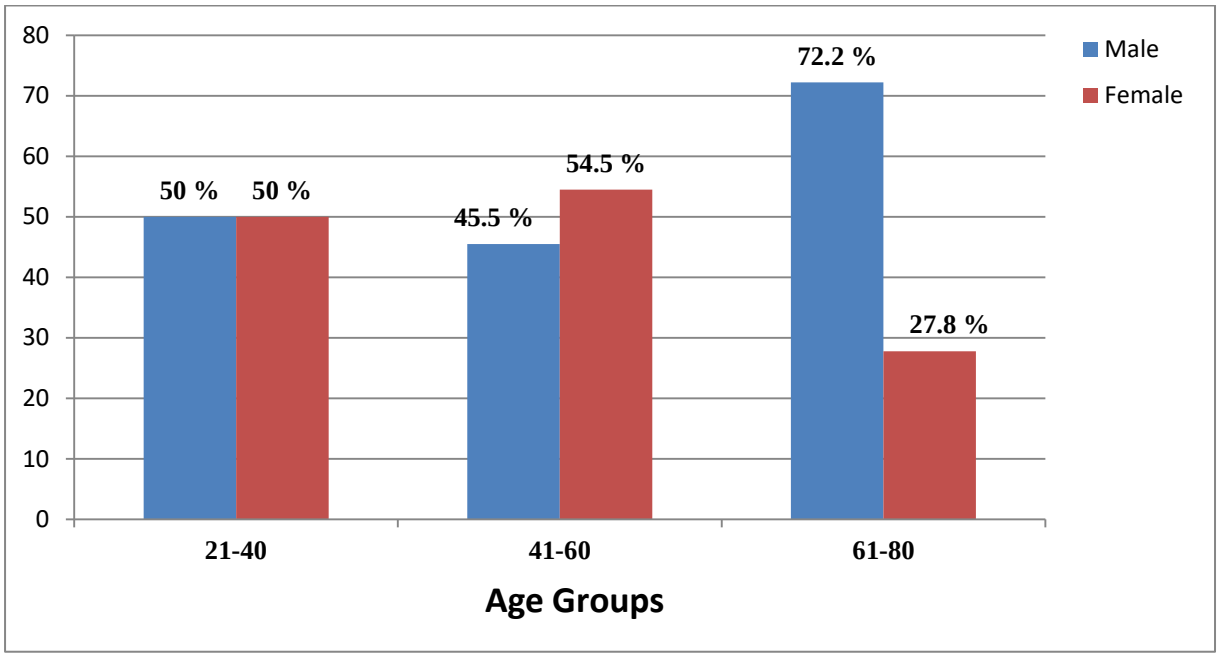

Figure (2): Distribution of patients with hepatitis C virus according to Gender and age.

Table (3): The correlation between HCV and TNF α according to ANOVA p-value = ≤ 0.05

TNF-C	Sum Of Squares	Df	Mean Square	F	Sig.
Between Groups	192585.180	2	96292.590	54.521	.000
Within Groups	114800.621	65	1766.163		
Total	307385.800	67			

Phylogenetic tree analysis

Thi-Qar Medical Journal (TQMJ):Vol.(25),No.(1),2023

Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq

ISSN (Print):1992-9218, ISSN (Online):1992-9218

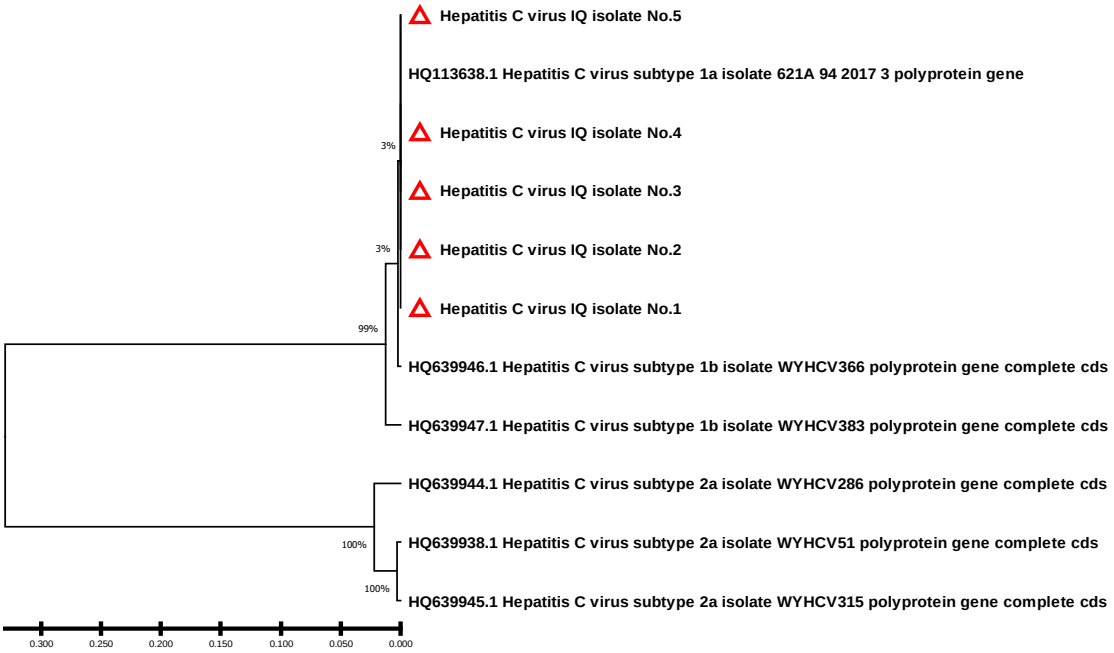

Figure (4): Phylogenetic tree analysis based on partial sequences of the UTR-polyprotein gene from local isolates of the Hepatitis C virus utilized for genotyping identification. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree) in (the MEGA 6.0 version) was used to build the phylogenetic tree. According to overall genetic alterations (0.3–0.05%), the local Hepatitis C virus isolates were closely related to NCBI-BLAST Hepatitis C virus subtype 1a (HQ113638.1).

Table (4) the NCBI-BLAST Homology Sequence identity (%) between local Hepatitis C virus isolates and NCBI-BLAST submitted related isolates:

Local Isolate No.	Gen Bank Accession Number	NCBI-BLAST Homology Sequence Identity (%)			
		Identical Isolate	Gen Bank Accession Number	County	Identity (%)
IQ.No.1	OQ843893	Hepatitis C Virus Subtype 1a	HQ113638.1	USA	99.41%
IQ.No.2	OQ843894	Hepatitis C Virus Subtype 1a	HQ113638.1	USA	99.41%
IQ.No.3	OQ843895	Hepatitis C Virus Subtype 1a	HQ113638.1	USA	99.61%
IQ.No.4	OQ843896	Hepatitis C Virus Subtype 1a	HQ113638.1	USA	99.61%
IQ.No.5	OQ843897	Hepatitis C Virus Subtype 1a	HQ113638.1	USA	99.61%

Discussion:

Using serologic assays, HBV and HCV are diagnosed. These tests look for human antibodies that are produced in response to HCV and HBV infection. Positive results for HCV antibodies could indicate a current infection, a previous infection, or a false-positive test [17]. The hepatitis C virus (HCV) is a blood-borne pathogen. The majority of epidemiological research has concentrated on populations at risk of infection, like thalassemic patients who have received numerous blood transfusions. Additionally, HCV is currently thought to be the world's most common cause of post-transfusion hepatitis. Thalassemic individuals must get repeated blood transfusions to survive; unfortunately, these transfusions increase the risk of contracting not only HCV but also hepatitis B virus (HBV), hepatitis G virus (HGV), and human immunodeficiency virus (HIV) [18].

The outcome was consistent with the earlier study, which demonstrated that birth cohort prevalence has traditionally been used to assess and publish age data for HCV prevalence. Those born between 1945 and 1965 (1.63%) had the highest rates, followed by those born after 1965 (0.51%), and those born before 1945 (0.21%) had the lowest rates, according to a recent analysis of birth cohort HCV prevalence estimates in the United States. These results are consistent with previously published data that showed the highest HCV prevalence was among those born between 1945 and 1965 [19,20].

The comparably high frequency of HCV infection among those born between 1945 and 1965 correlates with the large HCV incidence (new infections) among young people using or experimenting with injectable drugs in the 1970s and 1980s [21]. In recent years, the proportion of cases involving those born after 1965 has steadily increased.

Chronic kidney disease (CKD) and the beginning of renal disease have both been linked to HCV infection [22]. with advanced kidney disease [23]. compared to people who do not have HCV infection. HCV coinfection has also been linked to an increased risk of CKD in HIV-infected individuals, and older age is linked to more advanced CKD in HIV/HCV-co-infected individuals compared to HIV-mono-infected individuals [24]. In elderly HCV-infected patients, kidney failure frequently involves multiple factors. In addition to the less frequent immune-mediated kidney damage brought on by cryoglobulinemia, lifestyle choices include substance misuse [25], as well as concomitant illnesses linked to aging, like diabetes and hypertension [26], are also significant factors that influence how renal function changes in

HCV-infected people. Additional research The number of acute HCV infections reported in 2019 was larger in men (2,471) than in women (1,653), with men making up 60% of these infections [21]. According to estimates, 1.31% of adult males in the United States have HCV, which is 2.3 times more than the 0.57% estimate for adult females. This results in a male-to-female HCV prevalence ratio of 2.3 [27].

There are many genotypes and quasi-species of the hepatitis C virus (HCV) because its RNA frequently experiences genetic changes. Direct sequencing of the 5'-UTR for the detection of HCV genotypes is a terrific method because it employs amplified products from one step, no nested PCR, and does not require pattern processing procedures. Additionally, direct sequencing of PCR findings provides more comprehensive sequence information in comparison to alternative genotyping analyses. The HCV 5-UTR, which includes 324 to 341 nucleotide sequences, is the most conserved region, and it may be used to determine the HCV genotypes based on sequences, according to various studies [28].

The genetic fragment 5′ untranslated region (5′UTR) was target for the detection of HCV as it was characterized by highly conserved sequences for this organism (Kabakçı et al., 2014). The HCV 5′ untranslated region (5′UTR), responsible for the initiation of viral translation via an internal ribosome entry site (IRES), has been previously described to contain specific nucleotide substitutions when cultured in infected lymphoid cells. Sequence variability in this region has important implications for structural organization and the function of the IRES element and could correlate with HCV RNA concentration [29]. The 5'-UTR was selected because of its importance as an essential component of the Internal Ribosome Entry Site (IRES) that regulates Cap-independent translation of HCV as we have showed above

According to available information, the present study is the first of its kind in Iraq, which succeed in recording, New strains of the virus in the gene bank, and therefore we did not find any Iraqi strains registered can compare with them. But when compared with international strains, three of our isolates showed similar sequences to HCV strains from the United States of America, this may be because Iraq may have imported blood products from the United States or because of the US occupation of Iraq or as a result of the travel of Iraq is to the United States and therefore perhaps the transmission of the virus by one of the known transmission routes. The fact of the identity of all investigated samples was determined as they belong to the same viral identity, whether in terms of the viral HCV organism or genotype. However, this notion provided a further indication of the viral identity and

genotyping of these locally studied samples. HCV genotyping distribution has an important influence clinically on the morbidity, total costs, and duration of HCV treatment [30]. Therefore, for a better understanding of HCV epidemiology as well as the prevalence of its genotype pattern, performing HCV genotyping studies in Iraq is very important. In the study was conducted in Babylon Province this study gives a different estimation of HCV genotype distribution among infected HCV patients in Babylon from prevalent distribution in Iraq and Middle East Arab countries, but comparable to global distribution. Was reported (37%) genotype 1 was the most frequent genotype detected followed by 3 (27.3%), 4 (20%), and 2 (2.4%), while mixed genotypes were detected in 13.3% [31]. While, the study conducted in Baghdad by [32] reported another result, Genotype HCV-1b showed higher prevalent (52.9%) among the recipients of anti-D Ig therapy while genotype HCV-3a (6.6%) was the lowest.

In Istanbul, Turkey, the study aimed to determine the distribution of HCV genotypes in patients with HCV showed Genotype 1a (82.5%) was the dominant genotype [33].

This 5′-UTR -the based comprehensive tree has provided an extremely inclusive tool about the high ability of such 5′-UTR sequences to efficient identification HCV samples using this genetic fragment. This, in turn, gives a further indication of the power of the currently utilized 5′-UTR specific primers to discriminate among the currently investigated isolates.

TNF levels in hepatitis C virus patients

TNF is a pro-inflammatory cytokine that is secreted in response to pathogenic infections. TNF-converting enzyme separates soluble TNF from transmembrane TNF (TACE). The biological effects occur when the released TNF interacts with its receptors, in this case, TNFR1 and TNFR2 [34]. Because of TNF-'s widespread role as a pro-inflammatory agent, biologics that inhibit TNF- and associated cytokine pathways have been used to treat a variety of inflammatory and autoimmune diseases [35]. HCV patients have an elevated serum level of TNF-α, and this level is positively correlated with the severity of liver diseases [36]. The source of TNF-α is unclear, but it is generally assumed that it is produced by immune cells such as macrophages [37]. In this work, we demonstrated that TNF-α may also be activated in HCV-infected cells. Although the amount of TNF-α produced by HCV-infected hepatocytes might be lower than that produced by professional immune cells such as

macrophages [38]. It was enough to cause a reaction that prevented HCV replication. Effective antiviral immune response development requires TNF-alpha (TNF-) [39].

TNF- is a pleiotropic multifunctional cytokine that regulates Th-1 cellular immune response, phagocytic cell activity, and the production of cytotoxic cells, all of which aid in the maturation of the body's defense mechanisms. In the current investigation, it was discovered that serum TNF- levels were substantially (p 0.05) higher in the HBV patient group than in the control group (1-3). These findings are consistent with those of other authors, and it is concluded that monitoring TNF levels is indicative of liver damage even when liver enzyme levels are normal [40]. Any degree of liver inflammation is associated with significantly elevated blood levels of tumor necrosis factor (TNF), which raises the possibility that TNF could be utilized as a predictor of liver inflammation [41].

Conclusion:

A liver inflammatory disease called hepatitis C. Cytokines is chemical mediators that control immune cells' development, division, and function. The goal of this study was to determine the prevalence of [hepatitis C virus (HCV Ag)] among HCV surface antigen-positive (HCV Ag+) ELISA (enzyme-linked immunosorbent assay) and RT-PCR, respectively, were used to determine the results.

New strains of the virus are in the gene bank, and therefore we did not find any Iraqi strains registered that can compare with them. But when compared with international strains, five of isolates strains showed similar sequences to HCV strains from of the United States of America, this may be because Iraq may have imported blood products**.**

This 5′-UTR -the based comprehensive tree has provided an extremely inclusive tool about the high ability of such 5′-UTR sequences to efficient identification HCV samples using this genetic fragment. This, in turn, gives a further indication of the power of the currently utilized 5′-UTR specific primers to discriminate among the currently investigated isolates.

Recommendations:

1.The need for people to be aware of the seriousness of the disease.

2.Conduct a study survey of all provinces for the purpose of determining the dominant genotype and subtypes.

3. Since the disease has no vaccine to HCV so we need effective preventive measures.

4. Imposing a compulsory vaccination program for all and conducting periodic examinations for early detection of the disease and prevention to reduce the development of cases to advanced stages .

5. Expansion of the study of genotypes in Iraq for affected people to know the prevalence of the most common genotype, management of the clinical case, and appropriate treatments according to each genotype, especially for patients with progressive stages or coinfection or infected with autoimmune diseases.

Reference

1.BLACH, Sarah, Et Al. Global Change In Hepatitis C Virus Prevalence And A Cascade Of Care Between 2015 And 2020: A Modeling Study. The Lancet Gastroenterology & Hepatology, 2022, 7.5: 396-415.

2. ABATE, Maria Lorena; CAVIGLIA, Gian Paolo. Chronic Hepatitis C: Pathophysiology And Clinical Issues. Biology, 2022, 11.12: 1737.

3. HE, Juan, Et Al. The Relationship Between Tumor Necrosis Factor-A Polymorphisms And Hepatitis C Virus Infection: A Systematic Review And Meta-Analysis. Renal Failure, 2011, 33.9: 915-922.

4. CAVIGLIA, Gian Paolo, Et Al. Long-Term Hepatocellular Carcinoma Development And Predictive Ability Of Non-Invasive Scoring Systems In Patients With HCV-Related Cirrhosis Treated With Direct-Acting Antivirals. Cancers, 2022, 14.3: 828.

5. CAVIGLIA, Gian Paolo, Et Al. The Clinical Role Of Serum Epidermal Growth Factor Receptor 3 In Hepatitis C Virus-Infected Patients With Early Hepatocellular Carcinoma. Biology, 2021, 10.3: 215.

6. CHEEMERLA, Shantan; BALAKRISHNAN, Maya. Global Epidemiology Of Chronic Liver Disease. Clinical Liver Disease, 2021, 17.5: 365.

7. SELCUK, Haldun, Et Al. Distribution Of HCV Genotypes In Patients With End-Stage Renal Disease According To Type Of Dialysis Treatment. Digestive Diseases And Sciences, 2006, 51: 1420-1425.

8. JADOUL, M.; MARTIN, P. Kidney Disease: Improving Global Outcomes (KDIGO) Hepatitis C Work Group. KDIGO 2018 Clinical Practice Guideline For The Prevention, Diagnosis, Evaluation, And Treatment Of Hepatitis C In Chronic Kidney Disease. Kidney Int Suppl, 2018, 8.3: 91-165.

9. TIEGS, Gisa; HORST, Andrea K. TNF In The Liver: Targeting A Central Player In Inflammation. In: Seminars In Immunopathology. Berlin/Heidelberg: Springer Berlin Heidelberg, 2022. P. 445-459.

10. RAMSEYER, Vanesa D.; GARVIN, Jeffrey L. Tumor Necrosis Factor-A Regulation Of Renal Function And Blood Pressure. American Journal Of Physiology-Renal Physiology, 2013, 304.10: F1231-F1242.

11. ALZAHRANI, Alhusain J., Et Al. Detection Of Hepatitis C Virus And Human Immunodeficiency Virus In Expatriates In Saudi Arabia By Antigen-Antibody Combination Assays. The Journal Of Infection In Developing Countries, 2009, 3.03: 235-238.

12. FAN, Wenmei, Et Al. Nonstructural 5A Gene Variability Of Hepatitis C Virus (HCV) During A 10-Year Follow-Up. Journal Of Gastroenterology, 2005, 40: 43-51.

13. MARGRAF, Rebecca L., Et Al. Genotyping Hepatitis C Virus By Heteroduplex Mobility Analysis Using Temperature Gradient Capillary Electrophoresis. Journal Of Clinical Microbiology, 2004, 42.10: 4545-4551.

14. YUAN, H. J., Et Al. HALT-C Trial Group. Evolution Of Hepatitis C Virus NS5A Region In Breakthrough Patients During Pegylated Interferon And Ribavirin Therapy. J. Viral. Heptatit., 2010, 17.3: 208-216.

15. SOLER, Muriel, Et Al. Quasispecies Heterogeneity And Constraints On The Evolution Of The 5′ Noncoding Region Of Hepatitis C Virus (HCV): Relationship With HCV Resistance To Interferon-A Therapy. Virology, 2002, 298.1: 160-173.

10. VIRTANEN, Elina, Et Al. Genotyping Of Hepatitis C Virus By Nucleotide Sequencing: A Robust Method For A Diagnostic Laboratory. Methodsx, 2018, 5: 414-418.

17. ABDULKAREEM, Yasen Rashad, Et Al. Seroprevalence Of Hepatitis B And C Viruses Among Diabetes Mellitus Patients In Duhok Province. Medical Journal Of Babylon, 2021, 18.3: 219.

18. AL-SHARIFI, Liqaa Mohammed, Et Al. Prevalence Of Hepatitis B And C In Thalassemic Patients And Its Relation With The Type Of Thalassemia, Frequency Of Blood Transfusion, And Spleen Status. Med J Babylon, 2019, 16.3.

19. CASTREJÓN, Mariana, Et Al. Implementation Of A Large System-Wide Hepatitis C Virus Screening And Linkage To Care Program For Baby Boomers. In: Open Forum Infectious Diseases. Oxford University Press, 2017.

20. KONERMAN, Monica A., Et Al. Impact Of An Electronic Health Record Alert In Primary Care On Increasing Hepatitis C Screening And Curative Treatment For Baby Boomers. Hepatology, 2017, 66.6: 1805-1813.

21. TESHALE, Eyasu H., Et Al. Characteristics Of Persons Treated For Hepatitis C Using National Pharmacy Claims Data, United States, 2014–2020. Clinical Infectious Diseases, 2022, 75.6: 1078-1080.

22. PERICO, Norberto, Et Al. Hepatitis C Infection And Chronic Renal Diseases. Clinical Journal Of The American Society Of Nephrology, 2009, 4.1: 207-220.

23. CHEN, Yi-Chun, Et Al. A Nationwide Cohort Study Suggests That Hepatitis C Virus Infection Is Associated With An Increased Risk Of Chronic Kidney Disease. Kidney International, 2014, 85.5: 1200-1207.

24. FISCHER, Michael J., Et Al. Hepatitis C And The Risk Of Kidney Disease And Mortality In Veterans With HIV. JAIDS Journal Of Acquired Immune Deficiency Syndromes, 2010, 53.2: 222-226.

25. ABRAHAM, Alison G., Et Al. End-Stage Renal Disease Among HIV-Infected Adults In North America. Clinical Infectious Diseases, 2015, 60.6: 941-949.

26. YAMAKAWA, Y., Et Al. The Higher Elimination Rate Of Hepatitis C Virus Among Women. Journal Of Viral Hepatitis, 1996, 3.6: 317-321.

27. Hofmeister, Megan G., Et Al. Estimating Prevalence Of Hepatitis C Virus Infection In The United States, 2013-2016. Hepatology, 2019, 69.3: 1020-1031.

28. Ullah, Amin, Et Al. Phylogenetic Analysis Of The 5′ Untranslated Region Of Hcv From Cirrhotic Patients In Khyber Pakhtunkhwa, Pakistan. Scientific Reports, 2021, 11.1: 15023.

29. Barría, M.I.; González, A.; Vera-Otarola, J. And Et Al. (2009). Analysis Of Natural Variants Of The Hepatitis C Virus Internal Ribosome Entry Site Reveals That Primary Sequence Plays A Key Role In Cap-Independent Translation. Nucleic Acids Res. 37(3):957971.

30. Cuypers, Lize, Et Al. Genetic Diversity And Selective Pressure In Hepatitis C Virus Genotype 1–6: Significance For Direct-Acting Antiviral Treatment And Drug Resistance. Viruses, 2015, 7.9: 5018-5039.

31. Dlshad, A. H.; Sazan, Q. M.; Rastee, H. S. And Beston, F. N. (2018). Seroprevalence And Genotypic Distribution Patterns Of Hepatitis C Virus Among Infected Patients From Erbil Province: Kurdistan/Iraq. Diyala Journal Of Medicine. 14(1):84-93.

32.Karabulut, Nuran, Et Al. Distribution Of Hepatitis C Virus Genotypes In Istanbul, Turkey. Indian Journal Of Medical Microbiology, 2018, 36.2: 192-196.

33.Al-Kubaisy, Waqar, Et Al. Maternal Hepatitis C (Hcv) Infection And Anti-D Immunoglobulin Therapy: Study Testing Antibodies, Rna And Genotype Of Hcv In Baghdad. The Journal Of Maternal-Fetal & Neonatal Medicine, 2019, 32.20: 3464-3469.

34. Abady, Noor R.; Al-Khafaji, Zaytoon A.; Baay, Ali S. Clinical Features Associated With Severe Acute Respiratory Syndrome (Sars-Cov2). International Journal Of Health Sciences, 2022, 5679-5688.

35. Abady, Noor; Alkhafaji, Zaytoon A.; Baay, Ali Saleh. Genetic Detection And Phylogenetic Tree Study For Sars Cov-2 (N Gene) In Iraq. Journal Of Complementary Medicine Research, 2021, 12.4: 131-131.

36. Knobler, H.; Schattner, A. Tnf-A, Chronic Hepatitis C And Diabetes: A Novel Triad. Qjm, 2005, 98.1: 1-6.

37. Bradley, J. R. The Role Of Nuclear Organization In Cancer. J. Pathol, 2008, 214: 149-160.

38. Fletcher, Nicola F., Et Al. Activated Macrophages Promote Hepatitis C Virus Entry In A Tumor Necrosis Factor-Dependent Manner. Hepatology, 2014, 59.4: 1320-1330.

39. Abady, Noor R., Et Al. Detection Of Missense And Frameshift Mutations In Envelope Proteins Using Molecular Phylogenetic Sars-Cov-2. Journal Of Complementary Medicine Research, 2022, 13.1: 92-92.

40. Mourtzikou, Antonia, Et Al. Evaluation Of Serum Levels Of Il-6, Tnf-A, Il-10, Il-2, And Il-4 In Patients With Chronic Hepatitis. Inmunología, 2014, 33.2: 41-50.

41. Rajeswari, S.; Swaminathan, S. Inflammatory Markers In Infectious Diseases–An Update. Int J Curr Res Chem Pharma Sci, 2014, 1.7: 113-30.