Hepatitis C Virus genotypes in Nineveh Governorate

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Received: 8.2.2024 **Accepted :4.6.2024**

Abstract:

Background: Hepatitis C virus (HCV) infection is considered a major health problem worldwide. Despite the availability of an effective direct-acting antiviral (DAA) therapy; 58 million people still have chronic HCV globally. HCV had 6 major genotypes; identification of specific genotypes linked to appropriate DAA selection.

Aim of this study: To know the predominant HCV genotype in Nineveh governorate.

Patients and Methods: This is a descriptive cross-sectional study conducted in public health laboratory in Nineveh from March 2022 to February 2023, 107 patients with HCV antibodies were included in this study, they underwent HCV real-time polymerase chain reaction (RT PCR) and genotyping, all those 107 patients had positive HCV antibody by enzyme-linked immunosorbent assay (ELISA).

Results: From 107 patients with positive HCV antibodies, only 50 patients (46.7%) had positive HCV PCR, Genotype 4 is the most common HCV genotype in Nineveh (48 % and genotype 1a (26%) is the second common genotype, half of the current patients were young and HCV more common in male patients (62%).

Conclusion: The most prevalent HCV genotype in Nineveh governorate is genotype 4 followed by genotype 1a.

Keywords: Genotype, Hepatitis C Virus, Liver diseases.

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Introduction

Hepatitis C virus (HCV) belongs to the Flaviviridae family, and it is the only member of the genus Hepacivirus. Since the cloning of the HCV genome in 1989, the virus continues to be a major health problem (1,2).

In 2022, the World Health Organization (WHO) estimated that around 58 million people had chronic HCV infection (3).

HCV can cause acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Acute hepatitis seldom results in hepatic failure and is self-limiting, but it frequently causes chronic infection. Cirrhosis, hepatocellular cancer, and the requirement for liver transplantation are possible outcomes of chronic HCV infection, which frequently has a progressive course over several years (4,5).

HCV has been classified into six primary genotypes and around 50 subtypes; the most prevalent subtypes are 1a, 1b, 2a, and 2b (6,7). The seventh and eighth genotypes have been assigned to sporadic virus isolates that are unique from all others (8). These 6 genotypes had distinct

geographical distribution; genotype 1 is the most common worldwide, particularly in the USA and Europe, while in the Middle East genotype 4 is most prevalent (9,10). Knowing the HCV genotype is essential to determine the suitable treatment regarding duration and regimen dose.

HCV infections are rising over time in various regions of the world, which is a serious global health concern that affects many people globally and is a major factor that leads to chronic liver disease and hepatocellular carcinoma (11). The geographical distribution of various HCV genotypes must be determined for epidemiological research, clinical care, and vaccine development (12). HCV genotype prevalence varies globally owing to virus mutation, infection route, and demographic study (13).

At the beginning of 2020, it was estimated that the global prevalence of HCV was 0.7 percent (56.8 million people infected with chronic HCV) there was a drop in HCV prevalence when compared to 2015(63.6 million, 0.9% of the global population) (14).

HCV genotype 4 had the highest prevalence in the Middle East, including Arabic countries, with a rate of 74.7% followed by genotype 1 with a rate of 15.1%, genotype 3 with a rate of 4.2%, and genotype 2 with a rate of 1.7%. The 4.3% of the overall number of HCV infections in Middle Eastern nations were caused by uncommon genotypes such as 5 and 6, mixed HCV genotypes, and genotypes that could not be typed (15,16).

Treatment aims to eliminate HCV RNA, which can be foreseen by achieving a sustained virologic response (SVR), SVR is the absence of HCV RNA detected by polymerase chain reaction (PCR) 12 weeks after treatment has ended. Long-term follow-up following an SVR is associated with a 99 percent likelihood of eradicating HCV RNA, making it possible to declare that the virus has been cured (17,18). It has also been shown that achieving SVR leads to better clinical results.

An antibody test is usually the first step in the initial diagnostic evaluation or screening for chronic HCV. A negative antibody test often means that the patient does not have chronic HCV infection and does not require additional testing. A positive HCV-antibody test suggests an active (current) acute or chronic HCV infection, a previously treated infection, or in rare cases, a false-positive finding (19). 3.2% of the Iraqi adult population had positive HCV antibody (10).

HCV RNA testing should come after a positive or inconclusive/ambiguous antibody test. A detection threshold of 25 international units/mL or below is required for quantitative HCV RNA testing used to confirm the diagnosis. The existence of HCV infection is confirmed if HCV RNA is found (20).

Tests for HCV genotyping and sub-genotyping are helpful for predicting treatment response as well as epidemiological investigations (6).

Prior to beginning antiviral therapy, quantitative HCV-RNA and genotype testing were advised to ascertain baseline viremia (viral load), which may alter the DAA therapy. With the introduction of pangenotypic DAA regimens (effective and used for the same length of treatment regardless of the HCV genotype), HCV genotyping is no longer always necessary before starting treatment (21).

All patients, except for those whose life expectancy is less than 12 months owing to unrelated diseases, should be considered for therapy because it is safe, effective, and can lessen the devastating effects of this disease (22).

Treatment should be explored for all individuals who have chronic HCV infection, defined as a detectable HCV over six months. The treatment of HCV infection has been transformed by the development of DAAs, medications that specifically target nonstructural proteins of HCV and obstruct viral multiplication. Many drugs are available to treat chronic HCV, the new drugs have high potency to eliminate all HCV genotypes (23,24).

With the advent and widespread use of DAAs, which have the potential to provide very successful, interferon-free (and frequently, ribavirin-free) regimens for most HCV-infected people, antiviral therapy for HCV has been changing quickly. The

continuous examination of genotype in other patient populations was still important for treatment choices with some regimens (such as patients with cirrhosis or prior treatment failure) (25).

However, if limited funding prevents the distribution of pangenotypic DAA to all patients, doing HCV genotype before treatment had a fundamental role in selecting proper DAA that had activity against specific HCV genotypes, this is the situation in Iraq including Nineveh Governorate (26).

Patients and Methods:

This cross-sectional descriptive study was conducted in a public health laboratory in Nineveh between the periods of March 2022 to January 2023 and included 107 patients referred to the public health laboratory, those 107 patients already had positive HCV antibodies and had them again for HCV antibodies by VIDAS technique to confirm that these patients exposed to HCV.

After confirming the presence of HCV antibodies, all 107 patients underwent testing for quantitive HCV RNA (viral load) and genotyping.

Patients Characteristics:

Of those 107 patients who tested positive for HCV antibody, 50 patients (46.7%) had positive HCV RNA, and the remaining 57 patients had negative HCV RNS which means that they are cured of HCV and, to a lesser extent, had HCV RNA level below the level of detection by our technique. Those 50 patients with positive HCV RNA are tested for HCV genotypes.

Blood sampling and processing:

A 3 mL of venous blood sample was collected from each patient in an EDTA tube which was mixed well immediately and centrifuged at 800-1600 x g for 20 minutes to get plasma. The plasma was transferred to a 1.5 ml Eppendorf tube and stored at -20 °C deep freeze until the time of HCV viral load measurement.

Measurement of HCV viral load:

For the detection and measurement of HCV viral load, the patients' plasma samples were thawed at room temperature. HCV RNA was extracted from plasma using Zybio automated extraction system using Zybio's Nucleic Acid Extraction Kit (magnetic beads method) and following manufacturer guidelines. The extracted RNA samples underwent amplification in real time using HCV Real- TM Quant Dx kit (Sacace Biotechnologies) and Analytic Jena qTower3 thermocycler. By the means of calibrations samples, supplied with the kit, HCV viral load was calculated for each patient. The viral load was expressed in IU/mL, and the limit of detection (LOD) of the HCV Real-TM Quant Dx assay is 13 IU/mL.

Detection of HCV genotypes:

Following measurement of viral load, HCV genotypes were detected for the positive patients' samples after amplification of their RNA samples using HCV Genotype Plus Real-TM kit (Sacace Biotechnologies) and Analytic Jena qTower3 thermocycler. The kit was supplied with primers and probes for detection of 1b, 3, 1a, 2, 4, 5a and 6 genotypes of HCV.

Results:

Only 50 patients (46.7%)out of 107 patients had positive HCV RNA. The characteristics of those 50 patients included in the present study are mentioned in the table below (Table 1):

Table (1): Characteristics of the study sample.

Study parameters		No. of patients	Percentage
Sex	Male	31	62.0
	Female	19	38.0
Age groups	< 45 yrs	25	50.0
	45-65	22	44.0
	>65	3	6.0
Genotypes	Yes	41	82.0
	No	9	18.0
Types of genotypes	1a	13	26.0
	1b	2	4.0
	2	1	2.0
	3	1	2.0
	4	24	48.0

The mean age of the patients is 43.75 ± 16.37year (range 13-75), genotype specific ages are shown in Table (2).

Table 2: Mean age of each HCV genotype.

Genotype	Mean age ±SD (years)
1a	46.15 ± 13.23
1b	56±8.48
2	42
3	17
4	41.7±17.6
No genotype	47.75±17.83
All patients	43.75 ± 16.37

Thirty-one patients (62.0%) were males and 19 patients (38%) were females, with male to female ratio of 1.6:1, genotype 4 was more common in male patients 17 males (70.8%) Vs 7 females (29.2%) but there was no sex difference in genotype 1 (8 vs7) as shown in Figure (1), 50.0 % of present study's patients (25 patients) were young and less than 45 year old with just 3 patients (6.0%) over the age of 65 years which demonstrated in Figure (2).

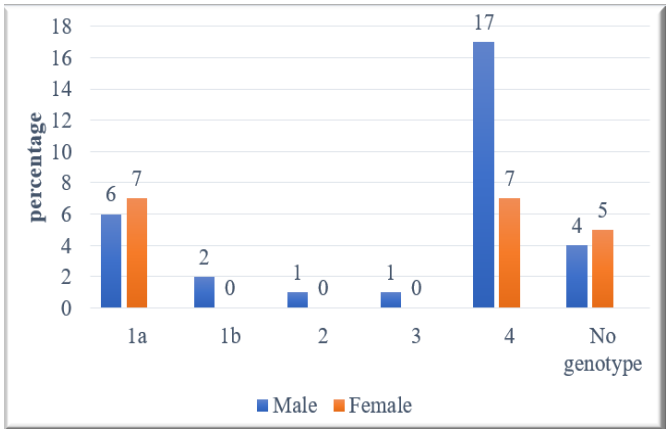

Figure 1: Gender-specific distribution of HCV genotypes.

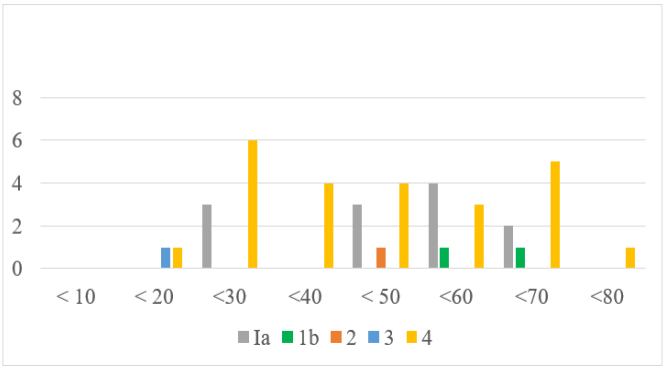

Figure (2): HCV genotypes and age distribution.

Regarding HCV genotyping, HCV genotype 4 was the most common in the current study which was found in 24 patients (48.0%), genotype 1a is the

second most prevalent genotype found in 13 patients(26.0%), genotype 1b was found in 2 patients (4.0%) and genotype 2 and 3 just in 1 patient in each genotype (2.0%). The remaining 9 patients (18.0%) despite having positive HCV RNA, had no specific genotypes shown in Figure (3).

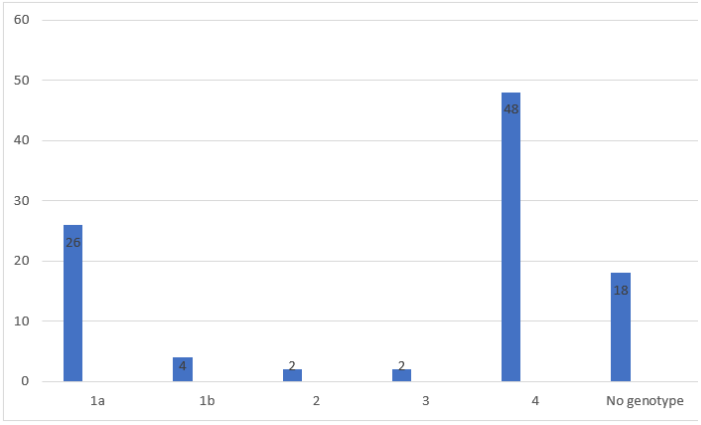

Figure (3): HCV genotypes distribution in Nineveh Governorate.

Discussion:

HCV genotype 4 is the most common in Iraq (15), this was in concordance with the current result which showed that 24 patients (48.0%) had HCV genotype 4 in Nineveh Governorate. The second common genotype is genotype 1 which was found in 15 patients (30.0%) with subtype 1a being more prevalent than 1b found in 13 patients, genotype 1a in 2 patients genotype 1b).

Most of the present study's patients were males 62.0% which is similar to the results from Duhok province (27), half of our patients were less than 50 years of age, which is in concordance with that published recently from Lebanon 75.0% of their patients were males and the peak age between 20-59 years (28).

Different HCV genotypes were identified in the nearby governorates, in Duhok the most common genotype is 1 and then genotype 4 (29), which is similar to the genotype prevalence in Nineveh governorate, while in Erbil the most prevalent genotype is1 followed by 3 (30), in Nineveh genotype 3 is the least prevalence. In Kirkuk genotype 4 is the most common followed by genotype 1, this is contrary to the prevalence of HCV genotype in Nineveh (31).

These differences in the HCV genotypes in the nearby cities may be due to differences in the ethnicity of their residents, as most of the Nineveh residents were Arabic, while in Erbil most people were Kurdish and in Kirkuk, people were a mixture of Arabic, Kurdish and Turkmen as demonstrated in figure(4) and table (4).

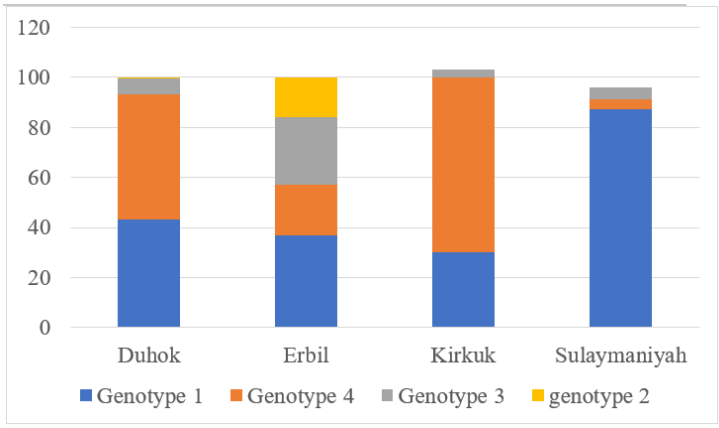

Figure (4): HCV genotype distribution in 4 governorates near Nineveh.

Table 4: HCV genotype distribution in 4 governorates near Nineveh.

Governorate	Genotype 1	2	3	4
Duhok 18	***	*	**	****
Erbil 17	****	*	***	**
Sulaymaniyah 16	****	***	*	**
Kirkuk 19	***			****

**** being the most common and * being the least common

The importance of this study comes from the fact that this is the first study about HCV genotypes in the Nineveh governorate.

78.0% of HCV-infected patients involved in this study are genotypes 4 and 1, more new data from different Iraqi governorates about the prevalence of HCV genotypes was needed as well as more patient numbers to get more information about HCV genotypes in Nineveh.

By comparing the genotype prevalence in the nearby countries, like as Syria, Jordan and Kuwait, genotype 4 is the most common followed by genotype 1 (25) which is in concordance with the HCV genotypes in Nineveh, while in Turkey (32) and Iran (33) genotype 1 is the most prevalent. The similarity of the HCV genotype of Nineveh with the nearby Arabic countries again may be related to the similarities in the Arabic ethnicity and mode of HCV spread, and the differences with Turkish and Iranian genotypes because of their different ethnicity.

Conclusion:

HCV genotype 4 is the most prevalent in Nineveh followed by genotype 1a, most of the present study's patients were young. More studies and bigger samples were required to delineate more about HCV in Iraq generally and in Nineveh specifically.

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النمط الجيني لفيروس التهاب الكبد Cفي محافظة نينوى

الخلاصة:

الخلفية :يعتبر فيروس التهاب الكبد C مشكلة صحية رئيسية على مستوى العالم .على الرغم من توفر علاج فعال ذو مفعول مباشر مضاد للفيروسات (DAA) ، لا يزال ٥٨ مليون شخص يعانون من التهاب الكبد الائي C المزمن على مستوى العالم .يحتوي فيروس التهاب الكبد الائي Cعلى ٦ أنواع رئيسية ؛ يرتبط تحديد الأنماط الجينية المحددة بالاختيار المناسب لـ.DAA

هدف الدراسة :التعرف على النمط الجيني السائد لفيروس التهاب الكبد الائي C في محافظة نينوى.

المرضى والطرق :هذه دراسة وصفية مستعرضة أجريت في مختبر الصحة العامة في نينوى للفترة من مارس ٢٠٢٢ إلى فبراير ٢٠٢٣ ، تم ضم ١٠٧ مريضًا مصابًا بأجسام مضادة للفيروس التهاب الكبد الائي C في هذه الدراسة ، خضعوا لفحص تفاعل البوليميراز المتسلسل في الوقت الحقيقي (RT PCR) وتحديد الأنماط الجينية ، وجميع هؤلاء المرضى البالغ عددهم ١٠٧ كانت نتيجة اختبار الأجسام المضادة للفيروس التهاب الكبد الائي C إيجابية بواسطة.ELISA

النتائج :من أصل ١٠٧ مريضًا لديهم أجسام مضادة إيجابية لفيروس التهاب الكبد الائي C ، لم يكن لدى ٥٠ مريضًا فقط (٤٦ (٪7.فحص PCR إيجابي لفيروس التهاب الكبد الائي C ، والنمط الجيني ٤ هو النمط الجيني الأكثر شيوعًا لفيروس التهاب الكبد الائي C في نينوى (٤٨٪ والنمط الجيني ١ (26٪) aهو ثاني أكثر الأنماط الجينية شيوعًا ، وكان نصف المرضى الحاليين صغارًا وكان فيروس التهاب الكبد الائي C أكثر شيوعًا بين الذكور (٦٢٪.(

الخاتمة :النمط الجيني السائد لفيروس التهاب الكبد الائي C في محافظة نينوى هو النمط الجيني ٤ يليه النمط الجيني ١.a

الكلمات المفتاحيه :النمط الجيني، فيروس التهاب الكبد C، أمراض الكبد.