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Hepatitis C Virus Genotypes and Subtypes in Chronically Infected Patients in Erbil Province, Kurdistan Region, Iraq

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ABSTRACT:

The hepatitis C virus (HCV) infection is a significant global public health issue because it can lead to liver diseases such as cirrhosis and liver cancer. This study aimed to determine the distribution of HCV genotypes and subtypes in Erbil, Kurdistan Region, Iraq. A total of 120 patients with chronic hepatitis C infection were enrolled, and blood samples were collected and tested using both serological methods (Enzyme-Linked Immunosorbent Assays (ELISA) and molecular techniques (nested Polymerase Chain Reaction(nested-PCR). Results from serodiagnosis using the EIAgen HCV antibody test showed 85% positive, 8% negative, and 7% equivocal results. Of 102 EIAgen-positive samples, 70 were genotyped and subtyped. The frequency of positive cases was higher in females (89.4%) than in males (82.2%), particularly in the 30–39 and 40–49 years age groups, where they reach 95% and 92.9%, respectively. The most common genotype was genotype 1 (32.86%), followed by genotype 3 (25.72%) genotype 2 (20%). Among subtypes, 1b was the most frequent (24.29%), then followed by 2a (20.00%). Finally, our work underscores the necessity of viral genotyping and subtyping in creating tailored antiviral therapies. The observed profile, which includes a high incidence of genotype 1 and subtype 1b, has significant epidemiological implications for the local HCV infection landscape.

Keywords: Chronic HCV, HCV Genotypes, HCV Subtypes, nested-PCR, seroprevalence of HCV.

1 INTRODUCTION

Hepatitis C virus (HCV) is considered a substantial cellular and global challenge, which may lead to multiple disorders, including liver cirrhosis, hepatocellular cancer, and death [1]. Prevalence and transmission of HCV were discovered in 1988 as an RNA virus that accounted for most cases of parenterally transmitted "non-A, non-B" hepatitis [2]. The virus is in the family of Flaviviridae. The icosahedral capsid is surrounded by a lipid envelope, 55-65 nm in diameter. The genome is a positive-sense, single-stranded RNA of 9.6Kb length and encodes structural and non-structural proteins [3, 4]. Approximately 58 million people are infected with HCV, and more than 290,000 die of HCV-related conditions annually. Hepatitis C virus prevalence is significantly higher in developing countries at approximately 2% compared to less than 1% in developed countries[5]. Hepatitis C virus (HCV) is mainly transmitted by blood exchange in the past, or iatrogenic source due to the unsterile glass syringes, even in the low-income countries[6]. In the Kurdistan Region of Iraq, HCV prevalence varies depending on the population studied. Among couples undergoing premarital screening, HCV prevalence was found to be 0.07%[7]. Higher rates were observed in hemodialysis patients in the region, with 9.2% HCV positivity, varying from 5.2% in Erbil to 12.9% in Sulaymaniyah [8].

Accurate genotyping is important for the selection of optimal antiviral therapy. Some genotypes, such as genotype 1, which is the most prevalent worldwide accounting for 46.2% of all HCV cases and is predominantly found in North America, Europe, and East Asia [9]. Known to have resistance-associated mutations (RASs) that may affect the potency of direct-acting antivirals (DAAs) [10]. By way of subtyping RASs could be detected to avoid inefficient treatments. For example, pre-existing RASs are common in genotype 1 and can influence drug choice [11]. Polymerase Chain Reaction (PCR) is an indispensable tool in the diagnosis of HCV, as it allows for increased sensitivity and specificity in a variety of clinical settings. The on-treatment management of treatment protocols is based on the transcription-mediated

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amplification assay, as the "gold standard" of HCV RNA diagnostics provides a measure of viral RNA through real-time PCR [12].

Significance and implications of the study, HCV genotyping and subtyping were considered important in Kurdistan region, as well as the increasing number of refugees, to determine the distribution of HCV[13, 14].The reported prevalence of HCV in Iraq ranges from 0.2% to 0.78% in the general population [15]. Among refugee populations in the region, Syrian refugees showed an HCV positivity rate of approximately 0.04% (1 positive case out of 2,750 examined samples)[16]. DAAs are the cornerstone of treatment for HCV, and include compounds that act directly on specific viral nonstructural proteins, including the NS3/4A protease, NS5A, and the NS5B polymerase. These DAA have transformed the treatment of HCV, with cure rates nowadays being high in the order of 95% or more. The emergence of resistance-associated variants (RAVs) in the viral genome is a significant hurdle; these drugs and alternatives will have to be devised [17, 18]. This research investigates the epidemiology and molecular characterization of hepatitis C virus (HCV) in Erbil, Kurdistan Region of Iraq. The primary aim is to determine circulating HCV genotypes and subtypes in persons with chronic infection. Due to the variability of HCV genotypes in their response to antiviral therapy, precise genotyping is essential for effective treatment planning. Nonetheless, genotyping resources in Kurdistan are still scarce. Comprehending local genotype distribution will enhance patient care, facilitate evidence-based healthcare planning, and inform regional HCV management measures.

2 METHODOLOGY

2.1 BLOOD SAMPLE COLLECTION

The study was performed on 120 chronic hepatitis C patients (73 males and 47 females), their age ranged (15-50 years), attending the following hospitals: Rizgari Teaching Hospital, Thalassemia Center, and Hawler Teaching Hospital in Erbil City, Kurdistan region, Iraq, from the period between September 2024 and January 2025, inclusive. Collected 5 ml of blood samples from HCV- infected patients were by syringe from vien and putted into a gel tube contains clot activator. The blood samples were allowed to clot at room temperature and separated plasma and clotted part by centrifugation at 3500 rpm for 15 minutes then stored in the Erbil Cardiac Center until uses. The exclusion criteria included patients with co-infections such as HIV, HBV, or other liver diseases, autoimmune liver diseases such as autoimmune hepatitis.

2.2 SEROLOGICAL ASSAY

For the detection of anti-HCV antibodies, anti-HCV antibody Screening EIAgen HCV Ab (v.4) commercial Kit (ADALTIS, Italy, EIA -6911 HCV) was used and is based on enzyme-linked immunosorbent assays (ELISA). The procedure was done according to the manufacturer's instructions.

2.3 GENOMIC RNA EXTRACTION

The HCV-seropositive ELISA tests were subjected to RNA extraction using the viral DNA/RNA Kit (Genesand Biotech) to isolate viral RNA. The extracted RNA was then analyzed for the presence of HCV RNA by nested PCR (Genekam Biotechnology, Germany, Ref.MK757), as well as to determine genotype and subtype.

The RNA extraction process involved adding Proteinase K to a centrifuge tube, followed by the sample and Buffer GSL. After vertexing and incubation at room temperature for 10 minutes, the solution was transferred to a spin Column RS for centrifugation. The nucleic acids were washed with Buffer GSW1 and GSW2, and residual impurities were removed. Finally, the RNA was eluted with RNase-Free water and stored at -80°C for long-term use.

2.4 PCR AMPLIFICATION

Following the extraction of RNA, the PCR method was subsequently performed to amplify the target sequences for genotyping of HCV RNA. The PCR method included one step reverse transcriptase nested PCR using HCV Genotyping Kit (Genekam Biotechnology, Germany, Ref.MK757). The process was carried out in compliance with the guidelines provided by the manufacturer. Primers, buffers, PCR master mix, positive and negative controls, a molecular marker, and loading dye are all included in a ready-to-use PCR kit. The interpretation of the PCR result (10µl) were examined using a UV transilluminator after being electrophoresed in 2% agarose with ethidium bromide present. The band size was determined by comparing directly with a 50-bp DNA marker. The presence of a (234 bp,139bp,190bp, 337bp, 176bp,208bp,232bp, 99bp, 320bp, 336bp) band on an agarose gel indicates that the sample is positive for HCV RNA. The run validity is dependent on the DNA buffer parameters and the negative control sample (NCS). Based on the genotyping for tube C, the desired fragment sizes were 234 bp-genotype 1b, 139 bp or 190 bp-genotype 2a, 337 bp-genotype 2b, and 176 bp-genotype 3b, whereas those for the genotype done with tube H were 208 bp-genotype 1a, 232 bp-genotype 3a, 99 bp-genotype 4, 320 bp-genotype 5a, and 336 bp-genotype 6a, respectively.

PCR Procedure for HCV Genotyping

Step A: First Round PCR

The genotyping procedure began with Step A, where one tube each of reagent's A, B, C, H, G, Y, D1, D2, E, and F was thawed and placed at 4°C for better stability, with unused kits stored at -20°C. Microtubes were labeled with sample numbers, positive control (+Ve), and negative control (-Ve). Seven microliters of reagent A, ten microliters of reagent B, and one microliter of reagent Y were added to each microtube, taking care to avoid touching the walls. To improve efficiency, a master mix was prepared by combining these reagents in advance: for ten reactions, 70 µL of A, 100 µL of B, and 10 µL of Y were mixed to make 180 µL total volume, from which 18 µL was distributed into each microtube. Two microliters of RNA template were then added to each labeled microtube using filter-equipped pipette tips, except for the control tubes, with a new tip used for each sample to prevent cross-contamination. The positive control (reagent D1) and negative control (tube D2) were added to their respective microtubes using new pipette tips, with care taken not to touch the walls. All tubes were centrifuged at 8000 rpm for 5 seconds to ensure proper mixing, though this step was optional but recommended for better results.

The thermocycler program was initiated after verifying that all components were added correctly and that the volume in each microtube was nearly identical. The PCR machine was programmed with the following cycles: initial incubation at 42°C for 3000 seconds, followed by 48°C for 600 seconds and 70°C for 600 seconds; then 20 cycles of 94°C for 60 seconds, 45°C for 60 seconds, and 72°C for 60 seconds; followed by another 20 cycles of 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds. Before starting the program, it was ensured that all tubes were properly sealed and in full contact with the metal block of the thermocycler to ensure optimal thermal transfer without air gaps. After completing this step, the microtubes were carefully removed from the thermocycler. Since no band was observed in the first round, electrophoresis was not performed at this stage, and the procedure proceeded directly to Step B.

Step B: Second Round PCR (Nested PCR)

Step B was initiated by marking new microtubes with sample numbers, positive control, and negative control designations. The tubes were divided into two groups labeled C and H, except for the negative control. Ten microliters of reagent B were added to each microtube, avoiding contact with the walls. Eight microliters from tube C were added to microtubes marked with C, while eight microliters from tube H were added to microtubes marked with H. To optimize time and resources, master mixes were prepared in advance: for ten reactions in the C group, 80 µL of tube C and 100 µL of B were combined to make 180 µL total, from which 18 µL was distributed into each microtube; the same approach was used for the H group with tube H reagent. The PCR product from Step A was diluted with tube G to ensure clear and distinct bands in the subsequent analysis. One microliter of the PCR product was diluted with 9 µL of tube G according to the labeling, and then two microliters of this diluted product were added to each tube according to its label.

The thermocycler program for Step B was run after confirming that all components were added correctly and volume levels were consistent across microtubes. The PCR machine was programmed for 30 cycles consisting of 94°C for 60 seconds, 62°C for 45 seconds, and 72°C for 60 seconds. Before starting the program, proper sealing of all tubes was verified, and it was confirmed that the microtubes were in full contact with the metal block without any air gaps or loose contact. After the PCR program was completed, the microtubes were removed and centrifuged briefly before proceeding to Step C.

Step C: Agarose Gel Electrophoresis

Following completion of the PCR step, agarose electrophoresis was prepared using 1.5% agarose in 1× TAE buffer. After the gel solidified, 1× TAE buffer was added to the gel chamber. Two microliters of dye from tube F were added to each sample (this step was omitted if the B tubes already contained a colored mixture). Ten microliters from each dye-added sample were pipetted into separate slots on the gel using a new pipette tip for each lane to prevent cross-contamination. Ten microliters of the marker (50bp) were added to the first, in the electrophoresis setup. A lane plan was created on paper according to the probes to facilitate later identification and interpretation of the results. The electrophoresis was then run to visualize the PCR products and determine the HCV genotypes based on the band patterns observed.

2.5 STATISTICAL ANALYSIS

Data were analyzed using the GraphPad Prism version 9.0. Chi-square test of association was used to compare the proportions. A p. value of ≤ 0.05 was considered statistically significant.

2.6 ETHICAL STATEMENT

Ethical considerations of the study were approved by the Research Ethics Committee of the College of Health Sciences, Hawler Medical University, Erbil No. 10A1 on 9/9/2024.

3 RESULT

The sociodemographic profile of the total 120 HCV suspected patients was as follows: the majority of respondents were in the 15–29 years age category 75 (62.50%), and a preponderance of males, 73(60.83%), as compared with females, 39.16%. Single status was reported by the majority of respondents 76 (63.33%), and in terms of living places, more than half were living in the city 62 (51.67%) but not in the surrounding area (48.33%). Alcohol use was an issue in only 1.67% with the majority not using it (98.33%). Regarding education, 38.33% were highly educated, and 85% were of intermediate socioeconomic status. With respect to occupation, the unemployed constituted 27.5%, the employed made up 32.5% while 36.67% of the population are students, as shown in Table 1.

Table 1. Descriptive statistics for demographic characteristics

Demographic characters		No.	%
Age (years)	15–29	75	62.50%
	30–39	20	16.66%
	40–49	14	11.66%
	50–59	7	5.83%
	≥ 60	4	3.33%
Sex	Male	73	60.833%
	Female	47	39.166%
Marital Status	Single	76	63.33%
	Married	43	35.83%
	Divorced	1	0.83%
Residency	Center	62	51.67%
	Surrounding	58	48.33 %
Alcohol Drinking	Yes	2	1.67%
	No	118	98.33%
Education	illiterate	12	10%
	primary	17	14.167%
	Secondary	45	37.5%
	Higher education	46	38.33%
Occupation	Unemployed	33	27.5 %
	Employed	39	32.5 %
	Retired	4	3.33%
	Student	44	36.67%
Socioeconomic State	Low	11	9.167%
	Intermediate	102	85%
	High	7	5.83%

The HCV seropositive test results revealed that 85% (102 individuals) tested positive for HCV antibody, while 8% (10 individuals) tested negative, and 7% (8 individuals) received equivocal results by using ELISA. This indicates a high prevalence of positive HCV results among the participants, as shown in Figure 1.

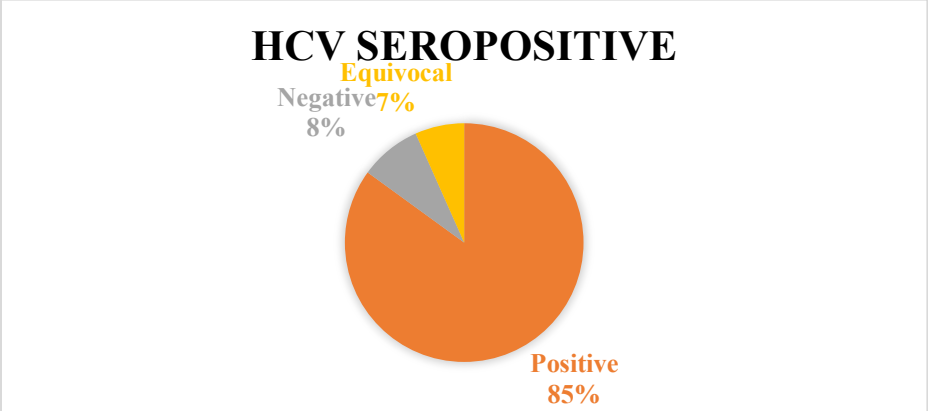

FIGURE 1. The Seroprevalence rate of HCV among study samples.

The distribution of HCV test results according to age group indicated that positive cases are widely distributed across age groups, including 95% and 92.9% in the 30–39 and 40–49 age groups, respectively, compared to Equivocal results that were observed exclusively in the youngest age group (15-29 years) with 8 cases (10.70%) and negative cases were identified in 7 participants (9.3%) in the 15-29 age group, 1 participant (5.00%) in the 30-39 age group, 1 participant (7.1%) in the 40-49 age group, with no significant correlation between age and the results of the EIAgen HCV antibody test, p=0.4446. In general, the test is highly positive across age groups. These findings are presented in Table 2.

Table 2. The distribution of HCV seropositive according to age groups.

Age Groups (Years)	Positive	Equivocal	Negative	Total	*p. value*
15–29	60 (80.00%)	8 (10.70%)	7 (9.3%)	75	0.4446
30–39	19 (95.00%)	0 (00.00%)	1 (5.00%)	20	
40–49	13 (92.90%)	0 (0.00%)	1 (7.1%)	14	
50–59	7 (100.0%)	0 (0.00%)	0 (0.00%)	7	
≥60	3 (75.00%)	0 (0.00%)	1 (25.00%)	4	

The sex-specific analysis of HCV test findings indicated some interesting patterns, but no significant difference was found between males and females with *p. value* of 0.5490. Positive results were 82.2% in men, 8.2% equivocal, and 9.6% negative; while in women, positive results were slightly higher (89.4%), with 4.3% equivocal and 6.4% negative. The prevalence of HCV positivity among patients according to the sex factor is represented in Figure 2.

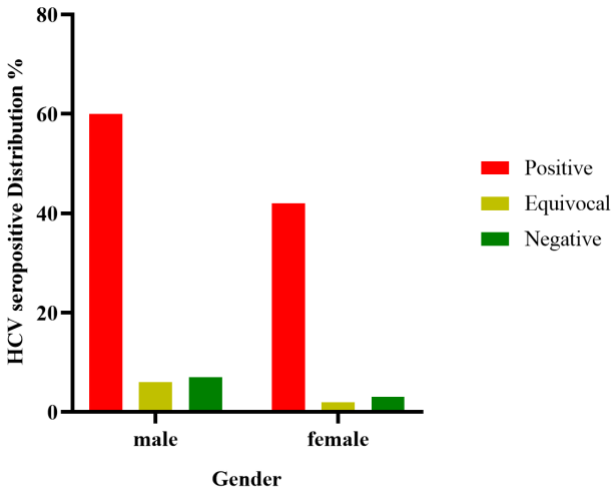

FIGURE 2. Distribution of HCV positivity across sex.

The analysis of HCV seropositive results reveals that a higher proportion of individuals residing in the center area tested positive for HCV compared to those in the Surrounding area. Specifically, the prevalence of positive results was notably higher in the Center, with 90.32% testing positive, compared to the surrounding area, which had a rate of 79.40%. In contrast, the rate of equivocal results was lower in the center (3.23%) compared to the surrounding area (10.30%), and similarly, the rate of negative results was slightly higher in the center. A statistical comparison between the two groups *p. value* 0.1968, indicates that there is no significant difference in HCV test outcomes between the two populations, as shown in Table 3.

Table 3. Distribution of HCV seropositive Results according to Resident.

Resident	Positive	Equivocal	Negative	Total	*p. value*
Surrounding	46 (79.40%)	6 (10.3%)	6 (10.30%)	58	0.1968
Center	56 (90.32%)	2 (3.23%)	4 (6.45%)	62	

The anti-HCV positive rate, equivocal rate, and negative rate were 76.2%, 9.5%, and 14.3% in current smokers, respectively. Among the never-smoked smokers, the proportions of positive, equivocal, and negative results were 85.9%, 6.5%, and 7.6%, respectively. Among never smokers, all (100%) were positive; no equivocal or negative results were noted. The statistical association between smoking status and HCV outcomes was non-significant, with a *p. value* of 0.6187 as shown in (Table 4).

Table 4. Distribution of HCV seropositive Results according to Smoking.

Smoking	Positive	Equivocal	Negative	Total	*p. value*
Currently	16 (76.2%)	2 (9.50%)	3 (14.30%)	21	0.6187
Never	79 (85.90%)	6 (6.50%)	7 (7.60%)	92	
Past	7 (100.0%)	0 (0.00%)	0 (0.00%)	7	

Distribution of HCV genotypes among HCV-seropositive cases showed that genotype 1 is the predominant genotype, the frequency rate was 32.86% of the total samples. Genotype 3 represented 25.72% followed by genotype 2 (20%). More rarely are HCV genotypes 6, 5, and 4, 5.71% to 8.57%, respectively. The total contribution of mixed genotypes 1 and 4 is the lowest of whole of the samples (1.43%), as **illustrated** in Table 5 (Figure 3).

Table 5. Frequency of the circulating genotypes.

Genotypes	Frequency	Percentage
1	23	32.856%
2	14	20.00%
3	18	25.72%
4	6	8.57%
5	4	5.71%
6	4	5.71%
1 and 4	1	1.43%
Totals	**70**	**100%**

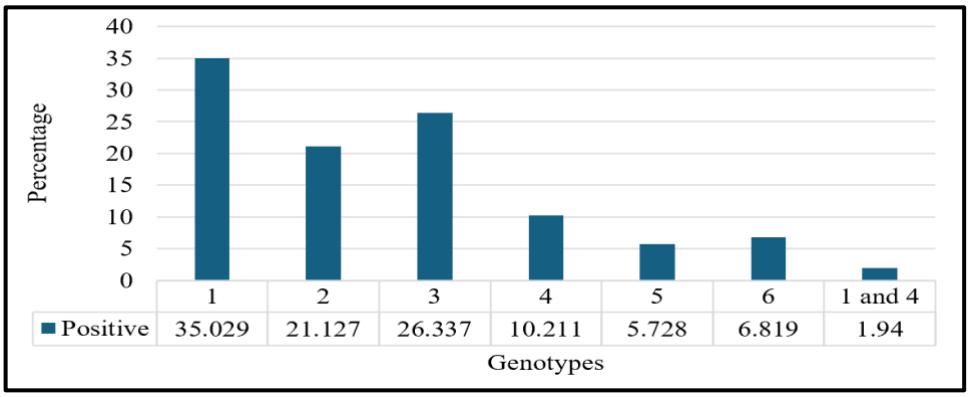

FIGURE 3. Distribution of HCV seropositive antibody results by Genotypes.

The statistical analysis indicates the prevalence of HCV diversity in the study group was, subtype 1b 24.29% the most frequent among the subtypes, of total HCV-positive cases, then followed by subtype 2a with 20.00%. Subtypes 3a and 1a were prevalent at 17.14% and 8.57% respectively, while each serotype 3b, 4, and 6a accounted for 8.57%, while subtype 5a was in 5.71%of the among HCV-positive cases. The pooled prevalence of subtypes 1b and 4 was 1.44%. There were 70 cases in total, and all subtypes spectrum of the patients was present in the sample, indicating an extensive distribution of HCV subtypes in the patients, as revealed in Table 6 (Figure 4).

Table 6: Frequency of the circulating Subtypes

Subtypes	Frequency	Percentage
1a	6	8.57%
1b	17	24.29%
2a	14	20.00%
3a	12	17.14%
3b	6	8.57%
4	6	8.57%
1b and 4	1	1.44%
5a	4	5.71%
6a	4	5.71%
Totals	**70**	**100%**

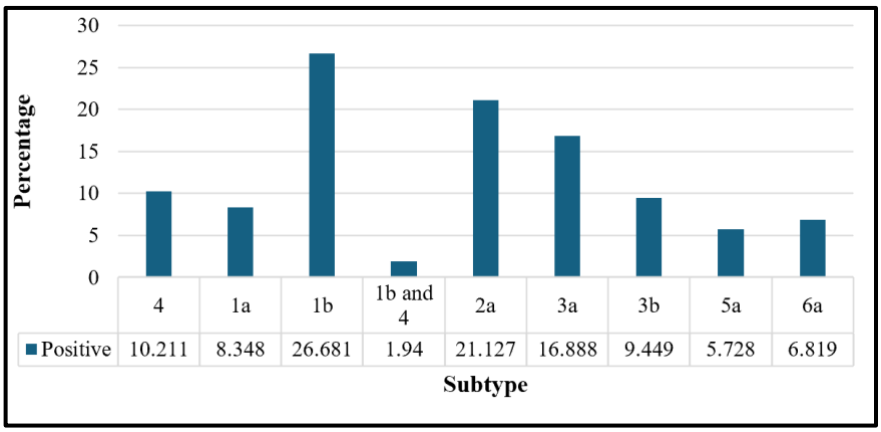

FIGURE 4. Distribution of HCV seropositive antibody results according to subtypes

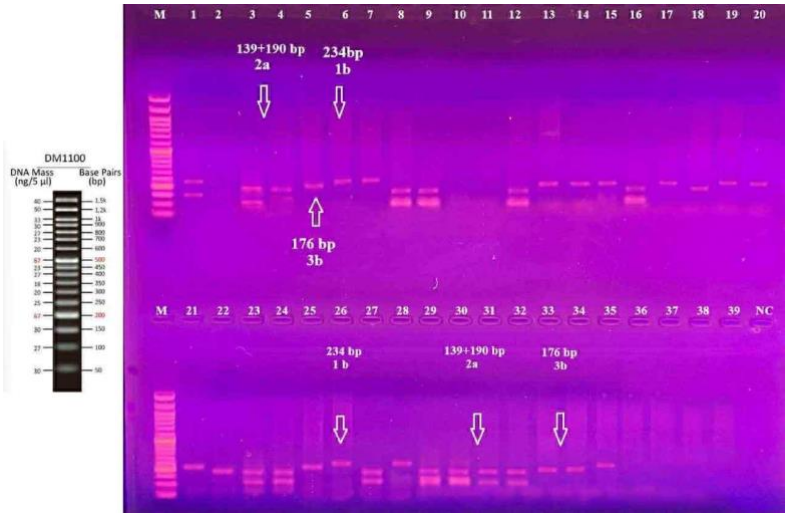

FIGURE 5. Electrophoresis gel profiles of second-round nested PCR types of HCV genotypes. Lane (M) Molecular weight DNA marker (50-bp ladder); lane 1, positive control; lane (3,4,8,9,12,16,22,23,24,27,29,30,31,32) genotype 2a (139+ 190 bp, the test was performed with tube C); lane (5,13, 18, 25, 33, 34,) genotype 3b (176bp, the test was performed with tube C); lane(6, 7, 14, 15,17, 19, 20, 21, 26, 28,35) genotype 1b (234 bp, the test was performed with tube C); and lane (NC), Negative control.

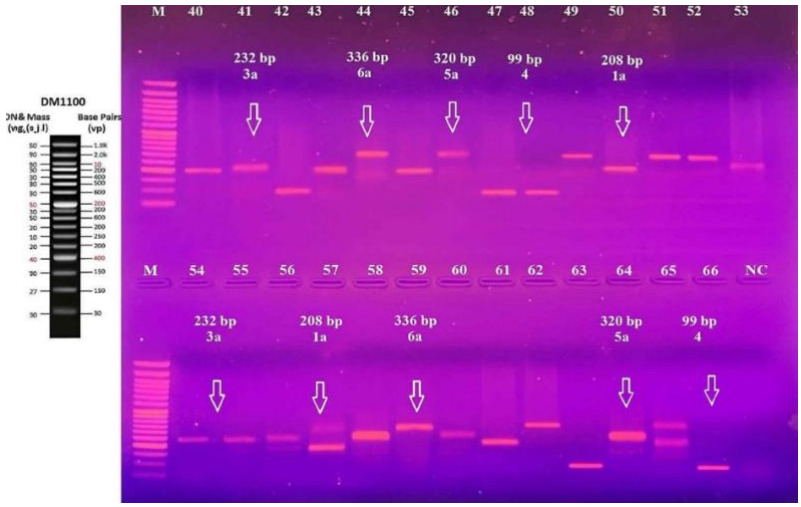

FIGURE 6. Electrophoresis of different HCV genotype PCR products (pattern of reaction in the second-round nested PCR). Lane (M), molecular weight DNA marker (50-bp ladder); lane 40, positive control; lane (41,43,45,51,52,54,55,56) genotype 3a (232bp, the test conducted with tube H); lane (44 ,59,62,65) genotype 6a (336 bp, the test conducted with tube H); lane (46,49,60,64) genotype 5a (320 bp, the test conducted with tube H); lane (42,47,48,63,66) , genotype 4 (99 bp, the test conducted with tube H); lane (50,53,57, 61), genotype 1a (208 bp, the test conducted with tube H) and lane (NC), blank control.

4 DISCUSSION

The present study investigated HCV seroprevalence among 120 participants with chronically infected patient. The HCV seropositive antibody test results revealed a notably high prevalence of HCV infection in this population. Specifically, 85% (102 individuals) tested positive for hepatitis C antibodies using ELISA, while 8% (10 individuals) tested negative, and 7% (8 individuals) received equivocal results. the results of this study cannot be generalized to the general population either from the Kurdistan Region or Iraq in which HCV infection is low endemic. For example, a community-based cohort study conducted in Erbil in 2020 showed that the HCV positive ratio was 6.93% [19]. Another community-based cohort study in Duhok among prisoner also indicated low prevalence at 0.09%[20]. Therefore, the high prevalence in our particular study should be compared with studies from other high-risk groups with similar exposure risks. For instance, our group has the similarity of 85% seroprevalence index to the 84.5% prevalence rate among hemodialysis patients in

Basra[21]. This continuing high prevalence clustered in the risk group pattern is repeated in other areas of Iraq: 9.2% in Kurdistan [8]. A meta-analysis and systematic review of the prevalence of HCV in Middle East hemodialysis patients found an overall prevalence of 25.3% (varying between 9% in Lebanon and 54% in Syria) and were studies from Iraq, where a prevalence of 20% to be among hemodialysis patients [22]. Most of Iraq's neighbors exhibit a low to moderate HCV prevalence in their general populations. For example, Iran is a low-prevalence area, where the prevalence is usually below 1% in the general population [23]. Also, in a recent 2024 cross-sectional study among 100,000 plus Saudi Arabian nationals, reported a very low general prevalence of 0.05% in the study population [24].The high prevalence among younger adults aged 15–29 years (62.50%) is in line with other Middle Eastern studies, where HCV transmission has mainly affected younger populations through healthcare-related inoculation during medical treatments in previous decades [25, 26]. in contrast, this age distribution differs from the picture of HCV infection in Western countries, where older adults are most infected, mainly due to injection drug use or blood transfusions before regular screening was introduced [27]. The male preponderance in our study, was found amongst 60.83% of cases as opposed to 39.16% for females which is a characteristic feature of HCV epidemiology in the Middle East. It has also been reported in studies in Iran [28] and Turkey [29]. The predominantly urban residential distribution (51.67% living in city centers) is consistent with the process of urbanization in the Kurdistan Region and could be indicative of the fact that health-seeking behavior might be more prevalent in urban areas where access to healthcare is presumed to be better, and hence may result in higher degree of awareness[29]. The positivity rate distribution from this study indicates the 30-49 years age groups had the most positive results, with more than 90% positivity observed in 30-39 and 40-49 years. This finding agrees with the international literature that reported HCV as a particular matter affecting individuals of middle age groups with past infections from years or even decades previously [30]. This is different from the findings of another study in Wasit Governorate, Iraq. In the Wasit study, which conducted a different sample population, the majority of cases of HCV infection were reported between the ages 21-30 years [31]. We found no relationship between sex and HCV infection, although a higher proportion of positive HCV tests was observed in females. This is in agreement with a local investigation which also reported no sex difference in HCV distribution [13]. The absence of male-biased prevalence, which is frequent worldwide as a consequence of higher rates of intravenous drug use, also imply the predominance of a different pattern, i.e., iatrogenic transmission by medical procedures [6, 32]. The analysis of HCV distribution compared center and surrounding areas. Positive rates were slightly higher in the center (90.32% vs 79.40%), but without statistical significance (p=0.1968), and equivocal results were lower in the center (3.23%) than surrounding regions (10.30%). These results are also consistent with the same regional trends in prevalence between urban and rural populations as reported in the studies of the Middle east where HCV prevalence of rural and urban areas was determined [33, 34]. These results are contrary to studies from other locations in which rural areas often had higher HCV rates. US data showed that the rates of rural acute HCV (1.8 per 100,000) are higher than urban (1.5 per 100,000), and maternal HCV is reported to be higher in rural counties [35]. These findings indicated the need for focused urban testing while maintaining universal access in order to meet WHO goals of elimination by 2030. The findings support geographically-informed HCV programs [36]. The analysis revealed no statistically significant relationship between smoking status and HCV outcomes (p=0.6187). Never smokers showed the highest positive rate (85.9%), followed by current smokers (76.2%), while all past smokers tested positive (100%, n=7). These results complement other studies found no association between smoking and HCV acquisition, such the absence of an association between HCV infection and smoking both among HCV positive and negative among NHANES (National Health and Nutrition Examination Survey) participants [37]. Nevertheless, inconsistent results exist among populations. Results from regional studies in surrounding countries are inconsistent. Turkish research conducted on injecting drug users who smoke reported smoking behaviors, but emphasis is being predominantly put on transmission pathways associated with drug use and it does not consider smoking as one of the HCV-specific risk factors [38]. Studies examining Syrian refugees in Lebanon identify multiple HCV risk factors but do not establish smoking as a primary transmission mechanism [39]. Epidemiological studies in Iran have shown disparate regional patterns in HCV prevalence but little evidence of an association with smoking [40]. Genotype distribution in the eligible patients, 32.86% were GT-1 followed by GT-3 (25.72%) and GT-2 (20.00%). The predominant HCV genotype detected was GT-1 among chronic HCV infected patients in Erbil province, Kurdistan Region of Iraq. This distribution was different from the previous reports in Iraq and provided valuable information about regional epidemiology of HCV infection in Kurdistan Region. The dominance of genotype 1 (32.86%) found in our study is different from what had been previously reported from the central part of Iraq in different populations. The high frequency of genotype 1 (32.86%) detected in our study ceases to cause compared to recent data from other Iraqi regions. According to the comprehensive analysis genotype 4 was identified as the dominant genotype in Iraq, consistent with patterns observed in other Arab countries of the Middle East and North Africa region [41]. More recent epidemiological studies from Iraq have confirmed this pattern, with genotype 4 being the most prevalent across central and southern regions [42]. Nevertheless, in the Northern Iraq studies, more genotypic heterogeneity was evident, some populations demonstrating the predominance of genotype 3, especially amongst multiply transfused patients[43]. This is in contrast to our findings where genotype 1 being the most prevalent, reflecting marked regional differences that do exist within Iraq specifically between Kurdistan Region and other regions of Iraq. Our findings demonstrate significant alignment with the genotype distribution patterns observed in neighboring non-Arab countries. According to a systematic review and meta-analysis of HCV genotypes in Eastern Mediterranean Regional Office (EMRO) countries, genotypes 1 and 3 were predominant in Iran and Pakistan,

while genotype 4 and 1 were the most common genotypes in the Middle East Arab countries and North African Arab countries [44]. Furthermore, studies in Iran have reported different regional distributions of HCV genotypes, which type 1a and 3a dominant, although their relative prevalence changes across the country. For example, data from Mashhad in northeast Iran has suggested that genotype 3a is the prevalent genotype in this part of the country that is inconsistent with some earlier studies in different parts of Iran [45], Meanwhile, according to other data from central Iran (Arak city), and southern/southwestern Iran different patterns were reported, where genotype 1 is more frequently observed than other genotypes in southern parts of the country particularly among high risk groups [46]. This geographic heterogeneity, the variation of the distribution of genotypes within Iran, makes up the complex epidemiology of non-Arab countries in the region. The high proportion of genotype 1 in our Kurdistan Region study (32.86%) confirms this regional trend of non-Arab countries albeit to a lesser extent when compared with that reported in Iran [47]. Results from an extensive systematic review in Saudi Arabia revealed that HCV genotype 4 is the predominant genotype, followed by genotype 1[48]. In Egypt, genotype 4 is predominant and represents, based on recent reports, about 94.1% of HCV cases in this Arab country [49]. Furthermore, studies have also concluded that genotype 4 followed by genotype 1 is the prevalent genotype in the Middle East Arab countries and non-Arab countries [50]. The high prevalence of genotype 3 (25.72%) in our population is remarkable and consistent with worldwide distribution in which genotype 3 is the second most common genotype (about 30.1% of all cases) especially in South Asia [9]. In a previous systematic review on the effects of conflict on infectious diseases, conflict settings were shown to be linked with the introduction and spread of a number of infections, including hepatitis epidemiology among displaced populations [51]. Furthermore, Syrian refugee studies in Lebanon revealed how massive force displacement impacts infectious disease patterns and healthcare resources, including viral hepatitis transmission dynamics [52]. The genotypic profile of our chronic HCV population is important to tailor HCV treatment in Erbil province. Considering that genotype 1 (32.86%) was the most prevalent genotype, followed by significant number of patients with genotype 3 (25.72%) and genotype 2 (20%), an adoption of pangenotypic DAA regimens e.g sofosbuvir/velpatasvir or glecaprevir/pibrentasvir, which has been shown to have greater than 95% sustained virologic response (SVR) rates across major genotypes, is what the majority of these patients stand to benefit from [53]. The high percentage of the subtype 1b patients (24.29%) is of special advantage for therapeutic responses, since this subtype usually responds extremely well to all currently available DAA combinations with few resistance issues [54]. The large number of genotype 3 patients, especially subtype 3a (17.14%), however, cannot be neglected, since prolonged treatment (16 weeks) or combination with ribavirin if the patient is cirrhotic as well, must be opted for, in order to maximize the SVR rates [55]. The genotype 2 population (20%) are a readily curable population with very high response rates to sofosbuvir containing regimens [56]. For the smaller percentages of genotype 4, 5 and 6 patients, the current pangenotypic therapies have maintained high response rates; patients with genotype 4 may also respond favorably with elbasvir/grazoprevir combinations [57]. The genotypic heterogeneity found among participants in the present study, emphasized the necessity of adopting broad screening strategies and treatment protocols, which would enable the spectrum of HCV types in the Kurdish area to be treated realistically, by purchasing several DAAs combinations to minimize the risk of inadequate therapeutic benefit in any case subgroup [58].

CONCLUSION

This study provides the first comprehensive analysis of HCV genotypes and subtypes distribution in Erbil Province, Kurdistan Region, Iraq. Of the 70 genotyped chronic HCV patients, genotype 1 (32.86%) was the most common, followed by genotypes 3 (25.72%) and 2 (20.00%), with subtype 1b being the most predominant (24.29%). This distribution is characterized by a lack of the normal predominance of genotype 4, and is distinct from the rest of Iraq, where genotype 4 is typically most common, highlighting unique regional transmission epidemiology.

The predominance of genotype 1, including subtype 1b, has significant clinical implications for the choice of DAA therapy and the monitoring of resistance in the region. The high representation of genotype 3 and the diversity of genotypes from 1 to 6 highlighted the importance of full-genome genotype determination before undertaking antiviral therapy. Such results would warrant regional HCV elimination strategies differing from national protocols in such settings, as well as support site-specific treatment guidelines implementation. This study provides critical baseline information not only for evidence-based practice clinical approach but also for health policy monitoring and planning in the Kurdistan Region, and thus supports the global HCV elimination target by 2030.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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