

Molecular And Phylogenetic Characterization of HCV Core Gene Among Hemodialysis Patients in Iraq

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Introduction

Hepatitis C virus (HCV) is a serious public health concern worldwide due to a high incidence of chronic infection and long-term liver-related sequelae. Although curative direct-acting antiviral medication is available, recent estimates suggest that tens of millions of people continue to live with chronic HCV infection globally. This is clinically essential as persistent HCV infection leads to progressive liver injury and is a leading cause of chronic hepatitis, cirrhosis, end-stage liver disease and hepatocellular carcinoma[1,2,3]. At the molecular scale, HCV is an enveloped, positive-sense single-stranded RNA virus of the family Flaviviridae with a genome of roughly 9.4–9.6 kb with a single large open reading frame encoding a viral polyprotein[4]. The clinical and epidemiological relevance of HCV is further compounded in patients on hemodialysis, a population that continues to be overrepresented in blood-borne infections[5,6]. Hemodialysis (HD) is the main renal replacement therapy to increase the survival and quality of life of people with CKD. However, the HD approach is associated with impaired cellular immune response in the patients and hence predisposes them to a variety of blood-borne viral infections. The risk of transmission of hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) in the HD population is complex. The important risk factors are percutaneous exposure, past blood transfusions, advanced age, longer duration of dialysis therapy and probable contamination of HD machines and associated apparatus[7]. HCV is considered both a cause and a consequence of chronic kidney disease (CKD). Besides HCV-linked glomerulonephritis, the virus causes CKD through multiple mechanisms, leading to incident CKD and progression to end-stage renal disease requiring hemodialysis or transplantation. The prevalence of HCV among patients on hemodialysis is substantially greater than in the general population with rates of 4-9% in high-income nations and higher and more variable rates in the Middle East, Africa, Asia and Eastern Europe. This additional burden is further worsened by an even greater diagnostic gap, with a considerable number of infected individuals remaining untreated outside of dialysis settings and uninformed of their infection[8]. Molecular data has also supported the nosocomial transmission concept in dialysis facilities, where phylogenetic grouping and even identical virus genomes have been detected among patients in some units. The regional data from Iraq showed a significant burden in dialysis settings as well. The seroprevalence of HCV was 36% in patients undergoing hemodialysis in Al-Muthanna and more in patients with extended duration of dialysis and previous history of transfusion [9,10,6]. These findings make HCV infection in dialysis patients not only a clinical problem but also an essential question in molecular epidemiology. The issue of molecular epidemiology is intimately linked to the high genetic heterogeneity of HCV. HCV shows wide sequence diversity[11,12]. due to the error-prone nature of RNA replication and the long-term evolutionary divergence of viral lineages. Globally, genotype 1 is most frequent, followed by 3 and 4; however, the distribution of genotypes varies widely by region and transmission network [13,2,1]. Genotype 4 is the most common regional genotype in the Middle East. Meta-analytic data reveal that genotype 4 predominance is also seen in Iraq with an estimated pooled prevalence of ~60% [14]. This variation is clinically relevant, as genotype and subtype still matter for molecular surveillance and can impact treatment selection, subtype assignment, and interpretation of naturally occurring resistance-associated variation even in the era of pangenotypic direct-acting antivirals[11,15]. The core gene is of particular interest for molecular and evolutionary studies inside the HCV genome because it encodes the nucleocapsid protein and lies in a structurally significant area adjacent to the 5' end of the viral open reading frame[12,16]. initial sequencing investigations established the core region as a conserved structural gene with enough inter-genotypic variability to identify major

viral lineages. Later studies have shown that phylogenetic analysis using core gene sequences is in agreement with analyses using other genomic regions and can generate trees that closely reflect broader genomic relationships and is therefore useful for genotyping, subtype classification and evolutionary inference [17,16,18]. More recent research using sequences has continued to exploit the HCV core domain for amplification, sequencing, and phylogenetic clustering of geographically diverse populations, emphasizing its practical value in molecular epidemiology[3]. Thus the methodological separation between ordinary diagnosis and molecular characterization is vital.

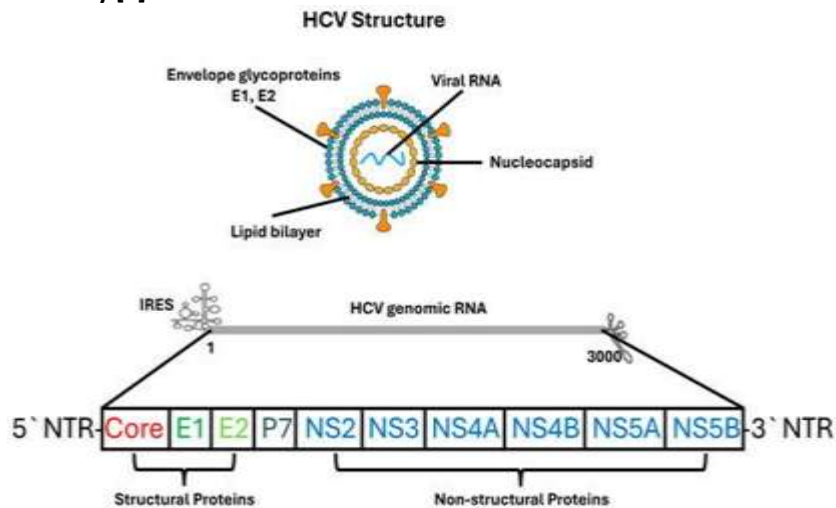
Serological assays, like anti-HCV testing, are commonly utilized for screening and prevalence investigations, including many dialysis-based surveys, although they do not identify active viremia, subtype identity, or evolutionary relatedness across strains [9,10]. Conversely, RNA-based molecular approaches, such as RT-PCR followed by sequencing, provide for the direct characterization of circulating viral genomes and support for phylogenetic analysis for lineage assignment and outbreak investigation[17,3,18]. Sequencing also increases the resolution of subtype determination as compared to some conventional genotyping tests which may be inadequate for reliable subtype characterization in several HCV genotypes [11]. This enhanced resolution is particularly relevant to dialysis populations as it allows distinguishing simple prevalence from potential transmission connections within units. Although clinically and epidemiologically important, there is a lack of genetic sequencing data on the HCV core gene among Iraqi hemodialysis patients. The available Iraqi data identified in the present literature set is focused mainly on seroprevalence and risk-factor assessment in dialysis populations rather than nucleotide sequencing or phylogenetic study of the core gene[10]. Genotype-distribution studies at the higher Middle Eastern level support the regional predominance of genotype 4 and include Iraqi data but do not clarify the precise evolutionary links of HCV strains circulating among Iraqi hemodialysis patients [14]. This gap matters because under-sampled areas can include genetically varied and clinically relevant lineages that are poorly described by current surveillance frameworks. Therefore, the present study aims to molecularly and evolutionarily characterize HCV core gene sequences obtained from hemodialysis patients in Iraq, specifically from Fallujah and Baghdad, to define circulating strains, clarify their phylogenetic relationships and strengthen the local molecular epidemiology evidence base[18].

Literature Review

Even with the breakthroughs in antiviral medication, hepatitis C virus (HCV) remains a leading cause of chronic liver disease globally. Estimates of the worldwide burden vary by technique but rigorous analyses continue to show a substantial burden from around 160 million people chronically infected to over 185 million people with anti-HCV antibodies in previous global assessments. This burden is clinically significant, as chronic HCV infection can lead to cirrhosis, hepatocellular cancer, liver failure, and liver-related death. The Middle East and North Africa remain among the most afflicted regions [20,21,22]. Genotype 1 is the most common genotype worldwide, followed by genotypes 3 and 4, as reported in global genotyping surveys, but exact amounts vary significantly between datasets [21,13,23]. The distribution of HCV genotypes is not uniform among populations but is substantially influenced by location and transmission history [21,24,25]. Such regional variation is of practical importance because genetic variety influences epidemiological monitoring, healthcare planning and treatment interpretation [21,23,14]. Pooled data from the Middle East show a characteristic genotype structure, with genotype 4 predominating in many Arab nations including Iraq, whereas genotype 1 is more prevalent in some non-Arab countries such as Iran and Turkey. Meta-analytical estimates indicated genotype 4 as the dominant genotype in the Middle East generally, representing 74.7% of infections, with Iraq being one of the nations where this genotype is dominant[26,14,27]. Further regional analysis of the sequence data revealed the commonality of subtype 1a in Iraq and the continued relevance of subtype 4a as a major circulating subtype across the entire Middle East and North Africa[28]. Patients on hemodialysis continue to be a high-risk population for HCV. Repeated exposure to blood and prolonged contact with the health care system provide continued opportunities for transmission. A worldwide meta-analysis of 407 studies with 1,302,167 patients estimated the incidence of HCV in patients on hemodialysis at approximately 21% and identified an association of infection with increased mortality. DOPPS data also found a prevalence of around 10% among prevalent hemodialysis patients, with some Middle Eastern settings approaching 20%, and showed that seroconversion clustered in units with higher baseline facility prevalence[29,30,31]. Molecular studies have demonstrated that sequence-based analysis increases comprehension beyond serology alone by refining genotype assignment and clarifying transmission pathways[25,30]. The core gene has been particularly effective for HCV characterization since it comprises both conserved and genotype-specific motifs and generates phylogenetic relationships similar to those derived from more variable genomic regions. Overall, the literature shows that HCV still remains a major public health

problem both globally and regionally; populations on hemodialysis remain at disproportionate risk of transmission; and molecular characterization is especially important in regions such as Iraq, where genotype 4 is predominant and local sequencing data are still limited [31,32].

FIG. 1 shows the structure of HCV (a lipid envelope with E1/E2 glycoproteins surrounding a core protein/RNA nucleocapsid) and its genome organization where a single ORF flanked by 5'/3' NTRs encodes structural proteins (Core, E1, E2, p7) for virion formation/entry and non-structural proteins (NS2–NS5B) for replication and assembly [4].



Significance of the Study

This study fills a well-defined gap in the genetic epidemiology of hepatitis C virus (HCV) among Iraqi hemodialysis patients. Iraqi hemodialysis populations have been repeatedly shown to harbor a substantial burden of HCV with local variability, but most evidence is still limited to seroprevalence surveys and conventional genotyping assays. Direct core-gene sequencing and phylogenetic characterization of circulating strains in this population have not been previously undertaken. The present study produced sequence-based data from hemodialysis patients in Fallujah and Baghdad and provides the first locally obtained molecular data on the genetic diversity and evolutionary linkages of HCV core gene isolates circulating in Iraqi dialysis environments.

The study is of clinical and public health importance outside the region. Core-gene sequencing provides a resolution not obtained by serology or routine PCR alone and allows for improved genotype and subtype assignment, identification of unusual or discordant strains and initial clues to possible transmission-relatedness among patients in the same dialysis units. Such information is directly useful to infection-control practice as it can assist in identifying sporadic infection from likely nosocomial dissemination. The findings also provide baseline sequence data that may be submitted to GenBank to aid future national and regional molecular surveillance initiatives, comparative genotyping research, and evidence-based infection-prevention strategies for hemodialysis facilities in Iraq.

Methodology

2.1 Subjects of the study

This study was a cross-sectional study done in the period between September 2025 and March 2026 in two main centers of hemodialysis in Iraq, which were the Iraqi Center for Dialysis at Baghdad Teaching Hospital and Fallujah Teaching Hospital.

2.2. Study Population

The study sample comprised 100 adult end stage renal disease (ESRD) patients on regular hemodialysis for at least six months. Eligible participants were >18 years of age and had been on hemodialysis for at least 6 months previous to participation. Those with a previous diagnosis or a history of treatment for hepatitis C virus (HCV) infection, and

patients co-infected with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), were excluded from the study.

2.3. Collection and Processing of Samples

Ten specimens were selected for further molecular analysis from the 24 positive results in the first RT-PCR screening. Blood samples were obtained before hemodialysis and centrifuged for 17 minutes at 3000-4000 rpm to get plasma. The plasma obtained after the separation was used to isolate viral RNA as indicated in Section 2.5.

2.4. Ethical Statement

The protocol of this research project was authorized by the research ethics committee of the College of Applied Sciences, University of Fallujah (Reference No.: AS-EC/0016, dated 13 January 2026). All subjects provided written informed consent before participation.

2.5. Extraction of viral RNA

Viral RNA was isolated from all specimens using the Geneaid Viral Nucleic Acid Extraction Kit II (Cat. No. VR100, Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. In brief, samples were lysed in VB Lysis Buffer, bound to a glass-fiber spin column in the presence of ethanol-supplemented AD Buffer, washed successively with W1 and Wash Buffers, and eluted in 50 µL of RNase-free water. The RNA was purified and kept at -20°C for subsequent analysis.

2.6. Nested RT-PCR Amplification of HCV Core Gene

Nested RT-PCR was used to amplify the HCV core gene area in two rounds of amplification with primers generated by NCBI Primer-BLAST based on conserved HCV sequences (Table 1). In the first round outside primers (HCVOUT-F/R) were used to obtain an amplicon of 400 bp. The product of the first round was diluted (1:10⁶) and used as a template for a second round reaction using inner primers (HCVIN-F/R) that generated a nested 300 bp amplicon specific for the core gene region. Reactions were performed in a total volume of 20 µL on a SaCycler-48 Real-Time PCR machine (Sacace Biotechnologies, Italy) and contained 10 µL PCR Master Mix, 5 µL RNase-free water, 0.5 µL of each primer and 4 µL of template RNA/cDNA. The thermal cycling included an initial denaturation step at 95°C for 5 min, followed by 45 cycles of denaturation (95°C, 5 s), annealing (60°C, 15 s) and extension (72°C, 15 s).

Table 1. Primer sequences used for nested RT-PCR amplification of the HCV core gene region.

HCV OUTER Primers

Primers set	Sequence (5'→3')	Length	Tm	GC%
HCVOUT-F	GTCTAGCCATGGCGTTAGTATGAGTG	26	63.13	50.00
HCVOUT-R	ACAAGTAACTCCACCAACGATCTG	25	61.25	44.00

Products length: 400 bp

HCV Inner Primers

Primers set	Sequence (5'→3')	Length	Tm	GC%
HCVIN-F	GCAGAAAGCGTCTAGCCATG	20	59.35	55.00
HCVIN-R	CTCGCAAGCACCTATCAGG	20	60.53	60.00

product length = 300 bp

2.7. Verification by Agarose Gel Electrophoresis

The nested PCR products were separated on a 1.5% (w/v) agarose gel in 1× TAE buffer for visibility after staining. Five microliters of each amplicon were combined with loading dye and electrophoresed with a 100 bp DNA ladder in a Cleaver Scientific OMNI-Sub™ XL system at 70 V for 60 min. Bands were observed under UV illumination and samples yielding the required 300 bp band were judged positive for the HCV core gene target and selected for subsequent sequencing.

2.8. Phylogenetic analysis and sequencing

Positive amplicons validated by nested PCR were sequenced by Sanger sequencing. The nucleotide sequences of the four HCV isolates (HCV_A1, HCV_A2, HCV_A4, and HCV_A5) were examined with Geneious Prime software. BLAST analysis against the GenBank/NCBI nucleotide database was performed to determine sequence identity and to find the closest international reference sequences. The Iraqi isolates were then aligned with selected homologous reference sequences from GenBank to detect conserved areas and locations of nucleotide variation by multiple sequence alignment.

The Neighbor-Joining method based on the Tamura-Nei genetic distance model was used to estimate the phylogenetic relationships in Geneious Prime. Bootstrap analysis with 1,000 replicates was used to test the statistical support for the inferred tree topology and the bootstrap values were annotated at the associated internal nodes. A hierarchically clustered identity heatmap was also used to depict pairwise identity values between the Iraqi isolates and reference sequences, showing patterns of genetic relatedness and divergence.

Result

3.1. Agarose Gel Electrophoresis Confirmation of Nested PCR Products

Four of the ten specimens moved forward for molecular characterization were positive by nested RT-PCR amplification of the HCV core-encoding region (HCV_A1, HCV_A2, HCV_A4, and HCV_A5). Agarose gel analysis showed a distinct band at the predicted amplicon size in all four samples (Figure 2), and these isolates were selected for Sanger sequencing.

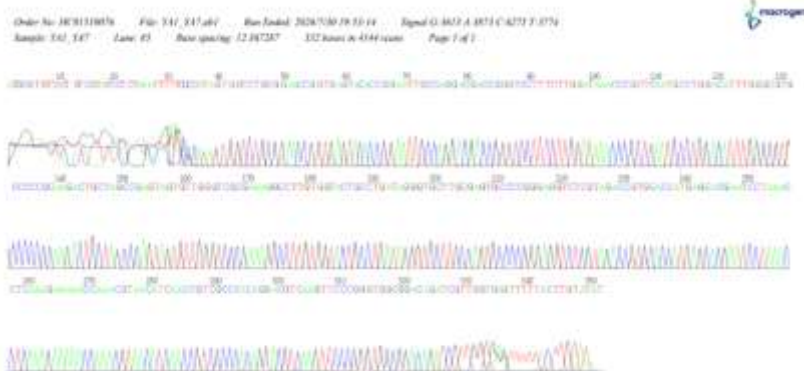
Figure 2. Agarose gel analysis of nested RT-PCR amplification of the HCV core-encoding region. Positive bands at the predicted size of the amplicon correspond to the isolates HCV_A1, HCV_A2, HCV_A4, and HCV_A5.



3.2. Sequence Identity and Genotyping Assignment by BLAST Analysis

Sanger sequencing chromatograms for all four isolates were of good quality, with the initial 20–25 bases showing the overlapping low-amplitude peaks typical of the primer-annealing region, followed by well-resolved, single-peak traces suitable for downstream analysis (representative chromatogram, Figure 3).

Figure 3. Representative chromatogram from Sanger sequencing of HCV isolate_A1.



A BLASTn search against the NCBI nucleotide database revealed that Hepatitis C virus isolate 8012_FU24 (accession EU362898.1) was the closest match for all four isolates used in this study with percent identities of 98.73%-98.74% and query coverage of 89-90% (Table 2). A second cluster of highly comparable reference sequences, ON630869.1, ON630865.1, ON630792.1, LC368327.1, and EU529676.1, was identified at somewhat lower identities (98.1–98.4%), consistent with all four isolates belonging to the same genetic ancestry. All four isolates were unambiguously positioned within HCV genotype 1, subtype 1a, with no hits below 98.1% identity in the top one hundred BLAST hits for each isolate. The strain HCV_A4 was closest to the reference EU362898.1 (98.74% identity), while the other three isolates had almost identical top-hit statistics, as they were derived from the same short and highly conserved section of the genome.

Table 2. Summary of BLASTn identity results of the four HCV core-region isolates.

Isolate	Closest GenBank match (accession)	% Identity	Query coverage	E-value	Genotype/subtype
HCV_A1	EU362898.1	98.73%	89%	2×10^{-154}	1a
HCV_A2	EU362898.1	98.73%	89%	2×10^{-154}	1a
HCV_A4	EU362898.1	98.74%	89%	4×10^{-156}	1a
HCV_A5	EU362898.1	98.73%	90%	6×10^{-154}	1a

3.3. Nucleotide variants in the sequenced region

Four polymorphic sites were discovered by multiple sequence alignment of the four isolates versus GenBank reference sequences (Table 3). All four isolates kept the consensus alleles (T and A, respectively) at locations 388 and 428, which were not among variant alleles reported for reference bacteria at these loci. By contrast, all four isolates shared a C variant at position 226 replacing the G consensus, which was a G>C transversion not associated with any single matching reference sequence in the database searched. This call is supported by the solitary chromatographic peaks identified at this location in all four isolates.

Table 3. Nucleotide variations (SNPs) found in the HCV core-region isolates sequenced in relation to the consensus allele and the GenBank reference sequences.

Position	Consensus	Variant	Variant type	Supporting reference(s)	A1	A2	A4	A5
388	T	C	Transition (T>C)	ON630869.1, ON630865.1, EF407442.1,	T	T	T	T

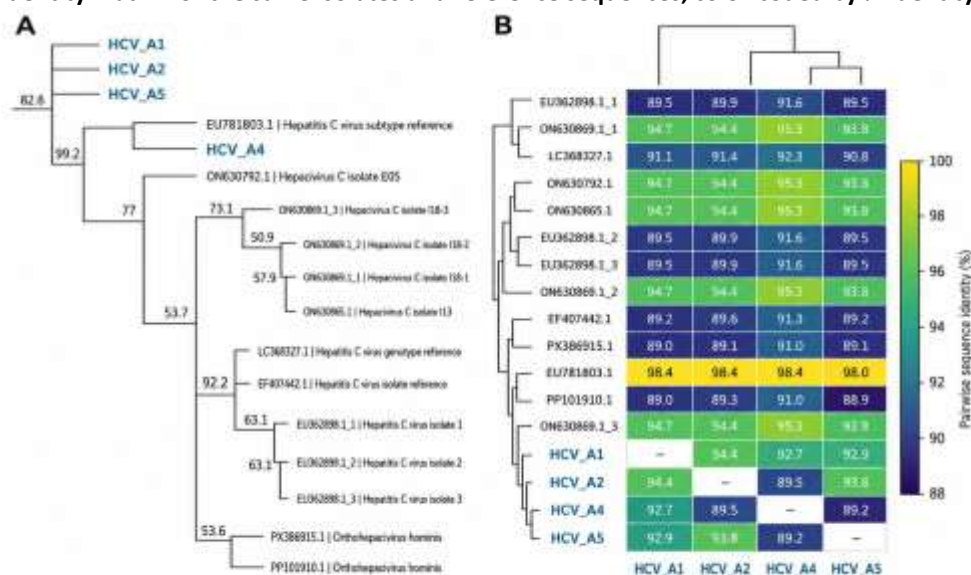
				PX386915.1, EU781803.1, PP101910.1				
428	A	C	Transversion (A>C)	LC368327.1	A	A	A	A
428	A	T	Transversion (A>T)	ON630792.1, PX386915.1, EU781803.1, PP101910.1	A	A	A	A
226	G	C	Transversion (G>C)	No matching reference identified	C	C	C	C

3.4. Phylogenetic Analysis and Pairwise Identity

The four Iraqi isolates grouped together with the closest GenBank reference sequences in a phylogenetic tree built using the Neighbor-Joining method (Tamura-Nei distance model, 1,000 bootstrap replicates) (Figure 4A). Three isolates (HCV_A1, HCV_A2 and HCV_A5) clustered in a well-supported clade, supporting the hypothesis that they share a recent common ancestor, while HCV_A4 split out from the others, closer to the reference sequence EU781803.1 than the other three study isolates.

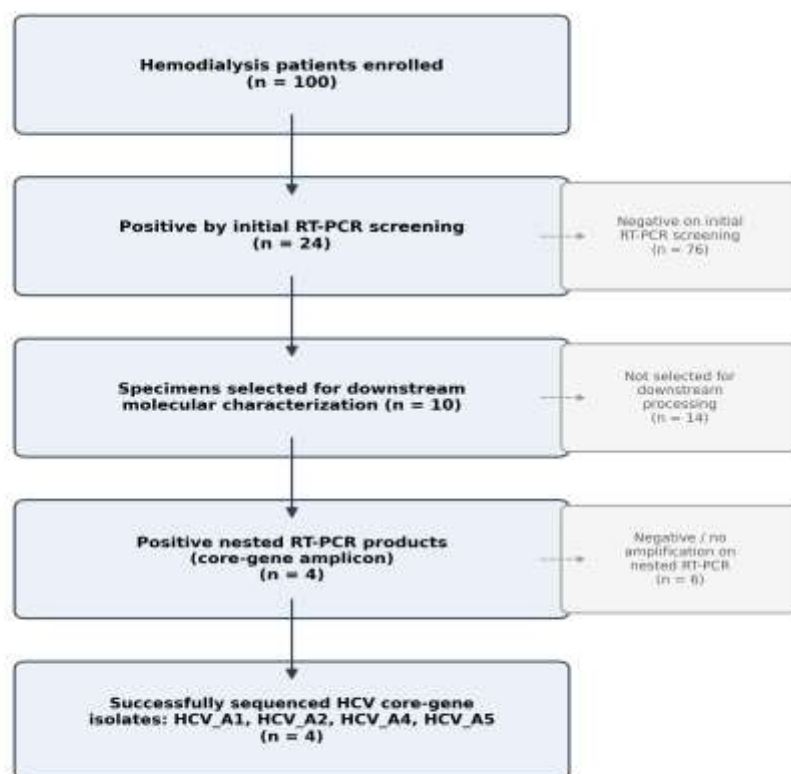
This topology was numerically corroborated by the pairwise identity matrix (Figure 4B) showing that all four isolates displayed their highest identity values against EU781803.1, rather than against each other, indicating that this reference sequence offers the closest overall match for the isolate group. Among the isolate set, HCV_A1 and HCV_A2 exhibited the highest identity with each other in line with their close clustering, whereas HCV_A4 showed a relatively lower identity to the other three isolates in line with its separate branching position. Pairwise similarities between the four isolates ranged from ~89 to 94%, suggesting that all isolates have a common genetic origin and subtype assignment, yet there is considerable sequence variability among locally circulating strains.

Figure 4. (A) Neighbor-Joining phylogenetic tree of the four Iraqi HCV isolates and the nearest reference sequences from GenBank with bootstrap support values (1,000 repetitions) at the internal nodes. (B) Pairwise sequence identity matrix for the same isolates and reference sequences, color-coded by % identity.



The graphic summarizes the complete method for sample selection and molecular characterization.

Figure 5. Flowchart of sample selection and molecular characterisation. Among 100 enrolled haemodialysis patients, 24 specimens were positive on initial RT-PCR screening. Of these, four specimens were positive for the HCV core gene region by nested RT-PCR and successfully sequenced (HCV_A1, HCV_A2, HCV_A4, and HCV_A5). Ten specimens were randomly selected for downstream molecular evaluation.



Discussion

The attached paper reports that all four successfully sequenced HCV core-gene isolates from hemodialysis patients in Fallujah and Baghdad were assigned to subtype 1a whereas phylogenetic analysis separated three isolates into one clade and placed HCV_A4 on a distinct branch closer to reference EU781803.1. It also finds 89%-94% pairwise identity in the four isolates and a shared G>C transversion at position 226 not matched to any reference sequence, showing subtype-level coherence with significant local variability rather than identical viral genomes.

Molecular and Phylogenetic Analysis

The present work shows that although there was noticeable sequence variability in this small sample, all four correctly sequenced HCV core-gene isolates from Iraqi hemodialysis patients were subtype 1a. Three isolates formed a phylogenetic cluster, but HCV_A4 branched individually and was closer to reference EU781803.1. Pairwise identities of roughly 89%-94% suggested that these viruses were linked at the subtype level but were not identical, arguing against a single uniform viral strain circulating throughout all studied patients. Additional support for a locally recurrent sequence characteristic that may represent a circulating subtype 1a variant in these dialysis-associated illnesses is provided by a common G>C transversion at nucleotide position 226 in all four isolates not matched to any searched reference sequence. Work has shown that this area contains enough evolutionary signal to differentiate HCV lineages and is conserved enough to be amplified robustly. Bukh et al. analyzed 52 isolates representing 14 genotypes and showed that the phylogenetic trees generated from the core gene were consistent with the

classification based on E1 and that the core region contains conserved, genotype-specific sequence patterns. They also observed a great deal of variation in nucleotide identity within the core gene, indicating that there can be a lot of diversity without losing the ability to recognize higher-level lineages. These observations are consistent with the view that the Iraqi sequences are robustly placed within subtype 1a in spite of internal divergence and a common on-reference polymorphism [16]. The pattern of internal branching seen is also consistent with what is known about variation within subtype 1a itself. Pickett et al. performed whole-genome analysis of subtype 1a sequences and showed that this subtype can be divided into at least two distinct clades. They further argued that HCV diversity is maintained via rapid replication and the error-prone viral polymerase, providing organized variation even within a single subtype. Importantly, the phylogenetic trees they generated did not demonstrate a strict link between site or time of isolation and clade membership, suggesting that subtype-level heterogeneity is a regular part of HCV evolutionary biology, not an outlier. Here, the dissociation of HCV_A4 from the A1/A2/A5 cluster is best explained as evidence for numerous related subtype 1a lineages rather than misclassification or methodological failure[34].

Regional Genotype Background

The exclusive detection of subtype 1a in the sequenced isolates appears, at first glance, to contradict the long-standing characterization of Iraq as a genotype 4–predominant regional zone. That bigger trend is real. In a systematic study and meta-analysis of genotype data from nations in the Middle East, Ghaderi-Zefrehi et al. discovered that genotype 4 was the most prevalent genotype in Iraq (estimated frequency of ~60%) and concluded that genotype 4 predominated in most of the region. They also noticed that there is more than one pattern of genotype distribution in the Middle East, where genotype 4 is predominant in many Arab countries, but genotype 1 is more prevalent in some nearby non-Arab settings [14]. Therefore, this research suggests genotype 4 as the background at the country level but does not exclude local enrichment of subtype 1a in some high-risk clinical populations [35,36]. More recent subtype-resolved research reconciles that apparent discrepancy. Athamneh et al. collected 6740 HCV sequences from 17 MENA countries and found that genotype 1 was somewhat higher than genotype 4 overall at the regional sequence level although subtype 4a was the most common single subgenotype. Of particular significance is the finding of subtype 1a in Iraq as well as in Iran and Palestine, in a regional context where genotype 4 remains predominant. Their analysis therefore shows that the dominance of genotype 4 and the high circulation of subtype 1a can coexist in the same country when data are analyzed at various taxonomic levels. The present findings are consistent with that model: the dialysis patients studied appear to be representative of one local subtype1a section of a more varied national HCV epidemic [28,5]. Recent Iraqi studies support this interpretation also. In the Diyala governorate, Khalaf and Hussein investigated 306 hemodialysis patients and detected HCV-positive cases of genotypes 4, 1a, 1b, and 2. The first genotype was the most prevalent, and subtype 1a represented 29.2% of the typed infections. Blood transfusion was also found to be the most significant risk factor in that dialysis cohort, further supporting the clinical importance of genotype data in people with repeated blood exposure. Khdeir et al. in Basra found that the most frequent HCV pattern overall was genotype 1a and it was also common among hemodialysis-unit patients. Taken together, this Iraqi research implies that subtype 1a is not incidental in the country and may be notably noticeable in specific medical populations and places [37,38].

Clinical and Transmission Consequences

These genetic findings are of particular epidemiological interest when considering the hemodialysis environment, since HCV infection in dialysis units remains closely related to repeated healthcare exposure. Jadoul et al. using international DOPPS data found that HCV prevalence remained close to 10% among prevalent hemodialysis patients in 2012–2015, and as high as 20% in the Middle East. They also observed that seroconversions were concentrated in a small number of units and that high HCV prevalence within the unit was an important facility-level risk factor for new infections. This finding was taken to imply that the local reservoir of infected individuals within dialysis facilities plays a significant role in onward transmission. In this setting, the grouping of three Iraqi samples in one lineage is consistent with a shared local transmission environment, although not sufficient by itself to indicate direct nosocomial dissemination [5]. This caution is supported by the evidence on wider outbreaks. Fabrizi and Messa conducted a systematic evaluation of 45 outbreaks of hemodialysis involving 335 patients and reported that nosocomial HCV infection was validated by phylogenetic analysis in roughly 69% of the reports. They also noticed that outbreaks commonly involved several breaches of infection-control measures but the precise route of propagation often was not identified even after investigation. An important parallel for the present work.

Phylogenetic relatedness may be a strong suggestion of healthcare-associated transmission, but sequence clustering alone does not reveal the specific route of distribution. The Iraqi findings thus support a valid transmission model, particularly for the A1/A2/A5 cluster, but epidemiologic linkage data are required before stronger epidemic claims can be made [36]. Other investigations of dialysis come to the same conclusion. Rinonce et al. reported a very high HCV prevalence in one dialysis unit in Yogyakarta with genotype 1a as the dominant HCV strain subtype. They also noted that numerous viral genomes were identical and concluded that their data were compelling evidence of nosocomial transmission. Conversely, subtype 1a was discovered in all sequenced isolates in the current study but pairwise identity values were only about 89% to 94%, which is suggestive of relatedness but not near-identity commonly observed in verified micro-outbreaks. The Iraqi results consequently support circulation of common subtypes rather than a verified single-source outbreak [6].

Limitations and Directions for Future Research

The main limitation of the present work is the small number of molecules in the sample. From a cross-sectional cohort of 100 hemodialysis patients, only four of ten selected PCR-positive specimens were available for sequencing, limiting the capacity to reliably assess subtype prevalence or rebuild transmission networks with certainty. A larger multicenter molecular study from southern India emphasizes the need for more extensive sampling. Marhoon et al. recruited 340 hemodialysis patients and reported a 17.35% prevalence of HCV. Genotype 1a was the most prevalent genotype with a frequency of 54.2% and next-generation sequencing was able to resolve the majority of previously uncharacterizable data. They also associated HCV infection with long-term dialysis and numerous transfusions. Compared to that size, the current Iraqi investigation should be considered an important initial molecular snapshot, not a comprehensive epidemiologic map [39]. A second drawback is that a short core-gene segment and neighbor-joining phylogenetics were used for the analysis. While short fragments can be useful for lineage classification, they provide less precision for analyzing recombination, fine-scale transmission chains or atypical subtype structure than multi-region or near-full-genome sequencing. Mellor et al. showed that short HCV sequences may still efficiently identify types, subtypes and isolates but also revealed considerable variability within single genotypes across geographical locations. While this confirms the validity of the current subtype classification, it also emphasizes that further sequencing of the core fragment may show more diversity than the current core segment. Future Iraqi research should therefore employ bigger sample sizes, richer epidemiologic metadata, and higher resolution sequencing to evaluate if subtype 1a represents a stable dialysis-linked reservoir, repeated importation, or a broader pattern of circulation among Iraqi centers [40]. In conclusion, the present data reveal that subtype 1a viruses circulate among Iraqi hemodialysis patients in Fallujah and Baghdad and that these viruses are related but genetically non-identical. This pattern is consistent with national and regional evidence suggesting that genotype 4 is the predominant genotype at the country level, and subtype 1a remains an established part of the Iraqi HCV landscape. Our study thus provides a valuable first molecular glimpse that dialysis-associated HCV in Iraq includes subtype 1a lineages that share and also have unique sequence features, warranting broader sequencing-based surveillance and tighter linkage of molecular findings to infection-control investigations.

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