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Ministry of Higher Education  
And Scientific Research  
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College of Medicine**



# **Detection and Genotyping of HCV among Hemodialysis Patients in Diyala Governorate**

**A Thesis**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ﴾

صَدَقَ اللَّهُ الْعَظِيمُ

سورة هود آية (88)

# *Dedication*

*I dedicate this work to ....*

*My dear mother*

*My dear father*

*My sisters, whom I love*

*My life partner*

*The two roses that filled my life as fragrances*

*My children Moomin and Mubeen*

*Shahad*

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*Shahad*

## Summary

Infection with the Hepatitis C virus is a major public medical condition that mainly affects the liver, causing liver cirrhosis, hepatocellular carcinoma, and liver failure if it is untreated or treated late. It is a major cause of morbidity and mortality in patients with kidney transplantation and hemodialysis patients due to continuous exposure to risk factors such as sharing the dialysis system, medical instruments, or blood transfusion that is necessary for these patients.

This study aims to determine the infection rate and genotyping of the HCV among patients with routine hemodialysis at the Dialysis Centers in Baqubah Teaching Hospital, and Khanaqin General Hospital. It also aims to study the correlation between infection rate and genotypes of the HCV with various parameters such as age, gender, residence, marital status, education level, history of blood transfusion, family history, smoking habit, drinking alcohol habit, tattooing, cupping, and duration of dialysis.

A cross-sectional study was based on the collecting and processing of blood samples from 306 hemodialysis patients, (177) males, and (129) females aged from (10-85years old), during the period 16<sup>th</sup> September 2020 till 16<sup>th</sup> December 2020. Plasma separated from blood and stored at (-20°C) to be used in determining the HCV antibodies by an enzyme immunoassay test, then viral RNA was extracted from hepatitis C positive samples and used for genotyping by reverse transcriptase-polymerase chain reaction technique.

The results showed that the infection rate of hepatitis C virus in hemodialysis patients in Diyala Governorate was 7.8% (24\306), with no differences between males and females (12 cases) for each gender. The age between 61-70 years old was more susceptible to infection (37.5%). Patients with primary education levels more frequently than others 9(37.50%). Blood transfusion was the most common risk factor of infection 19 (79.20%) in the study population. Other risk factors were less frequent such as cupping and tattoos. No positive cases were noticed within family

history, and drinking alcohol. The duration of dialysis ranging from 1-4 years was more frequent than other durations 21(87.5%).

The results of this study showed that genotype 4 was the most frequently detected (8 out of 24), followed by genotype 1a and 1b (7 out of 24) for each subtype, while genotype 2 was less frequent (2 out of 24) and genotype 3, 5, 6 were absent.

The study concluded that the HCV infection rate in hemodialysis patients in the Diyala governorate was low to moderate. Genotype 4 was the most frequent one in this population then genotypes 1a and 1b while genotypes 3, 5, and 6 were not noticed in this study. Blood transfusion was the most risk factor of infection in this population.

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## List of Abbreviations

Abbreviation	Meaning
AASLD	American Association for the study of liver diseases
ALP	Alkaline phosphate
ALT	Alanine aminotransferase
AST	Aspartate amino transferase
β	Beta
CD	Cluster of differentiation
cDNA	Complementary DNA
CKD	Chronic kidney diseases
CLDN-R	Claudin1 receptor
C <sub>T</sub>	Threshold cycle
CTL	Cytotoxic T-lymphocyte
DAAs	Direct acting antiviral
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
E1	Envelope protein- 1
E2	Envelope protein- 2
EASL	European Association for the study of liver
EDTA	Ethylenediaminetetraacetic acid



EGFR	Epidermal growth factor receptor
EIA	Enzyme Immunoassay
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
ESRD	End stage renal diseases
FDA	Food and drugs administration
GTs	Genotypes
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCWs	Health care workers
HD	Hemodialysis
HVR1	Hypervariable region 1
ICTV	International committee on taxonomy of viruses
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
ISGs	Interferon stimulating genes
IV	Intravenous
KT	Kidney transplantation

LDL	Low-density lipoprotein
LDLR	Low-density lipoprotein receptor
LVP	Lipoviral particles
MISC SET	Minimal instruction set computer
MSM	Men sex with men
NANB	Non-A non B hepatitis
NAT	Nucleic Acid Testing
NK	Natural killer cell
NNIs	Non-nucleotide inhibitors
NS	Nonstructural
OCLN-R	Occluding receptor
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
pH	Power of hydrogen
RdRp	RNA dependent RNA polymerase
RNA	Ribonucleic acid
RRT	Renal replacement therapy
RT-PCR	Real-time Polymerase chain reaction
SR-BI	Scavenger receptor class B type 1

TGF $\beta$ -R	Transforming growth factor receptor
Th	T helper
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
Treg	T- regulatory cell
USA	United States of America
VLDL	Very low-density lipoprotein
WHO	World health organization

## 1.1 Introduction

Hepatitis C virus (HCV) infection is a global community health challenge (Elghitany, 2019). Globally, more than 71 million patients with HCV infection worldwide, and 399,000 patients die each year due to HCV related cirrhosis or liver cancer (WHO, 2020). The pediatric population aged 0-18 years and the global estimate for viremia prevalence was 0.13% corresponding to 3.26 million in 2018 (Schmelzer *et al.*, 2020). There is no effective immunization against HCV; therefore prevention of this infection depends upon decreasing the risk of contact with the virus in healthcare settings and high-risk communities (WHO, 2019).

Hepatitis C virus is a small single-stranded with positive polarity RNA virus belonging to the family Flaviviridae and genus hepacivirus (Catanese *et al.*, 2013). It is classified into eight genotypes and several subtypes (Borgia *et al.*, 2018).

Hepatitis C virus is mainly transmitted by exposure to infected devices and tools, infected blood or blood products, hemodialysis, intravenous (IV) drug abuse, and organ transplantation (Ashkani-Esfahani *et al.*, 2017). Nearly 35% of subjects clear the virus spontaneously or after a self-limited asymptomatic acute hepatitis, whereas the remaining 65% progress to chronicity (Coppola *et al.*, 2019). Around 40% to 70% of cases of HCV infection are accompanied by extrahepatic manifestations such as autoimmune, metabolic, renal, cardiovascular, central nervous system, and lymphoproliferative disorders. Hepatitis C infection increases the morbidity and mortality rates in both dialysis patients and kidney transplanted (KT) recipients (Kim and Song, 2018).

Hemodialysis is the common method used to remove waste and toxic substances from the body. It is used to treat patients with different types of renal failure with the use of modern and effective dialysis machines for therapy (Mehmood *et al.*, 2019). It has been shown that infection risk increases with the duration of hemodialysis (Martin *et al.*, 2020). The prevalence of HCV among patients with hemodialysis

patients is considerably higher than in the general population, ranging from 10-50%, depending on the geographical region (Park *et al.*, 2018). Patients undergoing prolonged dialysis treatment display impaired adaptive immune responses and are vulnerable to HCV infection (Fabrizi *et al.*, 2007).

Patients on hemodialysis should be tested when they first start hemodialysis or when they transfer from another hemodialysis facility (Covic *et al.*, 2009). Initial testing either with enzyme immunoassay (EIA) or nucleic acid testing (NAT) is suggested, depending on the low or high prevalence of the virus in the country and the particular hemodialysis unit (Liu and Kao, 2011). On the other hand, several hemodialysis patients will test negative for anti-HCV antibodies while having detectable HCV viremia, so there is no doubt that detection of HCV-RNA by reverse transcriptase-polymerase chain reaction is the most sensitive and specific assay for HCV detection (Covic *et al.*, 2009).

Several studies conducted in different Iraqi towns about HCV frequency in hemodialysis patients and reported different rates such as the study of Ibrahim *et al.*, (2018) reported 4.3% in Duhok city. Muhrath (2018) found 5.66% in Diwaniyah city. Jasim and Athbi (2015) reported 6.6% in Holy Karbala governorate. Sinjari and Bakr (2018) recorded 9.2% in the Kurdistan region. Al-Ta'an and Khalid (2020) reported 20% in Mosul District, and Abdilazeem and Nasir (2019) found 46.36% in Al-Kindy Teaching Hospital in Baghdad. To our knowledge, there are no studies about this issue conducted in the Diyala governorate.

## **1.2 Aims of the Study**

The study aims to:

1. Determine the infection rate of the Hepatitis C Virus among hemodialysis patients in Diyala Governorate.
2. Molecular identification of Hepatitis C Virus genotypes of the study population.
3. Study the correlation between Hepatitis C Virus infection rate and genotypes with different parameters such as age, gender, residence, marital status, education level, history of blood transfusion, family history, smoking, drinking alcohol, tattooing, cupping, and duration of dialysis.