

## Frequency of Hepatitis C Virus among Hemodialysis Patients in Diyala Governorate

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### Abstract

**Background:** Hepatitis C Virus remains a significant risk for both patients and medical staff in hemodialysis centres. The current study aims to determine the infection rate of hepatitis C virus, and risk factors associated with hepatitis C virus in hemodialysis patients in Diyala Governorate\ Iraq. **Methods:** A cross-sectional study was based on the processing of blood samples from 306 hemodialysis patients with routine hemodialysis at the Dialysis Centres in the Baqubah Teaching Hospital, and Khanqin General Hospital, were collected during the period 16<sup>th</sup>September 2020 till 16<sup>th</sup>December 2020, after preparation of samples, enzyme-linked immunosorbent assay test was performed to detect on hepatitis C virus -antibody (Immunoglobulin G), then the seropositivity samples confirmed by real-time polymerase chain reaction technique. **Results:** The rate of hepatitis C virus infection among hemodialysis patients in Diyala Governorate was 7.8% (24/306), and there were no differences between males and females (12 cases) for each case. Age between 61-70 years was the most likely 9(37.5%). There were no differences between the accommodation of patients in Baqubah or around Baqubah (50%) for each one, and 22 married patients (91.70%) more than unmarried 2(8.30%). Patients with primary education were more frequent 9 (37.50%), hemodialysis duration ranging from 1-4 years showed more frequent than other periods 21 (87.5%). **Conclusion:** The rate of hepatitis C virus infection was moderate among hemodialysis patients in Diyala Governorate and there are no differences between gender and residence and the infection increases with the duration of dialysis.

**Keywords:** Hepatitis C, Hemodialysis, Frequency, Diyala.

### Introduction

Liver disease due to the hepatitis C virus (HCV) is a major public health concern that affecting millions of people globally (1). Lead to significant morbidity and mortality in developing and undeveloped countries. The clinical manifestation of viral hepatitis varied from subclinical to a life-threatening infection (2). The virus exists as an enveloped which is 50 nm in size, positive-stranded RNA virus is made up of 9.6 nucleotide bases and is covered by an icosahedra nucleocapsid which is further surrounded by a lipid bilayer containing two viral glycoproteins, envelope one (E1) and envelope two (E2) proteins (3) The high incidence of persistent infection is related to the high genetic diversity of HCV genotypes (4). Lack of an effective hepatitis C vaccine (5). Hemodialysis(HD)is the chosen treatment for end-stage hemodialysis patients and at the same time the patients at risk of developing blood infection infections, including hepatitis viruses synergy scale in accelerating progress to hepatic malformations(6). The frequency of HCV in hemodialysis units was detected all over the world. Risk factors include suppressed immunity for patients, for long periods exposure to blood vessels and multiple blood transfusions, invasive medical procedures, and share contribution to the environments of infected patients for viral hepatitis C prevalent between hemodialysis patients (7,8). Patients undergoing hemodialysis should be tested when they first start dialysis or when transferred from another dialysis facility(9). Initial testing is suggested with either an enzyme immunoassay (EIA) or a nucleic acid test, depending on the low or high prevalence of the virus in the country and the particular hemodialysis unit (10). Data from the national registries around the world show rapidly increasing numbers of ESRD 5 still there is a wide variation in prevalence rate, expressed as number of patients per million populations, among countries (11). Total number of

patients having ESRD on regular haemodialysis program in 18 centres in Iraq at January 2012, throughout the country was 2,445 patients with a prevalence of 74 per a million of population (pmp). The least number of ESRD patients on regular haemodialysis was seen in Thi-Qar province 38 (1.55%), while the highest number was reported in Baghdad 593 (24.25%). However, Diwaniya province has shown the highest prevalence which was 152 patients per a million population (pmp) followed by Al-Muthana and Karbalaa. While Thi-Qar has shown the least prevalence, 21 pmp, followed by Salah-Eldeen and Basrah (12). Several Iraqi studies were done in different cities about the frequency of hepatitis C virus among hemodialysis patients and reported different percentages such as 5.66% in Diwanayah city(13), 6.6% in Holy Karbala province(14), 20% in Mosul District reported(15)and 46.36%in Al-Kindy Teaching Hospital in Baghdad (16). To our knowledge, there are no studies about hepatitis C virus frequency among hemodialysis patients in the Diyala Governorate.

## **Methods**

### **Study design and samples**

A cross-sectional study was conducted on hemodialysis patients who were admitted to the Baqubah Teaching Hospital - Ibn Sina Dialysis Center and Khanqin General Hospital, during the period from 16<sup>th</sup> September 2020 until 16<sup>th</sup> December 2020.

### **Inclusion criteria**

The included blood sample was taken from hemodialysis patients from both genders, all age and from Diyala Governorate.

### **Exclusion criteria**

The excluded patients are those who positive for hepatitis B virus and human immunodeficiency virus

### **Sample collection**

Approximately (5 ml) of blood was collected from (306) hemodialysis patients (177 were males and 129 were females), the samples were put into a test tube containing an anticoagulant, ethylene amine acetic acid (EDTA), and expelled at 1500 rpm for ten minutes, the produced plasma was collected in Eppendorf tubes and stored in deep freezing at (-20 ° C) until it was used to identify IgG antibodies to hepatitis C virus by enzyme immunoassay, then samples were tested positive for IgG from HCV confirmed by using RT-PCR to detect the viral RNA in each sample. Data were collected through an interview with each patient after taken the permission from each one to perform research, through a structural questionnaire sheet which includes age, gender, marital status, residence, level of education, duration of dialysis.

### **Enzyme Immune Assay for Hepatitis C Virus**

All patients were screened for anti-HCV IgG antibodies by third-generation commercial ELISA test according to the manufacturer's instructions of Foresight EIA test kit (Cat. No. 1231-1031, USA) in the laboratory of Ibn-Sina Hemodialysis Center in Baqubah Teaching Hospital used for the qualitative detection of IgG antibodies to HCV in human plasma.

### **HCV-RNA Extraction and Hepatitis C Virus Quantification**

All positive samples are extracted for HCV-RNA by using ExiPrep<sup>TM</sup> Dx Viral DNA\RNA Kit (Cat. No K-4471/ K-4472/ K-4473, Korea), from the plasma according to the manufacturer's instructions. RNA was isolated from 400µl plasma using an automated system depending on the magnetic beads method in the isolation of the RNA. After the extraction of HCV-RNA, Anatolia Bosphore HCV quantification kit (Cat. No. ABHCQ1, Anatolia Gene works, Turkey) according to the manufacturer's instructions used for detecting and quantities HCV nucleic acid from all samples depending on the protocol of RT-PCR technique.

### **Statistical Analysis**

All data were analyzed using the Statistical Analysis Program (SAS) - 2012, version 22, number, percentage, and proportion, and chi-square was used to test the effect of different factors in the study, for a significant comparison of the percentage (0.05 and 0.01 probability) in this study.

## Results

The rate of infection of hepatitis C virus infection among hemodialysis patients in this study was 7.8% (24 of 306) according to the results of enzyme immunosorbent assay and RT-PCR techniques, as shown in Figure1.

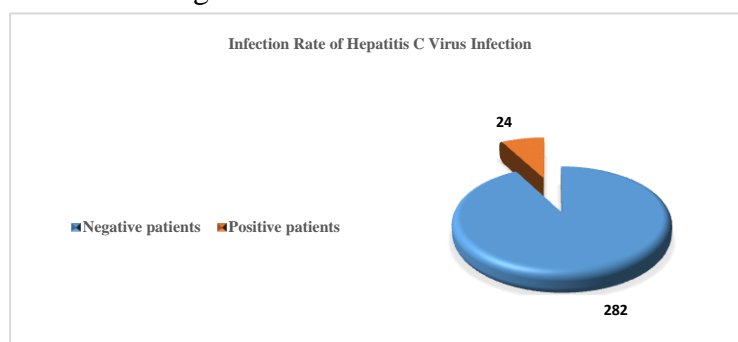


Figure 1: Infection Rate of Hepatitis C Virus Infection According to Enzyme Immunosorbent assay and Real Time-Polymerase Chain Reaction Techniques.

Among 24 hemodialysis patients, 12 (50%) males and 12 (50%) females were positive for hepatitis C virus with statistically significant differences ( $P = 0.0001$ ) between positive and negative patients. The infection was more frequent in the age group (61-70 years old) was 37.5% with highly significant differences ( $P=0.0087$ ) and there was an increase in HCV infection with increasing age with a mean age of  $51.6 \pm 14.16$ (SD) years. Half (50%) of the hepatitis C virus infection among dialysis patients lived in Baqubah district and 50% around Baqubah district (Muqdadiah, Khanqin, Balderoz, Al-Khalis) and there were statistically significant differences ( $P = 0.0001$ ). Regarding marital status, the highest incidence of viral hepatitis was observed in 22 married patients (91.7%) with statistically significant differences ( $P = 0.0074$ ), followed by unmarried 2 (8.3%) with significant differences ( $P = 0.0461$ ). Also, most of the negative cases were 196 (69.5%) married and 50 (17.7%) unmarried. Concerning educational levels in hemodialysis patients with HCV infection, 9 (37.5%) had primary education with significant differences ( $P = 0.0288$ ), 8 (33.3%) had secondary education with statistically significant differences ( $P = 0.0081$ ), While low cases 6 (25%) had illiterate education and 1 (4.2%) had higher education (had institute or college degree) without statistically significant differences, as shown in Table1.

**Table 1:** Demographic Characteristics among Hemodialysis Patients.

Variable factors		Positive No. (%)	Negative No. (%)	Chi-square*P-value **P-value
Gender type	Male	12(6.80%)	165(93.20%)	132.25 ** (0.0001)
	Female	12 (9.30%)	117(90.70%)	85.465 ** (0.0001)
Age groups (years)	1-12	1 (4.20%)	20 (7.10%)	3.026 NS (0.375)
	13-24	1 (4.20%)	30 (10.64%)	2.691 NS (0.331)
	25-36	5 (20.80%)	30(10.64%)	4.095 * (0.0466)
	37-48	5(20.80%)	50 (17.70%)	0.772 NS (1.026)
	49-60	3(12.50%)	70(24.80%)	4.821 * (0.0398)
Residence	Rural	9 (37.50%)	60(21.29%)	6.259 ** (0.0087)
	Urban	0(0.00%)	22(7.80%)	6.802 ** (0.0079)
Marital Status	Single	2 (8.30%)	50 (17.70%)	4.772 * (0.0461)
	Married	22(91.70%)	196 (69.50%)	7.205 ** (0.0074)
	Divorced	0 (0.00%)	5 (1.80%)	0.084 NS (0.933)
	Widow	0 (0.00%)	31 (11.00%)	4.659 * (0.0452)
Educational levels	Illiterate	6(25.00%)	68 (24.10%)	0.561 NS (0.692)
	Primary school	9 (37.50%)	148 (52.50%)	5.038 * (0.0288)
	Secondary school	8 (33.30%)	45 (16.00%)	6.502 ** (0.0081)

	High education	1 (4.20%)	21 (7.40%)	1.064 NS (0.329)
Total		24(100%)	24 (100%)	282(100%)

\* (P≤0.05), \*\* (P≤0.01), NS: Non-Significant.

The maximum duration of dialysis in positive HCV-infected patients was (7 years) while the minimum duration was (1 year) with a mean of  $3.38 \pm 1.3$  (SD) years. Hepatitis C virus was more common in patients on hemodialysis for (1-4 years), approximately 21 (87.5%) with statistically significant differences (P = 0.0054), and less common in patients on hemodialysis duration (5-7 years) were 3 (12.5%) with non-significant differences (P = 0.479), as shown in Table 2.

**Table 2:** Distribution of Positive and Negative Hepatitis C Virus According to Duration of Dialysis

Duration of Dialysis	Positive No. %	Negative No. %	Chi-square *P-value
Less than one year	0 (0.0%)	72(25.5%)	8.61 ** (0.0037)
1-4 years	21(87.5%)	173 (61.3%)	7.26 ** (0.0054)
5-7 years	3(12.5%)	30 (10.6%)	0.594 NS (0.479)
8-10 years	0(0.0%)	7 (2.5%)	0.558 NS (0.572)
Total	24 (100%)	282(100%)	---

\*\* (P≤0.01), NS: Non-Significant.

## Discussion

According to the results of the ELISA and RT-PCR techniques, the rate of HCV infection among dialysis patients at Baqubah Teaching Hospital and Khanqin General Hospital in Diyala Governorate was 7.8%. The result of this study is comparable to several Iraqi studies conducted in different cities such as 6.6% in Dhi Qar by using ELISA and RT-PCR techniques(17), 9.2% in the Kurdistan Region of by ELISA and PCR technologies were distributed in 5.2% in Erbil, 9.3% in Dohuk, and 12.9% in Sulaymaniyah (18). The similarities in the results of the current study and others due to geographical conditions, as all relevant studies were conducted in countries with similar genetic and ethnic factors, or they could be related to similarity of transmission or risk factors for the disease as well as the use of the same technique in detection. Moreover, the result of this study is relatively high compared to other Iraqi studies such as 2.1% and 4.3% respectively among hemodialysis patients in Duhok(19)(20).Also, with studies done on dialysis patients in neighboring countries such as 1.4% in paediatric hemodialysis centres in Iran(21), and 4.3% in Turkey (22). On the other hand, the result of this study was low when compared to the data received on dialysis patients by Iraqi researchers such as in Baghdad-Iraq were reported 13.4% as a percentage of HCV infection in pediatric hemodialysis centers (23), 14% in Kirkuk city(24), 20% in Mosul province (15), 21.2% in Al-Diwaniyah province(25), 43% in Baghdad Medical(26). The result of this study was low when compared to studies of neighboring countries such as 12.7% in Turkey (27), 71.8% in Aleppo city-Syria it reported a very high prevalence of HCV infection (28). These variations can be because the virus differs from region to region and year to year, or to the diversity of the research that characterized it, which can contribute to a successful age group, differences in the number of chronic kidney patients in each study, genetic factor, immune status of patients, detection technique sort, sample size, and cultural knowledge of exposure to a risk factor for transmission of the hepatitis C virus. According to gender, it was found that infection with hepatitis C virus is equal in males and females 12 (50%) with statistically significant differences (P = 0.0001) between positive and negative cases. The results of the current study agreed with other studies which were found there are no significant differences between both genders in viral hepatitis C infection among hemodialysis patients in different cities such as Baghdad (26)(29), Kurdistan (23) While disagreed with the study were reported a male had a statistically significant association with anti-HCV positivity than females such as in Al-Kindy Teaching Hospital-Baghdad (16), in ThiQar province (17), and disagreed with the results were reported the

infection was higher among females than males such as in Al-Najaf Al-Ashraf (30), in Iran (21), in Ibb city-Yemen (31). In this study, the infection rate was highest at ages (61-70 years) with significant differences. This may be attributed to the higher rate of renal disorders in the elderly, which has led to dialysis treatment, which is one of the high-risk factors for infection. These results were agreed with many studies which were revealed a significant relationship between HCV infection and relatively old age in the Kurdistan region (18), in Pakistan (32). On the other hand, the results of the current study disagreed with other studies in other countries such as Ibb City-Yemen where found the HCV infection was highest among age more than 45 years old (31), in East Azerbaijan, Iran (33) were reported there was no significant difference between HCV infection and age. According to the residence of patients in the study population, Hepatitis C virus infections in Baqubah and around Baqubah were the same (50% for each one) with highly significant differences ( $P=0.0001$ ). This is due to the similarities between these areas of the viral pattern and the nature of the area, as well as the mode of transmission and exposure among members of these communities to the same risk factors for this disease and health customs and traditions. These results when compared with other studies such as in Addis Ababa, Ethiopia reported hepatitis C infection more in rural than urban areas (34). This may be due to the disparity between rural and urban cultures, levels of education in rural areas, the degree of awareness of the rural population of the nature of the disease and its transmission factors, such as sharing razors or medical syringes between one person and another, as well as the economic and cognitive component of these societies. On the other hand, some studies have reported the infection in urban areas more than in rural areas (35)(36). Due to lack of knowledge and weakness in the high prevalence of hepatitis infection, living conditions in the area, and perhaps because of differences in the level of education and the level of cultures between the population and dangerous for infectious diseases or the way viruses are transmitted between them, and because of differences At the level of education and the level of cultures in the population. Regarding marital status, the highest infection rate of HCV was noticed with married patients 22(91.7%), followed by single patients 2(8.3%) with significant differences among these groups. These results agreed with several studies conducted in different areas such as Iran found a significant relationship between marital status and HCV infection in hemodialysis patients (37), in east Azerbaijan-Iran (33), in Makkah City-Saudi Arabia (38). While in Aleppo city- Syria found the marital status was not statistically significant regarding HCV infection among hemodialysis patients (28). The results relate to the fact that the virus is transmitted through sexual or intimate contact, that is, about 0.4-3% of its transmission (39). Concerning the educational levels in hemodialysis patients with HCV infection in this study, most patients in the study population 9(37.5%) had primary education, followed by secondary education 8(33.3%) with significant relation. These results agreed with the study in Tehran, Iran were found the infection of HCV in hemodialysis patients with primary education more than other educational levels (35). While disagreed with studies in Makkah City-Saudi Arabia (38), a study in Georgia was shown the hemodialysis patients with secondary levels were the highest infections with HCV (40). Also in different countries like Khartoum-Sudan (40), and the centre of Iran (33) were found no significant relationship between education level and HCV infection in hemodialysis patients. The differences between the current study and others may be due to a decreased knowledge of the nature of transmission, because they may not be interested in sharing sharp tools and equipment such as razors and pricing tools that can spread the infection from one person to another, and they may have little understanding of the risks of disease and risk factors for infection in these populations. The occurrence of hepatitis C infection due to the duration of dialysis in this study reported the duration (1-4) years more than other duration with highly significant differences. These results were agreed with results were reported in other Iraqi cities such as in the Kurdistan region (18), in Al-Najaf Al-Ashraf (30) which revealed a significant relationship between HCV infection and the longer duration of dialysis. Also agreed with studies

reported among hemodialysis patients in different countries such as Tehran (35), in Khartoum-Sudan (40) that showed there was a very strong statistical association between long duration of dialysis and HCV positivity in hemodialysis patients. The results of this study disagreed with the results of studies in other countries such as Egypt (41) where the infection rate was high in patients with less than 3 years of dialysis among Egyptian hemodialysis patients. Also disagreed with the study in Argentina that have reported the duration of hemodialysis was not a significant risk factor for HCV(42). These differences are caused by prolonged or short exposure to infection risk factors, such as the use of the same dialysis machines in patients with hepatitis C virus antibodies, insufficient disinfection and disinfection of environmental surfaces, and misuse of intravenous drugs.

### **Conclusion**

The rate of hepatitis C virus infection was moderate among hemodialysis patients in Diyala Governorate and there are no differences between gender and residence and the infection increases with the duration of dialysis.

### **Abbreviation**

HCV= Hepatitis C virus, E= Envelop, HD= Hemodialysis, EIA= Enzyme immunoassay (EIA), ESRD= End stage renal diseases, SD= Standard division.

### **Declaration**

We declare no company interests.

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### **Availability of data and materials**

The datasets generated and analyzed during current study are from the corresponding author (shahadkhudaeir@gmail.com) on reasonable request.

### **Authors' contributions**

Sample collection and processing was performed by first author, while others were equally involved in all parts of the manuscript, drafting, concept, design, writing, reviewing, editing, and approving the manuscript in its final form.

### **Ethics approval and consent to participate**

We conducted the research following the Declaration of College of Medicine -University of Diyala, Baqubah Teaching Hospital, Khanaqin General Hospital and after taken the permission from each patients.

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