Investigation of Hepatitis C Virus Infections by Serological and Molecular Methods in Haemodialysis Patients in Kirkuk City-Iraq

Nazhan Bakr Mahmood ALBAYATI[1], Serhat SİREKBASAN[2], Asmaa Mohammed Sulaiman Al-bayati[3]

[1] M.Sc. Molecular Biology, Kirkuk Health Directory, Iraq

[2] Doç. Dr. Eldivan Sağlık Hizmetleri Meslek Yüksekokulu, Çankırı Karatekin Üniversitesi.Turkey

[3] Northern Technical University, College of Medical Technology, Kirkuk , Iraq

E-mail: nnn1952005@gmail.com

Abstract

Background: Hepatitis C Virus (HCV) causes the development of both acute and chronic liver disease and increases the risk of cirrhosis and hepatocellular carcinoma at significant levels. Aim: In the present study, the purpose was to determine possible risk factors by determining the frequency of Hepatitis C in patients who are treated in the haemodialysis unit in Kirkuk, Iraq with serological and molecular methods. Materials and methods: This cross-sectional study was conducted in Kirkuk, Iraq, between April 2021 and January 2022. The study group consisted of 200 patients who were diagnosed with chronic kidney disease and applied to the Kirkuk State Hospital and underwent hemodialysis, while the control group consisted of donors who came to the blood bank of the same hospital for blood donation and were found to be free of any disease. Liver function tests and IL-6 cytokine were studied in all of the samples included in the study. In addition to biochemical tests, the presence of HCV antibodies and HCV RNA were examined. Results: When the presence of Anti-HCV antibodies was investigated in 200 cases from the Study Group and 50 cases from the Control Group with ELISA, Anti-HCV antibodies were detected in 36 cases in the Study Group, and no one was infected in the Control Group. HCV RNA was found to be positive in 30 of the 36 patients with positive anti-HCV in the Study Group. When the study and control group cases were examined in terms of serum ALT, AST, ALP, TSB, and IL-6 levels, it was determined that ALT, AST, ALP, and IL-6 levels were statistically significant between the groups. Conclusion: As a result, we believe that periodic studies will be useful in controlling the prevalence of HCV infection among hemodialysis patients and to uncover this relationship more clearly.

Keywords: Hemodialysis, Hepatitis C, HCV, Infection, Iraq

1. Introduction

When chronic kidney diseases are not detected at an early stage and treated appropriately, it is not possible to prevent the progression of the disease. In this case, the degree of renal failure increases in a way that cannot be ignored and patients need dialysis treatment (1, 2). Hemodialysis units have various risks in terms of transmission of different viral infections for both patients and health personnel working in these units. Among these blood-borne viral infections, HIV and hepatitis agents are the most dangerous. Especially in hemodialysis patients, hepatitis B virus (HBV) and hepatitis C virus (HCV) appear as causes of chronic hepatitis (3, 4). In addition, it has been observed that HCV, the most common infectious agent in hemodialysis patients, is closely associated with mortality and impairs quality of life (5). Due to the nature of the hemodialysis process, percutaneous interventions applied in each dialysis session increase the possibility of encountering these infections, creating serious safety concerns among the patients treated in the hemodialysis unit and the health personnel working in these units (6). Although the frequency of viral infections in hemodialysis patients has decreased in recent years, the transmission of HCV infection in dialysis units continues due to nosocomial spread. Inadequate practices in infection control measures against blood-borne pathogens are the most important cause of hospital-acquired transmission of hepatitis in hemodialysis units (7, 8). Additional measures beyond infection control practices are also mentioned to prevent HCV transmission in dialysis units. Foremost among these, it is recommended that all patients receiving hemodialysis be periodically screened for HCV twice a year and tested monthly to monitor ALT levels. However, screening for anti-HCV antibodies should not be considered as an alternative to the implementation of infection control precautions, especially in the hemodialysis setting, and inspections should be carried out meticulously by full compliance with infection control practices (6). In some studies, it has been reported that the introduction of stricter screening rules in dialysis units and strong adherence to infection control practices reduce the prevalence of HCV infection in the hemodialysis patient group (9-11). Our study, it was aimed to determine possible

doi.org/10.31838/hiv23.03.122 Received: 03.11.22, Revised: 07.12.22, Accepted: 10.01.23

risk factors by determining the frequency of Hepatitis C in patients who are treated in the hemodialysis unit in Kirkuk, Iraq with serological and molecular methods. In addition, IL-6 (Interleukin 6), ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase), TSB (Total serum bilirubin) levels were investigated between HCV-infected hemodialysis patients and healthy control group.

2. Material and method

This cross-sectional study was conducted in Kirkuk, Iraq, between April 2021 and January 2022. The patient population within the scope of the study consists of 200 patients who were diagnosed with chronic kidney disease and applied to Kirkuk State Hospital and underwent hemodialysis. As the control group, blood samples taken from 50 healthy individuals who came to the Kirkuk State Hospital blood bank for blood donation between the same dates and were found to be free of any disease were included. Demographic data of the groups (age, gender, place of residence, etc.) were collected with a follow-up form.

Procedures

Liver function tests (ALT [Alanine aminotransferase], AST [Aspartate aminotransferase], ALP [Alkaline phosphatase], TSB [total serum bilirubin]) and IL-6 cytokine were studied in all of the samples included in the study. A commercial kit (Genesis Diagnostics, Littleport, UK) based on the ELISA method was used to detect Anti-HCV antibodies against HCV antigens in serum samples. The presence of HCV RNA was extracted from plasma samples using the Quick-RNA™ Viral kit (Zymo Research Corp., USA) according to the manufacturer's recommendations. Obtained RNA samples were run with a SaCycler-96 Real-Time PCR system (Sacace Biotechnologies, Italy) using the HCV Real TM-Quant Dx kit (Sacace Biotechnologies, Italy) according to the manufacturer's recommendations.

Statistical Analysis

Descriptive statistics such as mean ± standard deviation (SD) were used in the analysis of data such as the age of the patients, and frequencies (n) and percentages (%) were used to define gender and test results. The conformity of the variables to the normal distribution was performed using visual methods (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov test). Values that did not show normal distribution within the groups were compared using the Kruskal-Wallis test.

Ethics Statement

Our clinical study was carried out in accordance with the approval decision of the Iraqi Ministry of Health, Kirkuk Provincial Health Office, dated 05.04.2021, and numbered 10939. All participants were informed about the study and set by signing the free will consent form. All patients and healthy volunteers gave written informed consent to participate in the study.

3. Results

The demographic data distributions of the cases in the study and control groups are numerically summarized in Table 1.

Table 1: Demographic data of study and control group cases

Parameters	Study Group	Control Group	*P* value
Number of cases	200	50	
Gender (Male/Female)	104/96	26/24	>0.05
Age, Mean±SD	52.98±12.76	49.64±9.69	>0.05
Age, Median (Min-Max)	56 (20-78)	52 (23-66)	

When the presence of anti-HCV antibodies in 200 cases from the study group and 50 cases from the control group were examined by ELISA; anti-HCV antibodies were detected in 36 cases in the study group, while no one in the control group was infected.

HCV RNA was found positive in 30 of 36 patients with positive Anti-HCV in the study group. The presence of HCV RNA was not observed in the patients in the study group who did not have anti-HCV antibodies. When the age distribution of the hemodialysis patients in the study group and the infection relationship were examined; the highest rate of HCV infection was found in the 65-74 age group (33.3%) (Table 2).

Table 2: The relationship between age distribution and infection in hemodialysis patients

Age Group	Study Group (n)	HCV RNA (+) (n)	HCV RNA (+) (%)
15-24	18	1	3.3
25-34	20	3	10.0
35-44	28	4	13.3
45-54	48	5	16.7
55-64	44	7	23.3
65-74	42	10	33.3
Total	200	30	100

When the relationship between the duration of hemodialysis and infection of the cases in the study group was examined; it was determined that the highest HCV infection rate was 46.7% in patients who had been on dialysis for more than 3 years and the HCV infection rate increased with the increase in the duration of hemodialysis (Table 3).

Table 3: The relationship between the duration of hemodialysis and infection of the patients in the study group				
Hemodialysis treatment time	HCV RNA			
	Positive (n:30)		Negative (n:170)	
	n	%	N	%
Less than 1 year	6	20.0	48	28.2
Between 2-3 years	10	33.3	55	32.4
More than 3 years	14	46.7	67	39.4
Total	30	100	170	100

Considering the relationship between HCV infection and transmission patterns, a statistically significant difference was found between hemodialysis patients who had undergone any previous surgical operation and those who had not undergone any previous operation (Table 4).

Table 4: The relationship between the surgical history of hemodialysis patients and the infection					
Surgical operation history	Hemodialysis patients				P
	HCV (+)		HCV (-)		
	n	%	n	%	
Yes	20	66,7	67	39,4	0.0055
No	10	33,3	103	60,6	
Total	30	100	170	100	

When the blood transfusion status of the cases in the study group was examined, no statistically significant difference was found between the hemodialysis patients who received a transfusion and those who did not receive any transfusion before ($p>0.05$). When the patients in the study group received dental treatment, a statistically significant difference was found between the hemodialysis patients who received dental treatment and the patients who did not receive any dental treatment before ($p<0.05$).

When the study and control group cases were examined in terms of serum ALT, AST, ALP, TSB, and IL-6 levels; ALT, AST, ALP, and IL-6 levels were found to be statistically significant between the groups. Despite that; when the groups were compared, no significant relationship was found between TSB levels (Table 5).

Table 5: Comparison of some biochemical parameters in the study and control groups					
	Study Group (n:200)		Control Group (n:50)		*P*
	Mean ± SD	Median	Mean ± SD	Median	
ALT	26.71±15.40	23	12.93±7.02	11	<0.001
AST	24.16±16.01	22	14.26±9.69	12.5	<0.001
ALP	100.79±52.48	84	64.38±25.74	69.1	<0.001
TSB	0.99±0.96	0.7	0.63±0.20	0.6	0.051
IL-6	24.84±19.19	21	16.90±9.63	16	0.026

When the serum ALT, AST, ALP, TSB, and IL-6 levels of the subjects with positive and negative HCV RNA in the study group were examined; ALT, AST, ALP, TSB, and IL-6 levels were found to be statistically significant between the groups (Table 6).

Table 6: Comparison of HCV RNA (+) and (-) cases in the study group in terms of some biochemical parameters					
	Study Group (n:200)				P
	HCV (+) (n:30)		HCV (-) (n:170)		
	Mean ± SD	Median	Mean ± SD	Median	
ALT	53.96±10.62	55	21.91±10.25	21.5	<0,001
AST	36.99±25.67	28.55	21.89±12.43	20	<0,001
ALP	209.63±25.24	201.7	81.58±25.68	78	<0,001
TSB	3.04±1.03	3.35	0.63±0.19	0.625	<0,001
IL-6	64.53±8.28	21	17.84±9.65	17.235	<0,001

4. Discussion

Although the incidence of HCV infection varies greatly between geographical regions, the prevalence of HCV infection in hemodialysis patients remains high, no matter where they are in the world. The prevalence rates of HCV infection are decreasing in hemodialysis units of most developed countries; however, this rate exceeds 80% in some countries (8). In our study, anti-HCV positivity was detected in 36 (18%) of our patients, and the presence of HCV RNA was detected in 30 (15%) of these patients. In a meta-analysis study investigating the prevalence of HCV infection among hemodialysis patients in the Middle East region, including our country, the overall prevalence was reported to be 25.3%. According to the results reported in this study, the overall prevalence of HCV infection among hemodialysis patients in Lebanon was reported as 9%, followed by increasing rates; in Iran (12%), Palestine (18%), Saudi

Arabia (19%), Iraq (20%), Turkey (23%), Jordan (35%), Yemen (42%), Egypt (50%) and Syria (54%) (12). It was observed that the results in Iran, Palestine, Saudi Arabia, Iraq, and Turkey were consistent with the rate we determined in our study. On the other hand, the rates reported in Jordan, Yemen, Egypt, and Syria are higher than our study results. Although the mechanisms responsible for the transmission of HCV infection in patients receiving hemodialysis are not yet fully known, we think that these high rates may be due to the lack of attention given to sterilization and infection control in dialysis units. Studies conducted in the United States report that the prevalence of HCV infection in hemodialysis centers varies between 8% and 16.8%, which is 5-10 times higher than the prevalence in the general population of the country (1.6%) (13-15). In developed countries of Europe such as Belgium, Germany, Spain, France, Sweden, Poland, Hungary, the United Kingdom, and Italy, the prevalence rates are reported as 6.8%, 4.6%, 7.5%, 15%, 3.1%, 4.4%, 0%, 0.4% and 14.7% respectively (16). Practices such as strict compliance with infection control strategies, routine screening for viral hepatitis during blood transfusion, use of separate dialysis machines for infected patients, and use of erythropoietin instead of blood transfusions may be the reason for the low prevalence rates in developed countries for HCV infection in hemodialysis patients in general. Diagnosis and follow-up of HCV is very important in hemodialysis patients. In these patients, first of all, anti-HCV test is performed in serum by ELISA method, and when positive results are obtained, the presence and amount of HCV RNA is investigated by molecular methods. In many studies, anti-HCV and HCV RNA positivity are detected at different rates, and it is recommended that anti-HCV and HCV-RNA follow-up be performed together in hemodialysis patients (17). In our study, HCV RNA was found positive in 30 of 36 patients who were positive for anti-HCV. The presence of HCV RNA was not observed in the patients in the study group who did not have anti-HCV antibodies. It is predicted that the detection of different results in the tests used in the diagnosis of HCV infection may be caused by many factors depending on the host and the diagnostic method used. Therefore, we support the view that it would be appropriate to study anti-HCV and HCV RNA together in the follow-up of HCV infection in hemodialysis patients. The main mode of transmission of HCV infection is by parenteral routes. Intravenous drug use, blood transfusion, hemodialysis, and surgical interventions also play an important role in HCV transmission (18, 19). When we look at our study results, a statistically significant difference was found between hemodialysis patients who had undergone any previous surgical operation and those who had not undergone any previous operation. On the other hand, in many studies, it has been determined that dental treatment is an important risk factor in the transmission of HCV (20, 21). In cases with HCV infection, the presence of a history of transfusion is frequently reported among the possible routes of transmission. In our study, a statistically significant difference was found between hemodialysis patients who received dental treatment and those who did not receive any dental treatment before, but no significant difference was found between hemodialysis patients who received and did not receive transfusion. In order for the transfusion to take place safely, the scans made on blood donors seem to give good results. In addition, considering that pre-transfusion nucleic acid amplification test methods have not gained enough place in the routine in many countries, it can be thought that the possibility of HCV transmission will continue. Some scientists have conducted controlled studies in patients with HCV and healthy individuals and analyzed the differences in routine liver function indicators, showing that HCV-infected patients have significant differences in liver function test results. The results of this study showed that ALT, AST, ALP, TSB, and IL-6 levels in HCV-infected hemodialysis patients were significantly higher than in HCV RNA-negative patients. Serum ALT, AST, and ALP as well as IL-6 levels were found to be significantly higher between the study and control group cases. In other words, we found that HCV infection significantly affects liver function and causes abnormal liver metabolism. Additionally, the recommendations that all patients receiving hemodialysis be tested monthly to monitor ALT levels are consistent with the results of this study.

In conclusion, our study showed that IL-6, ALT, AST, ALP, and TSB levels were found to be higher in hemodialysis patients with HCV infection compared to hemodialysis patients without HCV infection, and the lowest levels were found in the control group. These results support other studies carried out in the Middle East region in recent years. From these results revealed in the study, it is understood that the detection of some biochemical parameters such as liver function tests at high levels in hemodialysis patients may guide the diagnosis of HCV. We believe that comprehensive studies to be conducted on hemodialysis patients, especially those at high risk for HCV infection, will be beneficial in terms of controlling the prevalence of HCV and revealing this relationship more clearly.

5. Acknowledgement and/or disclaimers, if any

The authors declare that there is no conflict of interest regarding the publication of this paper.

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