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# Hepatitis C Viral İnfection Among Hemodialysis Patients and İts Effect on Interleukin-6

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## Abstract

**Background:** Chronic kidney disease (CKD) is a global public health issue characterized by a gradual loss of kidney function over time, poor outcomes, and substantial healthcare costs.

**Aim of the Study:** The aim of this study was to examine the prevalence of hepatitis C virus (HCV) infection in patients receiving hemodialysis (HD) and to determine if there is a link between HCV infection and interleukin-6 levels.

**Materials and Methods:** A cross-sectional study was conducted from April 1st to September 20th , 2024. The study involved 200 patients with chronic renal disease undergoing hemodialysis, aged 20-75 years, who were admitted to Al-Hussein Teaching Hospital in Dhi Qar. Additionally, 50 healthy control individuals aged 18-50 years, who visited the Main Blood Bank for blood donation and had no apparent disease, were included. Blood samples (5 ml) were collected via vein puncture from each participant for serological testing to detect HCV-specific antibodies, IL-6 levels using ELISA, and HCV viral load determination by PCR.

**Results:** Our results indicated that 18% of HD patients were infected with HCV. Among the HD patients, 83.33% of those who tested positive for HCV antibodies by ELISA were confirmed to HCV-positive by PCR. None of the ELISA-negative HD patients or individuals in the control group tested positive by PCR. The highest rate of HCV infection (44.44%) among patients had a viral load between 9,000 and 11,000 IU/mL. The study also demonstrated that HD patients with HCV infection had the highest mean levels of IL-6 compared to HD patients without HCV infection.

**Keywords:** Chronic kidney disease, kidney failure, Hemodialysis, Hepatitis C virus infection, IL-6

**Introduction:** Chronic kidney disease (CDK) is a growing global public health issue, with increasing prevalence and serious consequences, in addition to substantial financial costs. (CDK) can result in kidney failure and other complications like heart disease and impaired kidney function. Some of these negative outcomes may be preventable or delayed through early detection and treatment (Tonelli and Riella, 2014). However, identifying and treating chronic kidney disease is not always straightforward, leading to inadequate support for affected individuals. This is partly due to a lack of consensus on how to define and classify the different stages of disease progression, and how to utilize simple tests for detection and evaluation (Afolabi et al., 2009). Hepatitis C is a significant public health issue due to its high prevalence and the resulting illnesses and fatalities. Approximately 3 percent of the global population suffers from chronic hepatitis C (Ref). The hepatitis C virus (HCV) is mainly transmitted through blood, so individuals who undergo blood transfusions, use intravenous drugs, undergo hemodialysis, or engage in other medically-related activities are at higher risk of infection (Ref). The use of

synthetic erythropoietin to treat anemia caused by kidney failure and regular screening of blood donors for anti-HCV antibodies have significantly reduced the likelihood of HCV transmission through blood (Ref). This indicates that the primary mode of HCV transmission is now through nosocomial infections in individuals undergoing haemodialysis. Nosocomial infections occur due to the duration of dialysis, with or without blood transfusion (Dussol et al., 1995). People receiving haemodialysis at home have a lower risk of HCV infection compared to those receiving it at a medical facility (Noiri et al., 2001). To find out the origin of the virus, molecular, phylogenetic, and clinical analyses are necessary. However, in most cases, the specific mode of nosocomial transmission remains unknown. The hepatitis C virus is primarily transmitted through blood, such as when sharing needles or using contaminated medical equipment (Prati, 2006). Individuals who undergo hemodialysis are at a high risk of transmitting hepatitis C. These increased rates are mainly attributed to blood transfusions and the duration of dialysis treatment (Salama et al., 2000). Limited information is available regarding the prevalence and incidence rates of HCV among dialysis patients in developing countries (Olut et al., 2005). Based on data from these countries, the rates of HCV frequency and incidence tend to be significantly higher. This is likely due to HCV nosocomial infection in the HD setting, inadequate screening of blood and blood products for HCV, and a high overall HCV incidence in the community. The primary method of diagnosing HCV in individuals receiving dialysis is testing for antibodies to HCV proteins using enzyme-linked immunoassays (ELISAs) (Dussol et al., 1995). The objective of this study was to assess the prevalence of HCV in patients undergoing hemodialysis compared to healthy controls in Kirkuk city, and to investigate the role of IL-6 in the infection.

## Materıals and Methods

**The study design:** A cross-sectional study was conducted at Al-Hussein Teaching Hospital from April 1st to September 20th, 2024. The study included 200 patients, aged between 20 and 75 years, who had chronic renal disease and were undergoing hemodialysis. The interviews were conducted using a questionnaire specifically designed for this study. The questionnaire covered various demographic characteristics, including age, gender, and place of residence. Additionally, 50 healthy control individuals, aged 18-50 years and without any apparent diseases, were recruited from the Main Blood Bank in Kirkuk city during blood donation visits.

**Sample collection:** Five mL of blood were collected from each participant using a 5 mL syringe via venipuncture. The blood samples were divided into two tubes. A plain tube was left at 37°C for 30 minutes to clot, then centrifuged at 3000 rpm for 10 minutes. The serum obtained was aspirated using an automatic micropipette, transferred to Eppendorf tubes, and stored at -20°C for serological testing to detect specific HCV antibodies and IL-6 levels using the ELISA technique. A second tube containing the anticoagulant EDTA was used to obtain plasma, which was then aspirated using an automatic micropipette, transferred to Eppendorf tubes, and stored at -20°C for HCV viral load determination by PCR.

**Isolation of HCV RNA:** The isolation of HCV RNA was carried out using the Zymo Research Corp. kit. For buffer preparation, beta-mercaptoethanol was added to the viral RNA

buffer to achieve a final concentration of 0.5% (v/v). Twenty-four mL of 100% ethanol was added to the 6 mL viral wash buffer concentrate (R1034) to obtain 24 mL of viral wash buffer concentrate (R1035). Three volumes of viral RNA buffer were added to each plasma sample and mixed. The sample was then transferred to the Zymo-spin™ IC column 2 placed in a collection tube and centrifuged for 2 minutes; the flow-through was discarded. Viral wash buffer (500 µL) was added to the column and centrifuged for 2 minutes. The column was carefully moved to a DNase/RNase-free tube. DNase/RNase-free water (15 µL) was directly added to the column matrix and centrifuged for 30 seconds. The eluted RNA was used immediately or stored at -70°C for later use.

**Quantitative detection of HCV by Real-Time PCR:** The total reaction volume was 25 µl, with 12.5 µl of the RNA sample. Reagents were thawed, vortexed, and briefly centrifuged. Reaction tubes were prepared, including three extraction controls, a negative amplification control, and four standards. The entire contents of the tube with RT-PCR-mix-2-FRT were added to the tube with DTT and thoroughly vortexed. Tubes for samples, controls, and standards were prepared with the Reaction Mix as follows: (7.5 µL of RT-PCR-mix-1-HCV, 5 µL of RT-PCR-mix-2-FRT and DTT mix, 0.5 µL of Polymerase (TaqF), 0.25 µL of TM-Revertase (MMlv).
Equal volume (12.5 µL) of the reaction mixture and extracted RNA sample was added. Two standards, QS1 and QS2, were prepared for each run, adding 12.5 µL each and mixing by pipetting. The tubes were inserted into the thermocycler, and the instrument was programmed.

**Protocol and Data Analysis: Principle of Interpretation:** The signal of the internal control cDNA amplification product is detected in the FAM channel. The signal of the HCV cDNA amplification product is detected in the JOE channel. The results are interpreted based on the presence or absence of the intercept between the fluorescence curve and the threshold line, determining the Ct values of the sample. Using the Ct values and the specified values of the calibrators QS1 HCV and QS2 HCV, the calibration line will provide the number of HCV cDNA copies (JOE channel) and internal control cDNA copies (FAM channel) in a PCR sample. The HCV RNA concentration is calculated using the following formula: HCV cDNA copies per PCR sample divided by IC cDNA copies per PCR sample, then multiplied by coefficient A and coefficient B, which equals IU/ml of plasma. Specimens with Ct < 40 in the JOE channel are interpreted as positive for HCV, regardless of the FAM channel (IC) results. Specimens with Ct indicated as N/A in the JOE channel and with Ct < 34 in the FAM channel are interpreted as negative for HCV. Specimens with Ct absent or > 34 in both the FAM and JOE channels are interpreted as invalid.

**Results:** The results indicate a significant difference in the prevalence of anti-HCV antibodies between HD patients and the control group. Among the HD patients, 18% tested positive for anti-HCV antibodies, whereas none of the individuals in the control group tested positive (P-value 0.003), Table 1. This suggests that the prevalence of HCV infection is significantly higher in HD patients compared to the healthy control group. Our ELISA-positive results were confirmed by presence of HCV RNA in HD patients. Among HD patients who tested positive for anti-HCV

antibodies by ELISA, 83.33% were confirmed to have HCV RNA by PCR, indicating active HCV infection. None of the ELISA-negative HD patients had detectable HCV RNA, nor did any individuals in the control group. This significant finding demonstrates the reliability of ELISA as a preliminary screening tool for HCV infection in HD patients, with PCR confirmation highlighting the necessity for prompt and targeted antiviral therapy, Table 2.

**Table (1): Prevalence of HCV in patients under hemodialysis.**

| Anti-Hcv Ab (Elısa) | Hd Patients | | Control Group | | *P. Value* |
|---|---|---|---|---|---|
| | No. | % | No. | % | |
| Positive | 36 | 18 | 0 | 0 | 0.003 |
| Negative | 164 | 86 | 50 | 100 | |
| Total | 200 | 100 | 50 | 100 | |

**Table 2: Positive HCV RNA in HD patients.**

| Hcv Rna (Pcr) | Hd Patients | | | | Control Group | | | |
|---|---|---|---|---|---|---|---|---|
| | Elısa +Ve | | Elısa -Ve | | Elısa +Ve | | Elısa –Ve | |
| | No. | % | No. | % | No. | % | No. | % |
| Positive | 30 | 83.33 | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative | 6 | 16.67 | 164 | 100 | 50 | 100 | 0 | 0 |
| Total | 36 | 100 | 164 | 100 | 50 | 100 | 0 | 0 |

The distribution of HCV viral loads among the 36 HCV RNA-positive HD patients shows notable variation, with a significant proportion (44.44%) having viral loads between 9,000 and 11,000 IU/ml, indicating considerable viral replication in this subgroup. Additionally, 22.22% had viral loads ranging from 6,000 to 8,000 IU/ml. Both the lowest (< 6,000 IU/ml) and the highest (> 11,000 IU/ml) viral load categories each accounted for 16.67% of the patients. This data highlights the varying levels of viral replication among HCV-infected HD patients, with nearly half exhibiting high viral loads. The presence of patients with undetectable viral loads (< 6,000 IU/ml) may reflect early-stage infection or effective immune control in a subset of the population,Table 3. In the present study, it was found that a higher percentage of males (60%) were recorded with HCV infections compared to females (40%). The age group with the highest rate of HCV infection was 70-79 years, with a rate of 33.33%. Following that, the age group 60-69 had a rate of 23.33%. Additionally, the mean age of HD patients with HCV was 50.22 years, as shown in Table 4. It was also found that a higher percentage of males (60%) were recorded with HCV infections compared to females (40%).

**Table (3): Distribution of hepatitis C according to viral load.**

| Viral Load IU/Ml | No. | % |
|---|---|---|
| < 6,000 (Not Detected) | 6 | 16.67 |
| 6000-8000 | 8 | 22.22 |
| 9000-11000 | 16 | 44.44 |
| >11,000 | 6 | 16.67 |
| Total | 36 | 100. |

**Table 4: Relation of HCV infection with age of patients under hemodialysis.**

| Age Groups (Years) | Total No. (200) | HCV +Ve PCR (N:30) | |
|---|---|---|---|
| | | No. | % |
| 20-29 | 18 | 1 | 3.33 |
| 30-39 | 20 | 3 | 10 |
| 40-49 | 28 | 4 | 13.33 |
| 50-59 | 48 | 5 | 16.67 |
| 60-69 | 44 | 7 | 23.33 |
| 70-79 | 42 | 10 | 33.33 |
| Mean Age (Years) | | 50.22 | |

Then, we wanted to compare the levels of IL-6 in the study groups and determine the correlation between IL-6 and HCV infection. Our results indicated that the highest mean level of IL-6 was recorded in HD patients with HCV infection, compared to patients without HCV infection (67.53 vs. 40.26 pg/ml), and the lowest level was found in the control group (10.26 pg/ml) (p: 0.001), Table 5. Among hemodialysis patients, there was a strong positive correlation between hepatitis C viral load and IL-6 (r: 0.71, P<0.01), Figure 1. Furthermore, our results showed a significant positive correlation between hepatitis C viral load and ALT and between ALT and IL-6 among hemodialysis patients (r: 0.45, P<0.01), (r: 0.65, P<0.01), respectively, Figure 2 and 3.

**Table (5): Level of IL-6 in HD patients with and without HCV infection and the control group**

| Level Of IL-6 (Pg/Ml) | HD Patients | | Control Group | P. Value |
|---|---|---|---|---|
| | HCV +Ve | HCV –Ve | | |
| No. | 30 | 170 | 50 | 0.001 |
| Mean±SD. | 67.53±10.45 | 40.26±8.17 | 10.26±2.18 | |

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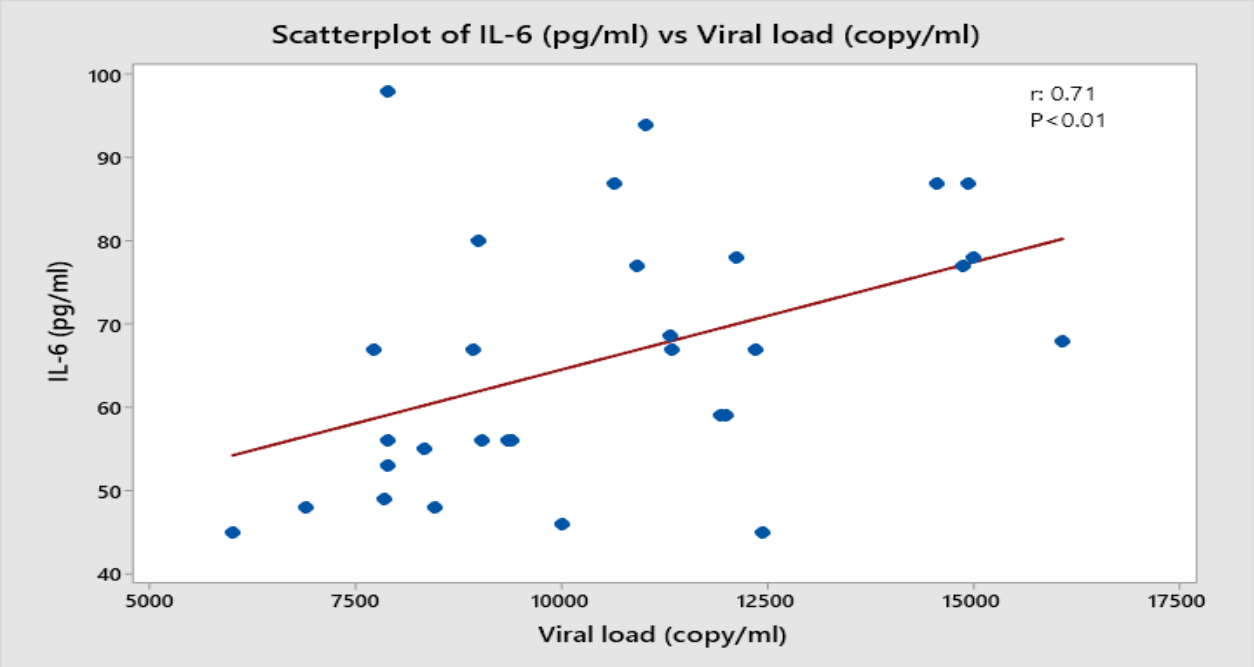


**Figure (1): Correlation between hepatitis C viral load ad IL-6**

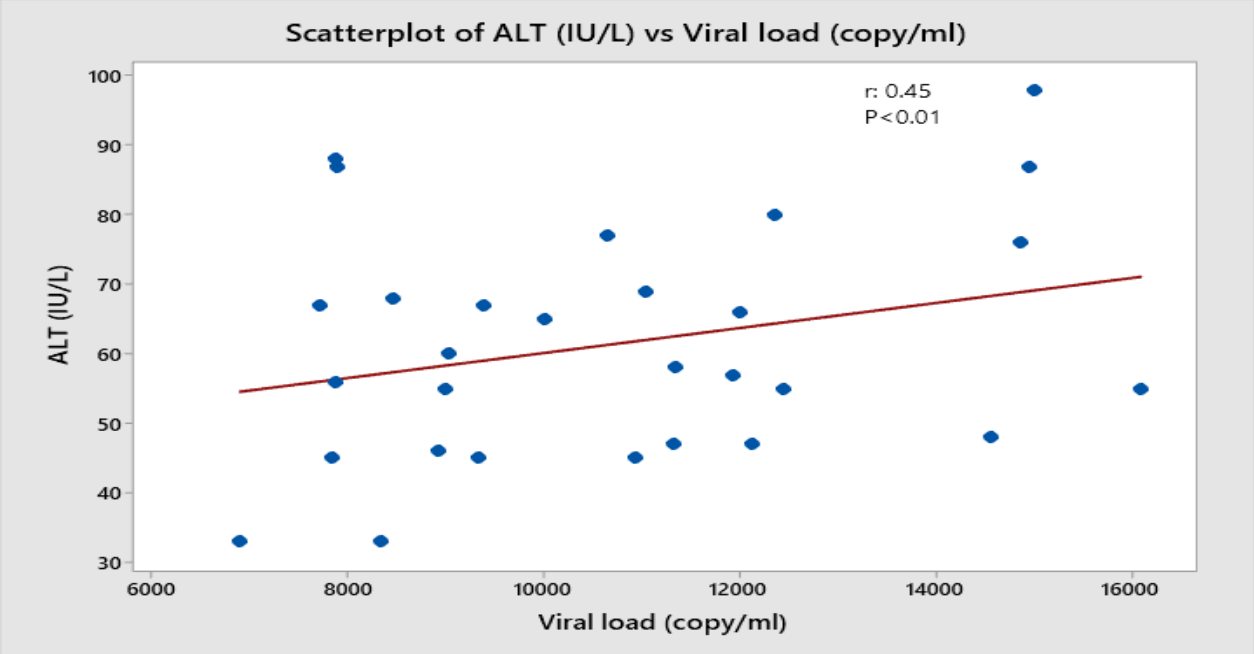


**Figure (2): Correlation between hepatitis C viral load and ALT**

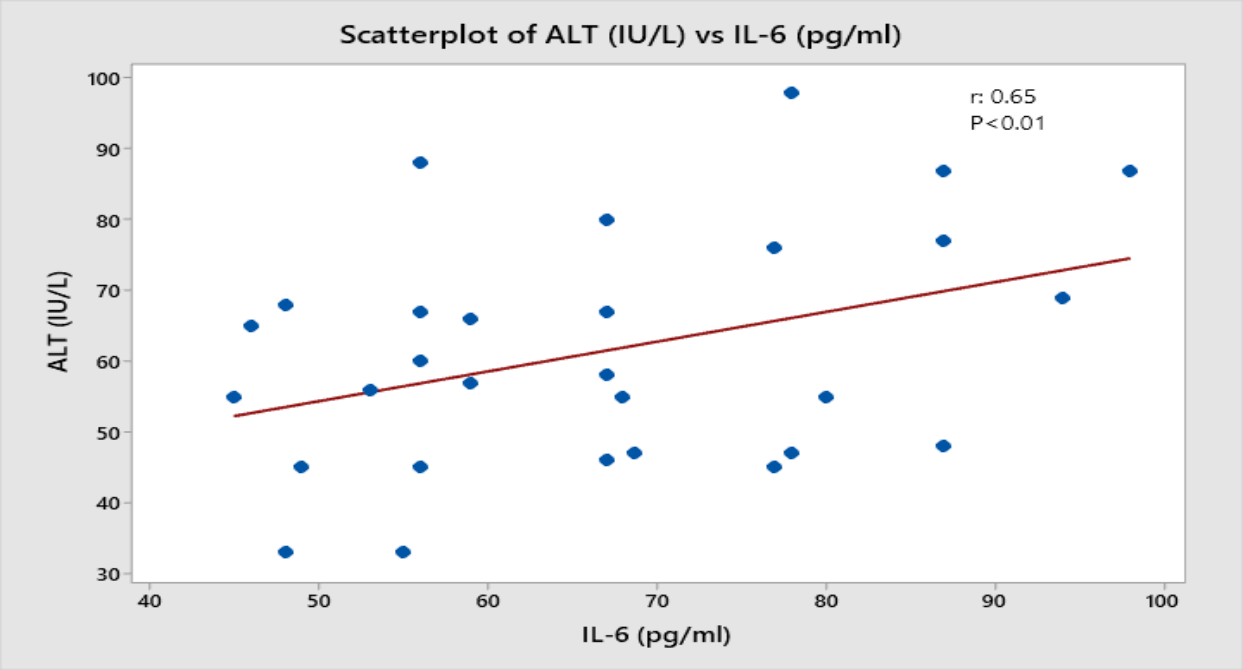


**Figure (3): Correlation between IL-6 and ALT**

**Discussion:** Even though strong rules are in place to stop the spread of infection in hemodialysis units, viral hepatitis is still a real threat to our country. Virus hepatitis C, which is not very common in Iraq, is especially dangerous (Ibrahim and Hashem, 2019). Hepatitis C virus infection is still a problem and a risk for people who need ongoing hemodialysis, including people who are on hemodialysis. It also greatly raises the risk of death. Diagnosing HCV viruses while they are still very active is important for starting antiviral treatment right away to lessen their effects. Early identification of HCV infection is also important to stop the infection from spreading, especially in hemodialysis patients who are more likely to get sick because they have to go through a lot of invasive treatments (Othman and Abbas, 2020). Ashkani-Esfahani et al. (2017) looked at how common HCV infections were among hemodialysis patients in some countries in the region. They found lower rates in Lebanon (9%), Iran (12%), Saudi Arabia (19%), Iraq (20%), Turkey (23%), and Palestine (18%). Still, this study agreed with Khattab et al.'s findings that anti-HCV antibodies were found in 12 (7.1%) of Iraqi hemodialysis patients and in 7 (6.3%) of the 112 (66.3%) patients who had never had a blood transfer.

Studies have talked about a rise in the viral load in patients' blood, but only 25% of patients were found to have a high viral load. In this study, however, 71.9% of patients had high viremia (Sidharthan et al., 2015). This difference had to do with the genotype of HCV, especially genotype 3 HCV, which was found to cause lower viremia. The cut-off number may also be different depending on the company and method (Wlassow et al., 2019). Also, the viral load changed depending on how long the sickness lasted; the highest levels of viremia were seen between 4 and 12 weeks after infection (Lee et al., 2014). Our results agree with those of Al-Qahtani et al. (2016), who also found that most of the infected cases were guys. According to the results of this study, the frequency was higher in older people. This could be because older

people are more likely to have kidney diseases. Also, the rate of hepatitis C was higher among patients who had hemodialysis for more than 5 years compared to those who had it for a shorter time. These results support what Ashkani-Esfahani et al. (2017) already said. In their 2018 study, Amen et al. found that 3.6% of the samples tested positive for HCV (5 males and 4 females, n=250) and 3.2% of the samples tested positive (3 males and 3 females, n=250). Across all cases, the age groups most affected were those between 31 and 40 years old (5 cases of control) and those between 41 and 50 years old (5 cases of HCV). Several studies have shown that T-cell immunoregulatory cytokines are very important for both the survival of HCV and the amount of damage to the liver. Some cytokines, like IL-6, may cause inflammation and can help T-cells become Th-1 immune cells (Sghaier et al., 2017). According to Shah et al. (2015), people who have a chronic HCV infection have much higher amounts of IL-6 cytokines in their blood. IFN treatment lowers these levels, but the HCV RNA load goes up. Salgüero et al. (2020) said that higher amounts of the Th1 cytokine IL-6 were linked to active liver damage in people with a chronic HCV infection. Some writers have said that a change toward the Th1 response might affect the result of the disease and how fast it spreads (Guzmán-Fulgencio et al., 2012). This big change in cytokine patterns in chronic hepatitis C towards Th1 pattern is more noticeable than in occult HCV infection. This suggests that the differences in symptoms and histology seen between occult and chronic hepatitis C may be due to the host's immune system and the cytokines it makes. This is because a shift in cytokines toward Th1 is linked to faster disease progression (Mourtzikou et al., 2014). There are two main ways that cytokines help the body fight off viral infections: indirectly, by deciding how the body will respond, and directly, by stopping the virus from replicating. On the other hand, cytokines may damage the liver during an inflammatory reaction to a virus (Sghaier et al., 2017). In this study, people with acute and chronic hepatitis had higher levels of IL-6 in their blood than healthy controls. The results were similar to those of other studies that found higher levels of IL-6 in people with hepattitis (Tarragô et al., 2014).

**Conclusions:** The study demonstrates a significantly higher prevalence of anti-HCV antibodies among hemodialysis (HD) patients compared to a healthy control group, with 18% of HD patients testing positive versus none in the control group. The distribution of HCV viral loads revealed notable variation, with a significant proportion exhibiting high viral loads, indicating varying levels of viral replication among patients. Additionally, the study found a higher prevalence of HCV infection in older age groups and a greater incidence among males. Elevated IL-6 levels were observed in HCV-infected HD patients compared to non-infected patients and controls, with a strong positive correlation between HCV viral load, IL-6, and ALT levels.

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**Competing interests:** The following authors have no competing interests.

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