

Immune Assay and Molecular Prevalence of Viral Hepatitis B and C among Hemodialysis Patients in Misan Province, Iraq

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Abstract

Background: In recent years, the number of kidney failure patients has increased, and due to a shortage of dialysis machines in Misan Province, the likelihood of hepatitis virus infection has risen due to frequent blood transfusions.

Objective: This study aimed to investigate hepatitis virus infection rates among hemodialysis patients and compare diagnostic results using Real-Time PCR and ELISA methods.

Methods: A cross-sectional study was conducted on 102 patients with kidney failure undergoing regular hemodialysis at the hemodialysis center in Al-Sadar Teaching Hospital, Misan Province, from January 2025 to December 2025. Serological and molecular assays were performed to detect HBV and HCV.

Results: Among the 102 hemodialysis patients, the prevalence of Hepatitis B virus (HBV) infection was 39.2% using ELISA and 16.7% using Real-Time PCR. Meanwhile, Hepatitis C virus (HCV) infection prevalence was 32.4% using ELISA and 14.7% using Real-Time PCR. Statistical analysis revealed no significant differences in infection rates based on sex or age groups across all tested parameters. Furthermore, comparison between ELISA and PCR demonstrated a clear discrepancy in positive cases, illustrating differing analytical sensitivities between serological and molecular techniques in detecting viral infections.

Conclusion: The study concluded that HBV prevalence is higher than HCV among hemodialysis patients. Demographic factors like age and sex showed no significant correlation with infection rates, indicating that healthcare-associated and dialysis-related exposures play a primary role in viral transmission. Single diagnostic methods are insufficient for comprehensive screening.

Keywords: Hemodialysis; hepatitis B and C; Kidney failure; RT-PCR; ELISA

1. Introduction

The primary kidney's function acts as filtration system; Kidney failure occurs when the kidneys are damage and no longer to do their functions. There are two types of Kidney failure according to the severity of kidney functions loss: Acute kidney injury (AKI) occurs when the kidneys' ability to purify blood of harmful wastes and excess salt is impaired [1]. Chronic Kidney Disease (CKD) is the last stage of kidney disease because it generally starts slowly, gradually, and catastrophically. In most cases, the patient no suffers signs that signal kidney damage or illness until it has progressed to the point of kidney failure., Diabetes, high blood pressure, some immunological diseases, some genetic diseases, and other factors can all contribute to chronic kidney disease failure [2]. End-stage kidney disease (ESKD) is a huge healthcare burden across the world, necessitating expensive renal replacement therapy such as dialysis or transplantation. However, the availability of healthy donors' limits kidney transplantation as a therapy option [3].

Hemodialysis HD is a life-sustaining therapy for those whose kidneys aren't functioning correctly. A catheter is inserted into a big vein and the individual is attached to a dialysis machine, which cleans the blood and returns it to

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the person [4]. As a direct result of uremia and its metabolic ramifications, patients on hemodialysis have immune system abnormalities, rendering them more susceptible to infections. Poor antigen processing, antibody production, and cell-mediated immune response are all affected by defects in neutrophils, lymphocytes B and T, and monocytes, for example, resulting in an increase in the incidence of microbial infections [5]. Patients are exposed to quantities of water ranging from 15000 to 20000L of dialysis fluid annually and non-selectively during treatment sessions that last between 4 and 6 hours, three times a week. As a result, all low-molecular-weight compounds in water have direct access to the patient's bloodstream [6].

Patients with kidney failure are at high risk to infected with Hepatitis B viruses (HBV) and hepatitis C viruses (HCV) because the using of needles always, blood transform, and hemodialysis apparatus leads to high possibility risk of contamination [7]. Globally, more 350 million of people are infected with HBV [8]. The prevalence of hepatitis viruses in Iraq considered an important health issue, despite the lack of specific national statistics but some studies in different cities showed continuous rise in the number of infections specially the southern cities [9],[10]. hepatitis B and hepatitis C viruses are extremely infectious and can spread from person to person by vertical, blood transfusions, and sexual contact. Most of these viral infections affect people who have kidney disease. Because kidney disease patients are unable to properly remove viral infections, immunodeficiency is linked to kidney disease. Patients with end-stage renal disease are at high risk to getting hepatitis B and hepatitis C infections due to their compromised immune systems [11] Hepatitis b virus is DNA virus that one of the hepadnaviruses, whereas the hepatitis c virus is RNA virus one of the flaviviruses. The mode of transmission of HBV and HCV are primarily via the blood [12], both viruses can be transmitted via needle-stick injury, but the risk for HBV is higher than HCV [13]. sexual transmission is another important rote for HBV and it is uncommon for HCV. Mother to child transmission during birth is another natural transmission for both viruses [14] This study aimed to determine the prevalence of hepatitis B viruses and C infection in hemodialysis patient in Misan Province, Iraq, by ELISA and real time PCR

2. Material and Methods

2.1 Study Design, Sample Collection and Processing

The cross-sectional study was conducted on the following study groups during the period Jan2025 to 30th of December 2025. Patients were recruited from hemodialysis center/ Al-Sadar teaching Hospital in Misan Province. A total of 102 participating patients were suffered from kidney failure and undergoing to dialysis. All patients attended for at least six months on hemodialysis were included in study records, reviewed and analyzed. A 5 ml of venous blood was collected from each participant. The blood sample was placed in a gel and EDTA tube for serum and plasma isolation.

2.2 Immunological study (Enzyme-linked Immunosorbent Assay (ELISA))

serum of Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibodies (HCV Ab) were tested using the commercial ELISA kit (Advanced HBsAg and HCV diagnostic kit, China) following the manufacturer's instruction.

2.3 Molecular study

2.3.1 Extraction of viral nucleic acid

HBV DNA and HCV RNA were extracted from each patient plasma using QIA amp commercial viral DNA extraction kit (Qiagen) and QIA amp commercial viral RNA extraction kit (Qiagen), respectively, following the manufacturer's instructions.

2.3.2 Real-time Polymerase Chain Reaction

Viral nucleic acid was amplified for HBV and HCV viral load using the artus HBV and HCV RG PCR-based RT-PCR (Qiagen) by the Real time PCR.

2.4 Statistical analysis

The data were analyzed using SPSS program. *P* values ≤ 0.05 were considered statistically significant. The statistical significance was assessed by Chi-square.

3. Results

3.1 Distribution of patients according to age and sex

A total of 102 patients; 47(46.1%) were female and 55 (53.9%) were male (Figure, 1). The patients were divided into 2 age groups, as shown in Figure, 1. The age groups with ages ≥ 50 years were represented 44(43.1%) patients, while, < 50 years were represented 58 (56.9%) patients.

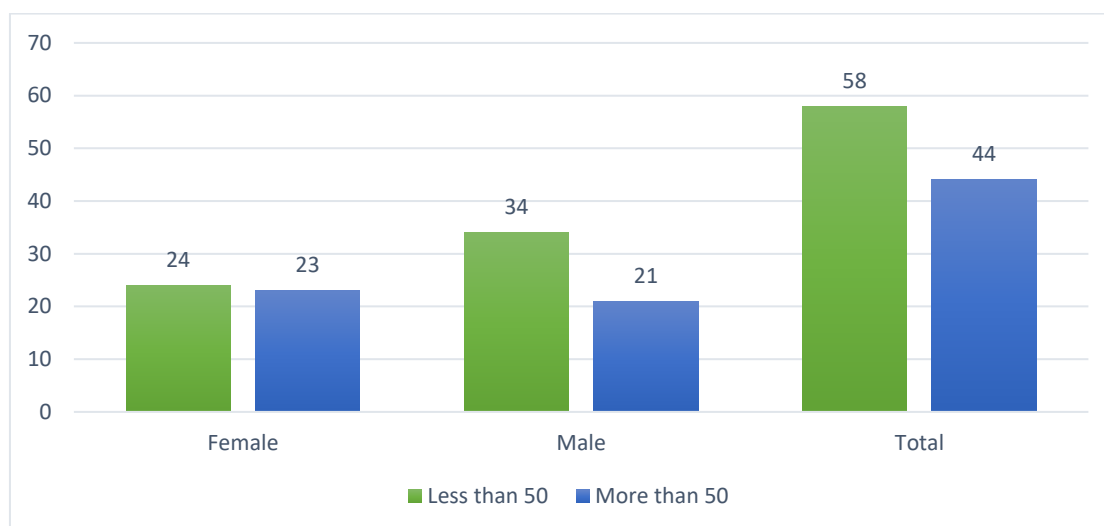


Figure (1) distributed of patient according to age and sex

3.2 ELISA Assay vs qPCR

3.2.1 HBV

The results revealed that HBsAg positive was 40 (39.2%) cases; 19 (47.5%) of them was female and 21(52.5%) was male (Figure, 2). Surprisingly, by real time PCR HBV DNA positive was 17(16.7%) cases; 5 (29.4%) of them female and 12 (70.6%) of them male (Figure, 3). There was no significant different between sex and HBV infection that tested by ELISA or real time PCR (P. value = 0.817 and P = 0.131 respectively). A total of 8 patients showed positive results of HBSAg and HBV DNA, and 53 patients were negative to ELISA assay and real time PCR. The age groups with ages ≥ 50 years with 19(47.5%) and 7(15.9%) cases were HBSAg positive and real time PCR HBV DNA positive, respectively, while, 21(52.5%) and 10(17.2%) cases were within < 50 years age groups of HBSAg positive and real time PCR HBV DNA positive, respectively. There was no significant different (P. value = 0.475 and P = 0.858) between age groups that tested by ELISA or real time PCR respectively.

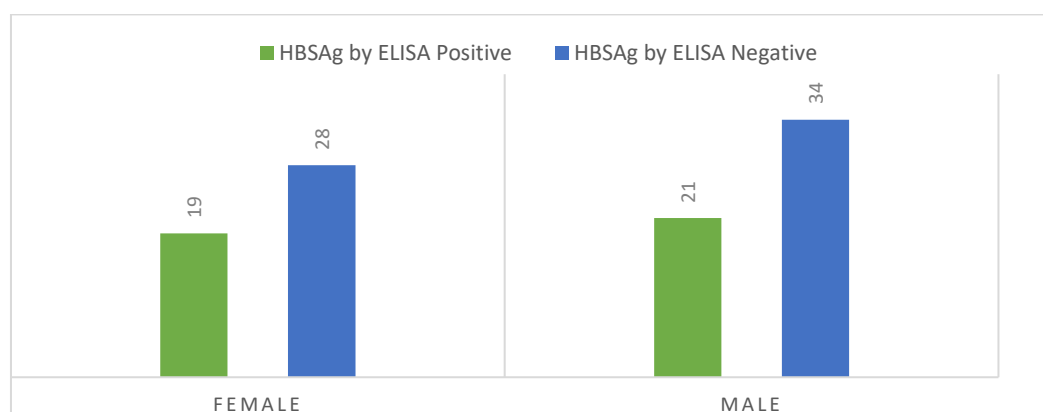


Figure (2) HBSAg positive according to sex

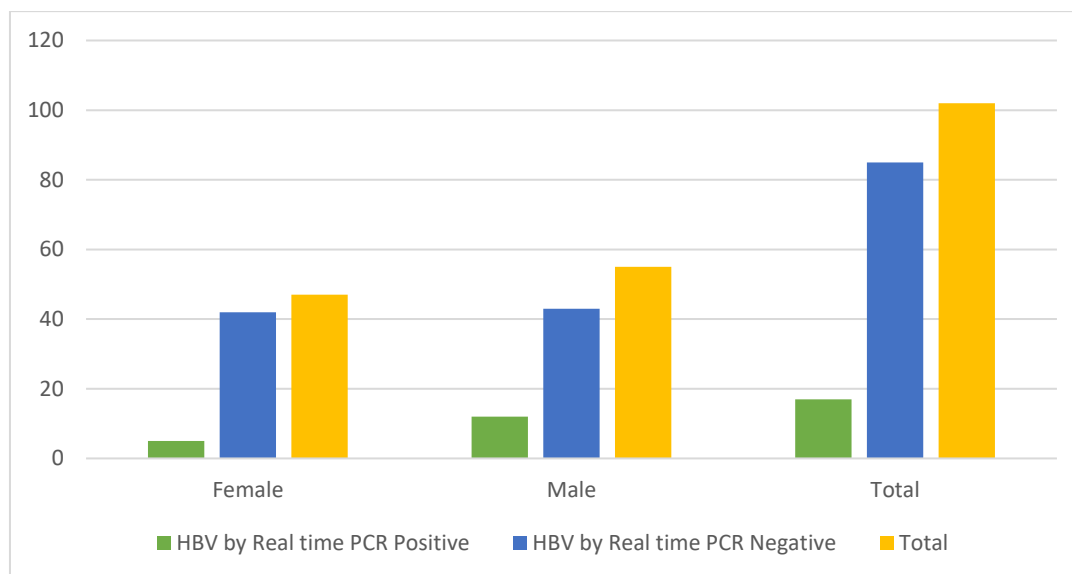


Figure (3) HBV positive by real time PCR according to sex

3.2.2 HCV

The results revealed that HCV Ab positive was 33(32.4 %) cases; 14(42.4%) of them was female and 19(57.6%) was male (Figure, 4). Furthermore, by real time RT-PCR HCV RNA positive was 15(14.7%) cases; 10(66.7%) of them female and 5(33.3%) of them male (Figure, 5). There was no significant different between sex and HCV infection that tested by ELISA or real time RT-PCR respectively ($P = 0.609$ and $P = 0.083$). HCV-Abs and HCV RNA were detected among 4 patients, while 58 patients were negative to ELISA assay and real time RT-PCR. The age groups with ages ≥ 50 years with 11(33.3%) and 6(40.0%) cases were HCV Ab positive and real time PCR HCV RNA positive, respectively, while, 22(66.7%) and 9(60.0%) cases were within < 50 years age groups of HCV Ab positive and real time RT-PCR HCV RNA positive, respectively. There was no significant different ($P = 0.167$ and $P = 0.791$) between age groups that tested by ELISA or real time RT-PCR respectively.

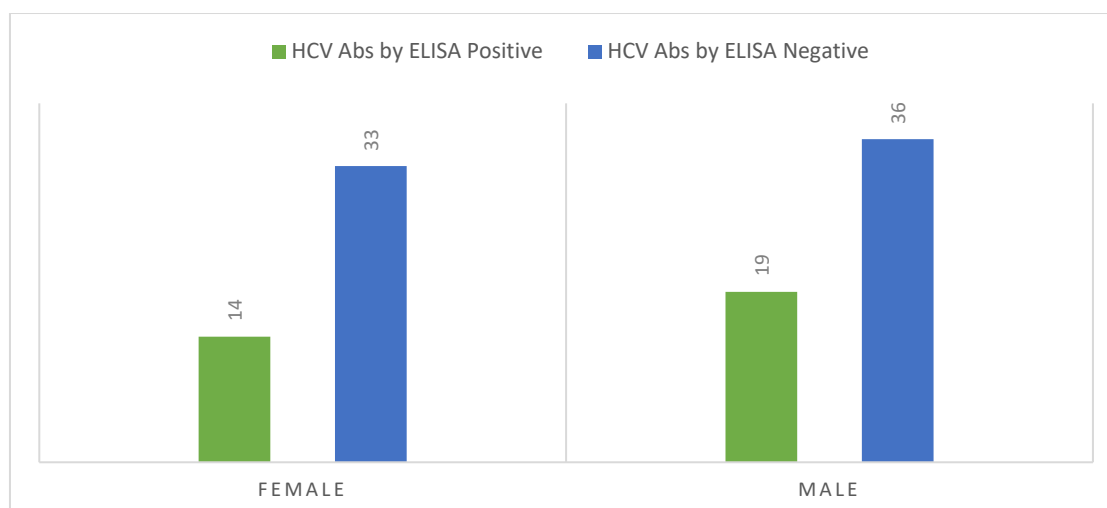


Figure (4) HCV Ab positive according to sex

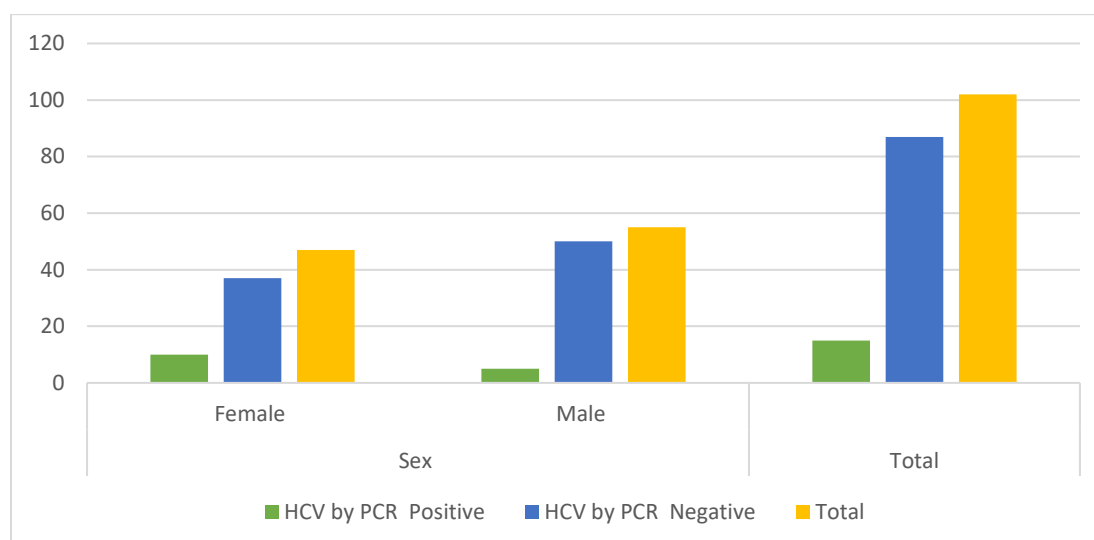


Figure (5)HCV positive by real time PCR according to sex

4. Discussion

This study identified a substantial burden of hepatitis viral markers among 102 patients receiving dialysis. HBsAg was detected in 39.2 % of participants, whereas HBV DNA was detectable in 16.7%; anti-HCV was detected in 32.4%, whereas HCV RNA was detectable in 14.7%. The most informative observation is therefore not the basic prevalence alone, but the pronounced discordance between immunological and molecular results. Immunological assays indicate exposure or antigenemia, while nucleic acid assays identify detectable circulating viral genomes at the time of sampling. Therefore, the two approaches consider as complementary rather than competing measures of a single identical state [15],[16] The HBV marker prevalence in the current study is markedly higher than pooled international estimates. A 2023 systematic review comprising 322 datasets and 795,623 haemodialysis patients estimated pooled prevalences of 7.57 % for HBsAg and 6.09% for HBV DNA, with very high between study heterogeneity [17]. Nonetheless, direct numerical comparison must remain cautious because the global synthesis combined different countries, calendar periods, laboratory platforms, sampling strategies, vaccination coverage, dialysis-unit practices, and patient risk profiles.

A recent multicentre study of 637 haemodialysis patients in Damascus reported HBsAg and anti-HCV prevalence of 3.2 % and 22.1% respectively [18]. The lower seroprevalence observed there underscores the considerable variation that can exist even within the Eastern Mediterranean region. Such variation may reflect differences in background endemicity, historical transfusion exposure, infection control implementation, patient turnover, vaccination uptake, and the duration of maintenance dialysis [19]. For HCV, the anti-HCV prevalence of 32.4 % is above the global pooled estimate of 24.5% but is close to the pooled estimate reported for Western Asia (34.9%). The HCV-RNA prevalence of 14.7% is below the pooled molecular estimate of approximately 23% [20]. These comparisons reinforce the importance of specifying the diagnostic target. KDIGO therefore recommends an immunoassay followed by nucleic acid testing when the immunoassay is reactive, while allowing initial nucleic acid testing in high-prevalence dialysis units [15]

4.1 HBV marker

The relationship between HBsAg and HBV DNA provides additional information beyond their individual prevalence. Eight patients had both detectable HBsAg and HBV DNA, representing the clearest evidence of surface antigen production accompanied by circulating viral DNA. These patients are likely to have ongoing HBV infection with measurable replication, although assessment of viral load, alanine aminotransferase, HBsAg status and liver fibrosis would be required to determine the phase and clinical activity of infection. In the same current study, 32 patients were HBsAg positive but HBV DNA negative. This pattern does not necessarily represent an inconsistent result because HBsAg and HBV DNA describe different aspects of HBV infection. HBsAg reflects the production of viral

surface proteins, whereas serum HBV DNA reflects the amount of circulating viral genetic material [21]. Surface antigen production may therefore continue when viral replication is low, intermittently detectable or suppressed by antiviral treatment. HBV DNA may also fall below the analytical sensitivity of the molecular assay, particularly in patients with low level infection. Information concerning recent vaccination, antiviral treatment, previous HBV results and quantitative HBV DNA would help differentiate suppressed infection, low replicative infection and transient antigen detection. The 2025 EASL guideline recommends resolving uncertain HBsAg patterns using anti HBc, HBV DNA, sequential HBsAg measurements and, where required, an HBsAg neutralisation assay [16]. Possible biological explanations include very low antigen concentrations, intermittent antigen expression or viral surface variants that are not efficiently recognised by the assay. This subgroup is clinically important because it would remain unidentified if screening depended exclusively on HBsAg. Previous studies reported occult HBV prevalence rates of 5 % among haemodialysis patients in Sana'a and 7.3% in a Ghanaian haemodialysis cohort [22-24]. These findings support the added value of molecular testing in high-risk dialysis populations.

4.2 HCV

Four patients were positive by both methods, indicating previous exposure accompanied by current detectable viraemia. The 29 anti HCV positive/HCV RNA negative patients probably represent a mixture of previously resolved infections, successful antiviral treatment and, less commonly, non-specific antibody reactivity [25]. This may indicate that the cumulative history of HCV infection in this dialysis population is considerably greater than the burden of current viraemia. KDIGO explicitly states that anti-HCV testing cannot distinguish resolved infection from current infection and that nucleic acid testing is required to identify viraemia [15]. Eleven patients were HCV RNA positive despite having negative anti HCV results. This pattern may occur during the early stage of infection, when viral RNA is already present but antibodies have not yet developed. It may also occur when antibody production is delayed or reduced, which is particularly relevant in haemodialysis because uraemia and chronic illness can impair humoral immune responses. KDIGO notes that an anti-HCV negative/HCV RNA positive profile strongly suggests acute infection in the appropriate clinical setting and that seroconversion may be delayed for several months among haemodialysis patients [15]. It would classify the 29 RNA negative participants as infected despite the absence of detectable viraemia, while failing to identify the 11 RNA positive participants who lacked detectable antibodies. Molecular testing therefore refines serological screening by separating historical exposure from current infection and by recognising infection before antibody development [26,27].

4.3 Age and sex distributions

HBV DNA was more frequently observed among men, whereas HCV RNA was more frequently observed among women. The findings were comparable between patients younger than 50 years and those aged 50 years or older. A younger patient who has undergone dialysis for many years, received several transfusions or attended multiple treatment centres may have a greater exposure history than an older patient who recently began dialysis. While older patients may have accumulated exposures through surgery, transfusion or previous medical care before starting dialysis [28]. These findings suggest that future analysis should prioritise dialysis specific and clinical variables rather than relying mainly on age and sex. Particularly relevant factors include duration of dialysis, number of weekly sessions, previous dialysis in another facility, history and number of blood transfusions, vascular access type, previous surgery, HBV vaccination response, antiviral treatment history and earlier hepatitis test results. The Damascus multicentre study similarly identified dialysis-related factors and vaccination history as more informative than demographic variables alone [18].

5. Conclusion

The study showed the prevalence of HBV among haemodialysis patients is more than HCV, and the real time PCR is more accurate than ELISA and detecting HBV and HCV by one method is insufficient, no significant was observed between most demographic variables (age and sex) and hepatitis infection, suggesting that dialysis-related factors may play a greater role in virus transmission.

6. Declaration

6.1 Acknowledgments : NIL

6.2 Ethical consideration: This is a cross-sectional study; no ethical approval is required.

6.3 Authors' contribution: All authors contributed to the study design, collection of data, analysis, and read and approved the final version of the manuscript.

6.4 Conflict of Interests: The authors declare no conflict of interest.

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