



# Comparative Study Between Serological and Molecular Diagnosis test for HBV and HCV in Chronic Renal Failure Patients on Hemodialysis in Nineveh Government/Iraq

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**Abstract:** Patients under hemodialysis treatment for chronic renal failure (CRF) are among the groups with the highest prevalence of hepatitis B and C viruses due to frequent blood transfusion and nosocomial transmission. A group of CRF patients living in Nineveh governments were tested with serological markers for hepatitis B and C using the ELISA Enzyme linked immunosorbent Assay test and Polymerase Chain Reaction (PCR). The validity parameters for the serological results were measured based on the PCR results. Of the 62 patients on hemodialysis during the study, 13 (21%) were HBsAg positive, 49 (79%) were anti-HBs positive 8 (61.5%) were anti-HBc positive and 16 (25.8%) were anti-HCV positive, the PCR tests results in 13 (21%) HBV-DNA positive, the mean viral load were 78950 copy/ml and 15 (24.1%) were HCV-RNA positive the mean viral load were 125000 copy/ml, the accuracy, sensitivity and specificity of Elisa for HBsAg were 90.6%, 50% and 94.8%, and the same parameters were 92.6%, 87.5% and 92.9% for anti-HCV. Based on the results just the negative predictive value for anti-HCV (98.2%) is reliable test in CRF patients on hemodialysis tests are the indicated methodology to diagnosis HBV and HCV infection in these patients. Serological and/or molecular tests are the indicated methodology to diagnosis HBV and HCV infection in these patients.

**Key Words:** hepatitis B and C, ELISA, PCR, HBsAg, HCV-RNA.

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# دراسة مقارنة بين طرق الفحص المصلية والجزئية من أجل الكشف عن رواشح التهاب الكبد (HBV) و (HCV) في مرضى الفشل الكلوي المزمن في محافظة نينوى - العراق

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**الخلاصة:** يعتبر مرضى الفشل الكلوي الذين يعتمدون على غسيل الكلى الأكثر تعرضاً للإصابة براشح التهاب الكبد B و C نتيجة حاجتهم الى نقل الدم واستخدامهم المستمر لأجهزة غسيل الكلى. استخدمت مجموعة من مرضى الفشل الكلوي من أجل الفحوصات المصلية لمؤشرات كشف راشح التهاب الكبد B و C باستعمال طريقة اليزا وكذلك استخدام فحص تفاعل سلسلة البوليميريز لمعرفة أيهما أنسب في الكشف عن الرواشح الكبدية. من خلال 62 مريضاً استخدم في الدراسة كان هناك 13 (21%) موجبين للمستضد HBsAg و 49 (79%) موجبين للمستضد anti - HB و 8 (16.5%) موجبين للمستضد anti - HBC و 16 (25.8%) موجبين للمستضد anti HCV - ، بينما كانت نتائج تفاعل ال PCR أن هناك 13 (21%) موجبين للمستضد HBV - DNA حيث كان عدد النسخ فيها 78950 نسخة / سم<sup>3</sup> و 15 (24.1) موجبين للمستضد HCV - RNA وعدد النسخ 125000 نسخة / سم<sup>3</sup>. بينت نتائج الفحص المصلي بأن الفحص المصلي بطريقة اليزا بين حساسية الفحص للمستضد HBsAg وبنسبة 90.6% و 50% و 94.8% وللمستضد anti - HCV كانت 92.6% و 87.5% و 92.9% بينما كانت النتائج سلبية (98.2%) للمستضد anti - HCV ، توضح النتائج أن من المهم استعمال الطريقتين في الكشف عن راشح التهاب الكبد B و C في مرضى الفشل الكلوي المعتمدين على أجهزة غسيل الكلى.

## Introduction

The hepatitis B Virus (HBV) and hepatitis C virus (HCV) are challenging blood-borne disease that are prevalent worldwide patient infected with chronic hepatitis B (CHB) and chronic hepatitis C (CHC) are at a greatly risk for developing cirrhosis and hepatocellular carcinoma (HCC) (1).

The world health organization (WHO) states that 2 billion people worldwide are currently infected with HBV alone and of those, 350 million are infected with CHB, which results in one million deaths per year (2).

As for HCV, the WHO reports that 170 million people are infected with CHC(3). The diagnosis of the viral hepatitis has demonstrated to not be as reliable in

CRF patients ongoing hemodialysis as in patients without CRF (4,5).

Intrinsic factors associated with renal failure and the hemodialysis process itself produces inconsistencies in the serological, biochemical, and molecular test results (6). Including qualitative and qualitative difference, thus, there is a high risk of patients with CRF being misdiagnosed with HBV or HCV, with threatens the whole CRF community and increases the nosocomial transmission risk for patients and health care workers (7,8).

Because little known about the current prevalence of HBV and HCV among CRF patients ongoing hemodialysis the aims of study is to measure the accuracy of the serological and molecular test to diagnosis HBV and HCV marker in CRF patients on hemodialysis.

## Materials and Methods

Sixty tow blood samples were collected from hemodialysis unit in Ibn Sina hospital from March 2012 until September 2012.

The blood was collected in the first hour before the hemodialysis session, the samples were separated in three tubes for each patients; one went to serological tests processed in virological laboratory in Mosul central blood bank, it was used third generation Elisa Kits, from (Plasmatic, UK), for the following visual markers: HBV surface antigen (HBsAg), antibody to surface antigen (anti-HBsAg) total antibodies to HBV core antigen (anti-HBCO), and total antibody to the HCV (anti-HCV).

The Polymerase chain reaction (PCR) test for the determination viral load for HBV and HCV were performed in Ibn-Alatheer hospital. PCR center, using real time PCR system (Q Tower-Germany, anatyic jena company), it was used primer disgn kit Germany, test were carried as per manufacturer instruction using the primers:

Hepatitis B

Care Region:

1763: 5'-GCT T TG GGG CAT GGA CAT TGA CCC GTA TAA-3'.

2032R: S'-CTG ACT ACT AAT TCC CTGGA& GCT GGG TCT3'.

Hepatitis C

Region 5' NCR

NCR: 5'-ATA CTC GAG GTG CAG GGT CTA CGA GAC Ct-3'.

PTC1: S'-CGT TAG TAT GAG TGT CGT GC-3'.

PTC3: S'-AGT GTC GTG CAG CCT CCA G6-3'.

NCR4: S'-CAC TCT CG A GCA CCC TAT CAG GCA GT-3'.

The prevalence and 95% confidence intervals (CI) were calculated using the "exact" confidence intervals, computed by the methods of clipper and Pearson. The validity parameters for the serological methods used to diagnosis hepatitis B and C in hemodialysis were analyzed using the molecular test methods as reference. The odds ratio methods was used to analyze the correlation between categorical variables and the X2 (Chi-squared) test with fisher exact test were used to test theories on the differences between the percentages, a level of significance of  $\alpha=0.05$  was applied to all tests, the statistics software used to process the data analysis were the Microsoft Excel 2007 and the SPSS.

## Results

Sixty tow hemodialysis patients on hemodialysis unit were enrolled in the study. The patients were 32 (51.6%) males and 30 (49.4%) females, with ages ranging from 16-75 years, average of 47.3 (SD=14.1) years and mean of 49.

The most prevalent serological marker was anti-HBS, follow by the anti-HCV, HBsAg and anti-HBCO show in table (1).

**Table (1): frequency of HBV and HCV serology marker in hemodialysis patients in hemodialysis unit**

No. Of patients	HBsAg		Anti-HBsAg		Anti-HBC		Anti-HCV	
	Positive%	Negative%	Positive%	Negative%	Positive%	Negative%	Positive%	Negative%
62	13 (21%)	49 (79%)	49 (79%)	13 (21%)	8 (61.5%)	51(38.5%)	16(25.8%)	46(74.2%)

For the 62 samples, the PCR results 13(2%) HBV-DNA positive mean viral load 78950 copy/ml and 15 (24.1%).

HCV RNA positive mean viral load 125000 copy/ml show in table(2).

**Table (2): Results of molecular markers for HBV and HCV in hemodialysis patients**

Results	HBV-DNA			HCV-RNA		
	No.	%	CI (95%)	No.	%	CI (95%)
<b>Positive</b>	13	21	(5.2-16.2)	15	24.1	(7.5-19.8)
<b>Negative</b>	49	76	(84-94.8)	47	75.9	(80.2-92.5)
<b>Total</b>	62	100.0		62	100.0	

Note: CI = Confidence interval.

The molecular test and serology test found the same number of HBV positive samples, 13 but not same results in HCV positive results, just 15 samples were

positive in both tests was shown in table (2).

The only validity parameter that reaches over 95% was the negative predictive value (NPV) for anti-HCV table (3).

**Table (3): Results of the analysis of the validity parameters of the serological tests for HBsAg and anti-HCV in comparison with the results of the PCR tests in hemodialysis patients**

Analysis	HCV-RNA		HBV-DNA	
	Anti-HCV (%)	(CI 95%)	HBsAg (%)	(CI 95%)
Accuracy	92.2 (86;96)		90.6 (84;95)	
Sensitivity	87.5 (60;98)		50.0 (22;78)	
Specificity	92.9(86;97)		94.8 (89;98)	
PPV	63.6(41;81)		51.3 (45;58)	
NPV	98.3(94;99)		66.7 (41;87)	

Note: PPV= Positive predictive value; NPV=Negative predictive value.

## Discussion

The implementation of blood borne disease transmission control protocols in hemodialysis units have been shown to decrease the prevalence of HBV and HCV in patients with CRF (9, 10). Such protocols require that all patients and staff to receive the vaccine for hepatitis B, enforce the use of individual protection equipment and provide hemodialysis and dialysis filters in a separate machine or room for viral hepatitis seropositive patients (9). The HBsAg and anti-HBC rates in the general population of Nineveh governorates is unknown, but the proportion of HBsAg positive samples in CRF patients on hemodialysis in Ibn-Sina hospital was higher than in the several population rate uncommon presentation of serological markers for HBV/HCV may hide the real diagnosis (table 3), these uncommon presentation are frequent finding in CRF patients hemodialysis (13). They maybe a results of a serious compromised immune system due to chronic uremia and also due to mutation in coding regions of HBsAg (S unit and core region) hindering seroconversion or reducing viral replication (5, 12, 14). Patients who present only the anti-HBC may present a risk since they may be at risk of acquiring HBV or maybe a source of infection for other patients, especially if they undergo hemodialysis treatment in machines shared by HBV negative patients, serial test, molecular and/or serological might help to confirm the accurate diagnosis (11, 15).

The HCV is the most prevalence chronic viral infection among CRF patients (19). The prevalence of anti-HCV infection by 3rd generation ELISA in this study (25.8%) was similar to the prevalence

found in Belo Horizonte (2%) (16). And lower than the prevalence found in Goiania (46%) (13).

The PCR is a very sensitive method for diagnosing HBV in patient without CRF (11). In this study the molecular test (PCR) failed to diagnosis 15% of HBV case. This results is in accordance with prior studies of the dynamics of the HBV load in hemodialysis patients (15). Following 29 HBsAg positive patients for 12 months with monthly HBV-DNA test, and found that 62.1% of the patients show intermittence in the HBV-DNA results (15, 17). Demonstrated that 15-48% of HBsAg positive patients were actually HBV-DNA undetectable, at any rates, these patients must be studied again, in order to identify possible mutation in HBV genome that may alter its serological patterns and viral load levels, however from an epidemiological point of view, the hemodialysis units must treat them as carriers.

The validity parameters found in this study for HBV serological tests (table 3) reflect the inconsistent findings of HBV serological markers in CRE patients on hemodialysis, and the potential cause of such inconsistency were discussed in the previous paragraph.

The discrepancies found between the serological results for HCV and PCR are discussed by several authors (16, 18, 19). The anti-HCV positive and HCV-RNA negative case may have been a result of elimination of HCV virus, but also of the low viral load frequently found in hemodialysis patients, generating intermittent results in 33% of the cases (20). The Anti-HCV negative and HCV-RNA positive results is present in the immune compromised and in immune tolerant conditions, the accuracy of serological anti-HCV and molecular (HCV-RNA PCR) results

found in this study projects are reliable negative were described by other authors (9, 22). However, Carniero et al., (13), showed a NPV of 90% for anti-HCV, indicating an error of 10% among the negative results; he suggests the addition of PCR for the detection of HCV to the test routine for CRF patients under hemodialysis treatment.

All the HBV or HCV patients identified by the study were referred to the ambulatory of chronic viral hepatitis for complementary test and follow up, further molecular studies will be set up to investigate the causes for the negative HBV results, this tests will aim variation on the HBsAg protein and HBV-DNA

PCR target, as well as potential mutations and immunological changes caused by the CRF disease or effects of long term ongoing hemodialysis in the HBV and HCV (15, 14, 17, 23, 24).

Until today neither the serological nor the molecular tests alone is a gold standard for HBV or HCV diagnosis for CRF patients on hemodialysis, effort must be made to optimize the operational guidelines at hemodialysis units, and to use more accurate diagnostic tools, for HBV/HCV by combining results, repeating tests or developing new exam.

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