The significance of hepatitis C RNA detection in hemodialysis patients for isolation and therapy efficacy assessment

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الملخص:

تعد عدوى فيروس التهاب الكبد الوبائي (سي) واحدة من المشاكل الرئيسية في وحدات غسيل الكلى (HD) في جميع أنحاء العالم. تعتمد معظم المعامل في ليبيا على الكشف عن الأجسام المضادة لفيروس التهاب الكبد سي (HCV-Ab) عن طريق تقنية الاستشراب المناعي (الاختبار السريع ICT) أو تقنية المقايسة المناعية بالارتباط الأنزيمي (ELISA). النتائج الإيجابية لاختبار الكشف عن الاجسام المضادة للفيروس تعني أن المريض اصيب بعدوى الفيروس ولكن ربما يكون قد نجح في التخلص من العدوى ولم يعد معديًا ولا حاجة إلى فصله لغسيل الكلى. هدفت هذه الدراسة إلى إيجاد الفرق بين المرضى ذوي اختبار الكشف عن الجسام المضادة الايجابي وأولئك الذين لديهم تبين وجود مورثة الفيروس لذيهم بواسطة تفاعل البوليميراز المتسلسل (PCR) وتقييم علاجهم.

تم تضمين 49 مريضاً (26 ذكور و 23 إناث) إيجابياً لاختبار الكشف عن الاجسام المضادة للفيروس لفيروس HCV-Ab بواسطة ELISA من وحدات مصراته للغسيل الدكوي HD, وتم اختبارهم بواسطة تفاعل البوليميراز المتسلسل للتحري عن المورثة الفيروسية (HCV-RNA) لديهم أظهرت النتائج أن 36 (73.5٪) 18 مريضًا من الذكور و 18 مريضًا من الاناث كانوا موجبين لاختبار التحري عن المورثة، تم علاج هؤلاء

الـ36 مريض باستخدام Zepatier (مضاد للفيروسات) وأعيد اختبار التحري عن مورثة الفيروس لهم فكان 4 فقط (11.1٪ من إجمالي المرضى المعالجين) من المرضى الذكور إيجابيين بعد العلاج وكان هناك فرق كبير في النتائج قبل العلاج وبعده ($p< 0.001$). خلصنا إلى أن اختبار التحري عن مورثة الفيروس HCV RNA بتقنية تفاعل البوليميراز المتسلسل PCR يجب أن يتم لجميع المرضى لفصل الإيجابين ، كما أن اختبار HCV RNA مهم لتقييم المرضى الذين يحتمل أن يكونوا معديين وكذلك لتقييم نجاعة المعالجة.

Abstract:

Hepatitis C virus (HCV) infection is one of the main problems in hemodialysis (HD) units worldwide. Most of the laboratories in Libya depend on HCV antibody (HCV-Ab) detection by Immunochromatography tests (rapid test) or Enzyme-Linked Immunosorbent Assay (ELISA). The positive HCV-Ab results mean that the patient is infected with HCV, but maybe he resolved the infection and the patient is not infectious anymore and no need to separate him for dialysis. This study aimed to find the difference between HCV Ab-positive patients and those who are HCV RNA-positive by PCR and to evaluate their therapy. 49 (26 males & 23 females) HCV-Ab positive patients by ELISA from Misurata HD units were included and were tested by PCR for HCV-RNA. Results showed that 36 (73.5%) 18 male and 18 female patients were HCV RNA positive, those 36 were treated with Zepatier (antiviral) and retested for HCV RNA. Only 4 (11.1% of total treated patients) male patients were HCV RNA positive after therapy, there was a significant difference between before and after treatment ($p< 0.001$). We concluded that the HCV RNA testing by PCR must be done for all patients to separate the positive, also the HCV RNA testing is significant for evaluating potentially infectious patients and therapy effectiveness.

Keywords: Hepatitis C virus, Hemodialysis, HCV-RNA, DAAs, Zepatier**.**

Introduction:

The hepatitis C virus (HCV) infection is one of the most common blood-borne infections worldwide (Lavanchy 2011) (Lavanchy 2011). Now, it is well known that HCV is of global importance, affecting all countries, leading to a major global health problem that requires widespread active interventions for its prevention and control (Smith DB. *et al.* 1995). As the measurement of incidence fails to produce reliable numbers, because of the mostly asymptomatic form of acute infection, most approximations are based on reviews of published prevalence data, The estimated total global HCV prevalence is over 180 million (3.0%) people worldwide (Alter MJ. 2007). The available estimates are 1.6% (104,736) of the population in Libya (Hepatitis C global prevalence country data 2010) (Lavanchy 2011).

HCV is transmitted by parenteral routes, most frequently through blood or blood products transfusion and intravenous administration of drugs, Other potential routes by which the HCV may be transmitted include tattooing, the use of intranasal cocaine, body piercing, and accidental injuries with infected needles (Alter MJ. 2007, Donahue JG. *et al.* 1992, and Ohto H. *et al* 1994) also HCV can be sexually transmitted (Lavanchy 2011). The main risk groups for HCV infection include (recipients of the possibly infected transfusions, intravenous drug users, hemodialysis patients, and health–care workers (Ahmetagić S. *et al.* 2006). In hemodialysis (HD) units, the most common faults leading to the infection are; contamination of dialysis machines, inadequate disinfection of environmental surfaces, incorrectly contact of the medical staff with equipment and patients, and mishandling of parenteral medications (Ozer Etik D. *et al*. 2015).

HCV infection is the most common hepatic viral infection that affects patients with renal failure on maintenance hemodialysis (MHD) and it is a significant cause of morbidity and mortality in patients with renal failure who is treated with dialysis or kidney transplantation (KT). The prevalence of HCV in MHD patients ranges from 6-60%**,** many risk factors have been identified for the high incidence of HCV infection in Hemodialysis (HD) patients; the most important ones are the number of blood transfusions, duration of the hemodialysis treatment, and nosocomial transmissions due to inadequate infection-control and prevention measures (Ozer Etik D. *et al*. 2015, and Madhavan A. *et al*. 2020)**.**

The diagnosis of Hepatitis C in chronic kidney disease patients because of many reasons is complicated and difficult, these reasons include the presence of nonspecific clinical signs and symptoms, being often asymptomatic; normal, or discrete (often fluctuating) levels of the alanine aminotransferase (ALT) enzyme in almost half of the patients with HCV infection; presence of possibly false-negative serology, in addition to the low viremia seen in these patients. ALT levels should be checked on admission to the dialysis unit and then monthly (Ghany MG. *et al.* 2009). Screening for hepatitis C should be performed in all patients who initiate the dialysis program or are transferred from other centers, initially with the immune assay, and if positive, confirmed by nucleic acid testing (NAT) to detect HCV-RNA by RT-PCR. However, in countries with a high hepatitis C prevalence, methods for the detection of HCV-RNA may be considered as the initial examination (Constancio NS. *et al.* 2019).Now, the laboratory diagnosis of HCV infection is based on these two categories of laboratory tests, detecting specific antibodies to HCV (anti-HCV) and assays that can detect, quantify, or characterize HCV RNA. These virological tests are the main in the diagnosis of infection,

therapeutic decision making, and assessment of virological response to therapy (Gupta E. *et al.* 2014). The "serologic window" between HCV infection and the detection of specific antibodies varies from patient to patient, seroconversion occurs on an average at 6-8 weeks after the infection. In patients who spontaneously resolve the infection, anti-HCV may persist throughout life, decrease slightly while remaining detectable, or gradually disappear after several years (Lefrère JJ. *et al.* 1997). While HCV-RNA can be detected as early as 1 week after the onset of infection, and at least 4-6 weeks prior to seroconversion as demonstrated in a number of transmission settings (Grebely J *et al.* 2012, Maheshwari A *et al.* 2008, and Kamal SM. 2008).

Patients with renal failure on hemodialysis are at higher risk for HCV infection if measures for effective control of HCV infection in the hemodialysis environment are not implemented. HCV transmission in the hemodialysis environment still represents a substantial problem in low-resource countries (Weber DJ. *et al.* 2016, and Ashkani-Esfahani S. *et al.* 2017). Hepatitis HCV causes significant morbidity and mortality among HD patients, the Prevalence of anti-HCV antibodies among HD patients is consistently higher than in the general population indicating an increased risk of acquiring HCV infection among HD patients (Fabrizi F. *et al.* 1997). The higher prevalence of HCV infection among hemodialysis is mainly due to underlying impaired cellular immunity. Also, hemodialysis patients are exposed to prolonged vascular access and exposure to contaminated equipment and handling by medical staff. In addition, HD patients required blood transfusion, frequent hospitalization, and surgery, which increase the opportunities for getting nosocomial infection exposure (Ozer Etik D. *et al*. 2015, and Khan S. *et al.* 2011). HCV has been included as a risk factor for death in HD patients, this call for the

treatment of patients who are infected with HCV irrespective of their liver fibrosis stage or those who will have a kidney transplant. Chronic HCV infection can be treated by effective antiviral therapies. HCV was treated with interferon (IFN) and ribavirin therapies. IFN-based therapies have poor efficacy and a high adverse event rate in HD patients. In addition, IFN-based therapies are associated with greater rates of allograft rejection after kidney transplants. The strategies for managing HCV have been revolutionized by the recent introduction of direct-acting antivirals (DAAs) that target specific nonstructural proteins of the virus and result in disruption of viral replication and infection (Ozer Etik D. *et al*. 2015, Fabrizi F. *et al.* 2018, and Yen HH. *et al.* 2022). The development of these DAAs, IFN-free or IFN-sparing regimens represents a step forward in the history of HCV treatment. It is expected that the treatment scheme for chronic HCV patients to change radically, as safe and potent therapies become more accessible. This will simplify the management of these cases and will open possibilities to include patient populations for which pegIFN is currently contraindicated. One of the limitations of some DAAs was the need to know the specific HCV genotype before initiating the therapy (Ozer Etik D. *et al*. 2015, and Yen HH. *et al.* 2022).. Zepatier (Elbasvir 50mg/grazoprevir 100mg) is one of the DAAs, a ribavirin-free combination of elbasvir (NS5A inhibitor) and grazoprevir (NS3/4A protease inhibitor), it is available as tablets, one tablet is taken orally for 12-16weeks, was approved by the Food and Drug Administration in 2016**,** this Antiviral Therapy is currently one of the recommended regimens for patients with renal failure and mostly treats patients with HCV genotype 1a, 1b, and 4 infections (Fabrizi F. *et al.* 2018, Early J, & Maxted G. 2017, Pagan J. *et al*. 2019, and Corson M. *et al* 2018).

Many studies were conducted to assess the relationship between seropositivity and potential infectivity, in one early study of these carried out at the Miami Veterans Affairs Medical Center and the University of Miami/Jackson Memorial Medical Center in the USA in 1990, sixty-three patients undergoing maintenance hemodialysis were tested for anti-HCV antibodies, 16 of 63 were anti-HCV-positive. Of these 16 anti-HCV positive patients, HCV-RNA was detected in 12 (75%). HCV-RNA was also detected in 2 (4.3%) of the 47 patients who were anti-HCV negative. The results showed that detection of HCV-RNA in anti-HCV negative patients and anti-HCV positive patients is the main indicator for potential infectivity, isolation, and need for treatment [(24)]. Another study Published in 2015 was done to assess an all-oral, ribavirin-free regimen in patients with HCV genotype 1 in the USA, Overall, 111patients with HCV genotype 1 infection and chronic kidney disease with or without hemodialysis dependence were included randomly assigned to receive Zepatier tablet (grazoprevir 100 mg and elbasvir 50 mg) once daily for 12 weeks, sustained virological response at 12 weeks was 94%, the combination of grazoprevir and elbasvir was demonstrated to be a safe and effective regimen for HCV-infected patients with advanced CKD and on hemodialysis. Dose adjustments were not needed for patients on hemodialysis, and no patients discontinued the regimen due to side effects. Efficacy was high in most patient populations [(25)]. In a study of HCV infection among hemodialysis patients in Kosovo in 2015, 668 HD patients were included in the study, they were screened for HCV-Ab by ELISA the overall seroprevalence of HCV infection was 53.0% (354/668), HCV RNA was detected in 323 samples of these (91.2%). the results showed a high prevalence of HCV infection in HD patients, also not all HCV-Ab positive patients are viremic and potentially infectious [(26)].

The aim:

The study was conducted to determine the presence of HCV-RNA in patients who are HCV-Ab positive as a marker for isolation and to evaluate their treatment efficacy with Zepatier.

Materials and Methods:

49 patients 26 males and 23 females at Misurata dialysis center and Alzarroug dialysis unit with renal failure on MHD who were positive for HCV-Ab by ELISA (HCV ELISA DiaSino China), were included in this study. By RT-PCR [Xpert® (Cepheid)], their blood samples were tested for HCV-RNA before treatment and after the treatment course with Zepatier.

Statistical analysis:

Because the study was an estimation study without a control group, and no planned formal hypothesis testing, the primary efficacy analysis prescribed by protocol estimated the proportion of patients. The variables were described by number and percent (N, %). A two-tailed $p < 0.05$ was considered statistically significant. All analyses were performed with the IBM SPSS 24 software.

Results:

As shown in the table 1, the 49 patients (26 males 53.1% & 23 females 46.9%) who were HCV-Ab positive by ELISA were tested for HCV-RNA before treatment, 36 (73.5%) of them were positive, 18 males (69% of total HCV-Ab positive males) and 18 females (78.3% of total HCV-Ab positive females), fig 1 illustrates these results.

Table 1: Sex of HCV-Ab positive patients and their PCR for HCV-RNA results before treatment.

	Males n(%)	Females n(%)	Total n(%)
HCV RNA negative	8 (31%)	5(21.7%)	13 (26.5%)
HCV RNA positive	18 (69%)	18(78.3%)	36 (73.5%)

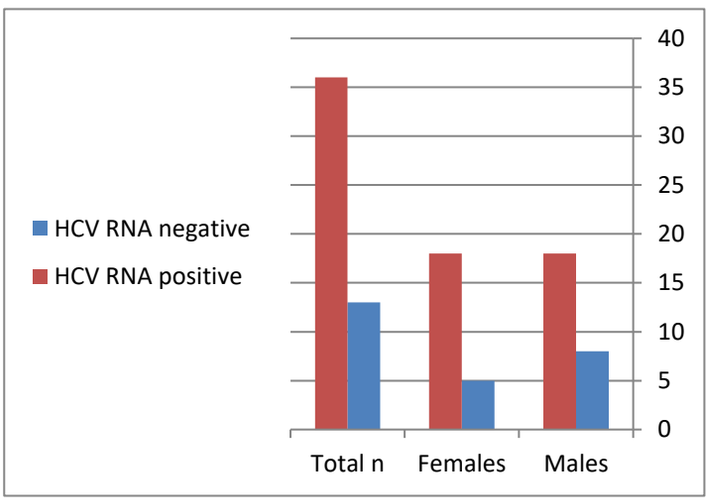

Fig 1: PCR results of the patients before treatment.

Those 36 positive HCV-RNA (18 males & 18 females) patients were treated with Zepatier one tablet daily for 12 weeks, after finishing the treatment course with Zepatier the 36 patients retested for HCV RNA, only 4 patients (11% of males) represents 11.1% of total treated patients were positive for HCV-RNA, all were males as summarized in table 2 and illustrated in fig 2:

Table 2: Sex of HCV-Ab positive patients and their PCR for HCV-RNA results after treatment.

	Males n(%)	Females n(%)	Total n(%)
HCV RNA negative	14(77.8%)	18(100%)	32 (89.1%)
HCV RNA positive	4(22.2%)	0(0.0%)	4 (11.1%)

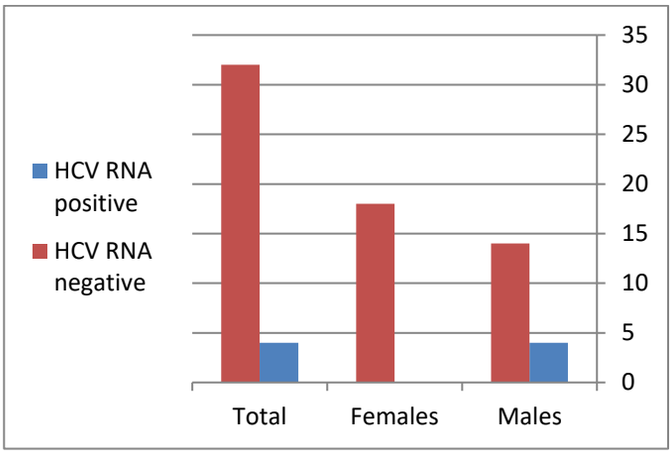

Fig 2: PCR results of the patients after treatment.

Discussion:

As mentioned patients with chronic renal failure on maintenance hemodialysis represent one of the main risk groups for hepatitis C virus infection which is still a persistent problem in hemodialysis units. HCV is the most frequent pathogen found in patients on maintenance HD.

Currently, Enzyme-Linked Immunosorbent Assay (ELISA) is the most used technique for the diagnosis and HCV-Ab screening in hemodialysis patients, its positive results indicate that the patient was infected with HCV but he may have recovered from the infection. To compare the serological results with the detection of the viral RNA and differentiate between those infectious and non-infectious HCV-Ab positive patients or viremic and non-viremic patients we have to detect HCV-RNA by a molecular technique (RT-PCR) that confirms the presence of the virus in the blood (viremia) (Timofte D, *et al.* 2020) to investigate the prevalence of HCV viremic patients to make the right decision about which patients should be isolated and treated.

Several studies have reported false-negative results of the serological tests which highlight the importance of viral RNA detection (Albuquerque AC. *et al.* 2005, Brandão AB. *et al.* 2000, and Rahnavardi M. *et al.* 2008), these false-negative results may be associated with the difficulty in diagnosis the infection during the initial phase before seroconversion has not yet occurred due to the time lapse between the time of HCV infection and the antibody detection in blood. This time span, called 'the serological window' varies from patient to patient (Albuquerque AC. *et al.* 2005, and Vidales-Braz BM. *et al.* 2015), or low level of the antibodies due to its low production as hemodialysis patients considered to be immunosuppressed (Vidales-Braz BM. *et al.* 2015).

The current study was carried out to report the prevalence of HCV RNA in HCV-Ab positive hemodialysis patients in Misurata to differentiate between potentially infective and non-infectious patients in comparison with other HD centers elsewhere. Our results showed that the prevalence of HCV RNA in HCV-Ab positive hemodialysis patients was 73.5% (36 out of 49 patients) and nearly the same in males and females. the prevalence of HCV RNA in our patients was lower than its prevalence in 21 Brazilian HCV-Ab positive HD patients included in a Study conducted in 2005 which was 90.5% (19/21 patients) (Albuquerque AC. *et al.* 2005). Similarly, its lower than the prevalence in 24 Danish HD HCV-Ab positive patients included in a Study conducted in 1990-1991 which was 95.8% (23/24 patients) (Bukh J. *et al.* 1993). The results of the current study show that 26.5% (13/49) of the patients get rid of the infection and recovered spontaneously, they became non-infectious and no need for their isolation and antiviral treatment. Since the HCV infection rate is higher in dialysis patients than in the general population; leading to poorer outcomes in HCV-infected than non-infected

dialysis patients. This situation increases the need for HCV infection treatment in dialysis patients (Akiba T. *et al.* 2012). Zepatier (Grazoprevir/elbasvir) is one of the oral combination tablets of HCV direct-acting agents (DAAs) that offers excellent treatment efficacy, safety, and tolerability in HCV treatment of patients with multiple genotypes, and it does not worsen kidney function in patients with preexisting renal disease and HCV infection. DAA agents now have a sustained virological response (SVR) rate of more than 95% (Corson M. *et al.* 2018, and Papudesu C. *et al.* 2017).

All HCV RNA positive patients undergo a treatment regime with Zepatier (elbasvir 50 mg plus grazoprevir 100 mg) once per day for 12 weeks, retested for HCV RNA after the end of treatment. There were only 4 (11.1% of total patients) patients positive after therapy, all were men representing 22.2% of total positive men (4/18) while all women were HCV RNA negative after treatment and get rid of the infection and recovered. The results reveal that patients had a generally favorable response to treatment. Efficacy was high in all patients, these data support the use of a fixed-dose combination of elbasvir plus grazoprevir in CRF patients on maintenance HD with HCV infection ($p< 0.001$). Several trials showed high efficacy, and safety of Zepatier for HCV-infected patients with various complications (Roth D. *et al*. 2015, Forns X. *et al.* 2015, and Buti M, *et al.* 2016). our results were nearly in concordant with a randomized study to see the efficacy of Zepatier in the treatment of patients with chronic kidney disease with or without hemodialysis in the united states in 2015, 122 patients with HCV infection were randomly assigned to receive Zepatier (grazoprevir 100 mg, and elbasvir 50 mg) once daily for 12 weeks, results show SVR of 94% (115/122 get rid of the infection and recovered) (Roth D. *et al*. 2015). Similarly, In an uncontrolled, non-

randomized, phase 3, open-label, single-arm study, for treatment of patients with chronic HCV infection and HIV co-infection, with or without cirrhosis, were enrolled from 37 centers in nine countries across Europe, the USA, and Australia All patients received grazoprevir 100 mg plus elbasvir 50 mg in a fixed-dose combination tablet once daily for 12 weeks. Between June 11, 2014, and Aug 29, 2014, 218 patients were enrolled and received grazoprevir plus elbasvir for 12 weeks, all of whom completed follow-up at week 12. SVR in week12 was achieved by 210 of 218 patients (96%) (Rockstroh JK. *et al.* 2015). Thus, the results of the current study indicated that treatment with Zepatier for 12 weeks was effective, leading to the cure of a high percentage of the treated patients.

Conclusion:

- Because of the presence of possibly false-negative serology associated with the difficulty in diagnosis of the infection before seroconversion or low level of the antibodies while having detectable HCV viremia, also detection of the HCV antibody in HD patients may indicate acute, chronic, or even resolved infection, we recommend RT-PCR the detection of HCV-RNA for all patients rather than screening for hepatitis C initially with serological assays for HCV antibody detection, and if positive, confirmed by RT-PCR testing to detect HCV-RNA.
- The HCV treatment regimen seems to be effective and well tolerated by patients. These data are consistent with previous trials of this regimen in infected patients. Our results indicate an overall HCV cure rate of 88.9% following treatment with zepatier as one of the DDA's. **T**hese results were in consent with previously reported global cure rates. This treatment of HCV infection among hemodialysis patients are both effective and safe.

Recommendations:

- HCV infection remains the major cause of chronic liver disease in HD patients, it presents histologically and clinically mild hepatitis that probably may be related to immunocompromised status and HD procedure. we recommend that patients on hemodialysis should be tested when they first start hemodialysis or when they transfer from another hemodialysis centers (patients should be screened, evaluated, and if necessary treated for hepatitis C).
- As in the general population, detection of the HCV antibody by ELISA in HD patients may be indicative of acute, chronic or even resolved infection. On the other hand, some hemodialysis patients will test negative for anti-HCV antibodies while having detectable HCV viremia, and the detection of HCV-RNA by RT-PCR is the most sensitive and specific assay for HCV detection, we recommend testing all patients for HCV-RNA by RT-PCR.
- Treatment of the infected patients who are positive for HCV-RNA with DAAs has proven to be effective, therefore, it is necessary to treat the infected patients to prevent the development of the hepatitis and its complications.
- It is essential to detect the virus genotypes and subtypes to identify non-responding types to choose another appropriate treatment regimen

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Conflict of interest:

All authors declare that they have no conflicts of interest.

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