Infectious Disease

Hepatitis C Virus Infection in Hemodialysis in Cameroon: Prevalence and Incidence

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Keywords

Hepatitis C Virus • Hemodialysis • Prevalence • Incidence rate • HCV-RNA

Summary

Background. *Hemodialysis (HD) is the world's most prevalent kidney replacement therapy for end-stage renal disease patients. Hepatitis C virus infection (HCV) is highly prevalent in patients undergoing HD. There are no reports of the epidemiology of HCV viremia and HCV incidence rate based on prospective studies in HD units in Cameroon. This study evaluated the prevalence and incidence rate of HCV based on anti-HCV antibody (Ab) and HCV-RNA detection.*

Methods. *We conducted a controlled prospective study in three hemodialysis centers in Cameroon, from October 2021 to January 2023. The HEXAGON HCV rapid kit was used to detect anti-HCV Ab, and HCV-RNA was searched using the Xpert® HCV Viral Load technology. We performed a Wilcoxon test and the chi-square or Fisher exact test in statistical analyses.*

Results. *A total of 205 HD patients were enrolled with a mean age of 47.7 ± 14.5 years and median hemodialysis duration of 36 months (IQR: 12-72). Anti-HCV Ab was positive in 59 patients, giving a prevalence of 28.8% (95% CI: 22.7-35.5). This Anti-HCV Ab infection was mainly found in patients with a relatively long period of HD (P = 0,00002). Of the 59 anti-HCV Ab-positive patients, HCV-RNA was detected in 42, with the HCV active infection prevalence of 71.2% (95% CI: 57.9-82.2). A high viral load (HCV RNA > 800,000 UL/ml) was detected in 31% (95% CI: 17.6-47.1). A total of 125 patients with a negative status at the start of the study (M0) were prospectively followed up. After seven months (M7), 15 [12%; 95% CI: 06.9-19.)] became HCV positive, with an incidence rate of 20.6 cases per 100 patients-years.*

Conclusion. *There is a high prevalence of HCV infection and HCV incidence rate in this study. Strategies aimed at decreasing HCV infection in HD centers in Cameroon are urgently needed.*

Introduction

Hepatitis C virus (HCV) is a ribonucleic acid (RNA) virus belonging to the family *Flaviviridae;* that is responsible for hepatitis C liver disease. He may be responsible for mild inflammation (acute hepatitis) or severe and persistent inflammation (chronic hepatitis) of the Liver. It is estimated that 50 million people have chronic HCV infection, and 1.0 million new infections occur each year [1]. Hemodialysis (HD) is the most prevalent kidney replacement therapy for patients with end-stage renal disease (ESRD) globally, accounting for approximately 69% of all kidney replacement therapy and 89% of all dialysis [2-4]accessibility, quality, and affordability**.** People undergoing HD are known to be at high risk of HCV infection [5HCV and HIV infections in patients with hemodialysis. Patients and methods: A retrospective study of 430 end-stage renal failure patients, referred to hemodialysis department at XXXX Teaching Hospital, Baghdad-Iraq from January-2015 to January-2017. Patients were investigated for HBs-Ag using enzyme-labeled antigen test (Foresight-EIA-USA-8]. Indeed, this high susceptibility of HD patients to acquire HCV infection is due to their frequent hospital admissions and the high frequency of invasive procedures [6]. The prevalence of HCV infection in HD units varies greatly across geographical regions and over time [9]. At the global level, this prevalence is estimated at 24.3%, with the highest observed in lower-middle-income economies (26.8%) [10]. Regarding the incidence rate of HCV infection, the Dialysis Outcomes and Practice Patterns Study (DOPPS) phase 1 study showed a decrease in the incidence of HCV from 2.9 to 1.2 per 100 patient-years in HD units in the United States, Europe, and Japan [11]. In contrast, this incidence remains high (4.44 per 100 patient-years) in the developing world [12]. As the number of patients with chronic kidney diseases requiring hemodialysis as a renal replacement therapy is growing, HCV infection and transmission are increasingly frequent and need to be emphasized in worldwide hemodialysis units. In Cameroon, only two studies have reported HCV prevalence in HD patients: the first at 19,2% in Buea and Bamenda HD centers (South West region and North West region) and the second at 26.3% in Yaounde University Teaching Hospital (Centre region) [13, 14]. However, these prevalences were only based on the antibody assay, which cannot differentiate HCV infection (anti-HCV antibody positive) from active HCV infection (viral RNA detection)

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which is reflective of replication and infectivity of the virus. Moreover, the burden of HCV remains unknown in HD centers in the South (Ebolwa), East (Bertoua), and North (Garoua) regions of Cameroon. In the setting where many reports have suggested a nosocomial patient-to-patient mode of transmission of HCV in HD units, HCV viremic patients could be the source of this transmission. A direct test for HCV viremia is thus necessary to identify infectious subjects in HD units. Some retrospective studies reported that the rate of HCV seroconversion in HD patients in Cameroon is between 7.1% and 25%, and only one study reported the HCV incidence rate at 3.6 per 100 patient-years [15Biorad-17]. In comparison to prospective studies, retrospective studies cannot demonstrate the real incidence rate because they are prone to different biases, particularly recall bias.

This study was conducted to determine the prevalence of anti-HCV antibody, active HCV infection, and HCV incidence rate in three hemodialysis centers in Cameroon, to stimulate health policies to implement strict infection control measures in Cameroonian hemodialysis units.

Methods

Study Design, Sites, and Population

This was a controlled prospective study conducted from October 17, 2021, to January 21, 2023, in three hemodialysis centers in three regions of Cameroon: The Ebolowa Regional Hospital Hemodialysis Centre (ERHHD, South Cameroon); Bertoua Regional Hospital Hemodialysis Centre (BRHHD, East Cameroon); and the Yaoundé General Hospital Hemodialysis Service (YGHHD, Centre Cameroon). The study was carried out over seven months in each center, and the samples were taken in two rounds: in month 0 (M0) and later in month 7 (M7). The study was carried out in the Cameroon hemodialysis patients' community. The included patients met the following criteria: 1) aged over 12 years irrespective of sex and ethnicity; 2) undergoing hemodialysis for end-stage chronic kidney disease (ESCKD-stage 5 GFR < 15 ml/min/1.73 m); 3) had been on hemodialysis for a minimum duration of 3 months; and 4) provided signed informed consent or parental assent. Demographic data (including age, gender, study level, marital status, and occupation) and hemodialysis data (including vascular access, HD duration, blood transfusion history, dialysis groups, and attending several dialysis centers) were obtained by direct interview of patients and completed by review of their medical records.

Sampling and hepatitis C virus assays

A volume of 5 mL of blood was collected either before the stated hemodialysis session (during patient connection); or thirty minutes before the end of the hemodialysis session, to avoid PCR inhibition by heparin. The blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and then centrifuged at 2500 rpm for 10 minutes. Thereafter, 1.5 mL of plasma was collected in Eppendorf tubes and then placed in an icebox (containing ice packs) for transportation to the Centre Pasteur of Cameroon (CPC), where all analyses were performed. At the CPC, the plasma was subsequently stored at -80°C. The HEXAGON HCV for rapid and qualitative detection of IgG antibodies to HCV in human serum, plasma, or whole blood with a sensitivity of 99.3% and a specificity of 99.5% was used. It is an immunochromatographic assay using recombinant antigens from structural (Core) and nonstructural (NS3, NS4, and NS5) regions of the HCV genome, known to be highly immunodominant. Briefly, 10 µl of plasma was applied onto the sample port (S) of the "TEST" device, and three drops of "DIL" solvent were added. The results were read within 5-20 minutes at a well-lit place. The detection and quantification of HCV-RNA were performed in all patients who tested positive for anti-HCV Ab. We used GeneXper Dx Systems, which uses the Xpert® HCV Viral Load (Cepheid Röntgenvägen 5, SE-171 54 Solna, Swede) technology with an assay sensitivity of 10 copies/mL. The Xpert® HCV Viral Load is an automated test, whose cartridge integrates HCV-RNA extraction, amplification, and detection of the target sequences (5' untranslated transcribed region) using real-time reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, slightly more than 1 mL of plasma was introduced into the sample chamber of the test cartridge using the transfer pipette included in the kit. The lid was then closed, and the cartridge was loaded into the GeneXpert Dx instrument.

Clinical data and definitions

Active HCV infection (virus infectivity) was defined by the presence of viral RNA in the plasma. High viral load was defined by HCV RNA > 800,000 UL/ml [18]. Newly HCV-infected patients: patients who were seronegative for anti-HCV Ab at the beginning of the study (M0), and became anti-HCV Ab positive at the end of 7 months.

Ethics Statement and Statistical Analyses

Our research protocol was approved by the Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH), N° 2021/07/91/CE/CNERSH/SP. The statistical analyses were performed using RStudio version 4.1.0 software. The Kolmogorov-Smirnov test allowed us to verify the normality of our quantitative variables that were expressed as the mean ± standard deviation or as the median interquartile range ((IQR): 1st-3rd) according to the normal distribution or not. Chi-square or Fisher exact tests were used to compare the proportions as applicable, and Wilcoxon tests were used to compare qualitative and quantitative variables. *P* values less than 0.05 were considered statistically significant.

Results

Demographic and hemodialysis data

A total of 205 HD patients (169 from YGHHD, 24 from ERHHD, and 12 from BRHHD) including 85 (41.5%)

Tab. I. Distribution of HCV positivity according to demographic and hemodialysis data.

Anti-HCV Ab Positive (N=59)						HCV-RNA Positive (N = 42)				
Data	Variables		a	b (95%IC)	P value	Variables		a	b (95%IC)	P value
Socio-demographic data	HD Centres	ERHHD (n=24)	14	58.3 (36.6-77.9)	**0.003***	HD Centres	ERHHD (n = 14)	10	71.4 (95%CI :41.9-91.6)	0.14
		YGHHD (n=169)	43	25.4 (19.1-32.7)			YGHHD (n = 43)	32	74.4 (95%CI :58.8-86.5)	
		BRHHD (n=12)	2	16.6 (2.1-48.4)			BRHHD (n = 2)	0	0 0 (95%CI :0-84.2)	
	Sex	M	37	62.7 (49.1-75)	0.44	Sex	M	25	59.5 (43.2-74.4)	0.61
		F	22	37.3 (25.1-50.9)			F	17	40.5 (25.6-56.7)	
	Age group	11-21	1	1.7 (0.0-9.1)	0.11					
		21-31	5	8.5 (2.8-18.7)						
		31-41	11	18.6 (9.7-30.9)						
		41-51	10	16.9 (8.4-29)						
		51-61	15	25.4 (15-38.4)						
		61-71	13	22 (12.3-34.7)						
		71-81	4	6.8 (1.9-16.6)						
		81-91	0	0 (0-6.1)						
HD Data	HCV Initial status	Positive	13	22 (12.3-34.7)	NA					
		Negative	17	28.8 (17.7-42.1)						
		Unknown	29	49.2 (35.9-62.5)						
	Blood transfusion	No	9	15.2 (7.2-27)	0.52					
		< 5	15	25.4 (15-38.4)						
		> 5	35	59.3 (45.7-71.9)						
	Vascular access	Catheter	9	15.2 (7.2-27)	0.77					
		Fistula	50	84.7 (73-92.7)						

a: Frequency; b: Percentage; BRHHD: Bertoua Regional Hospital Hemodialysis Centre; ERHHD: Ebolowa Regional Hospital Hemodialysis Centre; YGHHD: Yaounde General Hospital Hemodialysis Service; HD: Hemodialysis; NA: Not Applicable, *: Statistically significant.

females and 120 (58.5%) males, were included in this study. The mean age of the participants was 47.7±14.5 years (range: 12-86), and the hemodialysis duration ranged from 3 to 324 months with a median of 36 months (IQR: 12-72).

Prevalence of anti-HCV antibody and active HCV infection

Of the 205 included participants, 59 tested anti-HCV Ab positive, with a prevalence of 28.8% (95% CI: 22.7-35.5). This prevalence was significantly different between HD centers: 58.3% (95% CI: 36.6-77.9) at ERHHD, 25.4% (95% CI: 19.1-32.7) at YGHHD, and 16.6% (95% CI: 2.1-48.4) at BRHHD (P=0.003). The majority of anti-HCV Ab-positive patients were men [62.7% (95% CI: 49.1-75)], and the age range was 51-61 years [25.4% (95% CI: 15-38.4)] (table I). By comparing the median duration of HD between anti-HCV Ab positive patients [60 (IQR:36-84) months] and anti-HCV Ab negative patients [24 (IQR:12-60) months], we found that HCV infection mainly affected patients with a relatively long period of HD (P = 0,00002). Of the 59 anti-HCV Ab-positive patients, HCV-RNA was detected in 42, giving a prevalence of HCV active infection at 71.2% (95% CI: 57.9-82.2). This prevalence of HCV infectivity was very similar in ERHHD [71.4% (95% CI: 41.9-91.6)] and in YGHHD [74.4% (95% CI: 58.8-86.5)]. The median HCV RNA viral load was 207,000 IU/mL (IQR: 7,135- 863,322), and 31% (95% CI: 17.6-47.1) had a high viral load.

HCV incidence rate

At the start of this study (M0), 44 patients (21.5%; 95% CI: 16-27.7) were anti-HCV Ab positive, and 161 (78.5%; 95% CI: 72.3-84) were anti-HCV Ab negative. Among these 161 negative M0 patients, 125 were successfully followed up for a total duration of seven months (M7). Indeed, 36 patients were lost to follow-up for several reasons: death, impaired health leading to rejection of serological testing repeat, and transfer to another HD centers. At the end of the follow-up (M7), 15/125 [12%; 95% CI: 06.9-19.)] patients were confirmed to be newly HCV infected, for a total incidence rate of 20.6 cases per 100 patient-years (p-y). This incidence rate was 34.3 cases per 100 p-y in ERHHD [2/10 (20%; 95% CI: 02.5-55.6)], 21.1 cases

per 100 p-y in YGHHD [13/106 (12.3%; 95% CI: 06.7-20.1)], and 00.0 cases per 100 p-y [00/9 (00%; 95% CI: 0-33.6)] in BRHHD (P = 0.0398). Of the 15 newly HCV-infected patients, only one had undetectable levels of HCV RNA, and two had a high viral load (14,3%; 95% CI: 1.7-42.8).

Discussion

Currently, it is well known that HCV is the main viral infection found in patients undergoing HD. The burden of HCV infection among HD patients has been widely documented, but it is different across countries and between HD units in the same country. This study is the first to determine the prevalence of active HCV infection, and the HCV incidence rate among maintenance hemodialysis patients in Cameroon based on a prospective study.

The prevalence of anti-HCV antibody found in this study (28.8%) is slightly higher than those obtained by Luma *et al* in 2017 and Ndomgue *et al* in 2018 (19.2% and 26.3% respectively) [13, 14]. These results show that anti-HCV antibody prevalence among HD patients in Cameroon has been on the rise over the past six years. This can be explained by the fact that: because of the increasing rates of high blood pressure and diabetes mellitus in the population, the prevalence of end-stage renal disease is growing, which increases in the number of patients admitted for hemodialysis [19, 20] when the initiation of renal replacement therapy (RRT). Our prevalence is five times higher than those in the general population, blood donors, and sickle cell patients in Cameroon (6.5%; 2.5%; and 8.6% respectively) [14, 21]. Therefore, this study confirmed that patients on maintenance hemodialysis are at greater risk of acquiring HCV infection compared to the nondialyzed population. As shown in recent studies (1% in southern India, 5.4% in Iran, and 7.4% in China), the prevalence of anti-HCV antibody is low in most hemodialysis units in developed countries [22-24]. However, this study shows that this prevalence remains high in Cameroon, and this may reflect poor adherence to the standard infection control procedures recommended by the Centers for Disease Control and Prevention (CDC), and updated by the Kidney Disease: Improving Global Outcomes (KDIGO), regarding hepatitis C virus in hemodialysis units [25, 26]. Indeed, given the large population of individuals requiring hemodialysis therapy, many countries have deployed great efforts in the past few years to reduce HCV infections in this susceptible population. Implementing preventive measures to decrease HCV prevalence in HD units in Cameroon is an emergency.

We found that 71.2% (42/59) of the anti-HCV Ab-positive HD patients in this study were HCV-RNA viremic, which is consistent with studies performed by Miyasaka *et al.* (68.9%), and Albayati *et al.* (83.3%) [27, 28]. Our study confirms the assertion that most patients with anti-HCV Ab in HD units have active HCV infection. In this study, 31% (13/42) of HCV-RNA viremic patients had a high viral load (HCV RNA > 800,000 UL/ml) and could be the source of nosocomial transmission of HCV. Indeed, many studies that investigated local HCV infection outbreaks within dialysis settings found common evidence that suggests a nosocomial patient-to-patient mode of transmission [29-35]. It is therefore recommended that anti-HCV Ab-positive HD patients should undergo HCV RNA testing to confirm active HCV infection and identify and treat infectious subjects. Because, KDIGO does not recommend isolation and the use of dedicated machines for HCV-infected patients, the high percentages of active HCV infection and high viral load found in this study suggest improvement of hygienic precautions in Cameroonian HD units, especially in regions of high HCV endemicity.

A prospective follow-up of our HCV-negative HD patients allowed us to observe a total of 15 newly acquired infections, giving an incidence rate of 20.6 cases per 100 p-y. Our rate is very high compared to the 0.4-9.4 cases per 100 p-y range previously obtained in Cameroon, Lebanon, and Morocco [17, 34, 35]. This can be explained by the differences in time of follow-up and differences in initial HCV prevalence in the HD units. Determining the evolution of the HCV burden in the HD unit is crucial to implementing reduction and elimination policies. However, the best way to determine this burden is to compare the results of past and present incidence rates. Further studies should investigate the modes of spread of HCV among HD patients to propose solutions to limit the transmission of HCV in this population.

We acknowledge some limitations in this study. (1): HCV-RNA detection and quantification have been carried out only in anti-HCV Ab-positive patients, which could underestimate our prevalence of active HCV infection and incidence rate. (2): Sequencing and phylogenetic analyses were not performed in this study, so we did not search for the different HCV genotypes circulating in our HD units or establish the route of HCV spread.

Conclusions

This study reports a high prevalence of anti-HCV antibody, active HCV infection, HCV seroconversion, and incidence rate in HD patients in Cameroon. Strategies for preventing and eradicating HCV in our dialysis units are urgently needed.

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Ethics approval and consent to participate

Ethical approval (N° 2021/07/91/CE/CNERSH/SP) was obtained from the Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH). All samples were tested anonymously and coded, only the principal investigator had access to patients' data. All hemodialysis patients enrolled in this study signed a written informed consent, after an explanation of the objective, procedure, risks, and benefits of the project.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request**.**

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Conflict of interest statement

The authors declare that they have no competing interests.

Authors' contributions

RKM study conception, sampling, and data analysis and drafted the manuscript; MDN and RN study conception and design, supervision of data collection, laboratory experiments, and critical revision of the manuscript; MM, VSS, AKM, and GEA participated in study coordination, data collection, and manuscript revision; SWM, MZM, and SFL participated in laboratory experiments and manuscript revision. All authors read and approved the final version of the manuscript.

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