Accuracy of serology and molecular diagnosis tests for HBV and HCV in Chronic Renal Failure patients on hemodialysis, Porto Velho, Brazil

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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 transfusions and nosocomial transmission. A group of CRF patients living in Porto Velho were tested with serological markers for hepatitis B and C using the ELISA test and molecular biology techniques (PCR). The validity parameters for the serological results were measured based on the PCR results. Of the 128 patients on hemodialysis during the study, 12 (9.4%) were HBsAg positive, 69 (53.9%) were anti-HBc positive, 93 (72.7%) were anti-HBs positive, and 22 (17.2%) were anti-HCV positive. The PCR tests result in 12 (9.4%) HBV-DNA positive and 16 (12.5%) HCV-RNA positive. The accuracy, sensitivity and specificity of ELISA for HBsAg were 90.6%, 50% and 94.8%, and the same parameters were 92.2%, 87.5% and 92.9% for anti-HCV. Based on the results just the negative predictive value for anti-HCV (98,2%) is a reliable test in CRF patients on hemodialysis. Beside that, serial serological and/or molecular tests are the indicated methodology to diagnosis HBV and HCV infection in these patients.

Keywords*: CRF – Hemodialysis – HBV – HCV – Accuracy – Serology – ELISA – PCR.*

INTRODUCTION

The hepatitis B virus (HBV) and hepatitis C virus (HCV) are challenging blood-borne diseases that are prevalent worldwide[1]*. 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 Word 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]*.*

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The prevalence of HBV in Brazil varies geographically, ranging from 1-20%[4,5]. On the other hand, the prevalence of HCV is more uniform between regions in Brazil, orbiting around 1%[5]. However, in a study on a community near the Madeira river in Rondonia, Katsuragawa, HCV was found in 7% of the collected blood samples, and also 5.3% in the Acre state, within the Western Amazon[6].

There is a high prevalence of HBV and HCV infection in patients chronic renal failure (CRF) the prevalence is 2-61% for HBV and 10-65% for HCV[7]. In Brazil the annual seroconversion rate for anti-HCV is 15% among CRF patients[8,9]. The diagnosis of the Viral Hepatitis has demonstrated to not be as reliable in CRF patients ongoing hemodialysis as in patients without CRF[10,11]. Intrinsic factors associated with renal failure and the hemodialysis process itself produce inconsistencies in the serological, biochemical, and molecular test results[12], including qualitative and qualitative differences. Thus, there is a high risk of patients with CRF being misdiagnosed with HBV or HCV, which threatens the whole CRF community and increases the nosocomial transmission risk for patients and health workers[7,13].

The State of Rondonia presents a population close to 1.5 Million[14] and faces several public health challenges during its development. Some of the most striking health challenges were three times rapid increase of its population without property planning, drug trading, endemic malaria and intestinal parasitizes, and outbreak of cholera and dengue. Viral hepatitis has been one of the top public health issues in Rondonia[15,16], but little is known about the current prevalence of HBV and HCV. The seroprevalence among CRF patients ongoing hemodialysis has not yet been measured[15].

The objective of this study is to measure the accuracy of the serological and molecular test to diagnosis HBV and HCV marker in CRF patients on hemodialysis.

MATERIAL AND METHODS

A transversal analysis was performed looking at the prevalence of both serological and molecular markers for HBV and HCV in CRF patients on hemodialysis in Porto Velho, Rondonia between 2004 and 2005. The accuracy of the serological test was evaluated by comparison with the molecular test (PCR).

The study was approved by the committee for ethical research of Centro de Pesquisa em Medicina Tropical de Rondonia (CEP/CEPEM) and by the national committee for ethical research (CONEP) under the register number 027/05. All patients were informed about the study and verbal and written consent was obtained before the study was initiated.

Patients were recruited at the two hemodialysis units in Porto Velho. Inclusion criteria were CRF diagnosis and ongoing hemodialysis treatment for over 3 months. All patients answered a questionnaire and were submitted to physical exams.

The blood was collected in the first hour before the hemodialysis session. The samples were separated in three tubes for each patient: one went to serological tests and two tubes were frozen at -70º C for further qualitative or quantitative PCR, and genotyping tests. The serological tests were processed at the CEPEM's and Instituto de Pesquisa em Patologias Topicais de Rondonia's laboratory of serology. It was used third-generation ELISA kits from Diasorin® (Saluggia, Vercelli, Italy) for the following visual markers: HBV surface antigen (HBsAg); total antibodies to the HBV core antigen (anti-HBc); antibody to the HBV surface antigen (anti-HBs); and total antibody to the HCV (anti-HCV).

The polymerase chain reaction (PCR) tests for the detection of HBV and HCV material were performed in collaboration with the Instituto Adolfo Lutz's molecular team in Sao Paulo, Brazil, using the primers:

Hepatitis B

***Core Region**:*
1763: 5' - GCT TTG GGG CAT GGA CAT TGA CCC GTA TAA - 3'
2032R: 5' - CTG ACT ACT AAT TCC CTG GAT GCT GGG TCT - 3'
1778-E: 5' - GAC GAA TTC CAT TGA CCC GTA TAA AGA ATT - 3'
2017R-B: 5' - ATG GGA TCC CTG GAT GCT GCC TCT TCC AAA – 3'

S Region:
HBS1F: 5' - GAG TCT AGA CTC GTG GTG GAC TTC - 3'
HBS1R: 5' - AAA TKG CAC TAG TAA ACT GAG CCA - 3'
HBS2F: 5' - CGT GGT GGA CTT CTC TCA ATT TTC – 3'
HBS2R: 5' - GCC ARG AGA AAC GGR CTG AGG CCC – 3'

Hepatitis C

Region 5' NCR:
NCR2: 5' - ATA CTC GAG GTG CAC GGT CTA CGA GAC CT -3'
PTC1: 5'- CGT TAG TAT GAG TGT CGT GC –3'
PTC3: 5' - AGT GTC GTG CAG CCT CCA GG –3'
NCR4: 5' - CAC TCT CGA GCA CCC TAT CAG GCA GT –

The prevalence and 95% confidence intervals (CI) were calculated using the "exact" confidence intervals, computed by the method of Clopper and Pearson. The validity parameters for the serological methods used to diagnose hepatitis B and C in CRF patients on hemodialysis were analyzed using the molecular tests methods as reference. The odds ratio method was used to analyze the correlation between categorical variables and the χ^2(chi-squared) test with Fisher Exact test were used to test theories on the differences between the percentages. A level of significance of α=0.05 was applied to all tests. The statistics software used to process the data analysis were the Microsoft Excel® 2007 and the SPSS® 13.

RESULTS

One hundred twenty-eight CRF patients on hemodialysis were enrolled in the study. The the patients were 64 males and 64 female, with ages ranging from 16-77, average of 47.3 (SD=14.1) years, and a mean of 49.

The most prevalent serological marker was the anti-HBs, follow by the anti-HBc, the anti-HCV and the HBsAg (Figure 1).

For the 128 samples, the PCR results in 12 (9.4%) HBV-DNA positives and 16 (12.5%) HCV-RNA positives (Table 1).

The molecular test and the serology test found the same number of HBV positive samples, 12. However, just 6 samples were positive in both tests,

Figure 1. *Frequency of HBV and HCV serology marker in the CRF patients on hemodialysis in Porto Velho, Rondonia, 2004-2005.*

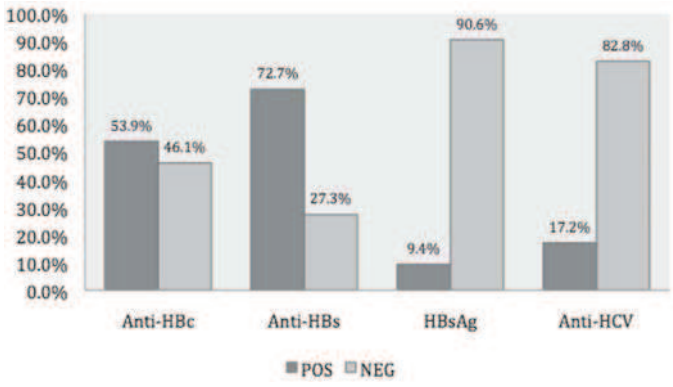

Table 1. *Results of molecular markers for HBV and HCV in CRF patients on hemodialysis, Porto Velho, Rondonia, 2004-2005.*

	HBV-DNA			HCV-RNA		
Results	**N**	**%**	**CI (95%)**	**N**	**%**	**CI (95%)**
Positive	12	9.4	(5.2 – 16.1)	16	12.5	(7.5 – 19.8)
Negative	116	90.6	(83.9 – 94.8)	112	87.5	(80.2 – 92.5)
Total	128	100.0		128	100.0	

Note: CI= Confidence interval.

and 18 samples were positive in a combination of the results of both tests.

The only validity parameters that reaches over 95% was the negative predictive value (NPV) for anti-HCV (Table 2).

DISCUSSION

The implementation of blood-borne diseases transmission control protocols in hemodialysis units has been shown to decrease the prevalence of HBV and HCV in patients with CRF[17,18]. 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[17].

The HBsAg and the anti-HBc rates in the general population of Rondonia is unknown, but population studies indicate that they might range between 3.4-7.8% and 32-68%, respectively[19,20,21,22]. The proportion of HBsAg positive samples in CRF patients on hemodialysis in Porto Velho (9.4%) was slightly below the average of other Brazilian units, and was higher than in the general population rate. The proportion of HBsAg samples found in Recife hemodialysis units was 12% and in Sao Paulo was 15%. In contrast, the proportion of anti-HBc patients (53%) in Porto Velho was similar to that the general population of Rondonia, and also similar to the anti-HBc rates found in other Brazilian hemodialysis units.

Uncommon presentation of serological markers for HBV/HCV may hide the real diagnosis (Table 3)[23, 24]. These uncommon presentations are frequent findings in CRF patients on hemodialysis[25]. They may be a result of a serious compromised immune system due to chronic uremia [24], and also due to mutations in coding regions of the HBsAg (S unit and core region), hindering seroconversion or reducing viral replication[11,26]. Patients who present only the anti-HBc may present a risk, since they may be at risk of acquiring HBV or may be a source of infection for other patients, especially if they undergo hemodialysis treatment in machines shared by HBV negative patients. Serial tests, molecular and/or serological, might help to confirm the accurate diagnosis[23,27].

The HCV is the most prevalent chronic viral infection among CRF patients[17]. The prevalence of anti-HCV identified by 3rd generation ELISA

Table 2. *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 CRF patients on hemodialysis in Porto Velho, Rondonia, 2004 - 2005.*

	HCV-RNA	HBV-DNA
Analysis	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: CI= Confidence interval; PPV= Positive predictive value; NPV= Negative predictive value.

Table 3. *Interpretation of ELISA results for HBV markers in CRF patients on hemodialysis in Porto Velho.*

N patients	*HBsAg*	*Anti-HBc*	*Anti-HBs*	*Outcome**
8	+	+	-	*HBV infection (classic)*
4	+	-	+	*HBV infection (?)*
0	+	-	-	*HBV infection (low AB⁺ detection)*
57	-	+	+	*Cured (classic)*
32	-	-	+	*Vaccine (or low anti-HBc detection)*
8	-	+	-	*Unknown*
19	-	-	-	*No HBV contact (or unknown)*

*Note: *KEEFFE et al.[38]; ⁺AB= Antibody.*

in this study (17.2%) was similar to the prevalence found in São Paulo (14.6%)[28] and Belo Horizonte (20%)[8], and lower than the prevalence found in Goiania (46%)[25]. However, the Brazilian prevalence is still high if it is compared to hemodialysis unit in the United States (7.8%)[29]. The PCR is a very sensitive method for diagnosing HBV in patients without CRF, but its power decreases in patients with CRF[23]. In this study the molecular test (PCR) failed to diagnose 33.3% of HBV cases. This result is in accordance with prior studies of the dynamics of the HBV load in hemodialysis patients. Fabrizi et al.[27] followed 29 HBsAg positive patients for 12 months with monthly HBV-DNA tests and found that 62.1% of the patients show intermittence in the HBV-DNA results. Moutinho et al.[30] and Fabrizi et al.[27] demonstrated that 14-58% of HBsAg positive patients were actually HBV-DNA undetectable. At any rate, these patients must be studied again, in order to identify possible mutations in the 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.

Therefore, the proportion of patients who are HBV carriers in Porto Velho by one time testing might best be estimated by counting the patients that were either HBsAg positive or HBV-DNA positive. This correction resulted that 14.2% CRF patients on hemodialysis in Porto Velho patients were HBV carriers. This higher value might be a more accurate prevalence of HBV in a hyper-endemic HBV area such as Porto Velho.

The validity parameters found in this study for HBV serological tests (Table 2) reflect the inconsistent findings of HBV serological markers in CRF patients on hemodialysis, and the potencial causes of such inconsistencies were discussed in the previous paragraphs.

The discrepancies found between the serological results for HCV and the PCR are described by several authors[8,31,32]. The anti-HCV positive and HCV-RNA negative cases may have been a result of elimination of the HCV virus, but also of the low viral load frequently found in hemodialysis patients, generating intermittent results in 33% of the cases[33]. The anti-HCV negative and HCV-RNA positive result is present in the immune compromised and in immune tolerant conditions.[34]

The accuracy of the serological (anti-HCV) and molecular (HCV-RNA PCR) results found in this study projects a reliable negative predictive value (NPV) for anti-HCV test. Similar results were described by other authors[17,35]. However, Carneiro et al.[25] 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.

Some considerations must be made. Before 2007 there was no commercial Kit for quantitative HBV-DNA registered in Brazilian Public Health Ministry. The "in house" PCR tests used were less expensive and comparable to the commercial kits. Most of CRF patients were in government funded public hemodialysis units, but molecular diagnosis methods were not available for these patients. Thus, for these CRF patients, the study provided molecular tests diagnosing HBV/HCV for the first time since the beginning of the 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 false negative HBV results. This tests will aim variation on the HBsAg protein and HBV-DNA PCR targets, 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.[11,26,30,36,37]

Much progress has been made to implement the blood-borne diseases transmission control protocols in hemodialysis units in Brazil, which has resulted in a decrease in the prevalence of HBV and HCV. Unfortunately, the risk of contamination outbreaks is still high[7,35].

Until today neither the serological nor the molecular tests alone is a gold standard for HBV or HCV diagnosis for CRF patients on hemodialysis. Efforts 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 exams.

ACKNOWLEDGEMENTS

We are grateful for the financial support from the Instituto de Pesquisa em Patologias Tropicais, the Centro de Pesquisa em Medicina Tropical de Rondônia and the State Department of Health of Rondônia, Brazil, as well as for stas well as for statistical help by Dr. Cor Jesus.

Precisão dos testes sorológico e molecular para diagnóstico do HBV e HCV em pacientes com Insuficiência Renal Crônica em hemodiálise, em Porto Velho, Brasil

Pacientes em tratamento com hemodiálise para a Insuficiência Renal Crônica (IRC) estão entre os grupos com maior prevalência da infecção pelo vírus da hepatite B e C, devido às transfusões de sangue e transmissão nosocomial. Um grupo de pacientes com IRC residentes em Porto Velho foi testado com marcadores sorológicos para hepatite B e C utilizando o teste ELISA e técnicas de biologia molecular (PCR). Os parâmetros para a validação dos resultados sorológicos foram medidos com base nos resultados da PCR. Dos 128 pacientes em hemodiálise durante o estudo, 12 (9,4%) eram HBsAg positivos, 69 (53,9%) eram anti-HBc positivos, 93 (72,7%) eram anti-HBs positivos, e 22 (17,2%) eram anti- HCV positivos. Os resultados dos testes com PCR foram de 12 (9,4%) HBV-DNA positivos e 16 (12,5%) HCV-RNA positivos. A precisão, sensibilidade e especificidade do ELISA para o HBsAg foram de 90,6%, 50% e 94,8%, e os mesmos parâmetros foram de 92,2%, 87,5% e 92,9% para anti-HCV. Com base nos resultados, apenas o valor preditivo negativo para anti-HCV (98,2%) mostrou-se confiável em pacientes renais crônicos em hemodiálise. Além disto, testes sorológicos e/ou moleculares representam a metodologia indicada para o diagnóstico da infecção pelo HBV e HCV nestes pacientes.

Palavras-chave: IRC – Hemodiálise – HBV – HCV – Precisão – Sorologia – ELISA – PCR.

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Recebido em / *Received*: 26/10/2009
Aceito em / *Accepted*: 28/11/2009