

Seroprevalence of Hepatitis C Virus and Hepatitis B Virus Among Dialysis Patients in Bahrain and Saudi Arabia

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ABSTRACT

Dialysis patients are at risk for contracting blood-borne infections, including hepatitis viruses (HBV and HCV). The aim of this study was to assess the prevalence of HBV and HCV infection among hemodialysis patients in Bahrain and Saudi Arabia. Study subjects comprised 81 Bahraini and 34 Saudi dialysis patients, and as control 7714 Bahraini and 2330 Saudi blood donors. Serologic markers of HBV (HBsAg, anti-HBc) and HCV (anti-HCV) were determined by EIA and confirmed by PCR (HBV) and RT-PCR (HCV). Higher prevalence of HCV (9.240% vs 0.300%, $P < .001$), HBsAg (5.88% vs 0.620%; $P < .001$), but not anti-HBc (1.7% vs 4.6%; $P = .01$) were seen in patients compared to controls, respectively. When compared to Bahrainis, higher prevalence of HBsAg (11.8% vs 3.7%) and anti-HCV (14.7% vs 7.4%) were seen among Saudi patients, respectively. Double HCV infection was frequent, and the most prevalent types were HCV1a/1b plus HCV4 in Bahraini, and HCV 2/2a plus HCV 4 among Saudi dialysis patients. Our results are the first report on viral hepatitis among dialysis patients in Bahrain, and the first to compare HBV/HCV rates among dialysis patients in the Eastern Arabian peninsula, and confirms other results that documented increased HBV and HCV infection among dialysis patients. Future studies aimed at assessing the status and to monitor the progress of viral hepatitis infection among dialyzed and transfused patients will have a strong impact on patient diagnosis, follow-up, and treatment.

ALTHOUGH dialysis is the treatment of choice for end-stage renal failure, dialysis patient are at risk for contracting blood-borne infections, including hepatitis viruses (HBV and HCV).¹ Viral hepatitis among dialysis patients is associated with significant severity and poor prognosis,² and both HBV and HCV synergize in accelerating the progression to hepatic anomalies,³ although some dispute any effect of combined HBV/HCV infection on acceleration of liver diseases.⁴ Previous screening for HBV and HCV infection relied on serologic tools (HBsAg and anti-HBs for HBV, and anti-HCV for HCV), and changes in liver enzymes.⁵ In view of the false-negatives inherent in serology testing,^{5,6} this highlighted the need for accurate means of detecting HBV/HCV, including polymerase chain reaction (PCR).^{6,7} Determination of the virus type and viral load serve an important diagnostic tool in the patient follow-up and treatment.^{2,7} In the present study, the prevalence of serologic markers of HBV and HCV, together with identification of the viral load of HBV and HCV, and

genotype were determined for dialysis patients in Bahrain and Saudi Arabia.

MATERIALS AND METHODS

Specimens

Hemodialysis patients from Bahrain ($n = 81$) and Saudi Arabia ($n = 34$), together with data collected for 7714 Bahraini and 2330 Saudi blood donors as controls, were recruited into the study. Predialysis samples were collected without preservatives, centrifuged at 4000 RPM for 10 minutes, and stored below -20°C . HBV and HCV nucleic acids were extracted using Trizol-LS (Life Technologies, Paisley, UK), or by Roche DNA/RNA extraction kits (Roche Molecular Systems, Mannheim, Germany), and were resuspended in nuclease-free water (Promega, Madison, Wisc, USA).

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HBV and HCV Analysis

Qualitative HCV was determined on COBAS/Amplicor automated PCR system (Roche Molecular Systems), and the assay sensitivity was 200 copies/mL. Qualitative HBV was assayed by PCR and agarose gel (2% w/v) electrophoresis (ProDect, Saluggia, Italy). HCV genotyping was performed by hybridization with genotype-specific and biotin-labeled DNA probes; genotypes screened were HCV-1a, -1b, -2, -2a, -2b, -3, -4, -5, and -6 (ProDect). Viral load (in copies/mL) were determined by quantitative PCR using COBAS/Amplicor system (Roche). Statistical analysis was performed using SPSS 11.5 software.

RESULTS

Serologic Profile of Blood Donors and Dialysis Patients

HBsAg (5.88% vs 0.31%) and HCV (9.24% vs 0.30%) were significantly higher among patients than donors ($P < .001$), while anti-HBc was higher among donors (4.60%) than patients (1.7%; $P = .01$). HBsAg, anti-HBc, and anti-HCV were compared between Saudi and Bahraini patients. Although its overall prevalence among dialysis patients was 6.1% (7/115), the prevalence of HBsAg were higher among Saudi (4/34; 11.8%) than Bahraini patients (3/81; 3.7%), but this was not statistically significant ($P = .10$), and 2 Bahraini but no Saudi patients were positive for anti-HBc ($P = .36$). HCV RNA was higher among Saudi (5/34; 14.7%) than Bahraini (6/81; 7.4%) patients. ALT levels among patients (20.9 ± 14.2) were lower than those of blood donors (35.1 ± 22.9 ; $P < .001$), indicating that most cases were asymptomatic.

HBV and HCV Viral Load and HCV Genotype Analysis

All HBV-positive cases were men, with average age of 46.8 years. HBV load among Bahraini and Saudi patients was 3200 to 171,000 IU/mL and 35,900 to 67,500 IU/mL, respectively. Of the 11 HCV-positive patients, 6 were Bahraini and 5 were Saudi, with similar mean age. Viral load showed heterogeneity among Bahraini and Saudi patients; most of the Bahraini patients (4/6) had high viral loads ($>500,000$ IU/mL), while Saudi patients had lower viral loads. All Saudi patients tested positive for HCV-4, and 2 each carried in addition HCV-2 or HCV-2a, while Bahraini patients were positive for HCV-1a and -1b, in addition to -2a and -4, and most cases (4/6) were double positive.

DISCUSSION

In this study, viral hepatitis markers were assessed in dialysis patients from Bahrain and Saudi Arabia. Higher prevalence of HBV and HCV were found among dialysis patients compared to blood donors, in agreement with previous studies, including those from Lebanon⁷ and Saudi Arabia.⁸ All anti-HCV- and HBsAg-positive samples were confirmed by PCR (HBV) and RT-nested PCR (HCV), resulting in the detection of HBV DNA in 7 (6.1%) and HCV RNA in 11 (14.7%) cases. Although this may contradict previous results on reduced sensitivity of serologic

versus PCR-based testing,⁹ both HBV and HCV samples had a relatively high viral titers. Although this was the first study on Bahraini subjects, previous studies on Saudi patients yielded a wide range of rates, which ranged from 11.2%¹⁰ to as high as 45.5%,⁸ and even 94.7%.¹¹ It is likely that the higher prevalence rates reported included local and foreign patients from countries endemic with HBV and HCV.¹¹ In our study, only native Bahraini or Saudi patients were included, and hence more accurately represent the HBV and HCV prevalence in both countries. All HCV- or HBV-positive patients were dialyzed on designated machines, thus minimizing the spread of the infection to HCV- and HBV-negative patients.¹² The HCV genotype distribution among Saudi patients appeared different from those previously reported,¹³ with a predominance of HCV4 and absence of HCV1a and HCV1b (common among Bahraini patients), and presence of type 2 and 2a.

HBV- or HCV-positive patients had normal ALT levels, indicating that most cases were asymptomatic, suggesting that altered ALT levels alone is not sufficient to monitor development and progression of hepatitis,¹⁴ and must be included in combination with other variables (serology and PCR) to determine patient clinical status. It should be noted here that silent cases in chronic dialysis patients, coupled with low viral load are the main problems of patient follow up, which necessitates the need for sensitive techniques that could detect low viral copies, including PCR, which is central for the follow up and management of dialysis patient.

REFERENCES

1. Puttinger H, Vychytil A: Hepatitis B and C in peritoneal dialysis patients. *Nephrol* 22:351, 2002
2. Fabrizi F, Lunghi G, Martin P: Recent advances in the management of hepatitis C in the dialysis population. *Int J Artif Organs* 25:503, 2002
3. Brechot C, Gozuacik D, Murakami Y, et al: Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). *Semin Cancer Biol* 10:211, 2000
4. Shiratori Y, Shiina S, Zhang PY, et al: Does dual infection by hepatitis B and C viruses play an important role in the pathogenesis of hepatocellular carcinoma in Japan? *Cancer* 80:2060, 1997
5. Caudai C, Padula MG, Bastianoni I, et al: Antibody testing and RT-PCR results in hepatitis C virus (HCV) infection: HCV-RNA detection in PBMC of plasma viremia-negative HCV-seropositive persons. *Infection* 26:151, 1998
6. Hitzler WE, Runkel S: Routine HCV PCR screening of blood donations to identify early HCV infection in blood donors lacking antibodies to HCV. *Transfusion* 41:333, 2001
7. Matar G, Sharara H, Abdelnour G, et al: Genotyping hepatitis C virus isolates from Lebanese hemodialysis patients by reverse transcription-PCR and restriction fragment length polymorphism analysis of 5' noncoding region. *J Clin Microbiol* 34: 2623, 1996
8. Al Nasser MN, al Mugeiren MA, Assuhaimi SA, et al: Seropositivity to hepatitis C virus in Saudi haemodialysis patients. *Vox Sang* 64:127, 1993
9. Furusyo NJ, Hayashi I, Ariyama Y, et al: Maintenance hemodialysis decreases serum hepatitis C virus (HCV) RNA levels in hemodialysis patients with chronic HCV infection. *Am J Trop Med Hyg* 95:490, 2000

10. al-Mugeiren M, al-Rasheed S, al-Salloum A, et al: Hepatitis C virus infection in two groups of paediatric patients: one maintained on haemodialysis and the other on continuous ambulatory peritoneal dialysis. *Ann Trop Paediatr* 16:335, 1996
11. Huraib S, al-Rashed R, Aldrees A, et al: High prevalence of and risk factors for hepatitis C in haemodialysis patients in Saudi Arabia: a need for new dialysis strategies. *Nephrol Dial Transplant* 10:470, 1995
12. Wreghitt TG: Blood-bore virus infections in dialysis units—a review. *Rev Med Virol* 9:101, 1999
13. Bosmans JL, Nouwen EJ, Behets G, et al: Prevalence and clinical expression of HCV-genotypes in haemodialysis-patients of two geographically remote countries: Belgium and Saudi-Arabia. *Clin Nephrol* 47:256, 1997
14. Milotic I, Pavic I, Maleta I, et al: Modified range of alanine aminotransferase is insufficient for screening of hepatitis C virus infection in hemodialysis patients. *Scand J Urol Nephrol* 36:447, 2002