Prevalence and risk factors of hepatitis B and C viruses among haemodialysis patients: a multicentric study

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Background Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important causes of morbidity and mortality among haemodialysis (HD) patients and create problems in the management of patients in the renal dialysis units, as patients with chronic renal failure do not absolutely clear these viral infections.
Aim The aim of the study is molecular detection of HBV and HCV and their possible risk factors among the HD patients in northern Pakistan.
Materials and methods A cross-sectional study was conducted from November 2013 to June 2014. The infections were investigated through serological and molecular techniques.
Results The overall prevalence of HBV among the five HD centres was 7.5%. The main risk factors were HD centre (26.66%), history of blood transfusion (20%), dental procedure (13.33%) and time duration on HD (6.66%). However, the overall prevalence of HCV among the five HD centres was 19.58%. The main risk factors included HD centre (25.53%), history of blood transfusion (25.53%), dental procedure (10.64%), surgical treatment (6.38%), patients treated abroad (6.38%) and time duration on HD (4.25%).
Conclusion The high prevalence of hepatitis viruses among HD patients of northern Pakistan indicates a close relation between HD centres and hepatitis virus transmission. Therefore, preventive control measures are essential to reduce hepatitis transmission in HD centres. Eur J Gastroenterol Hepatol 00:000–000

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are important causes of morbidity and mortality among haemodialysis (HD) patients and create problems in the management of patients in the renal dialysis units, because patients with chronic renal failure do not clear these viral infections absolutely. Approximately 200 million people are infected with HCV worldwide [1], where a 3–4% seroprevalence rate is reported in Asian countries, whereas in central Africa and Egypt, it is 10–20% [2]. Approximately 8% HBV prevalence rate was reported in South East Asia, sub Saharan Africa, China, Indonesia and Nigeria. In these developing countries, 70–95% population has serological markers against HBV such as Hepatitis B surface antigen and total antibodies to hepatitis B core antigen. Another similar study stated that 60% of the world population is present in high endemic zones [3,4]. Among Middle East, Eastern and Southern Europe, South America and Japan, the estimated HBV infection rate is ~10–60% and the chronic HBV rate is 2–7%. In the intermediate endemic region (Jordan), most infections develop in adults, but the rate of chronic infection is higher in infants owing to the early childhood exposure to HBV infection [5]. Similarly, the seroprevalence of HBV infection has been reported at ~5% in India [6]. The risk factors for HBV and HCV in dialysis patients include blood transfusions, total spent time on dialysis, intravenous drug use and a history of kidney transplantation. The dialysis-related risk is around 2%, varying according to the countries. Healthcare related HCV transmission can be eradicated with control measures planned to avoid transmission of blood-borne pathogens [7]. No documented data have been reported on the prevalence of hepatitis viruses among the HD patients in northern Pakistan, and to the best of our knowledge, this report is the first to address this issue. Therefore, the specific objectives of this study were to investigate the prevalence of HBV and HCV among the HD patients of northern Pakistan and to assess the major risk factors for transmission of these viruses among HD populations.

Materials and methods

All the five government HD centres of northern Pakistan were included in this study, and a total of 480 patients

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were tested during July 2013 to February 2014. The study principles and protocols were submitted to and approved by the committee of Ayub Teaching Hospital Abbottabad, District Head Quarter (DHQ) Hospital Haripur, DHQ Hospital Battagram, DHQ Hospital Kohistan and DHQ Hospital Mansehra. History was taken from each patient undergoing HD by using a detailed multiple-choice questionnaire to ensure proper data collection and prevent any misunderstanding.

Samples collection

Two blood samples were collected from each patient under aseptic conditions in a plain tube, which was labelled accordingly and kept at room temperature to clot. Serum from the first tube was tested within 2 h for HBsAg and anti-HCV antibodies. Serum from the second tube was frozen at −70°C in a sterile, DNAse-free and RNAse-free tightly capped tube and was later used for PCR analysis.

Virology

For the detection of HBsAg, HBsAg version 2.0 ICT (Immuno Chromatographic Techniques) kit (Healgen Scientific LLC, Houston, Canada) was used. Nonreactive serum samples were declared negative for HBsAg, whereas the reactive sample was retested to confirm the result. Serologically HBsAg-positive samples were tested individually through PCR. For anti-HCV antibodies detection, HCV version 3.0 ICT kit (Accurate Diagnostics, Healgen Scientific LLC, Houston, USA) was used. A nonreactive sample was declared negative HCV, whereas reactive sample was retested to confirm the result. Serologically, positive HCV samples were furthered subjected to PCR for confirmation.

Molecular screening for HCV RNA

RNA was extracted from 100-µl serum samples by using Gentra (Puregene, Minnesota, USA) RNA isolation kit according to the protocol described in the kit. Both cDNA synthesis and PCR amplification of the target sequences were performed in a single tube using the one-step RT-PCR kit according to the manufacturer-recommended protocols (Puregene). The primers were designed to specifically bind the 5′UTR of the HCV genome, which is highly conserved among different genotypes. Primer sequences were as follows: outer sense primer (5′-CCCTGTGAGGAACTWCT GTCTTCACGC-3′), antisense outer primer (5′-GGTGCA CGGTCTACGAGACCT-3′), inner sense primer (5′-TCTA GCCATGGCGTTAG TRYGAGTGT-3′) and inner antisense primer (5′-CACTCGCAAGCACCCTATCAGGCA GT-3′, W = A or T, R = A or G, Y = T) [8].

The reactions were carried out in 25-µl volumes using 10-µl RNA in the presence of 0.6 µmol/l of each HCV outer primer, 400 µmol/l dNTP and five units of RNase inhibitor. The reaction conditions were one cycle at 50°C for 30 min, one cycle at 95°C for 15 min followed by 40 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 1 min. The reaction cycles were complete at 72°C for 10 min. The second nested PCR was also performed to improve the detection capacity of the PCR test. The second PCR were carried out in 25-µl volumes using 5-µl DNA templates from first PCR with 1× PCR master mix (Promega, Madison, USA) and 0.4 µmol/l of HCV inner primers. The reaction cycling conditions were as follows: one cycle at 94°C for 2 min, followed by 35 cycles of incubation at 94°C for 30 s, 58°C for 45 s, and 72°C for 1 min and final extension was done at 72°C for 7 min. The products were analyzed on 2% agarose gel with 100-bp ladder and stained with ethidium bromide. The appearance of 298 and 235-bp bands was considered positive for the first and second PCR results. The present research data were collected, summarized, tabulated and analysed statistically by using statistical package, SPSS version 10.0 (SPSS Inc., Pearson, USA) and Graphpad Prism version 5.0 (GraphPad Software, Inc., La Jolla, USA). Percentages and ratios for all variables were calculated.

Results

During the study, 114 patients from Ayub Teaching Hospital Abbottabad, 36 patients from DHQ Hospital Battagram, 198 patients from DHQ Hospital Haripur, 24 patients from DHQ Hospital Kohistan and 108 patients from DHQ Hospital Mansehra were included. Of the 480 patients tested, 282 were male and 198 were female cases. By combining the results of both serological and molecular methods, 74 (15.41%) patients were positive for HBV and 140 (29.17%) were positive for HCV. Similarly, 30 (7.5%) patients were positive for HBV and 94 (19.58%) patients were positive for HCV through PCR. Of the positive cases for HBV and HCV, four (3.22%) patients were co-infected (Fig. 1).

Sex-wise distribution for HCV-positive cases was also determined. Of the 282 male samples, 16 (5.70%) samples were reported positive for HCV virus and 58 (20.57%) sample were positive for HCV through PCR. However, in case of 198 female patients, only 14 (7.07%) were reported positive for HBV and 36 (18.18%) were positive for HCV virus through PCR technique. The highest HCV-positive percentage was reported in male patients. Other risk factors reported during current study included blood transfusion, in which six (20%) cases were positive for HBV and 24 (25.53%) were reported positive for HCV. On the contrary, no HBV cases were reported in the patients with history of travelling abroad, whereas in case of HCV, there were six (6.38%) positive patients with such history. Among the patients with previous history of having dental procedures, four (13.33%) HBV-positive

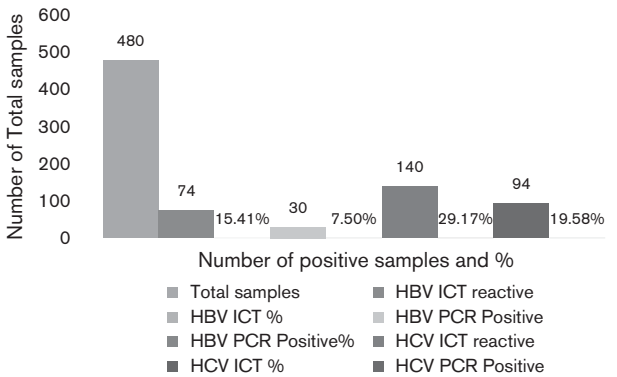

Fig. 1. The total number of samples, and the percentage of HBV PCR-positive, HCV-reactive and HCV PCR-positive samples. HBV, hepatitis B virus; HCV, hepatitis C virus.

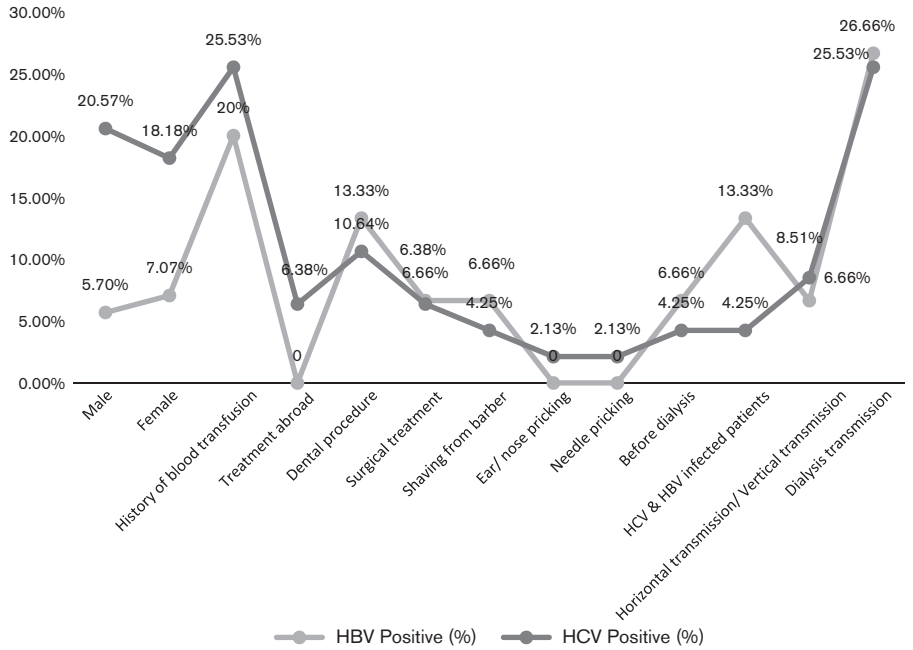

Fig. 2. Relationship of HBV, HCV infection and others risk factors of transmission. HBV, hepatitis B virus; HCV, hepatitis C virus.

cases were detected and 10 (10.64%) cases were reported for HCV. Similarly, the main risk factor for acquiring HBV and HCV infections during current study was because of visitation to dialysis centres and undergoing dialysis. After dialysis practices, we detected eight (26.66%) new HBV-positive cases, whereas 24 (25.53%) new cases were detected for HCV (Fig. 2).

District-wise distribution of HBV and HCV-positive cases among the HD patients of Hazara division was also carried out. A total of 114 patients were screened from Abbottabad; of which, 14 (12.28%) patients were positive for HBV through ICT method. However, 36 (31.57%) patients were found to be positive for HCV; of which, 18 (15.79%) were confirmed through PCR. Among the 36 patients of Battagram, only six (16.67%) were positive for HCV through ICT and PCR. Among the 198 Haripur patients, 20 (10.10%) were positive for HBV through ICT method and 14 (7.07%) were positive through PCR. However, six (3.03%) were positive for HCV through ICT method and PCR. Of 24 Kohistan patients, six (25%) were positive for HBV and HCV through ICT method and PCR. In 108 Mansehra patients, 34 (31.48%) were positive for HBV through ICT method and 10 (9.25%) were found to be positive through PCR. However, only 46 (42.59%) cases were detected for HCV through ICT method, of which 34 (31.48%) cases were also detected through PCR (Fig. 3).

The highest percentage for HBV was 25% in DHQ Hospital Kohistan followed by 9.25% DHQ Hospital Mansehra, and no cases were detected in both Ayub Teaching Hospital Abbottabad and DHQ Hospital Battagram. Similarly, for HCV, the highest percentage was 31.48% in DHQ Hospital Mansehra followed by 25% in DHQ Hospital Kohistan, 16.67% in DHQ Hospital Battagram, 15.79% in Ayub Teaching Hospital Abbottabad, and 15.15% in DHQ Hospital Haripur (Fig. 4). The appearance of ~180 and ~200-bp bands was considered positive for the HBV and HCV samples (Fig. 5).

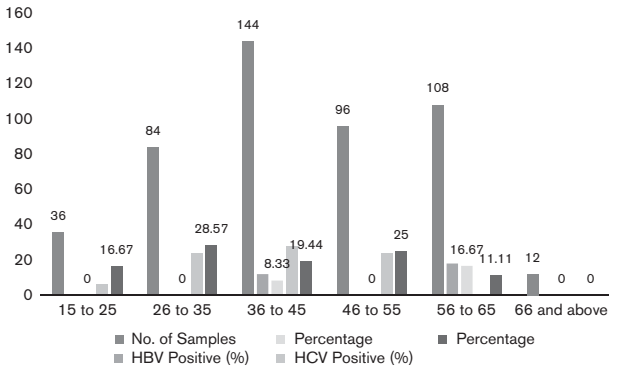

Fig. 3. The age-wise Distribution of HBV-positive and HVC-positive cases. HBV, hepatitis B virus; HCV, hepatitis C virus.

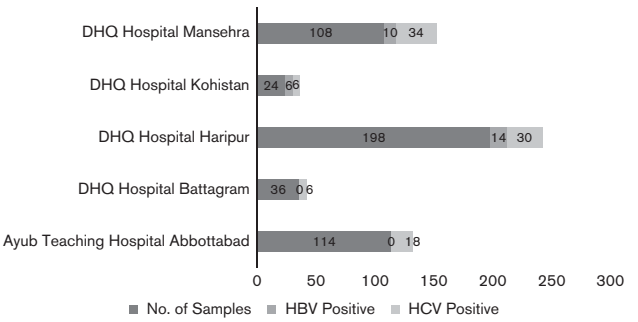

Fig. 4. The overall prevalence of HBV and HCV among all centres of HD patients. HBV, hepatitis B virus; HCV, hepatitis C virus; HD, haemodialysis.

Discussion

HBV and HCV infections are important causes of morbidity and mortality among HD patients, and they cause problems in the management of patients in the dialysis

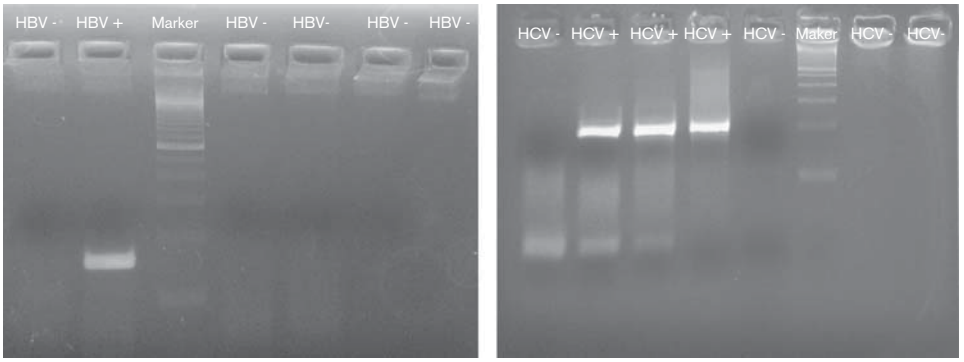

Fig. 5. The results of molecular screening of HBV-positive and HCV-positive patients after PCR. Lane marker: 2 kb DNA ladder, lane HBV: positive sample (~180 bp), lane HCV: positive sample (~200 bp). HBV, hepatitis B virus; HCV, hepatitis C virus.

units because chronic renal failure patients cannot clear these viral infections efficiently [9].

In our current research, the positive cases for HBV and HCV in 480 HD patients were 74 (15.41%) and 140 (29.17%), respectively. Our results are similar to study conducted by Ali et al, in which they found the prevalence of chronic HBV infection in the general population of Pakistan to be 1.4–11% in the nonblood donor population than the blood donor population (1.4–8.4%) and HCV seroprevalence was 0.3–31.9% [10]. Our results showed high positive results for HCV and HBV in the HD patients; we showed 20.57% HBV and 18.18% HCV frequency among the female and male HD patients. Among the total 124 positive cases detected through PCR, four (3.22%) patients were cohepatitis infected. A similar study was conducted by Lavanchy [11], which revealed high frequency of HBV and HCV among the patients with chronic liver disease (CLD). The prevalence of HBsAg and anti-HCV antibody in patients with CLD was 35.8 and 22.5%, respectively. Dual infection was also observed in three (2.5%) patients; the prevalence of HCV in patients with CLD is high as compared with the national prevalence of 1.9% in the general population. Hepatitis virus remains a major hazard for HD patients [12]. In the current study, prevalence of HBV in HD patients of Hazara division was 7.5%, which is higher than Jordan (5.9%) and lower than Saudi Arabia (10%) and Bahrain (11.8%) [8,13,14]. Prevalence of HCV in our studied group is 19.58%, which is supported by El-Kader *et al.* [15], who reported 19.1% of HCV cases in HD patients [16]. In our study, the prevalence of HCV was different among different HD centres; this difference may be because of the different degrees of commitment to universal precautions taken in each centre. Similar results were shown in previous studies in Jordan [17,18]. Blood transfusion was found to be a high risk factor in this study. The risk increased with the increase in the number of cases with blood transfusion. We found 25.53% positive cases for HCV and 20% for HBV. These results are in agreement with another study in USA [19]. RT-PCR has the highest sensitivity with a detection rate of 100 viral genomes per ml. Thus, prevalence data for HCV in HD patients were obtained by using third-generation ELISA assays for the detection of HCV antibodies and RT-PCR for HCV-RNA detection. We found HCV virus in 19.58% of HD patients, compared with ELISA, HCV virus was present in 64.9% of infected patients [20]. Our study shows high prevalence rate than other reports from different countries. According to Kalantari *et al.* [21], who also assessed the prevalence of HBV and HCV in 499 HD patients and risk factors for transmission of HBV and HCV among the HD patients in eight governmental HD centres in Isfahan, Iran, the prevalence of HBV and HCV infections was 1.2 and 5.2%, respectively. Age, sex and time duration on HD were not statistically significant in HBV and HCV patients. Similarly, the prevalence of HCV in 838 Iranian HD patients was found to be 13.2%. Alavian *et al.* [22] and Joukar *et al.* [23] showed the prevalence of HCV infection in HD patients was 11.9% in Guilan and seroprevalence of HBsAg was 1.4%. The prevalence of HBV in the HD patients in Khuzestan province has been reported to be 5.1% [24].

Conclusion

HBV and HCV infections are frequent among HD patients in northern Pakistan. The HD practices, blood transfusion and dental procedure appear to be the main risk factors for HBV and HCV infections. High prevalence of hepatitis viruses among the HD patients compared with the normal population of this region indicates a causative relationship among the HD centres and hepatitis virus transmission. Therefore, careful preventive control measures are needed to control hepatitis virus transmission in HD centres.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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