

# PREVALENCE AND RISK FACTORS OF HEPATITIS B AND C VIRUS INFECTIONS AMONG HEMODIALYSIS PATIENTS FROM PRIVATE HEMODIALYSIS UNITS IN SURABAYA, INDONESIA

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**Abstract.** The aims of the present study were to profile seroprevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and possible risk factors among hemodialysis (HD) patients in private hemodialysis units (HDU) in Surabaya, Indonesia. Sera were obtained from 180 HD patients in 4 different private HDUs and tested for hepatitis B surface antigen (HBsAg) and antibody to HCV (anti-HCV). Patients without HBsAg and anti-HCV at first sampling were followed serologically every 3 months for 9 months, while those with HBsAg or anti-HCV positive sera were subjected continually to PCR to detect HBV DNA and HCV RNA. The prevalence of hepatitis infections varied widely between the HDUs, from 0% to 8.1% of patients positive for HBsAg and 0% to 60.6% of those positive for anti-HCV, respectively. These values were markedly higher than those among the general population, but not as high as in governmental HDUs in Indonesia. New incidence of HBV was not detected in any HDU, whereas that of HCV was found in two HDUs, HCV-1b in one HDU and HCV-1a in the other. Inappropriate practices were observed, such as shortage of medical staff and malfunctions in infection-control committees. Prevalence of HBV and HCV infection among HD patients in private HDUs were high and varied among the HDUs. Isolation of both HBV- and HCV-infected patients and staff education should help to reduce the prevalence of hepatitis infections in HDUs.

**Keywords:** hemodialysis, hepatitis B virus, hepatitis C virus, private hemodialysis unit, Indonesia

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## INTRODUCTION

Hepatitis B (HBV) and C (HCV) virus infections are considered as major public health problems worldwide. Prevalence of HBV and HCV infections in patients on

maintenance hemodialysis (HD) is consistently higher than that in the general population because HD patients are at an increased risk of exposure to blood-borne viruses, including HBV and HCV (Fabrizi *et al*, 2002a,b; Su *et al*, 2013). Several outbreaks and sporadic cases of nosocomial HCV or HBV transmission in HD units (HDUs) have been linked to the poor disinfection of dialysis equipment and poor compliance with standard infection-control methods (Rinonce *et al*, 2013).

Prevalence of HBV and HCV among HD patients varies highly among countries and also among HDUs within a single country (Kosaraju *et al*, 2013). There is a high prevalence of HBV and HCV infections among HD patients in government hospitals in Indonesia (Soetjipto *et al*, 1996; Rinonce *et al*, 2013). It is suspected that long-term HD and frequency of blood transfusions are possible causes of the high prevalence of HCV infection among HD patients (Abacioglu *et al*, 2000; Alashek *et al*, 2012; Rinonce *et al*, 2013), which has remained high for a long period of time in Indonesia in spite of national dialysis practice guidelines and infection-control policies being enforced by the Ministry of Health and Association of Indonesian Nephrologists.

In addition to governmental HDUs, private HDUs, which have been established by religious bodies and foreign aid, also provide HD to patients in Indonesia. The status of hepatitis infections and management practice in private HDUs in Indonesia need to be determined in more detail. The aims of the present study were to profile the status of HBV and HCV infections and possible causes among private HDUs in Surabaya, Indonesia and to identify possible patient care practices associated with the high prevalence of hepatitis infections in HDUs.

## MATERIALS AND METHODS

### Patients

Four (HDU A - D) out of 14 private HDUs in Surabaya, Indonesia were included in this study. Sera were obtained from 180 HD patients in the four private HDUs between July 2012 and April 2014. Characteristics of the patients and the four private HDUs are shown in Table 1. Isolation program for HBV and HCV seropositive patients (HBsAg for HBV and anti-HCV for HCV) using dedicated areas and machines were implemented for HBV infection in all units, but in only one unit for HCV infection. The study protocol was reviewed and approved by the Ethics Committees of Airlangga University in Indonesia and Kobe University in Japan. We conducted the survey after obtaining written informed consent from all participants.

### Serological assays

Serological tests for HBV markers were performed on all 180 serum samples for HBsAg employing reverse passive hemagglutination assay (RPHA) (Mycell II HBSAg; Institute of Immunology, Tokyo, Japan) and enzyme immunoassay (EIA) (Espline HBsAg; Fuji Rebio, Tokyo, Japan), and for antibodies to HBsAg (anti-HBs) using passive hemagglutination assay (PHA) (Mycell II anti-HBs; Institute of Immunology). Anti-HCV antibodies were detected using particle agglutination method (Ortho HCV Ab PA test II; Ortho Clinical Diagnostics, Tokyo, Japan). Patients who were negative for HBsAg and anti-HCV at the first sampling were then serologically followed every 3 months until 9 months to investigate new incidence of hepatitis virus infections.

### Hemodialysis practice

Structured questionnaires were used to establish the status of precautions used

Table 1  
Characteristics of HD patients and HDUs.

	HDU-A (n = 12)	HDU-B (n = 62)	HDU-C (n = 35)	HDU-D (n = 71)
Age (years) (mean ± SD)	54 ± 14	52 ± 13	53 ± 12	46 ± 10
Sex (M)	10	36	22	46
Patient-to-staff ratio per shift	1.3	1.5	3	4
HB-vaccinated patients (%)	10 <sup>a</sup> (83%)	14 <sup>a</sup> (23%)	3 <sup>a</sup> (9%)	19 <sup>a</sup> (27%)
Year established	2003	1983	2010	2005
Dialyzer reuse	No	No	Yes <sup>b</sup>	Yes <sup>b</sup>
Glove change at every puncture	Yes	Yes	Yes	Yes
HCV patient segregation	Yes	No	No	No
	(transfer to other units)			
HBV patient segregation	Yes <sup>d</sup>	Yes <sup>c</sup>	Yes <sup>d</sup>	Yes <sup>d</sup>
Distance between beds (> 1 m)	Yes	Yes	Yes	No
Staff training (more than once a year)	Yes	Yes	No	Yes
Treatment of anemia	Epo	BT + Epo	BT + Epo	BT + Epo

BT, blood transfusion; Epo, erythropoietin. <sup>a</sup>*p* < 0.0001. <sup>b</sup>Manual washing. <sup>c</sup>Within the unit. <sup>d</sup>Transferred to a unit with a dialysis machine dedicated to HBV patients.

in each HDU. Each unit was asked to fill in a form detailing HD practices, such as distance of beds between patients, dialyzer reuse and nurse-to-patient ratio (Table 1).

#### Detection of HBV and HCV, sequencing, and phylogenetic analysis

HBV DNA was extracted from 200 µl of serum using a DNA extraction kit (QIAamp DNA Blood Mini Kit; QIAGEN, Tokyo, Japan). The presence of HBV DNA was assayed by PCR with P7 and P8 primer pairs located in the S region (Lusida *et al*, 2008). When PCR amplification was negative, second-round PCR was carried out using primers HBS1 and HBS2 (Lusida *et al*, 2008).

HCV RNA was extracted from 140 µl of serum using an RNA extraction kit (QIAamp Viral RNA kit; QIAGEN, Tokyo, Japan). NS5B region of HCV genome was amplified by nested RT-PCR using a Super Script III One-Step RT-PCR System with

specific primers (Akkarathamrongsin *et al*, 2010). If the result of nested RT-PCR amplification was negative, the extracted RNA was reverse transcribed and amplified using a set of primers located in 5'UTR region (Doi *et al*, 1996).

Amplicons were directly sequenced using Big Dye Deoxy Terminator cycle sequencing kit with an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA). Alignments were performed using CLUSTAL X software and phylogenetic trees were constructed using neighbor-joining method (Tamura-Nei model) with the 440-bp amplicon (HCV). In order to confirm reliability of the phylogenetic tree analysis, bootstrap resampling and reconstruction were conducted 1,000 times. These analyses were carried out using Molecular Evolutionary Genetics Analysis (MEGA) software program version 4.

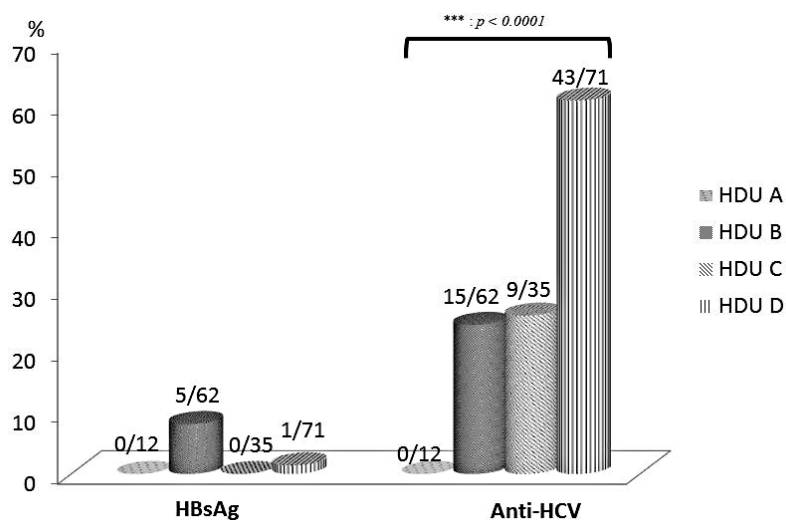


Fig 1—Comparison of positivity for hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (anti-HCV) among hemodialysis units (HDUs).

### Statistics

Statistical analyses were performed using chi-square or Fisher's exact test for categorical variables. Independent *t*-test was used for continuous variables. A *p*-value < 0.05 is considered significant.

### RESULTS

Twelve, 62, 35, and 71 patients from HDU-A, HDU-B, HDU-C, and HDU-D, respectively in Surabaya were included in this study and comprised 114 male and 66 female patients. Patient ages ranged from 22 to 80 years, and the mean ( $\pm$ SD) age for all patients was 50  $\pm$ 12 years.

Prevalence of hepatitis infections varied widely between the HDUs, ranging from 0-8% of patients positive for HBsAg and 0-61% of those positive for anti-HCV (Fig 1). HBV and HCV infections were not detected in HDU-A. The highest percent HBsAg was 8% (5/62) in HDU-B, followed by 1% (1/71) in HDU-D, with no cases be-

ing found in HDU-A or HDU-C (Fig 1). HBV DNA was detected in 80% (4/5) of HBsAg-positive patients in HDU-B, but was absent in HDU-D. HBV subgenotype was determined to be B3 in the 4 HBV strains (data not shown). No correlations were observed among prevalence of HBV infection and age, HD duration or number of blood transfusions. Hepatitis B vaccine had been administered to 45/180 (25%) patients

and antibodies to HBs were detected only in 14 (31%) vaccinated patients. The highest coverage was in HDU-A, followed by HDU-D (Table 1).

The highest prevalence of anti-HCV was 61% (43/71) in HDU-D, followed by 26% (9/35) in HDU-C and 24% (15/62) in HDU-B, with no cases being detected in HDU-A (Fig 1). Prevalence of HCV RNA was 61% (41/67). The highest percent HCV RNA was 93% (14/15) in HDU-B, followed by 53% (23/43) in HDU-D, and then 44% (4/9) in HDU-C. HCV infection is significantly associated with > 1 year HD duration in HDU-B and HDU-C, and > 5 blood transfusions in HDU-D (Table 2). The main HCV subtype was HCV 1a in HDU-C (100%) and HDU-D (94%), and HCV 1b in HDU-B (100%) (Fig 2). Phylogenetic analysis showed that HCV subtypes among HD patients had their own clusters between HCV 1a and HCV 1b, suggesting facility-related (nosocomial) infections (Fig 2).

Table 2  
Characteristics of patients and risk factors associated with anti-HCV positivity.

Characteristics and risk factors	HDU-A (0%; n = 0) <sup>a</sup>	HDU-B (24%; n = 15) <sup>a</sup>	HDU-C (26%, n = 9) <sup>a</sup>	HDU-D (61%, n = 43) <sup>a</sup>
Number of HCV RNA positive	0	14	4	23
Sex				
Male	0	11	6	28
Female	0	4	3	15
Age				
<45 years	0	6	5	19
≥45 years	0	9	4	24
Hemodialysis duration				
<1 year	0	4 <sup>b</sup>	1 <sup>c</sup>	14
≥1 year	0	11 <sup>b</sup>	8 <sup>c</sup>	29
Number of blood transfusions				
<5	0	13	3	18 <sup>d</sup>
≥5	0	2	6	25 <sup>d</sup>

<sup>a</sup> $p < 0.0001$ ; <sup>b</sup> $p = 0.046$ ; <sup>c</sup> $p = 0.033$ ; <sup>d</sup> $p = 0.045$ .

Two patients, 1 at HDU-B and the other at HDU-D, had a mixed infection of HBV and HCV. No new incidence of HBV was found at any HDU, whereas new incidence of HCV was found in 1/18 (6%) patient at the third sampling in HDU-C, and 2/18 (11%) and 2/15 (13%) at the second and third sampling, respectively in HDU-D.

## DISCUSSION

The prevalence of anti-HCV antibodies among HD patients in the majority of the private HDUs examined in this study was markedly higher than that among the general population, but was not as high as that in government HDUs in Indonesia (Soetjipto *et al*, 1996; Rinonce *et al*, 2013). Prevalence of both HBV and HCV infections varied widely among the four HDUs surveyed. HBV and HCV were not detected in HDU-A, suggesting that infection control in this facility was more effective than that in other three HDUs.

For example, administration of the hepatitis B vaccine and the segregation of both HBV and/or HCV infected patients were strictly practiced in HDU-A. Additionally, the patient-to-staff ratio was smaller than that in the other HDUs, suggesting that staff had opportunity to provide more detailed care to their patients. These results indicate that the staff-to-patient ratio greatly affected the prevalence of hepatitis infection in HDU. Prevalence of HBV-infected HD patients was higher in HDU-B than in other HDUs, which suggests that isolation practices within these units were still insufficient to control HBV infection, even though new incidence was not observed in HDU-B. HDUs free of or with a low prevalence of HBV should send HBV-infected patients to other HDUs equipped with HD machines dedicated only for HBV patients. Although HBV isolation within HDUs effectively reduces the prevalence of HBV infection (Kosaraju *et al*, 2013), sending HBV-infected patients to other better equipped HDUs also is



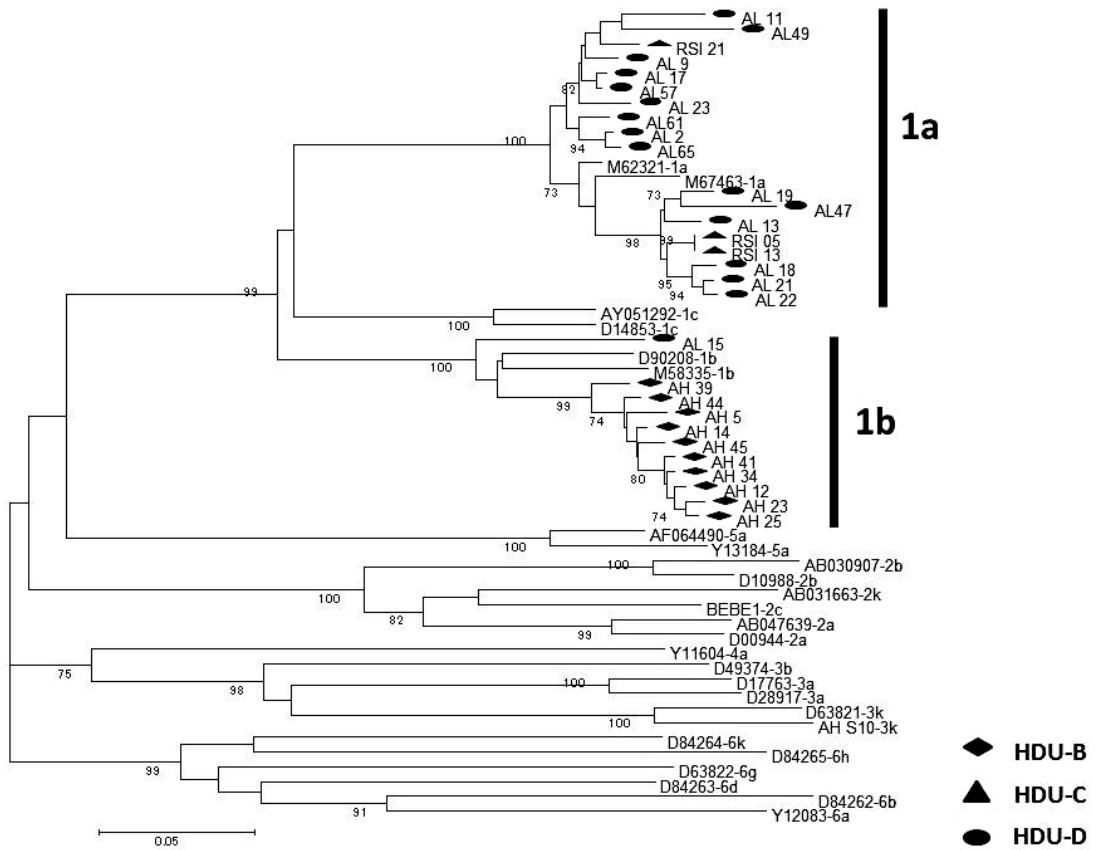


Fig 2–Phylogenetic tree based on NS5B region of HCV strains isolated from 29 hemodialysis patients compared with 26 references strains. Reference isolates from GenBank database are indicated together with their accession numbers. Number inside the tree indicates bootstrap reliability.

considered as an appropriate method in resource limited regions, including Indonesia.

HCV containment was performed in HDU-A by transferring patients to other HDUs, but such a measure was not carried out in other HDUs. Although the Centers for Disease Control (CDC), USA have not insisted on HCV isolation practice (CDC, 2001) isolation of HCV-infected HD patients may be an effective means for HCV control (Izopet *et al*, 1999; Saxena *et al*, 2003). Moreover, a previous study demonstrated that isolation of HCV-infected

patients during hemodialysis significantly decreases HCV seroconversion rate (Agarwal *et al*, 2009).

HBV and HCV are not able to pass through the dialyzer membrane to cause cross-infection between patients (Hubmann *et al*, 1995), so that dialyzer reuse should not be the cause of infection. However, contaminated blood may be transferred if dialyzers are not effectively sterilized between uses (Ahmad *et al*, 2005). As glove changing for staff was not strictly practiced in HDUs of government hospitals in Indonesia, in which

the prevalence of HCV infections was previously reported to be high, staff glove changing for every patient is strongly recommended in all HDUs.

No new infection was found in HDU-A or -B, suggesting that current protocols for infectious control in those units were adequate. Hepatitis B vaccinations, isolation of HCV-infected patients, duration of HD, and number of blood transfusions were associated with prevalence of hepatitis B and C infections shown in this study. Furthermore, patient-to-staff ratio, staff education on infection control, maintenance of dialyzer, glove changing, and distances between the beds were found to be potential risk factors for hepatitis infections in HDUs, but a larger cohort of patients are needed to attain statistical significant data.

Phylogenetic analysis suggested nosocomial transmission of HCV infections in HDU-B and HDU-D, as previously reported in various countries (Girou *et al*, 2008; Alashek *et al*, 2012; Rinonce *et al*, 2013). HCV-1a and 1b, the most common subtypes among HD patients in this study, also were previously identified as the main types in Surabaya (Soetjipto *et al*, 1996).

In conclusion, the prevalence of HBV and HCV infections among HD patients in private HDUs remains high and varies widely among the HDUs. HDU-A represents a model HDU for minimizing the prevalence and incidence of HBV and HCV infections. Segregation of both HBV and HCV infected patients may be a factor for reducing the prevalence of hepatitis infections in HDUs. Staff education on infection control in HDUs is also strongly needed to decrease hepatitis infection among HD patients. As the number of patients examined in this study was small,

analysis of a larger population is needed.

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