

Research Paper

Discrepancy between Serological and Virological Analysis of Viral Hepatitis in Hemodialysis Patients

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Abstract

Background and Aim: Viral hepatitis is a health threat for hemodialysis (HD) patients and it may be transmitted during treatment. Some patients categorized to have viral hepatitis were found to be non-viremic. To clarify the discrepancy between the serological tests in HD patients, we conducted the study.

Methods: A total of 1681 HD patients was included. Blood samples were analyzed for hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibody (anti-HCV). Detection of hepatitis B virus (HBV) DNA and hepatitis C virus (HCV) RNA were performed in either HBsAg (+) or anti-HCV (+) samples. HBV DNA/HCV RNA was also measured in a subset of HBsAg (-) and anti-HCV (-) patients. Liver function tests were analyzed and compared with the serological and virological tests.

Results: The serological tests showed that 230 patients (13.7%) were HBsAg (+) and 290 (17.3%) were anti-HCV (+). We were unable to detect HBV DNA in 97 of 230 (42.2%) HBsAg (+) patients, and HCV RNA could not be found in 76 of 290 (26.2%) anti-HCV (+) patients. In 167 HBsAg (-) patients, only one showed a trace amount of HBV DNA. None of 151 anti-HCV (-) patients showed detectable HCV RNA. The prevalence rate of viral hepatitis remains high in Taiwanese HD patients: 13.7% for HBV and 17.3% for HCV. However, virological analysis showed 42.2% non-viremic rate for HBsAg and 26.2% non-viremic rate for anti-HCV.

Conclusions: The findings might challenge the presently suggested principles of bed and machine dedication and the diagnosis of viral hepatitis in HD patients.

Key words: hepatitis B virus; hepatitis C virus; hemodialysis; viremia.

Introduction

Asia is classified as a high endemic area for hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (1). Routes of transmission for HBV and HCV might vary, but both could be transmitted through blood-borne and mucosal routes. Prevalence of both HBV and HCV are notably higher in hemodialysis (HD) patients than in the general population (2, 3), contributed mostly by blood transfusions in the pre-erythropoietin era and also the nature of extracorporeal blood exposure. By the year 2000, a survey of national HD cohorts in Taiwan showed a positive rate of 10.6% for hepatitis B surface antigen (HBsAg) and 26.5% for HCV antibody (anti-HCV) (4). It was suggested that the high prevalence rate of chronic HBV/HCV hepatitis explained the higher morbidity and mortality caused by liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in HD patients (5, 6). To lessen the health threat, the Taiwan Society of Nephrology launched practice guidelines for HD, and proposed strict infection control measures and suggested beds/machines dedication for both HBsAg (+) and anti-HCV (+) patients (7). Its beneficial results were reflected by the low annual sero-conversion rate in recent years: 0.25% for HBsAg and 0.72% for anti-HCV.

At present, the diagnosis of HBV and HCV infection is largely based on examining either the presence of circulating serological markers of HBV particles (most commonly HBsAg) or anti-HCV antibody before the 1990s, when the methods to detect HBV DNA and HCV RNA were unavailable (8, 9). With advancement of biotechnology, diagnosis of viral diseases has been brought to another level. Furthermore, it is the viral load in the blood that monitors the antiviral treatment response, but not the titers of viral particle markers or viral antibodies. Past literature had revealed significant false negative rates of the serological methods to diagnose active HBV and HCV infections (10-12). A serious issue is these false-negative but potentially infectious patients could be unintentionally assigned to the beds next to true hepatitis-free patients. Oppositely, false positive examinations were occasionally noted in some patients with positive serological tests but no detectable virus in their blood. Considering the utmost importance to prevent the transmission of viral hepatitis during HD, it is necessary to clarify the discrepancy between the serological tests and the more updated HBV/HCV virological examinations. Significance of the present study might urge nephrology practitioners to re-define the exact prevalence of viral hepatitis in HD patients. It would also be very useful to formulate a practical strategy of infection control in HD institutes,

especially for those areas with high prevalence rate of viral hepatitis and where bed/machine dedication has been suggested.

Patients and methods

Patient enrollment and blood tests for viral hepatitis

A total of 1902 patients who received HD for at least six months (ranging from seven months to twenty six years, with a median of 6.7 years) were recruited from fifteen institutes, including one medical center, three regional hospitals, and eleven dialysis clinics in southern Taiwan. Patients who rejected giving the informed consent and those who were in acute illness were excluded, and finally 1681 prevalent patients were included for this study. All patients received 3.5-4.5 hours per session, three sessions per week maintenance HD. Of the 1559 patients with known primary renal diseases, the causes of renal failure were as followed: chronic glomerulonephritis (n=644, 41.3%), diabetic nephropathy (n=579, 37.1%), hypertensive renal disease (n=88, 5.6%), lupus nephropathy (n=28, 1.8%), gouty nephropathy (n=25, 1.6%), obstructive nephropathy (n=23, 1.5%), polycystic kidney disease (n=28, 1.8%) and others (n=144, 8.6%). All the eligible subjects were tested for both anti-HCV and HBsAg examinations. The processing of all the samples in our study followed a regulated rule, and analyzed in a same laboratory by the same technician specialized in viral hepatitis nucleic acid measurement. Pre-HD blood samples were collected monthly in the period between January, 2011 and January, 2012. Only patients testing positive for HBsAg or anti-HCV at least twice within 6 months were eligible for the study. HBsAg was determined by using a standard chemiluminescent microparticle immunoassay (ARCHITECT HBsAg, Abbott Diagnostics). Anti-HCV was examined using a third-generation enzyme immunoassay (Abbott Laboratories, North Chicago, IL). HBV DNA was determined by a standardized automated quantitative polymerase chain reaction assay (COBAS TaqMan HBV test; Roche Diagnostics; detection limit 12 IU/ml) (13). HCV RNA was measured by a real-time polymerase chain reaction assay (RealTime HCV; Abbott Molecular, Des Plaines IL, USA; detection limit: 12 IU/ml) (14). All the above-mentioned tests were duplicated for each tested sample.

In a subset of the included patients (from Kaohsiung Municipal Hsiao-Kang Hospital), we also performed HBV DNA detection for 167 HBsAg (-) patients, and HCV RNA detection for 151 anti-HCV (-) patients in order to make sure if the possibility of false negative serological reaction existed as previ-

ously reported in the literature, probably because of using older versions of serological assays (10-12).

Ethics Statement

The study protocol was approved by the institutional review board of the Kaohsiung Medical University Hospital (KMUH-IRB-980083). Informed consents have been obtained in written form from patients and all clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. The patients gave consent for the publication of the clinical details.

Correlation of serological and virological examinations with liver function test

In Taiwan, HD patients receive routine monthly biochemical tests, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Results of the serological and virological tests were analyzed along with the six-month average AST/ALT levels.

Statistical analyses

Frequency was compared between groups using the χ^2 test, with the Yates correction, or Fisher exact test. Group means, presented as mean values standard deviation, were compared using analysis of variance and the Student *t*-test. The statistical analyses were performed using the SPSS 12.0 statistical package (SPSS, Chicago, IL, USA). All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$.

Results

Table 1 shows the demographic data of all the enrolled patients. Of the 1681 enrolled patients, 230 (13.7%) were positive for HBsAg and 290 patients (17.3%) were positive for anti-HCV. Also shown are the baseline characteristics of patients who were tested positive or negative for HBsAg or anti-HCV.

Patients positive for HBsAg or anti-HCV were older, had longer dialysis vintage and higher hepatic enzyme levels.

Difference between the serological and virological examinations

As shown in Table 2, HBV DNA was undetectable in 97 of 230 (42.2%) HBsAg (+) patients, and HCV RNA was undetectable in 76 of 290 (26.2%) anti-HCV (+) patients. The findings signified that the current updated serological methods to diagnose HBV/HCV hepatitis failed to demonstrate HBV viremia in 42.2% and HCV viremia in 26.2% of HD patients. In other words, the positive predictive value of HBsAg test to validate HBV viremia was 57.8%, and 73.8% for HCV viremia.

Samples in a subset of HBsAg (-)/anti-HCV (-) patients received virus detection tests in order to avoid potential virus transmission due to the possibility of false negative serological tests. None of the 151 anti-HCV (-) patients was found to have circulating HCV RNA. Only one out of 167 HBsAg (-) patients gave weak positive reaction of HBV DNA, but the copy number was only slightly above the detection limit. Repeated examination for this patient showed a negative reaction. Unlike the previous reports (10-12), the presently used serological assays did not report any incidence of unnoticed viremia, at least in this subset of patients.

HBsAg (+) and 290 anti-HCV (+) patients made a total of 520 in our cohort. Among them, 41 (7.88%) patients were HBsAg (+) plus anti-HCV (+). Twenty of the 41 (48.7%) were negative for HBV DNA and 12 of the 41 (29.2%) were negative for HCV RNA. Both of the percentages were higher than patients with only HBV (42.2%) and with only HCV (26.2%), but not very different. The AST/ALT levels in this dual-infected group of patients were similar to other patients with only HBV or HCV.

Table 1. Basic demographical, virological, and clinical features of the enrolled 1681 HD patients.

Parameter	All	HBsAg		Anti-HCV	
		(+), n=230	(-), n=1451	(+), n=290	(-), n=1391
Age (years), mean \pm SD	62.2 \pm 12.9	63.8 \pm 10.9	57.9 \pm 14.4	63.6 \pm 16.0	59.0 \pm 13.7
Male, n (%)	824 (49.0)	126(54.8)	698(48.1)	122(42.0)	702(50.5)
BMI (kg/m ²), mean \pm SD	22.5 \pm 4.0	25.5 \pm 14.2	22.1 \pm 5.5	22.0 \pm 6.2	22.9 \pm 4.7
AST (IU/L), mean \pm SD	20.6 \pm 11.2	25.5 \pm 14.2	19.8 \pm 10.1	26.2 \pm 13.5	19.4 \pm 10.3
ALT (IU/L), mean \pm SD	19.4 \pm 15.6	31.0 \pm 11.1	16.7 \pm 8.8	26.3 \pm 18.4	17.9 \pm 14.6
White blood cell count ($\times 10^3$ L), mean \pm SD	6.69 \pm 2.48	5.79 \pm 2.20	6.71 \pm 3.01	6.11 \pm 2.64	6.83 \pm 2.32
Hemoglobin (g/dL), mean \pm SD	10.5 \pm 1.3	9.7 \pm 2.0	10.7 \pm 1.6	11.0 \pm 2.2	10.1 \pm 1.5
Platelet count ($\times 10^3$ L), mean \pm SD	193 \pm 64	177 \pm 99	198 \pm 55	168 \pm 72	201 \pm 47
Diabetes, n/N (%) #	608/1560 (39.0)	78(33.9)	530(36.5)	115(39.6)	493(35.4)
Dialysis vintage(years), median	6.7	11.6	4.4	10.5	5.3

Note: SD: standard deviation; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase

clinical history available in 1560 patients.

Table 2. Correlation of HBsAg and HBV DNA, and of anti-HCV and HCV RNA with liver function tests.

	HBsAg (-)		P value	HBsAg (+)		P value
	(n=1451, 86.3%)	(n=230, 13.7%)		HBV DNA (-)	HBV DNA (+)	
AST (IU/L), mean±SD	19.8±10.1	25.5±14.2	<0.05	18.6±9.7	30.8±9.9	<0.001
ALT (IU/L), mean±SD	16.7±8.8	31.0±11.1	<0.001	17.2±12.0	34.3±10.3	<0.001
	Anti-HCV (-)		P value	Anti-HCV (+)		P value
	(n=1391, 82.7%)	(n=290, 17.3%)		HCV RNA (-)	HCV RNA (+)	
AST (IU/L), mean±SD	19.4±10.3	26.2±13.5	<0.001	21.4±10.4	27.8±14.1	<0.001
ALT (IU/L), mean±SD	17.9±14.6	26.3±18.4	<0.001	17.4±7.3	29.5±16.0	<0.001

* AST: aspartate aminotransferase; ALT: alanine aminotransferase

Correlation with AST/ALT

Traditionally, HD patients with either positive HBsAg or anti-HCV reactions were considered to be carrying HBV or HCV hepatitis. In this study, patients with sero-positive hepatitis markers (HBV or HCV) had significantly higher AST/ALT levels than did their sero-negative counterparts (Table 2). We further divided the patients into viremic and non-viremic groups according to the presence of viral nucleic acid. Patients who were viremic (either HBV or HCV) had significantly higher AST/ALT levels than patients who were non-viremic (Table 2). Finding of fatty liver is common (43%) and did not correlate with liver function in our series.

Discussion

It is well known that the overall prevalence rate of viral hepatitis in Asia, especially HBV and HCV hepatitis, remains higher than that in US and Europe, although some countries have become intermediate and low endemic areas of HBV infection because of their successful vaccination programs (15, 16). Taking Taiwan as an example, a population-based survey showed that the prevalence rate of HBsAg (+) still approached 12.9 % although the national HBV vaccination had successfully lowered the sero-positive rate in children (17); anti-HCV (+) was noted in 5.5%~8.6% of all citizens, with regional differences (18, 19). The issue of viral hepatitis is especially serious in some specific subgroups of patients, such as the ones undergoing maintenance HD. In 2008, a national survey by Taiwan Society of Nephrology showed that the HBsAg (+) rate in HD patients was 10.3%, but anti-HCV (+) rate was 18.5%, which is 3-5 times greater than that in the general population (20). In the US, the 2002 national surveillance showed an 8% of anti-HCV (+) rate in HD patients, five times greater than in the general population (21). Such a high prevalence was recognized to contribute to the HBV- and HCV-related liver diseases in these patients (22). Prevention of their transmission during HD is, there-

fore, one of the major strategies. The current recommendations by the Centers of Disease Control and Prevention relied on strict adherence to infection control measures for the prevention of hepatitis virus transmission in the HD setting. Isolation of patients or the use of dedicated machines for HBV/HCV infected patients was not recommended (23). However, no randomized controlled trials exist on the impact of isolation on the risk of transmission of HBV/HCV to hemodialysis patients and it remains uncertain if such recommendations fit every country for prevention of transmission (24), especially in the highly prevalent areas. Therefore, the Taiwan Society of Nephrology has suggested the use of dedicated bed and machine for patients of HBV and HCV infections, besides the universal measures of infection control, in the guidelines for HD practice since 1990s. It was also suggested patients with hepatitis be dialyzed in separate areas to avoid cross-contamination by staff. The achieved effect was reflected by the low annual sero-conversion rate, 0.25% for HBsAg and 0.72% for anti-HCV. In Japan, the annual sero-conversion rate for anti-HCV was approximately 1.04 % (25).

At present, the diagnosis of HBV and HCV infections in HD patients relies almost exclusively on HBsAg and anti-HCV serological tests. Comparative studies have questioned the accuracy of serological tests since the development of HBV/HCV nucleic acids analysis. Certain rates of either false negative or false positive of both HBsAg and anti-HCV have been reported in the literature (12, 26). For example, Cabrerizo et al. showed that they could detect HBV DNA in 58 % of HBsAg (-) HD patients (N=33) (27). Similar situations were also noted in non-HD populations (11). For HCV, the chance of detecting HCV RNA in anti-HCV (-) patients was much less (12), but still present. In the present era of molecular medicine, the persistent dependence on serological tests for the accurate diagnosis of HBV/HCV infection should be challenged, especially when it concerns the issues of disease transmission during HD (28). However, HBV/HCV nucleic acids quantification is not gener-

ally recommended by every national or international nephrology society, probably because international guidelines have to evaluate its application in countries with varied technical and economic competence. Nevertheless, 2008 KDIGO Guidelines has made a clear recommendation about the use of nucleic acid tests for units with a high prevalence of HCV (29). The current study is the first in Taiwan to include relative larger numbers of patients. The aim of this study is to demonstrate how the faithfulness of the presently used serological tests is when they are compared with the virological analysis. Our results showed that HBV DNA is absent in 42.2% of HBsAg (+) patients, and no HCV RNA can be detected in 26.2% of anti-HCV (+) patients. It was previously shown that background prevalence might influence the sensitivity and specificity of the serological tests (29). In high prevalent area, a high rate of false negative and a low rate of false positive might be expected (30). However, different from the reports in the literature (12, 27, 30), a negative result of the serological tests in our series is also accompanied by negative result of HBV/HCV nucleic acids in a subset of our cohort. Our findings showed that the so-called "silent hepatitis infection" patient, ie, actual existence of virus in the HBsAg (-) or anti-HCV (-) patients, was not present in our series. It raises a question concerning how we should define patients' status of hepatitis if they have a positive serological report, but do not have any detectable viral nucleic acid in their blood. In a strict sense of infection control, they are not infectious and will not transmit viral hepatitis. Yet, the answer would be uncertain if we consider the unstable and immuno-compromised states of the HD patients, and the basic difference of pathogenesis between HBV and HCV. Our study group has planned to follow these patients longitudinally in order to reassure our conclusions and clarify this disputable issue. The information from the result of the present (and the future) study may be especially helpful for those countries with higher prevalence rates of HBV/HCV hepatitis and for those where the dedicated bed/machine is an option.

In our series, HBV was with higher disproportion rate than HCV (42.2% vs 26.2%). We presume that the difference will mostly derive from the viral natures, DNA versus RNA virus. HBV viral genome can be incorporated into the human DNA in liver while HBV viral particles have been cleared from sera, and the viral genome may utilize the cellular machinery to constantly synthesize serum markers. On the contrary, HCV is an RNA virus and they do not hide in the human DNA. Immune system may still "remember" them, and keeps synthesizing anti-HCV antibodies after the HCV viremia is cleared.

It is well-recognized that chronic infection of

HBV and HCV contributes to potential higher risk of morbidity and mortality in the general population (1), and also in dialysis patients (2, 3). Clinical analysis in our study showed significantly higher six-month average levels of AST/ALT in patients with either "serological" or "virological" HBV/HCV. Chronic functional impairment potentially causes more serious clinical liver disease, that is, liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (31). In Taiwan, an endemic country of viral hepatitis, the positive rates of HBsAg and anti-HCV in the general population were approximately 15.1% and 5.5-8.6% (18, 19). For more severe liver disease, a population-based survey in Taiwan identified 0.97% prevalence for LC and 0.2% for HCC (32). Prevalence for LC in HD patients was 6.16% (33), at least six times higher than in the general population, and contributed a certain degree to their morbidity and mortality. Similar situations have been observed in other countries (6). Results of the present study are valuable in defining the truly infectious individuals and may help the nephrology societies in revising and adopting the management strategies toward HBV/HCV-infected patients.

Recently published papers concerning HCC in HD patients are more intriguing. HD patients had the same prevalence of HCC (0.2%) as in the general population (34), in spite of 2-3 times higher HCV carrier rate and 6-7 times higher LC rate. Furthermore, they do not have worse long-term survival compared with the non-dialysis counterparts (35). Discussion about this counter-intuitive phenomenon is out of the scope of this article but is definitely another topic of research interest.

The present study has limitations in several aspects. First, we did not perform viral nucleic acid detection for every included patient and therefore, could not demonstrate the sensitivity and specificity for either serological tests or virological tests. Second, we had only performed viral detection in a subset of HBsAg (-) and anti-HCV (-) patients. Extrapolation of our finding to exclude the existence of "silent hepatitis infection" by using the updated serological kits is presently not rational. Another weakness is the lack of longitudinal follow-up data. Therefore, we are unable to understand if the discrepancy between the serological and virological diagnostic tests of HBV/HCV would change over time. The present study cannot provide our own data of liver disease deterioration, ie, chronic liver disease to LC or HCC, and effect of anti-viral therapy on their outcome is not known (36). Another study is now underway and we might be able to answer some of the above-mentioned questions.

In summary, we found that the currently used serological tests (HBsAg and anti-HCV) show a high

non-viremic rate for the diagnosis of HBV/HCV infections (42.2% for HBV and 26.2% for HCV) when they are compared with the viral nucleic acid quantitative tests. In a subset of our cohort, no viral nucleic acid was detected in 151 HBsAg (-)/167 anti-HCV (-) patients. We encourage HD institutes to examine HBV DNA for HBsAg (+) patients, and HCV RNA for anti-HCV (+) patients and reconsider the allocation of patients. The test results can be used to confirm the potential of HBV/HCV transmission and to develop more efficient strategies in taking care of such patients, especially in countries with high prevalence rates of HBV/HCV.

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Competing Interests

There is no conflict of interest in the information contained in this paper.

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