

A Case-Control Study on Occult Hepatitis B Infection in Chronic Hemodialysis Patients from South-West of Iran

Seyed Jalal Hashemi¹, Eskandar Hajjani¹, Abdolrahim Masjedizadeh¹, Manoochehr Makvandi¹, Heshmatollah Shahbazian¹, Aliakbar Shayesteh¹, Mohammad Karimi¹

¹ Research Institute for Infectious Disease of Digestive System, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran

ABSTRACT

Background:

Blood born viral infections such as hepatitis B virus (HBV) are major concerns in chronic hemodialysis (CHD) patients and hemodialysis units. Undetected HBs Ag in the presence of viral DNA, occult HBV infection (OBI), is a concern in the care of CHD patients and hemodialysis unit as a mode of transmission.

Objectives

In this case-control study we compare the frequency of OBI in the CHD patients with the normal population.

Materials and Methods:

82 consecutive CHD patients and 82 healthy individuals without any risk factors for HBV infection were enrolled in this study. A selection criterion was negative serum HBs Ag by ELISA method. Subsequently, the sera were tested for HBV DNA by nested PCR method.

Results:

In the CHD group, 55 (67.1%) were male and 27 (32.9%) were female, with the overall mean age of 54.32 ± 13.67 years old. The mean age of control group was 32.65 ± 8.51 years old, with 26 (31.7%) male and 56 female (69.3%). HBV DNA was present in 9 (11%) CHD patients, 4 (8%) of whom were seronegative for anti-HBc and anti-HBs antibodies. No HBV DNA was identified in the control group ($p < 0.0001$). History of blood transfusion was present in all OBI CHD patients and 59 (80.9%) of non-OBI CHD patients. Duration of hemodialysis in OBI CHD and non-OBI CHD patients were 73.56 ± 39.53 and 44.24 ± 24.59 months, respectively ($p = 0.002$).

Conclusion:

The prevalence of occult HBV infection is relatively high in patients with chronic hemodialysis in our region. Duration of hemodialysis and history of blood transfusion are important risk factor for OBI infection. A more sensitive method, such as PCR, may need to be considered in this patient population.

Keywords: Adolescent; Adult; Age Factors; Biological Markers; Hepatitis B

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Corresponding author:

Seyed Jalal Hashemi, MD

Ahvaz Jundishapur University of Medical Sciences,
Ahvaz, IR Iran

Tel: +98 61 33330074

Fax: +98 61 33332036

E-mail: dorhashemi@gmail.com

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INTRODUCTION

Despite significant improvement in Anti-HBV vaccination and treatment, HBV infection continues to be a major global health problem. It has been reported that as much as 350 million carriers exist in the world and about one million of them will die annually (1). The prevalence of HBV infection in Iran ranged between 2.7 to 7.2 %, which declined below 2% following national vaccination (2). Patients on CHD are susceptible to HBV infection. In different proveniences of Iran the prevalence of HBV infection

in CHD patients with positive HBs Ag ranges from 0.38% to 6.5% (3-6). OBI defined as presence of HBV DNA in the serum, lymphocytes or hepatocytes with undetectable HBs Ag in the serum (7-9).

Association between OBI and hemodialysis, HCV infection, non-alcoholic liver disease, HIV infection, and progression of chronic liver diseases including alcoholic liver disease to hepatocellular carcinoma has been reported in different series (10-16). The frequency of OBI in the CHD patients is reported in a range of 0 to 58% in different series (17-19). In a large series of low-risk individuals in Iran the prevalence of OBI in the blood donors was 0.04%(20).

The prevalence of OBI in the high-risk population such as liver cirrhosis and CHD patients rang between 0 to 4.9%(21). The prevalence of OBI is ranged from 0-to 4.9% among HD patients in Iranian (22-24). The prevalence of OBI in the CHD patients in different regions of Iran has been reported between 0 to 7% in Guilan and Terhan, respectively Limited studies in the Khouzestan province reported a prevalence of HBs Ag seropositivity in the CHD patients between 7% and 16%(22,25). Thus this study was carried out to determine the frequency of OBI among the chronic HD patients in Ahwaz city.

Objectives

The present study compares the prevalence of OBI in CHD patients with the matched healthy individuals using nested PCR method.

MATERIALS AND METHODS

This case-control study was performed from May 2011 to June 2012 in the city of Ahvaz. Ahvaz is the capital of Khuzestan Province located in the southwest region of Iran with population of 1.5 million. Patients were recruited from the Hemodialysis Unit at the Imam Khomeini Hospital. The control group was selected from volunteered relatives of the patients without any risk factors for hepatitis or blood born infections. Demographic characteristics, duration of hemodialysis and history of transfusion were recorded for each patients. The Medical Ethics Committee and the Deputy of Research Affairs at Jondishapur University of Health and Medical Sciences approved the proposal.

Serology and biochemical tests

After obtaining an informed consent, blood was collected from the enrolled subjects. Each serum was studied for Alanin Aminotransferase (ALT), Asparate Aminotransferas (AST), Hbs Ag, HBs Ab,

anti HBc IgM and anti HBc IgG by enzyme-linked immunosorbent assay (ELISA) (Diagnostic BioProbes, Milan, Italy). For detection of OBI by nested PCR amplification assay, a portion of each serum sample was separated and stored at -80 C° (26).

DNA extraction

200µl serum was used for extraction of viral DNA using High Pure Nucleic Acid Kit (Roche Applied Science, Germany). The nested PCR was applied for amplification of the S region of HBV DNA gene using the following primer: FHBS1 (position 244 to 267), FHBS2 (position 255 to 278), RHBS2 (position 648 to 671) and HBS1R (position 668 to 691) (27, 28).

Statistical analysis

SPSS software version 16 (Chicago, IL, USA) was used for statistical analysis. Mean, standard deviation, Pearson Chi-Square test, Fisher's Exact Test were used for categorical variables, and students t test used for continuous variables. *p* value more than 0.05 considered statistically significant.

RESULT

A total of 82 CHD patients with mean age of 54.35 ± 13.67 years old (ranged from 22-78 years) were enrolled in the study. 55 (67%) patients were male and 27 (33%) were female. Duration of hemodialysis was 47.46 ± 27.87 months. 68 (82%) patients had history of transfusion. 82 healthy subjects including 26 (31%) male and 56 (69%) female with mean age of 32.63 ± 8 years were enrolled in the control group. Characteristics of CHD patients and healthy subjects are summarized in table-1.

HBV DNA was detected in sera of 9 (11%) CHD patients. Mean age of OBI patients was 48.22 ± 16.79 years old. 4 (44%) of were male and 5 (56%) were female. None of the healthy subjects had HBV DNA in their sera (*p*=0.002) by nested PCR. Sera of 9 (10.9%) patients in the CHD group and 8 of healthy individuals were positive for Anti-HBc antibody (*p*=0.1). Isolated Anti-HBc was detected in sera of 3 CHD and 2 healthy subjects. 1 of 5 cases with isolated Anti-HBc antibody showed detectable HBV-DNA in the serum.

All CHD patients with OBI and 59 (81%) CHD cases without OBI had history of blood transfusion. (*p*=0.16). Duration of hemodialysis was significantly more prolonged in infected patients than non-infected patients (73.56 ± 39.53 vs 44.24 ± 24.59; *p*= 0.002). Anti-HBs antibody was detected in sera of 4 (44.4%) OBI patients and 48 (65.8%) of CHD patients with negative HBV-

Table 1: Characteristics of hemodialysis patients and control group

Variable	Hemodialysis patients N=82	Healthy Control N=82	p value	Confidence interval
Mean Age (year)±sd	54.35 ± 13.67	32.63 ±8.14	0.001	18.98- 0.24.98
Male gender: N (%)	55(67%)	26(31.7%)	0.001	0.201 - 496
ALT IU/L(mean± sd)	19.13±7.44	28.26±11.76	0.0001	-12.8- 6.26
AST IU/L(mean± sd)	25.26± 10.12	24.45±8.41	0.58	-2.35 – 3.58
Anti-HBc: N (%)	10.9%) 9	8(10%)	0.100	0.001 - 174
Anti- HBs: N (%)	52(63.4%)	18(21.9%)	0.0001	0.238 – 0.561
OBI: N (%)	9(11%)	0 (0%)	0.002	0.161 - 0.306
History of transfusion	68(82%)	0(0%)	0.0001	

OBI: Occult HBV infection, ALT: alanine aminotransferase , AST:Asparate aminotransferase ,Anti HBc anti hepatitis core antibody

Table 2: Characteristics of hemodialysis patients with and without OBI

Variable	HBV DNA positive N=9	HBV DNA nega- tive N=73	P value	95% confidence interval Of the dif- ference
Male gender: N (%)	4(44.4%)	51 (69.9 %)	0.145	0.026 - 0.359
Age year(mean± sd)	48.22 ±16.79	55.11±13.18	0.15	2.66 - 16.44
ALT IU/L (mean± sd)	25.77±4.73	18.31±7.32	0.001	-12.45 - -2.466
AST IU/L (mean± sd)	31.44±6.65	24.49±10.24	0.016	-12.38—1.514
HBC ab: N (%)	1(11.1%)	8(10.9 %)	0.100	-0.164 - 0.360
HBs ab: N (%)	4(44.4%)	48(65.8%)	0.276	-0.344 – 0.056
Hx of transfusion	9(100%)	59(80.9%)	0.168	0.110 – 0.196
Duration of HD: mo	73.56± 39.53	44.24 ± 24.59	0.002	-47.91 - -10.70

OBI: Occult HBV infection, ALT: alanine aminotransferase AST:Asparate aminotransferase ,Anti HBc anti hepatitis core antibody HD:Hemodialysis

DNA in their sera ($p=0.84$) (table 2). 4 (44.4%) of our OBI cases were seronegative for Anti HBs Ab and Anti HBc Ab by ELISA. Mean level of ALT and AST was higher in infected cases.(Table 2)

DISCUSSION

In the present case-control study we investigated 82 hemodialysis patients and 82 healthy individuals to evaluate the prevalence of OBI in the two groups. We found a prevalence of 11% OBI in CHD patients. None of the subjects in the control group was positive for HBV DNA. Demographic characteristics of CHD patient with and without OBI were similar. History of transfusion in the OBI and non-OBI CHD patients was no statistically significant ($p=0.168$) table-2. Duration of hemodialysis in OBI CHD patients was significantly longer than the non-OBI CHD patients ($p=0.002$). There are several studies on the prevalence of OBI in Iran with different

results (29). Ramazani et al., reported the prevalence of 3% in 926 high-risk individuals while the prevalence of OBI in that control group was zero (30,31). Jokar et al., reported no HBV DNA in 507 CHD patients in Guilan province with negative HBS Ag(23). Aghakhani et al., evaluated 289 CHD patients to assess OBI in cases with isolated Anti HBc. They found 18 (6.2%) isolated Anti-HBc subjects, 9 of whom (50%) were positive for HBV DNA in their sera, equal to 3.1 % of the population(32). Niesi et al., reported a 4% OBI in CHD patients in Khuzestan province(22).

In our study we found 4 (44.4%) seronegative cases with OBI (negative for anti-HBc and HBsAb) and 1(11%) of OBI patients had Isolated Anti-HBc. 2 (2.4%) individuals in the control group had isolated anti-HBc, both of whom were negative for HBV-DNA. The prevalence of OBI in the CHD patients has been studied in many regions of the world. While Motta, et al

from Brazil reported a prevalence of 15% OBI among 100 CHD patients, Minuke estimated the frequency of OBI 3.8 % in a series in Canada(13). The prevalence of OBI reported 41.1% among IV drug abuser in Taiwan and 4.5% in female sex workers in Turkey(33,34). This finding suggests that epidemiology of OBI follows the seropositive HBV infection, and that vaccination program can prevent OBI.

Since the viral load in patients with OBI is very low the estimated prevalence can be affected by the method of HBV DNA testing. Nested-PCR is highly sensitive method for detection of low HBV DNA levels, but is subject to false positive results due to contamination (27). We included a control group to our study in an attempt to address the possibility of contamination and false positive results. We did not detect any positive HBV DNA in the control group.

There are several explanations for persistence of HBV infection without presence of HBs Ag in the serum samples, including mutation in the S gene causing deferent or no antigen production, Ag-antibody immune complex formation with secondary reduction in HBS Ag to undetectable level, HBV DNA integration into the genome of hepatocytes, and low sensitivity of the tests to detect low levels of antigenemia (35-38). Superimposed or concomitant infections with other viral agents such as hepatitis C virus and hepatitis D virus can decreased the HBs antigenemia to undetectable levels. During the serologic window period in acute HBV infection before appearance of anti-HBs, the HBS Ag may be undetectable (39,40).

In the OBI patients there is no HBV-specific T-Cell expansion, suggesting that very low HBV viral load is not sufficient for maturation of protective memory cells (41). Cellular and humoral immune responses exhibit specific and nonspecific defects in the patients with chronic renal failure. As a result, the anti HBs responses to vaccination in hemodialysis patients are suboptimal and estimated to be 45 – 60%. In our study we found 63.4% anti HBs response in CHD patients (42).

Patients with OBI infection may be the sources of HBV contamination in hemodialysis units and a possible source of HBV transmission. Role of OBI in the progression of the liver dysfunction and development of hepatocellular carcinoma has been demonstrated in some studies. In a metanalysis, Covolo suggested OBI increases likelihood of chronic liver disease by 8 folds (43). According to the European Association for

Study of the Liver guidelines, serologic tests are the recommended screening methods for HBV infection in hemodialysis unit (44).

Our study shows OBI is relatively common in CHD patients in Khouzestan province. The majority of these patients are seronegative. The duration of hemodialysis is the most important risk factor associated with OBI. The underestimation of HBV infection in the CHD patients can be explained by the immunocompromise states commonly seen in this population, as well as, the laboratory methods with suboptimal sensitivity for this population. Highly sensitive laboratory tests, such as PCR-based methods, may enhance detection of OBI in the screening workups of the hemodialysis patients and preoperative studies prior to the organ transplantation. The clinical significance of OBI in the hemodialysis patients may deserve additional and long-term prospective studies.

Limitation of the study:

The number of positive cases was not sufficient for comparing clinical implication of OBI on liver function and determining of other risk factors. We did not performed quantitative PCR to evaluate viral loads in the infected patients.

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