RESPONSE OF HEALTH CARE WORKERS WITH ISOLATED ANTIBODY TO HEPATITIS B CORE ANTIGEN TO HEPATITIS B VACCINE

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Abstract. Isolated hepatitis B core antibody (antiHBc) without hepatitis B surface antigen (HBsAg) or hepatitis B surface antibody (antiHBs) is found during routine screening for hepatitis B virus (HBV) markers. Isolated antiHBc may indicate immunity against HBV or occult infection. To determine the immune response of health care workers (HCWs) with isolated antiHBc, HCWs were divided into two groups. A single dose of recombinant hepatitis B (HB) vaccine was administered to HCWs with isolated antiHBc (n=36) and healthy HCWs (n=20) seronegative for HBsAg, antiHBc and antiHBs. One month later, the subjects were tested for antiHBs. Twenty-one of 36 HCW (58.3%) in the antiHBc group had antiHBs, while only 1 of 20 HCW (5.0%) in the seronegative control group had a detectable antiHBs titer exceeding 10 mIU/ml. The antiHBs response in HCWs with antiHBc was significantly higher than in the seronegative group. The subjects' sera were tested for HBV DNA by nested PCR. Of those with antiHBc, 4 had detectable HBV DNA (occult HBV infection). None of these 4 responded to the vaccine. Therefore, the response elicited by a single dose of HB vaccine administered to patients with antiHBc may serve as an indicator of occult HBV infection.

Keywords: hepatitis B virus (HBV), health care workers (HCWs), isolated hepatitis core antibody (isolated antiHBc), vaccination

INTRODUCTION

Occult hepatitis B infection (OBI) is suspected in patients who test negative for hepatitis B surface antigen (HBsAg) yet positive for hepatitis B virus (HBV) DNA from blood samples. Hepatitis B core antibody (antiHBc) and/or hepatitis B surface antibody (antiHBs) may not be detectable in serum (Ozaslan and Purnak, 2009; Gerlich *et al*, 2010; Hollinger and Sood, 2010; Pondé *et al*, 2010; Raimondo *et al*, 2010). Thus, OBI may inadvertently lead to HBV DNA transmission to blood

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and organ recipients (Gerlich *et al*, 2010), and may be associated with hepatocellular carcinoma in seronegative liver cirrhosis patients (Ikeda *et al*, 2009). Currently, diagnosis of OBI is based on the presence of HBV DNA in hepatic tissue (Gerlich *et al*, 2010; Hollinger and Sood, 2010; Raimondo *et al*, 2010), but liver biopsy is an invasive diagnostic method. HBV serological markers alone are insufficient to determine OBI in HBsAg negative patients because immunized populations express antiHBs and antiHBc is present in several clinical situations. AntiHBc is detected during acute HBV infection and usually persists as life-long immunity in recovered patients, as chronic HBV infection or OBI (Gerlich *et al*, 2010). One indicator for OBI is isolated expression of antiHBc, while serum HBsAg and antiHBs are both negative. Other causes for isolated antiHBc have been observed, such as false positive result on antiHBc, chronic HBV carriers with low HBV levels, HBV escape mutants, HBsAg-antiHBs immune complex formation, low titers of antiHBs from previous HBV infection and OBI (Hamkar *et al*, 2010; Pondé *et al*, 2010).

A previous study of the seroprevalence of hepatitis B infection among 548 health care workers (HCWs) at the Institute of Neurology, Thailand found that 29 HCW (5.3%) were positive for HBsAg, 40 (7.3%) expressed isolated antiHBc, or were positive for antiHBc with low titers of antiHBs (<10 mIU/ml) (Chiarakul *et al*, 2007).

The objective of this study was to determine the response to hepatitis B (HB) vaccination among health care workers (HCWs) positive for isolated antiHBc at the Institute of Neurology, Thailand, compared with a seronegative group in order to determine HBV status among individuals with isolated antiHBc.

MATERIALS AND METHODS

The present study was conducted after receiving approval from the Ethics Committee of the institute prior to enrolment. All participating HCWs were informed of the study's objective and written informed consent was obtained.

Population study

HCWs were divided into 2 groups: HCWs with isolated antiHBc and HCWs seronegative for HBsAg, antiHBc and antiHBs (control group). AntiHIV status was not examined among the participants in this study.

Laboratory examinations

A hepatitis B profile was determined for all collected sera. HBsAg, antiHBs and antiHBc were detected using enzyme-linked immunosorbent assay (ELISA) commercially available as a test kit, MEIA IMx (Abbott Laboratory, North Chicago, IL) according to the manufacturer's protocol. HCWs positive for antiHBc but negative for HBsAg and negative for antiHBs or with an antiHBs <10 mIU/ml, were classified as an isolated antiHBc group and subsequently subjected to tests for alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and the presence of HBV DNA (nested PCR). HBV DNA detection was first amplified by a previous method (Sa-nguanmoo *et al*, 2010) and nested by a primer set of fragment 2 (Suwannakarn *et al*, 2008). Prior to HB vaccination, the participants were interviewed and physically examined to exclude HCWs with a history of prior HB vaccination.

Hepatitis B vaccine administration

HCWs were injected intramuscularly with a single dose of recombinant HB vaccine (20 μg of Euvax-B, Aventis Pasteur Korea, Korea).

Table 1
Comparison of demographic data between cases and controls.

Demographic data	Isolated antiHBc (total *n*=39)	Seronegative (total *n*=20)	*p*-value
Sex			
Male	12 (30.8%)	3 (15.0%)	0.188
Female	27 (69.2%)	17 (85.0%)	
Age (years)			
< 40	7 (17.9%)	10 (50.0%)	0.01*
≥ 40	32 (82.1%)	10 (50.0%)	
Work history (years)			
≤ 5	3 (7.7%)	1 (5.0%)	0.697
> 5	36 (92.3%)	19 (95.0%)	
Body mass index (BMI) kg/m^2			
< 25	23 (58.9%)	15 (75.0%)	0.224
≥ 25	16 (41.1%)	5 (25.0%)	
History of smoking			
Yes	9 (23.1%)	2 (10.0%)	0.222
No	30 (76.9%)	18 (90.0%)	
History of liver disease			
Yes	13 (33.3%)	0 (0%)	0.002*
No	26 (66.7%)	20 (100.0%)	

* $p < 0.05$

One month later, HBsAg, antiHBs, and antiHBc were measured. The post-vaccination antiHBs levels and geometric mean titer (GMT) were compared between groups. A booster antiHBs response was defined as a level exceeding 10 mIU/ml one month after vaccination.

Data analysis

The data was recorded and presented as percentage of seroconversion. AntiHBs titer was shown as GMT. The chi-square and *t*-tests were used for statistical analysis. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Among 548 HCWs, 39 had antiHBc, of whom 20 were positive for antiHBc, negative for HBsAg, and negative for antiHBs, and 19 were positive for antiHBc but had a low antiHBs titer (<10 mIU/ml). Twelve HCW in the antiHBc group (30.8%) were males and 27 (69.2%) were females, with an age range of 27 - 60 years (mean 47.1). Twenty HCWs, who were seronegative for HBV markers (3 males and 17 females with a mean age of 40.4 years) were enrolled. In the isolated antiHBc group, 23 were in a high occupational risk group (2 dentists, 7 nurses, 1 laboratory technician, 8 patient assistants and 5 workers), 4 were in the moderate risk group (all technicians), and 12 were in the low risk group (1 pharmacist, 1 social worker, 2 electricians, 1 general administrator, 2 audio-visual technicians, 1 librarian and 4 clerks). Three of 39 HCWs (7.7%) had worked less than six years, 4 (10.2%) had worked 6-10 years, 7 (18.0%) had worked 11-20 years and 25 (64.1%) had worked >20 years. The isolated antiHBc group and

Table 2
History of liver disease among 13 HCWs in the isolated antiHBc group.

No.	Sex/age	Occupation risk group	Past history
1	M/48	High	History of jaundice 31 years ago.
2	F/59	Low	HCV cirrhosis s/p liver transplantation.
3	M/45	Low	Detection of HBsAg while donating blood 5 years ago.
4	M/29	Low	Acute viral hepatitis B, presented with jaundice 5 years ago.
5	F/38	Low	Acute hepatitis, unspecified, presented with jaundice 24 years ago.
6	F/58	High	Viral hepatitis, non-specified, 20 years ago.
7	M/36	Intermediate	Acute viral hepatitis B 20 years ago.
8	M/56	Intermediate	Jaundice 15 years ago.
9	F/47	High	HBV infection diagnosed from blood test > 10 years ago.
10	F/45	High	Acute hepatitis B during work 17 years ago.
11	F/41	High	Liver disease during childhood, unknown type.
12	F/58	High	Liver disease presented with fever, unknown type, 33 years ago.
13	F/40	High	Acute viral hepatitis C 8 years ago.

F, female; M, male

the seronegative control group were not significantly different in terms of sex distribution, body mass index (BMI), history of smoking (Table 1) and chronic disease (tuberculosis: 2.6% *vs* 0%, $p = 0.464$; diabetes: 7.7% *vs* 0%, $p = 0.197$; chronic renal failure: 2.6% *vs* 0%, $p = 0.464$; COPD 5.1% *vs* 0%, $p = 0.396$; malignant tumors 2.6% *vs* 0%, $p = 0.464$; immunosuppressive drug recipients: 5.1% *vs* 0%, $p = 0.296$).

Thirteen cases in the isolated antiHBc group (33.3%) had a history of liver disease as opposed to none in the seronegative control group. Of the HCW in the isolated antiHBc group with a history of liver disease, 6 reported acute viral hepatitis 5 - 24 years prior to the study; 3 had HBV infection (1 was a nurse who contracted hepatitis B after a needle stick injury on the ward 17 years prior to the study and was excluded from the study due to a history of prior HB vaccination) 1 was a hepatitis C virus (HCV) case, and 2 cases had hepatitis of unknown etiology. Two HCW with isolated antiHBc gave a history of jaundice 15 and 31 years prior to the study, respectively, without any further details given, 2 were positive for HBsAg 5 and > 10 years prior to the study, respectively, and 2 reported acute hepatitis without any further details given (1 case had hepatitis as a child and the other had hepatitis at age 25) and 1 was a known case of chronic HCV infection with liver cirrhosis who had received a liver transplant 4 years prior to the study (Table 2).

The mean AST of the isolated antiHBc group was 30.1 U/l (range 16 - 84 U/l) and the mean ALT was 27.4 U/l (range 4 - 114 U/l). Six (15.4%) had elevated transaminase levels (AST ≥ 45 U/l and/or ALT ≥ 45 U/l). Five had both AST and ALT elevated, 1 HCW in this group had a

Table 3
Details of isolated antiHBc group members who harbored HBV DNA.

No.	Sex/age/ occupation risk group	AntiHBs level (mIU/ml)		AST/ ALT	BMI (kg/m^2)	History of liver disease
		Pre-vaccine	Post-vaccine			
1	F/57/high	Neg	2.9	24/17	24.03	No.
2	M/36/intermediate	2.1	5.7	23/21	20.20	History of acute hepatitis 20 years ago.
3	M/44/low	Neg	3.4	76/38	22.06	Alcoholism.
4	F/59/low	Neg	Neg	17/17	19.88	HCV cirrhosis, s/p liver transplant.

Table 4
Post-vaccination antiHBs levels by group (excluding prior vaccination).

Group	Post-vaccination antiHBs level (mIU/ml)				Total
	Negative	< 10	10-50	> 50	
1. Isolated antiHBc					
Neg for antiHBs	8 (40%)	4 (20.0%)	4 (20.0%)	4 (20.0%)	20
With antiHBs <10	0	3 (18.7%)	6 (37.5%)	7 (43.8%)	16
Total	8 (22.2%)	7 (19.4%)	10 (27.8%)	11 (30.6%)	36
2. Control	15 (75.0%)	4 (20.0%)	1 (5.0%)	0	20

history of acute viral hepatitis B 20 years prior to the study; her BMI was 41.9 kg/m^2. One HCW, an alcoholic, had only an elevated ALT. Four of 39 (10.3%) had HBV DNA detected by nested PCR with primers specific for the *S* gene; 2 were male, 2 were female; 1 was in a high occupational risk group with no history of hepatitis; 1 had hepatitis C cirrhosis and was post liver transplantation; 1 had a history of acute hepatitis; and 1 was an alcoholic (Table 3). We excluded 3 persons of the isolated antiHBc group from the study because they had received HB vaccine in the past. At 1 month post-vaccination, 24 members of the isolated antiHBc group (61.5%) had an increase in antiHBs titer to ≥ 10 mIU/ml (10 had antiHBs levels between 10 and 50 mIU/ml and 14 had antiHBs levels > 50 mIU/ml), 7 had antiHBs levels < 10 mIU/ml, and 8 were still negative for antiHBs. Of the 14 cases with a antiHBs titer > 50 mIU/ml, 3 were excluded from further analysis due to prior HB vaccination.

Of the 4 cases with detectable HBV DNA by PCR (occult HBV infection), 1 was negative for antiHBs post-vaccination and 3 had antiHBs levels <10 mIU/ml. As for the post-vaccination antiHBs results in the control group, 15 (75.0%) were still seronegative, 4 had antiHBs <10 mIU/ml, and 1 had antiHBs ≥10 mIU/ml (Table 4).

Table 5
Screening for HBV DNA among each subgroup of the isolated antiHBc group.

Pre-vaccination subgroup	Post-vaccination antiHBs levels (mIU/ml)			
	Neg	< 10	10 - 50	> 50
AntiHBc pos, antiHBs neg	1	2	0	0
AntiHBc pos, antiHBs <10	0	1	0	0

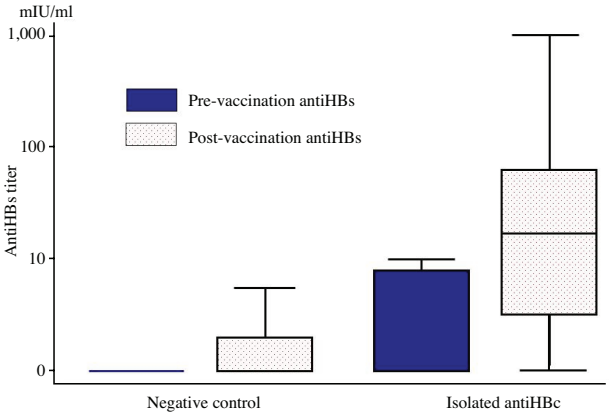

Fig 1–Pre- and post-vaccination antiHBs levels in 2 groups.

Comparison between the isolated antiHBc group and the seronegative control group after adjustment for age showed statistically significant differences in GMT of post-vaccination antiHBs levels (14.60 ± 7.19 *vs* 1.73 ± 2.84, $p < 0.001$) (Fig 1). The GMT of antiHBs changes from baseline were also different between the two goups (11.53 ± 8.01 *vs* 1.73 ± 2.84, $p < 0.001$).

Four members of the isolated antiHBc group with detectable HBV DNA had significantly different levels of antiHBs response compared with 35 members of the isolated antiHBc group who were negative for HBV DNA (GMT of antiHBs increased from baseline: 2.27 ± 1.74 *vs* 8.77 ± 6.54, $p = 0.01$) (Table 5).

DISSCUSSION

Isolated antiHBc is detected in various populations, such as HIV infected patients (Neau *et al*, 2004; Azadmanesh *et al*, 2008; Pérez-Rodríguez *et al*, 2009), chronic HCV infected patients (Helmy and Al-Sebayel, 2006) and hemodialysis patients (Aghakhani *et al*, 2010). The prevalence of isolated antiHBc in these patients ranges from 6.2% to 50.3% (Neau *et al*, 2004; Helmy and Al-Sebayel, 2006; Azadmanesh *et al*, 2008; Pérez-Rodríguez *et al*, 2009; Aghakhani *et al*, 2010). Studies conducted among the general population have shown an isolated antiHBc prevalence of 3.2% in Saudi Arabian blood donors (Panhotra *et al*, 2005), 8.9% in Koreans (Kang *et al*, 2010) and 20.1% in African immigrants (Gibney *et al*, 2008). Isolated antiHBc prevalence in this study was 7.3%. Several studies of HBV immune response in individuals with isolated antiHBc have been performed. A booster response had been observed in some isolated antiHBc patients (Draelos *et al*, 1987; McMahon *et al*, 1992; Ural and Findik, 2001; Coz Yataco *et al*, 2005; Koh *et al*, 2005). A study from Turkey in 2001 found that after 3 doses of HB vaccine at 0, 1 and 2 months, 89.6% of the isolated antiHBc group and 94% of the control group developed protective levels of antiHBs, with no significant difference between the two groups. A booster response to HB vaccine has been

observed in part of the isolated antiHBc group (41.6%) (Ural and Findik, 2001). The antiHBs response in our study to the single dose of HB vaccine in HCWs with isolated antiHBc was significantly higher than in antiHBc seronegative HCWs. The majority of HCWs with isolated antiHBc (58.3%) had a booster response (≥10 mIU/ml) after one dose of HB vaccine, which could have resulted from chronic HBV infection (Pondé *et al*, 2010). Of 36 HCWs with isolated antiHBc, four (11.1%) had OBI because they harbored HBV DNA (three expressed antiHBc only, one displayed antiHBc with antiHBs < 10 mIU/ml) but none of them showed a booster response after vaccination.

In conclusion, the antiHBs response to a single dose of HB vaccine in HCWs with isolated antiHBc was significantly higher than in antiHBc seronegative HCWs. About 60% of the isolated antiHBc group had a booster response to this single dose, except for those with OBI. A booster antiHBs response could be considered as evidence of chronic HBV infection. HBV vaccine response may discriminate between chronic HBV infection and OBI. This may serve as an indicator of OBI in patients with isolated antiHBc.

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