Immune Response to Hepatitis B Vaccine of Subjects with Isolated Antibody to Hepatitis B Core Antigen

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KEY WORDS:
Vaccine; Isolated anti-HBc; Primary response; Anamnestic response

ABBREVIATIONS:
Hepatitis B Virus (HBV); Antibody to Hepatitis B core antigen (anti-HBc); Antibody to Hepatitis B surface antigen (HBsAg); Antibody to hepatitis B surface antigen (anti-HBs); Immunoglobulin M (IgM); Radioimmunoassay (RIA); Hepatitis B e Antigen (HBeAg); Antibody to Hepatitis B e antigen (anti-HBe); Enzyme Immunoassay (EIA); Geometric Mean Titres (GMTs); Milli-International Unit per mL (mIU/mL); Polymerase Chain Reaction (PCR)

ABSTRACT

Background/Aims: For subjects with isolated antibody to hepatitis B core antigen, vaccination can help discriminate various diagnostic possibilities. The aim of this study was to evaluate the clinical significance of isolated antibody to hepatitis B core antigen.

Methodology: A total of 1403 hospital personnel were screened for hepatitis B surface antigen, antibody to hepatitis B core antigen, and anti-HBc. Thirty subjects were confirmed to have isolated antibody to hepatitis B core antigen, and 278 subjects lacked all hepatitis B virus markers. Twenty-five of the 30 subjects (group I) and 136 of the 278 subjects (group II) were vaccinated by hepatitis B vaccine at months 0, 1, 6, and 2 months after vaccination by checking antibody to hepatitis B surface antigen in geometric mean titres at months 1, 2, and 7.

Results: The geometric mean titres of antibody to hepatitis B surface antigen were higher in group I than in group II at month 1 (9.1 ± 8.6 vs. 3.2 ± 3.0, p < 0.05), and were lower in group I than group II at month 7 (267.2 ± 17.2 vs. 2315.3 ± 5.1, p < 0.05). Furthermore, primary response was higher in group II than group I (73.3% vs. 35.7%, p < 0.05), but anamnestic response and non-response were higher in group I than group II (50% vs 26.7%, p = 0.116 with a trend; 14.3% vs. 9%, p < 0.01, respectively).

Conclusions: For subjects with isolated antibody to hepatitis B core antigen, a strategy concerning sequential vaccination followed by checking antibody to hepatitis B surface antigen might be adopted to avoid any extra vaccine dosages and ineffective vaccination.

INTRODUCTION

Serologic studies have contributed greatly to the understanding of hepatitis B virus (HBV) infection. The interpretation of test result patterns will generally define the immune status and differentiate acute from chronic infection (1). For antibody to hepatitis B core antigen (anti-HBc), it can be detected in the sera of the patients whether individuals have acute or chronic HBV infection and may persist for life. Therefore, anti-HBc may act as a single serum marker for screening HBV infection prior to HBV vaccination in endemic areas (2).

However, the clinical significance of isolated anti-HBc remains unclear. Several studies have shown that 0.1-20% subjects screened for HBV markers have anti-HBc as the sole marker for HBV infection (3-16). Specifically, an isolated anti-HBc might represent: 1) acute HBV infection in a core-window period; 2) chronic "low-level" HBV carrier state with sub-detectable hepatitis B surface antigen (HBsAg); 3) remote infection with loss of measurable antibody to hepatitis B surface antigen (anti-HBs); 4) a false-positive test (1,5,17-19). Among them, vaccination could help discriminate between the various diagnostic possibilities associated with an isolated anti-HBc pattern (19). Individuals in a core-window phase can be identified by the presence of immunoglobulin M (IgM) of anti-HBc in serum. Persons who were chronic "low-level" HBV carriers should not respond to hepatitis B vaccine. Alternatively, persons who have recovered from previous HBV infection would develop a secondary or anamnestic response to a single dose of hepatitis B vaccine. Finally, persons who have a false-positive anti-HBc result would respond to hepatitis B vaccine as a primary response.

The aims of the present study were to investigate: 1) the response to hepatitis B vaccine in subjects with an isolated anti-HBc as compared with subjects negative for all HBV serologic markers; 2) the clinical significance of isolated anti-HBc as the initial test mark-
er for HBV vaccination in an endemic area.

METHODOLOGY
Screening Populations
A screening program for the medical and paramed-
ical health workers for hepatitis B vaccination was
carried out at National Cheng Kung University Hos-
pital. A total of 1403 adult subjects (median age 27
years old; range 20-63 years old) were screened. Pre-
vaccination screening included serum HBsAg, anti-
HBs, and anti-HBc by radioimmunoassay (RIA) (Aboott Laboratories, Chicago, IL, USA). Sera were
initially tested for HBsAg and anti-HBs; only those
that were negative for both were then tested for anti-
HBc. Thirty (2.1%) subjects were identified with iso-
lated anti-HBc and confirmed by repeated tests. Hep-
atitis B e antigen (HBeAg), antibody to hepatitis B e
antigen (anti-HBe) (RIA, Abbott Laboratories, Chi-
ca go, IL, USA), as well as IgM anti-HBc (RIA, Abbott
Laboratories, Chicago, IL, USA) were also tested in
subjects with isolated anti-HBc.

Vaccination and Follow-up of Laboratory
Assay
Hepatitis B vaccine (Heptavax; Merck Sharp &
Dohme), 20μg, was administered by intramuscular
injection into the deltoid muscle at months 0, 1, and 6.
Anti-HBs titres (Enzyme immunoassay, EIA, Abbott
Laboratories, Chicago, IL, USA) were also tested in
sera of those who were negative for both HBsAg and
anti-HBs at months 1, 2 and 7; and were expressed by
geometric mean titres (GMTs) that were calculated by
using the log-transformation of anti-HBs levels (seronega-
tive subjects have been given the value of 1 milli-
International Unit per mL (mIU/mL)) and taking the an-
tilog of the mean of these transformed values (20). Anti-
HBc was also checked at months 1, 2 and 7 for evalua-
tion of their persistence. HBV DNA levels were mea-
sure by the polymerase chain reaction (PCR) at base-
line and at month 7 (21). A set of nested primers from
HBV core gene was used, consisting of an outer primer
pair with a 471-base span: sense (5'-GAATTTG-
GAGCTACTGTGG-3'), and antisense (5'-GTGTT
GATAGATAGGGG-3'), and an inner primer pair with a 372-
base span: sense (5'-TGCCCTCTGACTTCTTTCC-3',
1958 nt. to 1976 nt.) and antisense (5'-GTGTT
GATAGATAGGGG-3', 2394 nt. to 2374 nt.), and an inner primer pair with a 372-
base span: sense (5'-GAATTTGGAGCTACTGTGG-3', 1924 nt. to 1942 nt.) and anti-
sense (5'-CGAGGGAGTCTCTCTAGG-3', 2394
nt. to 2374 nt.), and an inner primer pair with a 372-
base span: sense (5'-TGCCCTCTGACTTCTTTCC-3',
1958 nt. to 1976 nt.) and antisense (5'-GTGTT
GATAGATAGGGG-3', 2394 nt. to 2374 nt.).

Twenty-five subjects with isolated anti-HBc (defined as group I) and 136 subjects without any HBV
serological markers (defined as group II) were enrolled
into the vaccination program. Eleven subjects in group
I did not receive the checking of anti-HBs titres at
month 7 because they were not being followed up. Five
subjects in group II had no data of anti-HBs titres at
month 1. The rest of the individuals were given a com-
plete follow up for a total of 7 months.

Definition of Anti-HBs Responses after
Hepatitis B Vaccination
"Primary response" was defined as an anti-HBs
titre less than 10mIU/mL at month 1 and more than
10mIU/mL at month 7. "Anamnestic response" or "sec-

### TABLE 1 Clinical Characteristics and Responses to Hepatitis B Vaccine in Subjects Seropositive for only Anti-HBc (Group I) and Subjects Seronegative for All HBV Markers (Group II)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (n=25)</td>
<td>II (n=136)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16/5</td>
<td>105/30</td>
</tr>
<tr>
<td>Male</td>
<td>5/19</td>
<td>31/106</td>
</tr>
<tr>
<td>Age</td>
<td>29.2±5.6</td>
<td>27.7±4.6</td>
</tr>
<tr>
<td>Anti-HBs titre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13/25(52)</td>
<td>35/131(26.7)</td>
</tr>
<tr>
<td>GMT</td>
<td>9.1±8.6</td>
<td>3.2±6.0</td>
</tr>
<tr>
<td>Month 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17/23(73.9)</td>
<td>112/136(82.4)</td>
</tr>
<tr>
<td>GMT</td>
<td>30.70±8.5</td>
<td>61.6±7.0</td>
</tr>
<tr>
<td>Month 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12/14(85.7)</td>
<td>135/136(99.3)</td>
</tr>
<tr>
<td>GMT</td>
<td>267.2±17.2</td>
<td>2315.3±5.1</td>
</tr>
</tbody>
</table>

NS: non-significant; GMT: geometric mean titre. The number in parentheses indicates percentage. *Sex (female/male) and +positivity (percentage of subjects with anti-HBs more than 10mIU/mL) were compared between groups I and II by Chi-square test and Fisher's exact test. **Age and GMT (mean ± standard deviation) were compared between groups I and II by Student's t test.

### TABLE 2 The Differential Response Patterns to Hepatitis B Vaccine in Subjects Seropositive for only Anti-HBc (Group I) and Subjects Seronegative for All HBV Markers (Group II)

<table>
<thead>
<tr>
<th>Group</th>
<th>I (n=14)</th>
<th>II (n=131)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary response</td>
<td>5 (35.7)</td>
<td>96 (73.3)</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Anamnestic response</td>
<td>7 (50)</td>
<td>36 (26.7)</td>
<td>p=0.116</td>
</tr>
<tr>
<td>Nonresponse</td>
<td>2 (14.3)</td>
<td>0 (0)</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

The number in parentheses indicates percentage. *Comparison by Chi-square test and Fisher's exact test.

Statistics
The age and anti-HBs GMTs were expressed by
mean ± standard deviation. The comparison of clinical
characteristics and anti-HBs responses to vaccine
between the two groups was performed by Chi-square
test, Fisher's exact test, and Student's t test. A p value
less than 0.05 was regarded as statistically significant.

RESULTS
Of the 1403 subjects, 234 (16.7%) were HBsAg-
positive and 308 (22.0%) were negative for both
HBsAg and anti-HBs. Among subjects negative for
both HBsAg and anti-HBs, 30 subjects were con-
firmed to have isolated anti-HBc, and 278 subjects lacked all
hepatitis B virus markers.

None of the 30 subjects with isolated anti-HBc was
erpositive for HBeAg, HBeAb, and IgM anti-HBe.
Twenty-five subjects with isolated anti-HBc (19
females/6 males, 29.2±5.6 years old) were vaccinated.
Anti-HBc assayed by RIA consistently existed for sera
of baseline, months 1, 2, and 7; but HBV DNA was negative by PCR at baseline and at month 7.

Table 1 shows the comparison of clinical characteristics and anti-HBs responses between groups I and II. Anti-HBs GMT at month 1 was significantly higher in group I than in group II (9.1 ± 8.6 vs. 3.2 ± 6.0, p < 0.05). In contrast, anti-HBs GMT at month 7 was significantly lower in group I than in group II (267.2 ± 17.2 vs. 2315.3 ± 5.1, p < 0.05). Furthermore, the positivity at month 1 was significantly higher in group I than in group II (52% vs. 26.7%, p < 0.05). On the contrary, the positivity at month 7 was significantly lower in group I than in group II (85.7% vs. 99.3%, p < 0.05).

The anti-HBs response patterns after vaccination in both groups I and II are shown in Table 2. There were more subjects having primary response in group II than group I (73.3% vs. 35.7%, p < 0.05). In addition, there were more non-response in group I than in group II (14.3% vs. 0%, p < 0.01). Our results also showed the trend that more subjects had anamnestic response in group I than in group II (50% vs. 26.7%, p = 0.116).

DISCUSSION

The prevalence of isolated anti-HBc in various populations, ranging from 0.1% to 20% (3-16), can be attributed to the assay methods, the local HBV prevalence rates, and the screened cohort (14). RIA and EIA are commonly used for measurement of anti-HBc. From previous studies, EIA has a lower specificity than RIA, and thus yields a higher false-positive rate (14,18,22,23). The prevalence of isolated anti-HBc is parallel to the HBV prevalence rates among different areas. The lower prevalence is found in populations where HBV infection is endemic, such as Taiwan (10-15%), Hong Kong (11.9%), Shanghai (13.2%), and Senegal (20.1%) (3,4,6,14,15). In screened populations, the isolated anti-HBc is prevalent among older age groups (14). In our study, only 2.1% of isolated anti-HBc prevalence rate was found, far from what was reported in HBV endemic areas. In addition to assay of anti-HBc by more specific RIA, the age of screened populations may be another key factor influencing the prevalence rate of anti-HBc. In detail, the average age of the screened populations in our study was 28.3 ± 27 years old (mean/median), which was younger than in other studies with high prevalence rate of isolated anti-HBc, such as Wang et al. (mean age 31.3 years old) and Lok et al. (median age 33 years old) (5,14).

Unlike the low persistence of isolated anti-HBc seropositivity reported in some studies (14,16), our study showed that all subjects followed had persisted anti-HBc of serum for at least 7 months. In the study by Lok et al. (14), isolated anti-HBc seropositivity did not persist as frequently in those subjects aged 20 or less. It is likely that the difference in our study was due to the different populations studied as all subjects in the present study are adults, and the different methods used for assaying anti-HBc as RIA is more specific than EIA for detection of anti-HBc.

Isoalted anti-HBc might result from the four conditions: 1) acute HBV infection in a core-window period; 2) chronic “low-level” HBV carrier state with subdetectable HBsAg; 3) remote infection with loss of measurable anti-HBs; 4) a false-positive test cross-reactive to hepatitis B core antigen (1,5,17-19). All the subjects with isolated anti-HBc were seronegative of IgM anti-HBc, excluding vaccinees in a core-window period. Anamnestic response, the dominant response pattern in group I subjects, consisted of 50% of vaccinees, which implied that they belonged to remote infection with loss of measurable anti-HBs. Protective immunity might be achieved by a booster dose of vaccine. Five (35.7%) of the vaccinees in group I belonged to the primary response, which was regarded as possible false-positivity. Two (14.3%) of the vaccinees in group I belonged to non-response that implied in part to chronic low level HBV carrier state or in part to defects of humoral responses that failed to produce protective antibodies. In spite of the fact that chronic low level HBV carrier state cannot be excluded completely, the PCR for HBV DNA was negative. More sensitive techniques for detecting HBsAg or HBV DNA are needed to demonstrate this possibility. In addition, studying the cellular response to HBsAg would be another project for demonstrating the defects of humoral response in non-response subjects. The response patterns to hepatitis B vaccine were more different from some studies in which the primary response was dominant (6,7,10,11,19,24,25). Several factors were regarded as possible causes, including the age and area of populations, hepatitis B vaccine (plasma-derived or recombinant), assaying methods, and definitions of response patterns.

Anti-HBs GMTs expressed similar increasing trends for groups I and II according to the vaccination dosing numbers. However, anti-HBs GMTs in group I subjects were higher at month 1 and lower at month 7 than those in group II subjects, suggesting that there were more diverse response patterns in group I than group II that were HBV-naive subjects and mainly expressed by primary response.

In conclusion, we suggest that before HBV vaccination, anti-HBc should be checked if HBsAg and anti-HBs are both negative. For subjects with isolated anti-HBc, a strategy on serial vaccination followed by checking the titres of anti-HBs might be adopted since some portions of subjects responded adequately after the first dosing, but a few cases did not developed adequate anti-HBs even after a full 3-dose HBV vaccination.

REFERENCES


