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Isolated hepatitis B core antibody positive among vaccinated cohort in Malaysia

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BACKGROUND AND OBJECTIVES: Hepatitis B core antibodies (anti-HBc) are detected in almost every patient with previous exposure to hepatitis B virus (HBV). However, with this marker alone, one cannot understand the activity of the disease; therefore, this study aimed to identify the implication of isolated hepatitis B core antibody and evaluate the effect of hepatitis B vaccine booster in isolated anti-HBc among adults who received the HBV vaccine as infants.

DESIGN AND SETTINGS: A prospective cohort study of vaccinated undergraduate students of University Putra Malaysia.

PATIENTS AND METHODS: A total of 408 undergraduate students who received infant hepatitis B vaccination volunteered for this study; 5 mL of venous blood was taken from the volunteers. Hepatitis B surface antigen (HBsAg) and core antibodies were tested using a commercially available enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (DRG international Inc., USA). Molecular detection of hepatitis B viral DNA was performed using nested polymerase chain reaction.

RESULTS: The prevalence of isolated anti-HBc among the vaccinated cohort was found to be 5.0%, out of which 80% had a hepatitis B surface antibodies (anti-HBs) titer higher than 10 IU/L, while 20% had less than 10 IU/L anti-HBs titer. All the anti-HBc positivesubjects had detectable hepatitis B viral DNA in their serum. Anamnestic response was found to be 100% among isolated anti-HBc with negative antibody.

CONCLUSION: Isolated anti-HBc developed protective levels of anti-HBs after a single dose of recombinant hepatitis B vaccination. HBV DNA was detected in all isolated anti-HBc indicating occult chronic HBV infection with undetectable HBsAg.

Hepatitis B virus (HBV) vaccines were introduced in the early 1980s, while recombinant hepatitis B vaccines became available in the mid-1980s. More than 100 countries have adopted the national policy of infant hepatitis B vaccination. Hepatitis B Vaccination campaigns have been shown to adequately control the disease, even in endemic areas. The production of hepatitis B surface antibodies (anti-HBs) has been reported to confer long-term protective immunity; these antibodies are produced in response to vaccination or response to HBV infection. Hepatitis B core antibodies (anti-HBc), however, are detected in almost all individuals that have been previously exposed to the virus.[1] Infection with HBV can be acute or chronic with the immunoglobulin M and G indicating the acute and chronic stages, respectively. A good level of immunity to HBV has been reported in children vaccinated during infancy in high endemic countries.[2] A long-term protection can be predicted by measuring anamnestic response after the administration of a booster dose, HBV core antibody (anti-HBc), prevalence of infection in vaccinated cohort, and in vitro B and T cell activity testing.[3,4] With the inclusion of hepatitis B vaccine into an expanded program on immunization in Malaysia, the rate of hepatitis B surface antigen (HBsAg) positive decreases significantly from 5.5%[5] to 0.3.[6] Although, national vaccination programs were successful, clinical serological surveys conducted in different parts of the world including China shows that anti-HBs waned over time and iso-

lated anti-HBc was found in 1% to 9% of vaccines after 10 to 15 years of primary vaccination.[7]

Isolated anti-HBc refers to asymptomatic individuals with immunity as a result of previous natural infection or vaccination and who are anti-HBc positive. These individuals are classified into: type I (resolved HBV infection with low anti-HBs), type II (false positive anti-HBc), type III (occult chronic hepatitis B infection with undetectable HBsAg), and type IV (window period yet to produce anti-HBs with undetectable HBsAg).[8] However, the likelihood of isolated anti-HBc due to window period is extremely questionable with the existing sensitive HBsAg assays.[9] Some of the options available for this group of individuals include checking for anamnestic response after a "booster" dose of vaccine, complete vaccination, or no vaccination at all. However, whether isolated anti-HBc requires vaccination or not has remained a controversial issue. Therefore, this study aimed to investigate the implication of isolated hepatitis B core antibody (anti-HBc) and to evaluate the effect of hepatitis B vaccine booster in isolated anti-HBc among adults who received an HBV vaccine as infants.

PATIENTS AND METHODS

Study population

A total of 408 volunteers were randomly selected based on inclusion criteria from a vaccinated cohort of undergraduate students who were born in or after 1989, which was the year in which the hepatitis B vaccine was incorporated into the childhood vaccination program. Students who were vaccinated in the intervening years and those born before 1989 were excluded.

Sample collection

Venous blood (5 mL) was taken from 408 volunteers into a GD vacutainer tube containing clot activator (Lot.120510) using aseptic techniques; the samples were processed at the Laboratory of Medical and Molecular Virology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Serum was separated from the blood by centrifugation at 4000 rpm for 10 minutes at room temperature. Serum was kept at -80°C until further analysis. Vaccination status was assessed using self-administered questionnaire.

Laboratory testing

The anti-HBs status of the subjects was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (DRG international Inc., USA). Briefly, 50 µL of the serum samples and the controls were added to the microwell plates (90 wells) coated with purified HBsAg after which conjugate (horseradish peroxidase [HRP]) labeled with HBsAg was added to the serum and incubated at 37°C for 1 hour. The washing step was done using an immune washer (Model 1575; Bio Rad, Hercules, California), after which the absorbance was measured at 450 nm using the TECAN Magellan ELISA reader version 6.4 (Austria, Europe). The presence or absence of the protective anti-HBs antibody was read using optical density. The kit had a sensitivity of 10 IU/L and optical density greater or equal to 0.105. All anti-HBc positive and anti-HBs negative subjects were given 20 µg of hepatitis B recombinant vaccine (Euvax B, Sanofi Pasteur, Jeonbuk-do, Korea), which was given as an intramuscular injection. One month after the vaccination, sera from all volunteers were obtained and the anti-HBs level was tested for each of the volunteers. Anamnestic response was measured as anti-HBs titer equal to or greater than 10 IU/L post-booster vaccination.

Anti-HBc was tested by commercial EIAs (DRG international Inc., USA). A serum specimen was added to the microtiter wells together with HRP-conjugated anti-HBc antibody and incubated at room temperature, enabling the antibodies in the serum to compete with a fixed amount of conjugate; this was performed according to the manufacturer's instructions. The results were interpreted based on the cutoff value that was calculated from negative and positive controls. All sera were screened for HBV DNA with nested polymerase chain reaction (PCR) using published primers, as described previously.[10]

Ethical consideration

The ethical approval to carry out this study was obtained from the Institute for Medical Research, Malaysia, and Faculty of Medicine and Health Sciences ethical committee, Universiti Putra Malaysia. A written and informed consent was obtained from all respondents, and all of those who were found to not have protective ant-HBs were given free vaccination.

Data analysis

The prevalence of isolated anti-HBc, anti-HBs, and HBsAg as well as pre- and post-hepatitis B vaccination data were evaluated using SPSS, version 21.0 (SPSS Inc. Chicago, USA).

RESULTS

Of the 408 volunteers, 26.5% (108/408) were male and 73.5% (300/408) were female; the majority of them were Malay (66.3%), with the remaining being

Chinese 27.5% (112/408), Indian 2.5% (10/408), or other ethnicities 3.7% (15/408). The persistence of immunity against HBV was found in 62.5% (255/408) subjects, while 37.5% (153/408) did not have adequate anti-HBs in their circulation. The prevalence of isolated anti-HBc among the vaccinated cohort was found to be 5.0% (20/408) of the vaccinated cohort. A total of 80% (16/20) of the isolated anti-HBc had an anti-HBs titer more than 10 IU/L, while 20% (4/20) had less than 10 IU/L anti-HBs before booster administration. The nested PCR result showed that all of the anti-HBc positive subjects had detectable hepatitis B viral DNA in their serum (**Table 1**). The response to booster dose through anamnestic response was found to be 100% among isolated anti-HBc; however, they were all HBsAg negative.

Table 1. Summary of results for hepatitis B serological and molecular markers inasymptomatic volunteers who were vaccinated against hepatitis B virus as an infant. Pre: Pre hepatitis B vaccine booster; Post: post hepatitis B vaccine booster; the dash (----) indicate post vaccination anti-HBs not required.

Duration of vaccination (years)	HBsAg	Anti-HBs		Anti-HBc	HBV DNA
		Pre	Post		
21	Negative	Positive	---------	Positive	Positive
19	Negative	Positive	---------	Positive	Positive
19	Negative	Positive	---------	Positive	Positive
18	Negative	Positive	---------	Positive	Positive
20	Negative	Negative	Positive	Positive	Positive
19	Negative	Positive	---------	Positive	Positive
18	Negative	Positive	---------	Positive	Positive
20	Negative	Positive	---------	Positive	Positive
20	Negative	Positive	---------	Positive	Positive
20	Negative	Positive	---------	Positive	Positive
20	Negative	Positive	----------	Positive	Positive
21	Negative	Positive	-------	Positive	Positive
21	Negative	Negative	Positive	Positive	Positive
24	Negative	Positive	----------	Positive	Positive
23	Negative	Positive	----------	Positive	Positive
24	Negative	Positive	----------	Positive	Positive
19	Negative	Negative	Postive	Positive	Positive
19	Negative	Positive	Positive	Positive	Positive
20	Negative	Negative	----------	Positive	Positive
19	Negative	Positive	----------	Positive	Positive
Total	**20**	**20**	**4**	**20**	**20**

Pre: Pre hepatitis B vaccine booster; Post: post hepatitis B vaccine booster; the dash (----) indicates post-vaccination anti-HBs not required.

DISCUSSION

The prevalence of isolated anti-HBc varies in different populations ranging from as low as 0.1% to as high as 20%. 11-15 In this study, isolated anti-HBc prevalence was found to be 5%. A booster dose of hepatitis B recombinant vaccine generally leads to anamnestic response with detectable anti-HBs at a high concentration 1 month after the booster dose. The absence of anamnestic response in isolated anti-HBc may indicate low-level HBsAg carriers with immunological tolerance to surface antigens that are incapable of producing antibodies.[15] Hepatitis B vaccine is sensitive to freezing and heat, which can cause the vaccine to lose its potency, rendering it useless. Therefore, to maintain the potency of the vaccine, essential components of the cold chain system were maintained since its introduction in 1989, and the collection and transportation of the vaccines was done at temperature between +2°C and +8°C. This cold chain system in Malaysia was evaluated and found to be satisfactory in a previous study.[16] The perception of low-level hepatitis B surface antigens has been proven by reported cases of hepatitis B infection in the recipients of blood transfusion from isolated anti-HBc donors.[17, 18] The response to hepatitis B vaccine in isolated anti-HBc cases ranged from 56% to 100% in various studies.[12,19-21] This finding concurs with our study in which 100% of the isolated anti-HBc response to a single dose of recombinant hepatitis B vaccine indicates resolved HBV infection with anti-HBs loss. However, this is in contrast to a study conducted before introduction of national HBVvaccination in Taiwan in which most of the isolated anti-HBc showed no response to the hepatitis B vaccine booster dose.[22] The presence of isolated anti-HBc in this study might likely be a result of an occult HBV infection with vaccine escape mutants; therefore, there is a need for the deployment of a new vaccine that will be able to neutralize hepatitis B surface antigen mutants. Individuals with isolated anti-HBc are assumed to be in the so-called window period of resolving acute infection[23,24] or chronic late phase infection with HBV in the liver.[25] However, chronic infection is evident in individuals with detectable hepatitis B viral DNA measured by PCR. In our study, all of the isolated anti-HBc were positive for HBV DNA contrary to the findings in similar studies in Turkey[26] and Taiwan.[11] These differences may be the result of different sensitivities and specificities of the HBV DNA detection methods, studied populations, clinical specimens used, and differences in endemicity,[27,28] The high level of PCR sensitivity may easily lead to a false result due to minute contamination. However, precautions were taken to warrant the validity of our results by ensuring that there is consistency when PCR was repeated twice, and both

negative and no template controls were run in each experiment.

In conclusion, all of the isolated anti-HBc subjects developed protective levels of anti-HBs after a single dose of recombinant hepatitis B vaccination. HBV DNA was detected in all isolated anti-HBc indicating occult chronic HBV infection with undetectable HBsAg. The production of anti-HBs is attributed to the vaccine received in infancy and does not provide protection against mutant or occult strains of HBV. Even though there is no consensus over the clinical management of isolated anti-HBc, we strongly believe that they are due to occult hepatitis B infection with circulating antibodies and memory T-cells against the wild type. Therefore, they should be evaluated as such occult hepatitis B infection.

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