Significance of Response to Hepatitis B Recombinant Vaccine in Subjects with Isolated Antibody to Hepatitis B Core Antigen

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ABSTRACT

BACKGROUND

It is important to differentiate whether isolated anti-HBc is due to false positive results or the prior exposure to hepatitis B virus, because individuals with false-positive anti-HBc can benefit from vaccination and their blood can be safely transfused. To distinguish between these two conditions, we evaluated the serologic response to hepatitis B vaccine.

METHODS

Ninety subjects with isolated anti-HBc (cases) and 100 subjects with totally negative hepatitis B serologic markers (controls) were recruited to receive three doses of hepatitis-B (HB) vaccine. Thirty days after the first dose of the vaccine, anti-HBs titers were checked and individuals with anti-HBs titer >50 mIU/mL did not receive additional doses of the vaccine. However, others completed the vaccination course, and another blood sample was collected 30 days after the third dose to measure anti-HBs level.

RESULTS

Nineteen (21.1%) cases and three (3%) controls had no sero-conversion (anti-HBs titers <10 mIU/mL) 30 days after the third dose (p<0.0001). Primary response, defined as the development of anti-HBs antibody titers ≥10 mIU/mL 30 days after the third dose, was observed in 43 (47.8%) cases and 92 (92%) controls (p<0.0001). Also, 31.1% of cases developed anti-HBs titers ≥50 mIU/mL 30 days after the first dose of vaccine, but the rate was significantly lower (5%) in the control group (p<0.0001). Furthermore, half of the individuals with positive isolated anti-HBc developed protective levels of anti-HBs after three doses of HB vaccination.

CONCLUSION

More than 75% of individuals with positive isolated anti-HBc can benefit from vaccination and can be included in donor pool. Also, one fifth seemed to have occult HBV infection. So HB vaccination may be used as a diagnostic tool for clarifying the situation of the subjects with isolated anti-HBc.

KEYWORDS

Hepatitis B Core Antigens; Hepatitis B Vaccine; Blood Donors

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a potentially life-threatening liver disease with serious complications such as cirrhosis and hepatocellular carcinoma. Globally, more than 240 million people are infected with the virus. The prevalence of HBV infection varies widely, with rates ranging from 0.1% to 20% worldwide. Iran is an area of intermediate endemicity and the prevalence of chronic hepatitis B infection is reported to be 1.7% in general population.

Several serological markers are measured to distinguish hepatitis B infection status. It has been demonstrated that some HBs-Ag negative individuals with positive anti-HBc continue to replicate HBV. Thus, the absence of HBs-Ag in the blood of apparently healthy individuals may not be sufficient to ensure an HBV-free status. Anti-HBc can be detected throughout the course of both acute and chronic HBV infection. It persists lifelong after resolution, therefore the routine blood donor screening for anti-HBc has been implemented in some countries, resulting in a decrease in the risk of post-transfusion HBV infection.

In Iran, scanty reports dealing with isolated anti-HBc screening are available. The reported rates range from 5.1% in Hamadan and 6.5% in Shiraz to as high as 34% in Sistan-Balouchestan. Nevertheless, the associated figures for isolated anti-HBc prevalence in other studies were as low as 0.56% in the United Kingdom to as high as 76% in Ghana. However, most of previous studies reported isolated anti-HBc rates between 2-5%. Despite this fact, Iranian health policy makers have not introduced anti-HBc screening among blood donors, partly because of the lack of empirical data on the benefit of introducing anti-HBc screening in a donor population with low prevalence of HBV. On the other hand, the indefinite deferral of donors with false-positive anti-HBc is a major disappointing side effect of anti-HBc screening in countries with low endemicity of HBV infection. Therefore, anti-HBc screening of blood donations is still controversial.

It is of utmost importance to differentiate whether isolated anti-HBc is due to false positive results or the prior exposure to HBV, because individuals with false-positive anti-HBc can benefit from vaccination and their blood can be safely transfused. To distinguish between the aforementioned conditions, evaluation of response to HB vaccination has been proposed.

In the present study, we investigated the anti-HBs seroconversion in 2 groups of blood donors with isolated anti-HBc and their controls after HB vaccination. We also attempted to find the predictors of non-response status among individuals with isolated anti-HBc.

MATERIALS AND METHODS

This study was conducted in Zahedan Blood Transfusion Center. Ninety individuals with isolated anti-HBc positive test and 100 healthy persons with negative serological markers of hepatitis B were recruited in the study as case and control groups, respectively.

The exclusion criteria were: age > 64 years, previous HBV vaccination, organ transplantation, immunodeficiency disorders, hemodialysis, immunosuppressive therapy, contraindication for HBV vaccination (i.e. prior history of anaphylactic reaction to vaccine), positive results for HBs-Ag, anti-HBs, anti-HCV, or anti-HIV tests and aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels above 3 times normal cutoff values.

Also, none of the participants were positive for signs and symptoms of chronic hepatitis and cirrhosis. Prior to intervention, informed consents were obtained from all the participants, in accordance with the guidelines established by the Ethics Committee of Zahedan University of Medical Sciences.

All the participants were tested for HBV-DNA by qualitative polymerase chain reaction (PCR) that could detect as low as 100 copy/mL of viral genome. We strictly adhered to the standard guideline of PCR procedure. The following reagents were added to each tube on ice: 1X PCR mixture 15 ul and Taq-DNA polymerase 0.4 ul. Then the tubes were shaken...
and spun thoroughly. The PCR mixture contained primers amplifying 353 bp of the region of the core gene. One drop (20-25 ul) of mineral oil was also added to each tube. Then, 10 ul DNA (with the use of specified pipette for sampling of DNA) was added to the mixture and spun on microfuge for 3-5 seconds. The tubes were transferred to preheated thermocycler with the following program: 1 cycle of 60 seconds at 93˚C, 20 seconds at 61˚C and 40 seconds at 72˚C, followed by 35 cycles of 93˚C, 61˚C, and 72˚C for 20, 20, and 40 seconds, respectively. Finally, 10 IU/mL of amplified samples were directly analyzed in a 2% agarose gel without adding loading buffer. The presence of 353 bp fragments indicated positive test. Afterwards, all subjects received three doses of 20 μg of hepatitis B recombinant vaccine (Heberbiovac HBR, Havana, Cuba) at 0, 1, and 6 months, if applicable (See below). The vaccine was injected intramuscularly into the deltoid muscle.

In order to quantify anti-HBs antibodies, a blood sample was taken 30 days after the first dose of the vaccine. Individuals with high titer anti-HBs response (anti-HBs > 50 mIU/mL) did not receive additional doses of the vaccine. However, others completed the vaccination course, and another blood sample was collected 30 days after the third dose to measure anti-HBs level.

Anti-HBs titer ≥10 mIU/mL 30 days after the first vaccination was considered as early primary response. Also, late primary response was defined as anti-HBs titer ≥10 mIU/mL 30 days after the third dose of vaccines. Non-responders were individuals with <10 mIU/mL anti-HBs titers after receiving three doses of HB vaccine.

**Statistical analysis:**

Data were analyzed using SPSS for Windows software, version 11.5 (SPSS Inc., Chicago, USA) and STATA version 9 (STATA Corp., College Station, Tex., USA). Fisher’s exact test and Chi-squared test were used as appropriate. Odd’s ratios (ORs) were chosen to measure the association between dichotomous variables, and the results were adjusted for potential confounders by multivariate logistic modeling. Two-tailed significance tests were used in all statistical analyses. p values < 0.05 were considered as statistically significant.

**RESULTS**

The study population included 101 men and 89 women. Table 1 presents the demographic and important risk factors of the participants, comparing those with isolated anti-HBc to controls.

Participants of the case group were significantly older, and mostly married. The case group had also higher male/female ratio, and more history of transfusion and tattoo (p<0.05). However, history of infection in spouse, familial history of HBV infection, and serum AST and ALT levels was not significantly different between cases and controls.

Totally, 19 (21.1%) cases and 3 (3%) controls did not seroconvert (non-responder) after three doses of HB vaccine (p<0.0001). However, primary response was observed in 43 (47.8%) isolated anti-HBc positive cases and 92 (92%) controls (p<0.0001), of whom 15 cases and two controls seroconverted after the first dose of HB vaccine (early responder). Furthermore, anti-HBs titer ≥ 50 mIU/mL 30 days after the first dose of vaccine was significantly higher among cases (31.1% vs. 5%, p<0.0001). Two (2.2%) cases with positive isolated anti-HBc had detectable HBV DNA and were therefore, excluded from the analysis. Both of these two HBV DNA positive cases did not seroconvert after receiving 3 doses of vaccination. None of the cases and controls showed adverse effects after receiving recombinant HB vaccine.

Table 2 compares non-responders (excluding 2 HBV DNA positive cases) with primary responders (including early and late responders). According to univariate analysis, serum anti-HBc positivity, age, and marital status were significantly different between the two groups; however, in multivariable analysis, only anti-HBc positivity and age remained independently associated with non-responding status. Indeed, anti-HBc positive subjects were 12.2 times (95% CI: 3.2-46.4, p<0.0001) more likely to fail seroconversion. Similarly, age ≥ 50 years was
3.6 times more likely to lead in non-responsive state (95% CI: 1.0-12.3, \( p < 0.04 \)). Also, anti-HBc positive participants were more likely to develop anti-HBs titer ≥ 50 mIU/mL 30 days after receiving the first dose of vaccine (table 3).

As noted earlier, early response (anti-HBs titer ≥10 mIU/mL 30 days after the first vaccination) was found in 17 subjects. We compared early responders to late responders (the table is not included) and found a trend toward a higher seroprotective response rate after the first vaccination in the subjects with abnormal baseline ALT levels and those aged>50 years (ORs: 6.9 and 8, \( p < 0.02 \) and \( p < 0.001 \), respectively).

### DISCUSSION

In our setting, we vaccinated isolated anti-HBc as well as healthy blood donors and monitored their response to HBV vaccination. A total of 19 (21.1%) cases and 3 (3%) controls did not seroconvert after receiving 3 doses of HB vaccine. Prior investigators reported a wide range of non-responding state, Sunbul 9.1%, Cozyatacho 9.7%, Pereira 10%, Ural 10.4%, Silva 20%, Kabir 20.2% and Koh 29.4%. Therefore, at least 20% of isolated anti-HBc positive cases may suffer from occult hepatitis B infection, for whom a diminished ability to mount an anti-HBs response is proposed.

Furthermore, in our study, anti-HBs titer ≥ 50 mIU/mL 30 days after the first dose of vaccine was developed in 28 (31.1%) cases and 5 (5%) controls. Scanty studies have addressed anamnestic rates; however, in a study reported by Sunbul and colleagues, out of 33 (42.4%) subjects with isolated anti-HBc showed rapid high seroconversion, while in a report by Ural and co-workers, this rate was...
41.7% (20 out of 48). Our lower rate could in part be explained by lower prevalence of HBV in our region. In such patients, further vaccination may not be necessary. Finally, 43 (47.8%) subjects with isolated positive anti-HBc and 92 (92%) controls showed primary response after the vaccination protocol. The associated figures were 48.4% and 47.9% in Sunbul and Ural study, respectively.

Nevertheless, most of previous researchers assigned their subjects in two groups of responders (either primary or anamnestic) and non-responders. Accordingly, our cumulative responder rate would be 78.9% among subjects with isolated positive anti-HBc, which is in agreement with reports by Koh (70.6%), Kabir (79.8%), and Silva (80%), but lower than what reported by Ural (89.6%), Pereira (90%), Yatacho (90.3%) and Sunbul (90.9%).

Discrepancies in response rate to HB vaccination among isolated anti-HBc subjects can be explained by serologic kit sensitivity, defining cut off points, and inclusion criteria for borderline results. Therefore, in our region, nearly 80% of isolated anti-HBc subjects can be safely included in the donor pool, simply based on a vaccination protocol. Notably, the isolated anti-HBc subjects with false positive results (47.8% in our setting) are susceptible to hepatitis B infection and should receive a complete course of HB vaccine. However, they may lose HB vaccination because of misinterpretation of anti-HBc results.

On the other hand, our results revealed that individuals with isolated positive anti-HBc were significantly older, mostly married, had higher male/female ratio, history of transfusion, tattoo and abnormal serum AST and ALT levels when compared with controls. The higher rate of anti-HBc positivity among older subjects mirrors a cohort effect of HBV infections acquired decades ago when HBV

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**Table 2: Demographic and important risk factors of primary responders and non-responders**

|                          | Non-responders (%)(n=20) | Primary responders (%)(n=135) | p value | Odds ratio (95% CI) |
|--------------------------|--------------------------|-------------------------------|---------|---------------------|
| **Sex**                  |                          |                               |         |                     |
| Male                     | 10(50.0)                 | 68(50.4)                      | NS(2)   | 1.0 (0.4 – 2.5)     |
| Female                   | 10(50.0)                 | 67(49.6)                      |         |                     |
| **Age groups (yrs)**     |                          |                               |         |                     |
| ≤50                      | 13(65)                   | 121(89.6)                     | 0.04(1) |                     |
| >50                      | 7(35)                    | 14(10.4)                      |         |                     |
| **Marriage status**      |                          |                               |         |                     |
| Single                   | 1(5.0)                   | 49(36.3)                      | 0.005   | 0.1 (0.0–0.7)       |
| Married                  | 19(95.0)                 | 86(63.7)                      |         |                     |
| **Anti-HBc status**      |                          |                               |         |                     |
| Positive                 | 17(85.0)                 | 43(31.9)                      | 0.000   | 12.1 (3.4–43.6)     |
| Negative                 | 3(15.0)                  | 92(68.1)                      |         |                     |
| **History of infection in spouse** | |                               |         |                     |
| positive                 | 7(36.8)                  | 36(41.9)                      | NS(3)   | 0.8 (0.3– 2.3)      |
| negative                 | 12(63.2)                 | 50(58.1)                      |         |                     |
| **Familial history of HBV infection** | |                               |         |                     |
| Positive                 | 13(65.0)                 | 86(63.7)                      | NS(2)   | 1.1 (0.4–2.8)       |
| Negative                 | 7(35.5)                  | 49(36.3)                      |         |                     |
| **History of transfusion** |                          |                               |         |                     |
| Positive                 | 1(5.0)                   | 3(2.5)                        | NS(3)   | 2.3 (0.2–23.4)      |
| Negative                 | 19(95.0)                 | 132(97.5)                     |         |                     |
| **Tattoo**               |                          |                               |         |                     |
| Positive                 | 1(5.0)                   | 3(2.2)                        | NS(2)   | 2.3 (0.2–23.4)      |
| Negative                 | 19(95.0)                 | 132(97.8)                     |         |                     |
| **Serum ALT level**      |                          |                               |         |                     |
| Normal                   | 20(100.0)                | 128(94.8)                     | NS(3)   |                     |
| Abnormal                 | 0(0.0)                   | 7(5.2)                        |         |                     |
| **Serum AST level**      |                          |                               |         |                     |
| Normal                   | 20(100.0)                | 130(96.3)                     | NS(3)   |                     |
| Abnormal                 | 0(0.0)                   | 5(3.7)                        |         |                     |

1) Abnormal level was defined as levels > 1.5 times upper than normal limit
2) NS: Not significant
3) Linear by linear association (chi-squared for linear trend); Exact method.

* participants with anti-HBs titer ≥ 50 mIU/mL 30 days after the first dose of vaccine and those with positive HBV DNA were excluded
was moderately endemic in Iran and HBV National Vaccination Program had not been commenced. Furthermore, only two (2.2%) isolated anti-HBc positive cases had detectable HBV DNA. Recent studies suggest that 1-2% of anti-HBc reactive units contain low levels of HBV DNA. However, in studies conducted on 4930 healthy blood donor volunteers in Fars and Hamadan provinces, 6.5% and 5.1% were isolated anti-HBc positive cases, among whom 16 (12.3%) and 3 (1.2%) were HBV DNA positive, respectively.6,7

Having compared non-responders to primary responders (table 2), anti-HBc positivity, age, and marital status showed significant differences in univariable analysis. However, in multivariable analysis, only anti-HBc positivity and age (>50 years) remained independently associated with non-responding status. On the other hand, when we compared primary responders with those who developed anti-HBs titer ≥ 50 mIU/mL 30 days after the first dose of vaccine (table 3), positivity for anti-HBc and age >50 years were the most relevant factors in developing higher anti-HBs titers to HBV vaccine. Therefore, isolated anti-HBc subjects aged >50 years would more likely either develop anti-HBs titers ≥ 50 mIU/mL 30 days after receiving the first dose of vaccine or never respond to HB vaccine. As a result, one month after the first dose of

### Table 3: Demographic and important risk factors of primary responders and those with anti-HBs titers≥50 mIU/mL 30 days after receiving the first dose of vaccine

|                          | Anti-HBs titers≥50 mIU/mL (%, n=33) | Primary responders (%, n=135) | p value | Odds ratio (95% CI) |
|--------------------------|-------------------------------------|--------------------------------|---------|--------------------|
| **Sex**                  |                                     |                                |         |                    |
| Male                     | 21 (63.6)                           | 68 (50.4)                      | NS      | 0.6 (0.3–1.3)      |
| Female                   | 12 (36.4)                           | 67 (49.6)                      |         |                    |
| **Age groups (yrs)**     |                                     |                                |         |                    |
| ≤20                      | 3 (9.1)                             | 1 (7.6)                        | 0.006   | 3.4 (0.7–16.3)     |
| 21–30                    | 7 (21.2)                            | 26 (19.3)                      |         |                    |
| 31–40                    | 8 (24.2)                            | 22 (16.3)                      |         |                    |
| 41–50                    | 7 (21.2)                            | 26 (19.3)                      |         |                    |
| 51–60                    | 5 (15.2)                            | 11 (8.1)                       |         |                    |
| >60                      | 3 (9.1)                             | 3 (2.2)                        |         |                    |
| **Marriage status**      |                                     |                                |         |                    |
| Single                   | 7 (21.2)                            | 49 (36.3)                      | NS      | 2.1 (0.9–5.2)      |
| Married                  | 26 (78.8)                           | 86 (63.7)                      |         |                    |
| **Anti-HBc status**      |                                     |                                |         |                    |
| Positive                 | 28 (84.8)                           | 43 (31.9)                      | 0.0001  | 12.5 (4.3–33.3)    |
| Negative                 | 5 (15.2)                            | 92 (68.1)                      |         |                    |
| **History of spouse infection** |                       |                                |         |                    |
| positive                 | 9 (34.6)                            | 36 (41.9)                      | NS      | 1.4 (0.5–3.4)     |
| negative                 | 17 (65.4)                           | 50 (58.1)                      |         |                    |
| **Familial history of HBV infection** |                   |                                |         |                    |
| Positive                 | 18 (54.5)                           | 86 (63.7)                      | NS      | 1.5 (0.7–3.2)     |
| Negative                 | 15 (45.5)                           | 49 (36.3)                      |         |                    |
| **History of transfusion** |                                   |                                |         |                    |
| Positive                 | 3 (9.1)                             | 3 (2.5)                        | NS      | 0.2 (0.1–1.2)     |
| Negative                 | 30 (90.9)                           | 132 (97.5)                     |         |                    |
| **Tattoo**               |                                     |                                |         |                    |
| Positive                 | 2 (6.1)                             | 3 (2.2)                        | NS      | 0.4 (0.1–2.2)     |
| Negative                 | 31 (93.9)                           | 132 (97.8)                     |         |                    |
| **Serum ALT level1)**    |                                     |                                |         |                    |
| Normal                   | 30 (90.9)                           | 128 (94.8)                     | NS      | 1.8 (0.5–7.5)     |
| Abnormal                 | 3 (9.1)                             | 7 (5.2)                        |         |                    |
| **Serum AST level1)**    |                                     |                                |         |                    |
| Normal                   | 31 (93.9)                           | 130 (96.3)                     | NS      | 1.7 (0.3–9.1)     |
| Abnormal                 | 2 (6.1)                             | 5 (3.7)                        |         |                    |

1) Abnormal level was defined as levels > 1.5 times upper than normal limit
2) NS: Not significant
3) Linear by linear association (chi-squared for linear trend); Exact method
HB vaccine in isolated anti-HBc subjects aged >50 years, we can make a decision regarding whether to advise a strict follow-up (occult HBV infection) or assure the patient that he/she has a resolved HBV infection. Interestingly, when we compared the early and late responders (the table is not included), anti-HBc positivity, abnormal ALT level, and age >50 years were associated with a higher probability of early response. Therefore, anti-HBc positive subjects aged >50 years with abnormal ALT levels should be offered the first dose of HB vaccine and evaluated one month later in order to decide for their future follow-up.

Various molecular modalities have been developed to determine if isolated anti-HBc subjects could transmit HBV infection. However, they will further increase the testing costs and require trained personnel and equipment, which may not be possible in many blood banks around the country. Thus, studying the cost effectiveness of ‘monitoring the response to HB vaccination’ could be worth of considering as a diagnostic tool. We also suggest future studies focusing on evaluating the short-course vaccination protocols such as double-dose/double-time vaccination in order to make sooner decision.

In conclusion; using vaccination, we found that half of the subjects with isolated positive anti-HBc were falsely positive for the test and more than 75% of subjects with isolated positive anti-HBc could benefit from vaccination and could be included in donor pool. Moreover, one-fifth of subjects were non-responders and may have occult HBV infection. Because at least 5% of the donor population in Iran is anti-HBc positive, blindly rejecting anti-HBc positive donors would cause the exclusion of a consistent number of donors, most of whom could safely be included in the donor pool. Therefore, HB vaccination may be used as a diagnostic tool for clarifying the situation of the subjects with isolated anti-HBc and can help in reducing the blood supply problems.

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CONFLICT OF INTEREST
The authors declare no conflict of interest related to this work.

REFERENCES
1. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012;30:2212-9.
2. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004;11:97-107.
3. Poorolajal J, Majdzaheh R. Prevalence of chronic hepatitis B infection in Iran: a review article. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences 2009;14:249-58.
4. Yotsuyanagi H, Yasuda K, Moriya K, et al. Frequent presence of HBV in the sera of HBsAg-negative, anti-HBc-positive blood donors. Transfusion 2001;41:1093-9.
5. Kleinman SH, Kuhns MC, Todd DS, et al. Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-Hbc: implications for transfusion transmission and donor screening. Transfusion 2003;43:696-704.
6. Amini S, Mahmoodi MF, Andalibi S, Solati AA. Seropidemiology of hepatitis B, delta and human immunodeficiency virus infections in Hamadan province, Iran: a population based study. J Trop Med Hyg 1993;96:277-87.
7. Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Torab A, Moaddeh A. Anti-HBc & HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen in reducing risk of transfusion associated HBV infection. Indian J Med Res 2006;123:37-42.
8. Salehi M., Sanei-Moghaddam S, S. K. Seroepidemiological study of HBVand HCV infection in the total population of Zahedan region, Iran. Tabib-E-Shargh 2005;1:63-9.
9. Manzini P, Girotto M, Borsotti M, et al. Italian blood donors with anti-HBc and occult hepatitis B virus infection. Haematologica 2007;92:1664-70.
10. Grob P, Jilg W, Bornhau H, et al. Serological pattern “anti-HBc alone”: report on a workshop. J Med Virol 2000;62:450-5.
11. Allain JP, Candotti D, Soldan K, et al. The risk of hepatitis
B virus infection by transfusion in Kumasi, Ghana. *Blood* 2003;101:2419-25.

12. Prince AM, Lee DH, Brotman B. Infectivity of blood from PCR-positive, HBsAg-negative, anti-HBs-positive cases of resolved hepatitis B infection. *Transfusion* 2001;41:329-32.

13. Silva AE, McMahon BJ, Parkinson AJ, Sjogren MH, Hoofnagle JH, Di Bisceglie AM. Hepatitis B virus DNA in persons with isolated antibody to hepatitis B core antigen who subsequently received hepatitis B vaccine. *Clin Infect Dis* 1998;26:895-7.

14. Koh HJ, Kim SD, Choi JH, Kim SR, Lee JS. [A study of immune response to hepatitis B vaccine & HBV DNA in isolated anti-HBc positive subjects]. *J Prev Med Public Health* 2005;38:170-4.

15. McIntyre A, Nimmo GR, Wood GM, Tinniswood RD, Kerlin P. Isolated hepatitis B core antibody—can response to hepatitis B vaccine help elucidate the cause? *Aust N Z J Med* 1992;22:19-22.

16. Sunbul M, Leblebicioglu H, Esen S, Eroglu C, Barut S. Response to hepatitis B vaccine in HBsAg/anti-HBs negative and anti-HBc positive subjects. *Scand J Infect Dis* 2000;32:315-6.

17. Cozyataco A, Lozano Miranda A, Samalvides Cuba F, Antunez de Mayolo Ramis E. [Significance of the “isolated anti-HBc” serological pattern determined by the serological response to the vaccination against Hepatitis B]. *Rev Gastroenterol Peru* 2005;25:254-8.

18. Pereira JS, Goncales NS, Silva C, et al. HBV vaccination of HCV-infected patients with occult HBV infection and anti-HBc-positive blood donors. *Braz J Med Biol Res* 2006;39:525-31.

19. Ural O, Findik D. The response of isolated anti-HBc positive subjects to recombinant hepatitis B vaccine. *J Infect* 2001;43:187-90.

20. Kabir A, Keshvari M, Kashani AH, Alavian SM. Predicting response to HBV vaccination in people with positive anti-HBc but negative HBsAg and anti-HBs. *Hum Vaccin* 2008;4:379-83.

21. Katz L, Strong DM, Tegtmeier G, Stramer S. Performance of an algorithm for the reentry of volunteer blood donors deferred due to false-positive test results for antibody to hepatitis B core antigen. *Transfusion* 2008;48:2315-22.