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ORIGINAL ARTICLE

No evidence for occult HBV infection in hepatitis B vaccine non-responders

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ABSTRACT

Background and Objective: Although hepatitis B vaccine immunogenicity is high, certain risk factors such as age, tobacco consumption, obesity and genetic background have been associated with low responsiveness to HBV vaccine. We aimed to evaluate the role of occult hepatitis B virus (HBV) infection in non-responder adults to HBV vaccine in a low endemic area for HBV.

Material and Methods: A total of 52 subjects who were non-responder to HBV vaccine were enrolled in the study. HBsAg, anti-HBs and anti-HBc were tested in all subjects. The presence of HBV-DNA was determined in plasma samples by real-time PCR.

Results: A total of 52 cases with median age 34 years were enrolled in the study. 63.5% of patients were male and 36.5% were female. Isolated anti-HBc (HBsAg negative, anti-HBs negative and anti-HBc positive) was detected in 3.8% of cases. HBV-DNA was not detected in our cases.

Conclusion: This study showed no evidence of occult HBV infection in our HBV vaccine non-responders even in cases with isolated anti-HBc.

Keywords: HBV, HBV core antibody, HBV vaccine non-responders, Occult HBV infection

INTRODUCTION

Hepatitis B virus (HBV) infection is a main public health problem with 2 billion people infected throughout the world and 350 million suffering from chronic HBV infection and 75% of them are Asians (1). Iran is located in the low endemic area for HBV infection (2). The hepatitis B surface antigen (HBsAg) prevalence in Iran varied from 0 to 3.9% in different provinces (3).

The main strategy for effective control of the HBV infection is administration of three intramuscular doses of HBV vaccine. It has been reported that HBV vaccination elicits excellent immunogenicity and protective efficacy in more than 90% of healthy adults and more than 95% of infants, children and adolescents (4-6). However, approximately 5-10% of immunized individuals fail to develop detectable specific antibodies and remain at risk for hepatitis B infection (7). HBV vaccination has been implemented for newborns since 1992 in Iran (8). After 13 years of HBV vaccine administration, the vaccine coverage raised from 62% in 1993 to 94% in 2005 (2).

Although the overall rates of HBV vaccines efficacy

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are high, some factors have been associated with low responsiveness to HBV vaccine such as increasing age, male gender, obesity, tobacco consumption, administration of vaccine in the buttock instead of deltoid muscle and genetic factors such as certain HLA groups (9, 10). Moreover, an investigation showed that hepatitis B core antibody (anti-HBc) positivity is also associated with lack of hepatitis B surface antibody (anti-HBs) development after HBV vaccination and most of these cases assumed to have occult HBV infection (11).

Occult HBV infection is considered as the detection of HBV-DNA in the liver or serum with undetectable HBsAg with or without HBV antibodies (12). Occult HBV infection is a worldwide entity and its distribution is affected by the general prevalence of the HBV in various populations and geographical areas (13).

Occult HBV was well described in some high risk groups such as hemodialysis patients, HIV infected cases and injection drug users (14-16). Some studies have demonstrated that failure of HBV vaccine is associated with presence of HBV-DNA in sera (17, 18). Additionally, the emergence of HBV variants with mutations in the S gene, mostly developing in the ‘‘a’’ determinant region might be contributed in failure of HBV vaccine which had been reported in several regions of the world (19, 20).

Despite identification of risk factors for poor response to HBV vaccine in some population; the presence of occult HBV infection in adult subjects with HBV vaccine failure is not studied well. So we conducted this survey to assess the presence of occult HBV infection in adult populations who failed to respond to two full HBV vaccine series.

PATIENTS AND METHODS

In this cross-sectional study, 52 healthy employees who were non-responder to HBV vaccine (two full HBV vaccine series) were recruited in Tehran, Iran. Ethical clearance was taken from ethical committee of the Iranian Society for Support of Patients with Infectious Diseases and informed consent was obtained from all patients before enrollment.

A vaccine non-responder is a person who does not develop protective surface antibodies (anti-HBs titer < 10 IU/L) after completing two full HBV vaccine series [20 µg of the recombinant HBV vaccine (Pasteur Institute of Iran, Tehran, Iran) at 0, 1 and 6 months].

Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc) were tested with commercial electrochemiluminescence immunoassay kits (Roche Cobas e 411 analyzer; Roche Diagnostics, Indianapolis, IN, USA).

Subjects with isolated anti-HBc (HBsAg negative, anti-HBs negative and anti-HBc positive) were retested for reactivity to anti-HBc and HBsAg.

HBV-DNA was extracted using High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer’s instructions. HBV-DNA was determined by real-time PCR using Geno Sen’s HBV Real time PCR (Genome Diagnostics, New Delhi, India) on the Rotor-Gene 6000 real-time thermal cycler (Corbett Research, Sydney, Australia). The detection limit of the kit was 100 IU/ml according to the user manual.

Statistical Analysis. The Chi-square was used with the SPSS 16 Package program for statistical analysis (Chicago, IL, USA). Data are presented as median or, when indicated, as an absolute number and percentage.

RESULTS

A total of 52 HBV vaccine non-responders with median age 34 years were enrolled in the study. 63.5% of patients were male and 36.5% were female. Isolated anti-HBc was detected in two (3.8%) subjects. HBV-DNA was detected in none of our cases.

DISCUSSION

Presence of HBV-DNA with undetectable HBsAg, regardless of detection of other HBV serological markers, is defined as occult HBV infection (21, 22). The HBV-DNA could be detected with higher frequency in individuals with positive anti-HBc and negative anti-HBs (23). Description of occult HBV infection has been limited to blood or organ donors (21, 24, 25), hemodialysis patients and HIV infected cases (14, 15) and there is few published reports focusing on occult HBV infection in HBV vaccine non-responders.

The World Health Organization has recommended HBV vaccination to all nations since 1992 for

eliminating HBV infection. The coverage rate of HBV vaccine was estimated approximately 70% globally in 2009 (26). However breakthrough infections has been reported infrequently in vaccines and emergence of escape mutants, and low response to vaccine could be the causative factors (27- 29). Most of the breakthrough infections are relevant to wild type strain of HBV, but "a" determinant mutants of virus frequently reported in HBV vaccinated subjects from different regions of the world (19, 20, 30). Hsu study demonstrated a raising prevalence of S mutants 15 years after universal HBV vaccine implementation in infants in Taiwan (30). Therefore, vaccination can increase the possibility of selection pressure on the emergence of S mutants (31).

Limited studies are carried out on the presence of occult HBV infection in HBV vaccine non-responders. Yen *et al.* (11) evaluated the etiology of non-responsiveness to HBV vaccine in adults and showed that the lower response rate to HBV vaccine in isolated anti-HBc positive subjects may be due to occult HBV infection. Occult HBV infection was noted in 78.6% of non-responder subjects with isolated anti-HBc in this study. Although Yen *et al.* (11) could not find HBV-DNA in their cases and they assumed anti-HBe positive subjects as occult HBV infection.

In a study conducted by Su *et al.* (32), young adults with isolated anti-HBc who had been fully vaccinated with HBV vaccine at infancy were evaluated for response to HBV booster vaccine. No evidence of occult HBV infection was observed in participants. However we have to consider that isolated anti-HBc may be attributed to resolved HBV infection with loss of anti-HBs or false positive anti-HBc or presence of occult HBV infection.

The results of an investigation by Pande *et al.* (33) showed that 42% of infants born from HBsAg positive mothers develop occult HBV infection, which is not prevented by HBV vaccine administration to the newborn. HBIG administration at birth could not prevent occult HBV infection, although it prevented the overt HBV infection.

In another investigation by Mu *et al.* (34) occult HBV infection was documented in 10.9% of HBV vaccinated children. Our study is the first survey on occult HBV infection in HBV vaccine non-responders and showed that its frequency is negligible in non-responders to HBV vaccine even in subjects with isolated anti-HBc. There are some explanations for this situation. Firstly, occult HBV infection does not have a role in HBV vaccine non-responsiveness. Secondly, lack of occult HBV infection was expected in our cases because we also did not find occult HBV infection in our other recent studies in different groups such as blood donors (25), injection drug users (16) and dialysis patients (unpublished data). It may be due to the fact that our country is in the low endemic area for HBV infection and also improvement of people's knowledge about the risk factors of HBV transmission. Besides national vaccination program has decreased the HBV incidence in general population and other groups (2). Finally, isolated anti-HBc is an important marker for occult HBV infection so another cause for lower rate of occult HBV infection in this study can be attributed to low number of isolated anti-HBc in our cases (35). The national HBV vaccination program may be related to the low prevalence of isolated anti-HBc in Iran.

In conclusion, our investigation showed that occult HBV infection was not a risk factor for poor response to HBV vaccine in our population. This finding should be repeated in future studies with larger cohorts.

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REFERENCES

1. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733-1745.
2. Alavian SM, Fallahian F, Lankarani KB. The changing epidemiology of viral hepatitis B in Iran. *J Gastrointestin Liver Dis* 2007; 16: 403-406.
3. Merat S, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. *Arch Iran Med* 2000; 3: 192-201.
4. Hadler SC. Vaccines to prevent hepatitis B and hepatitis A virus infections. *Infect Dis Clin North Am* 1990; 4: 29-46.
5. Shapiro CN, Margolis HS. Hepatitis B epidemiology and prevention. *Epidemiol Rev* 1990; 12: 221-7.
6. Stevens CE. Immunoprophylaxis of hepatitis B virus infection. *Semin Pediatr Infect Dis* 1991; 2: 135-9

7. Goncalves L, Albarran B, Salmen S, Borges L, Fields H, Montes H, et al. The nonresponse to hepatitis B vaccination is associated with impaired lymphocyte activation. *Virology* 2004; 326:20-28.
8. Merat S, Rezvan H, Nouraie M, Jamali A, Assari S, Abolghasemi H, et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a population based study. *Arch Iran Med* 2009; 12:225-231.
9. Roome AJ, Walsh SJ, Cartter ML, Hadler JL. Hepatitis B vaccine responsiveness in Connecticut public safety personnel. *JAMA* 1993; 270:2931-2934.
10. Wood RC, MacDonald KL, White KE, Hedberg CW, Hanson M, Osterholm MT. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. *JAMA* 1993; 270: 2935-2939.
11. Yen YH, Chen CH, Wang JH, Lee CM, Changchien CS, Lu SN. Study of hepatitis B (HB) vaccine non-responsiveness among health care workers from an endemic area (Taiwan). *Liver Int* 2005; 25:1162-1168.
12 Said ZN. An overview of occult hepatitis B virus infection. *World J Gastroenterol* 2011; 17:1927-38.
13 Raimondo G, Caccamo G, Filomia R, Pollicino T. Occult HBV infection. *Semin Immunopathol* 2013; 35:39-52.
14. Azadmanesh K, Mohraz M, Aghakhani A, Edalat R, Jam S, Eslamifar A, et al. Occult hepatitis B virus infection in HIV-infected patients with isolated hepatitis B core antibody. *Intervirology* 2008; 51:270-274.
15. Aghakhani A, Banifazl M, Velayati AA, Eslamifar A, Ramezani A. Occult hepatitis B virus infection in hemodialysis patients: a concept for consideration. *Ther Apher Dial* 2012; 16:328-33.
16. Sofian M, Aghakhani A, Banifazl M, Azadmanesh K, Farazi AA, McFarland W, et al. A. Viral hepatitis and HIV infection among injection drug users in a central Iranian City. *J Addict Med* 2012; (4):292-296
17. Buchanan C, Tran TT. Management of chronic hepatitis B in pregnancy. *Clin Liver Dis* 2010; 14:495-504.
18. Ni YH. Natural history of hepatitis B virus infection. Pediatric perspective. *J Gastroenterol* 2010; 46:1-8.
19. Coleman PF. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis* 2006; 6:198-203.
20. Cooreman MP, Leroux-Roels G, Paulij WP. Vaccine- and hepatitis B immune globulin-induced escape mutations of hepatitis B virus surface antigen. *J Biomed Sci* 2001; 8:237-247.
21. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely 'occult'? *Hepatology* 2001; 34: 194-203.
22. Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? *Hepatology* 2001; 34: 204-6.
23. Urbani S, Fagnoni F, Missale G, Franchini M. The role of anti-core antibody response in the detection of occult hepatitis B virus infection. *Clin Chem Lab Med* 2010; 48:23-29.
24. Raimondo G, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; 46:160-170.
25. Sofian M, Aghakhani A, Izadi N, Banifazl M, Kalantar E, Eslamifar A, et al. Lack of occult hepatitis B virus infection among blood donors with isolated hepatitis B core antibody living in an HBV low prevalence region of Iran. *Int J Infect Dis* 2010;14(4):e308-e310
26. World Health Organization: vaccines and biologicals: WHO vaccine preventable disease monitoring system: global summary 2009 data up to 2008). Available at: ww.who.int/immunization/documents/WHO_IVB_2009/en/index.html.Assessed October 10, 2011.
27. Sande MABvd, Waight P, Mendy M, Rayco-Solon P, Hutt P, Fulford T, et al. Long-term protection against carriage of hepatitis B virus after infant vaccination. *J Infect Dis* 2006; 193:1528-1535.
28. Chang MH. Breakthrough HBV infection in vaccinated children in Taiwan. Surveillance for HBV mutants. *Antivir Ther* 2010; 15:463-469.
29. Ngui SL, Andrews NJ, Underhill GS, Heptonstall J, Teo CG. Failed postnatal immunoprophylaxis for hepatitis B: characteristics of maternal hepatitis B virus as risk factors. *Clin Infect Dis* 1998; 27:100-106.
30. Hsu HY, Chang MH, Ni YH, Chen HL. Survey of hepatitis B surface variant infection in children 15 years after a nationwide vaccination programme in Taiwan. *Gut* 2004; 53:1499-1503.
31. Sa-Nguanmoo P, Tangkijvanich P, Tharmaphornpilas P, Rasdjarmrearnsook AO, Plianpanich S, Thawornsuk N, et al. Molecular analysis of hepatitis B virus associated with vaccine failure in infants and mothers: a case-control study in Thailand. *J Med Virol* 2012; 84:1177-85.
32. Su FH, Bai CH, Chu FY, Lin YS, Su CT, Yeh CC. Significance and anamnestic response in isolated hepatitis B core antibody-positive individuals 18 years after neonatal hepatitis B virus vaccination in Taiwan. *Vaccine* 2012; 30:4034-4039.
33. Pande C, Sarin SK, Patra S, Kumar A, Mishra S, Srivastava S, et al. Hepatitis B vaccination with or without hepatitis B immunoglobulin at birth to babies born of HBsAg positive mothers prevents overt HBV transmission but may not prevent occult HBV infection in babies: a randomized controlled trial. *J Viral Hepat* 2013; 20:801-810.
34. Mu SC, Lin YM, Jow GM, Chen BF. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. *J Hepatol* 2009;50:264-72.
35. Ramezani A, Banifazl M, Eslamifar A, Aghakhani A. Serological pattern of anti-HBc alone infers occult hepatitis B virus infection in high-risk individuals in Iran. *J Infect Dev Ctries* 2010; 4:658-61.