Brief Report

The Prevalence of Hepatitis B Virus Markers among Students of Shiraz University of Medical Sciences

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Abstract

Background: Protection against hepatitis B virus (HBV) is based on the presence of antibodies against hepatitis B surface antigen (HBsAg). Vaccination of newborns is the most effective means of prevention. This study aimed to evaluate the frequency of anti-HBs antibody (anti-HBsAb), anti-HB core Ab (anti-HBcAb), HBsAg, and HBV DNA among university students in Fars province, Southern Iran. **Materials and Methods:** In this cross-sectional study, 272 students of Shiraz University of Medical Sciences, were enrolled. Venous blood (5 mL) was collected from each participant and centrifuged; the sera were stored at −20°C until use. Anti-HBsAb, Anti-HBcAb, and HBsAg were measured using a commercial enzyme-linked immunosorbent assay kit. HBV DNA load was also measured by a real-time polymerase chain reaction. **Results:** The mean age of the participants was 19 ± 1 years. There were 171 (62.9%) females and 101 (37.1%) males. Anti-HBsAb at a protective level (>10 mIU/mL) were detected in the sera of 104 (38.5%) of the cases. Of the anti-HBsAb seropositive participants, 82 were female and 22 were male; the difference between the gender and seropositivity to anti-HBsAb was statistically significant (P = 0.001, odds ratio: 3.3, 95% confidence interval = 1.89–5.79). Anti-HBcAb was detected in only one participant that was negative for both HBsAg and HBV DNA. **Conclusion:** Findings of the current study show that more than half of the students do not have a protective level of anti-HBsAb and might be susceptible to HBV infection, indicating the necessity of checking the level of anti-HBsAb as well as a booster dose in high-risk groups.

Keywords: *Hepatitis B Antibodies, Hepatitis B Surface Antigens, Enzyme-Linked Immunosorbent Assay, Hepatitis B Virus, Student, Vaccination*

Introduction

About 240 million people have chronic hepatitis B infections worldwide.[1] In 2015, hepatitis B virus (HBV) resulted in 887,000 deaths and also end-stage liver disease including cirrhosis and hepatocellular carcinoma.[2] HBV, as a blood-borne virus, could be transmitted through transfusion, sexual contact, perinatal, and horizontal route, but its transmission is preventable.[3] The estimation of injuries by sharp objects to health-care workers is 1.4%–9.5% per year.[4] Moreover, infected health-care workers (HCWs) can transmit HBV to their patients.[5] HCWs and medical students are at occupational risk of HBV infection through needle sticks or exposure to body fluids.[6]

Protection against HBV infection is based on the presence of anti-hepatitis B surface antigen antibodies (anti-HBsAb) at a titer of ≥10 mIU/mL.[7] Although the duration of the immunity against HBV has not yet been elucidated, vaccination of newborns is the best and most effective means of prevention.[8] In Iran, the rate of chronic HBV infection is 1.79% (1.67%–2.32%) and exposure to the virus varies from 4.17% in Isfahan province to 36.9% in Golestan province.[1,9] Iran has started mass vaccination of newborns against hepatitis B as the Expanded Program of Immunization since 1993.[8,10]

HBsAg is the major antigen of HBV and is commonly used as a marker of chronic infection. Anti-HB core Ab (anti-HBcAb) is the first antibody that can be detected, persists for a long time, and is the indicator of previous exposure to HBV.[11] In chronic HBV infection, both HBsAg and anti-HBcAb are positive. HBV genome detection test in HBsAg negative but anti-HBcAb positive individuals indicates occult HBV infection and they

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can transmit infection and are at risk of chronic liver diseases.[12]

Due to the fact that it is important to know the status of the immunity of medical students, as one of the high-risk groups in hepatitis B infection, and lack of such information in students of Shiraz University of Medical Sciences, the present study was conducted to evaluate the frequency of anti-HBsAb, anti-HBcAb, HBsAg, and HBV DNA among university students in Shiraz, Fars province, Southern Iran.

Materials and Methods

Subjects and sampling

In this cross-sectional study, 272 students from Shiraz University of Medical Sciences were enrolled. The study was carried out from 2014 to 2016 on the students of midwifery, nursing, laboratory sciences, pharmacy, medicine, anesthesiology, and dentistry. A questionnaire containing questions about gender, age, educational status, and history of vaccination was filled out by the participants. Informed written consent was obtained from each student and the study was approved by the Ethics Committee of Shiraz University of Medical Sciences (IR. SUMS. REC.1393.7591). Those who had a history of booster dose of HBV vaccine at any time were excluded from the study. Venous blood (5 mL) was collected from each student. The blood samples were centrifuged; the serum was separated and stored at –20 until use.

Detection of anti-hepatitis B surface antibody, anti-hepatitis B core antibody, and hepatitis B surface antigen

Sera samples were examined for anti-HBsAb and anti-HBcAb, using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Dia.pro Diagnostic, Italy) according to the manufacturer's protocol. HBsAg detection was performed, using an ELISA kit (Dia.pro Diagnostic, Italy), on the sample that was positive for anti-HBcAb to rule out the chronic HBV infection. The level of anti-HBsAb was expressed by mIU/mL, and a titer ≥10 mIU/mL antibody concentration was considered as the protective level. Anti-HBcAb was performed to follow the natural HBV infection in the recruited participants.[13]

DNA extraction and real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was performed to detect the HBV viral load in the serum sample of those participants that were positive for anti-HBcAb and negative for HBsAg to rule out the occult HBV infection. The viral nucleic acid was extracted from the sera samples, using a MagCore automated machine (RBC bioscience, Taiwan). Internal control was added to the samples to verify the viral DNA extraction and amplification process. The presence of HBV viral DNA in the samples was investigated using a TaqMan-based real-time PCR method by GeneProof HBV PCR Kit (GeneProof, Czech Republic), according to the manufacturer's instruction. The HBV DNA viral load was interpreted in comparison with the standard curve.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22) (Chicago, IL, USA). Chi-square was used to find the association between the HBV positivity markers and sociodemographic features of the subjects. P-value < 0.05 was considered as statistically significant.

Results

The mean age of the participants was 19 ± 1 years with a range of 18–20 years. All participants received a schedule of three doses of HBV vaccine at 0, 1, and 6 month intervals after birth. Out of 272 cases, 171 (62.9%) were female and 101 (37.1%) were male. Anti-HBsAb at a protective level (≥10 mIU/mL) was detected in the sera of 104 (38.5%) cases. Of the anti-HBsAb seropositive participants, 82 were female and 22 were male; and the difference between the gender and seropositivity to anti-HBsAb was statistically significant (P = 0.001, odds ratio: 3.3, 95% confidence interval = 1.89–5.79). Only one female student was positive for anti-HBcAb, but she was negative for anti-HBsAb. Moreover, the result of the real-time PCR assay showed that HBV DNA was not present in the sera of this case.

Discussion

HCWs are at higher risk of blood-borne viruses including HBV. It has been reported that the risk of HBV infection in HCW is up to three to six times greater than the general population.[5,14] Moreover, although HBV vaccination is included in the national program vaccination of many countries including Iran, some individuals do not respond to the HBV vaccine, and the titer of anti-HBsAb decreases by time.[10,15-17] Therefore, investigation of the state of immunity against HBV is necessary for those who are at risk of exposure to HBV, including medical students.

The current study demonstrated that 38.5% of students at Shiraz University of Medical Sciences had a protective level of anti-HBsAb. Consistent with this study, Aghasadeghi *et al*. reported a prevalence of 39.9% for anti-HBsAb in 1120 cases younger than 24 years.[18] Furthermore, Norouzirad *et al*. reported that 48% of the individuals at age of 18 had a protective level of anti-HBsAb.[17] Moreover, in a study in China, 38.02% of the participants aged 19 years had a protective level of anti-HBsAb.[16] Moreover, Melo *et al*. in a study in Brazil reported that 56.1% of teenagers with a mean age of 15 had an anti-HBsAb level higher than 10 IU/mL.[19] Furthermore, in a study in Italy, 38.1% of 871 students that had been vaccinated at infancy had anti-HBsAb titer <10 mIU/mL, and all were negative for both HBsAg and anti-HBcAb.[20]

It has been reported that the seroconversion rate after three doses of vaccination in Iranian children is 100%, but the

titer of anti-HBsAb decreases with time.[15] The results of the above studies showed that at age of 18, at least 50% of students did not have a protective level of anti-HBsAb; therefore, the high-risk groups including HCW should be aware of their anti-HBsAb titer and be revaccinated if necessary.[5]

In this study, anti-HBsAb was significantly higher in females and the chance of antibody loss was 3.3 higher in males in comparison with females. Therefore, gender might be an important factor in the duration of anti-HBsAb persistence. In this regard, Hassan *et al*. and Yu *et al*. reported that the level of anti-HBsAb was significantly higher in females than males.[8,21] On the other hand, some studies showed no difference between the genders in response to the HBV vaccine.[22,23]

The findings of the current study also showed that only one (0.27%) participant was positive for anti-HBcAb. Aghasadeghi *et al*. reported that only 6 (0.56%) out of 1120 cases younger than 24 years that had received the HBV vaccine during infancy were positive for anti-HBcAb.[18] Moreover, in a study in Italy, none of the 220 medical students was positive for anti-HBcAb.[4] Another study in Italy also showed that none of the 871 participants that had been vaccinated at infancy was positive for anti-HBcAb.[20] In Ghana, the prevalence of anti-HBcAb was 2.6% and 6.1% among pupils delivered after and before the HBV vaccine program introduction, respectively, which shows the effectiveness of the HBV vaccination program.[24] In Brazil, 29 out of 576 (5.0%) teenagers were reactive for anti-HBcAb.[19] In Iran, Moghadami *et al*. reported that the rates of anti-HBcAb were 5.5% and 7.4% among those vaccinated after birth and unvaccinated cohorts, respectively.[25] The presence of anti-HBcAb in the sera indicated the exposure of subjects to HBV. In this study, a very low frequency of anti-HBcAb indicates the effectiveness of the HBV vaccination program in Iran.

Moreover, the result of the current study showed that HBsAg was not detected in the sera of the participants that were positive for anti-HBcAb. In this regard, two studies from Italy showed that none of the students that had been vaccinated at infancy was positive for HBsAg.[4,20] In the same line, in a study in Tehran, Iran, on 1120 cases younger than 24 years, only 1 out of the 6 anti-HBcAb positive subjects was positive for HBsAg.[18] Moreover, in a study in Shiraz, Iran, the prevalence of HBsAg among vaccinated subjects was 0.6%.[25] Furthermore, 4 out of 29 Brazilian teenagers that were positive for anti-HBcAb were positive for HBsAg.[19] In a comprehensive study in China, the frequency of HBsAg in participants younger than 20 years old was 1.35%, which is lower than the time of the introduction of general HBV vaccination.[16]

The results of these studies indicated that the effectiveness of HBV vaccination in the prevention of the HBV chronic infection was very high (near 100%) and HBV vaccination decreased the rate of chronic HBV infection in Iran, as the rate of HBsAg positivity in different areas of Iran was decreased after general HBV vaccination.[25,26]

Finally, the result of highly sensitive real-time PCR assay on the sera of the participants that were positive for anti-HBcAb and negative for HBsAg was negative for HBV DNA and ruled out the occult HBV infection. In line with our study, in Aghasadeghi *et al*.'s study, HBV-DNA was not detected in HBsAg negative/anti-HBcAb positive specimens, indicating no or very low frequency of occult HBV infection in the Iranian population born after HBV mass vaccination.[18]

Although more than half of the participants aged 20 years do not have a protective level of anti-HBsAb, the very low frequency of anti-HBcAb and HBsAg in those that get HBV vaccination in infancy might be related to the function of cellular immunity. It has been reported that anti-HBsAb does not solely account for protection. It has been shown that a significant number of memory B and T cells exist in vaccines even in the absence of anti-HBsAb and if the exposure happens, memory T cells are able to help memory B cells to produce anti-HBsAb.[7]

In conclusion, the findings of the current study showed that more than half of the students did not have a protective level of anti-HBsAb and might be susceptible to HBV infection, indicating the necessity of checking the level of anti-HBsAb as well as a booster dose in high-risk groups. Moreover, none of the 272 students had chronic and or occult HBV infection, showing the effectiveness of the neonate HBV vaccination program in Iran.

Limitations of the study

The limitations of our study include the relatively low sample size. Lack of information about the students' living places can be mentioned as another limitation of this study.

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Conflicts of interest

There are no conflicts of interest.

References

1. Ghafari S, Sharifzadeh G, Jamali S, Taji B, Javadmoosavi SY, Ziaee M. Prevalence of hepatitis B and C among drug-abusing male prisoners in Birjand, South Khorasan, Iran. Arch Iran Med 2019;22:501-4.
2. Mani SKK, Andrisani O. Hepatitis B virus-associated hepatocellular carcinoma and hepatic cancer stem cells.

Genes (Basel) 2018;9-137.
3. Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin Infect Dis 2011;53:68-75.
4. Rapisarda V, Nunnari G, Senia P, Vella F, Vitale E, Murabito P, *et al*. Hepatitis B vaccination coverage among medical residents from Catania University Hospital, Italy. Future Microbiol 2019;14:41-4.
5. Alavian SM, Izadi M, Zare AA, Lankarani MM, Assari S, Vardi MM. Survey of the level of anti-HBs antibody titer in vaccinated Iranian general dentists. Spec Care Dentist 2008;28:265-70.
6. Yaghobi Y, Nikokar I, Sedigh Ebrahim Saraie H, Frahbakhsh M, Safavi M, Ansar R, *et al*. Serum level of anti-hepatitis B surface antigen among students in guilan, iran (brief report). Int J Sch Health 2015;2.
7. Bauer T, Jilg W. Hepatitis B surface antigen-specific T and B cell memory in individuals who had lost protective antibodies after hepatitis B vaccination. Vaccine 2006;24:572-7.
8. Hassan S, Ziba F. Antibody titer in Iranian children 6 years after hepatitis B vaccine administration. Vaccine 2007;25:3511-4.
9. Hajarizadeh B, Mesgarpour B, Nasiri MJ, Alavian SM, Merat S, Poustchi H, *et al*. Estimating the prevalence of hepatitis B virus infection and exposure among general population in Iran. Hepat Mon 2017;17-1-11.
10. Arefkhah N, Vafazadeh S, Shahriarirad S, Ghorbani F, Zoghi S, Emami M, *et al*. Serum levels of anti-hepatitis B surface antibodies among vaccinated children aged 1 to 12 years in a rural community in Fars Province, southern Iran. J Immunoassay Immunochem 2020;41:20-7.
11. Khorami F, Sobhani SA, Davoudian P, Khajeh E. Prevalence of HBc-Ab among HBs-Ag negative healthy blood donors in South of Iran. Electron Physician 2013;5:659-63.
12. Shirani K, Nokhodian Z, Kassaian N, Adibi P, Naeini AE, Ataei B. The prevalence of isolated hepatitis B core antibody and its related risk factors among male injected drug users in Isfahan prisons. Adv Biomed Res 2015;4:17.
13. Azarkeivan A, Karimi G, Shaiegan M, Maghsudlu M, Tabbaroki A. Antibody titration and immune response of Iranian beta-thalassemic patients to hepatitis B virus vaccine (booster effect). Pediatr Hematol Oncol 2009;26:195-201.
14. Khosravani A, Sarkari B, Negahban H, Sharifi A, Toori MA, Eilami O. Hepatitis B Infection among high risk population: A seroepidemiological survey in Southwest of Iran. BMC Infect Dis 2012;12:378.
15. Amin S, Andalibi S, Mahmoudi M. Anti-HBs response and its protective effect in children and adults receiving hepatitis B recombinant vaccine in Tehran; 2002.
16. Tao J, Zhang W, Yue H, Zhu G, Wu W, Gong W, *et al*. Prevalence of hepatitis B virus infection in Shenzhen, China, 2015-2018. Sci Rep 2019;9:13948.
17. Norouzirad R, Shakurnia AH, Assarehzadegan MA, Serajian A, Khabazkhoob M. Serum levels of anti-hepatitis B surface antibody among vaccinated population aged 1 to 18 years in Ahvaz city Southwest of Iran. Hepat Mon 2014;14:e13625.
18. Aghasadeghi M, Aghakhani A, Mamishi S, Bidari-Zerehpoosh F, Haghi Ashtiani MT, Sabeti S, *et al*. No evidence of occult HBV infection in population born after mass vaccination. Wien Medizinische Wochenschrift (1946) 2020;170:218-23.
19. Melo LV, da Silva MA, Calçada Cde O, Cavalcante SR, Souto FJ. Hepatitis B virus markers among teenagers in the Araguaia region, Central Brazil: Assessment of prevalence and vaccination coverage. Vaccine 2011;29:5290-3.
20. Verso MG, Lo Cascio N, Noto Laddeca E, Amodio E, Currieri M, Giammanco G, *et al*. Predictors of hepatitis B surface antigen titers two decades after vaccination in a cohort of students and post-graduates of the Medical School at the University of Palermo, Italy. Ann Agric Environ Med 2017;24:303-6.
21. Yu AS, Cheung RC, Keeffe EB. Hepatitis B vaccines. Clin Liver Dis 2004;8:283-300.
22. Viviani S, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, *et al*. Hepatitis B vaccination in infancy in The Gambia: Protection against carriage at 9 years of age. Vaccine 1999;17:2946-50.
23. Hollinger FB. Factors influencing the immune response to hepatitis B vaccine, booster dose guidelines, and vaccine protocol recommendations. Am J Med 1989;87:36S-40S.
24. Hagan OCK, Nsiah P, Obiri-Yeboah D, Yirdong F, Annan I, Eliason S, *et al*. Impact of universal childhood vaccination against hepatitis B in Ghana: A pilot study. J Public Health Afr 2018;9:721.
25. Moghadami M, Dadashpour N, Mokhtari AM, Ebrahimi M, Mirahmadizadeh A. The effectiveness of the national hepatitis B vaccination program 25 years after its introduction in Iran: A historical cohort study. Braz J Infect Dis 2019;23:419-26.
26. Alavian SM. Hepatitis B is a serious health problem in some parts of Iran; Sistan and Baluchestan Province. Int J Infect 2015;2:e17937.