Identification of occult hepatitis B virus (HBV) infection and viral antigens in healthcare workers who presented low to moderate levels of anti-HBs after HBV vaccination

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Abstract

Background Worldwide, healthcare workers (HCWs) show different levels of response to hepatitis B virus (HBV) vaccine. One of the factors associated with vaccine unresponsiveness may be the existence of current or past HBV infection. Regardless of the presence of HBsAg (overt infection), occult HBV infection (OBI, defined as presence of HBV DNA in the absence of HBsAg) might also account for some non- or hypo-response cases.

Methods Sera from 120 HBsAg-negative HCWs with low and moderate levels of anti-HBs, <10 IU/mL (group I) and <100 IU/mL (group II) respectively, were selected and were examined for OBI by sensitive real-time PCR regardless of HBV serological profiles. Direct sequencing on surface genes was carried out in OBI-positive cases.

Results Four (3.3%) were positive for OBI. All were negative for anti-HBc. Two of the positive cases had moderate levels of anti-HBs (>10 to <100 IU/mL). No significant differences were found between the two groups in terms of risk factors or serological data. No mutations were found in surface proteins of OBI cases.

Conclusion OBI in these subjects might be due to other factors rather than presence of “a” determinant mutations. Healthcare workers with inadequate to moderate levels of anti-HBs (<100 IU/mL) following vaccination, regardless of their serological profile for HBV, should be tested for the presence of HBV DNA by sensitive molecular tests. Anti-HBc is not a reliable marker for suspicion of OBI, especially in high-risk group individuals.

Keywords Healthcare workers, hepatitis B virus, HBV, HBV vaccine

Background

Globally, hepatitis B virus (HBV) is a major public health problem in both industrial and

developing countries; about 400 million carriers are at danger of liver disease complications caused by chronic infection.¹ Healthcare workers

Received: 30 August 2015; revised 01 December 2015; accepted: 01 December 2015

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(HCWs) are at the front line for acquiring blood-borne viruses such as HBV, HCV and HIV, and compared to the general population, the risk of acquiring HBV for HCW is estimated to be 16 to 30%.²⁴ In Iran, a vaccination campaign was implemented in 1993;⁵ it included HCWs and it encouraged all individuals at risk for HBV infection to get vaccinated against HBV. Despite a substantial decline in the incidence of occupationally-acquired HBV, residual infection in healthcare systems still exists due to both incomplete coverage of vaccination and non-responsiveness to vaccine among HCWs.⁶ Iran is an intermediate region for HBV infection with 2.45% and 5 to 8.2% prevalence of HBsAg and anti-HBc in the general population, respectively.^{7,8} Measurement of anti-HBs following vaccination showed that not all HCW were protected against HBV infection despite vaccine administration according to the standard schedule.⁹ Non-response to HBV vaccine has been reported in 5-20% of Iranian HCWs,¹⁰⁻¹² similar to international reports from non-endemic areas.¹³⁻¹⁵ In the general population, non-response to HBV vaccine has been attributed to host factors including: age, male gender, obesity, tobacco smoking, and HLA background.¹⁶ In HCW settings, other host cellular dysfunction factors associated with inadequate levels of anti-HBs following vaccination include: decrease in CD4⁺ T lymphocyte proliferation responses,¹⁷ suboptimal B cell response,¹⁸ low production of Th1 cytokines,^{19,20} etc. Other causes for non-response to HBV vaccination have been attributed to viral factors, such as past or current HBV infection,^{21,22} manifested by HBsAg and/or anti-

HBc positivity. The prevalence of these serologic markers in health settings worldwide is widely distributed and depends on the endemicity of HBV in those areas, the level of HCW coverage for HBV vaccine, etc.²³ On the other hand, occult HBV infection (OBI) is defined by negative HBsAg in the presence of HBV DNA, regardless of the positive test for either anti-HBc or anti-HBs.²⁴ OBI has been documented in 0% to 11% of vaccinated HCWs, who were anti-HBc positive but did not respond to the vaccine.²⁵⁻²⁸ Therefore, a particular number of non-responsive HCWs are unaware of their status for those markers.

The aim of our study was to determine the HBV infection status among HCWs from 3 hospitals in Tehran who had been fully vaccinated and were defined as being low- and moderate-responders.

Methods

In this study, 120 healthy HCWs were recruited from 3 hospitals (Tehran Cardiac Center, Mirza Koochak Khan and Bahrami). They were selected from the Infection Control database files as being low/moderate-responders to HBV vaccine, defined as having anti-HBs titers <10 IU/mL (Group I n=21) and 10-99 IU/mL (Group II n=99), respectively. They had received the full course of HBV vaccination in the past five years. They had no evidence of co-infection with other hepatitis viruses or HIV, and they were treatment-naïve. A questionnaire was provided for collection of demographic and laboratory information, and potential risk factors, including a history of blood transfusion, surgery, dentistry procedures, being hospitalized, imprisonment and sexual behaviors. The study included laboratory technicians (n=2), midwives (n=4), nurses (n=47), nurses' aides (n=9), surgery room technicians (n=5) and cleaning staff (n=53). The study was approved by the ethics committee of TUMS. Four milliliters of blood were obtained from each participant.

Laboratory methods

The samples were tested for serological markers of HBV infection (HBsAg, anti-HBc and anti-HBs) using commercially available enzyme-linked immunosorbent assay (ELISA) kits

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according to the manufacturer's instructions (Acon, San Diego, CA, USA).

HBV DNA extraction and PCR

Using Qiagen Mini Blood Kit (Qiagen, Hilden, Germany), HBV DNA was extracted from a 200 µL aliquot of serum according to the manufacturer's recommendation, stored at -20°C.

HBV DNA was initially determined in all samples by real-time PCR (Fast-Track Diagnostics, Luxembourg) regardless of serological results. Then, all positive samples were selected for surface genes by standard PCR using specific primers, as described previously.²⁹

Sequencing

Using 2 pmol of appropriate primers, sequencing of HBsAg was carried out on an automated sequencer (Genetic Analyzer ABI-3130 DNA Sequencer, Fostercity, CA, USA). The obtained electropherograms were modified using the Chromas program. Sequences of the surface gene were aligned using the BioEdit Package version 7.0.9. Iranian sequences obtained from GenBank, NCBI and from our own laboratory were compared with aligned surface genes, allocated ng for distinct HBV genotypes

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation. The comparison of categorical variables was made using χ^2 test or Fisher's exact test, as appropriate. Multiple logistic regression analysis was used to examine the independent contribution of each potential risk factor. For the statistical analysis IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA) was used and a p-value less than 0.05 was considered significant.

Results

Demographic features

Of the total 120 HCWs, 44 and 76 were male and female, respectively. Groups I and II consisted of 10, 34 males and 11, 65 females, respectively (P value>0.05) (Table 1). The mean age of participants was 38.1 ± 9.2 SD. The mean age of participants in groups I and II were 37.09 and 38.31, respectively (P value>0.05). No

significant association was found between demographic features and job descriptions between healthcare workers (results not shown).

Serological features

Table 1 shows the details of serologic data for all participants in groups I and II. All participants were negative for HBsAg. The total geometric mean titer (GMT) for anti-HBs was 32 IU/mL. The GMT in groups I and II were 2 and 45 IU/mL, respectively (P value<0.001) (Table 1). The total number of anti-HBc positive subjects was 17 (14%). In groups I and II, 3 (14%) and 14 (14%) subjects presented positive anti-HBc (P value>0.05). Anti-HBe was found in 4 (3.3%) patients, 2 cases in each of the two groups (P value>0.05) (Table 1). No correlation was found between the occupations of healthcare workers and serological finding (results not shown).

Risk factors

A history of needle-stick injury (at least one episode) was positive in 36 (30%) subjects, of which 5 (23.8%) and 31 (31.3%) in groups I and II, respectively (P value>0.05) (Table 1). Of the total 9 (7.5%) cigarette smokers among healthcare workers, 5 (23.8%) and 4 (4%) were included in groups I and II, respectively (P value=0.009) (Table 1). Other risk factors including family history of hepatic disease, history of hepatitis, trauma and transfusion were not statistically associated with differences between groups (results not shown).

Occult hepatitis B infection cases analysis

Four (3.3) out of 120 cases were positive for HBV DNA and were classified as occult HBV infection. All were genotype D, subtype *ayw2* (results not shown). The characteristic data of OBI positive cases are shown in Table 2. The levels of HBV DNA ranged between 30 and 126 copies/mL (mean 87.75 copies/mL). All four were positive for anti-HBe, however, none were anti-HBc positive. Two subjects in each group were OBI-positive. Two were males and two were females. The mean age of OBI-positive patients was 35 ± 3.69 years. The levels of ALT and AST of patients were around the normal range except for one case who displayed a slight increase above the

Table 1. Demographic and serologic characteristic of participants including individual groups studied.

Characteristic	All participants N=122	Group I N=21	Group II N=99	P-value
Mean age	38.1	37.09	38.31	>0.05
Gender				
Male	44	10	34	>0.05
Female	76	11	65	
Mean±SD anti-HBs	32±28	2±2	45±23	<0.001
Anti-HBc positive	17	3	14	>0.05
History of needle-stick injury	36	5	31	>0.05
Anti-HBe positive	4	2	2	>0.05
Smoking	9	5	4	0.009

Table 2. Demographic, serologic and molecular details of OBI-positive healthcare workers.

Sample code	Gender	Age	Anti-HBs, IU/mL	HBV DNA, copies/mL	ALT/AST, IU/L
1	M	34	1	126	54/36
2	M	26	80	30	9/10
3	F	36	40	118	13/17
4	F	44	2.5	77	17/17

normal ranges (Table 2). In direct sequencing of surface proteins no amino acid replacement was found in OBI-positive cases.

Discussion

National and international guidelines have been developed to help minimize the risk of blood-borne pathogen exposure in healthcare workers, and medical personnel have been recommended to receive vaccination against HBV, as well as to employ infection control measures such as using barrier methods to help prevent transmission of infectious agents to and from the patients during interventional procedures. Despite these, a significant proportion of HCWs have shown past or current results for positive HBV serological profiles. However, the data on the prevalence of OBI in this high-risk group is scarce. The aims of this survey were to evaluate the prevalence of HBV overt and occult infections and to assess the risk factors and occupational exposures to HBV infection.

None of the subjects in the study was positive for HBsAg; instead, 14% were positive for anti-

HBc, suggesting past HBV infection. Four cases (3.3%) were OBI-positive. However, none were positive for anti-HBc in this subgroup. Recent data have demonstrated that a proportion of OBI patients could be found in anti-HBc negative different clinical groups.^{24,30} This event might be related to certain factors. These cases could have been infected in the past, with the disappearance of anti-HBc; positive anti-HBe in these patients confirmed this possibility. These types of OBI-patients have been categorized as chronic HBV carriers without detectable HBsAg probably due to either spontaneous HBsAg seroclearance in a carrier³⁰ or its low levels.³¹ Another possibility could be the presence of mutations in “a” determinant of surface protein that are not (or are only poorly) recognized by antibodies in the assays.³² In some chronic HBV individuals, no infection markers other than HBV DNA can be detected, assigning them as “seronegative OBI”,^{33,34} indicating that in the process of chronicity they lose these markers, but continue to have a low-grade HBV infection.³⁴

Table 3. Reported prevalence of OBI from healthcare workers.

Author	Country	Number of samples	No. (%) of OBI positive cases	No. (%) of anti-HBc positive in OBI	Anti-HBs in OBI-positive cases (IU/mL)
Present study	Iran	120	4 (3.3)	0 (0)	50%>10 50%<10
Chiarakul /2011 ²⁵	Thailand	36	4 (11)	4 (100)	75%<10 1.3%: undetectable
Slusarczyk /2012 ²⁶	Poland	961	6 (4)	4 (100)	6%: <10 4%: 10<anti HBs<100 88.7%: >100
Shim/2011 ²⁸	Korea	334	0	0	≥50
Sukriti/2008 ³⁸	India	120	6 (5)	6 (100)	>10
Yen/2005 ²⁷	Taiwan	250	16 (6.4)	13 (81)	<10
Total		1821	33 (1.8)	27 (81.8)	

Four studies from Asia and one from Poland found a prevalence of 0% to 11% of OBI among 1821 HCWs, including the present study (Table 3). The mean prevalence of OBI was 1.8%. Unlike the finding in this study, a significant proportion of OBI-positive cases among HCWs were positive for anti-HBc. All subjects had received HBV vaccination before such investigations were undertaken. However, those study groups included versatile responders versus non-responders. Furthermore, two cases of OBI-positive patients displayed adequate levels of anti-HBs (>10 IU/mL), and two others were below protective levels (<10 IU/mL). The latter finding was not surprising, as previous studies showed the waning of HBs antibody sometimes after vaccine administration in adults (and healthcare workers). What came as a surprise was the occurrence of OBI in the presence of adequate anti-HBs in two of the occult cases.

We were not able to find any conclusive points about the relationship between the presence of moderate or low levels of anti-HBs and the incidence of OBI in those cases. Naïvely speaking, one would be surprised about the presence of protective levels of anti-HBs and the occurrence of OBI, however this is not the case. Rather, previous data indicated an incidence of HBV infection in healthcare workers who had been vaccinated against HBV despite either adequate or low anamnestic response to booster doses and emphasized that vaccinated HCWs may still be at the risk of acquiring infection when exposure occurs.^{6,35,36}

No mutations were found in “a” determinant domain of surface proteins in OBI-positive individuals. In fact, worldwide, not all OBI studies contain mutations that might be responsible for undetectability of HBsAg. We have discussed this issue in detail elsewhere.³⁷ In brief, with the exception of mutations, other reasons might be related to the very low levels of HBsAg expression due to low replication of HBV (all OBI cases in the present study had very low HBV DNA levels) and immune complex formation between anti-HBs and HBsAg (making it undetectable by current assays). Moreover, the presence of a mixture of wild type and mutant profile in these patients could not be ruled out, as we did not carry out cloning. Therefore, the true quasispecies profile of such isolates could not be revealed by direct sequencing.

Results from this study clearly showed that differentiation between low to moderate levels of anti-HBs (at least for HCWs) is problematic. No significant relationship was found between demographic and serological characteristics, nor for risk factors between those who had <10 IU/mL versus those who had 10> to <100 IU/mL (with the exception of smoking as risk factor). Precisely, this differentiation is a technical point of view rather than a functional issue. Therefore, we believe that a value of 100 IU/mL for anti-HBs could be considered as cut off for healthcare personnel. Undeniably, this level does not indicate that above this cut off no HBV infection could take place.

Conclusion

In conclusion, healthcare workers with inadequate anti-HBs (<100 IU/mL) following vaccination, regardless of their serological profile for HBV, should be tested for the presence of HBV DNA by sensitive molecular tests. Anti-HBc is not a reliable marker for suspicion of OBI, especially in high-risk group individuals.

Authors' contributions statement: All authors had equal contributions. All authors read and approved the final manuscript.

Conflicts of interest: All authors – none to declare.

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Please cite this article as:

Borzooy Z, Jazayeri SM, Mirshafiey A, Khamseh A, Mahmoudie MK, Azimzadeh P, Geravand B, Boroumand MA, Afshar M, Poortahmasebi V, Hosseini M, Streinu-Cercel A. Identification of occult hepatitis B virus (HBV) infection and viral antigens in healthcare workers who presented low to moderate levels of anti-HBs after HBV vaccination. *GERMS* 2015;5(4):134-140. doi: 10.11599/germs.2015.1081