Prevalence of Occult Hepatitis B Virus Infection in a General Adult Population in Korea

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Key Words

Prevalence • Hepatitis B surface antigen • Anti-HBc • Anti-HBs • Occult hepatitis B virus infection • Hepatitis B virus DNA

Abstract

Objectives: We investigated the prevalence of hepatitis B virus (HBV) markers and occult HBV infection in a general adult population in Korea. **Methods:** HBsAg, anti-HBs and anti-HBc were analyzed on 1,091 samples of routine medical check-up examinees by chemiluminescence immunoassay. Nucleic acid amplification test (NAT) was performed on 1,047 HBsAg-negative samples by multiplex real-time polymerase chain reaction (PCR) kit for simultaneous detection of HBV, hepatitis C virus, and human immunodeficiency virus (Cobas Taqscreen MPX) in pools of six and reactive pools were resolved to individual samples, and further discriminated by PCR-based assay for HBV (Cobas Ampliscreen HBV). **Results:** The prevalences of HBsAg, anti-HBc, and anti-HBs were 4.0, 39.3, and 75.4%, respectively. The prevalence of anti-HBc significantly decreased with decreasing age ($p < 0.001$). Occult HBV infection was found in 7 (0.7%) of 1,047 HBsAg-negative subjects, and 5 of them were anti-HBc-negative. Sequencing of HBV S gene in 3 cases revealed one wild-type and two mutant strains (W74S, F85Y; T63I, W74S, T131N substitutions).

Conclusions: This study helps to understand the current status of hepatitis B infection and the prevalence of occult HBV infection in a general adult population in Korea.

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Introduction

Hepatitis B virus (HBV) infection is highly prevalent in Asia and Africa, the infection rate ranges from 5 to 20%. South Korea is one of the endemic areas for HBV infection. In the 1980s, the seropositivity of hepatitis B surface antigen (HBsAg) was estimated as 7–9% and 60–70% of adults showed evidence of present or past HBV infection [1]. Over the past two decades, nationwide strategies have been adopted to eliminate hepatitis B virus infection and to prevent its transmission, including universal vaccination of infants and adolescents and screening of all pregnant women for HBsAg to prevent perinatal HBV transmission. Consequently, its prevalence had been decreased and it was reported by the 1998 National Health and Nutrition Survey that the prevalence of HBsAg was 5.1% in males and 4.1% in females with a low prevalence in those under 20 years old [2–4]. Although there are some recent reports on the prevalence of HBV markers in selected populations such as medical

personnel [5], pregnant women [6] and children [7], more data which is representative of the general Korean population is needed.

Occult HBV infection is defined by serologically undetectable HBsAg despite the presence of HBV DNA in the sera or liver [8]. Although the mechanism and clinical implications have not yet been elucidated clearly, occult HBV infection has its own risks of disease transmission and may contribute to acute exacerbation and development of HBV-associated diseases such as hepatic inflammation, cirrhosis, and hepatocellular carcinoma (HCC) [8, 9]. Many studies have shown that prevalence of occult HBV infection in HBV endemic areas is higher than in Western countries, and the differences in its prevalence might be closely related to the endemicity of HBV infection [10, 11]. In Korea, there have been some recent reports of occult HBV infection in outpatients of gastroenterology clinic [12] or in pregnant women [13]; however, data in general populations are not available. The data in general populations are also limited worldwide [11], especially for anti-HBc-negative healthy subjects. The aim of this study was to determine the seroprevalence of HBsAg, anti-HBs and anti-HBc and the prevalence of occult HBV infection in a general adult population in Korea.

Materials and Methods

Study Population

A total of 1,091 subjects who visited Health Promotion Center of Konkuk University Hospital for routine medical check-up during the period from July to August 2008 were investigated. The median age of 1,091 subjects enrolled was 42 years (range 14–72). A total of 637 subjects were males and 454 were females. This study was approved by the institutional review board of Konkuk University Hospital.

Serological Assays

Blood samples were centrifuged at 2,500 rpm and serum samples were used for serological tests within 4 h, and then the rest of serum samples were stored at -70° until thawed for HBV DNA test. HBsAg, anti-HBs and anti-HBc were analyzed on 1,091 samples by chemiluminescence immunoassay using Advia Centaur HBsAg and anti-HBs (Bayer Healthcare LLC, Diagnostics Division, Tarrytown, N.Y., USA) and Abbott Prism HBcore (Abbott Laboratories, Abbott Park, Ill., USA) reagents.

HBV DNA Test

To detect occult HBV infection, HBV DNA test was performed on 1,091 HBsAg negative samples. At first, Cobas TaqScreen MPX test for simultaneous detection of human immunodeficiency virus (HIV)-1/HIV-2/hepatitis C virus (HCV) RNA and HBV DNA by multiplex real-time polymerase chain reaction (PCR) method was performed in pools of six on the Cobas s 201 system (Roche

Molecular Systems, Branchburg, N.J., USA). For the pools which show positive reaction, individual serum samples were tested with Cobas TaqScreen MPX test. For the Cobas TaqScreen MPX-reactive individual samples, Cobas AmpliScreen HBV test (Roche Molecular Systems) was used for confirmation of HBV DNA. Testing was conducted according to the configuration recommended by the manufacturer.

HBV DNA Amplification and Sequencing

Among 7 cases with occult HBV infection (negative HBsAg and positive HBV DNA), 3 had enough remaining serum samples for sequencing analysis of HBV DNA. DNA was extracted using Qiagen Ultrasense Viral Mini kit (Qiagen, Hilden, Germany) from 1 ml of serum.

Two microliters of the purified DNA was used as a PCR template. To analyze substitution patterns in the major hydrophilic loop region of the S gene, a nested PCR protocol was used as previously reported [14]. First-round PCR was performed using the sense primer HB1F (5'-AAGCTCTGCTAGATCCCAGAGT-3') and the antisense primer HB2R (5'-CATACTTTCCAATCAATAGG-3') to yield a 972 bps amplicon between 18 and 989 nt of the HBV genome. Second-round PCR was performed using the sense primer P7 (5'-GTGGTGGACTTCTCTCAATTTTC-3') and the antisense primer P8 (5'-CGGTAWAAAGGGACTCAGAT-3') to yield a 541-bp amplicon between 256 and 796 nt of the HBV genome.

Amplification was performed using PTC-200 thermal cycler (PTC-200, MJ Research, Waltham, Mass., USA) in a 50- μ l PCR mixture containing 2.5 mM $MgCl_2$, 400 mM dNTP, and 2.5 U of LA Taq polymerase (Roche, Mannheim, Germany). The PCR products obtained were analyzed by electrophoresis on 2.0% agarose gels, stained with ethidium bromide, and visualized on a UV transilluminator. The PCR products were purified using a QIAEX II gel extraction kit (Qiagen, Hilden, Germany), and nucleotide sequences were determined using the both primers of secondary PCR, P7 and P8, and using ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, New York, N.Y., USA). For sequence comparisons of the genomic sequences of the three HBV isolates, sequence alignment was performed using the ClustalW2 [15]. We used the web-based genotyping program at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/projects/genotyping>) [16] to identify the genotype of hepatitis B viral nucleotide sequence.

Statistical Analyses

Analysis of the data was performed with statistics package SPSS/PC, v12.0 (SPSS Inc., Chicago, Ill., USA). For the comparison between males and females, χ^2 test or Fisher's exact test was used as appropriate. $p < 0.05$ was considered significant. In order to evaluate the trend of HBV markers over age, linear by linear association analysis (using $2 \times k$ tables) was used.

Results

Prevalence of HBV Markers

Forty-four subjects were HBsAg-positive, resulting in an overall prevalence of 4.0% (table 1). No significant dif-

ference was seen between males and females (4.4 vs. 3.5%). The positive rate of anti-HBc was 39.3% and was significantly higher in males than in females (43.3 vs. 33.7%, $p = 0.001$, OR = 1.5, CI 1.2–1.9). The positive rate of anti-HBs was 75.4%, and there was no difference between males and females. Among the 823 anti-HBs-positive subjects, 458 (55.7%) were anti-HBc-negative and 365 (44.3%) were anti-HBc-positive. The frequency of anti-HBs- and anti-HBc-positive subjects was significantly higher in males than in females (37.5 vs. 27.5%, $p = 0.0006$, OR = 1.6, CI 1.2–2.1). In contrast, the frequency of anti-HBs only-positive subjects was significantly lower in males than in females (38.1 vs. 47.4%, $p = 0.002$, OR = 0.7, CI 0.5–0.9).

When the seroprevalence of HBV markers were compared among different age groups, there was a steady increase in anti-HBc-positive rate with age ranging from 10.6% in <30 years group to 60.0% in ≥ 60 years group ($p < 0.001$) (table 2). The frequency of anti-HBs- and anti-HBc-positive subjects showed a steady increase with age ($p < 0.001$) and that of anti-HBs only-positive subjects decreased with age ($p < 0.001$). Twenty-two subjects (2.0% of 1,091 subjects) were anti-HBc-positive and anti-HBs-negative and were classified to the anti-HBc only group. The frequency of anti-HBc only-positive subjects also showed an increase with age ($p = 0.03$).

Anti-HBs level was compared between two groups, namely anti-HBs- and anti-HBc-positive group and anti-HBs only-positive group. Among 365 subjects positive for both anti-HBs and anti-HBc, 87 (23.8%), 199 (54.5%), and 79 (21.6%) had values of 10–99, 100–999, and $\geq 1,000$ IU/l, respectively. Among 458 anti-HBs only-positive subjects, 170 (37.1%), 222 (48.5%) and 66 (14.4%) had values of 10–99, 100–999, and $\geq 1,000$ IU/l, respectively.

The percentage of subjects with low anti-HBs titer (10–99 IU/l) was significantly higher in the anti-HBs only group compared to the anti-HBs- and anti-HBc-positive group (37.1 vs. 23.8%, $p = 0.00004$).

Occult HBV Infection

Among the 1,047 HBsAg-negative sera tested for the presence of HBV DNA by Cobas AmpliScreen MPX test with 6-pool, 7 cases of occult HBV infection were confirmed by the individual Cobas TaqScreen MPX and Cobas Ampliscreen HBV tests. The characteristics of these

Table 1. Seroprevalence of HBV markers in an adult Korean population

HBsAg	Anti-HBc	Anti-HBs	Males (n = 637)	Females (n = 454)	Total (n = 1,091)
+	+	+	1 (0.2)	0 (0.0)	1 (0.1)
+	+	–	26 (4.1)	16 (3.5)	42 (3.8)
+	–	+	0 (0.0)	0 (0.0)	0 (0.0)
+	–	–	1 (0.2)	0 (0.0)	1 (0.1)
–	+	+	239 (37.5) ^a	125 (27.5)	364 (33.4)
–	+	–	10 (1.6)	12 (2.6)	22 (2.0)
–	–	+	243 (38.1) ^b	215 (47.4)	458 (42.0)
–	–	–	115 (18.1)	88 (19.4)	203 (18.6)
+			28 (4.4)	16 (3.5)	44 (4.0)
	+		276 (43.3) ^c	153 (33.7)	429 (39.3)
		+	483 (75.8)	340 (74.9)	823 (75.4)

+ = Positive; – = negative. Figures in parentheses are percentages.

^a $p = 0.0006$, OR = 1.6 (CI 1.2–2.1); ^b $p = 0.002$, OR = 0.7 (CI 0.5–0.9); ^c $p = 0.001$, OR = 1.5 (CI 1.2–1.9).

Table 2. Seroprevalence of HBV markers according to age in an adult Korean population

Age	Total	HBsAg ⁺	Anti-HBc ⁺ ^a	Anti-HBs ⁺	Anti-HBc ⁺ , anti-HBs ⁺ ^b	Anti-HBs only ^c	Anti-HBc only ^d	All negative
<30 years	47	0 (0.0)	5 (10.6)	30 (63.8)	5 (10.6)	25 (53.2)	0 (0.0)	17 (36.2)
30–39 years	403	15 (3.7)	107 (26.6)	294 (73.0)	86 (21.3)	207 (51.4)	7 (1.7)	88 (21.8)
40–49 years	427	20 (4.7)	191 (44.7)	333 (78.0)	166 (38.9)	167 (39.1)	5 (1.2)	69 (16.2)
50–59 years	164	7 (4.3)	96 (58.5)	134 (81.7)	86 (52.4)	48 (29.3)	4 (2.4)	19 (11.6)
≥ 60 years	50	2 (4.0)	30 (60.0)	32 (64.0)	22 (44.0)	10 (20.0)	6 (12.0)	10 (20.0)
Total	1,091	44 (4.0)	429 (39.3)	823 (75.4)	365 (33.5)	458 (42.0)	22 (2.0)	203 (18.6)

Anti-HBs only = HBsAg[–]/anti-HBc[–]/anti-HBs⁺; anti-HBc only = HBsAg[–]/anti-HBc⁺/anti-HBs[–]. Figures in parentheses are percentages of each age group.

^a $\chi^2 = 78.9$, $p < 0.001$; ^b $\chi^2 = 62.1$, $p < 0.001$; ^c $\chi^2 = 38.5$, $p < 0.001$; ^d $\chi^2 = 10.5$, $p = 0.03$ by linear by linear association analysis.

Table 3. Characteristics of the subjects with occult HBV infection

Case No.	Sex	Age	HBsAg	Anti-HBc	Anti-HBs IU/ml	ALT IU/l	Mutations in S gene
1	M	59	–	+	+34.5	20	NA
2	F	41	–	+	+39.6	21	W74S, F85Y
3	F	53	–	–	+167.0	31	NA
4	F	53	–	–	+18.4	42	T63I, W74S, T131N
5	M	31	–	–	–8.6	26	NA
6	M	39	–	–	+24.7	25	wild type
7	M	44	–	–	+281.6	19	NA

ALT = Alanine aminotransferase; NA = sample not available.

subjects are summarized in table 3. Two of them were anti-HBc-positive and anti-HBs-positive. The remaining 5 cases were anti-HBc-negative and 4 of them were anti-HBs-positive. The titers of anti-HBs were <100 IU/l in 4 cases and >100 IU/l in 2 cases. The alanine aminotransferase (ALT) levels were within the normal range in all cases.

Sequencing Analysis of Hepatitis B Virus S Gene

A web-based NCBI retroviruses genotyping analysis was performed as previously described [16]. The query of the acquired sequences from the three sera into that web-based program resulted in genotype C/subtype *adr*.

After sequencing analysis of the HBV S gene with both reverse and forward direction, both sequences were arrayed in parallel and compared with the reference sequence of genotype C (gi|474959). One of the 3 cases was of the wild-type strain and the remaining 2 cases were mutant strains. One of the mutant strains showed W74S and F85Y substitutions and the other showed T63I, W74S and T131N substitutions (table 3).

Discussion

This study was conducted to investigate the current status of the prevalence of HBV markers and occult HBV infection in the general adult population in Korea. The prevalence of HBsAg was 4.0%, which was slightly lower than in previous studies [2–4]. The prevalence of HBsAg was highest (4.7%) in the 40s age group. Although the number of group was rather small ($n = 47$), no HBsAg-

positive subject was observed below the age of 30, which indicates the effectiveness of preventive measures for HBV infection performed in Korea, such as prenatal testing for HBsAg in pregnant women and a vaccination program since 1983. A recent report of low HBsAg prevalence (0.9%) in children [7] is in accordance with our results. The prevalence of anti-HBc decreased with decreasing age and was 10.6% in the below 30s age group, which also reflects the effect of those preventive measures for HBV infection in Korea. The proportion of anti-HBc and anti-HBs-positive subjects who may have acquired the anti-HBs through previous HBV infection was highest in the 50s age group (52.4%) and decreased with decreasing age. The proportion of anti-HBc-negative and anti-HBs-positive subjects who may have acquired the anti-HBs through HBV vaccination was highest in the below 30s age group (53.2%).

The prevalence of occult HBV infection was 0.7% in our study, which might represent the data in the general adult population in Korea. This was much lower than those of previous reports in Korea in selected populations, such as outpatients of a gastroenterology clinic [12] or in pregnant women [13]. Kim et al. [12] reported 16% (31/195) of occult HBV infection among 195 HBsAg-negative subjects with normal ALT levels using an in-house nested PCR method. Kwon et al. [13] reported 4% (8/202, by TaqMan PCR assay) and 11.4% (23/202, Cobas AmpliCor HBV Monitor test) of occult HBV infection among 202 healthy pregnant women using two specific quantitative HBV DNA tests. Considering the definition of occult HBV infection (HBsAg-negative and HBV DNA-positive), the prevalence of occult HBV infection can vary according to the sensitivities of detection methods of HBsAg and HBV DNA even in the same study population [9, 11]. Because the viral load of occult HBV infection has been known to be very low, a positive rate can be affected especially by the sensitivity of the HBV DNA test [9]. In addition, the in-house nested PCR method commonly used to increase the sensitivity of HBV DNA test was known to be vulnerable to contamination. Even in cases of using commercialized HBV DNA tests, a higher rate of occult HBV infection was reported on the same subjects [13] in the open system (11.4%) than in the closed system (4%). Our study was conducted on relatively healthy people from routine medical check-up examinees and used a closed system (MPX TaqScreen) and tested with 6-pool format, which reduces the sensitivity of the HBV DNA test. All the factors mentioned could affect the prevalence of occult HBV infection, and should be considered in further studies.

In our study, it is of interest that 5 of the 7 cases with occult HBV infection showed anti-HBc negativity: 4 cases were anti-HBc-negative and anti-HBs-positive, and 1 case was seronegative (anti-HBc- and anti-HBs-negative). Though anti-HBc has been known as a pathognomonic marker of HBV infection, the titer of anti-HBc can decrease after a long period of time [17], and anti-HBc negativities in a chronic HBV carrier were also reported in cases of decreased humoral immunity and mutants of HBcAg [18–20]. In an anti-HBc-negative and anti-HBs-positive lymphoma patient, reactivation of the HBV virus was reported after immunosuppressive therapy, which indicates that anti-HBc-negative occult HBV infection exists [21]. Because anti-HBc has been considered as a pathognomonic marker of previous HBV infection, most of the studies of occult HBV infection were conducted on anti-HBc-positive subjects. This is also due to the high cost of the HBV DNA test, and no sufficient data on anti-HBc-negative general populations are available. Some occult HBV infection cases which showed anti-HBc negativity have been reported in recently published look-back studies of blood donors. In Polish blood donors, among 28 cases of occult HBV infection, 3 cases of anti-HBc-negative and anti-HBs-positive and 4 cases of seronegative (both anti-HBc and anti-HBs-negative) subjects were reported [22]. In Japanese blood donors, among 158 cases of occult HBV infection, 60 were anti-HBc-negative [23]. In Taiwan, among 467 anti-HBc-negative blood donors, 11 showed positive HBV DNA test results [24]. In a North-American community-based study [25], 8.1% (33/407) of occult infection in a HBV-seronegative group was reported. In a recent study in outpatients of a gastroenterology clinic in Korea [12], 15% (7/47) of occult infection in the HBV-seronegative group was also reported. The frequen-

cy and mechanism of disappearance of anti-HBc in occult HBV infection are mostly unknown. Further studies are needed to elucidate the pathogenesis, frequency and clinical significance of anti-HBc-negative occult HBV infection in the general population.

In our study, among 3 cases with available sequencing data for the HBV S gene, one was wild type and two were mutant strains. One of the mutant strains showed W74S and F85Y substitutions, and the other showed T63I, W74S and T131N substitutions. Only one substitution (T131N) was detected in the major hydrophilic loop region of the S gene, which causes a change in the immunological epitope of HBsAg. Datta et al. [26] reported that about half of the subjects with occult HBV infection had no substitutions in the major hydrophilic loop region. It was suggested that besides the major hydrophilic loop region of the S gene, substitutions or deletions in the *pre-S* gene [27], core promoter gene [28] or reverse transcriptase domain in the *pol* gene [29] can affect the synthesis of viral envelope protein or suppress the replication of HBV DNA, thus result in the absence of circulating HBsAg. However, the results from previous studies are still not consistent and need further investigations.

In summary, the prevalence of HBV markers and occult HBV infection was investigated in relatively healthy medical check-up examinees, which might represent a general adult population in Korea. The positive rate of HBsAg was 4.0% and that of anti-HBc was significantly decreased with decreasing age, which indicates the effectiveness of preventive measures for HBV infection introduced in early 1980s in this country. The prevalence of occult HBV infection (0.7%) was much lower than those of recent reports in selected populations in Korea.

References

- Chun BY, Lee MK, Rho YK: The prevalence of hepatitis B surface antigen among Korean by literature review. *Korean J Epidemiol* 1992;14:54–62.
- Joo KR, Bang SJ, Song BC, Youn KH, Joo YH, Yang S, Kim KR, Chung YH, Lee YS, Suh DJ: Hepatitis B viral markers of Korean adults in the late 1990s: survey data of 70,347 health screenees. *Korean J Gastroenterol* 1999;33: 642–652.
- Jang MK, Lee JY, Lee JH, Kim YB, Kim HY, Lee MS, Park CK, Yoo JY: Seroepidemiology of HBV infection in South Korea, 1995 through 1999. *Korean J Intern Med* 2001;16: 153–159.
- Lee DH, Kim JH, Nam JJ, Kim HR, Shin HR: Epidemiological findings of hepatitis B infection based on 1998 National Health and Nutrition Survey in Korea. *J Korean Med Sci* 2002;17:457–462.
- Shin BM, Yoo HM, Lee AS, Park SK: Seroprevalence of hepatitis B virus among health care workers in Korea. *J Korean Med Sci* 2006;21:58–62.
- Kang HS, Song BC, Ji CX, Kim SY, Kim SK: Serologic markers of hepatitis B virus in pregnant women in Jeju Island. *Korean J Hepatol* 2004;10:191–196.
- An YW, Chung EH, Rheem IS: A study of the current (2003–2005) prevalence of anti-HBs and immunologic memory of hepatitis B vaccine in children from the central area of Korea. *Korean J Pediatr* 2006;49:630–634.
- Allain JP: Occult hepatitis B virus infection: implications in transfusion. *Vox Sang* 2004; 86:83–91.
- Torbenson M, Thomas DL: Occult hepatitis B. *Lancet Infect Dis* 2002;2:479–486.
- Liu CJ, Chen DS, Chen PJ: Epidemiology of HBV infection in Asian blood donors: emphasis on occult HBV infection and the role of NAT. *J Clin Virol* 2006;36:S33–S44.

- 11 Raimondo G, Pollicino T, Cacciola I, Squadrito G: Occult hepatitis B virus infection. *J Hepatol* 2007;46:160–170.
- 12 Kim SM, Lee KS, Park CJ, Lee JY, Kim KH, Park JY, Lee JH, Kim HY, Yoo JY, Jang MK: Prevalence of occult HBV infection among subjects with normal serum ALT levels in Korea. *J Infect* 2007;54:185–191.
- 13 Kwon CI, Hwang SG, Shin SJ, Chang SW, Kim SY, Ko KH, Hong SP, Park PW, Rim KS, Kang MS, Chung HJ, Hong SP: Occult hepatitis B virus infection in pregnant woman and its clinical implication. *Liver Int* 2008; 28:667–674.
- 14 Song BC, Kim SH, Kim H, Ying YH, Kim HJ, Kim YJ, Yoon JH, Lee HS, Cha CY, Kook YH, Kim BJ: Prevalence of naturally occurring surface antigen variants of hepatitis B virus in Korean patients infected chronically. *J Med Virol* 2005;76:194–202.
- 15 <http://www.ebi.ac.uk/Tools/clustalw2/>
- 16 Rozanov M, Plikat U, Chappey C, Kochergin A, Tatusova T: A web-based genotyping resource for viral sequences. *Nucleic Acids Res* 2004;32:W654–W659.
- 17 Arase Y, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Hosaka T, Sezaki H, Sato J, Kobayashi M, Ikeda K, Kumada H: Long-term presence of HBV in the sera of chronic hepatitis B patients with HBsAg seroclearance. *Intervirology* 2007; 50:161–165.
- 18 Zoulim F, Zhang X, Pichoud C, Trepo C: Heterogeneity of hepatitis B virus (HBV) core gene in a patient with HBV-associated cirrhosis and serum negativity for anti-HBc. *J Hepatol* 1996;24:155–160.
- 19 Lee JH, Paglieroni TG, Holland PV, Zeldis JB: Chronic hepatitis B virus infection in an anti-HBc-nonreactive blood donor: variant virus or defective immune response? *Hepatology* 1992;16:24–30.
- 20 Fiordalisi G, Primi D, Tanzi E, Magni E, Incarbone C, Zanetti AR, Cariani E: Hepatitis B virus C gene heterogeneity in a familial cluster of anti-HBc negative chronic carriers. *J Med Virol* 1994;42:109–114.
- 21 Awerkiew S, Däumer M, Reiser M, Wend UC, Pfister H, Kaiser R, Willems WR, Gerlich WH: Reactivation of an occult hepatitis B virus escape mutant in an anti-HBs positive, anti-HBc negative lymphoma patient. *J Clin Virol* 2007;38:83–86.
- 22 Brojer E, Grabarczyk P, Liszewski G, Mikulska M, Allain JP, Letowska M: Polish Blood Transfusion Service Viral Study Group. Characterization of HBV DNA+/HBsAg– blood donors in Poland identified by triplex NAT. *Hepatology* 2006;44:1666–1674.
- 23 Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K: Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. *Transfusion* 2007;47:1197–1205.
- 24 Liu CJ, Lo SC, Kao JH, Tseng PT, Lai MY, Ni YH, Yeh SH, Chen PJ, Chen DS: Transmission of occult hepatitis B virus by transfusion to adult and pediatric recipients in Taiwan. *J Hepatol* 2006;44:39–46.
- 25 Minuk GY, Sun DF, Uhanova J, Zhang M, Caouette S, Nicolle LE, Gutkin A, Doucette K, Martin B, Giulivi A: Occult hepatitis B virus infection in a North American community-based population. *J Hepatol* 2005;42: 480–485.
- 26 Datta S, Banerjee A, Chandra PK, Chowdhury A, Chakravarty R: Genotype, phylogenetic analysis, and transmission pattern of occult hepatitis B virus (HBV) infection in families of asymptomatic HBsAg carriers. *J Med Virol* 2006;78:53–59.
- 27 Cabrerizo M, Bartolomé J, Caramelo C, Barril G, Carreno V: Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen-negative cases. *Hepatology* 2000;32:116–123.
- 28 Kao JH, Chen PJ, Lai MY, Chen DS: Sequence analysis of pre-S/surface and pre-core/core promoter genes of hepatitis B virus in chronic hepatitis C patients with occult HBV infection. *J Med Virol* 2002;68:216–220.
- 29 Banerjee A, Chandra PK, Datta S, Biswas A, Bhattacharya P, Chakraborty S, Chakrabarti S, Bhattacharya SK, Chakravarty R: Frequency and significance of hepatitis B virus surface gene variant circulating among ‘anti-HBc only’ individuals in Eastern India. *J Clin Virol* 2007;40:312–317.