MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 1826-1835
DOI: 10.12659/MSM.905445

Received: 2017.05.23
Accepted: 2017.09.05
Published: 2018.03.29

Clinical Characteristics and Correlation Analysis of Subjects with Chronic Hepatitis B Virus (HBV) Infection and Sustained Low Levels of Hepatitis B Surface Antigen (HBsAg)

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2 **Jun Cheng***
BCD 2 **Yuzhu Dai***
BCD 2 **Li Yan***
BCD 2 **Huajun Zhou**
BCD 3 **Xujian Xu**
ABCDFG 2 **Changgui Sun**
BCD 1 **Zhongyong Wang**

1 Department of Clinical Laboratory, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, P.R. China
2 Department of Clinical Laboratory, The 117th Hospital of PLA, Hangzhou, Zhejiang, P.R. China
3 Department of Biotechnology, The University of Tokyo, Tokyo, Japan

* These authors contributed equally to this work

Corresponding Authors: Zhongyong Wang, e-mail: Jyk117@126.com; Changgui Sun, e-mail: sunchangguidoc@163.com
Source of support: This study was supported by the Medical Science Foundation of Nanjing Military Command (No.12MA117), and the Natural Science Foundation of Zhejiang Province (No.Y15H200001)

Background: The aim of this study was to investigate the clinical characteristics of individuals with chronic hepatitis B virus (HBV) infection with persistent low levels of hepatitis B surface antigen (HBsAg) and to undertake a correlation analysis of the clinical characteristics.

Material/Methods: The study included 1,204 subjects with chronic HBV infection. Serum HBsAg, HBV envelope antigen (HBeAg), and HBV core antigen (HBcAg) levels were measured using the chemiluminescent microparticle immunoassay (CMIA) and the neutralization test. HBV DNA was measured using real-time fluorescence quantitative polymerase chain reaction (RT-FQ-PCR).

Results: There were 1,023 subjects in the high-level HBsAg group (HBsAg level ≥10 IU/mL) and 181 subjects in the low-level HBsAg group (HBsAg level <10 IU/mL). In the low-level HBsAg group, the main serological pattern (93.37%) was HBsAg and HBeAg and HBcAg-positive (HBV-M2), and the asymptomatic carrier (ASC) status was 98.34%. The low-level HBsAg group had a lower HBV DNA-positive rate compared with the high-level HBsAg group (40.33% *vs.* 75.07%), with a normal distribution across all age groups (P>0.05). The low-level HBsAg group included an older age group. A low-level of HBsAg was positively correlated with a low level of replication of HBV DNA (r=0.452).

Conclusions: The findings of this study showed that individuals with chronic HBV infection and sustained low-levels of HBsAg were an older population and had a lower level of replicating HBV DNA when compared with individuals with high levels of HBsAg, and the majority (93.7%) were also HBsAg and HBeAg and HBcAg-positive.

MeSH Keywords: Hepatitis A Antigens • Hepatitis B virus • Statistics as Topic

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/905445

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Hepatitis B virus (HBV) infection is one the most widespread and severe forms of viral hepatitis. The presence of hepatitis B surface antigen (HBsAg) is one of the earliest serum proteins found in HBV infection, and because it persists, HBsAg is an important diagnostic marker of HBV infection. Other serum markers of HBV infection include HBV envelope antigen (HBeAg), and HBV core antigen (HBcAg). It is now known that during the process of HBV infection, some individuals will have persistent low serum levels of HBsAg, and it is important to test for the presence of HBsAg in the diagnosis of HBV infection. However, the clinical and epidemiological factors associated with low levels of HBsAg compared with high levels of HBsAg are unclear.

Therefore, serum testing for low levels of HBsAg in the serum may be important, as sustained low-level HBsAg [1–5], and replication of HBV nucleic acid [6–8] have been reported in some subjects with chronic HBV infection. Because these serological findings may be of relevance for the prevention and treatment of HBV, the study of serum HBsAg has attracted the attention of general physicians, hepatologists, epidemiologists, virologists, and molecular biologists [9–15].

Currently, correlations between replication of HBV DNA, and serological markers of HBV, including HBsAg, HBeAg, and HBcAg, have been reported in patients with chronic HBV infection [12,16–18]. However, little is known about the relationship between replication of HBV DNA, clinical associations, and associations with levels of other serological markers for HBV infection and the presence of persistent serum low-level HBsAg.

The aim of this study was to investigate the clinical characteristics of individuals with chronic HBV infection with persistent low levels of HBsAg and to undertake a correlation analysis of the clinical characteristics.

Material and Methods

Ethical approval, patients studied, and serum samples

This study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China.

A total of 21,217 adults, >18 years-of-age, who received routine health examinations were sequentially registered and recruited during 2015. There were 13,520 men and 7,697 women.

Blood samples were taken from the participants, and serum samples were analyzed for hepatitis B surface antigen (HBsAg), hepatitis B envelope antigen (HBeAg) and hepatitis B core antigen (HBcAg) levels using the chemiluminescent microparticle immunoassay (CMIA). Positive HBsAg sera underwent a further confirmatory neutralization test. Also, the medical records and laboratory findings were reviewed for all individuals who were HBsAg-positive. Clinical follow-up was performed in 1,204 subjects with chronic HBV infection as follows: re-examination was done once every three months during a 12-month period, to exclude transient low levels of HBsAg in the early or acute phase of HBV infection, or in the HBsAg/anti-HBsAg transition recovery phase.

A sustained low-level of HBsAg was defined as the level of serum HBsAg persistently lower than 10 IU/mL in HBsAg-positive subjects during the follow-up period. Individuals were excluded from the study if they had undergone medical treatments including immunomodulatory or anti-viral therapies within six months before the study commenced. All subjects who were positive for HBsAg received common chronic viral infection screening test including anti-HIV-Ag/Ab, anti-HCV-IgG, and anti-HDV-Ag/Ab. Among the study participants, there were five subjects with positive serum anti-HCV-IgG levels, one subject with a positive anti-HIV-Ag/Ab. Also, three subjects with combined infections with hepatitis C virus (HCV) and HBV, and five subjects with HBV infection and chronic active hepatitis (CAH) were excluded due to liver-protecting, enzyme-lowering, and anti-viral therapies. Finally, 1,204 subjects were enrolled in the study including two HCV infected subjects and one subject with combined infection with human immunodeficiency virus (HIV) and HBV. Serum samples were stored at –70°C.

Reagents and equipment

Architect i2000 automated chemiluminescence immunoassay instrument (Abbott Core Laboratory, USA), kits for the detection of HBsAg, anti-HBsAg, HBeAg, anti-HBeAg, HBcAg and anti-HBcAg (Abbott, USA), the StepOnePlus real-time polymerase chain reaction (PCR) system (ABI Applied Systems, USA), NP968 nucleic acid extraction system, nucleic acid extraction kit (Tian Long, China), anti-HBsAg (1000 IU/mL) for HBsAg detection, HBV DNA Fluorescence Quantitative Detection Kit (ACON, China), alanine aminotransferase (ALT) kit (Leadman, China) and Architect C8000 automatic biochemical analyzer (Abbott Diagnostics, USA) were used in this study.

Measurement of HBV markers and confirmation of serum HBsAg levels

CMIA was used to detect the anti-HBsAg, HBeAg, anti-HBeAg, HBcAg, and anti-HBcAg in HBsAg-positive serum using the Architect i2000 automated chemiluminescence immunoassay instrument (Abbott Core Laboratory, USA), according to the manufacturer's instructions. Positive results were determined if HBsAg was >0.05 IU/mL, anti-HBs was >10 IU/mL, HBeAg was >1.0 mIU, anti-HBe was <1.0 mIU and anti-HBc was >1.0 mIU. If the levels

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

of HBsAg in the sample was >250 IU/mL, the sample was diluted with normal saline to ensure that the HBsAg was <250 IU/mL. To ensure that the HBsAg test results were reliable, the HBsAg level was further validated using a neutralization assay.

Extraction of HBV DNA with immune beads and real-time fluorescence quantitative polymerase chain reaction (PCR)

The Np968 nucleic acid extraction system and nucleic acid extraction kit (Tian Long, China) were used to extract HBV DNA from serum samples, using a slight modification from the routine method and the enrichment method. In the routine method, serum samples (200 µl/well) and trypsin (20 µl/well) were used in the low-level HBsAg group and high-level HBsAg group of individuals. Also, in the low-level HBsAg group, an enrichment method was also used for the extraction of HBV DNA in 500 µl of serum samples with 50 µl of trypsin; other procedures were the same as those used in the routine method.

Grouping of individuals

Grouping was done according to the cut-off serum HBsAg level of 10 IU/mL [1,6,7]. There were 1,023 subjects in the high-level HBsAg group (HBsAg level ≥10 IU/mL) and 181 subjects in the low-level HBsAg group (HBsAg level <10 IU/mL).

On the basis of HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, HBcAg, and anti-HBc), subjects with chronic HBV infection were divided into six serological patterns that included:
HBV-M1 (HBsAg/HBeAg/anti-HBc positive);
HBV-M2 (HBsAg/anti-HBe/anti-HBc positive);
HBV-M3 (HBsAg/anti-HBc positive);
HBV-M4 (HBsAg/HBeAg/anti-HBe/anti-HBc positive);
HBV-M5 (HBsAg/anti-HBs/HBeAg/anti-HBc positive); and
HBV-M6 (HBsAg/anti-HBs/anti-HBe/anti-HBc positive).

According to serum ALT and clinical diagnostic criteria [19], subjects with chronic HBV infection were divided into asymptomatic carrier (ASC) with HBV, characterized by HBsAg that remained positive for more than six months, with low or undetectable serum HBV DNA levels and normal serum aminotransferases and chronic hepatitis B (CHB) characterized by positive serum HBsAg levels that remained positive for more than six months or an uncertain date of onset, periodic reactivation with a pattern of fluctuating levels of HBV DNA and aminotransferases and active hepatitis. On the basis of age, subjects were divided into the following age groups: <30 years; ≥30–40 years; ≥40–50 years; ≥50–60 years; and ≥60 years.

Correlations between HBV DNA with HBV markers and age

The logarithm of HBV DNA ($\log_{10}$ IU/mL) was used as a dependent variable (logarithm HBV DNA was 0 when HBV DNA was <30 IU/mL), and age, HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc) as independent variables for correlation analysis. The correlation between HBV DNA with HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc) and age were evaluated in subjects with HBV-M1, HBV-M2 or HBV-M3 in the high-level HBsAg group and in those with the HBV-M2 profile of the low-level HBsAg group.

Statistical analysis

Quantitative data were expressed as the mean ± standard deviation (SD) or median (range). Qualitative data were expressed as frequencies or rates. Comparisons of the HBsAg-positive rate, HBV DNA-positive rate, and composition ratio were made using the Chi-squared test among different groups. The means were compared among different groups using a t-test for equal variances assumed and not assumed. A Mann–Whitney U test for nonparametric data was used. The correlations of HBV DNA with age and HBV markers were evaluated with linear regression analysis. Figures were prepared with GraphPad Prism 6 for Windows, and statistical analysis was done using SPSS version 12.01 for Windows. A value of $P<0.05$ was considered statistically significant.

Results

Hepatitis B virus (HBV) markers and HBV DNA in 1,204 individuals with chronic HBV infection

In 21,217 adults who received routine health examination, chronic HBV infection with HBsAg-positive serology was found in 1,204 subjects (1,204/21217 or 5.67%), of whom there were 780 men (780/13,520 or 5.77%) and 424 women (424/7697 or 5.51%). There were no statistically significant differences between men and women in the HBsAg positive rate and the HBV DNA positive rate ($P>0.05$). However, the logarithm HBV DNA in males was significantly greater in men than in women ($P<0.05$). Also, no significant differences were observed in age, serum HBsAg, anti-HBsAg, anti-HBeAg and logarithm HBV DNA between women and men ($P>0.05$). In particular, the HBeAg level in men was significantly lower than in women ($P<0.05$) and the anti-HBcAg level in men was significantly greater than in females ($P<0.05$) (Table 1).

Serological patterns and clinical types in 1,204 subjects with chronic HBV infection

In the low-level HBsAg group, the main serological pattern was HBsAg and HBeAg and HBcAg-positive (HBV-M2) (169/181, 93.37%) and the major clinical type was the asymptomatic carrier (ASC) (178/181, 98.34%). In the high-level HBsAg group, the major serological patterns were HBV-M2 (725/103,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Markers of hepatitis B virus (HBV) infection and HBV DNA in 1,204 HBsAg- positive subjects with chronic HBV infection.

Parameters	Male	Female	P-value
HBsAg positive rate (%)	5.77 (780/13520)	5.51 (424/7697)	0.430[a]
Age (years)	44.83±12.35	46.32±13.07	0.051[b]
HBsAg (IU/mL)	677.28 (0.05–497000)	1018.40 (0.05–127801.8)	0.268[c]
Anti-HBs (mIU/mL)	0.22 (0–15.30)	0.18 (0–13.73)	0.213[c]
HBeAg (S/N)	0.40 (0.19–1933.04)	0.38 (0.17–2124.12)	0.000[c]
Anti-HBe (S/N)	0.01 (0.01~78.90)	0.01 (0.01–88.80)	0.885[c]
Anti-HBc (S/N)	12.77±2.22	12.23±2.95	0.001[d]
HBV DNA positive rate (%)	69.49 (542/780)	64.39 (273/424)	0.071[a]
HBV DNA (Log_{10} IU/mL)	3.10 (0–9.77)	2.84 (0–9.74)	0.025[c]

[a] Chi-square test; [b] t-test for equal variances assumed; [c] Mann-Whitney U test for nonparametric; [d] t-test for equal variances not assumed. %, mean ±SD, or median (range).

70.87%) and HBV-M1 (169/1023, 23.95%), and the major clinical types were ASC (909/1023, 88.86%) and chronic hepatitis B (CHB) (114/1023, 11.14%) (Table 2).

Compared with the high-level HBsAg group, the mean age of the subjects with major serological patterns and major clinical types and the overall mean age in the low-level HBsAg group were significantly greater than in the high-level HBsAg group (P<0.05); the HBsAg positive rate and the HBV DNA positive rate (by the routine method and the enrichment method) in low-level HBsAg group were lower than in the high-level HBsAg group (P<0.05). In the HBsAg-positive or HBV DNA-positive subjects, there was no significant difference in the proportions of men and women between the low-level HBsAg group and the high-level HBsAg group (P>0.05) (Table 2). In the low-level HBsAg group, the proportion of subjects with the HBV-M2 or ASC status was significantly greater than in high-level HBsAg group (P<0.05) (Figure 1). In the high-level HBsAg group, subjects with the HBV-M1 pattern were younger than those with the HBV-M2, HBV-M3 or ASC clinical pattern (P<0.05).

Distribution of HBsAg-positive subjects and HBV DNA-positive rate in different age groups

Subjects were grouped according to their age. The results showed that the high-level HBsAg group showed a normal distribution among the different age groups (Figure 2A), but a skewed distribution was observed in the low-level HBsAg group (Figure 2B). The HBV DNA positive rate of the high-level HBsAg group was significantly different among different age groups (P<0.05) and was positively associated with age (Figure 2C) (r=–0.981, P<0.05). However, the HBV DNA-positive rate in the low-level HBsAg group (by the routine method and the enrichment method) was comparable among age groups (Figure 2D) (P>0.05). Furthermore, the positive rate of HBV DNA by the routine method was less than that using the enrichment method in the low-level HBsAg group (P<0.05).

HBV DNA, HBV markers and correlation analysis for major serological patterns and clinical types in the high-level HBsAg group and the low-level HBsAg group

The major serological patterns and clinical types were compared, using the statistical t-test or the Mann–Whitney U test, between the high-level HBsAg group and low-level HBsAg group. In the high-level HBsAg group, the median logarithm of HBV DNA and mean levels of HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) in the HBV-M1 subjects were significantly different from the HBV-M2 and HBV-M3 subjects (P<0.05), and the logarithm of HBV DNA, HBsAg, and anti-HBs in CHB subjects were also significantly different from those in the ASC subjects (P<0.05). In the low-level HBsAg group, the major serological pattern and clinical types were HBV-M2 and ASC, respectively. The mean levels of HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) were comparable between the HBV-M2 subjects and the ASC subjects (P>0.05), but significant difference was observed in the median logarithm values of HBV DNA between the HBV-M2 subjects and the ASC subjects (P<0.05). The median logarithm of HBV DNA, HBsAg, HBeAg and anti-HBc of HBV-M2 subjects in the low-level HBsAg group were significantly lower than those in high-level HBsAg group (P<0.05), but anti-HBsAg and anti-HBeAg were significantly greater (P<0.05) (Table 3).

The correlations between HBV DNA with age and HBV markers for major serological patterns were further analyzed in both the high-level HBsAg group and the low-level HBsAg group. The results showed that the HBV DNA levels of subjects with

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Distribution of serological patterns and clinical subtypes in 1,204 subjects with chronic hepatitis B virus (HBV) infection.

Group	Parameters	Age (years)	HBsAg positive (n)			HBV DNA positive (n)		
		Mean ±SD	Male	Female	Total	Male	Female	Total
High-level HBsAg group (n=1,023)	Serological patterns							
	HBV-M1	39.70±10.38	169	76	245	169[#]	76	245
	HBV-M2	44.86±10.97[c]	465	260	725	323	174	497
	HBV-M3	44.92±9.82[c]	29	17	46	12	8	20
	HBV-M4	43.33±14.05	3	0	3	3	0	3
	HBV-M5	42, 47	2	0	2	2	0	2
	HBV-M6	48, 62	1	1	2	1	0	1
	Clinical types							
	ASC	43.84±10.87[c]	585	324	909	426	228	654
	CHB	41.82±11.49[d]	84	30	114	84	30	114
	Total	43.63±10.95	669	354	1023	510	258	768
Low-level HBsAg group (n=181)	Serological patterns							
	HBV-M1	63, 67	2	0	2	2[#] (2)*	0	2 (2)
	HBV-M2	54.99±16.76[a]	102	67	169 [e]	27 (43)	15 (22)	42 (65)[e]
	HBV-M3	58.86±9.77	4	3	7	2 (3)	0 (1)	2 (4)
	HBV-M4		0	0	0	0	0	0
	HBV-M5	25	1	0	1	1 (1)	0	1 (1)
	HBV-M6	48, 53	2	0	2	0 (1)	0	0 (1)
	Clinical types							
	ASC	55.09±16.45[a]	108	70	178 [e]	29 (47)	15 (23)	44 (70)[e]
	CHB	51.67±23.18	3	0	3	3 (3)	0	3 (3)
	Total	55.09±16.45[a]	111[b]	70 [b]	181 [e]	32 (50)[b]	15 (23)[b]	47 (73)[e]

[#] Routine method was used to detect HBV DNA in the low-level HBsAg group and the high-level HBsAg group. * The results in parentheses are detected by enrichment method in the low-level HBsAg group. [a] P<0.05: Compared with HBV-M2 (44.8±10.91), clinical type of asymptomatic carrier (ASC) (43.8±10.87) and overall mean age (43.6±10.95) in high-level HBsAg group. The other serological patterns and clinical types were not compared due to small number of subjects; [b] P>0.05: Compared with HBsAg positive subjects and HBV DNA positive subjects (routine method and enrichment method) in gender distribution of high-level HBsAg group; [c] P<0.05: Compared with HBV-M1 subjects in high-level HBsAg group in mean age; [d] P>0.05: Compared with subjects with HBV-M1 or asymptomatic carrier (ASC) in the high-level HBsAg group in mean age; [e] P<0.05: Compared with high-level HBsAg group in HBsAg positive rate (181/21217) and HBV DNA positive rate (47/181, 73/181) (routine method and enrichment method).

major serological patterns were positively related to HBsAg in both groups (r=0.207–0.452), and the correlation coefficient was greater in the low-level HBsAg group (0.452). The HBV DNA level was positively related to the serum HBsAg in the low-level HBsAg group. In the high-level HBsAg group, HBV DNA in subjects with the three major serological patterns was related to HBV markers, but to different extents. Furthermore, the HBV DNA levels of subjects with the HBV-M1 or HBV-M3 pattern was negatively related to age (P<0.05) (Table 4).

Discussion

China is one of the major countries with high prevalence of hepatitis B virus (HBV) infection, which is mainly caused by perinatal or early childhood transmission [20]. Since the initiation of a nationwide HBV vaccination program for neonates was launched by National Health and Family Planning Commission of the Peoples' Republic of China in 1992, the prevalence of serum HBsAg was reduced to 2.1% among all children, and to

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

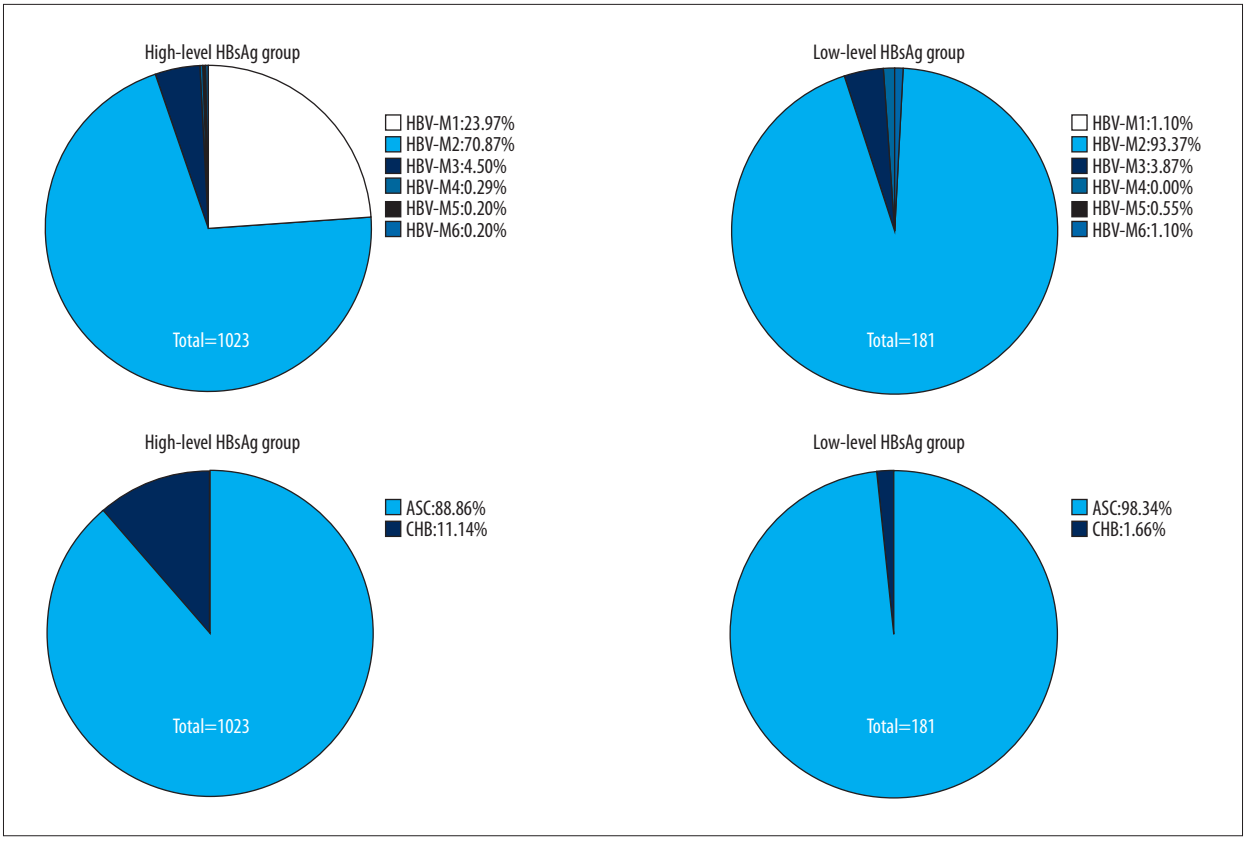

Figure 1. The proportion of hepatitis B virus (HBV) serological patterns and clinical types of HBV infection in high-level HBsAg group and low-level HBsAg group. The proportion of subjects with HBV-M2 or asymptomatic carrier (ASC) status: $P<0.05$ between the low-level HBsAg group *vs.* the high-level HBsAg group. HBV-M3, $\chi^2=0.145$, $P>0.05$. The other serological patterns and clinical types were not compared due to a small number of subjects.

1.0% among children born after 1999. Also, administration of universal HBV vaccination in infants has led to a dramatic decrease in HBV infection, with the prevalence of HBsAg declining from 9.75% in 1992 to 7.18% in 2006 [21]. The serum HBsAg positive rate in adults in China was 5.67% in 2006, and this low prevalence was due to universal HBV vaccination [21], wide application of clinical anti-viral therapy [22] and use of HBV infection detection methods with high sensitivity [1,23–25].

In the present study, we investigated 181 subjects with chronic HBV infection with a low level of serum HBsAg (HBsAg level <10 IU/ml) and their characteristics were compared with those from 1,023 subjects with chronic HBV infection with a high level of serum HBsAg (HBsAg level ≥10 IU/ml) in the same period. Subjects in the low-level HBsAg group accounted for 15.03% of HBsAg positive subjects (181/1204) and the male to female ratio was about 1.59: 1 (111: 70) which was similar to 1.89: 1 (669: 354) found in subjects in the high-level HBsAg group ($P>0.05$) (Tables 1, 2), However, the mean age (55.09±16.45 years) in the low-level HBsAg group was higher than in the high-level HBsAg group (43.63±10.95 years) (Table 2). The major serological pattern and clinical types were HBV-M2 (HBsAg and HBeAg and HBcAg-positive) (93.37%) and asymptomatic carriers (ASC) (98.34%), respectively, in the low-level HBsAg group unlike the high-level HBsAg group (Figure 1).

In subjects with low-level serum HBsAg showed a low replication of HBV DNA (Table 3) using the enrichment method, the median logarithm HBV DNA (0~4.86 IU/mL), with an HBV DNA positive rate of 40.33% (enrichment method, 73/181), which was lower than that in the high-level HBsAg group ($P<0.05$), and the detection rate of HBV DNA was comparable among different age groups in low-level HBsAg group ($P>0.05$) (Table 2, Figure 2D). However, the HBV DNA positive rate was negatively correlated with age in the high-level HBsAg group ($r=-0.981$, $P<0.05$) (Table 4, Figure 2C). In the low-level HBsAg subjects, the number of subjects in the advanced age group was greater (Figure 2B), while the number of subjects presented normal distribution in the high-level HBsAg group (Figure 2A). Correlation analysis showed that low serum HBsAg was positively correlated with low replication of HBV DNA ($r=0.452$) (Table 4). However, in the high-level HBsAg group, multiple clinical markers including age, HBsAg, HBeAg, Anti-HBe, Anti-HBc were related with HBV DNA level (Table 4).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

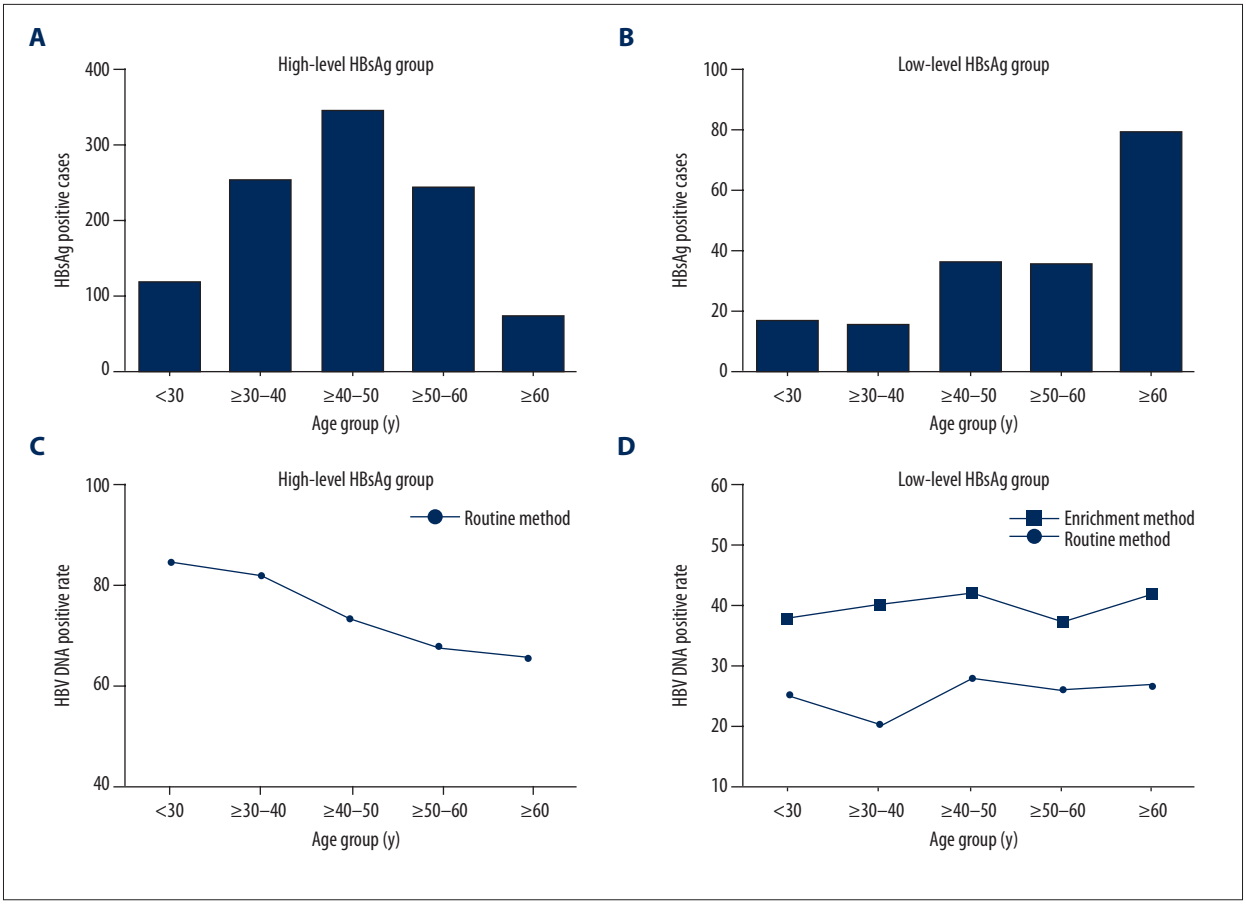

Figure 2. Distribution of HBsAg-positive subjects and hepatitis B virus (HBV) DNA positive rate in different age groups of high-level HBsAg subjects and low-level HBsAg subjects. In the high-level HBsAg group, the number of HBsAg positive subjects was greatest in the ≥40–50 year age group and a normal distribution was observed.(**A**); in the low-level HBsAg group, the number of HBsAg positive subjects was greatest in the ≥60 years group, and skewed distribution was found (**B**); In high-level HBsAg group, the number of HBV DNA positive subjects was significantly different among different age groups ($P<0.05$) and was negatively related to age (**C**), $r=-0.981$, $P<0.05$); in low-level HBsAg group, the number of HBV DNA positive subjects (routine method and enrichment method) was similar among different age groups (**D**), $P>0.05$), and the number of HBV DNA positive subjects determined with the routine method was less than that determined with enrichment method ($P<0.05$).

A previously reported study by our group showed that low-level HBsAg was not correlated with a low replication of HBV DNA [6], a finding that may be explained by the previous use of an immunoassay method with low sensitivity that was previously used for the detection of HBsAg, and a polymerase chain reaction (PCR) with a lower sensitivity to low HBV DNA levels (<1000 copies/ml) [26]. With the development of detection techniques for HBV markers and HBV DNA, the detection and precise quantification become feasible for serum samples with low-levels of HBsAg (<0.05 IU/mL) and low replication of HBV DNA (extraction of nucleic acids with immune beads: <30IU/mL) [17,18,27,28]. In the present study, a routine method and an enrichment method were employed to extract and detect HBV DNA in the low-level HBsAg group, and the positive rate was 25.97 (47/181) and 40.33% (73/181), respectively, which was greater than previously reported by the use of the direct method (10.3%) and the concentration method (34.6%). Constantly improving methods to evaluate subjects with low serum levels of HBsAg will reveal more clinical, epidemiological, and information.

Subjects with low-level HBsAg can be classified into HBsAg/anti-HBe/anti-HBc positive subjects (HBV-M2) according to the serological pattern, or to the asymptomatic carrier (ASC) status group, which may represent a non-active phase of HBV infection [19]. This study investigated the HBsAg level, serological pattern of HBV infection, clinical type of HBV infection, gender and age of the subjects with a low serum level of HBsAg: the male: female ratio of the ASC group was 1.54: 1 (108: 70) and 1.81 (585: 324) in the high-level HBsAg group and the low-level HBsAg group, respectively. There was no significant difference for gender ($P>0.05$). However, there was a significant

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The results of hepatitis B virus (HBV) DNA and HBV markers for major serological patterns and clinical types in the low-level HBsAg group and the high-level HBsAg group.

Group	HBV DNA (Log_{10} IU/mL)	HBsAg (IU/mL)	Anti-HBs (mIU/mL)	HBeAg (S/N)	Anti-HBe (S/N)	Anti-HBc (S/N)
High-level HBsAg (n=1,023)*	3.21 (0–9.77)	1302.00 (10.93–497000)	0.20 (0–14.13)	0.41 (0.17–2124.12)	0.01 (0.01–88.80)	12.80 ±2.51
HBV-M1 (n=245)[a]	6.72±2.02	5598.20 (22.08–497000)	0.06 (0.00–9.88)	612.82±591.01	32.16±23.86	11.51±2.35
HBV-M2 (n=725)[b]	2.81 (0.00–8.91)	738.00 (9.03–57775.00)	0.27 (0.00~7.94)	0.38±0.10	0.01 (0.01–0.97)	13.41±2.30
HBV-M3 (n=46)[b]	0.00 (0.00–3.68)	621.00 (12.14–6505.05)	0.00 (0.00–2.05)	0.65±0.24	1.50±0.26	10.27±2.33
ASC (n=909)[b]	3.05 (0–9.77)	1064.88 (10.93–497000)	0.21 (0–13.73)	0.41 (0.17–2124.12)	0.01 (0.01–88.80)	12.83±2.50
CHB (n=114)[a]	6.23±2.17	3957.50 (9.31–457240.00)	0.06 (0.00–14.13)	0.47 (0.19–1812.14)	0.15 (0.01–78.90)	12.57±2.53
Low-level HBsAg (n=181)[#]	0 (0~6.38)	1.47 (0.05–9.99)	0.24 (0–15.30)	0.33 (0.21~200.66)	0.01 (0.01~10.80)	11.32±2.17
HBV-M2 (n=169)[b]	0.00 (0.00~4.81)	1.37 (0.05~9.99)	0.23 (0–8.90)	0.33±0.04	0.01 (0.01~9.92)	11.49±1.77
ASC (n=178)[b]	0 (0~4.86)	1.44 (0.05~9.99)	0.24 (0–15.30)	0.33±0.04	0.01 (0.01~2.17)	11.33±2.18

[a] Results from all serum samples with hepatitis B virus (HBV) DNA ≥30 IU/mL; [b] Results from serum samples with HBV DNA <30 IU/mL and those with HBV DNA ≥30 IU/mL (0 IU/mL was used when HBV DNA was <30 IU/mL; the actual HBV DNA was used when HBV DNA was ≥30 IU/mL); * Detection of HBV DNA with routine method, other serological patterns (HBV-M4, HBV-M5, HBV-M6) were not analyzed due to small case numbers; [#] Detection of HBV DNA with an enrichment method, other serological patterns (HBV-M1, HBV-M3, HBV-M4, HBV-M5, HBV-M6) and chronic hepatitis B (CHB) were not analyzed due to small case numbers.

Table 4. Comparisons between the low-level HBsAg group and the high-level HBsAg group for the finding of hepatitis B virus (HBV) DNA with age and HBV markers for major serological patterns (r, P).

Group	Age (yrs)	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
High-level HBsAg[#]						
HBV-M1 (n=245)	–0.242, 0.000	0.226, 0.000	0.020,0.757	0.404, 0.000	0.488, 0.000	0.124, 0.053
HBV-M2 (n=725)	–0.001, 0.981	0.207, 0.000	0.066,0.077	0.077, 0.038	0.061, 0.098	0.194, 0.000
HBV-M3 (n=46)	–0.304, 0.040	0.370, 0.011	0.252,0.092	0.223, 0.136	0.288, 0.052	0.103, 0.497
Low-level HBsAg*						
HBV-M2 (n=169)	0.111, 0.150	0.452, 0.000	0.081, 0.298	0.012, 0.872	0.024, 0.760	0.096, 0.213

[#] HBV-M4, HBV-M5, HBV-M6 were not analyzed due to small case numbers. * Detection of HBV DNA with enrichment method, HBV-M1, HBV-M3, HBV-M4, HBV-M5, HBV-M6 were not analyzed due to small case numbers.

difference for age (43.84 ± 10.87 *vs.* 55.09±16.45, P<0.05). Among 178 subjects in the low-level HBsAg group, the main serum mode was HBV-M2 (94.4%, 168/178), while among the 909 subjects in high-level HBsAg group, the main serum modes were HBV-M1 (22.6%, 205/909), HBV-M2 (72.8%, 662/909), and HBV-M3 (4.6%, 42/909). There were significant differences in the serum mode distribution, HBsAg level, HBsAg frequency distribution in different age subgroups, HBV DNA positive rate, and HBV DNA positive rate distribution in different age subgroups, HBV DNA load, and correlation of HBV DNA with age and some HBV markers (P<0.05).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

There is evidence showing that low serum levels of HBsAg are related to the natural clearance of HBV in subjects with chronic HBV infection [29,30]. The findings of this study combined with those of our previous studies have shown other potential reasons for the low serum level of HBsAg. Most subjects with HBV infection, in the non-active phase, or subjects with asymptomatic HBV infection, have a low serum level of HBsAg [6,7], which may be attributed to the HBV *S*-gene mutations or variation. The alteration of the whole genome in HBV affects HBsAg expression, leading to low serum levels of HBsAg, possible due to an altered immune response that is unable to remove completely HBsAg and its immune complex after HBV infection, leading to a sustained low-level HBsAg, which induces a certain degree of immune tolerance to HBsAg.

Conclusions

The findings of this study showed that individuals with chronic hepatitis B virus (HBV) infection and sustained low-levels of HBsAg were an older population and had a lower level of replicating HBV DNA when compared with individuals with high levels of HBsAg, and the majority (93.7%) were also HBsAg and HBeAg and HBcAg-positive. The findings of this study have shown that individuals with chronic HBV infection with sustained low serum levels of HBsAg have special clinical characteristics, having mainly asymptomatic carrier (ASC) status, with HBV DNA that was slowly replicating and positively related to the serum HBsAg level. Although further studies are required to confirm these findings, the results of this study are clinically and epidemiologically important and suggest that improving immune tolerance in cases of chronic HBV infection may prevent HBV transmission.

Acknowledgements

The authors thank Mr. Qiang-Lin Duan for his editorial assistance and revision of the manuscript.

Conflict of interest

None.

References:

1. Chen Y, Wu W: Determination of low level HBsAg in serum by microparticle enzyme immunoassay. Hepatobiliary Pancreat Dis Int, 2002; 1: 262–64
2. Courouce AM, Drouet J, LeMarrec N et al: Blood donors positive for HBsAg and negative for anti-HBc antibody. Vox Sang, 1985; 49: 26–33
3. Fei CR, Ye AQ, Zhang J: [Evaluation of different methods in determination of low level HBsAg]. Zhejiang Da Xue Xue Bao Yi Xue Ban, 2011; 40: 436–39 [in Chinese]
4. Raafat A, Yates P, Sellers F et al: Benefit of dynamic over static incubation in the detection of a low-level HBsAg (chronic carrier) bone donor. Vox Sang, 1998; 74: 56–58
5. Satoh K, Iwata-Takakura A, Yoshikawa A et al: A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: An application of the method to the detection of occult HBV infection. Vox Sang, 2008; 95: 174–80
6. Cheng J, Sun CG, Chen Y et al: Molecular analysis on chronic hepatitis B patients with low-level HBsAg. Chin J Lab Med, 2009; 32: 1128–32
7. Cheng J, Sun CG, Chen Y et al: Analysis on clinical features and immunity in chronic hepatitis B virus infected patients with low-level HBsAg. African Journal of Microbiology Research, 2010; 4: 547–50
8. Jeffery-Smith A, Hubb J, Oliver A, Tong CY: An apparent low level of hepatitis B surface antigen (HBsAg) in the presence of significant viral replication. J Clin Virol, 2016; 77: 111–14
9. De Gascun CF, Fraher M, Crean M et al: The importance of being earnest: Following up a low level hepatitis B surface antigen (HBsAg) result. J Clin Virol, 2010; 49: 79–81
10. Huang G, Lau WY, Zhou WP et al: Prediction of hepatocellular carcinoma recurrence in patients with low hepatitis B virus DNA levels and high preoperative hepatitis B surface antigen levels. JAMA Surg, 2014; 149: 519–27
11. Martinot-Peignoux M, Lapalus M, Asselah T, Marcellin P: HBsAg quantification: Useful for monitoring natural history and treatment outcome. Liver Int, 2014; 34(Suppl.) 1: 97–107
12. Ozdil B, Cosar AM, Akkiz H et al: Negative correlation between viral load and HBsAg levels in chronic HBV-infected patients. Arch Virol, 2009; 154: 1451–55
13. Tseng TC, Liu CJ, Yang HC et al: Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology, 2013; 57: 441–50
14. Yang XH, Shi XF: [Significance of HBsAg quantification in guiding clinical treatment of chronic hepatitis B]. Zhonghua Gan Zang Bing Za Zhi, 2016; 24: 317–20
15. Li JM: Attention to the confirmative tests of weak reactive samples should be paid in serological testing for infectious diseases. Chin J Lab Med, 2006; 29: 577–80
16. Li MH, Xie Y, Qiu GH et al: [The relationship of serum HBsAg, HBeAg concentration and HBV-DNA load in chronic hepatitis B during IFN treatment]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi, 2011; 25: 26–28 [in Chinese]
17. Liu X, Chen JM, Lou JL et al: Correlation between hepatitis B virus DNA levels and diagnostic tests for HBsAg, HBeAg, and PreS1-Ag in chronic hepatitis B. Genet Mol Res, 2016; 15: 15028282
18. Zhu HY, Zhang XS: Relationship between HBV DNA load and levels of serum HBsAg in patients with chronic hepatitis B. Eur Rev Med Pharmacol Sci, 2016; 20: 2061–64
19. Hou JL, Lai W: [The guideline of prevention and treatment for chronic hepatitis B: A 2015 update]. Zhonghua Gan Zang Bing Za Zhi, 2015; 23: 888–905 [in Chinese]
20. Cui Y, Jia J: Update on epidemiology of hepatitis B and C in China. J Gastroenterol Hepatol, 2013; 28(Suppl. 1): 7–10
21. Liang X, Bi S, Yang W et al: Evaluation of the impact of hepatitis B vaccination among children born during 1992–2005 in China. J Infect Dis, 2009; 200: 39–47
22. Goyal A, Murray JM: The impact of vaccination and antiviral therapy on hepatitis B and hepatitis D epidemiology. PLoS One, 2014; 9: e110143
23. Chen Y, Wu W, Li LJ et al: Comparison of the results for three automated immunoassay systems in determining serum HBV markers. Clin Chim Acta, 2006; 372: 129–33
24. Krawczyk A, Hintze C, Ackermann J et al: Clinical performance of the novel DiaSorin LIAISON((R)) XL murex: HBsAg Quant, HCV-Ab, HIV-Ab/Ag assays. J Clin Virol, 2014; 59: 44–49
25. Louisirirotchanakul S, Khupulsup K, Akraekthalin S et al: Comparison of the technical and clinical performance of the Elecsys HBsAg II assay with the Architect, AxSym, and Advia Centaur HBsAg screening assays. J Med Virol, 2010; 82: 755–62
26. Karagoz E, Tanoglu A, Turhan V: Correlation between hepatitis B surface antigen titers and HBV DNA levels: What about the parameters affecting this correlation? Saudi J Gastroenterol, 2014; 20: 74

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

27. Karagoz E, Selek MB, Tanoglu A et al: Comparison of the Elecsys HBsAg II Assay and the Architect Assay for Quantification of hepatitis B surface antigen in patients with chronic hepatitis B. Infez Med, 2016; 24: 287–92
28. Karra VK, Chowdhury SJ, Ruttala R et al: Clinical significance of quantitative HBsAg titres and its correlation with HBV DNA levels in the natural history of hepatitis B virus infection. J Clin Exp Hepatol, 2016; 6: 209–15
29. Kim JH, Lee JH, Park SJ et al: Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: A prospective follow-up study. Hepatogastroenterology, 2008; 55: 578–81
30. Yuen MF, Wong DK, Sablon E et al: HBsAg seroclearance in chronic hepatitis B in the Chinese: virological, histological, and clinical aspects. Hepatology, 2004; 39: 1694–701

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]