ORIGINAL ARTICLE

Gut and Liver, Vol. 5, No. 2, June 2011, pp. 210-216

Distribution Characteristics of Hepatitis B Serological Markers in Hospitalized Children and Adolescents in Zhejiang, China between 2006 and 2010

Xuejun Chen*, Yuefang Shen†, and Wenqing Xiang*

*Departments of *Clinical Laboratory and † Clinical Pharmacy, The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China*

Background/Aims: To investigate serological patterns of hepatitis B based on electrochemiluminescent immunoassays and the distribution characteristics of these patterns in hospitalized children and adolescents in Zhejiang, China between 2006 and 2010. **Methods:** Five serological markers, including hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B e antigen (anti-HBe), and antibody to hepatitis B c antigen (anti-HBc), were chosen as a routine panel to monitor hepatitis B virus (HBV) infection and vaccination efficacy. A total of 33,187 children (21,187 boys and 12,000 girls) were selected using the following exclusion criteria: a previous diagnosis of hepatitis, age >16 years or an address outside of Zhejiang. **Results:** The average HBV vaccination coverage rates among 20,766 boys and 11,782 girls were 98.62% and 98.68%, respectively. Seventeen serological patterns of hepatitis B were found, and the dominant pattern was 'anti-HBs (+) alone' (62.03%) followed by 'negative pattern' (23.46%). The rates of the other 15 patterns ranged from 8.14% to 0.003%. Of 236 HBsAg-positive patients, the overall rate of seropositivity was 0.71%. The anti-HBs levels were grouped into 3 ranges (10-100 mIU/mL, 100-1,000 mIU/mL, and >1,000 mIU/mL) for all anti-HBs-positive children (36.08%, 43.43%, and 20.49%, respectively). **Conclusions:** A low HBsAg carrier rate and a relatively high anti-HBs positive rate are present in hospitalized children and adolescents in Zhejiang. The distribution of serological patterns is associated with age but is mostly independent of gender. **(Gut Liver 2011;5:210-216)**

Key Words: Hepatitis B antibodies; Chemiluminescent measurements; Immunoassay; Epidemiologic studies; Children

INTRODUCTION

Hepatitis B virus (HBV) infection is a public health problem and classification of an HBV infection requires the identification of several serologic markers.[1] There are five serological markers including hepatitis B s antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B e antigen (anti-HBe) and antibody to hepatitis B total c antigen (anti-HBc), which are valuable for the diagnosis and monitoring of hepatitis B infection and vaccination efficacy. Detection of serological markers has evolved from a cumbersome and time-consuming procedure by manual radioimmunoassay or enzyme-linked immunosorbent assay (ELISA) to procedures with systems that partially or fully automate the process. Electrochemiluminescence immunoassay (ECLIA) is a new quantitatively analysis technique with improved both sensitivity and measurement ranges, shortened reaction period (approximately 18 minutes) and random-access capabilities for specimen, has been gradually applied to detect these antibodies and antigens.[2-4]

The prevalence of HBV infection and HBsAg carrier rates vary with the particular population samples, ethnic groups studied and the detection methods used. China has had one of the highest rates of HBV endemicity in the world. Since a HBV vaccination program was implemented in 1992, the incidence of acute HBV infection in children has decreased dramatically. The HBsAg carrier rate decreased from 9.67% in 1992 to the current 0.96% in children 1 to 4 years old and to 2.42% in children 5 to 14 years old according to a serosurvey based on ELISA in 2006.[5] The hospital children and adolescents are special populations, the present seroprevalence of HBV infection (especially since 2006) among these based on ECLIA in China are still not well

Correspondence to: Yuefang Shen
Department of Clinical Pharmacy, The Children's Hospital of Zhejiang University School of Medicine, 57 Zhugan Lane, Yanan Road, Hangzhou 310003, China
Tel: +86-571-87061007 (Ext. 32451), Fax: +86-571-87020367, E-mail: webcxj@163.com
Received on September 5, 2010. Accepted on December 27, 2010.
pISSN 1976-2283 eISSN 2005-1212 DOI: 10.5009/gnl.2011.5.2.210

understood. In this study, we aimed to investigate and understand the present prevalence of hepatitis B infection and vaccination efficacy in Zhejiang children and adolescents based on the children's hospital data during 2006 to 2010, and indirectly evaluate the effect of mass HBV vaccination program locally since 18 years after was launched.

MATERIALS AND METHODS

1. Screening criterion and subjects

This study is a retrospective analysis based on common laboratory information system (LIS) data and no extra sera or tests were further required for the subjects. The investigation was approved by the Ethical Committee of Zhejiang University.

The Children's Hospital of Zhejiang University is the only comprehensive and teaching hospital in Zhejiang Province. It has 850 beds which mainly serves local pediatric patients from Zhejiang Province. No hepatitis clinic and wards are available in this hospital, so no congregation of HBV infection individuals who will artificially cause elevated HBV seroprevalence. HBV serological screening panel including five markers was routinely employed for most outpatients and inpatients in this hospital, not only specifically for patients who have suspected liver problems.

A total of 33,187 subjects were selected from LIS database during July 2006 to March 2010 according to the exclusion criterion consisting of non-first-time hepatitis results, >16 years older and whose living addresses are not in Zhejiang. Of 33,187 patients, 21,187 boys and 12,000 girls, age ranged from 0 to 16 years old. Of 33,187 subjects, 962 were outpatients, 32,225 were inpatients of whom 1,424 were from the Department of Intensive Care Unit, 574 Neonatology, 1,333 Hematology/Oncology, 7,642 General Surgery/Eye, Nose and Throat (ENT) Surgery, 2,918 Cardiothoracic Surgery, 2,947 Urology Surgery/Oncology Surgery, 3,581 Orthopedics/Neurosurgery/Burn and Plastic Surgery, 1,801 Gastroenterology, 543 Neurology, 1,465 Cardiology, 2,997 Nephrology/Ophthalmology, 2,628 Endocrinology, and 2,372 Respiratory Medicine. Location distribution among all children and adolescents according to their living addresses were as follows: Hangzhou City (46.00%), Jinhua City (12.69%), Shaoxing City (10.03%), Jiaxing City (7.82%), Taizhou City (5.79%), Ningbao City (4.85%), Huzhou City (4.17%), Wenzhou City (3.21%), Quzhou city (2.94%), Lishui City (1.94%), and Zhoushan City (0.56%). The constituent ratios of gender and age in entire subjects were shown in Table 1.

2. Detection of HBV serological markers

A routine test panel consisting of five serological markers was employed to monitor HBV infection or vaccination efficacy for patients in our hospital. If HBsAg, HBeAg, or anti-HBc was

Table 1. Constituent Ratios of Gender, Age, and HBV Vaccination Coverage Rates in 33,187 Children

Age	Total no.	Boys (constitution)		Girls (constitution)		Boys (vaccination)				Girls (vaccination)			
		No.	%	No.	%	Unk*	Vac	Unv	%†	Unk*	Vac	Unv	%†
0-1M	1,567	997	63.62	570	36.38	204	787	6	99.40	112	455	3	99.34
1M-1Y	8,352	5,215	62.44	3,137	37.56	37	5,148	30	99.42	18	3,106	13	99.58
1-2Y	4,665	3,090	66.24	1,575	33.76	12	3,052	26	99.16	5	1,556	14	99.11
2-3Y	2,675	1,677	62.69	998	37.31	12	1,650	15	99.10	8	981	9	99.09
3-4Y	2,141	1,335	62.35	806	37.65	11	1,311	13	99.02	2	797	7	99.13
4-5Y	1,938	1,201	61.97	737	38.03	15	1,174	12	98.99	8	723	6	99.18
5-6Y	1,816	1,164	64.10	652	35.90	11	1,140	13	98.87	7	634	11	98.29
6-7Y	1,737	1,128	64.94	609	35.06	13	1,103	12	98.92	6	593	10	98.34
7-8Y	1,469	918	62.49	551	37.51	14	893	11	98.78	4	540	7	98.72
8-9Y	1,423	866	60.86	557	39.14	21	825	20	97.63	6	539	12	97.82
9-10Y	1,208	728	60.26	480	39.74	17	692	19	97.33	11	455	14	97.01
10-11Y	1,255	814	64.86	441	35.14	17	769	28	96.49	10	418	13	96.98
11-12Y	1,141	768	67.31	373	32.69	11	733	24	96.83	7	354	12	96.72
12-13Y	841	603	71.70	238	28.30	14	571	18	96.94	8	222	8	96.52
13-14Y	522	396	75.86	126	24.14	6	367	23	94.10	4	116	6	95.08
14-15Y	261	177	67.82	84	32.18	4	160	13	92.49	1	77	6	92.77
15-16Y	176	110	62.50	66	37.50	2	101	7	93.52	1	61	4	93.85
Total	33,187	21,187	63.85	12,000	36.16	421	20,476	290	98.62	218	11,627	155	98.68

M, month; Y, year; Unk, unknown; Vac, vaccinated; Unv, unvaccinated.
*Subjects (421 boys and 218 girls) with unknown hepatitis B virus (HBV) vaccination; †The vaccinated numbers divided by sum of the vaccinated and unvaccinated numbers.

positive for any subjects, anti-HBc (IgM) would be tested further. Around 2 to 3 mL of venous blood for each subject was taken into vacuum-tube (BD, Franklin Lakes, NJ, USA) with coagulant, rested for at least half an hour at room temperature, then centrifuged for 3 minutes at 3,000 r/min to collect the sera. Five serological markers were detected on e601 analyzer (Roche Diagnostics, Mannheim, Germany) according to Roche's protocols by electrochemiluminescence immunoassay (ECLIA), where 'sandwich principle' for HBsAg, HBeAg, and anti-HBs, and 'competition principle' for anti-HBe and anti-HBc. The results for all samples reactive for HBsAg were confirmed by the confirmatory assays of Roche e601. All reagent kits, including calibrators and controls, were purchased from Roche Company and strictly used before the expiration date. Reference intervals for five serological markers were as follows: HBsAg <1 COI (cutoff index), HBeAg <1 COI, HBsAb <10 mIU/mL, HBeAb >1 COI, and HBcAb >1 COI.

3. Statistical analysis

Original data was sorted out by EXCEL 2003 (Microsoft Co., Redmond, WA, USA) and Statistical analyses were done by SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) software. Comparison of likelihood ratio was analyzed by using chi-square test and probability level less than 0.05 was considered statistically significant. For convenient and practical purpose of statistical analysis, anti-HBs concentration >1,000 mIU/mL was denoted 1,001 mIU/mL, <2 mIU/mL was denoted 1 mIU/mL. Additionally, age ≤29 days referred to <1M, 30 days to 12 months referred to 1M-1Y, by analogy in this work (D, day; M, month, Y: year).

RESULTS

1. HBV vaccination coverage rates

Brief HBV vaccination history is one of the routine contents in a case record for every patient in this hospital, but detailed three-dose HBV vaccination is not recorded. A total of 31,548 children were selected for the analysis of HBV vaccination coverage rates except 639 children (mostly neonates) with unknown HBV vaccination. Average HBV vaccination coverage rates among 20,766 boys and 11,782 girls were 98.62% and 98.68%, respectively (Table 1).

2. Seventeen serological patterns of hepatitis B

Seventeen serological patterns of hepatitis B were found out of 33,187 pediatric patients. Entire patterns and their distribution among these subjects were shown in Table 2. Four high-

Table 2. Distribution Characteristics of 17 Serological Patterns of Hepatitis B as Determined by ECLIA Assays in 33,187 Children

Sequence no.	Pattern*	Total		Boys		Girls		χ^2	p-value
		No.	%	No.	%	No.	%		
1	- + - - -	20,587	62.033	13,150	62.066	7,437	61.975	0.027	0.869
2	- - - - -	7,785	23.458	5,008	23.637	2,777	23.142	1.047	0.312
3	- + - - +	2,702	8.142	1,696	8.005	1,006	8.383	1.467	0.226
4	- + - + +	1,222	3.682	750	3.540	472	3.933	3.343	0.068
5	- - - - +	283	0.853	196	0.925	87	0.725	3.628	0.057
6	- + - + -	198	0.597	103	0.486	95	0.792	12.058	0.001
7	+ - + - +	158	0.476	117	0.552	41	0.342	7.168	0.007
8	- - - + +	104	0.313	69	0.326	35	0.292	0.284	0.594
9	- - - + -	56	0.169	34	0.160	22	0.183	0.238	0.626
10	+ - - + +	38	0.115	31	0.146	7	0.058	5.185	0.023
11	+ - + - -	25	0.075	18	0.085	7	0.058	0.721	0.533
12	- + + - -	13	0.039	8	0.038	5	0.042	0.000†	1.000
13	+ + + - -	5	0.015	3	0.014	2	0.017	0.000†	1.000
14	+ - - - -	4	0.012	2	0.009	2	0.017	0.003†	0.955
15	+ - - - +	4	0.012	1	0.005	3	0.025	1.202†	0.273
16	+ + - - -	2	0.006	1	0.005	1	0.008	0.000†	1.000
17	- - + - +	1	0.003	0	0.000	1	0.003	0.083†	0.773
Total		33,187	100	21,187	100	12,000	100		

All percentages in this table were reserved three decimal places but rounded to two decimal places when cited in the paper.
ECLIA, electrochemiluminescence immunoassay.
*Display sequence of five markers in a hepatitis B virus (HBV) screening panel: hepatitis B s antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe), and antibody to hepatitis B total c antigen (anti-HBc), respectively. +: positive, -: negative; †Corrected χ^2 value.

frequency patterns from pattern one to four were 'anti-HBs (+) alone' (62.03%), 'negative pattern' (23.46%), 'anti-HBc (+) anti-HBs (+)' (8.14%) and 'anti-HBe (+) anti-HBc (+) anti-HBs (+)' (3.68%). The positive rates of other thirteen low-frequency patterns from pattern five to seventeen varied from 0.003% to 0.86% and no HBcAb-IgM was found in any of subjects. Except for 'negative pattern', 'HBsAg (+) HBeAg (+) anti-HBc (+)', 'HBsAg (+) anti-HBe (+) anti-HBc (+)' and 'anti-HBe (+) anti-HBs (+)', no significant differences were found between the distribution ratios and corresponding gender groups by chi-square test ($p>0.05$).

3. Anti-HBs (+), 'negative' and HBsAg (+)

The detailed distribution and trend in three patterns of anti-HBs (+), negative and HBsAg (+) were shown in Table 3 and Fig. 1. A total of 260 subjects had HBsAg positive from 7 patterns (pattern 7, 10, 11, 13, 14, 15, 16) and the overall rate of seropositivity was relatively low (0.71%). The positive rate of HBsAg slowly went up with age, from 0.13% to 0.88% in the aged of ≤ 8 years and from 1.16% to 3.83% in the age of > 8 years, lowest in the age of <1M and highest in the age of 13 to14 years.

Of 24,730 anti-HBs positive subjects, 20,587 were 'anti-HBs (+) alone' (85.259%), and the highest positive rate was 92.35% in the age of 1 to 2 years, followed by 81.05% in the age of 2 to 3 years, while the lowest positive rate was 44.06% in the age of 14 to 15 years.

Of 7,785 'negative pattern' subjects, the lowest negative rate was 7.37% in the age of 1 day to 2 years, while highest negative rate was 51.34% in the age of 14 to 15 years. A trend of correspondingly reverse change between rates of 'anti-HBs (+)' and 'negative pattern' with age was found. Positive rate of anti-HBs increased with age after birth, peaked at 2 years of age, declined slowly up to 5 years of age and remained stationary up

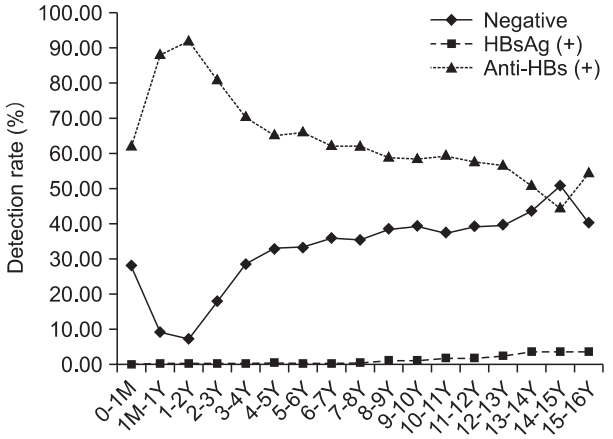

Fig. 1. Trends of the three hepatitis B virus patterns with respect to age.
M, month; Y, year; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to hepatitis B surface antigen.

Table 3. Distribution Characteristics of 'Anti-HBs (+)', 'Negative' and 'HBsAg (+)' Patterns among Children and Adolescents in Different Age Groups

Age	Total no.	Total anti-HBs (+)		Total HBsAg (+)		Negative	
		No.	%	No.	%	No.	%
0-1M	1,567	977	62.35	2	0.13	443	28.27
1M-1Y	8,352	7,372	88.26	19	0.23	778	9.31
1-2Y	4,665	4,308	92.35	16	0.34	344	7.37
2-3Y	2,675	2,168	81.05	10	0.37	488	18.24
3-4Y	2,141	1,509	70.48	12	0.56	612	28.58
4-5Y	1,938	1,271	65.58	17	0.88	642	33.13
5-6Y	1,816	1,202	66.19	8	0.44	602	33.15
6-7Y	1,737	1083	62.35	10	0.58	629	36.21
7-8Y	1,469	919	62.56	9	0.61	523	35.60
8-9Y	1,423	846	59.45	18	1.26	552	38.79
9-10Y	1,208	713	59.02	14	1.16	477	39.49
10-11Y	1,255	747	59.52	23	1.83	472	37.61
11-12Y	1,141	658	57.67	20	1.75	453	39.70
12-13Y	841	478	56.84	23	2.73	336	39.95
13-14Y	522	268	51.34	20	3.83	229	43.87
14-15Y	261	115	44.06	9	3.45	134	51.34
15-16Y	176	96	54.55	6	3.41	71	40.34
Total	33,187	24,730	74.52	236	0.71	7,785	23.46

M, month; Y, years; anti-HBs, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen.

Table 4. Distribution Characteristics of 24,730 Children and Adolescents with Total Anti-HBs Positive Markers in the Three Anti-HBs Concentration Ranges

Age	Total no.	10-100 mIU/mL		100-1,000 mIU/mL		>1,000 mIU/mL	
		No.	%	No.	%	No.	%
0-1M	977	265	27.12	531	54.35	181	18.53
1M-1Y	7,372	1,524	20.67	3,568	48.40	2,280	30.93
1-2Y	4,308	1,127	26.16	2385	55.36	796	18.48
2-3Y	2,168	1,081	49.86	919	42.39	168	7.75
3-4Y	1,509	874	57.92	502	33.27	133	8.81
4-5Y	1,271	718	56.49	377	29.66	176	13.85
5-6Y	1,202	623	51.83	367	30.53	212	17.64
6-7Y	1,083	480	44.32	327	30.19	276	25.48
7-8Y	919	391	42.55	315	34.28	213	23.18
8-9Y	846	329	38.89	330	39.01	187	22.10
9-10Y	713	305	42.78	268	37.59	140	19.64
10-11Y	747	354	47.39	285	38.15	108	14.46
11-12Y	658	322	48.94	255	38.75	81	12.31
12-13Y	478	250	52.30	157	32.85	71	14.85
13-14Y	268	163	60.82	85	31.72	20	7.46
14-15Y	115	61	53.04	42	36.52	12	10.43
15-16Y	96	55	57.29	29	30.21	12	12.50
Total	24,730	8,922	36.08	10,742	43.44	5,066	20.49

M, month; Y, years; anti-HBs, antibody to hepatitis B surface antigen.

to 15-18 years of age. While rate of 'negative pattern' decreased after birth, dropped to the nadir at 2 years of age, then went up slowly up to 5 years of age and remained stationary up to 15-18 years of age.

4. Distribution of anti-HBs concentrations among age groups

Detailed distribution characters of 24,730 children among three anti-HBs levels and age were shown in Table 4.

DISCUSSION

Hospital children and adolescents are special populations, whereas, the present seroprevalence of HBV infection and vaccination efficacy (especially since the nationwide serosurvey in 2006) among these populations based on ECLIA in China are still not well understood. In this study, seventeen serological patterns of hepatitis B were found among 33,187 pediatric patients, where the dominant pattern was 'anti-HBs (+) alone' (62.03%), followed by 'negative pattern' (23.46%), 'anti-HBc (+) anti-HBs (+)' (8.14%) and 'anti-HBe (+) anti-HBc (+) anti-HBs (+)' (3.68%). The positive rates of other thirteen low-frequency patterns varied from 0.003% to 0.86%. No significant differences were found between the distribution ratios and corresponding gender groups in most patterns by chi-square test.

Classification of an HBV infection requires the identification of several serologic markers or patterns which imply the different clinical significance. Interpretation of various serological profiles of hepatitis B has been described in some studies.[6-8] Pattern 1 is usually suggestive of successful hepatitis B vaccination where anti-HBs alone is present and pattern 3 indicates natural immunization due to the presence of anti-HBs antibodies along with anti-HBc (IgG). Pattern 2 shows individuals are free of HBV infection but are susceptible to HBV. Pattern 7, 10, 11, 13, 14, 15, and 16 mean active infection of HBV while pattern 3, 4, 6, 8, and 9 mean past infection of HBV. Pattern 5 is 'anti-HBc alone' which means probably active infection. Alhababi *et al*.[9] found 'anti-HBc alone' is a common phenomenon in the clinical virology laboratory but only a small proportion of samples had detectable HBV DNA. Knöll *et al*.[10] also pointed out that no evidence was found that HBV alone causes severe liver damage in individuals with 'anti-HBc alone.' Interestingly, there were three rare patterns, pattern 12 'HBeAg (+) anti-HBs (+)', pattern 13 'HBsAg (+) HBeAg (+) anti-HBs (+)' and pattern 16 'HBsAg (+) anti-HBs (+)', because technically HBsAg or HBeAg, and anti-HBs will not simultaneously present in same individuals. Wang *et al*.[11] unveiled that HBeAg can cross the human placenta and disappears from serum within 6 months in most babies. Qiu *et al*. found in the Hangzhou cohort of 638 mothers, hepatitis B positive rate was 6.0%.[12] Our investigation showed

that 13 children with pattern 12 were below 3 months of age and all were born to HBeAg (+) mothers. We speculated these HBeAg in these children came transplacentally from their mothers' sera and anti-HBs antibodies probably were the efficacy of vaccination. Therefore, the present of HBeAg in certain children aged below 6 months may not mean real HBV infection, making it is necessary to further monitor them. The coexistence of HBsAg and anti-HBs even HBeAg in pattern 13 and 16 were found, in principle, HBsAg and anti-HBs simultaneously present in same individuals is also rarely seen. In 3 cases, all anti-HBs were in 10 to 100 mIU/mL range while HBsAg was elevated. The real reason for this phenomenon was unclear but may be associated with infection of different HBV subtypes, transient HBsAg positive (mostly found at 6 days after hepatitis B immunization) or false positivity due to measurement methods.[2,13-15]

The dominant patterns of HBV were further discussed below. HBsAg is the first marker to appear in patient serum, and the presence of this antigen indicates an ongoing infection with HBV and is detectable in both acutely ill patients and chronic carriers of HBV, thus the importance of accurate testing for this marker. In this study, a total of 236 children had HBsAg positive and the overall seroprevalence of HBsAg was 0.71%. The trend of overall HBsAg carrier rate were found, slowly went up with ages, lowest in the age of <1M and highest in the age of 13 to14 years. Our overall HBsAg carrier rate was slightly lower than those in children 1 to 4 years old (0.96%) and 5 to 14 years old (2.42%) from a nationwide serosurvey conducted in 2006,[5] and significantly lower than those in Chinese adults (around 7.0%) as well.[5,16] Compared with international studies, our overall HBsAg carrier rate was lower than those in 1,500 Libyan neonates (0.9%),[17] 229 healthy Indonesian children born during 1994-1999 (3.1%),[18] and 251 Nigerian children (12.4%).[19] Obviously, HBsAg seroprevalence rate in Zhejiang children has decreased dramatically. The maternal HBsAg situations of 236 HBsAg positive children were investigated, 35 unknown, 149 HBsAg positive and 52 HBsAg negative, which indicated vertical transmission of HBV from mother to child is a main infection route in children.

Either an HBV infection or HBV vaccination can cause the anti-HBs marker to be positive. It is an immune globulin secreted by B lymph cells that can combine with the HBsAg to neutralize it. Along with other immune reactions, the anti-HBs protective antibody can eradicate the invading HBV from the body. This antibody can exist in the blood for a long time, gradually decreasing with age. In this study, a total of 24,730 children were anti-HBs positive (74.54%) and 20,587 were 'anti-HBs (+) alone' (62.03%). Our anti-HBs positive rate was similar to those in Taiwan where the anti-HBs-seropositive rates were 83.1%, 53.0%, and 53.5% for participants who were born before 1984 when universal hepatitis B vaccination program for newborns was launched, 1984-1986 and after 1986. The higher the level of anti-HBs, the longer the duration of persistence of anti-body. The persistence of anti-HBs depends on the peak antibody level achieved after three doses.[14] In present study, three kinds of anti-HBs levels (10-100 mIU/mL, 100-1,000 mIU/mL, and >1,000 mIU/mL) in anti-HBS positive children were 36.08%, 43.43%, and 20.49%, respectively.

'Negative pattern' denotes individuals are free of HBV infection but is susceptible populations to HBV. A total of 7,785 children harbored 'negative pattern' (23.46%), the lowest negative rate was 7.37% in the age of 1 to 2 years while highest negative rate was 51.34% in the age of 14 to 15 years. The present of 'negative pattern' in local children were associated with unresponsiveness to HBsAg, absence or insufficiency of vaccination, natural disappearance of anti-HBs. Liang *et al*.[20] reported the efficacy of hepatitis B vaccination with a timely birth dose was 88.3% in Chinese children, while a study in Israel showed that 22.9% of local children were nonresponser.[21] Unresponsiveness to HBsAg has been attributed to a number of environmental and genetic factors, the most important ones being the haplotype of HLA antigens and immunological tolerance.[21,22] Liang *et al*.[20] also found HBV vaccine coverage (3 doses) has increased to 93.4% for Chinese children born in 2005. Zhang *et al*.[23] revealed The weighted complete HBV vaccine coverage was 99.66%, 89.95%, and 13.21% in the age group of 1 to 4 years old, 5 to 14 years old, and 15 to 59 years old in Shandong Province, respectively. In this study, average HBV vaccination coverage rates among 20,766 boys and 11,782 girls were 98.62% (92.49-99.42%) and 98.68% (92.77-99.58%), respectively. We believed the lacking or insufficiency of HBV vaccination in Zhejiang children exists. We suggested a HBV vaccination catch-up campaign should be conducted in the older children in Zhejiang and further efforts should be made to improve HBV vaccine coverage among the high risk population for enhancement of hepatitis B control.

It is well known that HBV vaccination is most efficient measure to prevent and decrease HBV infection around the world.[16,22,24-26] China has been one of the highest rates of HBV endemicity in the world, However, Since HBV vaccination program was implemented in 1992, the HBsAg carrier rate in general population dropped from 9.75% in 1992 to 7.18% in 2006 and reduced to 1.0% among children born after 1999.[16,20] Reduced HBsAg prevalence was strongly associated with vaccination among all age groups.[16] China has now fallen from high endemic area with HBsAg seroprevalence ≥8% into the intermediate endemic area where HBsAg prevalence in general population is 2-7%, according to the WHO's category of HBV endemicity. Zhejiang Province is relatively developed area in the eastern China where the healthcare system is much better and more efficient than other provinces. Our study demonstrated that the rates of relatively high anti-HBs-positive and low HBsAg seroprevalence were present in hospital children and adolescents, which was also indirectly showed that the HBV vaccination program was successful and valuable to prevent

and decrease HBV infection in Zhejiang.

ACKNOWLEDGEMENTS

Conflicts of interest: None to declare.

REFERENCES

1. Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV. Global epidemiology of hepatitis B virus. J Clin Gastroenterol 2004;38(10 Suppl 3):S158-S168.
2. McCartney RA, Harbour J, Roome AP, Caul EO. Comparison of enhanced chemiluminescence and microparticle enzyme immunoassay for the measurement of hepatitis B surface antibody. Vaccine 1993;11:941-945.
3. Diepersloot RJ, van Zantvliet-van Oostrom Y, Gleaves CA. Comparison of a chemiluminescent immunoassay with two microparticle enzyme immunoassays for detection of hepatitis B virus surface antigen. Clin Diagn Lab Immunol 2000;7:865-866.
4. Huh HJ, Chae SL, Cha YJ. Comparison study with enzyme immunoassay and chemiluminescence immunoassay for hepatitis B virus surface antigen detection. Korean J Lab Med 2007;27:355-359.
5. Lu FM, Zhuang H. Management of hepatitis B in China. Chin Med J (Engl) 2009;122:3-4.
6. Mushahwar IK, Dienstag JL, Polesky HF, McGrath LC, Decker RH, Overby LR. Interpretation of various serological profiles of hepatitis B virus infection. Am J Clin Pathol 1981;76:773-777.
7. Irshad M. Interpretation of serological markers of hepatitis viruses. Trop Gastroenterol 1995;16:4-10.
8. Mujeeb SA, Ansari N, Ahmed A. Serological markers of hepatitis B infection: a study to reduce cost and improve interpretation. Infect Dis J Pakistan 2007;16:17-19.
9. Alhababi F, Sallam TA, Tong CY. The significance of 'anti-HBc only' in the clinical virology laboratory. J Clin Virol 2003;27:162-169.
10. Knöll A, Hartmann A, Hamoshi H, Weislmaier K, Jilg W. Serological pattern "anti-HBc alone": characterization of 552 individuals and clinical significance. World J Gastroenterol 2006;12:1255-1260.
11. Wang Z, Zhang J, Yang H, et al. Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies. J Med Virol 2003;71:360-366.
12. Qiu L, Binns CW, Zhao Y, Zhang K, Xie X. Hepatitis B and breastfeeding in Hangzhou, Zhejiang Province, People's Republic of China. Breastfeed Med 2010;5:109-112.
13. Patterson W, Werness P, Payne WJ, et al. Random and continuous-access immunoassays with chemiluminescent detection by Access automated analyzer. Clin Chem 1994;40(11 Pt 1):2042-2045.
14. Hadler SC, Margolis HS. Hepatitis B immunization: vaccine types, efficacy, and indications for immunization. Curr Clin Top Infect Dis 1992;12:282-308.
15. Köksal N, Altinkaya N, Perk Y. Transient hepatitis B surface antigenemia after neonatal hepatitis B immunization. Acta Paediatr 1996;85:1501-1502.
16. Liang X, Bi S, Yang W, et al. Epidemiological serosurvey of hepatitis B in China: declining HBV prevalence due to hepatitis B vaccination. Vaccine 2009;27:6550-6557.
17. El-Magrahe H, Furarah AR, El-Figih K, El-Urshfany S, Ghenghesh KS. Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. J Infect Dev Ctries 2010;4:168-170.
18. Utsumi T, Yano Y, Lusida MI, et al. Serologic and molecular characteristics of hepatitis B virus among school children in East Java, Indonesia. Am J Trop Med Hyg 2010;83:189-193.
19. Alikor EA, Erhabor ON. Seroprevalence of hepatitis B surface antigenaemia in children in a tertiary health institution in the Niger Delta of Nigeria. Niger J Med 2007;16:326-329.
20. 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.
21. Gold Y, Somech R, Mandel D, Peled Y, Reif S. Decreased immune response to hepatitis B eight years after routine vaccination in Israel. Acta Paediatr 2003;92:1158-1162.
22. Poland GA, Jacobson RM. Clinical practice: prevention of hepatitis B with the hepatitis B vaccine. N Engl J Med 2004;351:2832-2838.
23. Zhang L, Xu AQ, Yan BY. Analysis on hepatitis B vaccine coverage among the population of 1-59 years old in Shandong province. Zhongguo Yi Miao He Mian Yi 2009;15:159-162.
24. Lu JJ, Cheng CC, Chou SM, Hor CB, Yang YC, Wang HL. Hepatitis B immunity in adolescents and necessity for boost vaccination: 23 years after nationwide hepatitis B virus vaccination program in Taiwan. Vaccine 2009;27:6613-6618.
25. Zhou YH, Wu C, Zhuang H. Vaccination against hepatitis B: the Chinese experience. Chin Med J (Engl) 2009;122:98-102.
26. Dong Y, Liu SL, Zhai XJ, et al. A serological and molecular survey of hepatitis B in children 15 years after inception of the national hepatitis B vaccination program in eastern China. J Med Virol 2009;81:1517-1524.