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Scientific Letter

Molecular Characteristics of Hepatitis B Virus among Children Vaccinated with HBsAg Positive in Huzhou, China

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To the editor.

Chronic liver diseases such as chronic hepatitis B, cirrhosis, and liver cancer caused by hepatitis B virus (HBV) infection pose a serious threat to public health. Mother-to-child transmission is one of the main routes of HBV dissemination.[1] So far, immunoprophylaxis is an important strategy for preventing and controlling HBV infection.[2]

In China, the chronic HBV infection rate in children gradually decreased due to the universal implementation of the hepatitis B vaccine strategy since 1992.[3] However, some studies have reported that mutations within the major hydrophilic region (MHR) region within the surface gene of HBV result in immune escape and contribute to immunoprophylaxis failure.[4,5] Moreover, the genotype distribution is unequal in different areas in China.[6]

The HBV genotypes and surface gene mutation among children born after the universal HBV vaccination program have not been investigated in Huzhou, China. Thus, the present study was made up to delineate the molecular characteristics in vaccinated children with HBsAg positive in this area.

Materials and Methods. In the present study, 58 children vaccinated with HBsAg positive from the serum HBsAg screening were enrolled among 7342 children in the Huzhou Central Hospital. The study was approved by the ethics committee of Huzhou Central Hospital in accordance with the ethical guidelines of the Declaration of Helsinki.

Routine HBV serological markers, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were detected in the Department of Laboratory Medicine. HBV DNA was quantified, and the HBV surface gene region was amplified and sequenced as previously described methods.[7] Genotyping of HBV was performed by using an online tool (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). Serotype was determined as previously described.[8] In addition, the amino acid (AA) substitutions in the surface gene region were analyzed in comparison to the standard reference HBV isolates obtained from GenBank (Genotype B: AB073846, AB602818, D00329; Genotype C: AB014381, AY123041, X04615) by using MEGA 7.0 software.

Results. Fifty-six samples were sequenced successfully. The characteristics of the HBV-infected children are described in **Table 1**. Among these children, 45 cases were infected with HBV genotype B, and 11 were infected with HBV genotype C. There were no significant differences between children HBV infected with genotypes B and C at demographic and virological characteristics. Furthermore, three serotypes were found in the present study: adw (42), adr (11), and ayw (3).

Eighteen AA substitution sites in the MHR region were identified in 21 of 56 children (37.5%). Among these sites, 12 AA substitutions were found within the 'a' determinant. The mutation rate in the 'a' determinant region was 28.6% (16/56); these substitutions included K122R, I126T, Q129H, P142H, and T143M (**Table 2**). It's worth noting that eight children HBsAb positive (>10 mIU/mL) were infected with HBV; seven of these eight children (87.5%) were >9 years old. Among these HBV isolates, four (50%, 4/8) showed AA substitutions (P127T, T140I, T143M, G145R) within the 'a' determinant.

When we compared mutations within MHR between genotype B and genotype C, we found mutations in 33.3% of (15/45) children infected with genotype B and in 27.2% (3/11) children infected with genotype C. However, no statistical difference was found (*P=0.702*). Furthermore, frequencies of mutations in different regions between these two genotypes had no statistical differences ($P>0.05$) (**Table 2**).

The characteristics were compared between the children with and without MHR mutations (**Table 3**). Most children in the group without MHR mutations

Table 1. Characteristics of HBV infected children.

	Total (n=58)	Genotype B (n=45)	Genotype C (n=11)	*P* value
Age (years), Mean±SD	11.4±3.4	11.2±3.3	12.2±3.8	0.384
Gender(Male/Female)	36/22	27/18	8/3	0.508
Anti-HBs antibody (<10IU/ mL/>10IU/mL)	50/8	38/7	10/1	1.000
HBeAg status (Positive/Negative)	51/7	40/5	9/2	0.614
HBV DNA (log10IU/mL), Mean±SD	7.5±1.5	7.4±1.5	7.7±1.7	0.666
ALT(U/L), Median(P25, P75)	27.7(20.1,42.5)	28.4(19.5,45.6)	24.0(21.6,36.9)	0.657
AST(U/L), Median(P25, P75)	26.4(20.7, 35.2)	28.0(20.8,35.5)	21.8(19.0,30.6)	0.252
Clinical diagnosis(ASC/CHB)	45/13	33/12	10/1	0.426

Abbreviations: ASC, asymptomatic HBV carrier; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen.

Table 2. Amino acid substitutions in S region.

Region in S region	Amino acid substitutions sites (n)		Substitutions frequencies (%)		*P* value
	Genotype B (n=45)	Genotype C (n=11)	Genotype B (n=45)	Genotype C (n=11)	
N-terminal region(1-99)	N3D/E/I(4/3/1), I4C/T(4/1), A5C/G/S(1/3/1), S6A/C/D/T(6/2/2/1), G7A/R(3/14), L21S(1), I25V(1), N40S(2),G43E(2),V47E (3),L49R(1),Q56P(1), I57I(1),S59N(2),P62L(1), C64S(1), C76Y(1),F93I(1), L98V(1)	S3N(7), T5A(1) G7R(3), R24K(1), T47V(1), S61L(1), P62*(1), T47E(2), S53L(1), I68T(1)	1.46	1.84	0.339
MHR region(100-169)	I110L(1), K122R(3) P127T/S(1/1), Q129H(2), T131N(1), M133L(1), T140I(2), K141N(1), P142H(2), T143M(2) D144A(1), G145R(1), K160N(1), Y161S/F/T(1/1/1), L162Y(1), W163G(1), R169S(1)	I126T(2), R160K(1), F161Y(1), V168F(1),	0.83	0.65	0.820
A-determinant(124-147)	K122R(3) P127T/S(1/1), Q129H(2), T131N(1), M133L(1), T140I(2), K141N(1), P142H(2), T143M(2), D144A(1), G145R(1)	I126T(2)	1.67	0.76	0.274
The first loop region(124-138)	K122R(3) P127T/S(1/1), Q129H(2),	I126T(2)	1.33	1.21	0.902

The second loop region(139-147)	T131N(1), M133L(1), T140I(2), K141N(1), P142H(2), T143M(2), D144A(1), G145R(1)		2.22	0.0	0.130
C-terminal region(170-226)	S174T(1), W182*(3), V184A/E(3/16), P188H/T(17/2), L192P(1), I195K(1), W196*/L(3/1), W199*/C/S(1/5/1) F200Y(18), P203Q(1), S204R(1), L209M(1), M213I/T(1/1), F220L(1), C221S(1), W223S(1), V224A/L(1/1)	V184E(2) G185R(2), P188H(2) T189N(1), V190A(1), V194A(1), W196R(1), W199*(2)	3.23	1.91	0.073

Table 3. Relationship between MHR mutations and clinical characteristics

	Children with MHR mutations n=21	Children without MHR mutations n=35	*P* value
Age (years), Mean±SD	10.8±3.3	11.7±3.4	0.299
Gender (male/female)	10/11	25/10	0.075
Anti-HBs antibody (positive/negative)	4/17	4/31	0.456
HBeAg status (positive/negative)	18/3	31/4	1.000
HBV DNA (log10IU/mL), Mean±SD	7.3±1.5	7.6±1.6	0.589
Genotype(B/C)	18/3	27/8	0.664
ALT(U/L), Median(Q1, Q3)	22.0(18.5,57.6)	29.0(21.6,38.1)	0.393
AST(U/L), Median(Q1, Q3)	27.3(18.9.38.3)	26.3(21.0,35.2)	0.906

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen.

were boys (71.4%, 35/35), whereas, in the group with MHR mutations, only 47.6 % (10/21) of children were boys. Additionally, no statistical differences were observed in the other factors, including AST, ALT levels; HBeAg status; and proportion of genotype B between the two groups ($P > 0.05$).

Discussion. The present results showed that the predominant HBV serotypes and genotypes were adw and B among children in the Huzhou area. It differed from our previous study, which showed that the distribution of HBV genotype B was in 43.5% (78/179) of HBV-infected adults in the same area.[9] Considering the children in this study were all vaccinated and adults in our previous study were all not vaccinated, we assume that the B genotype of HBV may be more able to infect children vaccinated. Zheng et al.[10] reported that most HBV infections in vaccinated Chinese blood donors were genotype B, which supports this suppose. On the contrary, genotype C may lead to a higher rate of HBV breakthrough infection than genotype B, as reported in a Taiwan study.[11] However, more evidence must be collected to clarify the correlation between HBV genotype and HBV infection after vaccination.

MHR region is the main B-cell epitope, which may affect antibody immunogenicity. The 'a' determinant within MHR is the determinant antigen, and the target for the neutralizing antibody produced after the vaccine, many AA substitutions in the 'a' determinant affect the binding of neutralizing antibodies.[12] In the present study, 21 of 56 children (37.5%) were found to have AA substitutions in MHR. Furthermore, 28.6% (16/56) of children harbored mutations in the 'a' determinant. Many previous studies confirmed that mutations in MHR, especially within the 'a' determinant, contributed to immune escape of vaccine.[13,14] Therefore, present findings suggest that the risk of transmission of mutant HBV still exists in the Huzhou area. However, to our

knowledge, the K141N and P142H substitution found in this study was not mentioned previously; if these two mutations could lead to vaccine immune escape, further investigation needs.

The most common immune escape mutant G145R/A is in the second loop of the 'a' determinant. However, the G145R mutation was only found in one child in this study, indicating this mutation is not common in Huzhou. Of note, the proportion of girls (52.4%, 11/21) in children with MHR mutations was higher than that in children without MHR mutations (28.6%, 10/35). However, the difference was not statistically significant (P=0.075) due to the relative sample size. However, few studies focus on this issue. Whether girls are more susceptible to HBV infection with mutant HBV deserves further investigation.

Anti-HBs can neutralize the HBsAg and eliminate the HBV infection, a protective marker in vaccine recipients. But in the present study, eight children with positive level anti-HBs (<100mIU/mL) were infected with HBV, suggesting that presence of low-level anti-HBs could not completely prevent HBV infection. Furthermore, seven of eight children (87%) were older than nine years; this result was consistent with other studies, which revealed that the anti-HBs levels gradually decreased with age in some vaccinated children.[15-17] Previous studies have shown that people with anti-HBs remain at risk of HBV infection.[18-20] Another research reported that the incidence of occult HB infection (OBI) in infants with low anti-HBs (<100mIU/mL) was significantly higher than that in non-vaccinate infants, indicating the occurrence of OBI in infants may be due to the limited neutralizing capacity provided by low anti-HBs titers.[21] Therefore, we recommend that it is necessary to monitor and strengthen immunization in children with low-level anti-HBs to reduce the risk of HBV infection.

In summary, the present findings suggest that genotype B is the predominant genotype in children. It may be associated with the threat of HBV infection in vaccinated children, MHR mutations, and decreased levels anti-HBs in Huzhou. Further long-term prospective observation and functional analysis of mutant HBV strains in vitro and in vivo experiments are needed to confirm the findings in the present study. Nevertheless, the results may help a different vaccine improvement strategy, prevention, and control of HBV infection in children.

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