Transmission of “a” Determinant Variants of Hepatitis B Virus in Immunized Babies Born to HBsAg Carrier Mothers

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SUMMARY: Hepatitis B virus (HBV) surface antigen mutations may lead to immune escape and eventually cause failure of immunization. In this report, we identified immune escape variants in immunized babies born to hepatitis B surface antigen (HBsAg) carrier mothers. A total of 68 babies were followed up for 2 years after the full course of vaccination; 2.9% (2/68) of babies were found to be infected with the variant HBV in spite of preexisting antibody to surface antigen (anti-HBs) at 24 months post immunization. Both infants were positive for HBV-DNA; sequencing results of the “a” determinant region of the surface gene revealed that both babies had point mutations at a different nucleotide position resulting in various amino acid substitutions. In addition, an intriguing variant having an addition-deletion mutation was observed in one of the babies. This is the first report to show the addition-deletion variant of HBV in India. However, the immunological significance of the above HBV variants needs to be further elucidated.

The hepatitis B virus (HBV) envelope gene encodes the major surface antigen (HBsAg), which is composed of 226 amino acids. HBsAg contains antigenic sub-determinants (d/y, w/r, w1-w4 and q) (1). The antigen is grouped in nine serotypes according to the combinations of these epitopes (1). The highly conserved “a” determinant is present in all known serotypes and is the major immune target for antibodies either used for immuno-prophylaxis or in assays for the detection of HBsAg (2). The HBsAg “a” determinant (amino acids 124 - 147) (3) is located within the major hydrophilic region (MHR, amino acids 99 - 169) of the surface protein (4). A mutation within the “a” determinant affects the antigenicity of the protein which enables the virus to evade neutralizing antibodies (5). HBV isolates carrying such mutations have been reported to cause infection in infants and adults who have been vaccinated and/or received hepatitis B immunoglobulin (HBig) (4-6).

The most frequent mutation causing vaccine escape is the G145R substitution located in the “a” determinant region of HBsAg (4). This was first identified in successfully immunized infants who became HBV carriers, despite the presence of protective levels of antibodies to HBsAg (4). However, a wide variety of mutations affecting amino acids at positions 116, 120, 126, 129, 141, 142, and 144, either alone or in combination, were also isolated from vaccinated individuals who were HBV carriers (2). Thus, it is necessary to investigate whether current vaccines will continue to be effective in the future. The present study was executed to analyze the possible occurrence of infections caused by wild-type (wt) HBV or HBV “a” determinant mutants in immunized babies born to HBsAg carrier mothers. We followed up the babies for 2 years after a full course of vaccination, by analyzing their follow-up serum samples for HBV markers such as HBsAg, anti-HBs, anti-HBc (antibody to hepatitis B core antigen), HBeAg, and HBV DNA.

This study was approved by the institutional review committee of the Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras. The present study is a cross sectional study from a major vaccine evaluation study in which the vaccine efficacy of different commercially available hepatitis B vaccines to babies born to HBsAg carrier mothers was investigated (7). We followed up a total of 68 babies for 24 months who were successfully immunized with commercially available vaccines starting with the first dose at birth, with 0, 1, and 6 months schedule (6). Serial blood samples were obtained from the babies initially at 24 h after birth, and followed by blood samples obtained periodically at 1, 7, 12, and 24 months post birth. Blood samples were also collected from the respective mothers shortly before delivery. Serum samples of the baby and mother pairs were screened for HBsAg, HBeAg, anti-HBs, and anti-HBc by using commercially available Bio-Rad ELISA kits. Carriers of HBV variants were identified among vaccinated babies by the coexistence of HBsAg and anti-HBs, and were further investigated for the presence of HBV-DNA by PCR amplification of HBsAg encoding sequences.

The HBV-DNA was extracted from 200 μl of serum using a QIAamp blood kit (Qiagen, Valencia, Calif., USA). Serum HBV DNA was quantified by using the Cobas Amplicor HBV Monitor test (Roche Diagnostics, Branchburg, N.J., USA). According to the manufacturer’s protocol, the extracted DNA was amplified by using “S” gene specific primers: forward; nucleotide (nt) 412-433, 5’-CCT GCT GCT ATG CCT CAT CCT C-3’ and reverse: nt 799-778, 5’-CAG CCG GAT AAA

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GGG ACT CAC G-3' (GenBank accession no. AJ 428524 and AJ428525J) for HBV (388 bp). The PCR products were subsequently purified by using a commercial kit (QIA quick gel extraction kit; Qiagen) The purified PCR products were subjected to bidirectional sequencing with the Big Dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Foster City, Calif., USA) in an automated DNA sequencer (ABI Prism 377; Perkin-Elmer Applied Biosystems, Foster City, Calif., USA); sequence analyses were performed using an ABI Prism 377. The nucleotide sequences were aligned with the consensus sequences from the EMBL database utilizing multiple sequence alignment programs, Clustal X version 1.8 and Genedoc software.

In this study, approximately 97% of the infants born to HBsAg positive mothers who were successfully vaccinated at birth avoided HBV infection (7). Of the 68 immunized infants born to HBsAg positive mothers, two babies were found to be positive for HBsAg and anti-HBs after 2 years (Table 1), and the corresponding mothers were positive for the hepatitis B envelope antigen (HBeAg). This phenomenon of the persistent positivity of HBsAg with significant anti-HBs titers in immunized babies suggests the presence of hepatitis B variant infection. Analysis of the HBV-DNA of the two samples showed HBV-DNA positive for PCR using surface specific primers. The levels of serum transaminases, such as aspartate aminotransferase and alanine aminotransferase, were elevated in both the babies at the month 24. Nucleotide and amino acid sequences of the surface gene of HBV from these infants were compared with the HBV surface gene sequences from the corresponding mothers (Figure 1). The comparison revealed that both baby samples were infected with the HBV variants. The sequencing results of the “α” determinant coding sequences of the infants had point mutations at different nucleotide positions, resulting in various amino acid substitutions (Table 2).

Point mutations at various nucleotide positions which cause the amino acid substitutions were observed in the “α” determinant region of HBV compared with the respective mother’s sequences. Point mutations at nucleotide position nt-528 (Thr 125 Met), nt-531 (Ile 126 Thr), nt-533 (Pro 127 Thr), nt-555 (Phe 134 Tyr) were observed in sample S3 (DQ229962). However, many silent mutations were also observed in the surface region of the baby sample S3 at nt-562 (Ser 136 Ser), nt-574 (Ter 140 Ter), and nt-592 (Asn 146 Asn). On the other hand, in the baby sample S4 (DQ229963), in which the comparison of sequence analysis from mother and child revealed an addition of base “C” in between the nt-543 and nt-545 and deletion of base “C” in between the nt-546 and nt-548 region resulted in the amino acid changes which substitutes an amino acid asparagine in the place of theronine (Figure 1). Such an addition-deletion mutation is novel and intriguing, and is reported for the first time in India among immunized babies born to HBsAg carrier mothers. Apart from the mutations mentioned above, various point mutations were also observed at positions nt-531, nt-583 leading to the amino acid changes at Ile 126 Thr, Thr 131 Asn, and Ser 143 Thr in sample S4. In addition, silent mutations were also detected at nt-544 (Gly 136 Gly), nt-586 (Asp 144 Asp), and nt-592 (Asn

Table 1. Serological and molecular features of hepatitis B surface antigen (HBsAg) carrier mothers and corresponding babies post immunization

<table>
<thead>
<tr>
<th>Infant no.</th>
<th>HBsAg</th>
<th>Anti-HBs titer</th>
<th>Anti-HBC</th>
<th>HBeAg</th>
<th>HBV-DNA</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother of S3</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Not detected</td>
</tr>
<tr>
<td>Baby 1-S3</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1st m</td>
<td>–</td>
<td>22</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7th m</td>
<td>–</td>
<td>56</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>24th m</td>
<td>+</td>
<td>63</td>
<td>+</td>
<td>+</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td>Mother of S4</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Not detected</td>
</tr>
<tr>
<td>Baby 2-S4</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1st m</td>
<td>–</td>
<td>44</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>7th m</td>
<td>–</td>
<td>83</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>24th m</td>
<td>+</td>
<td>23</td>
<td>+</td>
<td>+</td>
<td>Detected</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Alignment of nucleotide and deduced HBsAg amino acid sequences of isolates S3 and S4 with corresponding mother’s HBV sequences. (A) Comparison of nucleotide sequence of mother and child pairs -HBsAg “α” epitope coding sequence. (B) Comparison of amino acid sequence of variant HBV strain with corresponding child pairs -HBsAg “α” epitope coding amino acid sequence.
In the present study, the classical vaccine escape mutation (G145R) was not detected. However, "a" determinant variants were detected in both the baby samples. Mutations at amino acid positions 126 and 136 observed in both samples. Mutations at position 144 of the second loop, this might influence the virus to evade the immune response and lead to HBV infection in the babies (Table 2 and Figure 1). However, the sequence analysis of the two infants does not show any point mutation determining the classical vaccine escape mutants G145R.

Antibodies against the group-specific "a" determinant, a complex antigenic structure with multiple immunogenic epitopes, normally neutralize virus and confer cross-protective immunity to all HBV subtypes. Historically, the secondary structure of this epitope is presented as a double loop, formed through disulfide bridges between cysteine residues 124 and 137 and residues 139 and 147 (8). Nucleotide substitutions that lead to amino acid changes within this region may result in reduced binding or failure to detect serum HBsAg in diagnostic assays with polyclonal and/or monoclonal antibodies (4). Amino acid changes have been described at positions 126, 129, 131, and 133 of the first loop of the "a" determinant and positions 141, 142, 144, and 145 of the second loop (4,8-10). The most common mutation that has been reported is G145R, which occurs either alone or in combination with others. This variant displays reduced levels of binding to anti-HBs antibodies, and is usually selected under immune pressure after administration of the HBV vaccine, with or without concurrent HBIG, or following treatment with polyclonal or monoclonal HBIG (11). Naturally occurring surface gene variants have also been reported around the world in persons who have not been immunized (11). In the present study, the classical vaccine escape mutation (G145R) was not detected. However, "a" determinant mutants were detected in both the baby samples. Mutations at amino acid positions 126 and 136 observed in both samples of the present study were already reported by Yamamoto et al. (12), Huang et al. (13), Harrison et al. (14), Oon et al. (15), and Lee et al. (16). Mutation at amino acid position 133 detected in the present study were already reported by Oon et al. (15) while studying the vertical transmission of babies born to HBsAg positive mothers. Apart from all the earlier reports, we had observed additional point mutations in amino acid positions 125, 127, 131, and 134, which are intriguing and are hitherto unreported in the "a" determinant region. In addition, a novel variant having an addition-deletion mutation was reported by us in baby S4 for the first time in this part of the region. However, the immunological significance of the above mutations needs to be further elucidated.

In the context of the above reports and the commentary of Zuckerman (3), the issue of hepatitis B vaccine escape mutants is creating concern among epidemiologists, virologists, and public health specialists, since all of them are interested in the control and global elimination of HBV infection. Given this context, our present study in India is a pilot report on HBV vaccine escape variants in immunized babies born to HBsAg positive mothers. With universal vaccination being implemented in all countries, it is of concern that atypical HBV vaccine escape variants occur even in people with protective levels of anti-HBs. The addition of appropriate HBsAg epitopes in the currently used vaccine or the inclusion of a protective pre-S epitope in addition to HBsAg may be a possible solution for preventing infections by vaccine escape variants (3).

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REFERENCES