Horizontal Transmission of a Hepatitis B Virus Surface Antigen Mutant

The envelope protein of hepatitis B virus (HBV) is referred to as the HBV surface antigen (HBsAg) (8). The antigenic “a” determinant is located from residue 124 to 147 of HBsAg and is involved in eliciting antibody production. Neutralizing antibodies (anti-HBs) target the “a” determinant and generally lead to the disappearance of HBV (1).

The coexistence of HBsAg and anti-HBs has been associated with mutations in the “a” determinant (2, 6). Displaying altered antigenic structures, these HBsAg mutants are capable of escaping detection and vaccination (4, 9). Some are infectious and are associated with liver diseases (3–5, 7). While some may be transmitted vertically (6), no horizontal transmission of these HBsAg mutants has previously been described.

We report the horizontal transmission of an HBsAg mutant (Asp144Ala) from an infected infant to his wild-type-HBV carrier Singaporean mother who gave birth to two identical twins in 1984. Despite vaccination at birth, both infants tested positive for HBsAg beginning at birth. The Asp144Ala mutant was detected in the serum of one twin (infant 1) by direct sequencing analysis. While only wild-type HBV was found in the maternal serum taken at delivery and in cord blood (Fig. 1A, panels 1 and 2, respectively), the Asp144Ala HBsAg mutant was detectable in maternal serum taken 6 years later (Fig. 1A, panel 3).

To rule out the possibility that a minor population of the Asp144Ala mutant that was undetectable by sequencing was present in the maternal serum at delivery, a PCR-based nucleotide analysis method was developed. The first PCR amplification resulted in a 420-bp fragment (position 420 to 840 of the HBV genome) in the three maternal samples (Fig. 1B, lanes 1 to 3) that were analyzed by the above-mentioned direct sequencing method (Fig. 1A). The second PCR using a nested primer (5'-AGGATGATGGGATGGGAATACAGGTGCTTTTTCC-3') with its 3'-end nucleotide immediately adjacent to the nucleotide mutation (GAC Asp to GCC Ala [mutation is underlined]), gave rise to a 200-bp fragment (position 420 to 440 of the HBV genome).
620 of the HBV genome) (Fig. 1B, lanes 5 to 7). The Asp144Ala mutation creates an internal EagI site (CGGCCG [Ala144 is underlined]) in the 200-bp PCR product, resulting in 170-bp (Fig. 1B, lane 7) and 30-bp (not shown) fragments upon EagI restriction. The presence of the 200-bp wild-type DNA (Fig. 1B, lane 7) despite restriction suggested a mixture of wild-type and Asp144Ala viruses in this maternal serum. Conversely, only the 200-bp fragment was observed in the maternal serum taken at delivery and in the cord blood (Fig. 1B, lanes 5 and 6, respectively) after prolonged EagI restriction, as well as by Southern blot analysis using digoxigenin-labelled HBsAg DNA fragment as the probe (data not shown). Similar analysis carried out on the other vaccinated twin (infant 2) showed the absence of Asp144Ala mutant virus in his serum from birth to 13 years of age (unpublished data). These results suggest the absence of the Asp144Ala mutant in the mother at delivery and in the second twin.

Our findings suggest the horizontal transmission of the HBsAg mutant, which has hitherto not been reported. It will be of interest to assess the transmissibility of other HBsAg mutants by using a similar sensitive method (expected to detect a 0.2% concentration of mutant virus), as some of them are now implicated in various aspects of childhood and adult liver diseases.

REFERENCES


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