Hepatitis B virus infection in children of HBV-related chronic liver disease patients: a study of intra-familial HBV transmission

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Abstract
Background HBV-infected patients are potential sources of intra-familial transmission. We studied HBV transmission and molecular characteristics within families of HBV-related chronic liver disease (CLD) patients.

Methods Family members [index cases (ICs), spouses, and 1–18-year-old children] of HBV-related CLD patients were tested for HBsAg, anti-HBc, and anti-HBs. HBsAg-positive subjects were tested for HBeAg/anti-HBe. Anti-HBc-positive children together with their family members were further investigated for HBV DNA. Sequences of positive isolates were analyzed over surface, precore (PC) and basal core promoter (BCP) regions.

Results Among 94 children of 46 ICs, the prevalence of HBsAg, anti-HBc, and anti-HBs was 10 (10.6 %), 19 (20.2 %), and 46 (48.9 %), respectively. Thirty-eight (40.4 %) children were seronegative, indicating susceptibility to HBV infection. HBV DNA was identified in all ICs, 4 spouses, and 16 children. Having both parents with HBsAg positive and at least two HBV carriers in the households were significant risk factors of intra-familial transmission. HBV genotype/subtype distributions were comparable between children and ICs/spouses, with predominance of genotype B. The majority of HBV DNA sequences found in children were identical to their corresponding ICs—particularly mothers—including mutation patterns in the surface, PC, and BCP regions. Recognized mutations associated with HBsAg detection and/or vaccination failure, T140I, T143S/M, G145R, and Y161F, were identified in 20 subjects; while mutations linked to HBeAg-defective variants, PC G1896A and BCP A1762T/G1764A, were found in 7 and 11 subjects, respectively.

Conclusions Children of HBV-related CLD patients were at increased risk of HBV infection through multi-modal

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transmission routes despite negative parental HBsAg and HBeAg status.

**Keywords** Hepatitis B in children · Hepatitis B virus · Intra-familial transmission · Transmission modes of HBV · Mutation

**Introduction**

Hepatitis B virus (HBV) infection is a major health problem. An estimated 2 billion people have been infected, and 240 million are chronic carriers with risks of liver disease progression to cirrhosis and hepatocellular carcinoma (HCC) and premature mortality in productive age [1]. The prevalence of HBV infection varies in different regions. Depending on the prevalence of hepatitis B surface antigen (HBsAg), HBV endemicity is classified into three categories: high (>8%), intermediate (2–8%), and low (<2%) [2]. In HBV endemic countries, most infections occur in infancy resulting from vertical or mother-to-child transmission (MTCT), while in low endemic countries, transmission occurs through horizontal mode, primarily among unvaccinated adults [3]. Indonesia has a moderate-to-high hepatitis B endemicity with average HBsAg prevalence of 9.4% (4–20.3%, varying across the archipelago) and anti-HBV core (anti-HBc) of 32.8% with a steady increase from 10.04% in the age group <5 years to 58.05% in the age group ≥60 years, indicating high infection rates through horizontal transmission [4].

Patients with chronic hepatitis B (CHB) are considered to be major reservoirs for HBV transmission [5, 6]. Within families, the HBsAg carrier rate in children is closely related to their parents’ HBsAg status [7, 8]. The acquisition age of infection and maternal hepatitis B ‘e’ antigen (HBeAg) status during pregnancy are important factors in determining chronicity in children [9]; chronic infection develops in 90% of infected neonates or infants and 25–50% of infected children in the age 1–5 years, but only 1–5% of infected adults [2]. Without HBV immunoprophylaxis, more than 90% of infants of HBeAg/HBsAg-positive mothers will become chronic HBV carriers, in contrast to <5% of those born to HBsAg-positive but HBeAg-negative mothers [2].

Although clinical histories and HBV serological markers of family contacts can reveal the possible routes of intra-familial HBV transmission in most instances, identification of transmission routes—particularly when both parents are HBsAg positive—is not possible on the basis of clinical and serological evidence alone. Instead, HBV genotyping and phylogenetic analysis followed by nucleotide sequence comparison from different viral strains have been used for this purpose [10, 11]. Studies of viral mutants also help in tracing intra-familial transmission, since HBV mutants—particularly of the core gene—remain stable for more than 2 decades in perinatally acquired familial infection [12].

This study aimed to investigate the molecular epidemiology of hepatitis B in children of HBV-related chronic liver disease (CLD) patients in Jakarta, Indonesia, and the intra-familial mode of HBV transmission by analysis of HBV genotypes and nucleotide sequences, including the specific mutations at the surface, precore (PC), and basal core promoter (BCP) regions of viral isolates of infected family members.

**Materials and methods**

This was a cross-sectional study on children of HBV-related CLD patients (i.e., CHB, liver cirrhosis, or HCC) who were treated at the Hepatology Outpatient Clinic, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Jakarta, during the period of July–September 2009. Patients enrolled as the index cases (ICs) were HBsAg positive for a period of more than 6 months. Study children were recruited from each patient’s household. Family members were eligible for inclusion if they had at least one child aged 1–18 years with no history of blood transfusion, intravenous drug use, and/or tattooing. After informed consent was obtained, each family was asked to complete a questionnaire on factors associated with HBV transmission to the children that included demography, perinatal events, and behavioral factors of the children, as well as medical histories of the ICs [13]. Serum samples were obtained from the patients/ICs, ICs’ spouses, and their children. Liver function was assessed directly for serum alanine transaminase (ALT), and other aliquots were stored at −70°C until used. This study was approved by The Committee of Medical Research Ethics of the Faculty of Medicine, University of Indonesia (no. 297/PT02.FK/ETIK/2009).

**HBV serological examination**

HBsAg, anti-HBs, and anti-HBc were tested on all subjects (ICs, ICs’ spouses, and children) using commercially available immunoassays (Elecsys HBsAg, Elecsys anti-HBs, and Elecsys anti-HBc Immunoassays; Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s instructions. Anti-HBs level ≥10 IU/l was considered positive [1]. All subjects who were positive for HBsAg and/or anti-HBc were further tested for HBeAg/anti-HBe status by Monolisa™ HBeAg Ag-Ab PLUS [Biorad, Marnes-la-Coquette, France] according to the manufacturer’s instructions.

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HBV DNA detection, genotype determination, and analysis of S gene

HBV DNA was extracted from 140 µl of serum using QIAamp DNA-MiniKit (Qiagen, CA, USA) according to the manufacturer’s instructions. The DNA fragment of S gene that encodes the HBsAg ‘a’ determinant was amplified by nested PCR using specific primer sets S2-1 (nt 455–474, sense; 5'-CAA GGT ATG TTT CCC GTT TG-3')/S1-2 (nt 704–685, antisense; 5'-CGA ACC ACT GAA CAA ATG GC-3') as outer primers and S88 (nt 462–481, sense; 5'-TGT TGC CTT GTC CTC TA-3')/S2-2 (nt 687–668, antisense; 5'-GGC ACT AGT AAA CTG AGC CA-3') as inner primers [14–16]. The amplification product was purified using a purification column (Qiagen, CA, USA) and subjected to direct sequencing reactions on DNA sequence analyzer ABI 3130xl (Applied Biosystems, Carlsbad, CA, USA). HBV genotypes were determined by phylogenetic tree analysis based on the S gene sequences together with HBV sequences of known genotypes retrieved from GenBank, using the neighbor-joining method and Kimura-2 parameter in Phylip v.3.68 software, with 1000 bootstraps (Suppl. Fig. 1). HBsAg subtypes (adw, adr, ayw, or ayr) were determined using deduced amino acid sequences of two pairs of mutually exclusive determinants, d/y and w/r, at amino acids 122 and 160 in the ‘a’ determinant region, respectively [17, 18]. S gene mutations were analyzed based on alignment against the wild-type reference sequence M54923 retrieved from GenBank. The nucleotide numbering was based on the EcoRI restriction site within the HBV genome.

The sensitivity of the nested PCR performed in this study was validated using a panel of sera with various HBV DNA titers tested by COBAS TaqMan 48 Real-Time PCR (Roche Molecular System, Branchburg, NJ, USA). The nested PCR was capable of detecting HBV DNA at titers lower than the detection limit of the COBAS TaqMan 48 Real-Time PCR (6 IU/ml) and thus met the sensitivity requirement for detection of occult HBV infection (OBI) of less than 10 IU/ml (Suppl. Table 1) [19].

Analysis of the BCP and PC region

BCP and PC regions were amplified from the extracted DNA by nested PCR using specific primer sets PC1 (nt 1554–1573, sense; 5'-CTG TGC CTT CTC ATC TGC CG-3')/PC2 (nt 1972–1949, antisense; 5'-AAA GAA GTC AGA AGG CAA AAA AGA-3') as outer primers and S12 (nt 1679–1699, sense; 5'-AAT GTC ACC ACC CGA CCT TG-3')/S13 (nt 1941–1919, antisense; 5'-TCC ACA GAA GCT CCA AAT TCT AA-3') as inner primers [15]. The purified products were sequenced as described above. The sequences were aligned with the wild-type reference sequence M54923 retrieved from GenBank.

Statistical analysis

The baseline data were summarized descriptively. Chi-square or Fisher’s exact test was used to determine the differences in categorical variables. Linear-by-linear association chi-square was used to assess the age-specific trend of HBV prevalence among the children [20]. Factors associated with transmission of HBV to the children were analyzed, and outcomes were reported as odds ratios (OR) with 95 % confidence intervals (CI). A p value of <0.05 was considered significant. All statistical analyses were two-sided and performed using the Statistical Program for Social Sciences (SPSS 15.0 for Windows; SPSS, Chicago, IL, USA).

Results

Forty-six (male/female 31/15) patients, their spouses, and 94 children (male/female 46/48) were included in this study. Among 46 ICs, 38 % had CHB and 39 % had cirrhosis. The recruitment of study subjects was performed as shown in Fig. 1.

Serological profile of 94 children

Among 94 children, 10 (10.6 %) were HBsAg positive, 19 (20.2 %) were anti-HBc positive, and 46 (48.9 %) were anti-HBs positive. Of 19 anti-HBc-positive children, 10 (52.6 %) were HBsAg positive and 9 (47.4 %) HBsAg negative. Of 46 anti-HBs-positive children, 9 (19.6 %) were HBsAg negative and anti-HBc positive, while 37 (80.4 %) were negative for both HBsAg and anti-HBc. The remaining 38 (40.4 %) children were negative for all three markers, indicating no past or present infection but susceptibility to HBV infection. The baseline characteristics and detailed serological profile of 94 children are shown in Suppl. Table 2.

There was no significant difference in the prevalence of HBsAg, anti-HBc, and anti-HBs between males and females. To assess the impact of age on the prevalence of these serological markers, the children were clustered into the age groups: 1–5, 6–12, and 13–18 years. In the three age groups, the prevalence of HBsAg was 10.0 % (2/20), 5.0 % (2/40), and 17.6 % (6/34), while that of anti-HBc was 15.0 % (3/20), 17.5 % (7/40), and 26.5 % (9/34), respectively. Although not statistically significant, escalation of anti-HBc-positive rates was seen with increasing age. The prevalence of anti-HBs among the three age
groups was 70.0% (14/20), 47.5% (19/40), and 38.2% (13/34), respectively, showing a decreasing trend of anti-HBs rates as age increased (p = 0.030) (Fig. 2).

In both univariate and multivariate analyses, there was no significant relationship between HBV exposure to the children (defined by positive anti-HBc) and risk factors for hepatitis B. However, two factors were associated with higher risk of HBV transmission: having both parents positive for HBsAg (p < 0.001) and having at least two HBV carriers in the household (p < 0.001) (Suppl. Table 3).

Analysis of 19 anti-HBc-positive children and their corresponding parents

Nineteen children of 14 ICs were anti-HBc positive, indicating evidence of HBV exposure. These children together with the 14 related ICs and ICs’ spouses were further investigated. Of 19 children (male/female 10/9), 10 (52.6%) were HBsAg positive and 9 (47.4%) were HBsAg negative. History of hepatitis B vaccination was obtained in 14 children, of whom 12 had completed three-dose series of vaccination during infancy. Two children obtained hepatitis B booster-dose vaccines at adolescence and had high anti-HBs levels (>1000 IU/l). Anti-HBs positivity was significantly higher in the HBeAg-negative than in the HBeAg-positive groups (p < 0.001). The characteristics of the 19 children are shown in Table 1.

Serological profile, HBV DNA detection, and molecular analysis of 19 anti-HBc-positive children and their ICs’ family members

Serological investigation of HBV markers (HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe) as well as HBV DNA detection, genotype determination, and mutation analysis were done in all ICs’ family members of the 19 anti-HBc-positive children. HBV DNA was detected in 14 ICs, 4 ICs’ spouses, and 16 children, with no significant difference between HBsAg-positive and HBsAg-negative groups. Two of the ICs’ spouses and six of the children with positive HBV DNA were HBsAg negative/anti-HBc positive. HBV genotype B was more prevalent than genotype C among ICs (10 vs. 4) and children (11 vs. 5), but equal among the ICs’ spouses. In HBV DNA-positive subjects, HBeAg-positive and HBeAg-negative cases were
equally distributed in the IC group; one HBeAg-positive and three HBeAg-negative among IC’s spouses; and ten HBeAg-positive, six HBeAg-negative among the children (p = 0.087). Both genotypes B and C were relatively comparable between HBeAg-positive and HBeAg-negative subjects (Suppl. Table 4).

Mutations in the HBV S, PC, and BCP regions

The possible routes of HBV transmission were traced by investigation of familial relations, HBV serology, genotypes, HBsAg subtypes, and analysis of nucleotide sequences of the HBV genome (Fig. 3; Suppl. Table 4). The majority of HBV DNA sequences found in children were identical to those of the corresponding ICs and ICs’ spouses, including some mutations in the S, PC, and BCP regions. Mutations in the S gene causing substitutions in the HBsAg ‘a’ determinant were detected in 20 subjects. Certain mutations were associated with genotypes: M133L with genotype B and T126I, T143S, and Y161F with genotype C.

In the PC region, G1896A mutation was found in 8 subjects, of whom 7 were HBeAg negative, while in the BCP region, the A1762T/G1764A mutation was identified in 12 subjects, with 5 showing HBeAg-negative phenotype. Significantly higher frequency of HBeAg negativity was observed in subjects with PC G1896A mutation (p = 0.029), but not in those with BCP A1762T/G1764A mutation (p = 0.710). Two subjects in two families showed deletion patterns within the BCP region (30 nucleotides at nt 1740–1770 and 20 nucleotides at nt 1754–1773, respectively), both with HBeAg positivity. The sequences generated in this study have been deposited in the GenBank database (accession no. KP123339-

Table 1 Characteristics of 19 anti-HBc-positive children from 14 families of HBV-related CLD patients

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Family no.</th>
<th>Index case</th>
<th>Age (year)</th>
<th>Gender</th>
<th>Hepatitis B vaccination History</th>
<th>HBsAg</th>
<th>Anti-HBs (IU/L)</th>
<th>ALT (U/L)</th>
<th>HBV DNA/ Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>8</td>
<td>Mother</td>
<td>13</td>
<td>M</td>
<td>–</td>
<td>+</td>
<td>1.99</td>
<td>164</td>
<td>+/C</td>
</tr>
<tr>
<td>17</td>
<td>9</td>
<td>Father</td>
<td>16</td>
<td>M</td>
<td>–</td>
<td>+</td>
<td>1.99</td>
<td>12</td>
<td>+/B</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>Mother</td>
<td>4</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>2.31</td>
<td>24</td>
<td>+/B</td>
</tr>
<tr>
<td>26</td>
<td>13</td>
<td>Mother</td>
<td>13</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>43</td>
<td>+/B</td>
</tr>
<tr>
<td>27</td>
<td>13</td>
<td>Mother</td>
<td>6</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>44</td>
<td>+/B</td>
</tr>
<tr>
<td>29</td>
<td>15</td>
<td>Father</td>
<td>18</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>18</td>
<td>+/B</td>
</tr>
<tr>
<td>56</td>
<td>28</td>
<td>Father</td>
<td>16</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>12</td>
<td>+/B</td>
</tr>
<tr>
<td>57</td>
<td>28</td>
<td>Father</td>
<td>11</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>20</td>
<td>+/B</td>
</tr>
<tr>
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<td>28</td>
<td>Father</td>
<td>3</td>
<td>M</td>
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<td>2.17</td>
<td>17</td>
<td>+/B</td>
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<tr>
<td>88</td>
<td>44</td>
<td>Mother</td>
<td>13</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>1.99</td>
<td>22</td>
<td>+/B</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>Father</td>
<td>16</td>
<td>M</td>
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<td>12.86</td>
<td>15</td>
<td>+/B</td>
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<td>7</td>
<td>4</td>
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<td>18</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>&gt;1000</td>
<td>11</td>
<td>+/C</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>Mother</td>
<td>7</td>
<td>M</td>
<td>+</td>
<td>–</td>
<td>161.8</td>
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<td>+/B</td>
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<tr>
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<td>M</td>
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<td>F</td>
<td>+</td>
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<td>–</td>
</tr>
<tr>
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<td>18</td>
<td>M</td>
<td>–</td>
<td>–</td>
<td>50.71</td>
<td>43</td>
<td>+/C</td>
</tr>
<tr>
<td>78</td>
<td>39</td>
<td>Father</td>
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<td>F</td>
<td>+a</td>
<td>–</td>
<td>&gt;1000</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
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<td>42</td>
<td>Father</td>
<td>6</td>
<td>F</td>
<td>+a</td>
<td>–</td>
<td>238</td>
<td>14</td>
<td>+/C</td>
</tr>
<tr>
<td>89</td>
<td>44</td>
<td>Mother</td>
<td>5</td>
<td>F</td>
<td>+</td>
<td>–</td>
<td>134</td>
<td>13</td>
<td>+/C</td>
</tr>
</tbody>
</table>

Children with positive HBsAg are highlighted. Anti-HBs levels ≥10 IU/l were considered positive

CLD chronic liver disease, HBsAg hepatitis B surface antigen, anti-HBs antibody against HBsAg, ALT alanine transaminase,
M male, F female

aIncomplete vaccination

Children with positive HBsAg are highlighted. Anti-HBs levels ≥ 10 IU/l were considered positive

CLD chronic liver disease, HBsAg hepatitis B surface antigen, anti-HBs antibody against HBsAg, ALT alanine transaminase, M male, F female

*Incomplete vaccination

The majority of HBV DNA sequences found in children were identical to those of the corresponding ICs and ICs’ spouses, including some mutations in the S, PC, and BCP regions. Mutations in the S gene causing substitutions in the HBsAg ‘a’ determinant were detected in 20 subjects. Certain mutations were associated with genotypes: M133L with genotype B and T126I, T143S, and Y161F with genotype C.

**Discussion**

Children with HBV infection are generally asymptomatic and do not require treatment, but they are at increased risk of developing complications and may become sources of infection for their entire lifetime [9, 21]. To our knowledge, this is the first study done in Indonesia exploring HBV infection among children of HBV-related CLD patients in the context of intra-familial transmission of this virus.

This study showed that the overall HBsAg prevalence in children from the families of HBV-infected CLD patients was 10.64 % (10/94), higher than in the general population of Indonesia (9.4 %) [4].

The prevalence of anti-HBc as evidence of HBV exposure was 20.2 % (19/94) with escalating rates by age, indicating the importance of horizontal transmission. Of 46 families, 14 (30 %) were identified to have at least one child with positive anti-HBc. Our finding was in accordance with studies on the familial occurrence of HBV infection reported from Northeastern Egypt, India, and Brazil, where HBsAg and anti-HBc prevalence ranged from 10.7 to 47.9 % and 23 to 26 %, respectively, among family members of CHB patients [22]. The decreasing trend of anti-HBs prevalence with age could indicate the waning antibody levels with time [23].

Based on the concordant HBV genotypes between carrier children and their parents, HBV infection in the 16 HBV DNA-positive children could be mostly transmitted from the mothers (8/16), followed by the fathers (3/16) (Fig. 3). The rest could be from either or both parents (3/16) or unidentified sources (2/16), as observed in family

![Fig. 3 Family trees of 14 HBV-related CLD patients. The transmission routes were investigated by identifying the concordant HBV genotype/subtype between carrier children and corresponding parents. Squares denote males, and circles denote females. B: genotype B; C: genotype C; adr, subtype adr; adw, subtype adw; ayw, subtype ayw.](image-url)
42, in which two distinct HBV clusters were identified, and family 44, where different HBV genotypes were identified in two daughters. This finding was similar to a study in Iranian children that indicated the possible HBV transmission sources as mothers (37.5 %), fathers (15 %), and the community (33.8 %) [24].

Data regarding the differences of transmission routes between HBV genotypes are scarce. Two studies in 2007 reported that genotype C had the strongest association with perinatal transmission compared to genotypes A, B, D, and F [25, 26]. This could not be demonstrated in our study; of eight mothers presumed to be the sources of transmission, six had genotype B and two had genotype C. However, a study in 2011 showed that both genotypes B and C could be transmitted from maternal and horizontal origins; the increased prevalence of genotype C was found because of the higher rate of breakthrough infection in immunized children born to genotype C mothers than those with genotype B [27]. The role of HBV genotypes in transmission routes remains a question and needs further global studies, considering the association between HBV genetic diversity with host ethnicity and geographical distribution [19, 22].

Mutations in the ‘a’ determinant of HBsAg, either singly or in combination, were identified in several paired sera of children and their corresponding parents, providing a stronger indication of intra-familial HBV transmission. The mutations were observed in both genotype B and C, with predominance of M133L in genotype B, and T143S and T126I in genotype C. These mutations were also reported in another study from Indonesia associated with HBsAg detection failure in blood donors [28].

The well-known mutation G145R—associated with vaccine escape and HBsAg detection failure [29]—was found in two subjects, one of which was HBsAg positive. Other important mutations associated with HBsAg detection and vaccination failure—M133L, T140I, T143S/M, Y161F—were also quite frequent. Intriguingly, the ‘a’ determinant mutations are more prevalent in those with genotype B than C (p = 0.01). Some of these mutations were found in six children and two spouses with HBsAg negative but anti-HBc and HBV DNA positive (OBI). This finding confirms that individuals with OBI could be ‘hidden’ sources of transmission to their spouses, children, siblings, and even the community [30, 31].

In CLD, HBeAg is a valuable marker for infectivity in HBV transmission [32]. However, mutations in the PC and BCP regions may downregulate or abolish HBeAg production, which mimics seroconversion to anti-HBe and immune clearance of the virus, while maintaining the presence of viremia known clinically as e-negative CHB [33]. The most common mutation in the PC region is G1896A, which abolishes HBeAg production by introducing a premature stop codon [34]. Reports from Europe indicated that G1896A was more common in genotype A-infected patients and associated with progressive liver diseases [34]. However, in Asia, this mutation was found in genotype B, C, and D and detected in similar percentages in asymptomatic carriers and patients with cirrhosis and HCC [15, 34–36]. In our study, a higher frequency of G1896A was observed in HBeAg-negative than in HBeAg-positive family members (p = 0.029) (Suppl. Table 4). Among BCP mutations, the most frequent is the double mutation A1762T/G1764A that decreases HBeAg expression but enhances viral replication [15, 37, 38]. This mutation is associated with more severe diseases, cirrhosis, and HCC [39, 40]. In this study, A1762T/G1764A was relatively more frequent in HBeAg-negative subjects and infection with genotype C.

One limitation of the study was the cross-sectional design that did not make it possible to determine the time of HBV exposure in the children and the precise duration of the infection. The closest approximation that could be made is by comparing the viral characteristics of the children with other infected family members [6, 10, 11]. Another limitation was the direct sequencing method for HBV DNA analysis that could not further characterize the viral quasispecies in the subjects with positive HBsAg but having mutation types associated with HBsAg detection failure. However, by performing genotyping/subtyping and comparison of HBV nucleotide sequences, combined with investigation of family relationships, this study could reveal the evidence of multiple transmission patterns of HBV infection among the family members of HBV-infected patients.

In conclusion, this study confirms that children of HBV-related CLD patients are at risk of acquiring hepatitis B infection, despite the negative HBsAg and HBeAg status of their parents. Having both parents with HBsAg positive and the presence of at least two HBV carriers in the household are significant risk factors of intra-familial HBV transmission. This intra-familial spread of HBV with high infection rates in children could contribute to the generation of pools of HBV-infected subjects that may become sources for further transmission to wider communities. This study reiterates that efficient preventive strategies that include routine infant hepatitis B vaccination, particularly the birth dose vaccine, and catch-up immunization targeted to unvaccinated children are of great importance for the children of HBV-related CLD patients.

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Compliance with ethical standards

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Conflict of interest All authors declare that he/she has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by The Committee of Medical Research Ethics of the Faculty of Medicine, University of Indonesia (no. 297/PT02.FK/ETIK/2009).

Informed consent Informed consent was obtained from all individual participants included in the study.

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