Chronic hepatitis B in pregnant women: is hepatitis B surface antigen quantification useful for viral load prediction?


*Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia
*Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta Pusat 10430, DKI Jakarta, Indonesia
*Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

ARTICLE INFO

Article history:
Received 27 July 2015
Accepted in revised form 20 October 2015
Accepted 4 November 2015
Corresponding Editor: Eskild Petersen,
Aarhus, Denmark.

SUMMARY

Background: New cases of hepatitis B virus (HBV) infection continue to occur worldwide. Most of these are due to mother-to-child transmission (MTCT), with maternal viraemia as the most important contributing factor. The hepatitis B surface antigen (HBsAg) level, which correlates positively with viral load, has been used for treatment monitoring in chronic hepatitis B. This study evaluated the usefulness of quantitative HBsAg for viral load prediction in HBsAg-positive pregnant women.

Methods: A total of 943 pregnant women in Makassar, Indonesia, were screened for HBsAg. Sixty-four women were HBsAg-positive and investigated. HBsAg level and hepatitis B e antigen (HBeAg)/hepatitis B e antibody (anti-HBe) status were determined serologically. Viral load was measured by real-time PCR. HBV DNA was sequenced and analysed for identification of genotype and basal core promoter (BCP)/precore (PC) mutations.

Results: Of 64 subjects, 12 (18.8%) were HBeAg-positive and 52 (81.3%) were HBeAg-negative. HBsAg and HBV DNA levels were significantly higher in the HBeAg-positive group (p < 0.001). HBsAg and HBV DNA levels were positively correlated in the HBeAg-positive group (r = 0.659; p = 0.02), but not in the HBeAg-negative group (r = 0.194; p = 0.168). Low HBsAg levels (<3.0 log10 IU/ml) corresponded with HBV DNA levels <6.0 log10 IU/ml (r = 0.404; p = 0.001), a recognized threshold for MTCT. Genotype C was more prevalent than genotype B, but not associated with HBsAg level, viral load, or HBeAg status. Two-thirds of HBeAg-negative subjects with high HBV DNA levels harboured BCP (A1762T/G1764A) and/or PC (G1896A) variants.

Conclusions: HBsAg levels provide a good viral load predictor in HBeAg-positive but not HBeAg-negative pregnant women. The HBeAg-negative group had a frequent occurrence of BCP/PC variants, which may have contributed to the lack of correlation observed. Samples with a low HBsAg level, which is associated with a low risk of MTCT, do not require HBV DNA measurement.

© 2015 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

More than 240 million people worldwide are chronically infected with the hepatitis B virus (HBV), and about 780,000 die from hepatitis B annually. In highly endemic areas of Asia, the Pacific, and Sub-Saharan Africa, most HBV infection occurs perinatally or during early childhood. This is associated with a high rate of persistent infection and increased risk of morbidity and mortality from cirrhosis and hepatocellular carcinoma later in life.

Efforts in the prevention of hepatitis B have focused on the immunization of infants implemented in 183 World Health Organization (WHO) member states. As of 2012, 94 member states including Indonesia had introduced the hepatitis B birth
dose immunization. This program has markedly decreased the disease burden, carrier rate, and HBV-related morbidity and mortality. However, 50 million new cases of HBV infection continue to be diagnosed annually, with the highest incidence due to mother-to-child transmission (MTCT). Despite the administration of hepatitis B immunization (active or active plus hepatitis B immune globulin (HBIG)) at birth, at least 10% of infants born to HBV-carrying mothers still suffer HBV infection. Several factors such as maternal serum HBV DNA level, hepatitis B e antigen (HBeAg) status, HBV S gene variation, mode of delivery, and neonatal immune deficiency have been related to MTCT. Of these factors, maternal HBV DNA level has been identified as the most relevant. Practice guidelines from major professional associations address the decision for antiviral treatment in pregnant women based on the HBV DNA threshold.

Assays for HBV DNA quantification with high sensitivity and specificity are currently available. However, the routine application of these methods for screening pregnant women is hampered by cost and limited resources. In recent years, the hepatitis B surface antigen (HBeAg) level, which correlates positively with viral load, has been used as a biomarker to predict disease status and to monitor the treatment response in chronic hepatitis B (CHB). The HBeAg level correlates with covalently closed circular DNA (cccDNA) in hepatocytes and can be considered a surrogate marker of infected cells. One attraction of the use of the HBeAg level is that the assay is less costly, is suitable for high-throughput screening, and its platforms are commonly used in many laboratories. However, some studies have reported that HBeAg quantification correlates poorly with the HBV DNA level. HBeAg and HBV DNA levels may vary during different phases of CHB and the correlation is associated with the HBeAg status of the patient. Certain HBV variants with basal core promoter (BCP) A1762T/G1764A or precore (PC) G1896A mutations could influence the synthesis of HBeAg. The presence of these variants needs to be taken into account since they often occur in endemic regions and may be associated with certain HBV genotypes that differ among geographical regions.

This study aimed to evaluate the usefulness of quantitative HBeAg as a viral load predictor in pregnant women with CHB in Makassar, Indonesia, and to analyse the association of the HBeAg level and viral load with the HBeAg status, as well as the molecular characteristics of HBV variants defective for HBeAg production.

2. Materials and methods

2.1. Study population

This cross-sectional study was carried out from January to July 2014 in the antenatal care units of Wahidin Sudirohusodo Hospital, Hasanuddin University Hospital, Fatimah Mother and Child Hospital, Pertwi Mother and Child Hospital, Labuang Baji Hospital, and Ibu Sina Hospital, as well as several maternity clinics, in Makassar, South Sulawesi. A total of 943 pregnant women were screened for HBV infection; 64 of whom were HBeAg-positive and considered eligible for enrolment. For inclusion it was required that the woman had been HBSAg-positive for >6 months without prior antiviral therapy. Subjects co-infected with hepatitis A virus, hepatitis C virus, or human immunodeficiency viruses, as well as those with evidence of liver diseases, were excluded. This study was approved by the Ethics Committee of the Faculty of Medicine Hasanuddin University, Makassar, Indonesia. Written informed consent was obtained from each patient.

2.2. Serological examination

HBSAg status was determined by VIDAS HBSAg immunoassay (bioMérieux SA, Marcy l’Etoile, France). HBeAg and hepatitis B e antibody (anti-HBe) were tested using Monolisa HBeAg-Ab PLUS immunoassay (Bio-Rad, Marnes-la-Coquette, France). HBSAg quantification was done using Elescys HBSAg Quant II (Roche Diagnostics, Indianapolis, USA) on a Roche Cobas e411 Immunoanalyzer following the manufacturer’s protocol.

2.3. HBV DNA detection and analysis

The HBV DNA level was determined from 500 μl of serum by quantitative real-time PCR (Cobas Taqman HBV Test; Roche Diagnostics, Indianapolis, USA) with a range of linearity between 6 and 1.1 × 10^5 IU/ml. HBV DNA for molecular analysis was obtained by extracting the DNA from 140 μl of serum using the QIamp DNA Mini Kit (Qiagen, Valencia, CA, USA) and amplification by nested PCR using primers S2-1/S1-2 for the first-round and S8R/S2-2 for the second-round (Supplementary Material, Table S1). Amplicons were purified using a PCR purification column (Qiagen, Valencia, CA, USA) and subjected to direct sequencing on a DNA sequence analyzer ABI 3130xl (Applied Biosystems, Carlsbad, CA, USA).

The HBV genotype was determined by phylogenetic analysis based on the 226-nucleotide sequences of the S gene compared with 70 reference sequences of known genotypes (A-H) retrieved from GenBank, using Phylip 3.68 software with the Kimura 2-parameter model, neighbour-joining algorithm, and 1000 bootstrapping.

2.4. Identification of BCP and PC mutations

Amplification of BCP and PC regions was done by nested PCR using primers PC1/PC2 for the first-round and S012/S013 for the second-round (Supplementary Material, Table S1). Amplicons were purified and sequenced as described previously. The sequences were aligned with reference sequence M54923 retrieved from GenBank.

2.5. Statistical analysis

The baseline data were summarized descriptively. Continuous and categorical variables were compared between groups using the Mann–Whitney test and Chi-square/Fisher’s exact test, respectively. Pearson’s correlation coefficient was used to describe the correlation between two continuous, normally distributed variables. Spearman’s correlation was used for categorical variables or continuous variables that were not normally distributed.

Statistical analyses were performed using IBM-SPSS v. 20 software (IBM Corp., Armonk, NY, USA). All statistical significance values were assessed at p < 0.05.

3. Results

3.1. Characteristics of study subjects

Among 943 pregnant women attending several antenatal clinics in Makassar, 64 (6.8%) were HBSAg-positive. Of these women, 12 (18.8%) were HBeAg-positive and 52 (81.2%) were HBeAg-negative. HBV DNA levels were significantly higher in the HBeAg-positive group (median 7.43 log_{10} IU/ml) than in the HBeAg-negative group (median 1.55 log_{10} IU/ml) (p < 0.001). Similarly, HBSAg levels were significantly higher in the HBeAg-positive group (median 4.21 log_{10} IU/ml) than in the HBeAg-negative group (median 2.91 log_{10} IU/ml) (p < 0.001). Age, alanine aminotransferase (ALT) levels, and HBV genotype distribution were comparable in the two groups (Table 1 and Supplementary Material Table S2).
Table 1
Baseline characteristics of HBsAg-positive pregnant women

| Parameter                          | Overall (n = 64) | HBeAg-positive (n = 12) | HBeAg-negative (n = 52) | p-Value
|-----------------------------------|-----------------|-------------------------|-------------------------|---------
| Age (years)                       | 29 (18-42)      | 28.5 (22-42)            | 30 (18-41)              | 0.564   |
| ALT (IU/L)                        | 24.5 (9-129)    | 27 (16-129)             | 24.5 (9-68)             | 0.129   |
| HBV DNA log_{10} IU/ml            | 1.71 (0.78-8.05)| 7.43 (1.54-8.05)        | 1.54 (0.78-6.48)        | <0.001  |
| HBeAg log_{10} IU/ml              | 3.03 (0.70-4.11)| 4.21 (3.25-4.91)        | 2.91 (0.70-4.11)        | <0.001  |
| Ratio HBeAg/HBV DNA log_{10} IU/ml| 1.25 (0.40-3.30)| 0.61 (0.56-7.356)       | 1.43 (0.90-2.78)        | <0.001  |
| Genotype (n = 47)                 | 8 (17.0%)       | 2 (22.2%)               | 6 (15.8%)               | 0.889   |

HBsAg, Hepatitis B e antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus; HBeAg, hepatitis B surface antigen.

* Results are reported as the median (minimum-maximum), or number (percentage of detected samples).

** Comparison between HBeAg-positive and HBeAg-negative groups (Mann-Whitney U-test or Chi-square test).

3.2. Distribution of serum HBV DNA levels among HBsAg-positive pregnant women according to HBeAg status

HBV DNA levels were categorized into <3.0 log_{10} IU/ml (close to 3.3 log_{10} IU/ml or 2000 IU/ml, which is the threshold to define inactive carrier state of CHB), 3.0-6.0 log_{10} IU/ml, and >6.0 log_{10} IU/ml (a level associated with HBV immunoprophylaxis failure). The proportions of pregnant women with HBV DNA levels <3.0 log_{10} IU/ml, 3.0-6.0 log_{10} IU/ml, and >6.0 log_{10} IU/ml in the HBeAg-positive group were 9% (1/12), 25% (3/12), and 67% (8/12), respectively; in the HBeAg-negative group, the proportions were 83% (43/52), 13% (7/52), and 4% (2/52), respectively (Figure 1A).

3.3. Distribution of serum HBeAg levels among the HBsAg-positive pregnant women according to HBeAg status

HBeAg levels were categorized into <3.0 log_{10} IU/ml (a level associated with a lower risk of CHB outcomes), 3.0-4.0 log_{10} IU/ml, and >4.0 log_{10} IU/ml. The proportions of subjects with HBeAg levels <3.0 log_{10} IU/ml, 3.0-4.0 log_{10} IU/ml, and >4.0 log_{10} IU/ml were 0% (0/12), 42% (5/12), and 58% (7/12), respectively, in the HBeAg-positive group, and 58% (30/52), 35% (18/52), and 8% (4/52), respectively, in the HBeAg-negative group (Figure 1B).

3.4. Correlation between HBeAg and HBV DNA levels

In all 64 HBsAg-positive subjects, serum HBeAg and HBV DNA levels showed a significantly moderate correlation (r = 0.513; p < 0.001) (Figure 2A). When analysed separately according to HBeAg status, there was a strong correlation between HBV DNA and HBeAg levels in the HBeAg-positive group (r = 0.659; p = 0.02) (Figure 2B), but no correlation was observed in the HBeAg-negative group (r = 0.194; p = 0.168) (Figure 2C).

There were four subjects (M150, M414, M415, and M818) with high HBeAg levels (>4.0 log_{10} IU/ml) but low HBV DNA levels (<3.0 log_{10} IU/ml). In contrast, there were four subjects (M167, M173, M258, and M810) with low HBeAg levels (<3.0 log_{10} IU/ml) but who had moderate viremia (3.0-6.0 log_{10} IU/ml). However, in most cases, low levels of HBeAg were associated with low levels of HBV DNA; HBeAg levels <3.0 log_{10} IU/ml were significantly correlated to HBV DNA levels <3.0 log_{10} IU/ml (r = 0.363; p = 0.003) and to HBV DNA levels >6.0 log_{10} IU/ml (r = 0.404; p = 0.001). No subjects with HBeAg levels <3.0 log_{10} IU/ml had HBV DNA >6.0 log_{10} IU/ml (Figure 3).

3.5. HBeAg and HBV DNA levels in genotype B and C

HBV genotype was successfully determined in 47 pregnant women based on the S gene sequences (sequencing was not possible in the other subjects because of insufficient HBV DNA content). The sequences generated have been deposited in the GenBank database (accession numbers KP241791-KP241837). Median HBeAg and HBV DNA levels were not significantly different in each genotype. Genotype C was more prevalent than genotype B (83% vs. 17%), but the proportions were comparable between the HBeAg-positive and HBeAg-negative groups (Figure 4).

3.6. Subgroup analysis for BCP and PC mutations in HBeAg-negative pregnant women

Sequencing of the BCP/PC region of the HBV genome was performed successfully in 12 HBeAg-negative subjects (sequencing was not possible in the other subjects because of low HBV DNA content). The sequences generated have been deposited in GenBank (accession numbers KP241838-KP241844 and KP241846-KP241850). Four subjects had the wild-type HBV DNA sequence at both the BCP and PC sites, four had the BCP mutation alone, and two had the PC mutation alone. Concurrent BCP and PC mutations were detected in two subjects. All subjects with the BCP mutation had HBV genotype C, while the PC mutation was detected in two subjects with genotype B and one subject with genotype C (Table 2).

4. Discussion

This study represents one of few reports of HBV infection in pregnant women from Indonesia. Of 943 pregnant women attending several antenatal clinics in Makassar, 64 (6.8%) were HBsAg-positive. This figure is higher than that reported recently from Jakarta (2.2%),20 and other places in Indonesia reported around 1985 (4.7% in West Java, 1.9% in Bali, 3.4% in Matalam).21,22 The wide variation in HBV infection rates may be associated with the general HBsAg prevalence in Indonesia (3.4-19.5%), geographical variation, and differences in cultural practices, as well as the methods used to detect HBV infection.23,24 This fact is of concern, because it occurs in pregnant women who tend to be in the immune-tolerant phase of CHB with normal physical/laboratory examinations and high-level viremia, but unaware of their HBeAg-positive status.

Varying thresholds of maternal HBV DNA have been discussed in association with MTCT and immunoprophylaxis failure. Wise- man reported that immunoprophylaxis failure occurred in infants when the maternal viral load was >8 log_{10} copies/ml (>1.7 x 10^7 IU/ml).25 Zou et al. showed that the immunoprophylaxis failure increased with higher levels of maternal HBV DNA.18 When the mothers’ HBV DNA levels were stratified to <6, 6-9.99, 7-7.99, and >8 log_{10} copies/ml, the corresponding rates of immunoprophylaxis failure were 0%, 3.2%, 6.7%, and 7.6%, respectively, and it was concluded that an antenatal HBV DNA level >6 log_{10} copies/ml (>200 000 IU/ml) was the most important predictor of MTCT.18
The European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL) guidelines recommend treating pregnant women when HBV DNA levels are $>2 \times 10^6$ IU/ml in the third trimester for the prevention of MTCT. In the present study, 15.6% (10/64) of all subjects had HBV DNA levels $>6.0 \log_{10}$ IU/ml, distributed in 67% (8/12) of the HBsAg-positive group and 4% (2/52) of the HBsAg-negative group. The fact that the subjects had a skewed distribution toward the higher levels of HBV DNA should be regarded as important, as this shows a higher possibility of MTCT.

There are few studies on the potential applications of quantitative HBsAg in the management of hepatitis B during pregnancy.27,28 To the best of the authors' knowledge, this is the first study to evaluate the relationship between HBsAg and HBV DNA levels with regard to HBsAg status in pregnant women with CHB in Indonesia. This study revealed that among all 64 HBsAg-positive pregnant women, the HBsAg level was correlated with the HBV DNA level regardless of age and viral genotype. When stratified based on HBsAg status, the correlation was strong in HBsAg-positive pregnant women but missing in the HBsAg-negative women. A possible explanation for this finding is that HBsAg synthesis has a pathway distinct from HBV DNA synthesis and under the influence of different immune-control mechanisms.27,28 HBsAg is present as a component of HBV virions but also as subviral particles, which exceed the number of virions.25 Pregnant women with an HBsAg-positive status could be in the...
immune-tolerant phase where HBV virions and their antigens are minimally subjected to the host immune reaction, while those with an HBeAg-negative status could be in the low-replicative phase of CHB where the number of virions has decreased as a result of successful immune control. Therefore, the reduction in HBsAg levels was not proportional to that of HBV DNA levels. The ratio of HBsAg/HBV DNA reflects the association between HBsAg production and HBV replication; this was significantly higher in HBeAg-negative subjects than in the HBeAg-positive group (median 1.43 vs. 0.61 log_{10} IU/ml, respectively).

Four pregnant women had high HBsAg levels with low HBV DNA. As explained, this could be due to the larger excess of HBsAg over the number of virions. This implies that a high level of HBsAg cannot be used to predict the HBV DNA level. The serum HBsAg level should thus be used together with, but not as a substitute for, HBV DNA. Four other subjects had low HBsAg levels but moderate viremia (>4 log_{10} IU/ml). Possible explanations for the decreased detection of HBsAg include (1) differences in analytical sensitivity and specificity in HBsAg detection of viruses of different genotypes; (2) mutations in the pre-S1 gene that cause HBsAg detection failure; (3) treatment-associated mutations that cause derangement of the P gene with subsequent alteration of the overlapping S gene; or (4) the concomitant presence of hepatitis B surface antibodies (anti-HBs) leading to the formation of immune complexes poorly displaced by HBsAg-capture antibodies. In the majority of subjects, however, low levels of HBsAg (<3.0 log_{10} IU/ml) correlated significantly with low levels of HBV DNA (<6.0 log_{10} IU/ml).

Table 2
<table>
<thead>
<tr>
<th>Subject code</th>
<th>BCP mutation</th>
<th>PC mutation</th>
<th>HBV DNA (log_{10} IU/ml)</th>
<th>HBsAg (log_{10} IU/ml)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>M177</td>
<td>-</td>
<td>+</td>
<td>6.48</td>
<td>3.46</td>
<td>C</td>
</tr>
<tr>
<td>M384</td>
<td>+</td>
<td>-</td>
<td>6.08</td>
<td>3.04</td>
<td>C</td>
</tr>
<tr>
<td>M167</td>
<td>-</td>
<td>+</td>
<td>4.55</td>
<td>2.69</td>
<td>B</td>
</tr>
<tr>
<td>M810</td>
<td>-</td>
<td>+</td>
<td>3.50</td>
<td>2.70</td>
<td>C</td>
</tr>
<tr>
<td>M218</td>
<td>+</td>
<td>-</td>
<td>3.20</td>
<td>3.55</td>
<td>C</td>
</tr>
<tr>
<td>M336</td>
<td>-</td>
<td>+</td>
<td>2.70</td>
<td>3.74</td>
<td>C</td>
</tr>
<tr>
<td>M508</td>
<td>-</td>
<td>+</td>
<td>2.80</td>
<td>3.40</td>
<td>B</td>
</tr>
<tr>
<td>M253</td>
<td>-</td>
<td>+</td>
<td>1.36</td>
<td>3.05</td>
<td>C</td>
</tr>
<tr>
<td>M819</td>
<td>+</td>
<td>-</td>
<td>1.34</td>
<td>4.11</td>
<td>C</td>
</tr>
<tr>
<td>M195</td>
<td>+</td>
<td>-</td>
<td>0.78</td>
<td>2.51</td>
<td>C</td>
</tr>
<tr>
<td>M212</td>
<td>+</td>
<td>-</td>
<td>0.78</td>
<td>3.55</td>
<td>C</td>
</tr>
</tbody>
</table>

Figure 3. Distribution of HBV DNA levels among pregnant women according to HBsAg levels. In most cases, low levels of HBsAg were associated with low levels of HBV DNA. HBsAg levels <3.0 log_{10} IU/ml were significantly correlated to HBV DNA levels <3.0 log_{10} IU/ml (r = 0.363; p = 0.003) and to HBV DNA levels <6.0 log_{10} IU/ml (r = 0.404; p = 0.001). No subjects with HBsAg levels <3.0 log_{10} IU/ml had HBV DNA levels >6.0 log_{10} IU/ml.

Figure 4. Distribution of serum HBV DNA (A) and HBsAg (B) levels in subjects with HBV genotype B (n = 36) and HBV genotype C (n = 39). HBV DNA and HBsAg levels were comparable in the two genotypes. Median values (max, min; log_{10} IU/ml) with 95% confidence intervals are shown.
log10 IU/ml) and an attendant lower risk of MTCT. This result suggests that HBV DNA quantification may not be necessary for pregnant mothers with low HBsAg levels.

Another important finding from this study was the presence of HBeAg-negative pregnant women with high viraemia. These women, with no evidence of liver disease, were apparently in the inactive carriers of CHB. However, some inactive carriers may have high HBV DNA levels accompanied by persistently normal ALT levels. Studies have documented that certain HBV variants with nucleotide substitutions in the PC and/or BCP regions could abolish or down-regulate HBeAg production. These variants may replicate rapidly in HBeAg-negative CHB, where HBsAg and HBV DNA levels are preserved. Analysis for the presence of BCP (A1762T/G1764A) and PC (G1896A) mutations was performed on HBeAg-negative subjects who had HBV DNA levels >2,000 IU/ml. A substantial proportion of isolates analysed, particularly from subjects with HBV DNA levels >6.0 log10 IU/ml, had BCP and PC mutations either alone or in combination. This finding is important because the emergence of these variants frequently occurs in regions with HBV endemcity. Notably, all BCP mutations identified had genotype C, which was prevalent among the subjects studied. It has been recognized that HBV genotype C and BCP mutations are independent risk factors for progression to severe liver disease.

Importantly, limitations of the present study include the relatively small number of pregnant women with chronic HBV infection. Wider clinical and community-based studies in different areas of the Indonesian archipelago will be necessary to estimate the true national burden of HBV infection in pregnant women. Also, the cross-sectional design of the study could not represent the fluctuating profile of CHB. Therefore, measurements of ALT levels are necessary to distinguish the true inactive carriers from active HBeAg-negative individuals. However, most studies have reported that ALT levels are lower during pregnancy and viral load is more likely to increase due to the natural immune suppression processes linked to pregnancy.

In conclusion, this study confirms that serum HBsAg level may be used as a predictor of the serum HBV DNA level in HBeAg-positive pregnant women, but not in HBeAg-negative pregnant women. The measurement of HBV DNA is not necessary if the level of HBsAg is low, as the probability of detecting a high viral load is low. An important role of BCP/PC variants influencing HBeAg status independent of viral load was also identified, providing a cautionary note to the interpretation of negative results of HBeAg tests when classifying HBV-infected individuals. These results offer the promise of practical guidance in using quantitative HBSAg as a tool to better manage HBV-infected pregnant women. Follow-up studies are needed to assess the impacts of the maternal and virological factors discussed in this study on HBV carriage and immunopathogenesis failure in the infants born to these HBSAg-positive mothers.

Acknowledgements

The authors would like to convey special thanks to the pregnant women who participated in this study. The authors would also like to express their gratitude to the Eijkman Institute for Molecular Biology in Jakarta for the molecular work support, the Hasanuddin University Medical Research Center (HUM-RC) for laboratory facilities, several maternity clinics in Makassar, and the Wahidin Sudirohusodo Teaching Hospital, as well as the Academic Health Center of Hasanuddin University with their staff for the recruitment of study subjects. Special thanks are given to Dr Kevin Baird from the Eijkman-Oxford Clinical Research Unit in Jakarta for proofreading this manuscript.

Funding: This study was supported by an Operational Support Grant for State University (BOPTN) from the Directorate General of Higher Education, Indonesian Ministry of Education (grant number 2058/UN4.20/PL.09/2013). PT Roche Indonesia donated the reagents used for HBsAg quantification.

Conflict of interest: No conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jiud.2015.11.002.

References


