Perinatal HBV viremia in newborns of HBsAg(+) mothers is a transient phenomenon that does not necessarily imply HBV infection transmission.

Vana Papaevangelou (Greece)
National and Kapodistrian University of Athens
Hepatitis B virus MTCT in the era of passive-active immunoprophylaxis

- **Failure of immunoprophylaxis**
  - 5%–10% of infants of HBsAg+ mothers
  - Recent meta-analysis (>7500 Chinese babies):
    - 4.87% in infants born to HBsAg(+) and 9.66% in infants born to HBeAg(+) mothers respectively

- **Why?**
  - HBV intrauterine infection
    - have an established infection at birth
  - Perinatal transmission post poor adherence to administration of immunoprophylaxis and/or timely administration of HBV vaccination

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Lin X et al PIDJ 2014
Hepatitis B virus MTCT in the era of passive-active immunoprophylaxis

- Transplacental (in-utero) transmission has been associated with:
  - HBeAg (+) mother,
  - **High maternal HBV DNA** (>10^6 copies/mL),
  - High maternal HBsAg titer,
  - HBV genotype B versus C,
  - male fetus, amniocentesis, pregnancy complications or prolonged labor,
  - antigenemia in siblings
Diagnosis of HBV infection in infants

- HBsAg (+) infants for > 6months

Shao ZJ, et al. JMV 2011
Chen T et al. BMC ID 2013
Diagnosis of HBV infection in infants

• HBsAg (+) infants for > 6 months

• HBsAg/HBeAg/HBV DNA positivity in the cord blood (? contamination)

• HBV seromarkers and HBV DNA in venous blood persist in older infants?

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Positive HBV markers at birth do not necessarily indicate in-utero transmission

- 385 neonates born to HBsAg (+) mothers followed for 8-12 months.
- Femoral vein (FV) and umbilical cord (UC) blood samples obtained before immunoprophylaxis.
Positive HBV markers at birth do not necessarily indicate in-utero transmission

- 385 neonates born to HbsAg (+) mothers followed for 8-12 months.
- Femoral vein (FV) and umbilical cord (UC) blood samples obtained before immunoprophylaxis.
- Immunoprophylaxis failure: 4.4% (17/385); all born to HBeAg(+) mothers whose HBV-DNA were > 6 log 10 copies/mL.
- Only 4/17 with high HBV-DNA at birth; In-utero infection less prevalent than appreciated??
Positive HBV markers at birth do not necessarily indicate in-utero transmission

- 385 neonates born to HbsAg (+) mothers followed for 8-12 months.
- Femoral vein (FV) and umbilical cord (UC) blood samples obtained before immunoprophylaxis.
- Immunoprophylaxis failure: 4.4% (17/385); all born to HBeAg(+) mothers whose HBV-DNA were > 6 log 10 copies/mL.
- Only 4/17 with high HBV-DNA at birth; In-utero infection less important than appreciated??
- HBV markers at birth cannot diagnose or exclude MTCT
Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mothers: 27% HBeAg (+), most high HBV-DNA levels
- All babies received combined immunoprophylaxis
- Neonates were found at birth: 28% HBsAg (+); 16% HBV-DNA(+) and 24% HBeAg(+)

**Figure 1** Correlation of HBsAg and HBV DNA between mothers and newborns. HBsAg(+) and HBV DNA(+) rates of the infants at birth in different levels of maternal HBsAg titer (A) and HBV DNA load (B) groups.
Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mother: 27% HBeAg (+), 26% high HBV-DNA levels
- Neonates were found at birth: 28% HBsAg (+); 16% HBV-DNA(+) and 24% HBeAg(+)

Figure 2 HBV DNA(+), HBsAg(+), HBeAg(+), anti-HBc(+), and anti-HBs(+) rates in infants. Positive rates of (A) HBV DNA, HBsAg, and HBeAg and (B) anti-HBc and anti-HBs at birth, 1 mo, 7 mo and 12 mo.

Chen T et al BMC ID 2013
Is there a marker that may identify HBV infected infants?

- 148 HBsAg(+) mother-infant pairs; 94% HBV genotype C
- Mother: 27% HBeAg (+), 26% high HBV-DNA levels
- Neonates were found at birth: 28% HBsAg (+); 16% HBV-DNA(+) and 24% HBeAg (+)
- Immunoprophylaxis failure: 9 infants (6.1%)

Table 3 Positive likelihood ratio of diagnostic indicators for chronic HBV-infected infants

<table>
<thead>
<tr>
<th>Diagnostic indicators</th>
<th>N</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Positive likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>False positive ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>True positive ratio</td>
<td></td>
</tr>
<tr>
<td>HBV DNA(+) at birth</td>
<td>21</td>
<td>9</td>
<td>9/9 = 1</td>
<td>12</td>
</tr>
<tr>
<td>HBsAg(+) at birth</td>
<td>41</td>
<td>9</td>
<td>9/9 = 1</td>
<td>32</td>
</tr>
<tr>
<td>HBV DNA- and HBsAg- positive at birth</td>
<td>18</td>
<td>9</td>
<td>9/9 = 1</td>
<td>9</td>
</tr>
<tr>
<td>Anti-HBs(−) at 1 month old</td>
<td>9</td>
<td>9</td>
<td>9/9 = 1</td>
<td>0</td>
</tr>
</tbody>
</table>

Chen T et al BMC ID 2013
Diagnosis of HBV infection in infants

• HBsAg (+) infants for > 6months
• HBsAg / HBV DNA positivity in the cord blood (? contamination)
• HBV seromarkers and HBV DNA in venous blood persist in older infants?

• Some of these infants may represent occult HBV infection?
• Definition: HBsAg (-) and HBV DNA (+)

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Diagnosis of HBV infection in infants

• HBsAg (+) infants for > 6 months
• HBsAg / HBV DNA positivity in the cord blood (? contamination)
• HBsAg / HBV DNA in venous blood and persists after the age of >3 months

• Some of these infants represent occult HBV infection?
• Definition: HBsAg (-) and HBV DNA (+)
• Most infants achieve protective levels of anti-HBs antibodies
• Most infants do NOT develop anti-HBc (+) antibodies

Shao ZJ, et al JMV 2011
Chen T et al. BMC ID 2013
Are these infants with true occult HBV infection? Are they really infected?
Occult HBV infection in immunized neonates born to HBsAg(+) mothers

Prospectively followed 32 infants diagnosed with OBI at 7 months: HBsAg(-)/anti-HBsAg(+) but HBV-DNA (+)

<table>
<thead>
<tr>
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<th>12 months (N=32)</th>
<th>24 months (N=32)</th>
<th>36 months (N=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA (+)</td>
<td>8/32 (25%)</td>
<td>7/32 (22%)</td>
<td>2/26 (7.7%)</td>
</tr>
<tr>
<td>Median HBV –DNA level (log IU/mL)</td>
<td>1.81 (1.28–2.91)</td>
<td>1.94 (1.23–2.58)</td>
<td>1.74 (1.59–1.89)</td>
</tr>
<tr>
<td>Anti-HBs (+)</td>
<td>30/32 (93.8%)</td>
<td>21/30 (70%)</td>
<td>14/17 (82.4%)</td>
</tr>
<tr>
<td>Median anti-HBs (mIU/mL)</td>
<td>239.2 (127.1–450.2)</td>
<td>26.7 (8.4–32.5)</td>
<td>34.3 (17.6–67.1)</td>
</tr>
<tr>
<td>Anti-HBc (+)</td>
<td>22</td>
<td>5</td>
<td>2</td>
</tr>
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- **Timely administration of the 1st dose of vaccine within 6 hours of birth** reduced the OBI rate from **38.2%** (13/34) to **15.3%** (19/124) (**p = 0.003**).
- No correlation with maternal HBeAg (+), HBsAg titers or HBV-DNA levels.
- No vaccine escape mutants found.
- **HBV infection is controlled in immunized infants**

Lu Y et al PLoS ONE 2016
Adequate levels of anti-HBs after vaccine and HBIG immunoprophylaxis eventually may clear the virus

• Prospectively followed 17/21 children with documented occult HBV infection post passive-active immunoprophylaxis

• At mean age 6.57 ± 2.75 years:
  • All remained HBsAg (-)
  • 16/17 were HBV DNA (-)
  • Two children developed anti-HBc antibodies
  • One child remained HBVDNA(+) with low viremia (50 copy/mL), carried the G145R mutation

Sadeghi A et al. JVH 2016
Pande C et al JVH 2013

Pande et al reported that development of anti-HBs >10 IU/mL at 18 weeks of age was associated with clearance of occult HBV infection
“Transient” occult HBV infection in immunized infants born to HBsAg(+) mothers

- HBsAg was detected in 3/77 (3.9%) babies.
- HBV DNA was detected in 28/77 (36.4%) HBsAg(-) infants
- The frequency of OBI decreased with age:
  - 48.4% <6 months to 18.2% at ≥12 months of age (p=0.06).

Zhou S et al. JMV May 2017

\[ p < 0.001 \]
"Transient" occult HBV infection in immunized infants born to HBsAg(+) mothers

Zhou S et al. JMV  May 2017
Population based study assessing OBI prevalence in <18yo

Table 3. Estimated Rates of OBI in HBsAg-Negative Subjects With or Without Infant Hepatitis B Immunization

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Prevalence rate of specific HBV marker profile in total enrolled children population in previous serosurveys, %

Prevalence rate of OBI in children with or without anti-HBc positivity who were sampled for analysis in the present study

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<td>HBsAg-negative children#</td>
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Hsu HY et al Hepatology 2014
Population based study assessing OBI prevalence in <18yo

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Rate of OBI in HBsAg (-) but anti-HBc (+) subjects§, %
- Rate of OBI in HBsAg (-) but anti-HBc (-) subjects¶, %

Estimated frequency of OBI per $10^4$ HBsAg-negative children#


Hsu HY et al Hepatology 2014
Population based study assessing OBI prevalence in <18yo

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Prevalence rate of OBI in children with or without anti-HBc positivity who were sampled for analysis in the present study

Rate of OBI in HBsAg (-) but anti-HBc (+) subjectsⅰ, %                                                                 | 1.7                  | 4.8               |

Rate of OBI in HBsAg (-) but anti-HBc (-) subjectsⅱ, %                                                                | 1.8                  | 0                 |

Estimated frequency of OBI per 10⁴ HBsAg-negative childrenⅳ                                                       | 160.7                | 11.5              |

Conclusions

• Breakthrough infections in immunized subjects seem to result in OBI while in unvaccinated subjects natural infection will ensue.

• In the postvaccination era, the presence of anti-HBc is a very useful marker for OBI screening in HBsAg-negative subjects.

• HBsAg(-) due to a very-low-level viral replication and HBsAg expression?
HBV viremia in newborns of HBsAg(+) predominantly Caucasian HBeAg(−) mothers

- HBV-DNA detected in 73.4% of the mothers (93% HBeAg-).
- HBV-DNA (+) detected in 27/109 (24.7%) newborns
  - 3/8 (37.5%) of HBeAg(+) mothers
  - 24/101 (23.8%) of HBeAg(-) mothers (p=0.39)
- No association with maternal viremia or maternal VL
- No association with mode of delivery
- Association between maternal HbeAg status and level of neonatal viremia.

Papaevangelou V et al JCV 2011
HBV viremia in newborns of HBsAg(+) predominantly Caucasian HBeAg(−) mothers

Upon follow-up

• At 9 months of age:
  • all children were HBsAg(-)
  • 97.2% had developed anti-HBs antibodies

• At 24 months of age:
  • all OBI re-examined were HBV DNA (-);
  • one child had developed anti-HBc antibodies

Papaevangelou V et al JCV 2011
What is the pathogenesis of HBV-DNA (other seromarkers) detection in infants?

- Using high sensitivity real time PCR, we are able to detect low levels of viremia that do not cause infection?

- Placenta leakage of maternal non-infectious antigens (Dane particles)?

- Maternally derived HBV infected PBMCs transferred?

- Perinatal transmission that is cleared by the infant post prophylaxis?

- HBV cccDNA long persists in hepatocytes, resulting in intermittent viraemia?

Shao Q et al. Arch Gynecol Obstet 2013
Are there any implications of the HBV-DNA detection in infants?

• Clinical significance not clear
• Infants develop adaptive cell mediated immunity
• Few children with persistent OBI

• Responses to vaccination?
  • Neonatal HBV viremia in HBsAg(−) infants is clinically important has been associated with vaccination failure

Badur S et al. JID 1994
Shi I et al ZEKZZ 2006
Conclusions

• Need to better differentiate:
  • Immunoprophylaxis failure (HBV infection)
  • Occult HBV infection (HBsAg-/HBV-DNA+/anti-HBc)
  • Transient HBV-DNA viremia

• Most likely these 3 outcomes are:
  • “exposure dependent” or
  • “infant immune response dependent”? the role of HBIG?
  • different stages of HBV infection?
Thank you for your attention
Backup slides
Administration of HBIG and occult infection

• Randomized 259 newborns to receive vaccine versus vaccine + HBIG
• 81% of mothers HBeAg(-)
• At 18 weeks of age 64% infants had OBI infection (HBsAg-/HBV-DNA+)
• OBI significantly more common in the HBIG group:
  • 76/106 (72%) versus 66/116 (57%); p = 0.025.
• Development of anti-HBs (+) at 18 weeks of age was associated with clearance of HBV-DNA in babies with occult HBV infection.
• At 24 months of age 42% infants had OBI infection

Pande et al. JVH 2013
Population based study assessing OBI prevalence in <18yo
Hsu HY et al Hepatology 2014

Table 3. Estimated Rates of OBI in HBsAg-Negative Subjects With or Without Infant Hepatitis B Immunization

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Prevalence rate of OBI in children with or without anti-HBc positivity who were sampled for analysis in the present study
Rate of OBI in HBsAg (-) but anti-HBc (+) subjects, %
Rate of OBI in HBsAg (-) but anti-HBc (-) subjects, %
Estimated frequency of OBI per 10^4 HBsAg-negative children
Conclusions

• Breakthrough infections in immunized subjects seem to result in OBI while in unvaccinated subjects natural infection will ensue.

• In the postvaccination era, the presence of anti-HBc is a very useful marker for OBI screening in HBsAg-negative subjects.

• A very-low-level viral replication and HBsAg expression, rather than surface gene mutations that may escape detection by HBsAg screening assays, is the major mechanism related to OBI.

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Estimated frequency of OBI per 10^4 HBsAg-negative children#

160.7 11.5

Hsu HY et al Hepatology 2014
The role of HBeAg

• Transplacental maternal HBeAg may induce immunologic tolerance in utero, thereby facilitating MTCT of HBV infection.

• HBeAg induces specific unresponsiveness of helper T cells to HBcAg and HBeAg in the neonates born to HBeAg (+) mothers.

• Therefore not only transmission but also chronicity rates of perinatal HBV infection increase:
  • 28.2% (17.4%–33.9%) in infants born to HBeAg(-) mothers
  • 64.5% (53.5%–100%) in infants born to HBeAg (+) mothers.

ZX Li et al. Emerging Microbes and Infections 2015.
Maternal viremia

- **Elesfsiniotis1**: Overall, 1.156% of women were HBsAg(+) and the majority of them (71.3%) were Albanian. The prevalence of HBsAg was 5.1% in Albanian women, 4.2% in Asian women and 1.14% in women from Eastern European countries. The prevalence of HBsAg in African (0.36%) and Greek women (0.29%) was very low. Only 4.45% of HBsAg (+) women were also HBeAg(+) whereas the vast majority of them were HBeAg(-)/anti-HBe(+). Undetectable levels of viremia (<200 copies/mL) were observed in 32.26% of pregnant women at labor and 29.03% exhibited extremely low levels of viral replication (<400 copies/mL). Only two pregnant women exhibited extremely high serum HBV-DNA levels (>10 000 000 copies/mL), whereas 32.26% exhibited HBV-DNA levels between 1 500 and 40 000 copies/mL.

- **Elesfsiniotis2**: Seroprevalence of HBsAg in 26,746 women at reproductive age in Greece and evaluation of HBeAg/anti-HBe serological status as well as serum HBV–DNA levels in a subgroup of HBsAg(+) women at labor. Only 2.67% of HBsAg(+) women were HBeAg(+). Of a subgroup of women in labor with available serum samples 28.6% had undetectable levels of viremia (<200 copies/ml) and 15.9% had extremely low levels of viral replication (<400 copies/ml). Only 12.7% of pregnant women evaluated at labor exhibited extremely high serum HBV–DNA levels (>10,000,000 copies/ml) whereas 42.8% of them exhibited HBV–DNA levels between 1500 and 40,000 copies/ml.

- **Conclusions**: The HBeAg(−)/antiHBe(+) serological status is a finding observed in the vast majority of HBsAg(+) women of our study population, and a significant percentage of them (approximately 44.5%) exhibit extremely low or even undetectable levels of viral replication at labor, suggesting possibly that only a proportion of HBsAg(+) women in Greece exhibit an extremely high risk of vertical transmission of the infection.

- Despite the predominance of HBeAg-negative serological status, about one-third exhibit significant (>10 000 copies/mL) or even extremely high (>10 000 000 copies/mL) viral replication levels at perinatal period, basically due to precore mutation (G1896A) of the HBV genome, a mutation that is frequently observed in the Mediterranean basin.
HBV intrauterine infection rates

Fig.1. HBV intrauterine infection rates of the infants born to mothers with different characteristics.
Outline

• Epidemiology/ evidence of viremia – studies
• How it happens – risk factors (genotype?, cs?)
• Why transient – evidence
• Innocent?
  • Association with infection
  • Association with immune response to vaccine
  • Implications for use of HBIG
  • Implications for deferred - delayed HBV vaccination
• Future research
Incidence of Acute Hepatitis B, by Age Group — United States, 2000-2014

Reported cases/100,000 population

Year

National Notifiable Diseases Surveillance System (NNDSS)

ACIP 2016
What is the role of HBsAg escape variants?

- HBsAg variants do not play a major role in OBI pathogenesis.
- “Breakthrough infections caused by S-gene mutants are occasionally reported but do not pose a serious threat to the established vaccination programs.”

Romano et al HVI 2015
Shahmoradi S et al J Hepatology 2012