(RESEARCH ARTICLE)

Blood HBV DNA in newborns from HBsAg-positive mothers might be predictive of immunization response

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Abstract

Background: In Ivory Coast, as in the rest of sub Saharan Africa, despite the introduction of anti-hepatitis B vaccine, toddlers and children remain a segment of the population frequently infected with Hepatitis B Virus (HBV). The conditions of early HBV transmission, and notably the materno-fetal route remain summarily documented in sub Saharan Africa.

Methods: From June to November 2018, we analyzed the socio-demographical, serological, and viral features related to HBV transmission in expectant mothers and their newborns who came for delivery at the CHU Cocody Abidjan.

Results: Out of 1628 contacted expectant mothers, a subset of 508 accepted to participate to the study, including 163 who were positive for HBsAg. All 56 mothers who transmitted either HBsAg or HBV DNA to their babies were presenting HBV DNA loads >5.0 log10 IU/mL Remarkably, fates of newborns either positive for HBsAg or HBV DNA who completed immune-prophylaxis were drastically different at 9-12 months. While a majority (90%) of HBsAg (+)-only babies became anti-HBs (+), 62.5% DNA-positive newborns failed to mount a proper anti-HBs response. Overall, 10.7% of newborns with at least one positive HBV marker at birth develop a *bona fide* chronic infection at 9 months.

Conclusions: In Ivory Coast, pregnant women presenting HBV DNA loads above 5.0Log10 IU/mL are at risk of maternofetal transmission. Presence of HBV DNA in the blood of newborns represents a better predictor of anti-HBV immunization failure than HBsAg presence. An adaptation of immunization procedures with a passive immune prophylaxis targeting specifically newborns at risk should be urgently considered.

Keywords: HBV; Sub-Saharan Africa; Viral load; Materno-fetal transmission.

1. Introduction

Persistent infection with the hepatitis B virus (HBV) still represents a very significant burden of severe pathologies and deaths in Sub-Saharan Africa and the Western part of this continent remains more affected than any other African sub-regions [1]. Reasons explaining precisely this situation are barely documented but could be plausibly linked either to a historically high endemic circulation of HBV in the region, to a greater infectivity of the local HBV genotype (mostly genotype E), or to the precarious hygiene conditions that prevails in a group of countries overall characterized both by a lower human development index than in the rest of Africa and by a low level of health expenditure *per capita* [2-5].

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Regarding HBV transmission, it is commonly transmitted by exposure to contaminated blood products, by sexual intercourse, by poorly defined horizontal transmission through casual familial contacts or quite often in the perinatal period, due to pre-delivery micro-transfusions and at delivery by contamination in the genital tract of the mothers [6]. The moment of lifespan at which transmission occurs is paramount as it conditions the capacity of the virus to install a persistent infection. It is considered that early infection with HBV i.e. *in utero*, at birth (pseudo-vertical transmission), or during the first 4 to 5 years of life, is at risk to become lifetime persistent in 70-90% of cases [7]. This situation is presumably due to the immaturity of children's immune system and to the intrinsically immune tolerant nature of the liver that has to accommodate the massive pro-inflammatory signals received during the establishment of the gut microflora in the first weeks of lifespan [8, 9]. For West African countries, it is, thus, a strategic aim for Public Health to break early life transmission of the virus, as young chronic carriers represent a renewed HBV reservoir that will sustain high local endemic levels [10]. Furthermore, and despite a usually mild disease, young patients are generally more infectious than older ones due to the commonly higher viral replication and corresponding DNA loads in their bloodstream [11].

In sub-Saharan Africa, the role played by vertical *sensu stricto* or perinatal transmission of HBV is still a matter of debate with authors considering that it is a major route of transmission while, for others, intra-household transmission through casual contacts between toddlers and infected members of the family is, in reality, preponderant [12, 13]. Vertical or perinatal transmission of HBV has been studied in great detail in Eastern Asia and it came to the conclusions that *in utero* infections are infrequent but that natural delivery represents a significant risk of infection when HBV DNA loads of the mother are high (>5.3log10 IU/mL). In such circumstances, the treatment of mothers with an antiviral before delivery is recommended. Within 24h following birth, administration to the newborn of anti-hepatitis B immunoglobulins (100IU) and of a dose of anti-hepatitis B vaccine will provide efficient protection against perinatal and subsequent HBV transmission risks [14]. This procedure suffers, however, from a certain proportion of failure that occurs essentially when a heavy HBV DNA burden affects the expectant mother and overflows preventive measures.

In West Africa, surveys of perinatal HBV transmission are scarce [15]. It remains nevertheless necessary to better define who among pregnant women is at risk to transmit the virus in order to make mothers and their babies benefit from the most appropriate prophylactic treatment. With the aim to better characterize clinical and biological determinants of perinatal HBV transmission in West Africa, we analyzed a series of 163 pregnant women seropositive for HBV surface antigen (HBsAg) together with their 169 babies.

2. Patients and methods

2.1. Patients

Between June and November 2018, a total of 1628 women came at the prenatal consultation in the Gynecology department of the Cocody University Hospital. A subset of 508 (31.2%) pregnant mothers accepted to participate to the study, including 163 (32.0%) who were positive for Hepatitis B surface antigen (HBsAg) and prospectively recruited. It is this latter group of expectant mothers that is exclusively analyzed in the present report. All mothers gave their written informed consent to participate in the study. None of the HBsAg (+) expectant mothers received antiviral therapy during pregnancy. The 163 seropositive mothers gave birth to 165 newborns as there were six twin pregnancies and four stillbirths. This study was approved by the National Committee for Ethics and Research (N/Réf:144/MSHP/CNER-kp), and complied with the rules of the Helsinki Convention. According to the guidelines applicable on the Ivorian territory at that time, all babies born from HBsAg (+) mothers received a first dose of anti-hepatitis B vaccine within 24h following birth. Anti-HBs immune globulins are not available in Côte d'Ivoire. Each newborn enters subsequently the regular vaccination program at 6, 10 and 14 weeks and benefits from a post-vaccination check-up at 9 months of life. Serological status of the babies including the search for AgHbs, anti-HBs immunoglobulin and total anti-HBc are recommended in this occasion. All babies with anti-HBs above 10 IU/mL are considered immune to viral hepatitis B.

2.2. Serological and viral analyses

Blood sampling of the mothers took place before delivery. Newborn's blood samples were collected before receiving the first dose of the anti-hepatitis B vaccine. The blood samples were centrifuged to collect 250 to 500 µl of sera and/or plasma which were stored at -80°C before further analyzes. Markers of infection with HBV (HBsAg, total anti-HBc) were investigated in neonates using Cobas® 6000 (Roche) and Architect plus i1000sr (Abbott). Viral loads were determined in mothers using the Roche CAPCTM automaton. Viral DNA was extracted from plasma using an Invitrogen kit according to the manufacturer's recommendations. The DNA extracts were eluted in tubes and stored at -20 ° C before PCR analysis with the GenAmp PCR System 9700 (Applied Biosystems, USA) using a 25 µL reaction mixture.

A nested PCR was carried out to amplify and genotype HBV strains. This method uses an in-house primer pair for outer PCR primers and the method of Farazmandfar *et al*. [16] for the inner PCR and genotype determination steps. Primers are listed in Table 5.

2.3. Statistical analyses

Data collection were performed by using a standardized questionnaire and statistical analyses were done with a Prism 8.1.2 statistical package (San Diego, CA). Numerical variables were summarized by their median, mean and range according to their distribution types. Comparisons were made either with a Student T-test or a Mann-Whitney test as appropriate. Categorical variables were summarized as frequencies that were compared using Fischer exact test. All tests were two-sided and the level of significance was set at $p<0.05$.

3. Results

The mean age of the 163 HBsAg (+) expectant mothers was 27.4 ± 4.9 years. Demographical characteristics of participants are described in Table 1. In brief, a minor subset of them did not benefit from any schooling (20.8%). Occupations of the mothers were primarily housewives (34.3%) and shopkeepers (29.4%). A large majority has Ivorian citizenship (>80%) and the more represented ethnolinguistic groups were Akan (33.1%) and Mandé (28.8%) distantly followed by Gur languages-speaking women (19.6%). The most prevalent risk of infections was excision (27.6%) followed by the sharing of different categories of objects (weaving tools and toiletries, 17.7%, Table 2). Taken together, all iatrogenic risks (surgery, transfusion, dental care, cesarean section) of infection reached 20.0% of cases. No notion of familial hepatitis was recovered among participants.

Table 1 Demographical features of HBsAg (+) mothers

Age (Years, mean±SD)	27.4±4.9
Education (%)	
No schooling	20.8 (34/163)
Primary school	19.6 (32/163)
Secondary school	44.2 (72/163)
University	14.7 (24/163)
Professions (%)	
Housewives	34.3 (56/163)
Shopkeepers	29.4 (48/163)
Hairdressers	9.2 (15/163)
Students	7.9 (13/163)
Needlewomen	5.5 (9/163)
Accountants	3.0 (5/163)
Teachers	1.8 (3/163)
Secretaries	1.8 (3/163)
Miscellanous	6.7 (11/163)
Citizenship (%)	
Ivorian	80.3 (131/163)
Burkinabe	7.9 (13/163)
Malian	3.6 (6/163)
Beninese	0.6 (1/163)
Guinean	1.2 (2/163)

Nigerian	3.6 (6/163)
Nigerien	2.4 (4/163)
Languages (%)	
Afro-asian	3.6 (6/163)
Akan	33.1 (54/163)
Gur	19.6 (32/163)
Kru	9.2 (15/163)
Mandé	28.8 (47/163)
Yoruba	3.6 (6/163)
Miscellanous	1.8 (3/163)
Familial situation (%)	
Singles	20.8 (32/163)
Cohabitants	55.8 (91/163)
Married	23.3 (38/163)

Table 2 Risk factors in HBsAg (+)

Infectious Risk factors	% (n)
Excision	27.6 (45/163)
Weaving instruments sharing	9.8 (16/163)
Toiletries sharing	7.9 (13/163)
Surgical antecedents	8.5 (14/163)
Scarification	6.1 (10/163)
Dental treatment	6.1 (10/163)
Cesarean section	5.5 (9/163)
Blood transfusion	1.2 (2/163)
Jaundice antecedents	0.6 (1/163)
Homosexuality	0.0 (0/163)
IV Drug use	0.0 (0/163)
Tatooes	0.0 (0/163)

The mean child number per mother was 1.3±1.6 before the delivery that led to inclusion in the current study (Table 3). The mean pregnancy length was 38.0±2.7 weeks. All women were HBsAg carriers but infection with HIV was infrequent (4.9%). More than 90.0% of mothers were carrying anti-Rubivirus IgG while only 20.0% were immune-reactive to *Toxoplasma*. Mean and median HBV surface antigen levels were 3.5±0.9 log10 IU/mL and 3.6 log10 IU/mL (Inter-quartile range, IQR=2.8-4.2) respectively. The corresponding values for HBV DNA were 4.5±1.3 Log10 IU/mL and 4.2 Log10/mL (IQR=3.7-5.1). All deliveries occurred through natural routes.

Concerning the viral features observed in newborns, 21.2% were seropositive for HBsAg (n=35/165) whereas 10.3% (n=17/165) presenting detectable HBV DNA at birth in their plasma could be scored as intrauterine infections (Table 4). Three newborns (1.8%) were presenting both markers. Overall, 29.6% (n=49/165) of the newborns carried at least one of the two markers investigated. All HBV strains were belonging to genotype E.

**Table 3** Biological features of HBsAg (+)

Clinical features of Expectant mothers	
Gravidity (mean±SD)	**2.8±1.8**
Parity (mean±SD)	1.3±1.6
Pregnancy length (weeks, mean±SD)	38.0±2.7
Blood Groups (%, n)	
A	25.3 (38/150)
B	23.3 (35/150)
AB	9.3 (14/150)
O	42.0 (63/163)
Rhesus	94.6 (142/150)
Serologies (%, n)	
HBsAg(+)	100.0 (163/163)
anti-HBc(+)	100.0 (163/163)
anti-HIV(+)	4.9 (8/163)
anti-Rubella virus	91.3 (137/150)
anti-*Toxoplasma gondii*	20.0 (30/150)
anti-*Treponema pallidum*	0.0 (0/150)
HBsAg quantification (Log10 IU/mL, mean±SD)	3.5±0.9
HBV DNA (Log10 IU/mL, mean±SD)	4.5±1.3

Table 4 Clinical and biological features of Newborns

Clinical features of Newborns	
Sex ratio M:F	1.3 (96:73)
Stillbirth (%, n)	2.3 (4/169)
Twins (%, n)	3.7 (6/163)
Cranial perimeter (cm, mean±SD)	32.0±2.3
Birthweight (g, mean±SD)	2944±634
Size (cm, mean±SD)	48.2±3.4
APGAR score 5' (mean±SD)	7.6±1.5
HBsAg(+) (in %)	20.7 (35/169)
anti-HBc(+) (in %)	99.4 (168/169)
HBV DNA (%)	10.0 (17/169)

Table 5 Sequences of primer pairs used for nested PCR

| Primer IDs	Séquences d'ADN (3'-5')	Positions	Polarité
MD-S	CCGCGTCGCAGAAGATCTCAATC	2417-2440	Sens
MD-R	CCGGAACTGGAGCCACCAGCAGG	56-79	Anti-sens
Common1-F (A,D,E)	AGTATTCCTTGGACTCATAAGGTGG	2457-2481	Sens
E1-R (E)	CTAGGGGCAAATATTTCGTAGAGA	2659-2682	Anti-sens
D1-R (D)	AGGTGTCCTTGTTGGGATTGTAA	2948-2970	Anti-sens
A1-R (A)	GGCAGGAGGAGGAATTGTTGA	3118-3138	Anti-sens
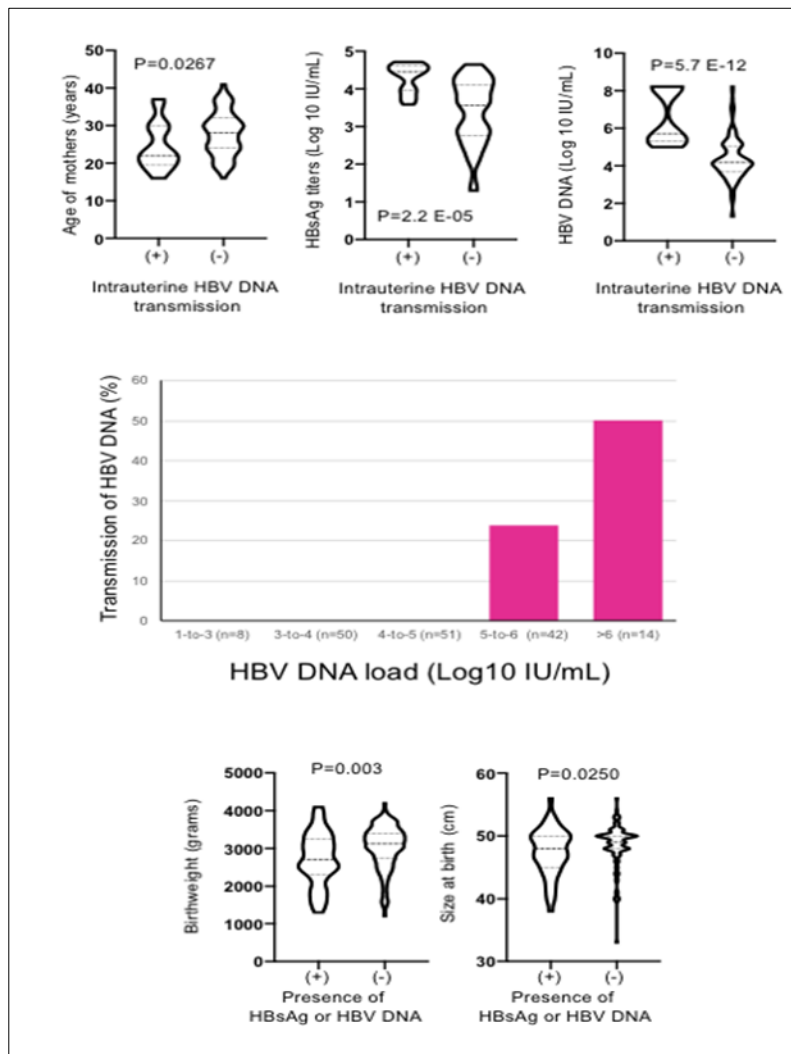
Figure 1 Intra-uterine transmission of HBV DNA according to age of mothers, AgHbs title and Birthweight

We decided to explore the clinical and/or biological bases explaining the passage of HBV markers in newborns bloodstream. We first intend to characterize the maternal features associated with the presence of either HBsAg or HBV DNA in the plasma of newborns. No socio-demographical features or medical antecedents were associated with the early transmission of HBV biomarkers from mother to child. While HBsAg sero-reactivity of newborns was not associated with any variables in mothers, in the case of HBV DNA detection in the plasma of the baby, various clinical and biological traits were different in the transmitting mothers and non-transmitting ones. Mothers of HBV DNA (+) newborns were four years younger (24.5±6.2 years vs 28.0±6.0 years, P=0.0267) and tended to be more often, albeit

non-significantly, anti-HIV (+) (17.6 vs 4.1%, OR=4.83, 95%CI=0.6792-28.1649, P=0.0604, ns). We observed that none of them was carrying anti-toxoplasma IgG (0.0% vs 25%, P=0.0223). Parameters defining HBV infection were markedly more severe in these mothers. In mothers who transmitted HBV DNA, circulating HBsAg levels were significantly higher (4.2±0.4 Log10, IU/mL vs 3.4±0.8 Log10 IU/mL, P=2.2 E-05) just as were HBV DNA loads (6.5±1.4 Log10 IU/mL vs 4.3±1.0 Log10 IU/mL, P=5.7 E-12). The rate of transmission was 24% for mother carrying HBV DNA loads between 5.0 to 6.0 log 10 IU/mL, but it was as high as 50% for those above 6.0log10 IU/mL (see figure). All mothers who transmit HBV DNA presented loads above 5.0 Log10 IU/mL (100 000 IU/mL). Overall, above 5.0 log10 IU/mL, the transmission rate of HBV DNA was 30.3%.

Concerning the clinical consequences for the newborns, positivity for either HBsAg or HBV DNA were associated with a lower birthweight (2720±703g vs 3035±582g, P=0.003) and a slightly lower body size (47.4±3.7cm vs 48.6±3.3cm, P=0.0490).
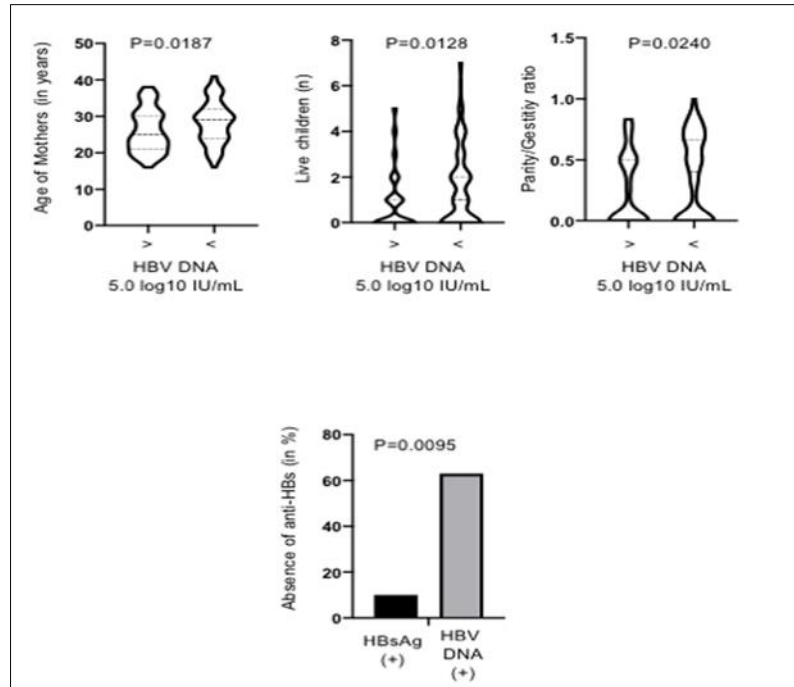
Figure 2 HBV DNA log evolution according to age of mothers, parity/gestity ratio and live children

HBV DNA load above 5.0 log10 IU/mL was thus the most important parameter conditioning viral transmission from mothers to newborns. This subset of expectant mothers represents in our study a bit more than one-third of the series (n=56/163, 34.3%). We thus wonder whether these women differ from other participants for any of the socio-demographical, ethnolinguistic or clinical parameters. The mean age of mothers with HBV DNA>100 000 IU/mL was three years lower than other mothers (25.5±5.8years vs 28.2±5.8years, P=0.0187) (Fig1). For these mothers, the number of already born live children before the investigated delivery was lower than in other women (0.9±1.3 vs 1.5±1.7, P=0.0128). Interestingly, the ratio of successful pregnancies (ratio parity/gravidity) was lower for mothers of the upper median for HBV DNA loads (0.24±0.29 vs 0.36±0.33, P=0.025). Interestingly, both age (Spearman r=-0.21, P=0.0054), parity (Spearman r=-0.23, P=0.0028), and the ratio of successful pregnancies (Spearman r=-0.22, P=0.0032) were inversely correlated with HBV DNA loads. As expected, circulating HBsAg levels were significantly higher in the upper median for HBV DNA (4.3±0.3 Log10 IU/mL vs 3.6±0.7 log10 IU/mL, P=5.9 E-22). Finally, we observed that Akan ethnicity tended to be, albeit not significantly, more represented in the upper median of HBV DNA loads (42.8% vs 28.2%, P=0.0791, ns).

We next wonder whether each of the marker values (HBsAg or HBV DNA) would have a predictive value concerning the future status of the newborn concerning HBV persistence later in life (Fig 2). Out of the subset of 49 newborns presenting either marker, one died shortly after birth while 20 others were lost in follow-up. Among the 28 remaining babies brought back for consultation by their parents between 9 and 12 months, 20 (71.4%) were only seropositive for HBsAg, 7 (35.0%) were positive for HBV DNA and a single one (3.6%) was presenting both markers at birth. All newborns completed immune-prophylaxis against hepatitis B. Remarkably, the fates of newborns either positive for HBsAg or HBV DNA at birth were drastically different. A majority (90%, n=18/20) of HBsAg (+)-only babies became

anti-HBs (+) while 2 (10.0%) remained positive for HBsAg. By contrast, among those presenting HBV DNA in their blood at birth, 62.5% (n=5/8) failed to mount detectable anti-HBs response with one of them affected with an overt persistent HBsAg (+) infection (n=1/8, 12.5%). Despite the small size of the series analyzed, the difference in the capability to mount a sufficient anti-HBs response according to the nature of HBV marker in blood at birth was, thus, very significant (OR=15.0, 95%CI=2.1-89.1, P=0.0095) and HBV DNA presence could be considered as a predictor of poor response to vaccination. Unfortunately, we did not have the possibility to check the response to anti-HBV vaccine in a series of newborns without HBV DNA in plasma despite a birth from mothers with HBV DNA>5.0log10 IU/mL. However, four newborns with HBsAg in plasma but no HBV DNA and born from mothers with HBV DNA>5.0log10 IU/mL were received in consultation between 9 and 12 months of age. All of them (n=4/4) developed a satisfying anti-HBs response post-immunization. This observation suggests that HBV DNA presence in the plasma of newborns is a better predictor of poor response to immunization than high maternal viral loads.

Overall, 10.7% (n=3/28) of newborns who presented at least one HBV biomarker at birth and completed the immunization schedule, were chronically infected between 9 and 12 months of age.

4. Discussion

Our study involved 1628 women i.e. a population similar to previously published studies conducted in sub-Saharan. Indeed, a total of 2105 mothers-pairs child were included in Togo (2017) [17], 743 participants in Tanzania (2020) [18] and 240 mother-child pairs in Burkina-Faso (2018) [19]. The respective importance of HBV transmission routes is still a matter of debate in sub-Saharan Africa with authors considering the intrauterine transmission represents the major mode of transmission while others estimate that perinatal (mostly *per partum*) or even subsequent horizontal transmission between various members of the household is predominant [13, 15, 20]. It is, however, possible that, due to the varieties of conditions of living, HBV strains virulence and hosts susceptibility, these different routes are present but unevenly important throughout the different regions of sub-Saharan Africa. To try to answer this question, we explored the conditions of intrauterine HBV transmission in a large Maternity department of West Africa.

The rate of HBsAg seroprevalence at birth in Abidjan (21.2%) in newborns from HBsAg (+) mothers was lower than the rate observed in Libya (60.9%) on a small series of cases (n=23) and similar to that measured in Upper Egypt (28%) [21, 22]. In our study, HBV DNA loads in mothers were a crucial parameter conditioning viral DNA transmission to newborns while it was not the case for HBsAg titers. We observed that all mothers who transmit HBV DNA present HBV DNA load threshold above 5.0 Log10 IU/mL (100 000 IU/mL). This observation confirmed several other studies performed on transmitting mothers and conducted in Canada (median viral load=5.6 log10 IU/mL), China (>6.0-6.9 log10 IU/mL) or Australia (>8.0 log10/IU/mL) than emphasized the importance of high HBV DNA loads [24-28]. It suggests, nevertheless, that the threshold for virus transmission is lower from several logs in sub Saharan Africa than in geographic locations like Eastern Asia or North America. The transmission rate (30% when HBV DNA>5.0log10 IU/mL) was, however, below the levels observed by Candotti *et al*. in Ghana (55% when HBV DNA>4.0log10 IU/mL *versus* 15.9% in our series) but in keeping with the observation of Sellier *et al.* on a multiethnic cohort in Paris (27% for HBV DNA>5.0log10 IU/mL) [23-30].

Our study revealed that HBV DNA presence in the blood of newborns is a better predictor of anti-HBV immunization failure than HBsAg presence in newborns plasmas and possibly than high HBV DNA loads (>5.3log10 IU/mL) in mothers. This observation is of special interest in countries with limited resources where all newborns from HBsAg (+) mothers could not have access to the costly passive immune prophylaxis with anti-HBs. Albeit more investigations are needed, especially on newborns without any detectable HBV biomarkers at birth, our data indicate that it might be possible to focus on newborns with HBV DNA in plasma (10%) rather than on babies from mothers with HBV DNA >5.0 log10 IU/mL (34%).

Our study suffers, of course, from several weaknesses. Among them, the lacks of HBeAg, anti-HBe measurements are important as they prevent any comparisons with most previous studies that used these biomarkers as read-outs for risk assessment. At the molecular level we did not performed HBV PCR on umbilical cord blood, a technique commonly used to detect intrauterine infection in the past. Finally, we did not have the possibility to perform a PCR targeting HBV DNA on the plasma of the nine months-old infants, a technique that would have informed us about the prevalence of an eventual occult B infection.

5. Conclusion

In conclusion, a subset of Ivorian mothers (34.3%) with high HBV DNA loads (>5.0log10 IU/mL) are at risk of maternofetal transmission of HBV DNA. Although, this marker is not the invariable sign of a future chronic infection, it is associated with a poor response to anti-HBV immunization, and, therefore to a prolonged susceptibility to infection. Overall, when measured at birth, the rate of intrauterine transmission of HBV is grossly in keeping with previous report from the region. However, at 9 months of age, only 10% of infants develop a *bona fide* chronic infection. Further experiments are now warranted to determine whether occult B infection can be found in this population of young patients despite the completion of anti-hepatitis B immunization.

Compliance with ethical standards

Acknowledgement

Disclosure of conflict of interest

No conflict of interest to disclosed.

Statement of informed consent

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

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