Intrahepatic cccDNA and Circulating Hepatitis B Markers among Persons with Untreated HBeAg-negative Chronic Infection in Senegal

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Background

- Liver biopsy is needed to measure covalently closed circular DNA (cccDNA), the template for hepatitis B virus (HBV) transcription. Novel HBV markers in the blood may reflect cccDNA activity and be used to monitor HBV cure.
- We aimed to determine the relationship between intrahepatic HBV cccDNA and plasma HBV DNA, quantitative HBsAg (qHBsAg) and HBV RNA levels in persons with chronic HBV infection from the SEN-B cohort in Senegal

Methods

Study setting and population

- We included 50 HBeAg-negative participants with paired core liver biopsies and serum/plasma samples enrolled in SEN-B.
- SEN-B includes individuals aged >18 years with a positive HBsAg followed at the Infectious and Tropical Disease Service and the Ambulatory Treatment Center at Fann University Hospital, Dakar, Senegal.
- We excluded participants with HIV co-infection and those exposed to HBV antiviral therapy.
- Sociodemographic, clinical and laboratory individual data as well as liver stiffness measurements were collected at the time of liver biopsy.

Laboratory measurements

- **qHBsAg** and plasma **HBV DNA** were measured on site using e411 cobas® and cobas®/TaqMan® (Roche Diagnostic Systems, Basel, Switzerland)
- Circulating **HBV RNA** levels were measured from cryopreserved plasma samples using cobas® 8800 investigational assay (Roche Molecular Systems) with a lower limit of quantification (LLOQ) of 10 copies/mL. Results with detectable target below the LLOQ were assigned an arbitrary value of 5 copies/mL.
- **HBV cccDNA** was extracted from snap-frozen liver biopsies using modified Hirt protocol, followed by ExoV nuclease treatment, and quantified by an HBV specific droplet digital PCR

Statistics

- Participant characteristics were compared between those with and without detectable circulating HBV RNA using the χ^2 test for categorical variables and the Wilcoxon rank-sum test for continuous ones.
- The individual correlation between levels of **intrahepatic cccDNA** and i) plasma **HBV DNA**, ii) **qHBsAg** and iii) circulating **HBV RNA** was assessed using Spearman's rank correlation coefficient.

Results

Characteristics of study population

- Fifty SEN-B participants were included with a median age of 31 years (interquartile range 26-37) and 16/50 (32%) were female
- HBV genotype A was found in 33/50 (66%) individuals and gt E in 17/50 (34%)

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Main findings

- Only one half of antiviral therapy-naïve persons with HBeAg-negative infection had detectable HBV RNA levels in the plasma
- We found a moderate correlation between intrahepatic cccDNA and plasma HBV RNA among HBeAg-negative persons with detectable RNA levels
- No significant correlation was found between plasma HBV DNA or qHBsAg levels and intrahepatic cccDNA.

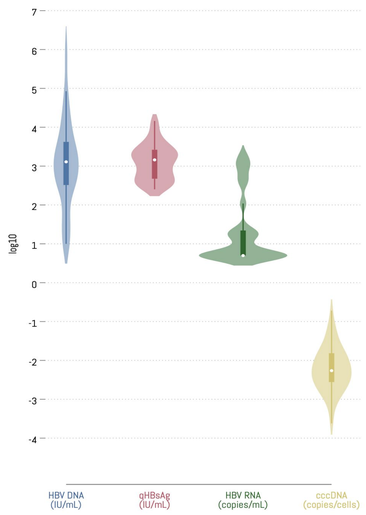

Figure 1: Distribution of plasma HBV DNA, qHBsAg, HBV RNA and intrahepatic cccDNA levels.

Peripheral HBV markers and cccDNA (Figure 1)

- Median intrahepatic cccDNA was 0.0055 copies/cells (IQR 0.0028-0.0153)
- Circulating HBV RNA was detected in 26/50 (52%) participants, of whom 12/50 (24%) had >10 copies/mL.
- HBV DNA >2,000 IU/mL was found in 19/50 (38%) participants.
- Median qHBsAg was 1447 IU/mL (IQR 474-2635) and 28/50 participants had qHBsAg >1,000 IU/mL

Characteristics by circulating HBV RNA levels (Table 1)

- One individual (3%) had ALT >40 IU and 13/50 (26%) had liver fibrosis. The median levels of ALT, LSM and qHBsAg were similar between participants with and without detectable HBV RNA in the plasma
- The proportion of individuals with HBV DNA <20 IU/mL was higher among HBV RNA-negative compared to HBV RNA-positive individuals (17% vs 0%; p<.001)
- Median cccDNA ($\log_{10}$copies/cells) was similar between HBV RNA-negative and positive individuals (p=0.74)

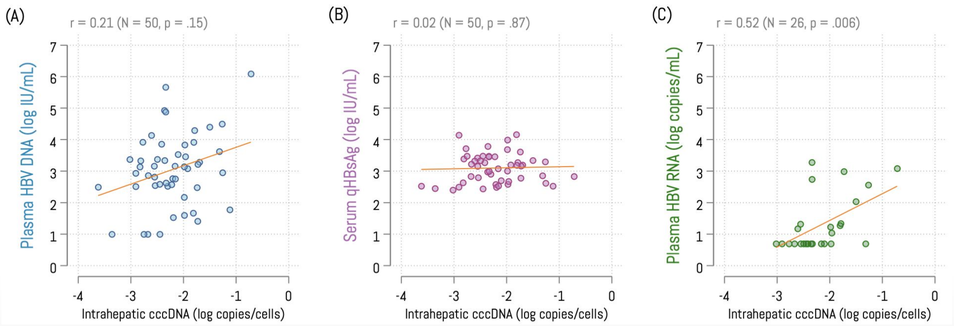

Figure 2: Correlation of intrahepatic HBV cccDNA and (A) plasma HBV DNA (IU/mL), (B) qHBsAg (IU/mL) and (C) plasma HBV RNA (copies/mL) in HBeAg-negative individuals

Table 1: Characteristics of study participants, by plasma HBV RNA levels

Characteristics	HBV RNA negative* N = 24	HBV RNA positive N = 26	p-value
Female sex	9 (38%)	7 (27%)	0.42
Age, years	31 (25-36)	32 (27-37)	0.36
ALT, IU	17 (14-22)	19 (16-24)	0.31
<40	24 (100%)	25 (96%)	0.33
41 - 80	0 (0%)	1 (4%)	
LSM, kPa	5.5 (4.5-6.1)	5.7 (5.0-6.6)	0.25
<7.0	22 (92%)	21 (81%)	0.27
7.1 - 11.0	2 (8%)	5 (19%)	
HBV DNA, IU/mL	330 (44-1055)	3140 (1390-13700)	<0.001
<20	4 (17%)	0 (0%)	<0.001
20-2,000	18 (75%)	9 (35%)	
>2,000	2 (8%)	17 (65%)	
qHBsAg, IU/mL	880 (362-2945)	1610 (682-2193)	0.47
<50	0 (0%)	0 (0%)	0.08
51-1,000	13 (54%)	9 (35%)	
>1,000	11 (46%)	17 (65%)	
cccDNA, copies/cells	0.0061 (0.0019-0.0136)	0.0046 (0.0028-0.0156)	0.74

*Data are presented as median (IQR) for continuous measures, and n (%) for categorical measures. * plasma HBV RNA levels below the limit of detection.*
Abbreviations. *LSM: Liver stiffness measurements; qHBsAg: quantitative HBsAg; cccDNA: covalently closed circular DNA, kPA: kiloPascals*

Correlation between cccDNA and peripheral HBV markers (Figure 2)

- We observed no significant correlation between the intrahepatic **cccDNA** and plasma **HBV DNA** levels (r=0.21, p=0.15)
- We did not find any correlation between intrahepatic **cccDNA** and serum **qHBsAg** levels (r=0.02, p=0.87).
- There was a moderate positive correlation between **cccDNA** and total plasma **HBV RNA** among persons with detectable plasma **HBV RNA levels** (r=0.52, p=0.006)

Conclusions

- Our study provides some of the first data on the relationship between intrahepatic and peripheral HBV markers in sub-Saharan Africa.
- One half of participants with HBeAg-negative chronic infection had undetectable HBV RNA despite detectable HBV DNA in the plasma.
- HBV RNA was the only circulating marker which correlated with intrahepatic cccDNA in our well-characterized group of persons with HBeAg-negative HBV infection in Senegal.
- Further research is needed to better understand the underlying mechanisms and implications of this correlation.

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