Journal of Clinical Virology 111 (2019) 42–47

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Analysis of serum hepatitis B virus RNA levels in a multiethnic cohort of pregnant chronic hepatitis B carriers

Nishi H. Patel[a], Shivali S. Joshi[a,b], Keith C.K. Lau[a,b], Eliana Castillo[a], Carla S. Coffin[a,b,*]

[a] Department of Medicine, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
[b] Department of Microbiology, Immunology and Infectious Diseases, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

ARTICLE INFO

Keywords:
CHB
Pregnancy
MTCT
Serum HBV RNA

ABSTRACT

Background: Hepatitis B virus (HBV) flares have been reported due to alterations in the immune system during pregnancy. Recent studies in non-pregnant chronic hepatitis B (CHB) carriers have indicated that serum HBV RNA is a novel viral marker to assess treatment response and risk of disease flares.
Objectives: To analyze serum HBV RNA levels in association with established HBV markers in pregnant and/or post-partum CHB carriers.
Study design: In this prospective cohort study, serum and plasma were collected from 46 pregnant and/or post-partum CHB patients. Clinical data included demographics, hepatitis B e antigen (HBeAg) status (Abbott), quantitative hepatitis B s antigen (qHBsAg) levels (Abbott), HBV DNA (Abbott, sensitivity 10 IU/mL), alanine aminotransferase (ALT), liver stiffness measurement (LSM, post-partum), and treatment regime. Serum HBV total RNA and pre-genomic (pg)RNA were quantified using in-house assays, and HBV genotype was determined by direct population sequencing of HBV surface gene. Parametric and non-parametric statistical methods were used for analysis.
Results: In this study, we found that serum HBV total RNA levels correlated with the HBeAg status, HBV DNA, qHBsAg, ALT, and LSM while serum HBV pgRNA levels did not ($p < 0.05$, $N = 46$). Additionally, HBV total RNA & pgRNA levels increased, HBV DNA levels decreased, and qHBsAg levels remained unchanged throughout tenofovir disoproxil fumarate (TDF) treatment ($N = 2$).
Conclusions: The associations between serum HBV total RNA with other validated markers indicates it may be a complementary HBV marker to monitor liver disease and HBV replication during pregnancy.

1. Background

Globally, 2 billion people have been infected with hepatitis B virus (HBV), and 257 million people have been diagnosed as chronic carriers [1–3]. Chronic hepatitis B (CHB) carriers are at a risk of developing hepatocellular carcinoma (HCC) and/or cirrhosis, which has resulted in 1.19 million deaths in 2015 [3]. Approximately 50% of CHB infections are acquired through mother to child transmission (MTCT) [4]]. Therefore, the prevention of MTCT is critical to reduce the global burden of CHB. Expert clinical guidelines recommend that all infants born to HBV-positive mothers should receive HBV immune globulin (HBIG) and vaccine [5]. Despite receiving complete passive/active immunoprophylaxis, some studies have reported that ~9% of newborns still acquire HBV infection, especially in highly viremic mothers [6]]. To further reduce MTCT risk, guidelines from the American and European Association for the Study of Liver Disease (AASLD and EASL) recommend antiviral therapy to mothers with high HBV DNA levels ($> 2 \times 10^5$ IU/mL or 1×10^6 virus copies/mL); yet the Asian Pacific (APASL) guidelines recommend treatment at ˜1-log (10) higher cutoff (i.e., 2×10^6 IU/mL) [7–9].

There are several established HBV viral tests in CHB, including HBV DNA, quantitative hepatitis B surface antigen (qHBsAg), hepatitis B e antigen (HBeAg) status, and genotype [10–14]. Additionally, liver disease is also assessed by alanine aminotransferase (ALT), and non-

Abbreviations: CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; MTCT, mother-to-child transmission; WHO, World Health Organization; HBIG, hepatitis B immune globulin; NUC, nucleos(t)ide analog; TDF, tenofovir disoproxil fumarate; HBV pgRNA, HBV pre-genomic RNA; HBeAg, hepatitis B e antigen; qHBsAg, quantitative hepatitis B s antigen; HBV cccDNA, HBV covalently closed circular DNA; ALT, alanine aminotransferase; LSM, liver stiffness measurement; IQR, interquartile range

* Corresponding author at: Liver Unit, Division of Gastroenterology and Hepatology, Cumming School of Medicine, University of Calgary, 6D21, Teaching, Research and Wellness Building, 3280 Hospital Drive N.W., Calgary, T2N 4Z6 AB, Canada.
E-mail address: cscoffin@ucalgary.ca (C.S. Coffin).

https://doi.org/10.1016/j.jcv.2019.01.002
Received 10 October 2018; Received in revised form 24 December 2018; Accepted 7 January 2019

Table 1
Summary of demographic, clinical, and virological data of the 46 enrolled pregnant/ post-partum CHB participants.

Median Age (IQR, range), years		32 (7.5, 21–43)
Ethnicity	Asian	27/46 (59%)
	African	13/46 (28%)
	Caucasian	3/46 (6.5%)
	Other	3/46 (6.5%)
HBV Genotype[†]	A; B; C; D; E; F	5/46 (11%); 13/46 (28%); 11/46 (24%); 6/46 (13%); 5/46 (11%); 1/46 (2%)
Median ALT (IQR, range), U/L, pregnant vs. post-partum		21 (16, 5–47) vs. 24 (25, 1–111)
Median HBV DNA (IQR, range), log_{10} copies/mL, pregnant vs. post-partum		3.7 (3.31,1.7–9.5) vs.3.4 (1.5, 0.7–7.7)
qHBsAg (IQR, range), log_{10} IU/mL, pregnant vs. post-partum		3.4 (1.53, 0.1–5.1) vs. 3.2 (1.6, 0.1–4.3)
Serum HBV Total RNA (IQR, range), log_{10} copies/mL, pregnant vs. post-partum		2.75 (2.47, 0.20–5.76) vs. 1.11 (0.795, 0.33-1.45)
Serum HBV pgRNA (IQR, range), log_{10} copies/mL, pregnant vs. post-partum		5.52 (1.335, 4.57–7.91) vs. 6.32 (1.335, 5.24–6.59)
HBeAg status, N (%)	**HBeAg positive**	11/46 (24%)
	HBeAg negative	35/46 (76%)
Antiviral therapy, tenofovir disoproxil fumarate (TDF), N (%)		12/46 (26%)
Median Liver Stiffness measurement by transient elastography or FibroScan® (IQR, range), kPa, post-partum		3.6 (1.9, 2.2–8.5)

N, number; IQR, interquartile range.
[†] HBV genotype was unable to be determined in 5 participants.

invasive markers such as liver stiffness measurement (FibroScan®) [15]. However, the ideal serum viral marker should assess activity of HBV intrahepatic cccDNA, levels the resilient intranuclear HBV template. Our prior studies have documented HBV immune (i.e., cytokine) and ALT flares in pregnancy, and HBV DNA correlation with qHBsAg levels, especially in HBeAg positive mothers with HBV DNA levels (i.e., $> 2 \times 10^6$ IU/mL) [16]. Other studies have also noted that qHBsAg may be useful in predicting MTCT risk, hence EASL recommends to test both HBsAg and HBV DNA pregnancy; yet this data has recently been questioned [17,18]. As such, increased understanding of HBV replication and associated risk of host immune mediated or virological flares during pregnancy and post-partum will help guide clinical management in pregnancy.

Currently approved nucleos(t)ide analogue (NUC) therapy is effective at suppressing HBV DNA, yet off-treatment virological flares are common due to minimal NUC effect on intranuclear viral template, HBV covalently closed circular (ccc) DNA [19]. Some data suggest that qHBsAg levels may help predict the risk of viral relapse following NUC therapy discontinuation, but larger studies especially within multi-ethnic / multi-genotype population are needed to confirm this data. In patients with underlying advanced liver disease, HBV reactivation and biochemical flares could lead to decompensation. Recent studies have indicated that serum HBV RNA can be used as a new viral marker to assess HBV cccDNA activity and monitor the risk of viral relapse following NUC discontinuation [20,21]. Serum RNA may also be used to detect recently discovered serum HBV particles with encapsidated pregenomic (pg)RNA [20,22]. In the current study, we aim to analyze serum HBV RNA levels in pregnant and post-partum CHB carriers, and its possible association(s) with established HBV viral markers.

2. Study design

2.1. Patient recruitment and serological tests

Ethics approval for this project has been granted by the University of Calgary Conjoint Health Research Review Board (Ethics ID # 25084). In this study, 46 pregnant and/or post-partum CHB carriers were enrolled. In 7 carriers, blood samples were available in both pregnancy and post-partum for serum HBV total and pgRNA analysis. All the pregnant CHB carriers were assessed in second trimester and followed post-partum (N = 40, including 7 with serial blood samples collected during pregnancy and post-partum). Demographic and clinical data such as age, ethnicity, ALT levels, LSM, HBV DNA (viral load, assessed by clinical real time TaqMan PCR, sensitivity 10 IU/mL or 50 virus copies/mL, Abbott Architect), qHBsAg levels (Abbott Architect), HBeAg status (Abbott Architect), and antiviral therapy regime were collected at baseline for all the enrolled participants except for two post-partum participants (collected while on TDF therapy, **Supplementary Tables 1 & 3**). Liver fibrosis was assessed by liver stiffness measurement (i.e., LSM via Transient Elastography or FibroScan®) for participants during post-partum follow-up.

2.2. HBV genotype determination and quantification of serum HBV total RNA and pgRNA

HBV genotype was determined in 41/46 (89%) participants by commercial line probe assay (Inno-LiPa HBV Genotyping, Fujirebio Europe N.V.) or in-house nested PCR using primers specific to the HBV surface gene, followed by Sanger sequencing, according to previously published protocols (**Supplementary Table 2**) [23]. Serum HBV total RNA quantification was completed using Rapid Amplification of Complementary DNA (cDNA) Ends technique using primers and probes complementary to poly-A region in HBV X gene (N = 46, **Supplementary Table 2**) [24]. HBV pgRNA quantification was completed for all the participants using primers and probes complementary to HBV basal core promoter (N = 46, **Supplementary Table 2**) [25]. Serial dilutions of a plasmid with a single copy of HBV genome were used as the standard (1×10^0 - 1×10^7 copies/mL) [24].

2.3. Statistical analysis

The collected data was analyzed using paired *t*-test, Wilcoxon Mann-Whitney test, multiple linear regressions, Pearson's correlation test, and correlation *t*-test ($p < 0.05$ considered significant).

3. Results

3.1. Summary of demographic and clinical data

Demographic and clinical data of the 46 enrolled participants are summarized in Table 1. In total, 10/46 of the participants started TDF therapy after baseline blood collection was performed; and 2 participants were on TDF therapy when post-partum blood collection was performed (not included in the main data analysis, **Supplementary Tables 1 & 3**). The median age of all the participants was 32 years (IQR 7.5, range 21–43, Table 1). 59% of all the participants were of Asian ethnicity, 28% were African, 6.5% were Caucasian and 6.5% were other (Table 1). Of the 46 participants, 35 (76%) were HBeAg negative, and

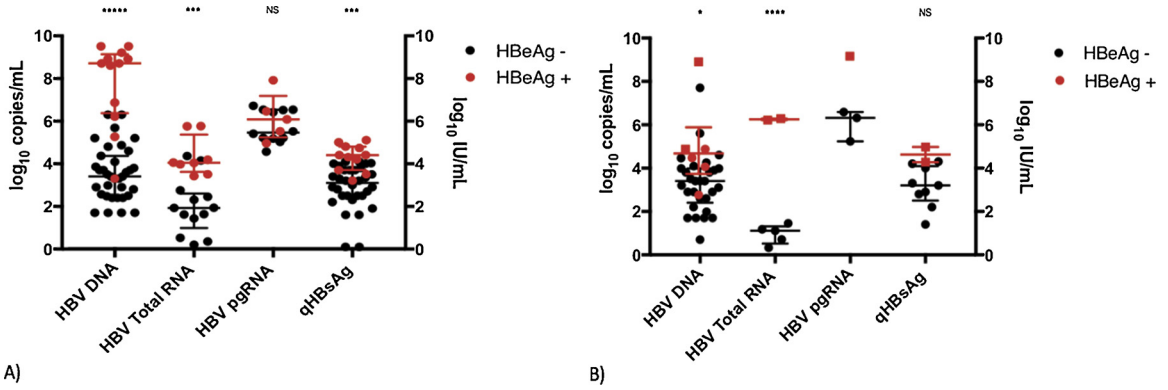

Fig. 1. Comparison between HBV DNA, serum HBV total RNA & pgRNA, and qHBsAg levels in HBeAg negative (black) and HBeAg positive participants (red) in (A) pregnant (N = 40) and (B) post-partum CHB carriers (N = 13, the squares represent 2 participants on TDF therapy). HBV DNA levels were available in 22 pregnant CHB carriers during their post-partum follow-up visit. The left y-axis is for HBV DNA, HBV total RNA, and HBV pgRNA levels. The right y-axis is for qHBsAg levels. Medians with IQR bars are shown. Two tailed t-test was performed, where NS = non-significant ($p > 0.05$), *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$, and *****= $p < 0.00001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

11 (24%) were HBeAg positive (Table 1). HBV DNA, qHBsAg, and ALT levels were comparable in pregnancy vs. post-partum (Table 1). Serum HBV total RNA levels were higher in pregnancy than post-partum, but serum HBV pgRNA levels were lower (Table 1).

3.2. HBeAg status correlates with serum HBV total RNA and pgRNA and qHBsAg in pregnancy

Serum HBV total RNA and pgRNA levels were analyzed in relation to HBeAg status, HBV DNA, and qHBsAg levels for pregnant and post-partum CHB carriers (two tailed *t*-test, $p < 0.05$, Fig. 1). In pregnancy, HBeAg status correlated with HBV DNA, serum HBV total RNA, and qHBsAg levels. In post-partum, HBeAg status correlated with HBV DNA and serum HBV total RNA, but not with qHBsAg levels. Serum HBV pgRNA levels did not correlate with HBeAg status in pregnancy and post-partum.

3.3. Comparison of serum RNA levels in CHB patients with genotype BC vs. ADEF

It was observed that for pregnant CHB carriers (HBeAg negative and positive), serum HBV total RNA, serum HBV pgRNA, HBV DNA, and qHBsAg levels were not significantly different between genotypes BC vs. ADEF (two tailed *t*-test, $p > 0.05$, Fig. 2A-B). However, in HBeAg negative post-partum CHB carriers, serum HBV total RNA levels were significantly different between genotypes BC vs. ADEF (two tailed *t*-test, $p < 0.05$, Fig. 2C).

3.4. ALT, LSM (FibroScan® Score), HBV DNA, and qHBsAg correlations

Pearson's test was performed to determine the overall correlation between serum HBV total RNA and pgRNA levels to established clinical and viral markers (i.e., ALT, LSM, HBV DNA, and qHBsAg) for pregnant and post-partum CHB carriers (Fig. 3A and **Supplementary Fig. 1**, respectively). The ALT levels, LSM, HBV DNA, serum HBV total RNA, and qHBsAg levels of pregnant CHB carriers correlated with one another at varying strengths (Fig. 3A). The serum HBV total RNA levels correlated most strongly with HBV DNA ($r = 0.65$, $p = 0.01$), but showed a lower association to qHBsAg ($r = 0.50$, $p = 0.07$), LSM ($r = 0.43$, $p = 0.12$), and ALT levels ($r = 0.17$, $p = 0.56$; Fig. 3**B–E** respectively). Serum HBV pgRNA levels did not correlate with any other clinical or viral marker in pregnancy. In post-partum samples, only 7 cases had serum RNA/pgRNA measurement, which showed no correlation with other viral / clinical tests (Supplementary Fig. 1). However clinical HBV DNA testing and qHBsAg correlated with ALT, with strong association between ALT levels and LSM for CHB carriers assessed post-partum (Supplementary Fig. 1).

4. Discussion

Current guidelines recommend several established viral (HBV DNA, HBeAg status, HBV genotype) and clinical tests (ALT, liver histology or non-invasive tests, FibroScan®) to determine the need for antiviral treatment and assess liver disease progression [20]–[22]. A number of studies have found that qHBsAg may also be a useful viral marker, especially in predicting the risk of viral relapse following NUC discontinuation, yet the data is limited in certain genotypes, and may be less accurate in CHB carriers due to presence of integrated HBV producing HBsAg [26]. The ideal serum viral marker should assess activity of HBV cccDNA, the resilient intranuclear HBV template.

Recent studies have indicated that serum HBV RNA (total RNA and pgRNA) may be useful complementary tests for HBV clinical management [20,21]. It may be used to detect recently discovered HBV virions with encapsidated HBV pgRNA instead of HBV DNA [20,22]. Despite the promising nature of serum HBV RNA / pgRNA as a potential new HBV viral marker, it has not been analyzed in pregnant and/or post-partum CHB carriers.

In this prospective multiethnic study, serum HBV total RNA and pgRNA levels in pregnant and post-partum CHB carriers were analyzed to determine potential correlations with established HBV viral markers and liver disease tests. We noted a significant correlation between serum HBV total RNA and HBeAg status. Additionally, similar to HBV DNA and qHBsAg, we observed that serum HBV total RNA levels for HBeAg positive pregnant and post-partum participants were higher than that of HBeAg negative participants ($p < 0.05$, Fig. 1), as expected given the wealth of literature in non-pregnant CHB carriers [27,28]. Secondly, it was observed that serum HBV total RNA and pgRNA levels were not significantly different for pregnant HBeAg positive and negative CHB participants with HBV genotypes BC (prevalent in Asia) vs. ADEF ($p > 0.05$, Fig. 2). This finding is not consistent with previously performed studies that have found that serum HBV total RNA and pgRNA levels correlate with HBV genotype in non-pregnant CHB carriers [27,28]. Thirdly, we found that serum HBV total RNA correlates better to other established markers (i.e., HBV DNA, qHBsAg, ALT, and LSM) than HBV pgRNA in pregnant CHB carriers (Fig. 3). Based on correlation studies performed in non-pregnant CHB carriers, significant correlations were expected between serum HBV total RNA levels and HBV DNA, qHBsAg, and ALT levels [27–29]. In addition, we report a significant correlation between serum HBV total RNA levels and LSM, which has not been previously reported.

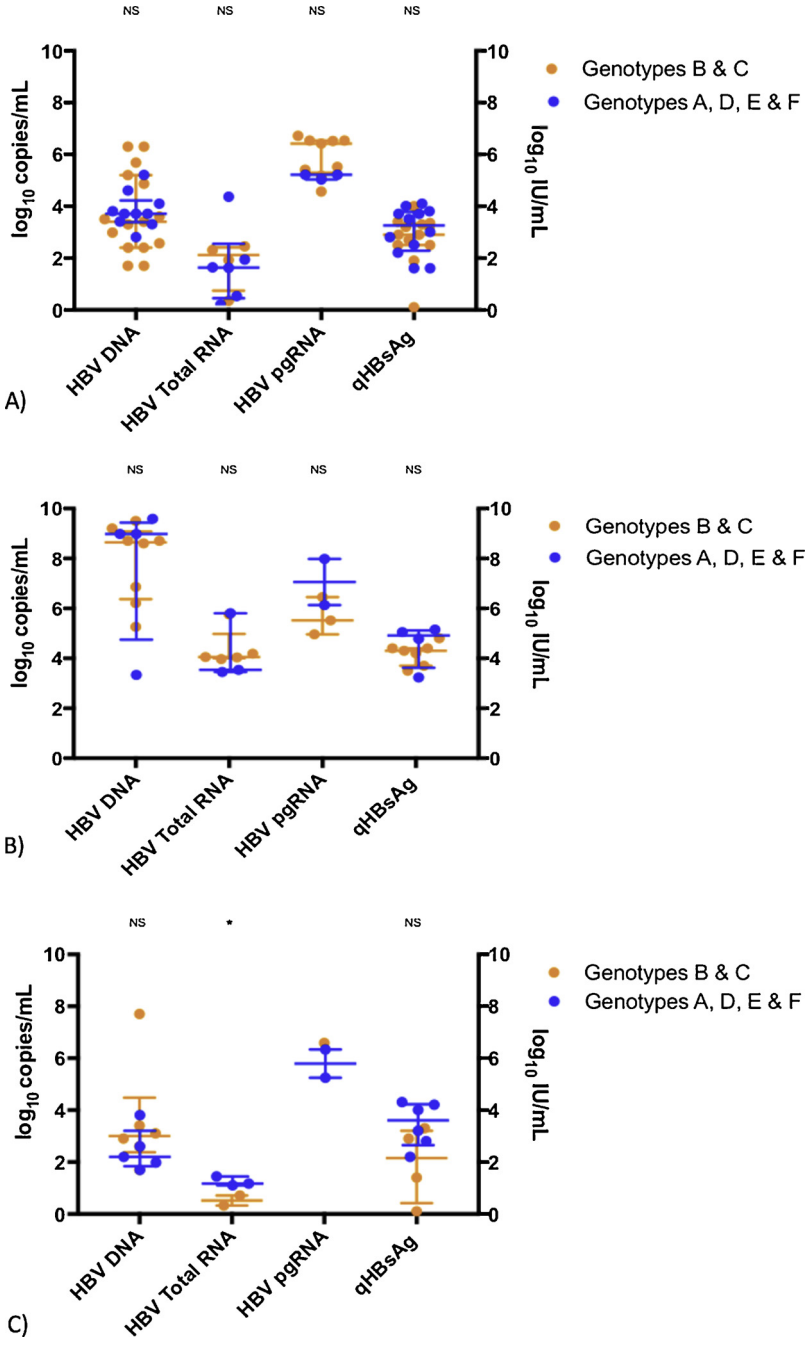

Fig. 2. Comparison of HBV DNA, serum HBV total RNA & pgRNA, and qHBsAg levels between HBV genotypes BC vs. ADEF in A) pregnant HBeAg negative (N = 29, unable to be determined in N = 5), B) pregnant HBeAg positive (N = 11), and C) post-partum HBeAg negative CHB carriers (N = 11). The left y-axis is for HBV DNA, HBV total RNA, and HBV pgRNA levels. The right y-axis is for qHBsAg levels. Medians with IQR bars are shown. Two tailed *t*-test was performed, where NS = non-significant $p > 0.05$ and *$= p < 0.05$. Data for HBV genotype in post-partum HBeAg positive CHB carriers is not shown due low cohort size (N = 2).

In the current study, we found no significant correlations between serum HBV total RNA and pgRNA levels (Fig. 3 and Supplementary Fig. 1), but acknowledge the limited sample size, hence further analysis is recommended. Lastly, for the two post-partum CHB carriers that were on TDF treatment, we observed that serum HBV total RNA and pgRNA levels increased while HBV DNA decreased and qHBsAg levels remained unchanged (**Supplementary Table 1**). This is expected given the known effect of NUC on inhibiting reverse transcription of pgRNA into HBV DNA, but no direct effect on cccDNA and its transcription. In future, further analysis should be performed regarding changes in serum HBV total RNA and pgRNA due to TDF treatment during pregnancy and post-partum. Also, further validation on the relationship between serum HBV pgRNA levels and other markers is required due to the small sample size of pregnant HBeAg positive carriers enrolled in this study (N = 5).

5. Conclusion

Serum HBV total RNA and pgRNA levels have not been analyzed in pregnant and post-partum CHB carriers. In this study, we show that serum HBV total RNA levels correlate with HBeAg status, HBV DNA, qHBsAg, ALT, and LSM. Additionally, HBV total RNA levels are not significantly different between pregnant CHB participants with HBV genotypes BC vs. ADEF. This suggests that serum HBV total RNA has the

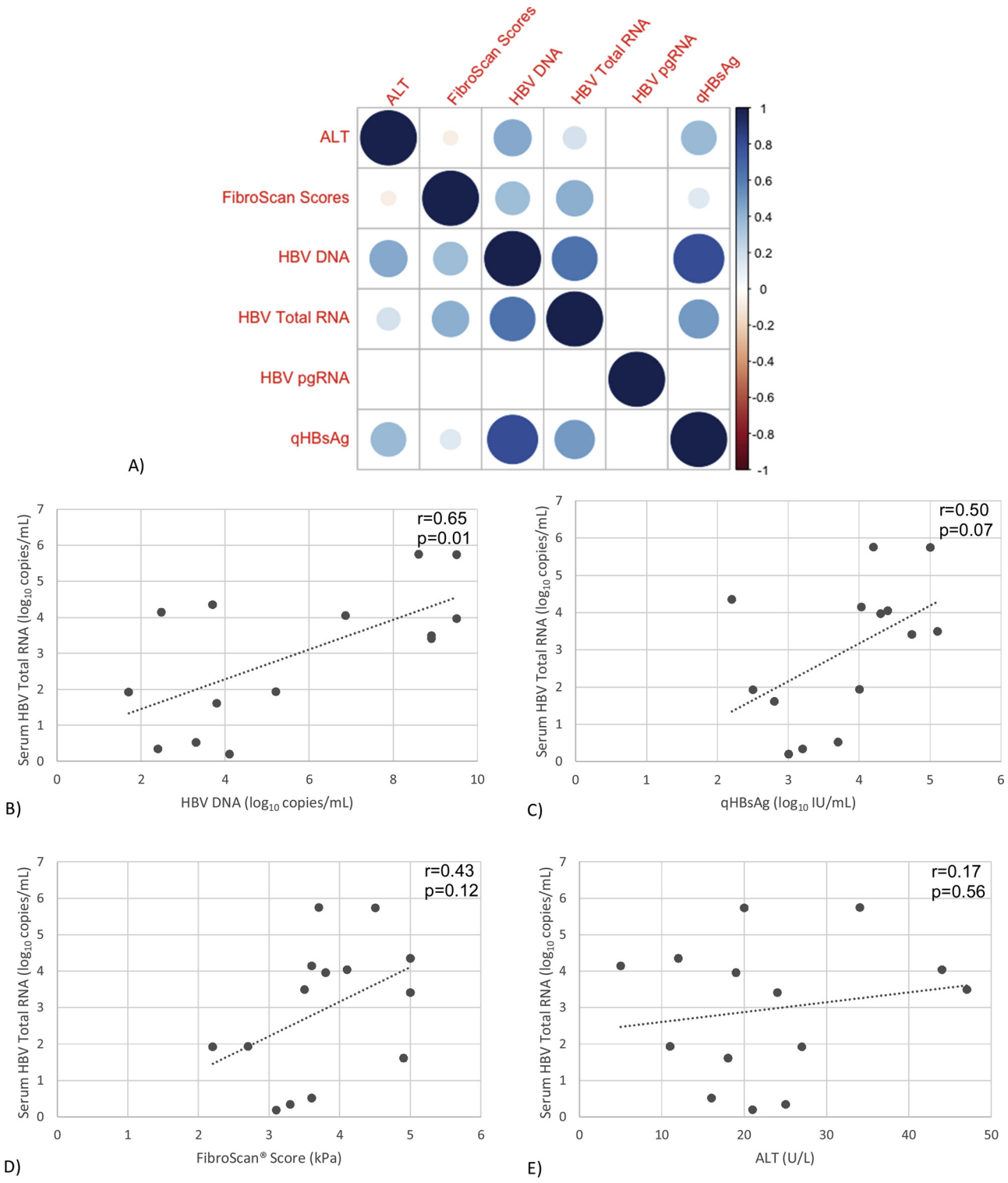

Fig. 3. A) Correlation matrix of virological and clinical markers that were available/ quantifiable for pregnant CHB carriers (N = 14). The circle size represents values for Pearson's test. Blue indicates positive correlation, and red indicates negative correlation. The larger the circle, the greater the p value. B) C) D) E) Scatterplots of serum HBV total RNA levels (y-axis) vs. HBV DNA, qHBsAg levels, Liver Stiffness Measurement (i.e., FibroScan®, kPa), and ALT (x-axis) in pregnant CHB carriers, respectively. r = Pearson's correlation coefficient; p = p value of correlation *t*-test. Overall, serum HBV total RNA correlates with HBV DNA, qHBsAg, ALT, and liver stiffness measurement; whereas serum HBV pgRNA does not. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

potential to serve as a complementary HBV biomarker to monitor and assess HBV replication and liver disease in pregnant CHB carriers.

Conflict of interest

Nishi H. Patel, Shivali S. Joshi, Keith C.K. Lau, Eliana Castillo: No conflicts of interests.

Carla S. Coffin: Investigator Initiated Research Grant/ Research Materials - GSK, Gilead Sciences, Arbutus Biopharma, Bristol-Myers Squibb; Educational Grants - Merck, Gilead Sciences, Janssen Inc. Advisory Board - Merck, Gilead Sciences, GSK; CTPC committee - Springbank Pharmaceuticals; Local Site PI participation in Clinical Trials - Gilead Sciences, Springbank Pharmaceuticals, Transgene.

Ethical approval

Approved. Reference CHREB# 14-1966.

CRediT authorship contribution statement

Nishi H. Patel: Data curation, Formal analysis, Writing - original draft. **Shivali S. Joshi:** Data curation. **Keith C.K. Lau:** Data curation, Writing - review & editing. **Eliana Castillo:** Data curation, Resources, Writing - review & editing. **Carla S. Coffin:** Conceptualization, Project administration, Formal analysis, Supervision, Resources, Funding acquisition, Writing - review & editing.

Acknowledgement

We would like to thank Ms. Gurmeet Bindra for her assistance with patient recruitment.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.01.002.

References

[1] N.P. Nelson, P.J. Easterbrook, B.J. McMahon, Epidemiology of hepatitis B virus infection and impact of vaccination on disease, Clin. Liver Dis. 20 (4) (2016) 607–628.
[2] World Health Organization, Hepatitis, Available at: (2013) http://www.who.int/immunization/diseases/hepatitisB/new_vaccine/en/index3.html.
[3] World Health Organization, Global Hepatitis Report, Available at: (2017) http://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf;jsessionid=F57994D28C4B5BD2EFF94CAF4042A2A3?sequence=1.
[4] B. Navabakhsh, N. Mehrabi, A. Estakhri, M. Mohamadnejad, H. Poustchi, Hepatitis B virus infection during pregnancy: transmission and prevention, Middle East J. Dig. Dis. 3 (2) (2011) 92–102.
[5] M.H. Chang, D.S. Chen, Prevention of hepatitis B, Cold Spring Harb. Perspect. Med. 5 (3) (2015) 1–12.
[6] P. Yi, R. Chen, Y. Huang, R.-R. Zhou, X.-G. Fan, Management of mother-to-child transmission of hepatitis B virus: propositions and challenges, J. Clin. Virol. 77 (2016) 32–39.
[7] N.A. Terrault, A.S.F. Lok, B.J. McMahon, K.-M. Chang, J.P. Hwang, M.M. Jonas, R.S. Brown Jr., N.H. Bzowej, J.B. Wong, Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance, Hepatology 67 (4) (2018) 1560–1599.
[8] European Association for the Study of the Liver, EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection, Hepatology 67 (2017) 370–398.
[9] S.K. Sarin, M. Kumar, G.K. Lau, Z. Abbas, H.L.Y. Chan, C.J. Chen, D.S. Chen, H.L. Chen, P.J. Chen, R.N. Chien, A.K. Dokmeci, E. Gane, J.L. Hou, W. Jafri, J. Jia, J.H. Kim, C.L. Lai, H.C. Lee, S.G. Lim, C.J. Liu, S. Locarnini, M. Al Mahtab, R. Mohamed, M. Omata, J. Park, T. Piratvisuth, B.C. Sharma, J. Sollano, F.S. Wang, L. Wei, M.F. Yuen, S.S. Zheng, J.H. Kao, Asian-pacific clinical practice guidelines on the management of hepatitis B: a 2015 update, Hepatol. Int. 10 (2016) 1–98.
[10] Centers for Disease Control and Prevention, Hepatitis B FAQs for Health Professionals, Available at: (2018) https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm.
[11] Centers for Disease Control and Prevention, Hepatitis B Questions and Answers for the Public, Available at: (2016) https://www.cdc.gov/hepatitis/hbv/bfaq.htm.
[12] C.L. Lin, J.H. Kao, New perspectives of biomarkers for the management of chronic hepatitis B, Clin. Mol. Hepatol. 22 (4) (2016) 423–431.
[13] Z.N.A. Said, An overview of occult hepatitis B virus infection, World J. Gastroenterol. 17 (15) (2011) 1927–1938.
[14] T.C. Tseng, J.H. Kao, Evolution of viral biomarkers in predicting outcomes of chronic hepatitis B patients: from DNA to surface antigen, Tzu Chi Med. J. 25 (2) (2013) 75–81.
[15] R. Huang, N. Jiang, R. Yang, X. Geng, J. Lin, G. Xu, D. Liu, J. Chen, G. Xhou, S. Wang, T. Luo, J. Wu, X. Liu, K. Xu, X. Yang, Fibroscan improves the diagnosis sensitivity of liver fibrosis in patients with chronic hepatitis B, Exp. Ther. Med. 11 (5) (2016) 1673–1677.
[16] S.S. Joshi, D. Wong, E. Castillo, M.G. Swain, C.S. Coffin, Peripartum cytokine flares in a multiethnic cohort of chronic hepatitis B carriers does not correlate with hepatitis B virus suppression or increased risk of liver disease, Am. J. Reprod. Immunol. 78 (4) (2017).
[17] C. Thilakanathan, G. Wark, M. Maley, S. Davison, J. Lawler, A. Lee, N. Shackel, V. Nguyen, K. Jackson, A. Glass, S.A. Locarnini, M.T. Levy, Mother-to-child transmission of hepatitis B: examining viral cut-offs, maternal HBsAg serology and infant testing, Liver Int. 38 (7) (2018) 1212–1219.
[18] T. Kushner, P.A. Shaw, A. Kalra, L. Magaldi, P. Monpara, G. Bedi, K. Krok, S. Centkowski, K. Dalldorf, J. D'souza, D. Halegoua-De Marzio, D.S. Goldberg, S. Trooskin, L.D. Levine, S.K. Srinivas, J.D. Lewis, K.A. Forde, V. Lo Re, Incidence, determinants and outcomes of pregnancy-associated hepatitis B flares: a regional hospital-based cohort study, Liver Int. 38 (5) (2018) 813–820.
[19] M.-L. Chang, Y.-F. Liaw, Hepatitis B flares in chronic hepatitis B: pathogenesis, natural course, and management, Hepatology 61 (6) (2014) 1407–1417.
[20] J. Wang, T. Shen, X. Huang, G.R. Kumar, X. Chen, Z. Zeng, R. Zhang, R. Chen, T. Li, T. Zhang, Q. Yuan, P.C. Li, Q. Huang, R. Colonno, J. Jia, J. Hou, M.A. McCrae, Z. Gao, H. Ren, N. Xia, H. Zhuang, F. Lu, Serum hepatitis B Virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound, Hepatology 65 (4) (2016) 700–710.
[21] M. Tsuge, E. Murakami, M. Imamura, H. Abe, D. Miki, N. Hiraga, S. Takajashi, H. Ochi, C. Nelson Hayes, H. Ginba, K. Matsuyama, H. Kawakami, K. Chayama, Serum HBV RNA and HBeAg are useful markers for the safe discontinuation of nucleotide analogue treatments in chronic hepatitis B infections, J. Gastroenterol. 48 (10) (2013) 1188–1204.
[22] Y.W. Huang, K. Chayama, J.H. Kao, S.S. Yang, Detectability and clinical significance of serum hepatitis B virus ribonucleic acid, Hepatobiliary Surg. Nutr. 4 (3) (2015) 197–202.
[23] T. Deressa, D. Damtie, K. Fonseca, S. Gao, E. Abate, S. Alemu, Y. Aleka, M.G. Swain, G. Van Marle, C.S. Coffin, The burden of hepatitis B Virus (HBV) infection, genotypes, and drug resistance mutations in human immunodeficiency virus-positive patients in Northwest Ethiopia, Public Library Sci. 12 (12) (2017) e0190149.
[24] F. Van Bommel, A. Bartens, A. Mysickova, J. Hofmann, D.H. Kruger, T. Berg, A. Edelmann, Serum Hepatitis B Virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors, Hepatology 61 (1) (2014) 66–76.
[25] F. Lebosse, B. Testoni, J. Fresquet, F. Facchetti, E. Galmozzi, M. Fournier, V. Hervieu, P. Berthillon, F. Berby, I. Bordes, D. Durantel, M. Levrero, P. Lampertico, F. Zoulim, Intrahepatic innate immune response pathways are downregulated in untreated chronic hepatitis B, Hepatology 66 (5) (2017) 897–909.
[26] C.R. O'Neil, S.E. Congly, M.S. Rose, S.S. Lee, M.A. Borman, C.L. Charlton, C. Osiowy, M.G. Swain, K.W. Burak, C.S. Coffin, Long-term follow-up and quantitative hepatitis B surface antigen monitoring in north american chronic HBV carriers, Hepatology Ann. Hepatol. 17 (2) (2018) 232–241.
[27] Y. Li, L. He, Y. Li, M. Su, J. Su, J. Hou, J. Deng, S. Wang, Q. Wang, X. Xu, H. Zhuang, T. Li, Characterization of serum HBV RNA in patients with untreated HBeAg-Positive and -negative chronic hepatitis B infection, Hepat. Mon. 18 (2) (2018) e62079.
[28] M.J.H. Van Campenhout, F. Van Bommel, M. Pfefferkorn, J. Fischer, D. Deichsel, A. Boonstra, A.J. Van Vuuren, T. Berg, B.E. Hansen, H.L.A. Janssen, Host and viral factors associated with serum hepatitis B virus RNA levels among patients in need for treatment, Hepatology (2018), https://doi.org/10.1002/hep.29872.
[29] L. Jansen, N.A. Kootstra, K.A. Van Dort, R.B. Takkenberg, H.W. Reesink, H.L. Zaaijer, Hepatitis B virus pregenomic RNA is present in virions in plasma and is associated with a response to pegylated interferon Alfa-2a and nucleos(t)ide analogues, J. Infect. Dis. 213 (2) (2015) 224–232.